MEIOTIC BEHAVIOUR AND SPERMATOGENESIS IN MALE MICE
HETEROZYGOUS FOR TRANSLOCATION TYPES
ALSO OCCURRING IN MAN

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VOORWOORD

Hèt stukje privé in dit boekwerkje; zoveel te zeggen maar hoe? Laat het voor iedereen bij deze duidelijk zijn dat zonder de kritiese begeleiding van Peter de Boer en mijn promotor Prof. J. Sybenga, dit boekje zo nooit tot stand zou zijn gekomen. Zij zijn het bovendien in de eerste plaats geweest, die indertijd besloten mij voor dit onderzoek aan te trekken en daar ben ik dankbaarder voor dan ik mogelijk de afgelopen jaren heb laten blijken. "Daadwerkelijke en welhaast dagelijkse begeleiding van het promotieonderzoek" als de voorwaarde voor het co-referentschap, als jij dáár niet aan voldaan hebt Peter! Niets menselijks was ons beiden vreemd de afgelopen jaren en hoe leerzaam zijn onze diskussies ook daarover geweest. Ja, boeiend blijft het verschijnsel mens. Mijn promotor heeft zich steeds grote moeite getroost de betoogtrant in de verschillende hoofdstukken kort en helder te houden en mogelijk ben ik daarin geen goede leerling geweest. Ik blijf mijn best doen.

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Vader en moeder, ziehier mijn eerste schreden op 't biologiese pad. Dus toch nog!

Marja, 't viel niet altijd mee, hè?

STELLINGEN

I

In studies met betrekking tot meiotische non-disjunctie moet meer aandacht besteed worden aan celfysiologische parameters.

II

De aanwezigheid van een translocatie multivalent kan een verhoogde non-disjunctie frequentie van de normale bivalenten in de eerste meiotische deling tot gevolg hebben.

dit proefschrift.

III

Tijdens de eerste meiotische deling is de 3:1 segregatie frequentie van een quadrivalent veroorzaakt door een reciproke translocatie tussen een meta- en acrocentrisch chromosoom hoger en de adjacent 2 segregatie frequentie lager dan in het geval de multivalent wordt veroorzaakt door dezelfde translocatie tussen twee acrocentrische chromosomen.

dit proefschrift.

IV

De waarneming dat voor de categorie van bivalenten met 1 chiasma de plaats ervan steeds blijkt samen te vallen met de plaats van overkruising, zichtbaar tussen differentieel gekleurde chromatiden na broomuridine incorporatie in het DNA, is onvoldoende voor de conclusie dat in de mannelijke muis chiasma terminalisatie niet plaatsvindt gedurende de meiose I. De waarneming zal zich ook moeten uitstrekken tot bivalenten met 2 of meer chiasmata, terwijl de invloed van het geïncorporeerde broomuridine op de meiose I dient te worden nagegaan.

Kanda, N. en H. Kato (1980) Chromosoma 78, 113-121.

V

Het gebruik van testsystemen met een hoge spontane meiotische non-disjunctie frequentie voor de studie naar de effecten van endogene en exogene factoren op deze frequentie, vergroot zowel het bruikbare meetgebied als ons inzicht in deze materie en verdient om die redenen ruimere toepassing.

dit proefschrift.

VI

Een veredelingsinstituut annex genenbank van parasieten en predatoren van insecten en mijten is van wezenlijk belang om geïntegreerde bestrijdingssystemen op grotere schaal toepasbaar te maken.

VII

Het valt toe te juichen dat in studies betreffende de reikwijdte van de psychotherapie, opgevat als een methode om menselijk gedrag te veranderen, nu weer meer dan in de afgelopen decennia de rol van erfelijke factoren wordt benadrukt.

VIII

Het emancipatiestreven, gericht op een evenwichtige rolverdeling tussen vrouw en man binnen een gezin, wordt in niet geringe mate gefrustreerd doordat in de sociale zekerheidswetgeving het inkomen van de kostwinner de basis is voor de inkomensgaranties die deze wetgeving biedt.

Brünott, L.A.M. (1981) Sociale zekerheid en kostwinnerschap, Intermediair 37.

IX

De belangrijkste doelstelling bij het opvoeden is het kind te leren de angsten in het leven niet te laten omslaan in angst voor het leven.

X

Het in de handel brengen van een geparfumeerd chloorbleekmiddel bestemd voor huishoudelijk gebruik is een belediging voor het gezond verstand.

J.H. Nijhoff

Meiotic behaviour and spermatogenesis in male mice heterozygous for translocation types also occurring in man.

23 oktober 1981.

CONTENTS

GENERAL INTRODUCTION

A central theme of this thesis is the comparison of the meiotic behaviour of two translocations in the mouse which are structurally comparable with those in man, in order to determine the extent to which the study of mouse translocations can be used as a substitute for the study of the meiotic behaviour of translocated human chromosomes.

During the last decade the value of the laboratory mouse for cytogenetic studies has greatly increased. Since all its chromosomes are acrocentric, and the human has both metacentric and acrocentric chromosomes, it has become clear that the accumulated knowledge concerning the meiotic behaviour of reciprocal translocations between acrocentric mouse chromosomes (Searle et al., 1971; Eicher & Green, 1972; de Boer, 1976; Searle & Beechey, 1978) could not readily be applied to man. In man the great majority of reciprocal translocations are formed by translocations either between one metacentric and one acrocentric or between two metacentric chromosomes (c.f. Jalbert et al., 1980). To gain more insight in the segregational behaviour during meiosis of reciprocal translocations between metacentric and acrocentric chromosomes, such a translocation has been constructed by us in the mouse, by recombination within a common segment of a reciprocal translocation and a (metacentric) Robertsonian translocation. Besides a more detailed description of the behaviour of this type of translocation per se, a comparison with a group of principally identical translocations in man can now be made (Chapter 4).

In man, Robertsonian exchanges, which involve the fusion of two acrocentrics to yield one metacentric chromosome, are probably the most frequently observed structural rearrangements. Their incidence among 11148 newborn children was estimated to be 0,1% compared to 0,085% for the reciprocal translocations (Nielsen & Sillesen, 1975). The ascertainment of Robertsonian translocation carriers in man is mostly done retrospectively through "reproduction failures". This implies that this type of translocation - when in the heterozygous condition - tends to be associated with the production of aneuploid gametes through association of one of the acrocentric chromosomes with the "Robertsonian" chromosome during anaphase of the first meiotic division. The same phenomenon occurs in the mouse. In this species one Robertsonian translocation in male

carriers has been reported in this thesis in an attempt to elucidate the mechanisms of non-disjunction (Chapters 2 and 3). A brief comparison of the meiotic behaviour in Robertsonian translocation heterozygotes in man and mouse will be made in the "General discussion".

In addition, male murine carriers of the Robertsonian translocation were used to determine the effects of two types of irradiation, administered at relatively low doses 2-3 hours before prometaphase-metaphase II (which probably coincides with metaphase-anaphase I). The working hypothesis was that a meiotic system with a high initial incidence of aberrant chromosome behaviour at anaphase I, may be prone to a changed cellular environment as induced by irradiation (Chapter 3). This project was set up in an attempt to develop a sensitive test system for non-disjunctional events in general. Such a test system with a high initial non-disjunction frequency may be strictly necessary when a substitute for experimentation on man is sought, as the extent of "spontaneous" meiotic non-disjunction in mammals makes clear that man has a greater proneness to this phenomenon than any of the experimental animals studied so far (Chapter 1).

After the introduction of translocations involving metacentric and acrocentric chromosomes, a significant rise in the incidence of the normal bivalent non-disjunction after anaphase I was found (Chapter 4). This finding makes this mouse translocation system attractive as a model system for the future study of the endogeneous and exogeneous factors on the meiotic process, because extrapolation from mouse to man may now become more warranted.

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SPONTANEOUS MEIOTIC NON-DISJUNCTION IN MAMMALS

A study evaluating the various experimental approaches.

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1. General introduction

This report, in the first place, reviews and evaluates the various methods and techniques used for measuring the incidence of spontaneous meiotic non-disjunction in mammals generally and particularly in mouse and man. It also gives the principal results obtained with these methods and techniques and consequently, in a sense, is also a review of the incidence of meiotic non-disjunction in these species. The incidence of non-disjunction is only given for normal meiosis. Studies involving chromosomal aberrations, e.g. of translocation carriers, have not been included.

Meiotic non-disjunction normally denotes the failure of chromosomes associated in meiotic configurations (bivalents or multivalents) or not so associated to separate "regularly" at first meiotic division, or of sister chromatids to do so at the second division. This failure will, in the great majority of cases, result in (complementary groups of) numerically and genetically unbalanced gametes. If such an abnormal gamete is involved in fertilization the consequence will be embryonic or fetal death (the majority of cases) or more or less severe phenotypic effects in live born progeny.

That meiotic non-disjunction is an important problem in man can be concluded from a) the occurrence of an extra chromosome in approximately half of the spontaneous abortuses (which constitute 15-20% of all recognized conceptions; Boué et al., 1975) and b) the fact that at least 4.5% of the babies dying perinatally and half of the recognized chromosome anomalies - in total making up 0.6% of the live births - in newborns can be accounted for by "meiotic disjunctional errors" (Hook and Hamerton, 1977). "Conceptus derived data" are the principal source of information on the chromosome constitution of human gametes. The determination of the true incidence of meiotic non-disjunction from such data is hampered by a number of distorting factors such as chromosome or chromatid loss during the anaphases of the meiotic divisions, mitotic non-disjunction during the first - or subsequent - cleavage division (often resulting in "mosaics") as well as selection against unbalanced progeny at stage(s) of development earlier than that diagnosis take place. To enlarge our knowledge of the causes of meiotic non-disjunction itself and to get a better insight in the various "distorting factors" other mammalian assay systems than man have been introduced.

Table 1. The experimental approaches to assess the incidence of spontaneous meiotic non-disjunction in mammals

Stage examined	Parameters studied	Characteristics and derived data	Their use in man
1. prophase-MI in both sexes	- synaptonemal complex - chiasmata, their number and position - incidence of univalents	- only of importance for knowledge of mechanisms leading to non-disjunction - no direct relation between non-disjunction and the phenomena studied has yet been determined	studied predominantly in male via testes biopsies
2. MII in both sexes	- number of chromosomes - premature centromere division	- level of non-disjunction in AI can be determined - chromosomes characterized by heteropycnotic behaviour or deviating morphology may be identified	few data from oocytes cultured in vitro; no results in male due to technical problems
3. early spermatids till mature spermatozoa	- F-bodies - heterochromatic spots - amount of DNA per nucleus	- summed incidence of non-disjunction in AI and II can be determined for specific chromosomes. Reliability of first two parameters still disputed, while the third may not discriminate sharply enough	most techniques in first place developed to study mature human sperm
4. metaphase of the first clevage division	- number of chromosomes in both male- and female-derived chromosome complements - karyotype analysis and chromosome identification are possible	- selection against chromosomally unbalanced gametes before fertilization (in vivo or in vitro) may be determined - the best estimate for determining the level of incidence of the primary event in both sexes	not studied; chromosome complement in male pronucleus can be studied on limited scale after interspecific fertilization in vitro
5. pre-implantation stage	- karyotype analysis with the aid of suitable banding techniques	- process of selection - in terms of elimination - against chromosomally unbalanced progeny with proceeding development can be determined	not studied
6. post-implantation stage	- see 5	- see 5	in abortion material (also possible via amniocentesis)
7. post natals	- see 5	- see 5	not widely used

During both meiotic stages morphological aspects are studied predominantly. Some can be considered to be probable causative phenomena related to anaphase originating non-disjunction: a) reduced pairing of the homologous chromosomes, b) low chiasma frequency, chiasma localization, and c) occurrence of lagging univalents during Meiosis I or "unpartnered chromatids" at Meiosis II. Direct information about the true incidende of non-disjunction should give the study of the separate chromosome sets at both meiotic anaphases, but these cells are rare (a consequence of the relative shortness of these periods) and have not been studied in a quantitative way in mammals. In principle Metaphase II - meiocytes are used to determine anaphase I (AI) originating non-disjunction, while the incidence at anaphase II (AII) must be assessed via the chromosome constitution of cells from "later developmental stages" (from zygote to live born progeny); each stage with its own limitations of biological (as mentioned above) and/or technical origin.

In this review the emphasis is on the technical aspects of the various experimental approaches and on the discussion of their significance for the determination of the (true) incidence of meiotic non-disjunction in man and other mammals (mainly the mouse). No detailed discussion of the cellular processes underlying the phenomenon was attempted, except in the sections dealing with the meiotic stages themselves, primarily in view of the paucity of solid experimental results.

The text has been subdivided according to the various stages studied (table 1). The data published on the incidence of spontaneous non-disjunction have been tabulated for each stage separately. Results concerning induced non-disjunction in mammals are left out of consideration.

2. Meiosis I (table 2)

2.1. Introduction

The course of meiosis is fundamentally the same in both sexes although two relevant differences can be noted: 1) In the fertile male, gametogenesis is a continuous process which implies that in randomly chosen pieces of testicular material, if not too small, all stages can be found. On the other hand no continuity is found in the mammalian female. Contrary to the male where meiosis starts around puberty, in the female this stage is entered by all cells during fetal development. Oocyte development stops just before diakinesis in a modified diplotene called the dictyate stage, which is reached around the time of birth. In the mature female, gametogenesis is resumed periodically and for a few cells only (the number depends on the mammalian species) shortly before ovulation. After ovulation oocyte development again stops at the MII-stage and meiosis is resumed only after the spermatozoon-head had touched the vitelline membrane. 2) Whereas in the female only one of the four meiotic products develops into an oocyte (the others become polar bodies with no function in propagation), in the male in principle all four products of the two meiotic divisions mature into spermatozoa. Moreover, in mammalian females the majority of dictyate oocytes appear to degenerate after birth and are consequently never used for propagation.

As regards the stages prior to anaphase I (the meiotic prophase and metaphase) investigations, naturally, concentrate in both sexes on possible mechanisms leading to non-disjunction at AI. The parameters studied must be correlated with the incidence of AI-originating non-disjunction as determined at metaphase II (MII), because due to its relatively short duration cells at AI are rare.

From a morphological point of view four major events take place at the mammalian meiotic prophase: a) pairing of the homologous chromosomes (or chromosome segments) called synapsis, b) exchange of genetic material (recombination) between non-sister chromatids seen as chiasmata at later stages, and c) the process of co-orientation followed by d) segregation of the homologous centromeres (John, 1976).

Pairing at early prophase stages (zygotene, pachytene) can be studied via the Synaptonemal Complex (S.C.), a tripartite structure which consists of two lateral and a central element and is found between

the closely associated chromosomes or chromosome segments. Besides as a parameter for pairing behaviour, the S.C. is sometimes also studied for establishing the sites of recombination which are assumed to be marked by (recombination) nodules: solid bodies sometimes found to connect the lateral elements (rat: Moens, 1978). For reviews concerning the S.C. e.g. Moses (1968) and Gillies (1975).

The S.C. itself is predominantly studied by electron microscopy (E.M.) but with the aid of "silver staining" it is also possible with the light microscope. Morphological aspects at later stages of meiosis I (end diplotene to anaphase I), like for instance chiasma-frequency and -localization as well as the occurrence of prematurely dissociated bivalents at metaphase I,are exclusively studied with the light microscope.

In most mammals (among which man and mouse) these phenomena cannot be followed for individual bivalents throughout the whole of meiosis I. During this stage the chromosomes (bivalents) cannot be distinguished by differential staining while, moreover, during early prophase stages (leptotene to diakinesis) cells with sufficiently spread chromosome complements are rare.

2.2. Experimental Methods

2.2.1. Methods to study male meiosis I

Early prophase to diplotene

E.M. techniques to study the S.C. are : 1) the serial section approach and 2) the whole mount spread technique.

In the first method the testicular and cellular structures are left intact by a careful fixation of whole testes, or pieces thereof (biopsies), immediately after removal. Smaller pieces taken from the fixed material are subsequently stained (usually with uranyl acetate) dehydrated in a graded alcohol series and embedded in polymerizing resin. Serial sections from relevant nuclei are used for electron microscopical investigation. The series of electron micrographs made of each nucleus can be used for three dimensional reconstructions of pairing configurations (e.g. man: Holm and Rasmussen, 1977).

Contrary to the first method, the spatial relationships of the chromosomal configurations are not left intact in the second approach. The Counce and Meyer (1973) technique (Locusta) is now used more often to

visualize the S.C. in mammalian cells (for example Moses et al., 1975).

For liberating the spermatogenic cells, the mass of seminiferous tubules, taken from fresh testis material, is gently macerated in physiological saline. One microdrop (~ 1 µl)of the suspension of separate cells so derived is spread on the surface of a hypotonic spreading solution and allowed to stabilize. The cells are fixed immediately after having been picked up on a copper grid, dried and stained with ethanolic phosphotungstic acid. To facilitate the time consuming E.M. investigation, good cells can be traced beforehand with the light microscope (hamster: Moses, 1977).

The S.C. in whole mount spreads can also be made accessible to light microscopical investigation by preferential staining of this structure (rich in proteins) with positively charged silver ions: Many staining procedures, all variations of the "AgI and AgAS techniques" are presently available (for example Bloom and Goodpasture, 1976). Critical information concerning pairing-irregularities however, lies at the border of light microscopical resolution (hamster: Dresser and Moses, 1980).

Diakinesis to Anaphase I

The standard method for light microscopical analysis of primary spermatocytes at later stages of prophase is the "Evans air-drying technique" (mouse: Evans et al., 1964). Here the intratubular cell associations are disrupted by macerating the seminiferous tubules in iostonic saline. After hypotonic pretreatment (to ensure a better final spreading of the chromosome complement) the cells are fixed and dried on a slide. High numbers of analysable cells, representing all stages of spermatogenesis can be obtained, although the standard preparation appears to be enriched in diakinesis/metaphase I- (and possibly metaphase II-) spermatocytes. Simply by using other isotonic- and hypotonic-solutions, numerous pachytene spermatocytes and relatively few cells at diakinesis/metaphase I can be obtained (mouse: de Boer and Branje, 1979). An alternative to air-drying is squashing of small pieces of the seminiferous tubule. The method allows the study of spermatocytes in restricted stages of meiosis but, because the results are less repeatable, is hardly used (but see for example Ohno et al., 1959, analysing mouse spermatocytes at AI after squashing).

To facilitate the analysis of the chromosome complements, preferential staining of the constitutive centric heterochromatin (C-banding) can be performed after air-drying (man: Sumner, 1972). This standard staining technique is widely used in combination with light microscopical analysis of meiocytes at diakinesis and later meiotic stages.

2.2.2. Methods to study female meiosis I

Early prophase: zygotene to diplotene

For reasons outlined in section 2.1., oocytes at these stages have to be liberated from the fetal ovary. Fetuses from laboratory animals can be taken from females at the appropriate stages of pregnancy whereas in man this requires the use of abortion material between the third and seventh month of gestation (Uebele-Kallhardt, 1978, p. 3). In man air-dried preparations of such oocyte stages are made from suspended ovarian material, avoiding the hypotonic pretreatment to preserve the original chromosome structure and association patterns as well as possible (Luciani et al., 1974). Slides made according to this procedure contain high numbers of oocytes but in a variety of early meiotic stages (Uebele-Kallhardt, 1978, p. 99).

In the mouse, whole fetal ovaries can be taken and are hypotonically treated and fixed after which small pieces of ovarian material are carefully smeared on slides. After staining the chromosomes can be studied with the light microscope (Jagiello and Fang, 1979).

Till now S.C. studies in female meiosis have not been reported although silver staining of this structure in "pachytene oocytes" can in principle be carried out (E.P. Evans, personal communication; and also personal observation of P. de Boer).

Diplotene to metaphase I

In a variety of mammals, including man, these stages can be studied in oocytes matured in vitro after selected culturing times (Edwards, 1965; Donahue, 1968; Chandley, 1971; Basler, 1978). The "dictyate oocytes" have to be liberated first from their follicles. This can be done by puncturing the ovaries in isotonic saline or with the aid of collagenase and deoxyribonuclease (mouse: Eppig, 1976). Oocytes with

Table 2. Mean chiasma frequency and incidence of univalents in relation to age, estimated in gametes at prophase till metaphase of the first meiotic division

Reference	Species (strain)	Stages analysed	Age in months	Mean nr. of chiasmata/cell		% cells with true univalents	
				female	male	female	male
1. Henderson & Edwards, 1968	mouse (CBA+C57B1)	diakinesis	1.5	24.75	-	-	-
	C57B1	diakinesis	12	23.0	-	12.0	-
	CBA	diakinesis	12	-	-	42.0	-
	CBA	diakinesis	13.5	20.4	-	-	-
2. Luthardt et al., 1973	mouse (ICR+C57B1/6J)	metaphase I	1-3	22.9	-	-	-
		metaphase I	11-14	20.0	-	-	-
		metaphase I	1-6	-	-	0.5	-
		metaphase I	7-15	-	-	1.9	-
3. Polani & Jagiello, 1976	mouse (CSI/Ash+CFLP)	diak./MI	0.5-18	24.5	23.2	-	-
		diakinesis	1-18	-	-	-	4.6
		metaphase I	1-18	-	-	-	13.9
		metaphase I	1-4	-	-	0.9	-
		metaphase I	6-18	-	-	24.0	-
4. Speed, 1977	mouse (Q,C57B1+CBA)	metaphase I	2	27.8	-	-	-
		metaphase I	15	24.7	-	-	-
		metaphase I	2-15	-	23.7	2.5	2.5
5. Jagiello & Fang, 1979	mouse (Swiss-CAMM)	diplotene	16-18 days gest. age	27.2	-	3.1-9.0	-
		diak./MI	1.5-12	25.8	-	-	-
		diplotene	1-25	-	22.9	-	4.0
		diak./MI	2-24	-	22.3	-	-
6. Lange et al., 1975	human	diak./MI	-	-	52.4	-	-
7. Uebele-Kallhardt, 1978	human	metaphase I	-	42.5	-	-	-

a normal appearance, showing a clear germinal vesicle, are selected for microculture in a few drops of a suitable medium, often under a sterile paraffine oil layer. The microcultures have to be incubated in a gas phase, usually 5% carbondioxide in air. After a chosen culture time, which depends on the desired nuclear stage to be analysed, the oocytes are transferred to a hypotonic solution and subsequently fixed directly on a slide with a few drops of fixative (Tarkowski, 1966).

2.3. Results and Discussion

Most phenomena studied during the meiotic prophase are difficult to correlate directly with AI-originating non-disjunction. In fact only the occurrence of single, unassociated, chromosomes (univalents) at Metaphase I is assumed to lead to a distorted segregation due to a random orientation which may result in homologous chromosomes moving to the same spindle pole. Phenomena more or less directly related to univalence, although observed at earlier stages of Meiosis I, are discussed later on in this section.

Univalents

Experimental evidence to sustain the assumption that univalence at Metaphase I is indicative for non-disjunction is scanty. Polani and Jagiello (mouse, 1976) found no parallel between the percentage of MI-cells with univalents and the percentage of aneuploid MII's. This comparison may not be completely valid because of a) the interpretation of so called "ambiguous univalents", homologues supposedly attached in a non chiasmatic fashion at MI (mouse: Speed, 1977) and b) the finding that MI-cells which contain high levels of univalents are associated with cell death at that stage (human: Pearson et al., 1970 c, mouse: Purnell, 1973). A parallel between the occurrence of univalents at MI and AI-originating non-disjunction is found in aged translocation homozygous T70H/T70H female mice for the small marker bivalent: an eight-fold increase of the univalent frequency at MI is here accompanied by a nine-fold increase of non-disjunction for this chromosome estimated at MII (de Boer and van der Hoeven, 1980).

In all cases, the number of MI-cells showing univalents will be dependent on the quality of the preparation.

Chiasma frequency

Homologous chromosomes at diplotene to MI are held together by their chiasmata. Consequently a possibly distorted segregation at AI due to the occurrence of single, unassociated, chromosomes at a premature stage of meiosis I can be related to the formation, localization and terminilization of chiasmata. If chiasmata are not formed this may be caused by failing or incomplete pairing (synapsis) and can possibly be studied via the S.C. (see later on in this section). A smaller number of chiasmata at the moment of analysis, however, can also be the consequence of reduced formation or of precocious loss of these structures (desynapsis) for reasons other than failing, or incomplete, pairing.

Chiasma-frequency and -localization (from late diplotene to metaphase I) have been studied in connection to ageing especially of the mammalian female. Henderson and Edwards (mouse, 1968) observed a decline in number of chiasmata per MI-oocyte with increasing maternal age and proposed their " production line theory" to explain this phenomenon. Basic to this theory are the assumptions that a) the later the oocytes are formed during fetal development the lower will be the number of cross-over events (chiasmata) and b) that this order in formation is reflected in the ovulation order during reproductive live. The theory got some experimental support from Jagiello and Fang (mouse, 1979) who observed a higher mean chiasma frequency in diplotene oocytes from early (day 16) mouse fetuses than from late (day 18) ones. Their results are disputable, however (mouse: de Boer and van de Hoeven, 1980).

The alternative remains that ageing as such leads to an increase of irregular bivalent behaviour with regard to chiasma terminalization, chromosome orientation and centromere separation. Concerning the last point an increase with age is sometimes found in the incidence of mouse secondary oocytes with separated chromatids, a phenomenon that may find its origin before AI (mouse: Uchida and Freeman, 1977; de Boer and van der Hoeven, 1980).

In man an increase in, for example, "trisomy 21" among the progeny of women of 35 and over is a well documented fact (Erickson, 1978) but

the data on chiasma frequencies in human females are insufficient to draw conclusions about the age effect on this parameter (Uebele-Kallhardt, 1978).

Ageing does not appear to affect the mean chiasma frequency in the mammalian male. In the mouse even a slight, though non-significant increase with age was observed in some studies (Henderson and Edwards, 1968; Speed, 1977). A slight downward trend with increasing age is suggested by the results derived from 183 human males (gathered from the literature by Lange et al., 1975), but this trend is non-significant.

The range of chiasma counts in the human female (42 to 50 per cell; Uebele-Kallhardt, 1968) is equal to or somewhat lower than that in the male (overall mean frequency of 52.3; Lange et al., 1975). Studies on map distances in most linkage groups in man however, show an excess of recombination in the female (for example the ratio of female male distance for chromosome 1 is: 1.86; Cook and Hamerton, 1979). Because the numbers of chiasmata are mostly determined in cells at diakinesis/MI, this discrepancy may be explained by a more rapid loss of chiasmata in the female which in its turn may be the consequence of the in vitro maturation technique used for oocytes.

In general the morphology of the chromosome (degree of contraction) in MI-cells is dependent on the quality of the preparations which will directly influence the observations concerning presence and positions of chiasmata: Lange et al. (1975) observed significant differences in the chiasma frequencies found by various authors studying this subject in the human male, a phenomenon which may however also be conditioned by genetic variation.

Synaptonemal complex

Close pairing is believed to be a prerequisite to crossing over, while irregularities in pairing can be traced via E.M. studies of the S.C. Whether the pairing phenomena are indeed "irregular" can, among others, only be concluded if the exact meiotic prophase stage of the cell is known (as defined by light microscopy). Recently these stages have been described accurately for the male mouse (Oud et al., 1979). The method makes use of "selected cell killing" by which a restricted gametic population (with all cells at the same stage) passes the meiotic prophase. This approach appeared to be useful for describing

Table 3. Incidence of spontaneous AI non-disjunction, estimated in MII-spermatocytes from air-dried preparations

Reference	Species (strain)	% non-disjunction in N cells †	
		%	N
1. Tettenborn & Gropp, 1970	Mus musculus (NMRI)	1.93	207
	Mus m. poschiavirus	0	233
2. Beatty et al., 1975	mouse (13 strains)	0.38 ± 0.12	5200
3. Szemere & Chandley, 1975	mouse (Q)	0	200
4. Polani & Jagiello, 1976	mouse (CFLP and CSI/Ash)	1.43	1533

† incidence based on 2 times (n+1) chromosomes containing cells

pairing behaviour via E.M. studies of the S.C. in the right sequential order (Morsink and de Boer, unpublished results). At present no quantitative E.M. studies of the S.C. in whole mount spreads have been undertaken in a mammalian meiotic system showing a high frequency of MI's with univalents.

S.C. nodules were suggested to have a function in crossing over although their exact role is still not understood (rat: Moens, 1978). Fragments of the S.C. (S.C. remmants) are found at the sites of chiasmata in diplotene (mouse: Solari, 1970) but have not been related directly to chiasma position in man (Holm and Rasmussen, 1977). So, in conclusion, studies of the S.C. have not yet yielded much relevant information in connection with AI-originating non-disjunction (Holm et al., 1979).

3. Meiosis II (tables 3 and 4)

3.1. Introduction

An interkinesis followed by a prophase II stage (if present at all) is generally not distinguished during meiosis II of the mammalian male and female. Prometaphase II appears to follow AI very quickly. Metaphase II (MII) cells of both sexes are the major source of information for assessing the overall frequency of AI-originating non-disjunction simply by counting the number of chromosomes per cell. Unfortunately technical problems caused by insufficient spreading of the haploid chromosome set prevent analysis of secondary spermatocytes in man.

Anaphase II (AII)-cells are rare (the stage must be of short duration), so MII's are also studied for factors possibly leading to AII-originating non-disjunction: Chromatids of precociously split MII-chromosomes are assumed to segregate randomly to one of the two spindle poles and may consequently lead to non-disjunction (Rodman, 1971). Both premature centromere division during Meiosis II and separation of sister chromatids during Meiosis I can underly this phenomenon although the latter process has seldomly been observed (mouse: Polani and Jagiello, 1976).

The best assessment of AII-originating non-disjunction can be made by comparing MII counts (non-disjunction at AI) with the counts of the, still separate, haploid sets in the zygote at the first cleavage division (see section 5). In the latter case the summed AI plus

Table 4. Incidence of AI non-disjunction, estimated in MII-oocytes

Reference	Species (strain)	Technique	Matured in vivo/vitro	Super-ovulated	% non-disjunction in N-cells †		Femage age in months
				+/-	%	N	
1. Röhrborn, 1972	mouse (C_3H)	Tarkowski	vivo	+	4.8	335	-
				-	4.6	128	
2. Hansmann, 1974	mouse (C_3H)	Tarkowski	vivo	+	4.8*	347	2.5-3
3. Uchida & Lee,	mouse (C_3HxICR/Swiss)	Tarkowski	vitro	-	0	1054	3-6
4. Reichert et al.,1975	mouse (NMRI)	Tarkowski	vivo	+	0	143	-
5. Martin et al., 1976	mouse (CBA)	Tarkowski	vitro	-	2.6*	458	2-12
6. Polani & Jagiello,1976	mouse (CFLP and	Polani	vitro	-	0.5*	409	1-6
	CSI/Ash)				0.5*	403	9-18.5
7. Becker & Schöneich,1977	mouse (NMRI)	Tarkowski	vivo	-	1.4	144	3
					2.3	266	5
8. Golbus, 1977	mouse (Swiss-Webster)	Tarkowski	vitro	-	4.8		1.5-2
					7.2		13-15
9. Speed, 1977	mouse (Q)	Tarkowski	vitro	-	3.4*	50	12
10. Uchida & Freeman,1974	mouse (C_3HxICR/Swiss)	Tarkowski	vitro	-	1.2	1293	12
11. Hansmann & Probeck,1979	Syrian hamster	Tarkowski	vivo	+	0.6	307	
	Chinese hamster				0	334	

† incidence based on 2 times(n+1)chromosomes containing cells

* incidence based on 2 times hyper-haploid (more than n chromosomes containing)cells

AII-originating non-disjunction is estimated; selection against unbalanced gametes is assumed not to occur during this period.

3.2. Experimental Methods

Preparations of MII-spermatocytes can be made with the standard air-drying technique (Evans et al., 1964; see Meiosis I, experimental methods).

Mammalian MII-oocytes from laboratory animals can be obtained after culturing in vitro (see section 2.2.2.) or in vivo. In the latter case MII-oocytes can simple be flushed from the oviduct because they will stay at that stage if no fertilization takes place. To increase the number of oocytes as well as to time oocyte maturation more accurately females are frequently "superovulated" with the aid of both pregnant mares serum (PMS) and human chorionic gonadotrophin (HCG) (mouse: Edwards and Gates, 1959). If exogeneous gonadotrophins are to be ommitted, timing of ovulation requires careful study of oestrus or may be determined via pairing with a vasectomized or otherwise sterile male. In the mouse the obtained number of MII-oocytes appears to be strain and age dependent (Evans, 1979).

As at Meiosis I, no general banding technique is known to recognize the individual chromosomes at Meiosis II: Selective staining of the constitutive heterochromatin (C-banding) is in both stages the most widely applied staining method to facilitate the distinction of the chromosomes.

3.3. Results and Discussion

The frequency distribution of number of chromosomes in second metaphase spreads is, in most preparations,asymmetrical in favour of the hypo-haploid group. The conventional and likely assumption is that the excess of cells containing less than the haploid set of chromosomes is caused by artifactual chromosome loss during preparation. The estimation of the incidence of AI-originating non-disjunction will in those cases be based on the hyper-haploid cells (male mice: Beatty et al., 1975).

From the MII-data estimated for the mouse (tables 3 and 4) it can be concluded that AI-originating non-disjunction is low in the male and somewhat higher (and increasing with rising maternal age) in the female.

In man no data are available for the male and only few for the female: All 37 MII-oocytes analysed contained the complete haploid chromosome set (Uebele-Kallhardt, 1978).

4. The spermatid and spermatozoon stage (table 5)

4.1. Introduction

In the mammalian male no direct chromosomal analysis of the combined AI- plus AII-originating non-disjunction can be made in the spermatid stage. On the other hand, much effort has been made, predominently concerning the human, to get an idea about the level of numerically unbalanced spermatozoa. These methods range from estimating the amount of nuclear (or spermatozoal) material in individual cells to methods approximating the frequency of meiotic non-disjunction for specific chromosomes, characterized by deviating staining properties (human: Barlow and Vosa, 1970; Pearson et al., 1970b; Geraedts and Pearson, 1973; Microtus: Tates, 1978). In the latter approaches extrapolation is needed to quantify for the whole genome. While in the first group of methods the estimation of the level of ploidy is inherent to the approach, care must be taken to avoid this disturbing factor when the incidence of non-disjunction is based on the presence of marker chromosomes. In these cases the visually assessed size of the sperm head or the spermatoid cell is possibly an useful parameter: In man for instance, the group of relatively larger sperm heads consists predominantly of diploid cells (Beatty, 1977).

4.2. Experimental Methods

4.2.1. Determination of nuclear DNA content

Cytophotometric determination of the relative DNA-content of individual sperm heads has been carried out by measuring their light-absorption 1) "directly" or 2) after Feulgen staining. In the first approach DNA-absorption at 260 nm is estimated. The values obtained have to be corrected for protein absorption at 280 nm and often for so called "non-specific" absorption at 310 nm (bull: Blackshaw and Salisbury, 1972; mouse: Stolla and Gropp, 1974). Spermatozoa have to be smeared on quartz slides and covered with glycerol and a quartz

cover glass. A fixed beam or scanning microspectrophotometer can be used. Results presented make it unlikely that discrimination between nuclei containing n and n $\pm$ 1 chromosomes will be possible at the moment (mouse: Stolla and Gropp, 1974).

For Feulgen staining, sperm heads have to be fixed (important step), air-dried on slides, hydrolysed and stained according to the Feulgen method. Nuclear absorption is determined at about 556 nm but must be corrected for background with the aid of an adjacent blank area. Automated scoring of 20-60 cells per hour via a computer assisted scanning and integrating microspectrophotometer with field limiting system is possible (van der Ploeg et al., 1977).

Alterations of DNA-content have also been diagnosed via determination of the relative fluorescence intensity of nuclei after staining with a "DNA specific and quantitative fluorochrome". Examples of fluorescent dyes used are: ethidium bromide plus mithramycin (man: Meistrich et al., 1978b), propidium iodide (rat: Libbus and Schuetz, 1978), acriflavine (man: Sarkar et al., 1978; Levinson et al., 1978) and acridine-orange (bull: Blackshaw and Salisbury, 1972). For the automated analysis applied in most procedures cells flow in a narrow stream across an intense beam of exciting light after which the emitted fluorescent light pulses are automatically analysed (up to 10^5 cells/min, can be done with flow cytofluorometry, mouse: Meistrich et al., 1978a). The approach requires highly purified, unaggregated suspensions of one cell type only.

In sperm heads smeared on a slide, the dry mass of both the protein and DNA-content can be determined via integrated optical path differences (refractive index times thickness) with an integrating microinterferometer (Goldstein and Hartmann-Goldstein, 1974). A linear relation between the amounts of DNA and dry mass seems to exist (man: Sumner and Robinson, 1976). The method is claimed to be more sensitive than the one based on Feulgen-DNA staining (coefficients of variation about 9% here against 15% for the Feulgen method; man: Sumner et al., 1971) but on the other hand, more specilization is required to operate the system.

4.2.2. Determination via chromosome markers

These approaches are all based on the 1:1 representation of the chromosomes in question by differentially stained spots visible in the sperm heads.

a. The human Y chromosome

When a washed and fixed human sperm sample is stained with an aqueous solution of quinacrine or quinacrine mustard, fluorescent spots (F-bodies) can be observed in about half of the nuclei (Barlow and Vosa, 1970; Pearson and Bobrow, 1970). This is supposed to be caused by the fluoresceing distal region of the long arm of the Y chromosome. Therefore the frequency of, in this case, AII-originating non-disjunction of this chromosome may be obtained by determining the incidence of 2F-bodies containing sperm cells. This incidence appeared to be extremely high and prompted the alternative that one single "bifid Y" may also give rise to two (closely attached) F-bodies (Pearson et al., 1975; Beatty, 1977).

b. Human autosomal markers

Autosomal chromosomes (no. 1 and 9), polymorphic in their amount of constitutive heterochromatin, are also used for determining meiotic non-disjunction frequencies in spermatozoa. Fixed and air-dried on slides the sperm cells are either pretreated with barium hydroxide at pH 11 and stained with Giemsa (G11-method used to recognize chromosome 9, Bobrow et al., 1972) or denatured in boiling 0.9% NaCl followed by a treatment for 10 min with 0.2 M CsCl before staining with Leishmans's solution (for recognition of chromosome 1; Geraedts and Pearson, 1973).

c. The sex chromosomes in Microtus oeconomus(Northern vole)

These chromosomes can be distinguished, also from each other, in the round nuclei of early spermatids after C-banding. Air-dried preparations of the whole content of the seminiferous tubules are made according to the "Evans technique" (Tates, 1979). Early spermatids of normal size containing two heterochromatic dots in their nuclei are considered to be sex chromosomal non-disjunction products of the XX, XY or YY type respectively.

4.3. Results and Discussion

For a number of reasons the results derived at this stage of spermatogenesis are not very useful at the moment:

1. Stoichiometric staining of the DNA content in mature sperm cells is hampered by their asymmetric shape and the very condensed state of the nuclear chromatin (Meistrich et al., 1978b). These problems may be overcome using a pure sample of round spermatids at the same stage of development. The fraction can be obtained by combining the "HUT"-selective cell killing system (mouse: Oud et al., 1979) and the "STA-PUT"-sedimentation system (mouse: Chandley et al., 1977). Spermatids must all be at the same stage of development because the degree of DNA condensation, a process that normally takes place during sperm maturation, may influence both the amount of light absorbed in Feulgen stained cells and the fluorescence intensity after staining with a fluorochrome (Salisbury et al., 1978). Both methods proved to be sensitive enough to resolve X and Y bearing human sperm cells with or without an F-body showing a difference in DNA content of $\sim$ 3.5% (Evans, 1971; Sumner et al., 1971; Meistrich et al., 1978b). However, the more continuous and partly overlapping distributions prevent an absolute discrimination between individual X and Y bearing cells and, generally, between cells containing n and n $\pm$ 1 chromosomes.

2. The results of DNA measurements, via absorption at 260 nm, in unstained sperm heads of a meiotic system known for high levels of aneuploid gametes reveal that this approach too cannot dinstinguish unambiguously between cells containing n and n $\pm$ 1 chromosomes (mouse: Stolla and Gropp, 1974).

3. In man, the special staining properties of the autosomes 1 and 9 as well as the Y chromosome are doubted to be reliable markers. The meiotic non-disjunction frequencies for these chromosomes are extremely high when estimated via this method, leading to an overall frequency of about 40% of aneuploid spermatozoa when extrapolated to the whole genome (Geraedts and Pearson, 1973; Beatty, 1977 and 1978). This conclusion may, in case of the human Y chromosome, be sustained by the finding that the dry mass of spermatozoa containing 2 F-bodies is, contrary to the expectations, still significantly lower than that of X-bearing cells without an F-body (Sumner and Robinson, 1976).

Table 5. Incidence of spontaneous meiotic non-disjunction for special chromosomes estimated in the post-meiotic cell stage of the male or for any chromosome in the pronuclei

Reference	Species (strain)	Material and methods	% of non-disjunction in N-cells		Remarks
			%	N	
1. Sumner et al., 1971	human, male	ejaculate, incidence of 2 F-bodies in morphologically normal looking sperm cells	1.2		only the non-disjunctional level for the Y-chromosome (= F-body) may be determined in this way.
2. Geraedts & Pearson, 1973	human, male	ejaculate, incidence of 2 heteropycnotic spots in individual sperm cells	1.2		only the non-disjunctional for chromosome 1 may be determined in this way.
3. Beatty, 1977	human, male	see 1	3.0	7000	see 1; 2 Y-bodies not accepted as reliable parameter for Y-chromosome non-disjunction.
4. Kapp et al., 1979	human, male	see 1	1.2	6000	see 1
5. Tates et al., 1979	Microtus oeconomus, male	early spermatids, analysed on the incidence of 2 heteropycnotic spots	0.001	16.10^4	nuclei with 2 spots are assumed to represent XX, YX or YY nuclei.
6. Rudak et al., 1978	human, male	chromosome complement in the male pronucleus after fertilization in vitro of hamster	3.3	60	non-disjunctional level of all chromosomes are mentioned here, based on 2 times (n+1) chromosomes containing human complements.
7. Phillips & Kaufman, 1974	mouse, female	haploid cleavage phase; oocyte has been stimulated to pass AII in vitro	5.8	172	see 6
8. Maudlin & Fraser, 1978	mouse, male and female (T0)	first cleavage phase, fertilization in vitro			
		- female derived complement maternal age: 2-4 months	1.8	434	level of meiotic non-disjunction of any chromosome based on 2 times % pronuclei with (n+1) chromosomes
		- female derived complement maternal age: 8-10 months	6.4	375	
		- male derived complement	1.4	712	
9. Fraser & Maudlin, 1979	mouse, male and female (T0)	first cleavage phase, fertilization in vitro			
		- female derived complement	0.94	1925	see 8
		- male derived complement parental age: 2-4 months	0.86	1880	
		first cleavage phase, fertilization in vivo			
		- female derived complement	0.86	935	

In spite of this serious criticism the "F-body approach" has been used in practice to trace Y-chromsomal non-disjunction in dibromochloropropane exposed workmen (Kapp jr. et al., 1979).

5. The first cleavage division (table 5)

5.1. Introduction

This stage offers the best opportunity for tracing the share of both sexes in the production of chromosomally aberrant progeny. The egg, arrested at MII, will complete its second meiotic division subsequent to the penetration of the sperm cell. Male- and female-derived gametic nuclei form pronuclei, replicate their DNA and enter the first cleavage prophase almost simultaneously. Due to different states of contraction the maternally- (most contracted of the two) and paternally-derived chromosome sets can be distinguished and cytologically analysed (mouse: Donahue, 1972a, Hansmann, 1973; Fraser and Maudlin, 1979). Banding techniques for recognition of individual chromosomes can be successfully applied at this meiotic stage, so the incidence of meiotic non-disjunction can be estimated for individual chromosomes as well. This stage cannot be studied in man, but the approach proved to be promising for analysing the chromosome content of human sperm after interspecific fertilization in vitro (Rudak et al., 1978).

5.2. Experimental Methods

In most studies using mammalian assay systems, irrespective of the fertilization system chosen, females are induced to superovulate after injecting gonadotrophins (PMS followed by HCG; see also section 3.2.). The advantages are a greater number of oocytes and a more accurate timing of the stages from maturation till the first cleavage division (mouse: Edwards and Gates, 1959; Donahue, 1972b).

5.2.1. Fertilization in vivo

Females, induced to superovulate, are allowed to mate. Ovulation will take place a specific time interval after the last hormone injection (in mouse about 12 hours after application of HCG, Edwards and Gates, 1959) so the "one cell embryo" can be recovered from the oviduct a specific number of hours later. This time interval is of particular importance because, if the cells are transferred too early to a culture medium, also containing a spindle inhibitor, especially male chromosome condensation will be stopped too prematurely to allow

a proper analysis (mouse: Fraser and Maudlin, 1979). Good preparations are also obtained after 24 hours culturing in the presence of a mitotic inhibitor of oocytes removed from the oviduct a few hours after fertilization (C.V. Beechey, personal communication, 1978).

5.2.2. Fertilization *in vitro*

The eggs are released from the oviducts as soon as ovulation is completed and are put directly into a sperm suspension. After fertilization, as determined by the presence of second polar bodies, the eggs are transferred to a culture medium with a mitotic inhibotor. For good results, well suspended sperm cells are an important condition, a datum which appears to be genetically influenced: For this purpose TO-strain male mice (used in the studies of Fraser and Maudlin, 1979; Maudlin and Fraser, 1977, 1978) proved to be suitable.

The *in vitro* fertilization technique has recently been used to study the chromosome content of mature human sperm. Golden hamster eggs released from the oviducts of superovulated females were, enzymatically freed from cumulus cells and zona pellucida before incubation with human sperm, after which the condensation of the chromosomes of the penetrated male germ cells made these accessible to analysis. Chromosome identification took place after Q-banding (quinacrine staining) and lacto-acetic orcein staining (Rudak et al., 1979).

5.3. Results and Discussion

The results with both fertilization systems in mouse indicate that the *in vitro* system in general may be preferable to the *in vivo* system: As a consequence of a better synchrony of fertilization and subsequent chromosome condensation more preparations are suitable for analysis with the first (80%) than with the latter (50%) system (Fraser and Maudlin, 1979). Good results are also obtained after combining the *in vivo/in vitro* techniques (see section 5.2.1.).

Because ovulation is induced with exogeneous gonadotrophins in all the studies cited in this section, it is of relevance to check its possible influence on the level of aneuploidy. Although PMS seems to enhance the level of polyploidy proportional to the dose given, the level of (non-disjunction caused) aneuploidy appears not to be affected in zygotes derived after induced ovulation (mouse: Maudlin and Fraser, 1977). Analogous to the reasoning given for the MII counts, preparation caused chromosome loss may make it necessary to base the estimate of

the incidence of meiotic non-disjunction on the hyperhaploid group, for selection against unbalanced gametes is not assumed to occur (mouse: Fraser and Maudlin, 1979).

In general this stage has not been studied on a large scale until now. The reason seems to be the fact that 1) it requires a relatively time consuming procedure as experimental animals in general demonstrate low levels of spontaneous non-disjunction and 2) the procedure is of no use for man due to a lack of human oocytes. Rudak et al. (1978) were able to analyse the chromosome constitution of human spermatozoa via "interspecific fertilization". Due to technical pitfalls their data are far from being quantitative: A maximum of 10 sets of sperm chromosomes could be analysed with the aid of 150-200 hamster eggs (see also table 5).

6. The pre-implantation stage (table 6)

6.1. Introduction

Reasons for establishing the incidence of aneuploidy at this early stage of embryogenesis may be the following: 1) By flushing the oviducts and/or uterine horns embryonic material uncontaminated with maternal cells can be derived easily in experimental animals. 2) Selection against unbalanced progeny is assumed to start during this stage. The latter can be concluded for instance from the pre-implantation loss data derived after mating Robertsonian translocation heterozygous male mice (with high levels of aneuploidy at MII) with chromosomally normal females (Ford and Evans, 1973; Gropp et al., 1974). Assuming a 100% fertilization, pre-implantation losses are estimated by subtracting the sum of dead and living implants from the total number of ovulated eggs (as number of corpora lutea in the ovaries). Normally the quantitative assessment of the incidence of meiotic non-disjunction in this period is based on trisomics only because it is assumed that part of the monosomics have already been lost (mouse: Ford and Evans, 1973). The importance of analysis of this stage is that it permits the ascertainment of the moment of death of genetically unbalanced (predominantly monosomic) embryos.

Table 6. Incidence of spontaneous meiotic non-disjunction estimated in pre-implantation embryos

Reference	Species (strain)	Material (age of embryo)	Matured in vivo/vitro	Super-ovulated +/-	% of non-disjunction in N-cells %	N	Remarks
1. Donahue, 1972	mouse (CF1)	first cleavage	vitro	+	1.8	110	based on two times % of cells with (n+1) chromosomes
2. Kaufmann, 1973	mouse (CFLP)	first cleavage	vivo	+	1.04	193	see 1
3. Hansmann, 1973	mouse (C_3H)	first clevage	vivo	+	12.5	32	see 1
		2 cell stage	vivo	+	19.0	(21 embryos)	based on hypo- plus hyper-ploid embryos
4. Maudlin & Fraser, 1977	mouse (TO)	first cleavage	vivo	+	1.8	(655 embryos)	see 1
			vitro	+	1.2	(311 embryos)	
5. Vickers, 1969	mouse (PDE)	(3-4 days)	vivo	+	0.6	(309 embryos)	based on hypo- plus hyper-ploid embryos
6. Gosden, 1973	mouse (CBA/H-T6)	(3-5 days)	vivo				
		(a) maternal age: 1-7 months			(a)0.9	(110 embryos)	see 5
		(b) maternal age: 8-12 months			(b)6.9	(58 embryos)	
7. Fechheimer & Beatty, 1974	rabbit	(5.75 days)	vivo	+	0.9	(463 embryos)	all trisomic (2n+1) individuals
8. Shaver, 1975	rabbit	(6 days)	vivo	-	0	(74 embryos)	see 5
9. Yamamoto, 1972	Syrian hamster	(3 days)	vivo		0.7	(135 embryos)	see 5
10. Binkert & Schmid, 1977	Chinese hamster	(3-3.5 days)	vivo	-	0.9	(226 embryos)	see 5

6.2. Experimental Methods

In principle, approaches identical to those mentioned for first cleavage cells can be used here. In practically all studies females are induced to superovulate. After fertilization has taken place in the natural way, the embryos can either be flushed from the oviducts soon after fertilization or are allowed to develop until just prior to implantation. In the first case, embryos at the two-cell-stage are removed from the oviducts a defined time after mating (Chinese hamster, 72 hr; Basler and Röhrborn, 1977; mouse, 44 hr; Basler et al., 1976) and cultured *in vitro* in medium covered with a sterile oil layer. Development can be followed with an inverse microscope. A few hours before chromosome preparations are made, a mitotic inhibitor is added to enhance the number of metaphases.

In case the embryonal development until implantation takes place *in vivo*, embryonic cell division is stopped by incubating embryos for a short time in a medium with a mitotic inhibitor or by injecting the pregnant female with colchicine. Chromosome spreads from metaphase cells can be made according the standard procedure (mouse: Tarkowski, 1966).

6.3. Results and Discussion

Although methods for *in vitro* culturing of pre-implantation embryos have been improved (Chinese hamster: Basler and Röhrborn, 1977; mouse: Bürki and Sheridan, 1978), up to now most data have been derived with the *in vivo* approach. Late morula/early blastocyst stages are the most suitable for analysis: Towards implantation, which takes place in mammals at late blastocyst stage, the metaphase chromosomes of, for instance, mouse embryos become fuzzy.

The incidence of meiotic non-disjunction estimated at the pre-implantation stage (AI- and AII-originating non-disjunction, table 6) can be compared with the MII-data combined for both sexes (non-disjunction only AI in origin, tables 3 and 4). The incidence of non-disjunction, contrary to the expectations, appears to be higher after AI than after AI and AII combined. To avoid problems with regard to negative selection against genetically unbalanced zygotes and/or pre-implantation embryos, the incidence is in most cases based on hyperhaploid MII's, respectively trisomic embryos (see tables 3, 4 and 6, groups assumed not to be affected by selection before implantation (mouse: Ford and Evans, 1973; Ford, 1975). The comparison is restricted however by differences in strains or in animal age (an increase in the

incidence of non-disjunction can be observed with increasing-maternal age; mouse: e.g. Fraser and Maudlin, 1979; de Boer and van der Hoeven, 1980; man: e.g. Erickson, 1978). The possibility exists that the differences in outcome can be ascribed to non-random sampling of embryonic cells at metaphase in favour of the, more rapidly dividing, numerically and genetically balanced embryos.

No data concerning pre-implantation losses are known for man, because embryonic death at this stage normally remains unrecognized. Some preliminary conclusions may be drawn from a study by Williamson and Miller (presented at the Wessex symposium, Southampton, 1979) who used the level of beta HCG in urine and plasma for early recognition of conception. Of 149 conceptions an extremely high percentage, 31.5%, appeared to be lost before they were recognized by current criteria. The contribution of numerically unbalanced products to this level is, of course, unknown but possibly high.

7. The post-implantation stage (table 7)

7.1. Introduction

In man, this is the second stage (after MI) yielding cytological information of a sufficient quantity to make comparison with experimental animal-derived data possible.

In general data concerning the karyotypes of "implantation embryos" at a very early stage are meagre especially in man. For experimental animals the reason is the difficulty of separating the minute quantity of embryonic cells from the uterine wall without contamination with maternal cell material.

In man karyotype analysis of embryos and fetuses can be done via ammiocentesis or after abortion. In both cases the majority of the material is obtained between the 12th and 16th week of gestation (post-menstrual age) and here again care must be taken to avoid contamination with cells of maternal origin.

7.2. Experimental Methods

7.2.1. Laboratory animals

At known periods after mating pregnant females are dissected. The uterine content can be subdivided into dead and living implants. If the embryo dies soon after implantation, only a local swelling of the

uterine wall will be seen (deciduomal reaction). Died at later stages, the moment of death can roughly be judged from the state of resorption. The source of embryonic cell material to be used for analysis depends on the stage of development: a) When the number of dividing cells is low chromosome preparations are made from whole implants (membranes plus embryo). b) Karyotype analysis at later stages of fetal development can be carried out on metaphase cells taken from either rapidly dividing organ tissue or from the embryonic membranes. Generally the number of metaphases is enhanced in both approaches, by a short time incubation of fetal tissue in medium with mitotic inhibitor (mouse: Evans et al., 1972) but also, prior to dissection, by injecting pregnant females with this compound. However, not all chemicals will pass the placenta (mouse: Jojena and Leliever, 1973). The subsequent treatment of the cells prior to air-drying includes the usual hypotonic swelling- and fixing-steps (method of Evans et al., 1972).

7.2.2. Man

In case of a terminated pregnancy chromosome preparations are made primarily of cultured cells derived from embryonic tissue or chorion and amnion but in case of still dividing tissue, for example after induced abortion, culturing can be omitted (Yamamoto et al., 1975). In these cases, possible contamination with maternally derived cell material is often histologically verified (Laurtisen, 1976).

Intra uterine diagnosis is also possible however. Amniocentesis (sampling of amniotic fluid cells) is normally performed transabdominally between the 14th and 16th week of gestation (calculated from the first day of the last menstruation e.g. Leschot et al., 1979).

Both extraembryonic explants and amniotic fluid cells, are cultured in standard medium under a "5% carbondioxide in air gas phase" at 37 ^{o}C, untill a considerable number of cell divisions can be observed. Metaphase spreads for karyotype analysis are made according to the standard air-drying technique (Evans et al., 1972). Chromosome identification can be done via a number of banding techniques of which Q, G or R-banding are most widely applied.

Table 7. Incidence of spontaneous meiotic non-disjunction estimated in post-implantation embryos

Reference	Species	Material	% non-disjunction	Number of fetuses	Remarks
1. Yamamoto et al., 1973	mouse	10.5 days old fetuses (a) maternal age: 3-5 months (b) maternal age:11-16 months	 (a) 0 (b) 2.6	 149 156	 - 1.9% trisomics, 0.7% monosomics
2. Basler et al., 1976	mouse	9.5-13.5 days old fetuses	0	106	-
3. Max, 1977	mouse	9.5-12,5 days old fetuses	0.94	213	all trisomics
4. Chandley & Speed,1979	mouse	9-10 days old fetuses	0.36	561	all trisomics
5. Hansmann, 1978	mouse	9.5 days old fetuses	0	90	-
6. Yamamoto & Ingalls, 1972	Syrian hamster	9 days old fetuses	0	124	-
7. Adachi & Yamamoto,1978	golden hamster	10 days old fetuses (a) maternal age: 3-6 months (b) maternal age:13-16 months	 (a) 0 (b) 0.91	 175 110	 - a monosomic
8. Yamamoto et al., 1975	human	induced abortions gestation age: 28-84 days	4.6	500	3.2% trisomics, 1.4% monosomics
9. Tsuij et al., 1978	human	induced abortions gestation age: 42-84 days	5.1	256	all trisomics
10.Kajii et al., 1973	human	spontaneous abortions	41.4	152	33.6% trisomics, 7.9% monosomics
11.Boué et al., 1975	human	spontaneous abortions	42.4	1498	33.0% trisomics, 0.4% monosomics
12.Creasy et al., 1976	human	spontaneous abortions	22.6	941	15.4% trisomics, 7.2% monosomics
13.Lauritsen, 1976	human	spontaneous abortions during first 16 weeks	41.6	255	25.9% trisomics (0.4% double tr.) 15.7% monosomics
14.Hassold et al., 1978	human	spontaneous abortions	34.6	234	22.6% trisomics (1.3% double tr.) 12.0% monosomics
15.Milunsky, 1979	human	amniocentesis, maternal age 35 (world wide survey data)	2.28	17859	2.26% trisomics, 0.02% monosomics

7.3. Results and Discussion

Mouse data reveal that a great deal of trisomic fetuses survive at least until day 10, that is halfway the pregnancy while the majority of monosomics have supposedly been eliminated at that stage (Ford and Evans, 1972; Gropp et al., 1974). The percentage of primary trisomics in the mouse that survive until after days 13-14 of gestation is very low, but some of them (some trisomics for chromosomes 14 or 19) may develop up to term but will die soon after birth. In man, at least 80% of all spontaneous abortions occur in the first 14 weeks of gestation calculated from the first day of the last menstrual cycle (Boué et al., 1975; Hassold et al., 1978). In both man and mouse the monosomic progeny (an important exception has to be made for monosomy (45(XO) in man: Lauritsen, 1976; Carr and Gedeon, 1977; Hassold et al., 1978) probably die off in the peri-implantation stage (Ford, 1975).

A higher frequency of aneuploidy for specific chromosomes in human abortion material is a well documented fact: Sex chromosomes aneuploidy and trisomy for chromosome 16 but also for the acrocentrics 14, 15, 21 and 22 occur relatively more often (Carr and Gedeon, 1977; Hassold et al., 1978). To some extent this may be caused by negative selection at earlier stage of gestation against aneuploids for the other chromosomes.

A hypothesis to explain the higher incidence of an extra acrocentric chromosome in trisomics is based on non-homologous association during meiosis via their nucleoli possibly a parallel of satellite association in mitotic metaphases (Hansson, 1979). In this context differences in bivalent arrangements in pachytene oocytes from mice and men have also been related to the known difference in level of spontaneous non-disjunction between both species (Stahl et al., 1980). However, looking at the even greater frequency of trisomics for chromosome 16, nucleolar fusion may not be the major cause of association, leading to non-disjunction. Heterochromatin may well play a significant role.

To extend the data concerning the parental origin of the trisomy in human abortions, possible chromosome heteromorphism (Jacobs, 1977) in the parents can be used (Lauritsen and Friedrich, 1976; Niikawa et al., 1977; Hassold, 1980). This approach has been applied to post-

natals but up to now hardly to the more frequently occurring cases of aneuploidy among abortion material.

The incidence of meiosis-originating aneuploidy is different when estimated after either spontaneously or induced abortion (table 7). The first category is a highly selected sample of aberrant progeny, because spontaneous abortion is often the consequence of chromosomal abnormality in the fetus. The frequency of aneuploidy caused by non-disjunction therefore, is high in this group (≈ 40%). Differences between the results of the various studies may be explained by previous selection in some studies for the presence of a fetus or fetal sac before culturing (in favour of the chromosomally normals) and variation in success during culturing (chromosomally abnormals are more difficult to culture; man: Hassold et al., 1978). On the basis of the two data that (a) 15 to 20% of all recognized (implanted) human conceptions abort spontaneously (Boué et al., 1975) and (b) 40% of these abortions are due to mitotic non-disjunction, an overall frequency of 6 to 8% for "non-disjunctional products" which still implant can be calculated. This is in line with the non-disjunction level estimated after induced abortion (see below and table 7).

The reasons to induce an abortion are many. Those cases in which abortion is induced for "socioeconomic reasons", represent a more or less random sample of human progeny between 5 to 12 weeks of gestation (counted from the first day of the last menstruation). Because earlier occurring spontaneous abortions are not taken into account, the level of 5% non-disjunctional products must be considered as a minimum value (table 7). The incidence of aneuploid fetuses that can be ascribed to meiotic non-disjunctional errors is even lower when diagnosis has taken place via amniocentesis (2.3%, Milunsky, 1979). This percentage however is determined during the 14th to 16th week of gestation (post-menstrual), so after the majority of spontaneous abortions have already taken place (before the 14th week of gestation, Boué et al., 1975). One has to recognize the fact that prenatal diagnosis by amniocentesis is normally applied for a non-random group at higher risk (among the principal indications of relevance here, are high maternal age and recurrence risk for trisomy 21), so a good comparison cannot be made.

The overall frequency of 0.4 to 2.0% aneuploid mouse embryos, predominantly trisomics, due to meiotic non-disjunction is of the same order as those frequencies estimated at earlier stages (compare with tables 3, 4, 5 and 6). This indicates a) that the majority of trisomics in the mouse survive at least untill days 9/10 after conception and b) that in man meiotic non-disjunction apparently occurs relatively more frequently than in mouse. Also at this stage, a rise in aneuploid progeny with maternal age is indicated in some studies (mouse: Yamamoto et al., 1973; Adachi and Yamamoto, 1978; man: Tsuji et al., 1978).

8. The post-natal stage (table 8)

8.1. Introduction

Quantitative data concerning the incidence of "meiotic non-disjunction products" which survive untill after birth are available in man. However, chromosome heteromorphisms visible after staining and/or of marker genes can be used to reveal, in the informative cases, the parental origin of the chromosomal aneuploidy.

In the mouse post-natals are used, in some experimental set-ups, to estimate the incidence of meiotic non-disjunction for specific chromosomes marked with phenotypically expressed genes.

8.2. Experimental Methods

Usually blood samples are taken to perform karyotype analysis on banded chromosomes from lymphocyte metaphases. The samples are cultured *in vitro* in the presence of a mitogen to stimulate lymphocyte division. Preparations are made according the usual air-drying technique.

Several banding techniques are standardly performed on mitotic metaphase chromosomes: Q-, G-, R- and C-banding are the most frequently applied ones.

Throughout the human population, heteromorphisms for several chromosomes can be found. The majority have been made visible after fluorescence staining (Jacobs, 1977). Such heteromorphisms have in some cases rendered information on the parental origin of the aneuploidy in post-natals. The parental origin may also be revealed by biochemical analysis for specific marker gene products; for example

Table 8. Data derived from live born progeny concerning the incidence of spontaneous non-disjunction on their parental origin

Reference	Species (strain)	Parameters studied	Results
1. Nielsen & Sillesen, 1975	human	11148 karyotypes of newborns	0.135% autosomal trisomics 0.284% sex-chromosomal trisomics 0.019% sex-chromosomal monosomics (45,XO)
2. Hook & Hamerton, 1977	human	56952 karyotypes of newborns	0.14 % autosomal trisomics 0.16 % sex-chromosomal trisomics 0.010% sex-chromosomal monosomics (45,XO)
3. Langenbeck et al., 1976	human	62 Down's syndrome patients: parental origin of the extra chromosome 21 with the aid of chromosomal variants	maternal* 69.3% (I: 33.9%, II: 27.4%, I or II: 8.1%) paternal* 30.6% (I: 9.7%, II: 17.7%, I or II: 3.2%)
4. Magenis et al., 1977	human	see 3, 31 patients	maternal 77.4% (I: 74.2%, II: 3.2%) paternal 22.6% (I: 16.1%, II: 6.5%)
5. Hansson & Mikkelsen,1978	human	see 3, 26 patients	maternal 73.1% (I: 34.6%, II: 23.1%, I or II: 15.4%) paternal 26.9% (I: 11.5%, II: 15.4%)
6. Goodlin, 1965	mouse (BALB/cx129)	karyotypes of 756 newborns; mothers at least 15 months old	0% chromosomal anomalies
7. Russell, 1976	mouse	X-chromosome anomalies via phenotypic expression of X-linked	maternal: $0X^{P}_{M}$ (0.05%); $X^{M}X^{M}Y$ (0%)** paternal: $0X^{M}$ (0.3 %); $Y^{XP}X^{M}$ (0.01%)

* Extrapolation of these data to the population level has led the authors to conclude that Down's syndrome as a whole is 5-10 times more often caused by AI- then by AII-errors

** Difference statistically significant

I, II: Meiosis I, II, respectively

for the Xg blood type in Klinefelter's Syndrome (karyotype 47, XXY; Race and Sanger, 1969).

Concerning primary aneuploidy in mice, some of the trisomics for the autosomes 14 or 19 survive until shortly after birth, but generally only aneuploids for the sex chromosomes are found among post-natals. X-linked coat colour markers can be used in these cases to trace both the incidence and parental origin of meiotic non-disjunction (Russell, 1976). To estimate the incidence of meiotic non-disjunction for the autosomes in live born progeny a special experimental set up is required. The method makes use of the complementation of unbalanced gametes during fertilization and consequently requires a high incidence of non-disjunction in both sexes for the same autosome(s), marked with recessive genes affecting the coat colour, as for example in mice heterozygous for certain Robertsonian translocations (Lyon et al., 1976).

8.3. Results and Discussion

In man some of the consequences of disjunctional errors during meiosis are still traceable among the live born progeny. Various types of trisomy for both sex chromosomes (0.22% of the newborns) and some of the autosomes (0.14% of the newborns; Hook and Hamerton, 1977) survive to birth. The last group predominantly consists of trisomics for chromosome 21 (about 0.1%) and the chromosomes 13 and 18 (each constituting about 0.01% of the newborns).

With respect to the parental origin of these "chromosomal anomalies" the following can be concluded:

1) Autosomal trisomics: In good quality preparations of lymphocyte metaphases Down's syndrome (47, + 21) probands sometimes show fluorescence polymorphisms for chromosome 21. The origin of this extra chromosome appeared to be maternal in about 70% and paternal in about 30% of the informative cases (Langenbeck et al., 1976; Magenis et al., 1977; Hansson and Mikkelsen, 1978). In "trisomic abortions" preliminary results indicate the origin to be maternal in about 88% (n = 33) of the cases (Hassold, 1980). Newborns surveys indicate a rise in trisomy 21 among the progeny of mothers older than 35 (Erickson, 1978).

2) Sex chromosomal anomalies: In man, the majority of aneuploids coming to birth belong to this category; in mice almost all (see 8.2.). This denotes either a relatively lower fetal wastage for this kind of anoma-

lies or/and a higher liability of these chromosomes for disjunctional errors. The latter statement finds support in the considerable level of mosaics among sex chromosomal aneuploids in both man and mouse (see for example Russell, 1976 table 7). In man, X-linked marker genes reveal the parental origin of the extra X chromosome in some Klinefelters (karyotype 47, XXY): maternal in about 60% and paternal in about 40% of the cases (Race and Sanger, 1969).

9. General conclusions

In spite of the considerable amount of work that has been done to study meiotic non-disjunction in mammals, the mechanisms leading to this phenomenon remain poorly understood. In comparison to other mammals man shows an exceptionally high incidence of meiotic non-disjunction events. This high level may be allocated to factors of (1) biological and/or (2) environmental (exogenous) origin, although in most individual cases a sharp distinction cannot be made.

In man biological factors known to be of importance to the level of non-disjunction are 1) the parental age as well as sex and 2) other factors, either chromosome specific (for example the level of trisomy 16 appears hardly affected by maternal age, Kajii et al., 1977) or having to do with chromosome pairing, chiasma formation, spindle formation, attachment of the spindle threads to the centromeres, etc. Although the human female appears to be more prone to disjunctional errors during meiosis than the male, which become even more pronounced with increasing age (Erickson, 1978), non-disjunction is not rare in the human male (Langenbeck et al., 1976; Magenis et al., 1977; Hansson and Mikkelsen, 1978). Consequently, studies on the male side must not be neglected.

When quick scoring systems are to be applied, cytophotometric methods using round spermatids may be useful in man. However, till now the more continuous and partly overlapping DNA frequency distributions (obtained by studying meiotic systems with varying degrees of spontaneous non-disjunction) limit its usefulness For a proper development of these systems a cytological check of the cytophotometric techniques will remain necessary. In experimental male animals (but not yet in man, due to unsufficient spreading of the metaphases II), first anaphase non-disjunction can be studied relatively simply and on a quanti-

tative base in secondary spermatocytes. If first cleavage eggs are studied in addition, anaphase II non-disjunction may also be quantified. Of fundamental value for man are the studies on the male pronucleus chromosomes after interspecific fertilization in vitro (Rudak et al., 1979, section 5.3.).

Concerning the mammalian female, anaphase I-originating non-disjunction can be determined cytologically in oocytes at Metaphase II (for humans these data are almost absent).

Since at first cleavage division male and female derived pronuclei can be distinguished morphologically, both sexes can be studied simultaneously at this stage with respect to the incidence of meiotic non-disjunction.

Studies in man, making use of chromosome heteromorphisms or marker genes, indicate that the female is more prone to disjuntional errors during meiosis than the male and also that AI-originating non-disjunction may at least be 5 to 10 times more frequent than AII-originating errors (Langenbeck et al., 1976; Jacobs and Morton, 1977 and table 8). Therefore, future research should focus more on factors affecting the ovum prior to ovulation, as in the mammalian female, meiosis I is completed before the shedding of the eggs. In this context irregularities in the duration of oocyte maturation may be of importance (mouse: Butcher and Fugo, 1967; Karp and Smith, 1975; human: Jongbloet, 1975). An irregular menstrual cycle is most frequently found in women at the start and at the end of their fertile years and indeed a relatively higher incidence of trimsony 21 is found among the progeny of women belonging to both age groups (Erickson, 1978). Age dependent non-disjunction during MI has been attributed to varying factors like spindle malfunction slowerdispersion of nucleolar material and a decreasing chiasma frequency which may also be the result of an aberrant chiasma-terminalization process. The higher incidence of disjunctional errors may also find its origin in the presumed genetic diversity in man (Hook and Porter, 1977) when compared to laboratory animals. The latter are held under standard environmental conditions and in relatively small groups, often selected among others for good reproduction capabilities.

Epidemiological studies remain necessary in man to enhance our knowledge about clearly exogenous factors (food additives, radiation for diagnostic purposes, medicine, etc.) which may influence the meiotic process in man. That environmental conditions are suspected may follow from the fact that - in contradiction to the expectations - the frequency of chromosomally aberrant progeny born to nowadays younger parents at lower risk is not declining (Matsunaga and Fujita, 1977).

For more detailed information on aberrations of the meiotic process in both sexes, experimental animals will still be needed in the future.

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A FIRST EXPLORATION OF A ROBERTSONIAN TRANSLOCATION HETEROZYGOTE IN THE MOUSE FOR ITS USEFULNESS IN CYTOLOGICAL EVALUATION OF RADIATION-INDUCED MEIOTIC AUTOSOMAL NON-DISJUNCTION

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Summary

In this report some data concerning the male meiotic system of mice heterozygous for Rb(11.13)4Bnr are presented and compared with those of a chromosomally normal Swiss random-bred stock.

Change of the genetic background from a C3H/Swiss hybrid situation to the fourth backcross generation (to the Swiss random-bred stock), did not alter the average frequency of aneuploid secondary spermatocytes. This was confirmed by studies on post-implantation loss.

Spermatogenic characteristics of Rb4/+ mice, such as testis weight, sperm production and the number of diplotene-metaphase-I figures found in stage XII of the seminiferous epithelium, suggest delay and cell death during this period. These data support our working hypothesis that such an aberrant chromosome system may be more prone to radiation effects and therefore is promising in our cytological studies into the causes of spontaneous and induced autosomal non-disjunction during meiosis in the mouse.

From cytogenetic work in humans, the conclusion seems to be valid that man is prone to chromosome non-disjunction during meiosis. In human abortuses (which constitute 15—20% of all recognized conceptions) the occurrence of an extra chromosome accounts for about half of the cases (Boué et al. [4]). A small part of the trisomic embryonic population survives to birth (i.e. mainly those for chromosome 21, causing Down's syndrome) and, in population surveys, accounts for 0.135% of live births (Nielsen and Sillesen [25]). Thus, among human zygotes the incidence of monosomics and trisomics must be of a significant percentage, although this cannot be given accurately because of the unknown ratio between recognized and unrecognized conceptions.

Langenbeck et al. [21] have studied the parental origin of Down's syndrome, making use of chromosomal polymorphisms. In 30.6% the origin was male and in 69.4% it was female. Moreover, they concluded that errors at first meiotic divisions are between 5 and 10 times more important in causing aneuploid gametes than errors at second division (see also Hansson and Mikkelsen [16]).

Switching to the mouse data, we may conclude that the frequencies of both anaphase I and II non-disjunctions are much lower here than in man. The frequency of mainly autosomal non-disjunction at first meiotic division is believed to be very low throughout life in male mice (0.38 ± 0.12%, Beatty et al. [1]; and also Ohno et al. [26]). The data in the female, with an equally low rate in young adults, are not uniform at an older age. Uchida and Freeman [32] found an increase with age whereas other investigators did not (Martin et al. [23]; Polani and Jagiello [27]). Indirect evidence — through study of the chromosome complement of the first cleavage division — indicates that, under normal conditions, non-disjunction originating at anaphase II in mice must also be infrequent (Donahue [9]).

Despite the gap between man and mouse with regard to spontaneous non-disjunction, the mouse is the experimental animal from which most of our knowledge about the incidence of mutational events after exposure to physical and chemical mutagens has accumulated. To date, no sensitive cytogenetic test system has been evolved (Searle [30]) for non-disjunctional events concerning the autosomes.

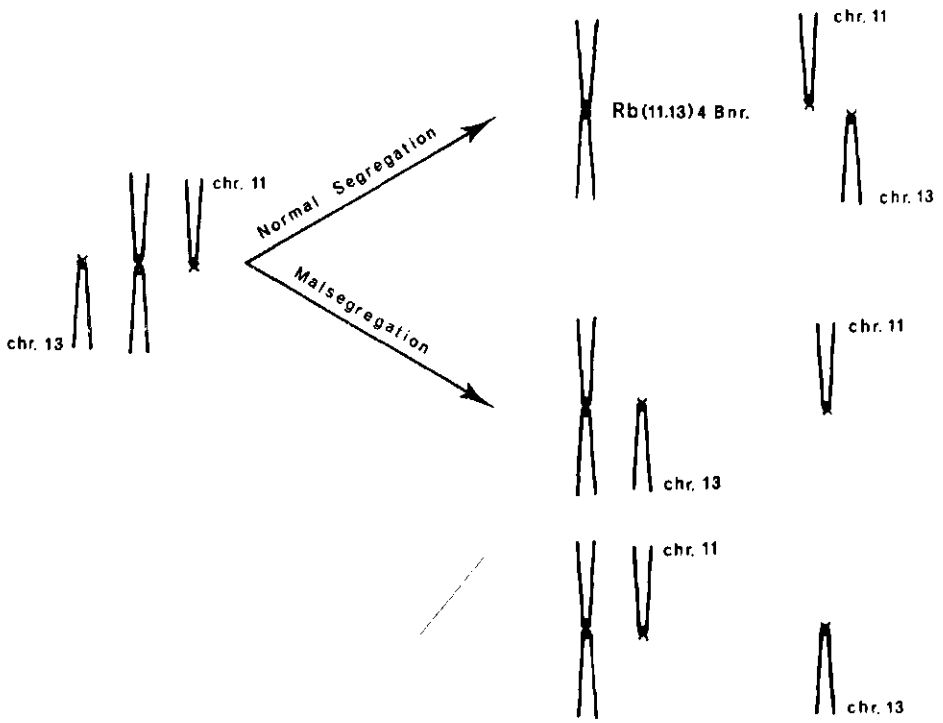

Fig. 1. Segregation possibilities of the trivalent caused by the Rb(11.13)4Bnr chromosome at anaphase of the first meiotic division.

In the present contribution, some observations concerning the male meiotic system of Rb(11.13)4Bnr, which shows high spontaneous non-disjunction for chromosomes 11 and 13, are presented. The Rb4Bnr heterozygote has shown relatively the highest level of aneuploid secondary spermatocytes, of the group of tobacco-mouse-derived metacentrics studied: ≃25% aneuploid secondary spermatocytes are caused by mal-segregation of the trivalent (Fig. 1) formed at prophase of the first meiotic division (≃29%, Cattanach and Moseley [7]; ≃25%, Gropp et al. [14]).

The working hypothesis is that a meiotic system, showing a high spontaneous abnormality percentage, after anaphase I disjunction, is more prone to a changed cellular environment, for instance induced by low doses of radiation during meiotic prophase till anaphase I. These systems may give us a better understanding of the minimal detectable dose, and dose—effect relationships are probably more easily obtained.

Before aneuploidy-induction studies will be carried out, we considered it desirable to gain a better insight into the "spontaneous" spermatogenic and spermiogenic situation within Rb4 heterozygotes. Thus, data concerning the amount of aneuploidy originating at anaphase I (due to the translocation) are presented here. Furthermore, an approach is made in describing spermatogenesis quantitatively, and indications for meiotic delay are examined.

Another reason for focusing on a Robertsonian translocation is the relative abundance of this type among human translocations, involving D- and G-group chromosomes (Hamerton [15]). (For all different types of Robertsonian exchange see John and Freeman [18]; Daniel and Lam-Po-Tang [8]; and Lau and Hsu [22]).

The work described here is part of a larger comparative study on chromosomal behaviour in male (and female) meiosis in the mouse that makes use of (a) reciprocal translocations (de Boer [3]) and Robertsonian translocations (Nijhoff, in preparation), and (b) combinations of these two when there is a partial homology (Nijhoff, in preparation).

Material and methods

The Rb(11.13)4Bnr heterozygous male mice used for this report were from two sources. One represents the F1 between Rb4Bnr homozygotes on a C3H genetic background (obtained from Prof. A. Gropp) and the Swiss random-bred

TABLE 1

BREEDING RESULTS FOR Rb4Bnr TRANSLOCATION HETEROZYGOTES AND THEIR CHROMOSOMALLY NORMAL CONTROLS

	Number of males tested	Number of females mated	Total number of implantations	Mean number of implantations	Post-implantation loss (%)
Rb4/+(F_1) × +/+	26	33	513	15.5 ± 2.3	18.5
+/+(B_1) × +/+	13	24	394	16.4 ± 3.1	5.3
Rb4/+(B_4) × +/+	18	34	571	16.8 ± 2.7	19.6
+/+(B_4) × +/+	18	34	654	19.2 ± 2.4	8.4

TABLE 2

CHROMOSOME ARM COUNTS IN METAPHASE II CELLS OF Rb4Bnr TRANSLOCATION HETEROZYGOTES AFTER ONE (F_1) AND FIVE (B_4) TIMES OUTCROSSING WITH SWISS Cpb:SE(S) RANDOM-BRED STOCK

Results are given for each male separately.

	Number of cells scored	Arm counts					19 + 21 (%)	2 X 21 (%)
		less than 19	19	20	21	more than 21		
RB4/+ (F_1)	100	3	9	80	8	—	17	16
$n = 5$	100	—	18	75	7	—	25	14
	100	2	13	71	14	—	27	28
	100	1	13	70	15	1	28	30
	100	4	8	79	9	—	17	18
							22.8 ± 4.8	21.2 ± 6.5
Rb4/+ (B_4)	100	2	7	80	11	—	18	22
$n = 5$	100	—	10	79	10	1	20	20
	100	1	11	76	12	—	23	24
	100	1	10	79	10	—	20	20
	100	—	10	74	15	1	25	30
							21.2 ± 2.5	23.2 ± 3.7

stock (Cpb:SE(S)). The other consitutes the fourth backcross generation to this Swiss stock. The two will be designated F1 and B4 throughout this text.

The data about the percentage of unbalanced male gametes — M II counts and embryonic plus foetal loss data — are derived from both genetic backgrounds (F1 and B4, Tables 1 and 2). The chromosomally normal controls (+/+) were the most closely related animals: for the F1 their +/+ sons and for the B4 generation full brothers were used.

The meiotic preparations were made according to the Evans technique [10], when the animals were between 2 and 5 months of age. The preferential staining of the constitutive centric heterochromatin (C-banding) of the meiotic chromosomes was routinely performed by a modification of the BSG method described by Sumner [31].

The embryonic plus foetal loss data were determined by caging males with a 2—3-month-old virgin Swiss Cpb:SE(S) random bred female from a line selected for high litter size (Schreuder [28]). For most of the males this was done twice, but never in the same week. The day the vaginal plug was recorded was designated as day 1. Autopsy was carried out on the 19th day of pregnancy, and the numbers of big and small moles as well as live embryos were counted.

The data on spermatogenesis — testis weights, epididymal sperm counts, fraction of abnormally shaped sperm and the number of cells in late diplotene-metaphase I, in randomly selected regions of the seminiferous tubules with stage XII of spermatogenesis — were obtained from Rb4/+ F1 males (Tables 3 and 4). Here +/+ Swiss random-bred males were used as controls. The animals were ≃4 months old when examined. Epididymal sperm was obtained and counted according to a method described by Searle and Beechey [30]. For

TABLE 3

TESTIS WEIGHTS AND SPERM DATA OF SWISS Cpb:SE(S) RANDOM BRED (+/+) AND Rb4Bnr C3H/+ Cpb:SE(S) TRANSLOCATION HETEROZYGOTES

Line	Mean testis wt. (g)	Mean sperm count $\times 10^6$	% of abnormally shaped sperm heads and tails (10 animals contributing)
RB4/+ (F_1) $n = 15$	0.074 ± 0.009	2.75 ± 1.06	14.8 ± 10.5 ($n = 500$)
+/+ $n = 15$	0.122 ± 0.011	6.52 ± 1.11	7.9 ± 4.7 ($n = 500$)

the determination of sperm morphology, the method of Bryan [6] has been taken. The subdivision into different types of sperm abnormalities was adapted from Bruce et al. [5].

Preparations of seminiferous tubules for counts of diplotene-metaphase I cells were made as follows. The testes were brought into an isotonic 2.2% aqueous sodium citrate solution, and the tunica albuginea were removed. With the aid of two needles, long pieces of tubules were selected and fixed in Carnoy (3 : 1, methanol : acetic acid) after they had been given a hypotonic pretreatment for 20 min in a 0.7% aqueous solution of sodium citrate. From a chosen tubule, successive pieces of ≃4 mm were separated. Their accurate lengths were first determined, with the aid of a microscope drawing attachment, while still in a drop of Carnoy's fixative. Air-dried preparations of each piece were made by releasing the entire contents of the tubule into a small drop of 45% acetic acid and subsequent spreading by placing the slide on a pre-warmed hot plate of ≃50°C. If adjoining pieces also contained meiotic figures, they were all considered to belong to one spermatogenic "clone" (contained by one stage XII) and were summed.

To determine the mean distance between subsequent stages XII, we chose the following approximation. Per male the summed length of all pieces of seminiferous tubule examined was divided by the total number of stages XII found in this animal. This probably gives an underestimate of the real mean value.

Results

Table 1 shows the effect of the F1 and B4 genetic backgrounds on post-implantation loss caused by heterozygosity for Rb(11.13)4Bnr. These values,

TABLE 4

COUNTS OF FIRST MEIOTIC FIGURES WITHIN STAGE XII OF THE SPERMATOGENIC WAVE

Line	$n_{stages\ XII}$	Mean number of MI's at stage XII	Mean distance (mm) between subsequent stages XII
Rb4/+ (F_1) $n = 5$	23	397 ± 334	19.72 ± 2.54
+/+ $n = 5$	23	269 ± 296	22.22 ± 6.97

corrected for their nearest related chromosomally normal controls, showed no major differences: the Rb4/+ condition caused 13.2% post-implantation loss in the F1 and 11.2% in the B4 generation.

The Rb4 chromosome appeared to affect the mean number of implantations only significantly ($p < 0.01$) in the B4 generation when compared with the control data.

The cytological results on aneuploidy in secondary spermatocytes are shown in Table 2. The percentage of cells with either 19 or 21 arms are considered to be the complementary result of non-disjunction of the chromosomes involved in the trivalent formed by the Rb4Bnr chromosome with its two homologues at the first meiotic division (see Fig. 1). Arm counts of 18 and less were considered to be artifacts caused by preparation and as such have not been taken into account. The percentages found were reasonably low (av. 1.3%) and give some indication of the reliability of the values presented in this table.

Cell breakage may also lead to arm counts of 19. For this reason the estimate of the frequency of aneuploid spermatozoa was calculated twice: based on the sum of metaphase-II cells with 19 and 21 (this may theoretically tend to be an overestimate), as well as on the 21 arms containing cells only (this may give an underestimate). Comparing these procedures, we found no significant difference, again showing that the percentage of broken cells may be considered low in these preparations (see also Table 2). The few cells shown in this table to contain more than 21 arms are considered to be the result of a simultaneous non-disjunction for the trivalent, as well as for a normal bivalent.

In agreement with the post-implantation loss data, presented in Table 1, the difference in genetic background did not seem to influence the average percentage of aneuploid secondary spermatocytes caused by non-disjunction of the trivalent at anaphase I. It is noteworthy, however, that the variance of the frequency of aneuploid secondary spermatocytes seems to be less in the B4 generation than in the F1 generation (Table 2). In the B4 generation, the coefficient of variation was 11.8%, which is comparatively low for a biological trait and a useful characteristic when induced non-disjunction responses have to be measured. Monosomy for chromosomes 11 or 13 is not usually compatible with survival to the beginning of implantation (Gropp et al. [14]). For this reason there is a discrepancy in the values presented for post-implantation loss caused by Rb4/+ and presented at the beginning of this section and for the percentages of aneuploid secondary spermatocytes.

In the investigation into the causes of reduced fertility of the Rb4/+ male, testis weights and sperm data of the F1, as well as of chromosomal normals of the Swiss random-bred stock, were determined, as shown in Table 3. The mice that carried Rb4Bnr showed a significant lower testis weight ($p < 0.05$, Tukey test) than the +/+ animals and their sperm count was only 42.1% of the value found in the Swiss +/+ stock, which, again, is a significant difference ($p < 0.05$, Tukey test). Testis weights and sperm counts were highly correlated in the Rb4/+ group ($r = 0.95$, $p < 0.001$), and a similar positive relation was found within the Swiss +/+ animals ($r = 0.58$, $p < 0.05$). Within Rb4/+(F1), this relation may be even more pronounced when only morphologically normal spermatozoa are considered; a negative correlation coefficient between the sperm count and the percentage of abnormally shaped sperm was calculated ($r = -0.61, p < 0.05$).

Table 4 presents data on the numbers of meiotic figures found at stage XII of spermatogenesis and the wavelength of the seminiferous epithelium for Rb4/+ F1 and Swiss random-bred males. The number of cells at late diplotene-metaphase I was determined to give an indication of the sizes of the individual spermatogenic "clones". In spite of the variety in sizes, the mean number of MI's at stage XII counted in Rb4/+ showed a significant difference compared with the mean number found in the +/+ mice ($p = 0.055$, Mann-Whitney U test). No such difference was found with regard to the wavelength of the seminiferous epithelium.

Discussion

The foregoing results lead to the following information about the influence of the heterozygous condition for Rb(11.13)4Bnr on the fertility of the male mouse.

It has been stated that gross genome imbalance does not seem to influence the fertilizing capacity of mouse spermatozoa, nor the development of the zygote till implantation (Ford and Evans [13]). In spite of this, the percentage of numerical unbalanced secondary spermatocytes is not concordant with post-implantation loss when corrected for the control values. All post-implantation data were determined at day 19 of gestation, and at this stage of embryonic development all aneuploid individuals may be considered to be eliminated (Gropp et al. [14]). So it seems plausible to attribute the differences to pre-implantation loss of monosomic embryos. Cytological proof of this phenomenon is as yet lacking. There is the possibility, of course, that a gradual disappearance of very early resorptions at this late developmental stage may more likely affect the Rb4/+ data.

It has been suggested indirectly, by Ford and Evans [12], that translocation heterozygosity may exert an influence on the fertilizing capacity of the Rb4/+ male, as this may be true for another Robertsonian translocation in mouse (Evans et al. [11]). This seems less likely here, because the sperm counts of the Rb4/+ males, corrected for morphologically abnormal spermatozoa, are sufficiently high to ensure normal fertilization (Searle and Beechey [30]) which is also reflected in the high mean numbers of implantations.

It is tempting to try and relate the deviant spermatogenic characteristics ("clonal" size in stage XII, testis weight and epididymal sperm count) of Rb4Bnr heterozygotes to the production of aneuploid gametes. To do so, the following expression might be helpful, although we realize the right-hand side parameters may be interrelated in a more complicated way:

mean sperm count = mean number of MI's at stage XII × number of stages XII/mm × total tubule length (mm) × c

With the length of the spermatogenic wave being about the same for translocation and non-translocation carriers, an increase of the population of diplotene-metaphase I figures per stage XII, at first sight, means an increase of the sperm production. It is known that the total length of the tubules is an important parameter for testis weight (Hayward [17]). The testis weight of Rb4/+ is 61%

of the control value, while the sperm count amounts to 42%. So it seems less likely that "clonal" size, as defined here, shows a positive linear relationship with the sperm production. In fact, the increase of the population of cells at diplotene-methaphase I indicates that at least a fraction of the cells spend a longer duration at this stage, which we believe is a sign of meiotic breakdown of this category. Support for this view comes from an autoradiographic study where preliminary results indicate the period pre-meiotic S-phase to diplotene to be prolonged as well, when one Rb4Bnr chromosome is carried in the male. Compared with the data presented by Kofman-Alfaro and Chandley [20] for Q-strain +/+ males, our data suggest a delay of $\simeq$18 h. It does not seem likely that one can attribute this delay to genetic background differences only (see for instance Bianchi and Tiglao [2]).

At present, research is in progress to follow the cells from metaphase I to metaphase II. If an increased time lapse is discovered here as well, an analogue will have been found with female meiosis "in vitro" in the mouse where Karp and Smith [19] found that low temperature caused delays induced by first meiotic non-disjunction.

More research is needed, however, before the relation between deviant meiotic timing and behaviour within Rb4/+ male mice is fully understood. If the relation has more of a causal than a casual component, Rb4/+ males seem to constitute a useful system to study delay-induced non-disjunction. It should be reported here that the [^{3}H]thymidine autoradiographic experiments mentioned earlier show the period between metaphase I and metaphase II to be a matter of hours (<3 h). In this light, a mitotic delay of a few hours (3—5 h), in mouse spermatogonia of all types, when exposed to 100 R of X-rays, is already of significance (Monesi [24]).

Although we do not believe this to be of a major effect, it has to be indicated here that besides a partial difference in the genetic background between +/+ and Rb/+ animals, the difference in origin of the Y-chromosome between the two can also influence testis weights (Hayward [17]). Mean aneuploidy values between Rb4/+ F1 and Rb4/+ B4 males were not different (Table 2), however.

Despite the signs of meiotic breakdown in Rb4/+ males, indicated by the present results, the yield of secondary spermatocytes, when normal cytological procedures are followed, is sufficiently high to allow inspection of a sufficient number of cells within a reasonable amount of time. Careful cell-to-cell inspection will remain necessary with this procedure.

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RADIATION-INDUCED MEIOTIC AUTOSOMAL NON-DISJUNCTION IN MALE MICE

THE EFFECTS OF LOW DOSES OF FISSION NEUTRONS AND X-RAYS IN MEIOSIS I AND II OF A ROBERTSONIAN TRANSLOCATION HETEROZYGOTE

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Summary

Male mice, heterozygous for the Rb(11.13)4Bnr translocation, were irradiated for 14.5 min with either a dose of 15-rad fission neutrons or 60-rad X-rays. Animals of this karyotype are known to show high levels of spontaneous autosomal non-disjunction (20—30%) after anaphase I. The effects of the irradiation on this process were determined after 2 and 3 h in air-dried preparations.

The length of the period from the end of meiosis I till the end of meiosis II was assessed autoradiographically, with the aid of cells showing a labelled Y chromosome only and appeared to last less than 3 h. Inter-mouse variation with regard to the duration of the period "last premeiotic S-phase till diakinesis/metaphase I" prevented a more accurate estimate.

On the basis of this 3-h datum, the induced effects were studied at intervals of 2 and 3 h after the start of the irradiation. The influence of irradiation was assessed by scoring: (1) univalents in primary spermatocytes, (2) deletions, aneuploid chromosome counts and precocious centromere separation in secondary spermatocytes, and (3) chromatid gaps and breaks in both cell types. Both radiation types induced comparable levels of chromosomal damage. A neutron-X-rays RBE value for these parameters was calculated to be 5.4 for the MI stage and 3.3 for the MII stage. The significantly higher incidence of cells showing damage at MII than at diakinesis/MI is not believed to indicate a difference in radiation sensitivity, but is believed to be merely the consequence of the different chromosomal processes taking place during the irradiation-fixation time interval.

Only the 15-rad neutron irradiation decreased the level of aneuploid secondary spermatocytes signifcantly, which is best explained by selective cell killing rather than protection against non-disjunction. Earlier data about the meiotic system of Rb4Bnr have indicated delay and cell death during diakinesis/metaphase I, and it is hypothesized that the selective cell killing by neutrons is determined by the length of delay occurring in each individual cell in the uninfluenced situation. The effect found underlines the possibility that delay and proneness to anaphase-I non-disjunction are interrelated, which can only be observed in a meiotic system already showing high levels of spontaneous autosomal meiotic non-disjunction.

Low doses of radiation, administered for diagnostic purposes, are suspected as one of the possible "environmental factors" increasing the level of spontaneous meiotic non-disjunction in man. This may be of importance with regard to the increasing evidence that the lowering of the mean age of child-bearing mothers nowadays is, in contradiction to expectation, not attended with a decline in the incidence of Down's syndrome patients among the newborns (Matsunaga and Fujita, 1977; Uchida, 1979). An alternative explanation for this phenomenon may be a better ascertainment of patients in recent years (Mikkelsen et al., 1976). That meiotic non-disjunction is an important problem in man is reflected by the fact that of spontaneous abortuses (constituting 15—20% of all recognized conceptions) trisomy accounts for about half of the cases (Boué et al., 1975). To what extent both sexes contribute to this level is still unknown. However, fluorescence polymorphisms for chromosome 21 make it possible to trace the parental origin of the extra chromosome in some Down's syndrome patients and indicate a paternal origin in at least one-third of these informative cases (Langenbeck et al., 1976; Jacobs and Morton, 1977; Magenis et al., 1977; Hansson and Mikkelsen, 1978). Extrapolation of these data to the population level has led Langenbeck et al. (1976) to the conclusion that Down's syndrome as a whole is 5—10 times more often caused by AI than by AII errors. Both conclusions may, however, not hold true for all autosomes.

When the mouse is used as a mammalian assay system for studying meiotic non-disjunction, a possibly relevant difference between man and mouse may be this high level of spontaneous meiotic non-disjunction in the former in comparison with the latter. In male mice, incidences of aneuploid segregation products have been determined at the MII stage (0.38 ± 0.12%, Beatty et al., 1975) and at the first-cleavage division (from 0.5 to 0.9%, Fraser and Maudlin, 1978).

Working with mice, therefore, we considered it promising to carry out a study of the effects of low doses of irradiation on a male-mouse meiotic system spontaneously showing a high level of meiotic non-disjunction. The reasons for choosing male mice heterozygous for Rb(11.13)4Bnr (~25% of aneuploid MII's) have been outlined in a previous report (Nijhoff and De Boer, 1979). X-Rays may induce meiotic non-disjunction during mouse spermatogenesis, as could be the conclusion from the studies of Russell and Saylors (1963), Szemere and Chandley (1975) and Hasmann et al. (1979).

Two radiation types, fission neutrons and X-rays, were chosen for a study of their effects at low doses on the disjunctional process at metaphase—anaphase

I. Both stages are known to be of short duration and to allow a good comparison between both radiation types equal exposure times of 14.5 min were given. As the time lapse between the successive meiotic metaphases was not well known, the length of this period was first assessed autoradiographically before the recording of the incidence of aneuploid secondary spermatocytes.

In addition to numerical aberrations, chromosomal damage was assessed, so that the possible correlation between these two radiation effects might be traced.

Searle et al. (1969) estimated a fission neutron-X-rays RBE of about 3.7, when results from 50—400 R X-rays (induction of translocations in mouse spermatogonia) are compared with the results from 25—50 rad neutrons. Oakberg (1975) mentioned an RBE value of 6.5 for 1—2 MeV fission neutrons in the range of 18—101 rad when their effectiveness in killing mouse spermatogonia was measured. To obtain comparable levels of effectiveness in these irradiation experiments, doses of 15-rad fission neutrons (mean energy 1.7 MeV) and 60-rad X-rays were chosen; so a neutron-X-rays RBE value of 4 was assumed.

Material and methods

The Rb(11.13)4Bnr heterozygous male mice used for these studies were either of the 6th or 7th backcross generation of the original Rb4Bnr homozygotes (on a C_3H genetic background) with the Swiss Cpb SE(S) random-bred stock. The 2 generations will be designated B6 and B7 throughout the text.

Autoradiography

The mean durations of the stages between the metaphases of both meiotic divisions (MI and MII) were recorded autoradiographically in the B6 generation. For this purpose 9 mice, between 5 and 7 months old, were injected intraperitoneally with 5 μCi/g body weight of [6-^{3}H]thymidine (Amersham, specific activity 28 Ci/mmole) and killed after various time intervals (see Table 1). Autoradiographs were made and interpreted according to the method described by Kofman-Alfaro and Chandley (1970).

Scoring was done as follows. Primary spermatocytes — from late diplotene till metaphase I — were allocated to 3 classes of chromosome spiralization according to criteria described elsewhere (De Boer and Groen, 1974). Only primary spermatocytes allocated to "spiralization class 3" (cells showing chromosomes in their most contracted form) were taken into consideration here, to approximate the metaphase-I stage as precisely as possible. In the group of secondary spermatocytes no subdivision was made.

To avoid problems with the interpretation of the labelling patterns, cells showing a labelled Y chromosome only — i.e. those incorporating label at the end of the last premeiotic S phase — were used to compute the time lapse between MI and MII (see Figs. 2A and 2B). This choice has several advantages: the group making up the front of labelled cells is clearly defined and represents a never-returning stage during the uptake of label into the chromosomes. By this method the profile of labelled cells against time should approach a humped curve with resemblance to a true pulse.

TABLE 1

LABELLING OF METAPHASE-I AND METAPHASE-II SPERMATOCYTES DERIVED FROM Rb4Bnr TRANSLOCATION HETEROZYGOTES (B6 OR B7 GENERATION) OR +/+ ANIMALS (SWISS Cpb SE(S) RANDOM-BRED STOCK), AT DIFFERENT TIMES AFTER THE i.p. ADMINISTRATION OF [6-^{3}H]THYMIDINE

	Time after ^{3}H injection		MI			MII		
	days	h	Number of cells scored	Percentage labelled	Percentage with only a labelled Y	Number of cells scored	Percentage labelled	Percentage with only a labelled Y
Rb4/+ (B6)	10	18	50	0	0	50	0	0
	11	0	100	6	3	100	0	0
	11	3	100	62	8	100	8	2
	11	6	100	60	7	100	23	7
	11	7.5	100	99	0	100	72	1
	11	9	100	61	1	100	17	1
	11	12	100	61	3	100	9	0
	11	15	100	95	5	100	55	4
	11	18	100	99	0	100	98	1
+/+ (Swiss)	10	18	75	0	0	75	0	0
	11	0	100	30	5	100	1	1
	11	6	100	87	4	100	42	2
	11	12	50 *	96	0	50 *	56	2
Rb4/+ (B7) ** (a)	11	2	100	1	0	100	0	0
(a)	11	2	100	19	0	100	0	0
(b)	11	2	100	23	1	100	0	0
(b)	11	2	100	75	5	100	37	5

* Male with small testes and limited analysable material.

** Animals had been irradiated 2 h before with 15-rad neutrons (a) or 60-rad X-rays (b).

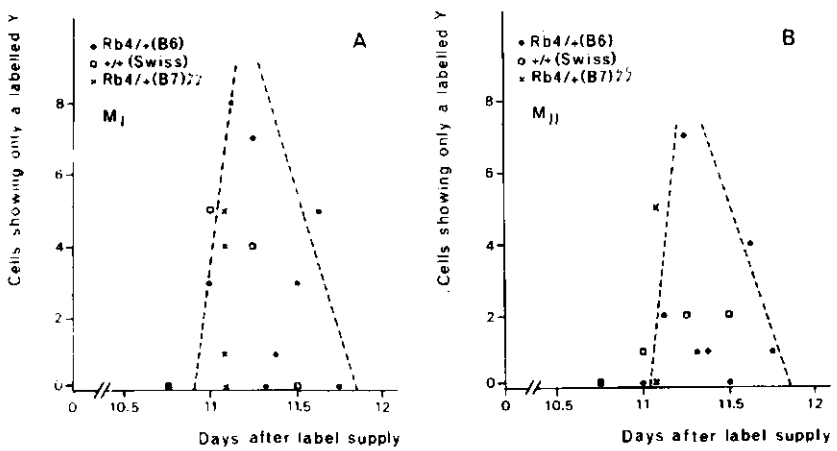

Fig. 1. (A) Frequencies of metaphase-I spermatocytes (spir. class 3), as determined in Rb4Bnr/+ and +/+ mice, with only a labelled Y chromosome at various intervals following the injection of [^{3}H]thymidine. See also Table 1. Dashed lines indicate trend for Rb4/+ (B6) mice. (B) Same frequencies in metaphase-II spermatocytes.

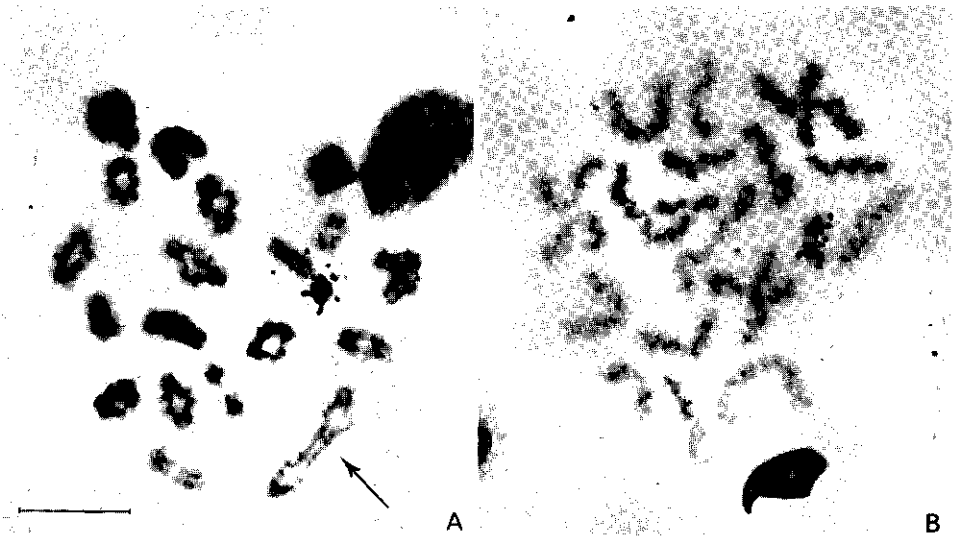

Fig. 2. (A) Metaphase I spermatocyte (spir. class 3) of an Rb4Bnr heterozygote showing a labelled Y chromosome. The arrow indicates the trivalent caused by the Rb(11.13) 4 Bnr chromosome. (B) Metaphase-II spermatocyte showing a labelled Y chromosome. The bar represents 10 μm.

For tracing the effect of the "Rb4Bnr/+ karyotype" on the MI—MII time lapse, 4 +/+ Swiss CpbSE(S) males were studied.

Radiation experiments

Rb4Bnr/+ males of the B7 generation were used, about 5 months old at the moment of irradiation.

2 types of radiation were used at equal exposure time. The first group consisted of 6 animals exposed to 15.2-rad fast neutrons (mean energy of the fission spectrum 1.7 MeV, exposure time 14.5 min) including 1.9-rad γ-contamination from the ITAL reactor (Wageningen, The Netherlands). The second group of 6 was exposed to whole-body X-irradiation (300 kV, HVL 3.1 mm Cu, exposure time 14.5 min). Thus, dose rates were ~1 rad/min for the fast neutrons and ~4 rad/min for the X-rays. During both procedures the mice were kept in plexiglass cages with sides 2 mm thick.

Identical procedures were followed for both groups thereafter. From each group 4 animals were killed at 2 h and 2 animals at 3 h after the beginning of irradiation, the timing being based on the autoradiography experiments (see Results).

To trace a possible irradiation effect on the progression of the spermatocytes from MI to MII, 2 animals from the 2-h sub-group were also labelled with [6-^{3}H]thymidine (5 μCi/g, injected intraperitoneally) exactly 11 days before the start of the irradiation. At 11 days the front of labelled cells was presumed to be at metaphase I (see Results). The preparations from these animals were treated and scored as described in the previous section. They were also used for the estimation of the irradiation effects on the chromosomal level (following section).

For an accurate scoring of the irradiation effects, meiotic preparations made

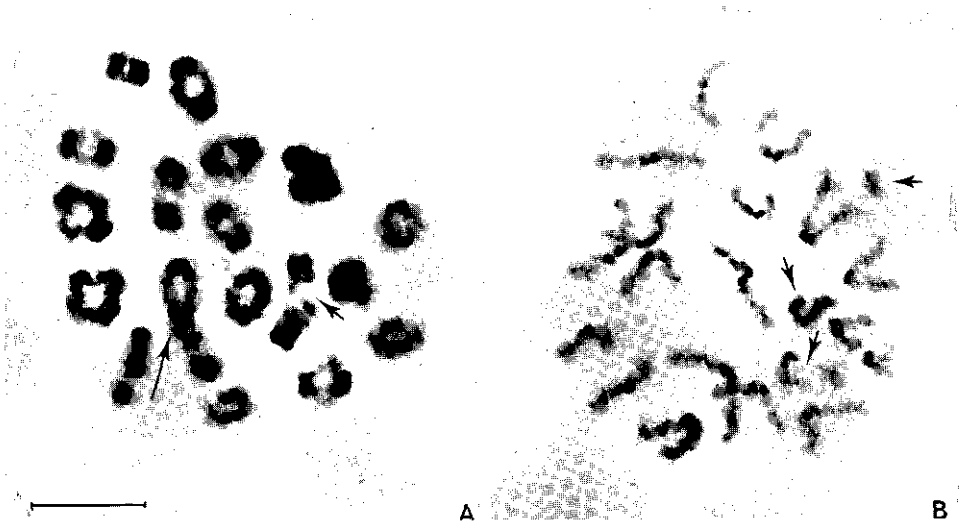

Fig. 3. (A) Metaphase-I spermatocyte (spir. class 3) of an Rb4Bnr heterozygote showing a chromatid gap (short arrow), 2 h after 60-rad X-rays. The long arrow indicates the trivalent. (B) Metaphase-II spermatocyte showing chromatid breaks in an autosome and the X-chromosome as well as the separated X-chromosome fragment (arrows), 2 h after 60-rad X-rays. The bar represents 10 μm.

according to the Evans technique (Evans et al., 1964) were subsequently C-banded with a slight modification of the BSG method described by Sumner (1972). Without the subdivision of the "MI stage" as carried out in the previous section, all cells from late diplotene till metaphase I were included in the MI fraction here. The number of chromosome arms as well as the presence of the metacentric Rb4Bnr marker chromosome were determined in MII cells. Both MI and MII cells were scored for radiation-induced damage (including chromatid breaks or gaps and deletions; Figs. 3A and 3B) as well as for the incidence of univalents at MI or unpartnered chromatids at MII. Gaps were included in this study because they may be considered as incomplete breaks (Brecher, 1977). The radiation-induced damage was scored to test its possible influence on the process of non-disjunction. Control data on all these parameters were derived from 6 unirradiated Rb4Bnr heterozygotes (5—7 months old) of the same B7 generation.

Statistical tests were mainly carried out by the Wilcoxon signed rank test. The differences in sample variances (Bartlett test) did not always allow the use of a Student *t* test.

Results

Autoradiography

Table 1 presents the results of the different animals processed for various periods of time after label supply. From these results the following can be concluded. (1) There appear to exist between-mice variations with regard to the time cells spend between the last premeiotic S phase and either metaphase I (spiralization class 3) or metaphase II. (2) The front of labelled cells at MI and

MII, resp., was found in animals killed 3 h apart, a first indication of the length of the period "start of MI (spiralization class 3) to start of MII".

A graphical presentation of the data in Table 1, concerning the fraction of cells at MI or MII with only their Y-chromosome labelled, is given in Figs. 1A and 1B. Dashed lines are drawn to indicate the most likely trend for the "Rb4Bnr/+ (B6) points". Comparison of the points of interception with the ordinate of the dashed lines to the left in both graphs indicates a duration of the period "start of MI (class 3) to start of MII" also of about 3 h.

Needed however, is the datum about the duration of the period "end of MI to end of MII". The percentage of cells with only the Y-chromosome labelled at MI and MII respectively is determined by both the time of autopsy as well as the duration (i.e. the total number of cells) of each stage. So, theoretically, the MI/MII ratio for the "maxima" in Figs. 1A and 1B will give the inverse ratio for the durations of both stages. Before this comparison can be made, the value for the maximal fraction of MII cells showing only a labelled Y has to be corrected by multiplying by a factor 2 because, in contrast with the MI's, only half of the MII's is Y-chromosome containing. An exact determination of the maximal levels is not possible (see Fig. 1), but the shapes of both "curves" indicate an MI/MII ratio (after correction) of 1 or less. So it was concluded that MII does not last any longer than MI (spiralization class 3), and the latter was estimated to last about 3 h. On this basis, periods of 2 and 3 h after irradiation have been chosen for tracing its effect on the disjunctional process at metaphase I—anaphase I.

Also shown in Figs. 1A and 1B, and in Table 1, are the labelling data derived from both chromosomally normal (+/+) Swiss Cpb SE(S) males and Rb4Bnr heterozygotes (B7) irradiated 2 h before autopsy. From the +/+ group, autoradiographs with time intervals shorter than 10 days 18 h were also obtained with no sign of labelled cells at the diakinesis/MI stage. It cannot be deduced from these data whether there are important differences between these groups and the Rb4/+(B6) mice with regard to the progress of spermatogenesis.

Chromosome-arm counts in secondary spermatocytes

The results on the levels of aneuploidy in secondary spermatocytes are shown in Table 2. Owing to differences within radiation treatments the results of the 2 irradiation-autopsy intervals (2 and 3 h) could not be compared. In comparisons between irradiation treatments and controls, they have been pooled.

Theoretically, in Rb4Bnr/+ males half of the MII's will contain the metacentric marker chromosome. But in controls as well as in the irradiated mice a significant excess of cells without a metacentric marker was found ($2p < 0.01$, Wilcoxon test). When the irradiation effect on this parameter was tested, the mice exposed to the dose of 15-rad neutrons showed a significantly higher percentage ($2p < 0.01$, Wilcoxon test) of cells without the Robertsonian translocation marker chromosome than the control group. No such significant effect can be deduced from the data presented for the X-rays group.

The frequency of aneuploidy was calculated in 2 ways. To avoid the problem of preparation-caused cell breakage, predominantly resulting in cells containing 19 chromosome arms, the level of non-disjunction was also based on twice the

TABLE 2

CHROMOSOME ARM COUNTS IN METAPHASE-II SPERMATOCYTES OF Rb4Bnr TRANSLOCATION HETEROZYGOTES (B7 GENERATION) 2 OR 3 h AFTER IRRADIATION WITH 15-rad NEUTRONS OR 60-rad X-RAYS AND OF UNIRRADIATED MALES

Results are given for each male separately.

	Number of cells scored	Arm counts in cells								19 + 21 (%)	2 × 21 (%)
		Without metacentric marker				With metacentric marker					
		less than 19	19	20	21	less than 19	19	20	21		
15-rad neutrons	100	2	11	51	5	—	2	21	8	26	26
	100	—	8	55	4	—	6	23	4	22	16
	100	—	12	48	4	1	—	28	7	23	22
	100	—	10	51	—	—	—	32	7	17	14
	100 [b]	1	9	18	3	—	—	24	5	17	16
	100 [b]	1	12	45	3	—	4	28	7	26	20
		$N = 393 \simeq 65.5\%$				$N = 207 \simeq 34.5\%$				21.8 ± 4.4	19.0 ± 4.5
60-rad X-rays	100	—	16	46	2	—	1	21	14	33	32
	100	—	11	43	2	—	2	33	9	24	22
	100	—	12	45	3	—	4	30	6	25	18
	100	—	15	40	5	2	2	24	12	34	34
	100 [b]	3	16	40	—	—	3	29	9	28	18
	100 [b]	—	12	48	4	—	1	27	8	25	24
		$N = 363 \simeq 60.5\%$				$N = 237 \simeq 39.5\%$				28.2 ± 4.4	24.7 ± 6.9
Control group	100	—	8	51	2	—	3	26	10	23	24
	100	—	14	44	1	—	1	27	13	29	28
	100	—	14 + 1 [a]	43	2	—	—	29	11	27	26
	100	—	18	35	1	—	—	29	17	36	36
	100	1 [a]	16	36 + 2 [a]	3	—	1	25	16	36	38
	100	—	12 + 2 [a]	38	4	—	—	37	7	23	22
		$N = 348 \simeq 58\%$				$N = 252 \simeq 42\%$				29.0 ± 5.9	29.0 ± 6.5

[a] Cells showing the indicated number of chromosomes plus an extra single chromatid.
[b] Males sacrificed 3 h after irradiation.

number of cells with 21 chromosome arms. This last method, on the other hand, may lead to an underestimate. Although no significant differences between the outcomes of the 2 methods can be found, for both irradiated groups lower values were calculated when the incidence of meiosis I nondisjunction was based on the 21-arm-containing MII's only. Cells showing chromosome counts deviant from a whole number (caused by an extra single chromatid and only seen in the control group) were not included in these calculations.

Significant differences, concerning the levels of 19—21-arm-containing cells were found between the mice exposed to 15-rad neutrons and both the 60-rad X-rays and control group (in both cases $2p < 0.05$, Student t test). The variance of the frequency of 19—21-chromosome-arm-containing secondary spermatocytes appeared to be the highest for the control group; coefficients of vari-

ation ranged from 20.3% (control group) to 18.8 and 15.6% for the groups exposed to 15-rad neutrons or 60-rad X-rays.

With regard to the percentages of 21-chromosome-arm-containing cells, the 15-rad neutrons group only showed a significant difference when compared with the control group ($0.01 < 2p < 0.02$, Student t test). No significant effect was found for this parameter after irradiation with a dose of 60-rad X-rays when compared with the control group.

The mean incidence of aneuploid MII's for the untreated control mice (B7 generation) was significantly higher than the value presented earlier for Rb4Bnr heterozygotes of the combined F_1 and B_4 generation (Nijhoff and De Boer, 1979). When the 19 + 21-chromosome-arm-containing MII's were compared, the level of significance was $0.01 < 2p < 0.02$; for the 21-arm-containing MII's this value was $0.02 < 2p < 0.05$ (Student t test).

Structural chromosome damage

Results on the incidence of radiation-induced chromosomal aberrations at diakinesis/MI spermatocytes 2 or 3 h after irradiation are shown in Table 3. To test the effect of the time of harvest on the parameters studied, mice and irradiation treatments were pooled within the 2-h and 3-h groups, both groups being

TABLE 3

FREQUENCY OF ABERRATIONS IN DIAKINESIS/METAPHASE-I SPERMATOCYTES OF Rb4Bnr TRANSLOCATION HETEROZYGOTES (B7 GENERATION) 2 OR 3 h AFTER IRRADIATION WITH 15-rad NEUTRONS OR 60-rad X-RAYS AND OF UNIRRADIATED MALES

From each male, 100 cells were scored.
Results are given for each male separately.

	Percentage of cells with less than 40 chomosomes [a]	Percentage of cells showing dissociation of			Percentage of cells showing chromosomal damage		
		XY-biv.	trivalent	autosomal biv.	gaps	breaks, fragments	in general
15-rad neutrons	1	2	2	9	2	1	3
	2	14	2	10	2	1	3
	5	17	2	2	13	2	15
	3	18	1	2	4	0	4
	3 [b]	15	2	1	7	0	7
	6 [b]	13	4	6	2	1	3
	3.3 ± 1.9	11.5 ± 5.8	2.2 ± 2.0	5.0 ± 3.9	5.0 ± 4.4	0.8 ± 0.7	5.8 ± 4.7
60-rad X-rays	11	10	3	3	1	0	1
	3	14	1	9	2	1	3
	11	11	1	4	0	1	1
	3	13	2	14	2	1	3
	11 [b]	16	0	3	10	1	11
	— [b]	10	0	9	6	1	7
	6.5 ± 5.0	12.3 ± 2.4	1.2 ± 1.2	7.0 ± 4.4	3.5 ± 3.8	0.8 ± 0.4	4.3 ± 3.9
Control group	2	4	2	1	2	1	3
	0	12	0	1	0	0	0
	0	18	3	6	0	1	1
	1	9	2	4	1	0	1
	0	9	2	2	0	0	0
	0	9	3	5	1	0	1
	0.5 ± 0.8	10.2 ± 4.6	2.0 ± 1.1	3.2 ± 2.1	0.7 ± 0.8	0.3 ± 0.5	1.0 ± 1.1

[a] Assumed to be caused by (mechanical) cell damage.
[b] Males sacrificed 3 h after irradiation.

TABLE 4

FREQUENCY OF ABERRATIONS IN METAPHASE-II SPERMATOCYTES OF Rb4Bnr TRANSLOCATION HETEROZYGOTES (B7 GENERATION) 2 OR 3 h AFTER IRRADIATION WITH 15-rad NEUTRONS OR 60-rad X-RAYS AND UNIRRADIATED MALES

From each male, 100 cells were scored.

	Percentage of cells showing single chromatids	Aberrations per 100 cells				Percentage of cells showing chromosomal damage		
		chromatid gaps	iso-chromatid gaps	fragments	deletions	gaps	fragments, deletions	in general
15-rad neutrons	6	22	1	10	3	20	10	29
	12	38	1	21	0	34	19	44
	5	28	5	14	1	24	10	31
	2	26	11	19	2	25	16	36
	6 [a]	41	3	10	4	34	9	39
	4 [a]	27	6	28	2	20	22	35
	5.8 ± 3.4	30.3 ± 7.4	4.5 ± 3.8	17.0 ± 7.0	2.0 ± 1.4	26.2 ± 6.4	14.3 ± 5.5	35.7 ± 5.4
60-rad X-rays	12	28	1	15	0	23	13	34
	2	32	2	16	3	27	16	37
	1	26	1	28	2	22	22	40
	4	24	2	37	0	18	26	40
	9 [a]	42	6	33	9	39	31	59
	1 [a]	57	3	18	1	44	16	53
	4.8 ± 4.6	34.8 ± 12.6	2.5 ± 1.9	24.5 ± 9.4	2.5 ± 3.4	28.8 ± 10.3	20.6 ± 6.9	43.8 ± 9.9
Control group	2	7	1	4	1	7	3	10
	3	5	1	1	2	6	3	8
	2	6	3	2	0	7	2	9
	0	11	4	0	0	11	0	11
	4	17	3	2	0	14	2	16
	3	18	3	0	1	18	1	18
	2.3 ± 1.4	10.7 ± 5.5	2.5 ± 1.2	1.5 ± 1.5	0.7 ± 0.8	10.5 ± 4.8	1.8 ± 1.2	12.0 ± 4.0

[a] Males sacrificed 3 h after irradiation.

considered as homogeneous. No significant difference was found between the 2 harvest times in respect to the percentages of cells showing "radiation damage in general".

A significant effect of 15-rad neutrons irradiation can be seen in the percentage of cells showing chromosomal gaps ($2p < 0.01$, Wilcoxon test) whereas the mice exposed to 60-rad X-rays showed a non-significant difference ($0.05 < 2p < 0.10$, Wilcoxon test) for this parameter, when compared with the control group. Table 3 shows that these differences also find expression in the percentages of cells with radiation damage in general (gaps and/or breaks and/or fragments), and at the same levels of significance.

Both types of radiation appeared to affect the percentage of aneuploid MI cells when data of treated and control mice were compared ($2p = 0.01$ for the 15-rad neutrons and $0.01 < 2p < 0.05$ for the 60-rad X-rays groups). The 2 radiation treatments did not differ in any of the parameters of induced damage in Table 2.

Table 4 shows the incidence of radiation-induced aberrations found at the chromosomal level in secondary spermatocytes (MII cells). Both groups, the 60-rad X-rays as well as the 15-rad neutrons group, consisted of animals killed either 2 or 3 h after the start of the irradiation procedure. Testing for an effect of harvest time (2 vs. 3 h) did not yield a significant difference, and both groups were considered homogeneous.

A comparison of the percentages of cells showing chromosomal damage irrespective of radiation type or parameter studied, indicates a significant effect of the radiation treatment ($2p < 0.01$, Wilcoxon test). It appears that both types of radiation predominantly induce chromosome fragments and gaps of the chromatid type: the irradiated groups differed significantly from the group of untreated mice, concerning these 2 parameters, when expressed as total number per hundred cells ($2p < 0.01$, Wilcoxon test). The finding of gaps of the isochromatid type was unexpected. Radiation was administered after replication, so if the gaps really were a radiation effect, this finding must be explained as a 2-hit event on both sister chromatids and on similar positions. No significant radiation effects were found, however, when the number of isochromatid gaps or chromosome deletions per 100 cells were compared with the control values.

As to the percentage of cells showing unpartnered chromatids, the 15-rad neutrons irradiation enhanced this level significantly when compared with the control group ($2p < 0.05$, Wilcoxon test). The 2 radiation types mutually showed no significant differences between their effects on any of the "MII parameters" presented in Table 4.

The level of radiation-induced chromosomal aberrations was low and independent of the type of radiation; for both gaps and fragments plus deletions this level was 1.3 per cell. In the majority of cases both categories were not found in the same cell (Table 4).

The neutrons-X-ray RBE values (for the number of breaks, fragments and deletions) were 5.4 for the MI and 3.3 for the MII stage (Tables 3 and 4).

Discussion

The results presented in Table 1 show that inter-mouse variations exist with regard to the duration of the meiotic prophase in Rb4Bnr/+ males. To some

extent the variable results can be explained by the rapid increase with time of the percentage of labelled cells (see also Figs. 1A and 1B).

However, another factor must be of importance. In an earlier study — a comparison of the number of diakinesis/metaphase I cells found in separated groups along the seminiferous epithelium and the epididymal sperm counts between +/+ and Rb4/+ males (Nijhoff and De Boer, 1979) — it was concluded that in Rb4/+ males at least a fraction of cells spend a longer duration at diakinesis/MI and eventually some die. Such a phenomenon will influence the fraction of labelled cells at both MI and MII. On the other hand, the large variation in sperm counts found for Rb4/+ males (Cattanach and Moseley, 1973; Nijhoff and De Boer, 1979) indicates that this factor may vary between males of this karyotype. Had the "Y-labelled fraction" been found during a significantly longer period in the diakinesis/MI stage of Rb4/+ (compared with +/+) males, this would have supported the hypothesis of delay at this stage of spermatogenesis in Rb4/+ males. Owing to a shortage of +/+ data this cannot be deduced from the results shown in Table 1, but an indication for it can be seen in Fig. 1A.

It is tempting to relate the variation in the duration of meiotic prophase between Rb4/+ males to the incidence of aneuploid secondary spermatocytes as well as to the epididymal sperm counts, but this aspect has not been included in this study. Too many results remain doubtful — the maximal levels of "Y-labelled cells" at both MI (spiralization class 3) and MII, the exact time of first appearance of these "Y-labelled cells" at both stages — to justify a more accurate calculation of the MII-stage duration (~3 h, see Results). Nevertheless, we believe a duration of 3 h for the MII to be a maximal value for the following reasons.

(1) The fraction of cells spending a longer duration at diakinesis/MI may only have enlarged the assumed MI/MII ratio with regard to the maximal levels of the "Y-labelled cells" (see Results). (2) In the literature, only little clarity exists about the presence of an interkinesis in between the 2 meiotic divisions in the mouse. In the rat the duration of this phase has been determined as 4 h (Hilscher and Hilscher, 1969), whereas recently Oud et al. (1979) did not mention its existence in their sequential analysis of male mouse meiosis. But if present, the calculated durations of the "spiralization class 3" MI and consequently the MII stage both include this resting phase. The duration of MI (class 3) is of no importance in physiological terms.

Another aspect of the spontaneous meiotic behaviour of the Rb4Bnr/+ translocation in male mice that emerges from this study is the higher fraction of aneuploid secondary spermatocytes found here in the B7 generation when compared with the F_1/B4 generations (Nijhoff and De Boer, 1979). This can either be ascribed to the changing genetic background or must be considered as a random effect.

The difference in the percentage of cells showing radiation-induced chromosomal damage at either the diakinesis/MI or the MII stage is striking (compare Tables 3 and 4, last columns). This phenomenon may reflect (1) a difference in radiation sensitivity between cells at different stages of spermatogenesis or (2) the lack of apparent radiation damage in cells at diakinesis/MI when diagnosed at a 2- or 3-h interval after irradiation.

Concerning the first point, it is hardly credible that primary spermatocytes at either prometaphase—metaphase I or at mid/late diakinesis (which at the moment of diagnosis are supposed to be MII and prometaphase I cells resp.) differ to such an extent in radiation sensitivity. However, it may be that cells at the MII stage are more sensitive to irradiation and in that case the differences found must be explained by assuming a radiation-induced delay of cells already in MII at the moment of irradiation. The significant fraction of MII cells with chromosomal fragments (which normally get lost during cell division) supports this explanation (Table 4). On the other hand, irradiation affects the level of non-disjunction at the first meiotic division (Table 2), so an unknown proportion of cells comes through this division after being irradiated. Moreover, no clear argument for delay can be deduced from the labelling results of the irradiated Rb4Bnr/+ males (B7 generation) when compared with the results derived from the unirradiated B6 generation (both, Table 1).

We believe in the second alternative: the radiation damage is induced, but simply not (fully) shown at diakinesis/MI after an irradiation—fixation interval of 2 or 3 h. Chromosomal processes (spiralization, bivalent separation) taking place during this interval may be of major importance.

Oakberg and DiMinno (1960) found that, in the mouse, MI was the most sensitive stage of meiosis to induce chromosome breakage. They estimated that a dose of 138 ± 6 rad X-rays would produce 50% abnormal anaphase II cells. This is in line with our 60-rad results considering the percentage of MII's showing fragments and deletions (Table 4).

Klášterská et al. (1977) did not mention any sign of affected diakinesis/MI cells when fixation took place 3 h after X-irradiation with doses from 100 to 1000 rad. However, when an interval of 6 h was chosen, the effect of 200 rad X-rays was claimed to become apparent in "almost every cell" at diakinesis/MI. The same "time effect" can be deduced from the results obtained at this diakinesis/MI stage after a dose of 200 rad X-rays at a 4.5-h interval: at least 12% of the cells at metaphase I show chromosomal damage (Walker, 1977), and at a 6-h interval, 79.5% of abnormal diakinesis/MI cells were diagnosed (Chandley, unpublished results). The last author found 45.5% after the cells had been exposed for 6 h to a dose of 100 rad X-rays.

Our conclusion is that when late first meiotic stages are irradiated, the time of harvesting is of critical importance for assessing radiation damage.

The neutrons-X-rays RBE value of 4 accepted here when we chose the doses of 15-rad neutrons and 60-rad X-rays appeared to be of the right order: no significant difference with regard to any of the "chromosomal damage parameters" was found between the irradiated groups (Tables 3 and 4).

A major part of our investigation was tracing the influence of the irradiation on the processes of chromosome separation at anaphase I. Only the dose of 15-rad neutrons induced a significant decrease in the incidence of aneuploid secondary spermatocytes. This finding may be explained in terms of "protection" by the neutron irradiation against non-disjunctional events during the first meiotic division. We believe, however, that arguments can be offered to explain the decline in percentage of aneuploid secondary spermatocytes in terms of a selective cell killing by fast neutrons of the primary spermatocytes which will give rise to aneuploid daughter cells.

Male mice, heterozygous for Rb4Bnr, are known to show a high level of spontaneous meiotic non-disjunction (Cattanach and Moseley, 1973; Gropp et al., 1974; Nijhoff and De Boer, 1979; and this report). Secondly, indications for delay and cell death during the diakinesis—metaphase I stage have been found in males of this karyotype when compared with +/+ males of a similar genotype (Nijhoff and De Boer, 1979). These 2 phenomena may offer the basis for the following explanation of the supposed selection after irradiation with 15-rad neutrons. The occurrence of the spermatogenic wave in the seminiferous epithelium of the mouse (Oakberg, 1956) implicates a strong synchrony in the course of development within groups of cells at the same stages of spermatogenesis. For Rb4/+ on the other hand, we hypothesize that during the final period of the primary spermatocyte stage, cells show a continuous distribution with regard to the extent of delay: cells with a strong delay will die off while the fraction showing only minor delay will survive in the unirradiated situation but with an increased risk of producing aneuploid daughter cells. The results indicate that, in terms of cell death, the "victims" of the 15-rad neutron irradiation predominantly have to be searched for in this last category. This suggests an interaction between the "neutron irradiation effect" and cell delay. The basis for this interaction remains unknown. The level of chromosomal damage per MI cell is independent of the type of radiation at the doses used here, which makes it unlikely that this parameter has a bearing on the selective cell death found only after the neutron irradiation. One has to keep in mind, however, that compared with the controls only the neutron irradiation enhanced the frequencies of gaps at MI (and premature centromere divisions at MII). The relation between delay and proneness to first meiotic non-disjunction may also be deduced from a study in vitro by Karp and Smith (1975): a significantly enhanced percentage of aneuploid secondary oocytes was found after a "low-temperature interval" during meiosis I.

In contrast with X-rays, neutron-irradiation may cause premature centromere division. We found a significantly higher percentage of MII's with unpartnered chromatids and an apparent absence of the metacentric marker chromosome when compared with the controls. This last finding suggests that the Robertsonian translocation chromosome is more prone to centromere division induced by neutron irradiation than are the other autosomes. An enhanced incidence of anaphase II originating non-disjunction as a consequence of the random orientation of the single chromatids may also be an effect of the treatment with 15-rad neutrons but has not been considered here.

Earlier studies showed a rise in aneuploids after X-irradiation of the meiotic prophase of male mice (Russell and Saylors, 1963; Szemere and Chandley, 1975; Hansmann et al., 1979). Differences in prophase-stages irradiated, exposure rates or X-ray spectra may in the first place underly the difference with our results.

In conclusion, with regard to the cellular mechanism leading to non-disjunction, the present study with Rb4/+ mice has indicated a possible relation between cell delay during male meiosis I and the enhanced chance for the occurrence of aneuploid secondary spermatocytes. This may be an important finding for the human situation as well. It has still to be shown that this relation has a general validity.

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THE MEIOTIC BEHAVIOUR OF TRANSLOCATIONS INVOLVING ACROCENTRIC AND METACENTRIC CHROMOSOMES IN THE MOUSE AND THEIR EFFECTS ON SPERMATOGENESIS

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Summary

A reciprocal translocation between an acrocentric and a metacentric chromosome was constructed by combining the T(1;13)70H reciprocal translocation between the acrocentric chromosomes 1 and 13 with the RB(11.13)4Bnr Robertsonian translocation followed by crossing-over in the interstitial segment of 13. Backcrossing of the double heterozygote (T70H,+/+,Rb4Bnr) with Rb4Bnr/+ produced the recombinant type T(1;11.13S),+/+,Rb4Bnr. The double heterozygote (T70H,+/+,Rb4Bnr) was also included in the analysis. Sperm counts from the capita epididymi were 20-34% of the level of T70H/+ male mice whereas the level of abnormal spermatozoa ($\tilde{\sim}$ 14%) was slightly increased compared with +/+ and T70/+ males. The spectrum of multivalent configurations in males of the two T70H,Rb4Bnr containing karyotypes did not differ appreciably. Chains, produced through a missing chiasma in the short interstitial segment of the acrocentric chromosome were most prominently found in both karyotypes. For chromosome association in the interstitial segment of chromosome 13, a difference was noted, however, with a lacking chiasma in 12.5% of the T(1;11.13S),+/+,Rb4Bnr primary spermatocytes against 2.7% in T70H,+/+,Rb4Bnr. The segregation behaviour of the multivalents was analysed on the basis of the chromosome counts and the presence of marker chromosomes in secondary spermatocytes. For T70H,+/+Rb4Bnr, excluding chromosome 11, the percentages were following: alternate segregation, 28.7%, adjacent 1, $\tilde{\sim}$ 24%, adjacent 2, 23.4% and "3:1" segregation, 22.6%. Non-disjunction for normal bivalents occurred in $\tilde{\sim}$ 14% of the anaphases I. For T(1;11.13S),+/+,Rb4Bnr, these percentages were 76.8% for alternate/adjacent 1 segregation (undistinguishable here), 12.3% for adjacent 2, 10.9% for "3:1" segregation and 23.2% for non-disjunction of normal bivalents. In agreement with the high level of

"3:1" segregating multivalents in T70H,+/+,Rb4Bnr, a considerable fraction (27.5%) of tertiary trisomic live young was obtained from these males. For T(1;11.13S),+/+,Rb4Bnr, a correlation between non-disjunction for the normal bivalents and adjacent 2 segregation of the translocation multivalent was observed. For T70H,+/+,Rb4Bnr, non-disjunction for chromosome 11 often coincided with adjacent 2 segregation. For both karyotypes, the agreement between the frequencies of post implantation deaths at day 18 of pregnancy and the estimates based on the severely imbalanced spermatozoa as deduced from the secondary spermatocyte data, was good.

1. Introduction

The laboratory mouse is characterized by an "all acrocentric" karyotype. Most human reciprocal translocations occur between metacentric chromosomes or between a metacentric and an acrocentric one (Boué et al., 1979; Jalbert et al., 1980). In order to create mouse translocations of a more "human-like" nature, the following karyotypes have been constructed: T(1;13)70H,+/+,Rb(11.13)4Bnr and T(1;11.13S),+/+,Rb(11.13)4Bnr. The T(1;11.13S) translocation (the nomenclature indicates a reciprocal rearrangement between chromosome 1 and the relative shortest arm 13 of the metacentric; Lyon, 1977 and Mouse News Letter 1979, 61) was produced during meiosis I of the former karyotype by recombination in the non-translocated (interstitial) segment of chromosome 13. The multivalents at meiosis I in both karyotypes are highly comparable (see the Figs. 1 and 2) and thus useful for a comparative analysis of the factors influencing chiasma position and frequency in the various multivalent segments. These may have their impact on the mode of segregation at anaphase I. A cytological study of the segregation behaviour of the T70H reciprocal translocation has been performed by de Boer (1976) and Oshimura et al. (1976).

According to the classical theory, the meiotic multivalent configurations formed in heterozygous carriers of a reciprocal translocation may segregate:

a) alternate: alternate centromeres move to the same pole
b) adjacent 1: adjacent but non-homologous centromeres move to the same pole
c) adjacent 2: adjacent but homologous centromeres move to the same pole
d) 3:1: three chromosomes move to one pole and one to the other.

For human carriers of a reciprocal translocation,data with respect to the modes of segregation of the multivalent configurations at the first meiotic division are primarily derived from karyotype analysis of their live-born offspring. The category of reciprocal translocations, involving a metacentric and an acrocentric chromosome, is probably overrepresented when the method of ascertainment is based on malformed, i.e. imbalanced, progeny after birth. Translocations in man listed on the basis of this criterium, show a ratio of the meta/acro: meta/meta types of 4.25:3 (N=146; Jalbert et al., 1980), whereas when the method of ascertainment was based on more general criteria - like malformed infants, recurrent

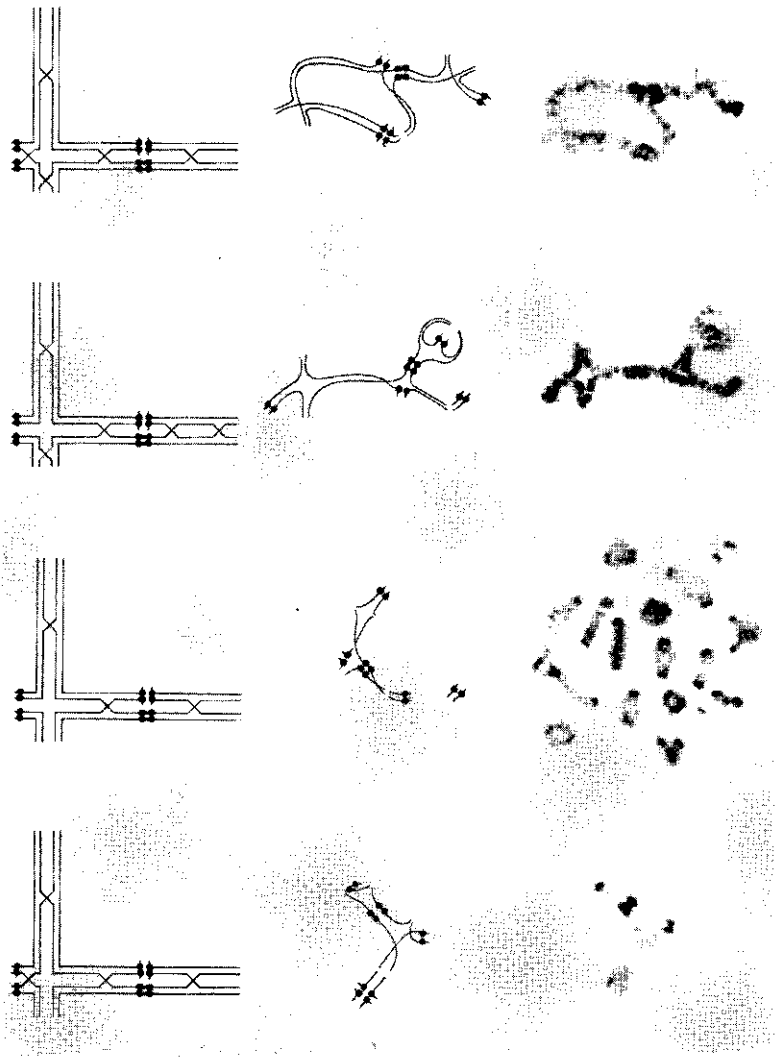

Fig. 1 Multivalent configurations in diakinesis spermatocytes of T70H,+/+, Rb4Bnr (third column), explained via their chromatid diagrams and pachytene crosses with chiasma positions (second and first column, respectively). Shown are from upper to bottom row a V, a V_{1_i}, a $IV+I_{(1_i+13_t)}$ and a V_{13_t} configuration (see also the Materials and Methods (ii)).

spontaneous abortions or family history - a ratio of 3:6.8 (N=196; Boué et al., 1979) was found. The need, also in view of the increasing demand for genetic counselling, to clarify the meiotic behaviour of the multivalent caused by translocations of the meta/acro type has hereby clearly been indicated.

The observations at meiosis have been related to the relative fertility scores of the males of both T70H,Rb4Bnr karyotypes, as expressed by the testis weights, the number of sperm in the caput epididymi, the sperm morphology and the survival profile of normal oocytes, fertilized by a sperm population of translocation heterozygous origin. An integration of the results is presented in the Discussion.

2. Materials and Methods

(i) Animals

Male mice of different karyotypes but of a comparable genetic background were used. The T(1;13)70H/+ data, to which will be referred throughout the text, were given earlier by de Boer (1976 and 1979) and were obtained from males of a Swiss Cpb(SE)S random bred genetic background. The metacentric Robertsonian translocation Rb(11.13)4Bnr, originally kept in a C3H genetic background, was transferred to "Swiss Cpb(SE)S random bred" by introgressive breeding. The data for Rb4Bnr/+ were obtained from F_1 as well as B_4 (fourth backcross) generation males and some have been published earlier (Nijhoff & de Boer, 1979). The Rb4Bnr/+ (B_4 generation) males were crossed with T70H/T70H females, Swiss Cpb(SE)S random bred, to obtain the T(1;13)70H,+/+,Rb(11.13)4Bnr males: Among the progeny of the cross T70H,+/+,Rb4Bnr times Rb4Bnr/+ (B_5) the recombinant T(1;11.13S),+/+,Rb4Bnr males were found (see section ii of the Results).

All males were of comparable age: The T70H,+/+,Rb4Bnr and T(1;11.13S),+/+,Rb4Bnr mice were processed when 2-7 months old while the T70H/+ and Rb4Bnr/+ data had been obtained from males of the same age interval.

(ii) Mitotic and meiotic preparations

The offspring of the cross between T70H,+/+,Rb4Bnr males and Rb4Bnr/+ females was karyotyped by means of a peripheral blood culture

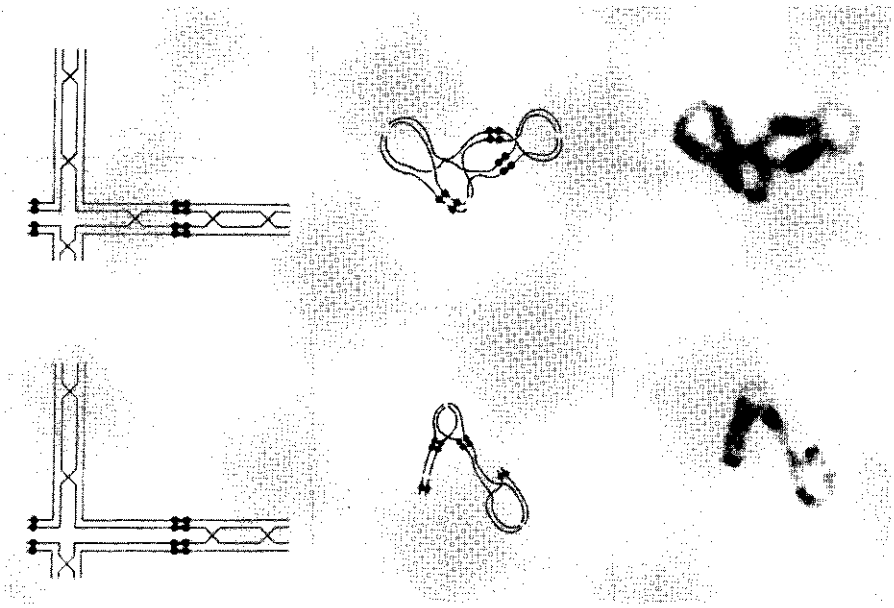

Fig. 2 Multivalent configurations in diakinesis spermatocytes of T(1;11.13S),+/+,Rb^{4}Bnr (third column). The pachytene pairing cross with the chiasma positions and the chromatid diagrams are given as well. Shown are a IV_{1_i} and a $IV_{1_i+13_i}$ configuration (see also the Materials and Methods (ii)).

procedure as described by de Boer et al. (1977). Chromosome preparations of the spermatocytes were made according to the standard air-drying technique (Evans et al., 1964). The chromosomes were C-banded following the BSG method (Sumner, 1972). From each male 200 primary and a maximum of 100 secondary spermatocytes were analysed. For T(1;11.13S),+/+,Rb4Bnr this number of spermatocytes II was not reached and varied from 62 to 83 cells per male.

(iii) Notations and computations with regard to meiotic chromosomes

In meiosis I cells with sufficiently contracted chromosomes to make analysis possible (late diplotene to metaphase I), the unassociated segments and the chiasma positions in the associated ones were scored in the multivalents. Moreover, in order to relate these parameters to the stage of meiosis, the analyzable spermatocytes I were grouped into three subjective classes of chromosome morphology. Class 1 contained the least contracted (late diplotene-early diakinesis), class 3 the most contracted chromosomes (metaphase I, for criteria see de Boer & Groen, 1974). Roman numerals are used to indicate the number of chromosomes in the various multivalent configurations. The interstitial and translocated chromosome segments of concern to chiasma formation are indicated with the subscripts i and t added to the chromosome number respectively. Thus, V stands for a pentavalent with no segment unassociated, $IV+I_{(1_i+13_t)}$ for a quadrivalent plus univalent with segments 1 interstitial and 13 translocated unassociated and $III+II_{(1_i+13_i)}$ for a trivalent plus bivalent with segments 1 interstitial and 13 interstitial unassociated. This nomenclature, illustrated in the Figs. 1 and 2 was adopted from de Boer (1976).

The chiasma position in the interstitial segment of chromosome 13 and in chromosome 11 was subjectively scored as proximal (p), intermediate (p/d) or distal (d) according to criteria proposed by de Boer (1979).

Notations of the chromosomes during the second meiotic division give the chromatids separated by a semi-colon. Four types of marker chromosomes could be recognized during prometaphase-metaphase in secondary spermatocytes:

a) reciprocal translocation chromosomes clearly shorter or longer than

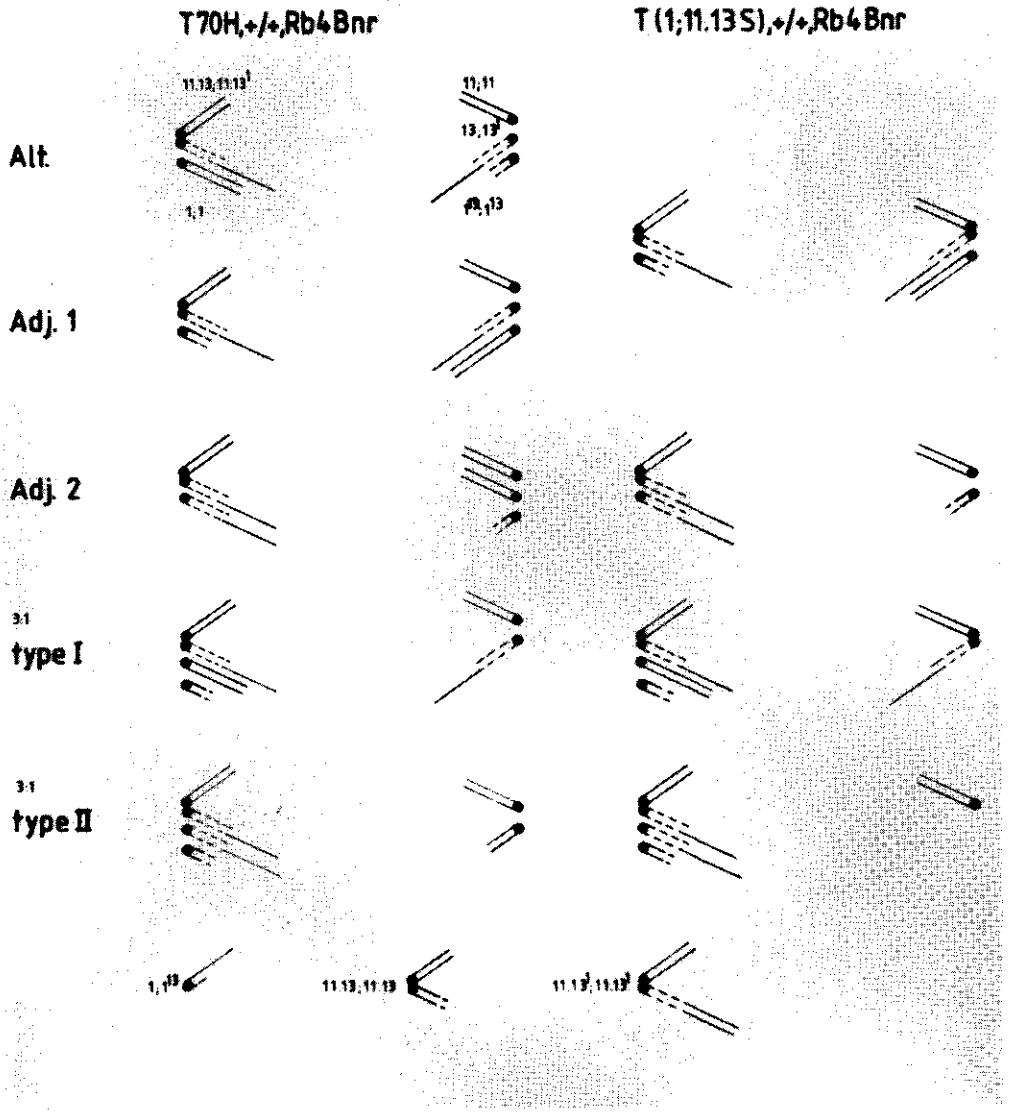

Fig. 3 The modes of segregation of the translocation multivalents of the two karyotypes concerned. For T(1;11.13S),+/+,Rb4Bnr, alternate and adjacent 1 cannot be distinguished in secondary spermatocytes. Three more marker chromosomes of secondary spermatocytes, created by rare cross-over situations or by the absence of it have been given at the bottom of the figure. Chromosome 13 parts are interrupted.

the shortest or longest acrocentric mouse chromosomes (examples $1^{13};1^{13}$ and $13^{1};13^{1}$),

b) the Robertsonian translocation chromosome 11.13;11.13,

c) acrocentric chromosomes with chromatids of unequal length, produced through a chiasma in an interstitial segment (examples $13;13^{1}$ and $1;1^{13}$),

d) the "Robertsonian" combination of the categories b and c, i.e. $11.13;11.13^{1}$, a metacentric chromosome with chromatids of unequal length in one arm.

These examples are illustrated in Fig. 3. T(1;11.13S),+/+,Rb4Bnr resembles T(1;13)70H/+ in that the alternate and adjacent 1 products of multivalent segregation are indistinguishable at Meiosis II if there is one chiasma in the interstitial segment of chromosome 13. Then, adjacent 2 segregation products are marked by either two $11.13;11.13^{1}$ or no metacentric chromosomes while the alternate/adjacent 1 category contains one such metacentric per secondary spermatocytes (see Fig. 3). Contrary to T70H/+, lack of a chiasma, or alternatively two reciprocal or two complementary chiasmata in segment 13 interstitial do not obliterate the "segregational origin" of the cell, due to the fact that both chromosomes $11.13^{1};11.13^{1}$ and 11.13;11.13 are "markers". Both through 3:1 segregation of the four multivalent involved chromosomes and through non-disjunction of the other bivalents, secondary spermatocytes with deviant chromosome counts can be obtained. In order to unravel the roles of these two mechanisms for producing hyper- and hypomodal chromosome counts at Meiosis II, the supposition has been made that the chiasma in segment 1_{t} always leads to coorientation of the two attached chromosomes i.e. 1 and 11.13^{1} (compare Figs. 1 and 2). Only four anaphase I products characterized by a combination of chromosome arm count and the presence or absence of (a) marker chromosome(s), produced by two types of 3:1 segregation (see Fig. 3) can be distinguished now. Combinations of "non-disjunction for other bivalents" and alternate/adjacent 1 segregation or adjacent 2 segregation of the multivalent will produce the same four "marker cells" and four more which are unique with respect to chromosome arm number and marker chromosome(s). For the reasons that a) the two daughter cells of the primary spermatocytes, irrespective of alternate/adjacent 1, adjacent 2 or 3:1 segregation, do occur in equal frequencies and b) within each daughter dyad, chromosome losses and gains due to

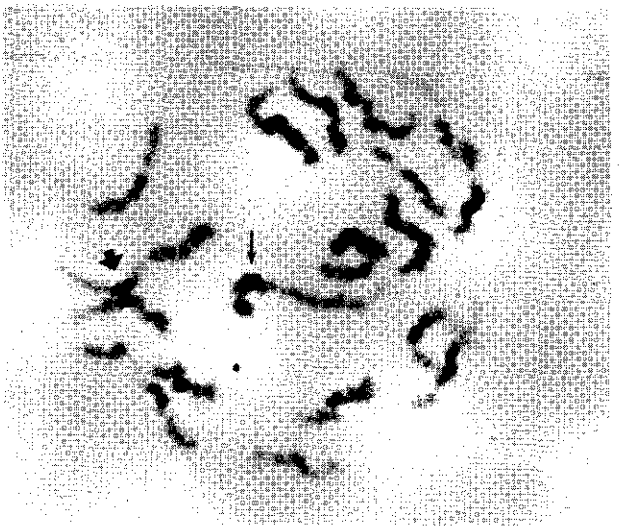

Fig. 4 An adjacent 2 derived metaphase secondary spermatocyte of T70H,+/+, Rb4Bnr origin showing the $13;13^{1}$ (arrow) and $11.13;11.13^{1}$ (bold arrow) marker chromosomes.

non-disjunction for normal bivalents are expected to be equally frequent, the frequencies of these 4 unique combinations have been used to separate the total production of aneuploid secondary spermatocytes with regard to their mode of origin. For extracting the segregational history of the secondary spermatocytes of T70H,+/+,Rb4Bnr, the same principles have been applied (see also Fig. 3). One complication of relevance to this karyotype is that chromosome 11 and 13 when combined with Rb(11.13)4Bnr can fail to coorientate with the metacentric in a considerable frequency of anaphase I spermatocytes (29%, Nijhoff and de Boer, 1980). The class of secondary spermatocytes, characterized by non-disjunction for chromosome 11 must be added to "3:1 segregation" and non-disjunction for normal bivalents as mechanisms by which aneuploid secondary spermatocytes can be produced. As with Rb(1;11.13S),+/+,T70H, the multivalent segregation frequencies are based on the frequencies of "marker spermatocytes" and the fact that complementary secondary spermatocytes (daughter dyads) must occur in equal frequencies.

(iv) Fertility estimates

Post-implantation losses in females mated with T70H+/+Rb4Bnr and T(1;11.13S),+/+,Rb4Bnr males were estimated with the aid of 2-3 months old, +/+ virgin Swiss Cpb(SE)S random bred females from a line selected for high litter size. For some males a first mating was not successful, but a second female was never given during the same week. The numbers of large and small moles (indicative for the period of death after implantation) and the number of live foetuses were recorded 18 days after finding the vaginal plug.

Weights of the testes were determined as so called "wet weights". Epididymal sperm counts from both capita were obtained according to the method of Searle & Beechey (1974). From each male the mixed contents of both capita were smeared on a slide and the spermatozoa were subsequently stained according to Bryan (1970) and their morphology was diagnosed according to Bruce et al. (1974). Sperm count and morphology data were obtained from males which were removed from female cage mates five days before autopsy.

Table 1. The distribution (percentages) among the various spiralization classes of late diplotene-metaphase I spermatocytes of the T70H/+, Rb4Bnr/+ and the T70H,+/+,Rb4Bnr and T(1;11.13S),+/+, Rb4Bnr karyotypes. Class 3 contains cells with the most contracted bivalents

Karyotype	N	Class I	Class 2	Class 3
T70H/+*	1459	10.6	60.9	28.5
Rb4Bnr/+**	1000	3.4	64.3	32.3
T70H,+/+,Rb4Bnr	1000	3.4	46.0	50.8
T(1;11.13S),+/+,Rb4Bnr	1000	4.2	29.8	66.0

* From de Boer (1976)

** From Nijhoff et al. (in preparation)

3. Results

(i) Meiosis I

Primary spermatocytes have been subjectively allocated to three classes reflecting the development from late diplotene (class 1) to metaphase I (class 3). The results, compared with the T70H/+ data from de Boer (1976) and with Rb4Bnr/+ (Nijhoff et al., in preparation) are given in Table 1. It appears that primary spermatocytes with more contracted bivalents dominate in the two T70H,Rb4Bnr containing karyotypes. Table 2 gives the spectrum of multivalent configurations found for T70H,+/+,Rb4Bnr and T(1;11.13S),+/+,Rb4Bnr, some of which are illustrated in Figs. 1 and 2. Again, a comparison with T70H/+ has been made. Table 3 extracts some of the information of Table 2 and gives the frequencies with which the chromosome segments of the multivalents are devoid of a chiasma. The most striking finding here is that a relatively great fraction of meiosis I cells in T(1;11.13S),+/+,Rb4Bnr (12.5%) lacks a chiasma in segment 13_i, which cannot be ascribed to a precocious disappearance with proceeding meiosis. The alternative is to attribute this phenomenon to the presence of the metacentric structure in homozygous condition in this karyotype. Another difference between the karyotypes is that within the T70H,Rb4Bnr containing karyotypes, the presumed chiasmate association within the short translocated segment 13_t (see Figs. 1 and 2) is found more often than in T70H/+. When late diplotene - early diakinesis cells are considered only (spiralization class 1), the differences have vanished. Thus, chiasma terminalization with proceeding meiosis is observed less frequently within segment 13_t for the two Rb4Bnr containing karyotypes than for T70H/+.

The positions of the usually single chiasmata in segment 13_i and in chromosome 11 have been subjectively scored (see the Materials and Methods (ii)); the results are presented in Table 4. Only multivalent configurations with chromosome 1^{13} associated with chromosome 13, which make up the large majority of the late diplotene - early diakinesis spermatocytes, have been taken into consideration. For T70H/+ and T(1;11.13)S,+/+,Rb4Bnr, the results of all three spiralization classes have been pooled, because no change in chiasma position has been found. For segment 13_i, the chiasma within T70H,+/+,Rb4Bnr multivalents was found in a more distal position when meiosis proceeded. So here, only the results of spiralization class 1 (late diplotene - early diakinesis) have been given. It appears that the position of the 13_i chiasma is more

Table 2. Multivalent configuration frequencies in diakinesis/metaphase I cells. For this subdivision only the chromosome segments involved in the multivalent of T70H/+ males were considered. For the nomenclature used, see the Materials and Methods and Figs. 1 and 2

T70H/+* (N = 1459)		T(1;11.13S),+/+,Rb4Bnr (N = 1000)		T70H,+/+,Rb4Bnr (N = 1000)	
Configuration	%	Configuration	%	Configuration	%
IV	2.55	IV	1.2	V	2.4
IV_{1_i}	61.55	IV_{1_i}	67.4	V_{1_i}	72.9
IV_{13_t}	1.1	IV_{13_t}	1.2	V_{13_t}	2.3
		IV_{13_i}	0.5		
$III+I_{(1_i+13_t)}$	33.65	$III+I_{(1_i+13_t)}$	16.7	$IV+I_{(1_i+13_t)}$	16.8
$II+II_{(1_i+13_i)}$	0.95	$IV_{(1_i+13_i)}$	10.7	$III+II_{(1_i+13_i)}$	2.3
		$III+I_{(1_i+1_t)}$	0.7	$IV+I_{(1_i+1_t)}$	1.4
		$IV_{(13_t+13_i)}$	0.2		
$II+I+I_{(1_i+13_t+13_i)}$	0.15	$III+I_{(1_t+13_t+13_i)}$	1.1	$II+II+I_{(1_i+13_t+13_i)}$	0.4
$II+I+I_{(1_i+13_t+1_t)}$	0.05	$II+I+I_{(1_i+13_t+1_t)}$	0.3	$III+I+II_{(1_i+13_t+1_t)}$	1.5

distal in T70H,+/+,Rb4Bnr compared with the other two karyotypes. When the position of the single chiasma in chromosome 11 also is taken into account, T70H,+/+,Rb4Bnr resembles Rb4Bnr/+ with respect to chiasma formation in the 11.13 chromosome, whereas for the T(1;11.13S),+/+,Rb4Bnr originating structurally homozygous region of this chromosome, a more proximal chiasma in 13_i accompanies a more distal one in chromosome 11. These observations suggest negative chiasma interference between these two chromosome segments with respect to chiasma position and possibly - formation, both in the structurally heterozygous and homozygous situation.

(ii) Chromosome segregation at anaphase I

As stated in the Materials and Methods (ii), the T(1;11.13S),+/+, Rb4Bnr males were produced by crossing T70H,+/+,Rb4Bnr males with Rb4Bnr/+ females. On the basis of the karyotype analysis of 98 young produced by this cross, Table 5 gives the interpretation, in terms of meiotic segregation, of the frequencies of the various types of male and female gametes leading to viable progeny. For the modes of segregation of the translocation multivalent in T70H,+/+,Rb4Bnr males see Fig. 3. The frequency of progeny, derived through complementation of nullisomic and disomic gametes of the chromosomes 11 and 13 can be shown to be low and was not of quantitative importance for the interpretation given in Table 5. It can be seen that the Rb4Bnr/+ karyotype can be produced in two ways: adjacent 1 segregation in the male combined with a Rb4Bnr containing oocyte and alternate segregation with a + oocyte. On the basis of the other frequencies, a best fit for these categories has been obtained. Summing the frequencies of the male and female gametes leads to the following conclusions:

a) In the female meiosis of Rb4Bnr/+, Rb4Bnr containing oocytes are found less frequently than + containing ones though not significantly so ($\chi_1^2 = 2.57$).

b) In T70H,+/+,Rb4Bnr males, alternate orientation within the translocation multivalent occurs less frequently than adjacent 1 orientation ($\chi_1^2 = 6.31$, $p < 0.025$).

c) A considerable frequency of tertiary trisomic offspring is produced. In all cases, an association of chromosome 1^{13} with Rb4Bnr was found. Association of chromosomes 11, 13 and 1^{13} in male meiosis could only have occurred if chromosomes 1;1 and 13;13^1 had not dis-

Table 3. The frequencies of the diakinesis/metaphase I multivalents with the indicated chromosome segments unassociated

	1_i(%)	13_t(%)	13_i(%)	1_t(%)	11(%)
T70H/+* N = 1459	96,4	34.9	1.2	0	-
T(1;11.13S),+/+,Rb4Bnr N = 1000	96.9	19.5	12.5	1.0	3.0
T70H,+/+,Rb4Bnr N = 1000	95.3	21.0	2.7	2.9	1.2
Rb4Bnr/+ (F_1+B_4 gen. N = 1456	-		1.6**	-	0.5

* From de Boer (1976)

** Concerns the entire chromosome 13; Nijhoff et al. (in preparation)

joined. Introducing the modes of 3:1 segregation of the translocation multivalent in the Materials and Methods (ii) we had excluded this possibility, a supposition which in the light of the absence of +/+Ts(1^{13}) offspring, i.e. 11,13,1^{13} gametes, seems to be correct.

Of 5 male T70H,+/+,Rb4Bnr carriers, 5 x 100 secondary spermatocytes were analysed for chromosome arm count and the presence of marker chromosomes. Fig. 4 gives an example of an adjacent 2 segregation product. The chromosomes 11.13;11.13 or 13^{1};13^{1}, indicative for zero or two reciprocal chiasmata in segment 13_i were found in 3.2% of the cells. This percentage is in agreement with 2.7% of the primary spermatocytes of this karyotype lacking a chiasma in this segment (Table 2). In 4 cells the marker chromosome 11.13^{1};11.13^{1} was found, indicative for two complementary chiasmata in segment 13_i. As the meiosis II products resulting from two reciprocal (see above) and two disparate chiasmate cannot be distinguished from those resulting from zero or one chiasma, respectively, an overall frequency of 6.4%(4x(2x4)/500) of meiosis I cells with two chiasmata, can be calculated on the basis of these 4 cells. Cytologically, however, 2 chiasmata have never been observed in this segment. Marker chromosome 1;1^{13}, illustrative for a chiasma in segment 1_i was observed only once, contrary to expectation (see Table 2). Equational separation of chromosome 1^{13} in two 1^{13} chromatids at anaphase I was only observed in 0.6% of anaphases I, compared with 5.3% in T70H/+ (de Boer, 1976).

For T70H,+/+,Rb4Bnr, hypo- and hyperploid chromosome arm counts were in good agreement with each other: 87 cells with 19 arms versus 91 cells with 21 and 9 cells with 18 arms versus 3 with 22. With regard to the reliability of the marker chromosomes, 1^{13};1^{13} was found in 52.8% of the cells and the metacentric Robertsonian chromosome, in 46.2%. The marker structure 13;13^{1} was contained twice by 73 cells and was not found in 107 cells, which indicates an overrepresentation of the latter category (χ^2_1 = 6.5, p<0.02).

Table 6 gives the segregational origin of the secondary spermatocytes of T70H,+/+,Rb4Bnr for the four chromosomes involved in the T70H-caused part of the multivalent. Altogether 480 cells could be used for this classification. As was noted in the Materials and Methods, non-disjunction for chromosome 11 with the 11-containing Robertsonian chromosome could lead to cell types indiscernable from "3:1" segregation

Table 4. The positions of single chiasmata of the chromosome segments 13_i and 11, found in the various karyotypes. p = proximal, d = distal, p/d = all positions in between

	Chromosome 13 (interstitial segment)				Chromosome 11			
Karyotype	N	p	p/d	d	N	p	p/d	d
T70H/+*	515	49.9	44.5	5.6				
T(1;11.13S),+/+,Rb4Bnr	674	42.1	55.3	2.5	616	0.3	20.0	79.7
T70H,+/+,Rb4Bnr	29	24.1	75.9	0	683	0.3	61.0	38.6
Rb4Bnr/+**					1408	2.3	52.1	45.7

* From de Boer (1979)

** From Nijhoff et al.(in preparation)

(see Fig. 3). Because of the fact that the production of tertiary trisomic offspring (see Table 5) outnumbered the maximal possible frequency based on the secondary spermatocyte cell types (27.5 versus 15.8 percent), chromosome 11 non-disjunction (which leads to prenatal lethals) was considered negligible when alternate segregation was concerned. As has been specified in the Materials and Methods, section iii, the frequencies of the classes produced by "3:1" segregation, 11 non-disjunction and non-disjunction for normal bivalents are found by substraction. Therefore, standard deviations are not given and the percentages given here are best assessments only. When the frequencies for the main 6 types, describing anaphase I segregation in T7OH,+/+,Rb4Bnr males are calculated, the following values are derived: "alternate" segregation, 28.7%, "adjacent 1" segregation, 23.7-25.3%, "adjacent 2" segregation, 23.4%, "3:1" segregation, 22.6%, 11 non-disjunction, 6.2%, non-disjunction for normal bivalents, 13.5-15.2%. It should be noted (Table 6), that no associations between the various phenomena are found unless for 11 non-disjunction which predominantly occurs in adjacent 2 segregating cells.

For T(1;11.13S),+/+,Rb4Bnr, the following observations were made. Secondary spermatocytes with marker chromosomes 11.13;11.13 and 11.13^{1}; 11.13^{1} were indicative of either two reciprocal or zero chiasmata in segment 13_{i}. The primary spermatocyte frequency for these two events, based on the secondary spermatocyte markers was 6.2% (N = 357) which is lower than the frequency of primary spermatocytes lacking a chiasma in segment 13_{i} of 12.5% (N = 1000, see Table 3; χ^{2}_{1}=12.1,p<0.001). The chiasma frequency of segment 1_{i} was 0.8% based on the $1;1^{13}$ marker chromosome in secondary spermatocytes and 3.1% based on association in primary spermatocytes (see Table 3). Again, the frequency with which chromosome 1^{13} devided equationally at anaphase I must be very low (none found). With regard to the production of aneuploid secondary spermatocytes, a slight excess of hypoploid (79) versus hyperploid cells (50) was found. Chromosome 1^{13} was found in 52.1% of the secondary spermatocytes. Both with regard to the presence of the $13;13^{1}$ marker structure and with regard to the presence of the metacentric Robertsonian chromosome, the data indicate that these markers were not always seen (for the 13^{1} chromatid, 27 cells with two compared with 52 cells without, $\chi^{2}_{1} = 3.9$, $p < 0.05$ and for the metacentric chromosome 13 with two metacentrics relate to 48 without any, $\chi^{2}_{1} = 20.1$, $p < 0.001$). In total 357 cells have been used to derive the percentages for the segregational behaviour

Table 5. Interpretation, in terms of meiotic segregation, of the frequencies of the karyotypes (N = 98) among the viable progeny of Rb4Bnr/+ ♀ times T7OH,+/+,Rb4Bnr ♂. For the modes of segregation in the ♂, see Fig. 3. The two adjacent 1 gametes are expected 1:1 as are the two alternate gametes. Aneuploid ♀ gametes produced through non-disjunction are excluded because frequency of viable progeny derived through complementation is not of quantitative importance. For details see Results (ii).

♂	Adjacent 1		Alternate		3:1 segregation
	1:1		1:1		
♀	T(1;11.13S)	+	Rb4Bnr	T7OH	$Rb4.1^{13}$
Rb4Bnr	9.2	9.1*	3.1	6.1	11.2
+	15.3	17.3	6.2*	6.1	16.3
	50.9		21.5		27.5

* These percentages have been assessed as described in the Results (ii)

of this mutant given in Table 7. For the reasons that a) the frequencies of "3:1" segregation and non-disjunction for other bivalents are obtained after subtraction (see Materials and Methods) and, b) that some markers did not have full observational penetrance (see above), the percentages have to be viewed with some caution and standard deviations are omitted. Due to the incomplete recovery of the marker chromosomes, adjacent 2 might be slightly overestimated. The summed frequency of alternate/adjacent 1 segregation is 76.8%, adjacent 2 segregation, 12.3%, "3:1" segregation, 10.9% and non-disjunction for normal bivalents, 23.2% (Table 7).

Non-disjunction for normal bivalents occurs more frequently among primary spermatocytes with adjacent 2 than with alternate/adjacent 1 plus 3:1 segregation of the translocation multivalent ($\chi^2_1 = 7.9$, $p<0.01$). Of the 22 cells which most probably descend from primary spermatocytes without a chiasma in segment 13_1, half show non-disjunction for a normal bivalent as well.

(iii) Fertility estimates

The testis weights of the two T70H,Rb4Bnr containing karyotypes and of Rb4Bnr/+ are of the same order but significantly lower than the weights found for +/+ males with the same genetic background (Student's t test, 2 p << 0.001, Table 8). Within the Rb4Bnr containing karyotypes, significantly higher sperm counts are found for Rb4Bnr/+ when compared to T70H,+/+,Rb4Bnr (Student's t test, $p < 0.01$). It must be noted that the sperm counts for Rb4Bnr/+ are considered to be an underestimate because these males had not been kept with females, contrary to all other males.

Sperm cells with deviating morphology are found with the same frequencies in the three Rb4Bnr containing karyotypes. These levels are significantly higher when compared to +/+ (for instance Rb4Bnr/+: Wilcoxon test, $p < 0,05$, Table 8).

Post-implantation loss can be subdivided in early and late deaths (small and large moles, respectively). For T70H,+/+,Rb4Bnr these levels are 71.3% and 5.1% (n = 313), for T(1;11.13S),+/+,Rb4Bnr 53.2% and 6.3% (n = 111) of the total number of implantations, respectively (see Table 9). When the secondary spermatocyte cell types which form the

Table 6. The segregation of the multivalent of the T70H,+/+,Rb4Bnr translocation and of the normal bivalents as derived from observations of metaphase secondary spermatocytes (N = 480). For illustrations of the segregation types, see Fig. 3

Segregation of the multivalent	Segregation status of chromosome 11 and normal bivalents		
	Normal	11-non-disjunction	Non-disjunction for normal bivalents
Alternate	21.8%	0.6%	6.3%
Adjacent 1	21.6-23.2%	-	2.1%
Adjacent 2	15.3%	5.6%	2.5%
3:1 segregation type I	13.9%	-*	1.9-3.5%
3:1 segregation type II	6.0	-*	0.8%

* Assumed not to be of significance

basis for Tables 6 and 7 are used for predicting the fraction of gametes with duplications and/or deficiencies of sufficient size to lead to a prenatal lethal, total death before birth would amount to 73.6% for T70H,+/+,Rb4Bnr and to 67.5% for T(1;11.13S),+/+,Rb4Bnr. The assumption has been made that most if not all tertiary trisomic conceptuses for chromosome 1^{13} survive up to birth. Especially for T(1;11.13S),+/+, Rb4Bnr, part of the adjacent 2 segregation products (those with a deficiency for chromosome 11 and segment 13 interstitial) might lead to pre-implantation lethals. The combined effects of hypoploidy through non-disjunction for a normal bivalent and the alternate/adjacent 1 products with a deficiency for the greater part of chromosome 1 (segment 1_t) can also have this developmental effect. For T70H,+/+,Rb4Bnr, pre-implantation genetic lethals due to hypoploidy for almost two chromosomes can be expected to a lesser extent. In agreement with these expectations, the fit between the frequency of genetically imbalanced gametes and the amount of post-implantation lethals is better for T70H,+/+,Rb4Bnr than for T(1;11.13S),+/+,Rb4Bnr.

4. Discussion

Contrary to the spermatogenic process in T70H/+ males, which is hardly affected by the presence of the translocation (de Boer, 1976), spermatogenesis is severely affected when the Rb(11.13)4Bnr chromosome is combined with T70H. Table 8 suggests that the sperm counts of the T70H,+/+,Rb4Bnr and T(1;11.13S),+/+,Rb4Bnr karyotypes are even lower than those of Rb4Bnr/+. Thus, complicating the meiotic pairing of Rb4Bnr homo- and heterozygotes by introducing T70H/+ at the telomeric end of chromosome 13 (see Figs. 1 and 2) leads to a further reduction of the quantitative effectiveness of the meiotic process. De Boer & Nijhoff (1981) have shown that a relative increase in the number of primary spermatocytes with short contracted bivalents (spiralization class 3) correlates highly with a decreasing sperm count. Furthermore, they make plausible as does Oshimura (1980) that the greater part of these cells are in fact degenerative and never reach the secondary spermatocyte stage. Table 1 shows that the two translocation heterozygotes of concern here show increased levels of primary spermatocytes with short

Table 7. The segregation of the multivalent of the T(1;11.13S),+/+,Rb4Bnr translocation and of the normal bivalents as derived from observations of metaphase secondary spermatocytes (N = 357). For illustrations of the segregation types, see Fig. 3

Segregation type multivalent	Segregation of normal bivalents	
	Normal	Non-disjunction
Alternate/adjacent 1	61.1%	15.7%
Adjacent 2	7.3%	5.0%
3:1 segregation type I	7.3%	2.5%
3:1 segregation type II	1.1%	

contracted bivalents. Thus, the population of primary spermatocytes scored for multivalent configurations may not be entirely representative for the population of secondary spermatocytes on which the genetic (i.e. through the mode of segregation) consequences of the translocations studied, was based. For instance, in both the T70H,+/+,Rb4Bnr and T(1;11.13S),+/+,Rb4Bnr karyotypes, the cytological proof for a chiasma in segment 1_i was found less frequently in the secondary spermatocytes (i.e. the $1;1^{13}$ marker chromosome was hardly present) than indicated by the chiasma frequency for this segment in the primary spermatocytes (Table 3).

As given in the Results (i), the chiasma in segment 13_t was found to terminalize less often in the T70H,Rb4Bnr containing karyotypes than in T70H/+. When we combine this finding with the chromosome morphology of the cells scored and with the relevant sperm counts (see above), we conclude that the lack of loss of a chiasma in segment 13_t is a sign of spermatocyte degeneration. However, when T70H/+ was made oligospermic on a hybrid genetic background with M. musculus molossinus, and death of germinal cells predominantly occurred around diakinesis-metaphase I, this phenomenon was not found (de Boer & Nijhoff, 1981).

Unlike T70H/+ and T(1;11.13S),+/+,Rb4Bnr, the alternate and adjacent segregation products can be distinguished in T70H,+/+,Rb4Bnr, both in the secondary spermatocytes (Table 6) and in the offspring (Table 5). In the offspring, adjacent 1 products are found more frequently while on the basis of the secondary spermatocytes, the two estimates do not differ. Limited sample size of the offspring seems to be the most plausible reason why the two alternate/adjacent 1 ratio's do not agree.

When the segregation behaviour of the multivalents in the two T70H, Rb4Bnr containing karyotypes (in T70H,+/+,Rb4Bnr with respect to the four chromosomes involved in the T70H caused part of the multivalent) is compared with the findings on T70H/+ (de Boer, 1976), a few trends emerge. When tabulated in accordance with the rules given in this paper, the T70H/+ secondary spermatocytes are characterized by 26.3% adjacent 2 segregation, 3.5% 3:1 segregation and 3.6% non-disjunction for normal bivalents (de Boer et al., in preparation). Thus, in the structurally heterozygous combination of T70H/+ and Rb4Bnr/+, adjacent 2 remained at approximately the same level, whereas 3:1 segregation sharply increased. The level of non-disjunction for normal bivalents seems to be

Table 8. Testis weights and sperm data of males of various karyotypes. The number of males, and for the sperm morphology also the total number of sperm scored (N), are given in parentheses

Karyotype	Testis wt. (g) ± s.d.	Sperm count ± s.d.	Abnormally shaped sperm (%) ± s.d.
T70H/+*	-	414 ± 111 (32)*	4.3*
T(1;11.13S),+/+,Rb4Bnr	0.080 ± 0.007 (7)	142 ± 84 (8)	14.0 ± 3.6 (4; N = 400)
T70H,+/+,Rb4Bnr	0.071 ± 0.017 (14)	86 ± 86 (14)	14.6 ± 10.0 (5; N = 500)
Rb4Bnr/+ (F_1 gen.)	0.074 ± 0.009 (15)**	176 ± 68 (15)***	14.8 ± 10.5 (10;N = 500)**
+/+	0.122 ± 0.011 (15)**	512 ± 125 (55)*	7.9 ± 4.7 (10;N = 500)**

* From de Boer (1976)

** From Nijhoff and de Boer (1979)

*** From Nijhoff and de Boer (1979). Males were not kept with females, thus the sperm count underestimated by a factor of ± 0.75

elevated also, a trend more readily visible in the T(1;11.13S),+/+, Rb4Bnr karyotype where the greater part of the Rb4Bnr chromosome is structurally homozygous. In this karyotype, we see a reduction of the adjacent 2 segregation frequency and, compared to T70H,+/+,Rb4Bnr and T70H/+, an intermediate frequency of 3:1 segregation has been found (Table 7). The reduction of the adjacent 2 segregation frequency in this karyotype seems to follow the general observation on translocations between metacentric chromosomes when an interstitial chiasma is formed (Sybenga, 1975). In T(1;11.13S),+/+,Rb4Bnr, a 13_i chiasma occurred in 87.5% of the primary spermatocytes while based on marker chromosomes in the secondary spermatocyte stage this would be close to 94% (see the Results (ii)). In a previous report concerning T70H/+, a correlation was found between adjacent 2 segregation and a proximal position (i.e. close to the centromere) of the single chiasma in segment 13 interstitial (de Boer, 1979). It can easily be seen (Tables 4, 6 and 7) that this rule does not hold when an extra pairing segment (chromosome 11) is introduced adjacent to the 13 arm (Figs. 1 and 2). The reverse rather seems to be the case with T70H,+/+,Rb4Bnr where a more distal 13 interstitial chiasma is observed to lead to a high adjacent 2 frequency. As can be seen in Table 6, chromosome 11 usually coorientates with chromosome 11.13. We explain the high adjacent 2 frequency of T70H,+/+,Rb4Bnr by assuming that the 11-chiasma is more prominently found in the equator than the 13_i chiasma, thus creating a situation in which the two 13 centromeres (including the 11.13 one) are positioned in one half of the spindle, which in turn increases the chances of adjacent 2 segregation. In T(1;11.13S),+/+,Rb4Bnr, both the 13_i and 11 chiasmata can be imagined to occupy the equator, leading to the predominant coorientation of the two 11.13 centromeres (for the various modes of segregation in both karyotypes, see Fig. 3).

An alternative explanation for the differences in adjacent 2 frequencies between the karyotypes here noted is offered by Vosselman (1981) in his study on the meiotic segregation in translocation heterozygotes of the onion fly. He hypothesized a relation between the segregational pattern of the multivalent and the distance between the centromeres and the nearest attachment point, i.e. chiasma position, and introduced the term "Coorientation Determining Distance" (CDD).

The extent to which CDD's differ between homologous and non-homologous

Table 9. Fertility of the two karyotypes, after pairing with +/+ females of the same background genotype, based on the uterine contents on day 18 of pregnancy (day of finding the vaginal plug is day 0)

		Fertile matings		
Male karyotype	Sterile mating (%)	N males	Post-impl. loss (%)	Mean number of impl. ± s.d.
T(1;11.13S),+/+,Rb4Bnr	9.1 (1/11)	10	59.5 (66/111)	11.1 ± 4.5
T70H,+/+,Rb4Bnr	28.6 (10/35)	25	76.4 (239/313)	12.5 ± 5.4

centromeres, will consequently be of influence for the segregational pattern of the multivalent. In line with this hypothesis, in T7OH,+/+, Rb4Bnr - in comparison to T(1;11.13S),+/+,Rb4Bnr - a more distally positioned 13_i chiasma, thus longer CDD for the two 13 centromeres, coincides with a higher adjacent 2 frequency (23.4% versus 12.3%). The hypothesis does not hold when both karyotypes are compared with T7OH/+ (26.3% adjacent 2 segregation) with respect to the relative position of the 13_i chiasma (see Table 4). A complicating factor here may be the role of the adjacent chromosome 11 segment (see explanation above).

When the two karyotypes studied are compared with T7OH/+ with regard to 3:1 segregation of the translocation multivalent, the sequence - in increasing frequency - is T7OH/+, T(1;11.13S),+/+,Rb4Bnr and T7OH, +/+,Rb4Bnr. The conclusion seems warranted that by introducing the extra pairing segment of chromosome 11, "3:1" segregation is increased. Human translocations between acro- and metacentric chromosomes which show 3:1 segregation have been reported in a review on 3:1 segregating human translocations by Lindenbaum & Bobrow (1975). They largely substantiate Hamerton's view (1971) that the risk for 3:1 segregation is high in human translocations between acrocentric and metacentric chromosomes having one short interstitial segment and furthermore characterized by translocated segments of strikingly unequal length. The pairing diagram of the Figs. 1 and 2 precisely fits such a description. In our material, the tertiary trisomic product survives into adulthood and thus, we can say that there is congruency between one mode of segregation of the translocation complex (3:1) and the appearance of deviant offspring. The frequent recovery of the tertiary trisomics is due to the limited size of the extra material in the tertiary disomic gametes produced. Jalbert et al. (1980) more or less assume parallelism between the segregation pattern (adjacent 1, adjacent 2 or "3:1") as derived from the frequency of birth of genetically imbalanced offspring and the frequency of the underlying meiotic event. This leads to their erroneous statement that a given reciprocal translocation produces "genetic imbalances" by one way of segregation only. Our results allow the conclusion that the factors predisposing for 3:1 segregation and the zygotic life expectancy of the resulting tertiary trisomic product produced coincide (see above). Thus, human translocations recognized through an aneuploid proband probably are the only translocations to

show 3:1 segregation in addition to the other modes of segregation which at the meiotic level cannot be studied, thus cannot be excluded in man.

A remarkable observation in the meiotic studies reported was the high level of non-disjunction for normal bivalents, especially for the T(1;11.13S),+/+,Rb4Bnr karyotype. The observation that this karyotype seems to show this phenomenon more frequently than the T70H,Rb4Bnr double heterozygote, together with the observation, that, within the former karyotype, primary spermatocytes without a chiasma in segment 13_i have a tendency to produce even more of it (see the Results (ii)), lead us to suppose that long and/or rigid structures around the equatorial plate of the primary spermatocyte interfere with the segregation process of the bivalents present. Beatty et al. (1975) reviewed the incidence of first anaphase non-disjunction in the male for a large number of karyologically normal stocks and strains in the mouse. The percentage of 0.38 they produced seems to be lower than the level found in T70H/+ males (3.6%) and in T70H,+/+,Ts(1^{13})70H translocation trisomic males (6.0%, de Boer et al., in preparation). Gropp et al. (1981, in the press) and Gropp & Winking (personal communication) do not seem te find reasons to suppose that heterozygosity for one or more Robertsonian translocations leads to a higher level of non-disjunction for normal bivalents. In T(1;11.13S),+/+,Rb4Bnr the combination of a Robertsonian translocation (in the homozygous state) with a reciprocal one (heterozygously) in one structure leads to a significant increase of non-disjunction for the remaining bivalents. It should be noted here that Cattanach and Moseley (1973) and Gropp et al. (1981) found indications for normal bivalent non-disjunction in male homozygotes for Rb4Bnr.

For T(1;11.13S),+/+,Rb4Bnr (this paper) and for T70H translocation trisomics (de Boer et al., in preparation), a correlation was observed between adjacent 2 segregation of the translocation and non-disjunction for normal bivalents. For T70H,+/+,Rb4Bnr, non-disjunction of chromosome 11 with Rb11.13;11.13^1 predominantly occurred with adjacent 2 segregating multivalents. The mechanism at work in the former two karyotypes probably is not similar to that operative for chromosome 11. Two statements concerning a coincidence of this nature can be made.

a) There is an interaction between the two.
b) The general physiological status of the cell influences both the segregation mode of the multivalent and the chances for mis-

segregation of the normal bivalents. Within the frame work of this paper, it is impossible to choose between these two alternatives, and the solution of this problem must await an experimental approach. The second possibility is in line with the hypothesis put forward by Nijhoff and de Boer (1979 and 1980) to explain the high level of non-disjunction for the chromosomes 11 and 13 in Rb(11.13)4Bnr heterozygotes. Here, a correlation was sought between non-disjunction for these chromosomes and the detrimental effect the Rb4Bnr/+ structure had on the spermatogenic process, possibly finding its origin during the pachytene stage through incomplete pairing of the two acrocentric chromosomes with the metacentric one (preliminary unpublished results). The negative effect of a lack of meiotic pairing on the spermatogenic process is well documented (Miklos, 1974; de Boer and Searle, 1980).

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GENERAL DISCUSSION

Meiotic non-disjunction is the failure of chromosomes to separate regularly at the first, or chromatids to do so at the second meiotic division. In spite of the considerable amount of work done, the causative mechanisms are poorly understood. Their elucidation is important, as is clear from the observation that in man 15-20% of all recognized conceptions end in a spontaneous abortion, approximately half of these due to the occurrence of an extra chromosome (Boué et al., 1975). For obvious (ethical) reasons, a fundamental study of the causes of non-disjunction cannot be undertaken in the human. However, as it appears that man is far more prone to meiotic non-disjunction than any suitable experimental animal, such as the mouse, no good experimental substitute is available for the human.

The usefulness of the mouse as a meiotic model for man has been analysed in this thesis by using mouse carriers of translocation types also common to man. A comparison of the meiotic behaviour of such translocations can then be made between man and mouse. Moreover, the highly increased level of non-disjunction in males heterozygous for one of these types has been used to determine the effects of irradiation on this process. Such a test system with a high initial non-disjunction frequency may be strictly necessary when a substitute for experimentation on man is sought, as the extent of "spontaneous" meiotic non-disjunction is greater in man than in mouse (see above). The general concept behind the studies described in this thesis has been outlined in the General Introduction.

In the following discussion, the comparability of man and mouse with respect to incidence and general causes of non-disjunction in the spontaneous situation, i.e. when no chromosomal rearrangements are involved, will be treated (points 1.1 and 1.2). The translocation types used are discussed next, with respect to structural comparability with those in man, their frequency of occurrence in man and their general effects on meiotic segregation in both man and mouse (point 2). In point 3 a general conclusion of the studies described in this thesis will be outlined.

1 Normal meiosis in man and mouse

1.1 Incidence of non-disjunction

A direct estimate for the incidence of spontaneous meiotic non-disjunction in man cannot be given (Chapter 1). Insufficient spreading of the chromosome sets of the secondary spermatocytes hampers a measurement of the incidence of anaphase I non-disjunction for the human male. At the spermatozoal level, the hitherto most credible approximation of the summed anaphase I plus II non-disjunction frequency of 3.3% was obtained on the basis of the analysis of the chromosome complements from 60 sperm heads, possible after their fusion with hamster oocytes in vitro (Rudak et al., 1978). For the human female, a direct estimate of the summed anaphase I plus II non-disjunction frequency is not available. Studies on the parental contribution to the incidence of trisomy 21 (Down's syndrome) among the live born progeny indicate a male: female contribution of 3:7 (Langenbeck et al., 1976, Magenis et al., 1977, Hansson & Mikkelsen, 1978). The extrapolation of this ratio to the whole genome is possibly not justified, but by making this assumption and the assumption that this ratio is basically the same immediately after fertilization, 11% (3.3% + 7/3 x 3.3%) of the zygotes would be aneuploid in man. It is not unlikely that this frequency is too low (c.f. Chandley, 1981). In mice the frequency of aneuploid zygotes after fertilization in vivo, based on the summed frequencies of aneuploid male and female derived chromosome complements in the pronuclei, was estimated to be 1.4% (N=1679 pronuclei; Fraser & Maudlin, 1979). So with respect to the incidence of normal bivalent non-disjunction at conception, there are clear indications for a considerable gap between man and mouse. Parallel findings for the two species with respect to some of the causes of meiotic non-disjunction also exist.

1.2 Causes of non-disjunction

For both man and mouse the female sex shows higher incidences of meiotic non-disjunction (for man see the preceeding paragraph), which becomes more pronounced at an older age. Newborn surveys indicate a sharp rise in trisomy 21 among the progeny of mothers older than 35 (c.f. Erickson, 1978). For mice the female contribution to the incidence of aneuploid zygotes was only somewhat higher than that of males when they are 2-4 months old (Maudlin & Fraser, 1978; Fraser & Maudlin, 1979) but the difference was evident when 8-10 months old females were used

(male: female contribution = 1 : 4.6; Maudlin & Fraser, 1978, Table 5, Chapter 1). These data suggest that factors operate at the systemic and cellular levels. On the other hand chromosome specific, i.e. species specific, factors may also be involved. The level of trisomy 16 among the human progeny after implantation for example is hardly affected by the maternal age (c.f. Chandley, 1981).

In order to study the possible causes of non-disjunction at the two meiotic stages, laboratory animals (most often the mouse, see Chapter 1) were used. Studies have been concentrated on meiosis I because the incidence of anaphase I non-disjunction can well be estimated at meiosis II and is, moreover, most probably the principal cause of aneuploid gametes in the male as well as the female mouse (compare Chapter 1, Tables 3, 4, 5 and 6). Studies in man, using chromosome heteromorphisms indicate that non-disjunction at anaphase I may at least be 5-10 times more frequent than at anaphase II (Langenbeck et al., 1976; Jacobs & Morton, 1977; c.f. Table 8, Chapter 1). Moreover, the direct estimate of the frequency of anaphase II non-disjunction in man is hampered by the fact that analysis at the gamete level is not well possible.

Non-disjunction at anaphase I has been attributed to a variety of factors like spindle malfunction, slower dispersion of the nucleolar material and a decreased chiasma frequency (ultimately leading to an increased univalence frequency), the latter possibly including a precocious chiasma terminalization. Ageing in the female mouse was often found to be accompanied by a decline in the chiasma frequency in oocytes (Henderson & Edwards, 1968; Luthardt et al., 1973; Polani & Jagiello, 1976; Speed, 1977), although this could not be sustained for the long 13^{1} bivalent in T70H/T70H translocation homozygous females (de Boer & van der Hoeven, 1980). On the level of the normal bivalents, meiosis I observations have not yielded much information on anaphase I non-disjunction. Polani and Jagiello (1976), for example, found no evidence for the relation between univalence and non-disjunction. On the other hand a good parallel between the occurrence of univalents at metaphase I and non-disjunction is found in aged translocation homozygous T70H/T70H female mice for the small marker bivalent 1^{13}. Here an eight-fold increase of the univalent frequency is accompanied by a nine-fold increase of non-disjunction for this chromosome estimated at meiosis II (de Boer & van der Hoeven, 1980). Another alternative to explain the increase of non-disjunction in ageing female mammals is to relate this phenomenon

to the poorer physiological condition within the oocytes. In this context Crowley et al. (1979) hypothesized an interaction between the hormonally governed rate of meiosis - which possibly is influenced by the age of the mammalian female - and the timing of chiasma terminalization. This alternative fits the observation of Karp and Smith (1975) who transferred primary mouse oocytes in culture for 12 h to 23 °C with re-incubation at 37 °C thereafter. They noted an increase in the percentage of hyperploid secondary oocytes from 0 to 9.6%. Indications that this explanation may be of a more general importance to non-disjunction are offered in our own work concerning meiosis in translocation carrying male mice (Chapters 2, 3 and 4).

2 The chromosome rearrangements used; a man-mouse comparison

A Robertsonian translocation - Rb(11.13)4 Bnr/+; Chapters 2 and 3 - and a reciprocal translocation between a metacentric and an acrocentric chromosome - T(1;11.13S),+/+,Rb4Bnr; Chapter 4 - have been selected as rearrangements which also occur in man. For the estimate of the frequency of occurrence of these types in man, see the General Introduction.

The term Robertsonian translocation refers to a rearrangement combining the long arms of two acrocentric chromosomes, or to the fusion of two telocentrics, whereby one new (sub)metacentric chromosome is formed. It is assumed that such an event usually is not a true fusion but a reciprocal translocation. Depending on the positions of the breakpoints, the newly arisen metacentric chromosome may be mono- or dicentric (John & Freeman, 1975). Within the former category the single centromere may be derived from either of the two constituent chromosomes or alternatively may be a compound centromere (Hsu et al., 1973). The decisive method to elucidate the structure of the centric region has not yet been found but on the basis of the presence of two C bands, most Rb translocations are assumed to be dicentric in man (c.f. Niebuhr, 1972; Mattei et al., 1979), According to this criterium, the Rb(11.13)4Bnr translocation chromosome is also dicentric (unpublished personal observation).

Among the group of Rb translocations in man, a preferential involvement of the acrocentric chromosomes of the D and G groups is observed. In newborn surveys D/D type Rb translocations prevail, among which the Rb(13.14) translocation is most frequently found (Nielsen & Sillesen, 1975). This is contrary to the mouse, where the involvement seems to

follow a more or less random pattern (c.f. Mouse News Letter 62, 1980; 59-60, containing a list of 101 Robertsonians in mice from wild populations). Moreover, in man but not in the mouse, Rb translocations involving both homologous acrocentrics as for example Rb(21.21) are encountered though with a low frequency (Chamberlin & Magenis, 1980). Although the data suggest that in man Robertsonian translocations can be produced through more mechanisms than in the mouse,Rb4Bnr seems to be a good choice, representing a major fraction of this type of chromosomal rearrangement in man.

A comparison of the general features accompanying Robertsonian translocation heterozygosity in man and mouse reveals the following. Segregation distortion of the Rb chromosome, i.e. selection against this chromosome, has been observed after meiosis I in mice for many, though not all female Rb/+ conditions, but this phenomenon was absent in Rb/+ males (Winking & Gropp, 1976; Gropp et al., 1981). Segregation distortion for the Rb chromosome was also concluded to take place in human Robertsonian translocations, but in comparison to the mouse with two notable differences. Firstly, it seems to work in the opposite direction, thus in man selection in favour of the Rb chromosome was concluded, while secondly, it occurs in both sexes (Boué, 1979) and possibly even preferably in Rb/+ males (Hamerton, 1968). It is unknown to what extent the human data are influenced by selection at later stages than meiosis because for man the conclusions were not based on meiotic observations (like the mouse) but on the analysis after implantation only. Karyotyping via amniocentesis of pregnancies of parents carrying Rb translocations, presented evidence for elevated non-disjunction levels of the Rb involved chromosomes in man. The frequencies of unbalanced products differed considerably, however, for the individual Rb translocation types and ranged from 1.1% (N=94) for Rb(13.14) to 8.9% (N=90) for Rb(14.21) and 17.6% (N=17) for Rb(21.22) (Boué et al., 1979). When no chromosomal rearrangements are involved, these frequencies ranged from 2.3-5.1%, depending on the way - after amniocentesis or induced abortion - the material has been obtained (Chapter 1, Table 7). The differences between the human Robertsonians may in the first place be attributed to differences in the stage(s) of pregnancy at which elimination of the unbalanced products will take place (Chapter 1) but, on the other hand, may also reflect the level of non-disjunction intrinsic to the type of

Rb translocation. In mice a wide range in the rate of anaphase I non-disjunction (on the basis of meiosis II data) and clearly dependent on the type of Rb translocation is a well known fact (Cattanach & Moseley, 1973; Gropp et al. 1974, 1975 and 1981). It is interesting in this respect, that Rb translocations with analogous arm composition but from different feral origin can give rise to different rates of non-disjunction when in heterozygous condition in the laboratory mouse. Examples are Rb(10.11)8Bnr/+ and Rb(10.11)5Rma/+ males showing 5.0% (N=600) and 13.8% (N=600) aneuploid secondary spermatocytes, respectively. It is unknown wether this finding must be contributed to minor structural differences around the centromeres or to differences of undefined genetic origin. For both man and mouse, Robertsonian translocation heterozygotes follow the general rule that females are more prone to non-disjunction than males. In man this was most apparent for Robertsonians which involve chromosome 21 (man: Boué, 1979; mouse: Gropp et al., 1981).

A comparison of the meiotic behaviour of reciprocal translocations between one metacentric and one acrocentric chromosome in man and mouse was hitherto not possible a) because this type of translocation has, till now (Chapter 4), not been studied in the mouse and b) data for man are merely based on the karyotype analysis of the live born progeny and thus have been greatly influenced by preceeding selection. The category of reciprocal translocations, involving a metacentric and an acrocentric chromosome, is probably overrepresented when the method of ascertainment is based on "malformed", i.e. unbalanced, progeny after birth. Translocations in man listed on the basis of this criterium, show a ratio of the meta/acro: meta/meta types of 4.25 : 3 (N=146; Jalbert et al., 1980), whereas when the method of ascertainment was also based on additional criteria - like malformed infants, recurrent spontaneous abortions, family history - a ratio of 3 : 6.8 (N=196; Boué et al., 1979) was found. The need, also in view of the increasing demand for genetic counselling, to clarify the meiotic behaviour of the multivalent caused by translocations of the meta/acro type has hereby clearly been indicated. Chapter 4 of this thesis makes plausible, that the observations on a mouse translocation between a meta- and an acrocentric chromosome T(1;11.13S),+/+,Rb(11.13)4Bnr) show parallels with those of the comparable human type. Studies of

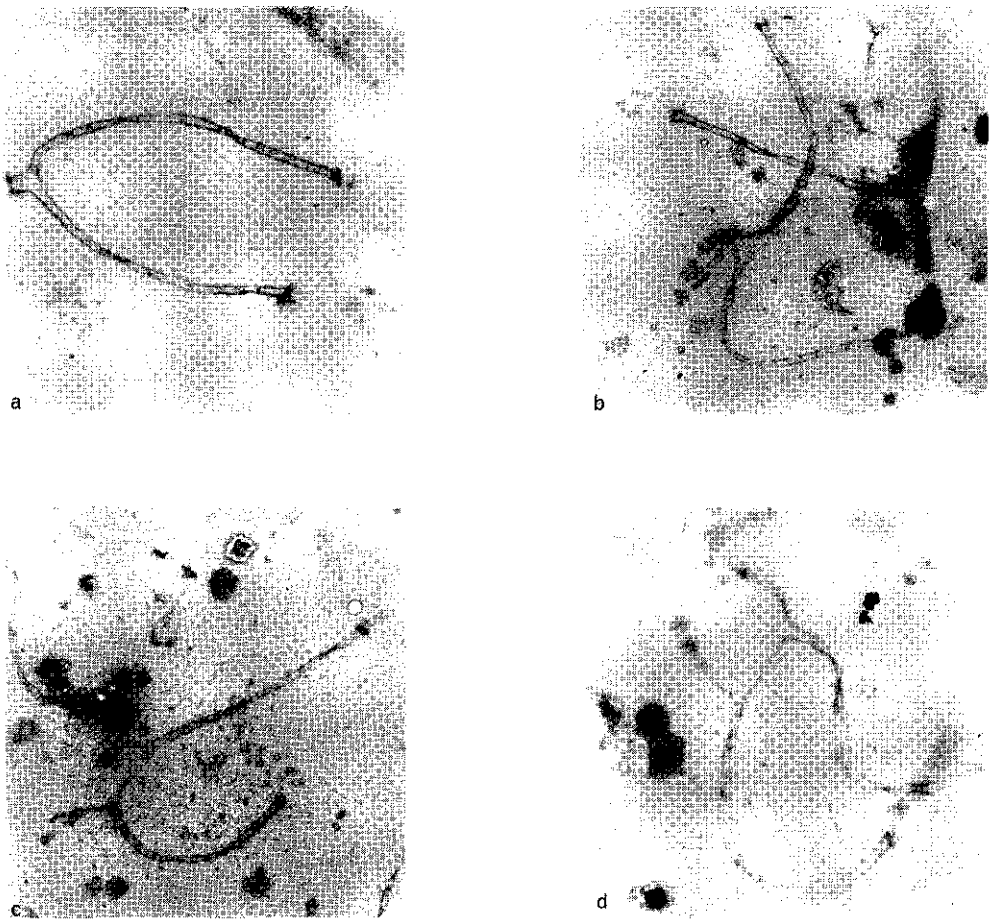

Fig. 1a-d Four electronmicrographs of representative synaptonemal complexes (SC) from trivalents in surface spread pachytene spermatocytes of Rb(11.13)4Bnr/+ mice, made and stained according to the method described by Moses (1977). The structures consist of 1) one long lateral element continuous along the SC trivalent, corresponding to the metacentric Rb4Bnr chromosome and 2) two shorter lateral elements paired with the arms of the metacentric and corresponding to the two acrocentrics.

In the electronmicrographs a and b, the two non-homologous pericentric regions of the chromosomes 11 and 13 have paired and in technically favourable preparations, a central element can be seen as proof of the true SC structure of this segment. In c and d the two pericentric regions have not paired thus creating an unpaired region in the lateral element of the metacentric chromosome as well. This area contains the centromere(s), but no sign of these can be noted.

such mouse translocations, therefore, may be of considerable medical importance.

3 One possible cause of non-disjunction

The argument to study the meiotic behaviour of a reciprocal translocation between a metacentric and an acrocentric chromosome in the mouse was to explore its usefulness as a model system for the situation in man. The unexpected and highly significant rise of normal bivalent non-disjunction after anaphase I in this meiotic system in the mouse (Chapter 4) introduces a parallel with man of normal karyotype. In T(1;11.13S),+/+Rb4Bnr male mice a correlation was observed between adjacent 2 segregation of the multivalent chromosomes (homologous centromeres move to the same pole) and non-disjunction for the normal bivalents. This suggests either an interaction between the two phenomena or alternatively, its coincidence may be an effect of the general physiological status of the cell. The second possibility is in line with what was discussed in section 1.2 of this Chapter and with the hypothesis put forward in Chapters 2 and 3 to explain the high level of non-disjunction for chromosomes 11 and 13 in Rb(11.13)4Bnr heterozygous male mice. Here a correlation was sought between non-disjunction for these chromosomes and the detrimental effect the Rb4Bnr caused multivalent had on the meiotic process. This effect probably finds its origin in pachytene through incomplete pairing of the centromeric regions of the two acrocentric chromosomes with the metacentric one (see Fig. 1), a phenomenon more generally observed in relation to Rb translocation heterozygosity (mice: Gropp et al., 1981; lemurs: Moses et al., 1979). In this respect, the negative effect of reduced meiotic pairing on the spermatogenic process is well documented (Miklos, 1974; de Boer and Searle, 1980).

Taken together, these data and those presented in the "Summary and conclusions", warrant more detailed investigations of translocation carrying mice, especially considering their obvious usefulness for the understanding of human meiosis.

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SUMMARY AND CONCLUSIONS

In this thesis a start was made with meiotic observations of mouse translocation types - a Robertsonian translocation and a translocation between a metacentric and an acrocentric chromosome - which also occur in man. It is generally accepted that, when no chromosomal rearrangements are involved, man shows a higher level of non-disjunction than the mouse. When the meiotic behaviour of translocations in mouse and man would be more similar than that found for the autosomal bivalents (and sex bivalent) of these species - Chapter 1 reviews the methodology to establish this difference - mouse systems characterized by chromosome aberrations would become more attractive as models for the study of endogeneous and exogeneous factors on the meiotic process and extrapolation from mouse to man would be facilitated. As it appeared in these studies that not only the translocation complex but also the normal bivalents contribute to a significant extent to the non-disjunction rates in the heterozygote for a translocation of the meta/acro type, this material appeared even more favourable as an experimental substitute for man than was initially expected.

Meiosis in male mice, heterozygous for the Robertsonian translocation Rb(11.13)4Bnr, has been studied in an attempt to elucidate the mechanism(s) of non-disjunction for the trivalent formed at meiosis I by the Rb chromosome and the two acrocentric homologues 11 and 13 (Chapters 2 and 3). As an exogeneous factor of possible influence, the meiotic effects of two types of radiation, administered at relatively low doses 2 and 3 hours before prometaphase-metaphase II (probably during metaphase-anaphase I), were determined in Rb4Bnr/+-males (Chapter 3).

The segregational behaviour of the multivalent at anaphase I has been compared in T(1;11.13S),+/+,Rb4Bnr, structurally homozygous for the metacentric Rb(11.13)4Bnr chromosome whereby arm 13 of one of the two metacentrics is involved in a reciprocal translocation with the acrocentric chromosome 1, and T(1;13)70H,+/+,Rb4Bnr (Chapter 4). The multivalents formed by the latter karyotype at meiosis I are highly comparable to those of the former one; for details see section III below.

The results obtained, and presented below, warrant a further investigation of these mouse meiotic systems as models for the situation in man as is for example indicated by the significant increase of normal bivalent non-disjunction found in the T(1;11.13S),+/+,Rb4Bnr and the

T70H,+/+,Rb4Bnr karyotypes.

In the next sections the chapters on which the conclusions are based, have been indicated between parentheses.

I Indications for the relation between delay before and around anaphase I and non-disjunction in male Rb4Bnr Robertsonian translocation heterozygotes

- For Rb4Bnr/+ males, inter mouse variation was noted with respect to the duration of the meiotic prophase, i.e. the period "end of the premeiotic S phase-metaphase I". This was concluded on the basis of the frequency of late diplotene/metaphase I cells with labelled chromosomes recorded autoradiographically in mice killed various time intervals after the i.p. administered injection with ^{3}H-thymidine (3).

- For Rb4Bnr/+ males, compared to their +/+ controls, a greater mean number of late diplotene/metaphase I cells was counted in the separate groups along the seminiferous epithelium, whereas the mean distance between the subsequent groups was smaller. As these data are in contradiction with the reduction by 58% of the epidydimal sperm count for Rb4Bnr/+ compared to +/+, the greater number was thought to be an accumulation effect due to delay during diplotene/metaphase I for at least a fraction of the cells. Implicitly a causal relation between delay and cell death before the spermatozoal stage is also suggested (2).

- The duration of the period "end meiosis I (metaphase I) - end meiosis II" was approached autoradiographically for Rb4Bnr/+- males with the aid of cells showing a labelled Y chromosome only. The best assessment of the duration of this period was 3 hours or less. Inter mouse variation with respect to the duration of the period "end of the premeiotic S phase-metaphase I" (see above) prevented a more accurate estimate (3).

- Irradiation of Rb4Bnr/+ males for 14.5 minutes with a dose of 15.2 rad fast neutrons (mean energy 1.7 MeV) significantly

decreased the incidence of aneuploid secondary spermatocytes, scored 2 and 3 hours after application. This was explained by selective cell killing of those cells giving rise to aneuploid daughter cells after anaphase I, rather than by protection against non-disjunction by the irradiation dose, as follows: For Rb4Bnr/+ during the final period of the primary spermatocyte stage, cells show a continuous distribution with respect to the extent of delay. Cells with a strong delay will die off before the secondary spermatocyte stage, while the fraction showing only minor delay will survive (in the unirradiated situation) but with an increased risk of producing aneuploid daughter cells. The results suggest that, in terms of cell death, the victims of the 15.2 rad neutron irradiation predominantly have to be searched in the last category. A causal relation between delay and ensuing non-disjunction at anaphase I, and between delay and the neutron irradiation effect respectively, is thus suggested but the basis for these interactions remains unknown (3).

II Irradiation induced damage during meiosis in male Rb4Bnr Robertsonian translocation heterozygotes

- After irradiation of Rb4Bnr/+ male mice for 14.5 minutes with either a dose of 15.2 rad neutrons or 60 rad X rays, only neutrons affected, viz. significantly decreased (see above), the incidence of aneuploid secondary spermatocytes estimated 2 and 3 hours after irradiation. The reason for this difference remained unknown and could not be explained on the basis of a difference in the levels of radiation induced damage. The two radiation types induced comparable levels of chromosome damage, i.e. numbers of breaks, fragments and deletions per cell, when compared in either late diplotene/metaphase I or metaphase II cells. For these parameters the neutrons: X ray RBE ratios estimated were 5.4 for meiosis I and 3.3 for meiosis II cells (3).

- After an irradiation-fixation interval of 2 to 3 hours, the chromosome damage (i.e. numbers of breaks, fragments and deletions) induced by either 15.2 rad neutrons or 60 rad X rays was

5-10 times higher when scored in metaphase II than in diakinesis/ metaphase I cells. At the moment of irradiation the two categories of cells were, most probably, at prometaphase/metaphase I and late diplotene/early diakinesis, respectively. The difference in chromosome damage was argued to be merely the consequence of differences in chromosomal processes taking place during the irradiation-fixation interval and not a reflection of a difference in radiation sensitivity between the two meiosis I stages (3).

III The meiotic behaviour in T(1;11.13S),+/+,Rb4Bnr and T70H,+/+,Rb4Bnr male mice, also with reference to the behaviour of the normal bivalents

The T(1;11.13S),+/+,Rb4Bnr karyotype includes the metacentric Rb(11.13)4Bnr chromosome in homozygous condition whereby the 13 arm of one of the metacentrics is involved in a reciprocal translocation with the acrocentric chromosome 1. The T70H,+/+,Rb4Bnr karyotype is double heterozygous for the T(1;13)70H reciprocal translocation and the metacentric Rb(11.13)4Bnr chromosome. Its multivalent at meiosis I differs from the one in the former karyotype in one respect: The (acrocentric) chromosome 11 is here involved once separately and once as one of the arms of the metacentric, i.e. attached to the 13 arm, whereas in the former karyotype the two chromosomes 11 are involved each in a metacentric.

- For the short translocated segment 13_t, in both T(1;11.13S),+/+, Rb4Bnr and T70H,+/+,Rb4Bnr males, chiasma terminalization with proceeding meiosis I was observed less frequently compared with T70H/+. It was argumented that this is probably due to spermatocyte I degeneration taking place to a higher extent in the former two karyotypes as in both, in comparison to T70H/+, a greater frequency of meiosis I cells with short contracted bivalents was observed. The latter phenomenon was in an earlier study shown to correlate highly with a decreasing sperm count (4).

- In T(1;11.13S),+/+, Rb4Bnr a chiasmate association of the long interstitial segment 13_i was absent in 12.5% (N=1000) of the meiosis I cells, but only in 2.7% (N=1000) of these cells in T70H,+/+,Rb4Bnr males. A cause for this discrepancy could not

be given - the phenomenon could not be contributed to a precocious "disappearance" of the 13_i chiasma - and seems to be an effect of the metacentric chromosome present in homozygous condition in the former karyotype (4).

- A significant increase of the 3:1 segregation of the multivalent chromosomes after anaphase I in comparison to T70H/+ (3.5%) was found for T(1;11.13S),+/+,Rb4Bnr (10.9%) but most significantly for T70H,+/+,Rb4Bnr (22.6%). In the latter karyotype this refers only to the four chromosomes involved in that part of the multivalent caused by the T70H translocation. In T70H,+/+,Rb4Bnr, a preference of the small marker chromosome 1^{13} to segregate with the metacentric Rb4Bnr chromosome was indicated and this could be sustained by the high frequency of Ts(1^{13})70H tertiary trisomics among the progeny of males of this karyotype. The results suggest that factors predisposing for 3:1 segregation and life expectancy of the tertiary trisomic product produced among the progeny may coincide (4).

- In T70H,+/+,Rb4Bnr males the alternate and adjacent 1 segregation products could be distinguished at meiosis II and almost equal frequencies were found for both. In the offspring however, karyotypes resulting from adjacent 1 were found more frequently but this disagreement is probably due to the limited sample size of the offspring karyotyped (4).

- In both T(1;11.13S),+/+,Rb4Bnr and T70H,+/+,Rb4Bnr male mice, the normal bivalent non-disjunction after anaphase I appeared to be highly elevated in comparison to +/+ and T70H/+. For the former karyotype a correlation was observed, moreover, between non-disjunction for the normal bivalents and adjacent 2 segregation of the multivalent chromosomes. This suggests either an interaction between the two phenomena or, alternatively, their coincidence may be an effect of the general (poor) physiological status of the cells. A sharp distinction between these alternatives cannot be made. The indication of spermatocyte I degeneration (see first point of this section) in both karyotypes puts weight

on the latter hypothesis, assuming an interaction between the poorer physiological status of the cell and adjacent 2/normal bivalent non-disjunction.

SAMENVATTING EN KONKLUSIES

I Het verband tussen non-disjunctie en meiotische vertraging voor en tijdens de anafase I in mannelijke Rb4Bnr Robertsonische translokatie heterozygoten

- In mannelijke muizen heterozygoot voor de Robertsonische translokatie Rb4Bnr (Rb4Bnr/+) werd variatie tussen de dieren gevonden in de duur van de periode "eind premeiotische S fase - metafase I". Dit was gebaseerd op de frequentie van laat diploteen/metafase I cellen met radioactief gemerkte chromosomen na verschillende tijdsintervallen na het toedienen van ^{3}H thymidine (3)*.

- In Rb4Bnr/+ mannelijke muizen, vergeleken met hun +/+ kontroles, werd een gemiddeld groter aantal laat diploteen/metafase I cellen gevonden in de afzonderlijke groepen langs het spermatogene epitheel, terwijl de gemiddelde afstand tussen de groepen kleiner was. Omdat deze gegevens in tegenspraak leken met de reduktie met 58% van de gemiddelde telling van het epidydimaal sperma voor Rb4Bnr/+, vergeleken met +/+, werd het groter aantal toegeschreven aan ophoping als gevolg van vertraging tijdens diploteen/metafase I voor tenminste een gedeelte van de cellen. Impliciet wordt zo ook een oorzakelijk verband tussen vertraging en celdood nog voor het volwassen sperma stadium gesuggereerd (2).

- De duur van de periode "eind meiose I (metafase I) - eind meiose II" in Rb4Bnr/+ mannelijke muizen werd autoradiografisch vastgesteld met behulp van cellen die alleen een radioaktief gemerkt Y chromosoom bevatten. De beste schatting voor de duur van deze periode was 3 uur of minder. Variatie tussen muizen met betrekking tot de duur van de periode "eind premeiotische S fase - metafase I" (zie boven) verhinderde een preciesere benadering (3).

* Tussen haakjes: nummers betreffende hoofdstuk

- Bestraling van Rb4Bnr/+ mannelijke muizen gedurende 14.5 minuten met een dosis van 15.2 rad snelle neutronen (gemiddelde energie 1.7 MeV) verlaagde de frequentie van aneuploide secundaire spermatocyten, 2 en 3 uur na bestraling vastgesteld, significant. Dit werd verklaard door het selectief doden door de straling van die cellen die aanleiding zouden hebben gegeven tot aneuploide dochter cellen na anafase I (en niet door bescherming tegen non-disjunctie door de bestraling) op de volgende wijze: In Rb4Bnr/+ vertonen de cellen, gedurende de laatste periode van het primaire spermatocyt stadium, een continue verdeling met betrekking tot de mate van vertraging. Cellen die een relatief sterke vertraging ondervinden zullen afsterven voor het secundaire spermatocyt stadium, terwijl de fraktie welke slechts een geringe vertraging vertoont zal overleven (in de onbestraalde situatie), maar met een verhoogde kans tot het produceren van aneuploide dochter cellen. De resultaten suggereren dat, in termen van cel dood, de slachtoffers van de bestraling met 15.2 rad snelle neutronen voornamelijk moeten worden gezocht in de laatste kategorie. Een oorzakelijk verband tussen vertraging en erop volgende non-disjunctie tijdens de anafase I, respektievelijk tussen vertraging en het effekt van de neutronen bestraling, wordt hiermee gesuggereerd maar de basis voor deze interakties blijft onopgehelderd (3).

II <u>Stralings geïnduceerde schade tijdens de meiose in mannelijke Rb4Bnr Robertsonische translokatie heterozygoten</u>

- Na bestraling van Rb4Bnr/+ mannelijke muizen gedurende 14.5 minuten met een dosis van 15.2 rad neutronen of 60 rad Röntgen stralen bleek alleen de neutronen straling de frequentie van aneuploide secundaire spermatocyten, 2 en 3 uur na bestraling vastgesteld, beïnvloed (dat wil zeggen significant verlaagd, zie boven) te hebben. Een verklaring voor dit verschil werd niet gevonden; het kon niet worden toegeschreven aan een verschil in het nivo van door de straling geïnduceerde schade. Beide stralings types induceerden vergelijkbare nivo's van chromosoom-

schade (aantallen breuken, fragmenten en deleties per cel) wanneer werd vergeleken in laat diploteen/metafase I of metafase II cellen. Voor deze parameters werden de volgende neutronen: Röntgen stralen "RBE waarden" voor dit dosis gebied gevonden nl. 5.4 voor de meiose I en 3.3 voor de meiose II cellen (3).

- Na een interval van 2 tot 3 uur tussen bestraling en fixatie van de cellen, was de chromosomale schade (zie boven) geïnduceerd door zowel 15.2 rad neutronen als 60 rad Röntgen stralen 5-10 maal zo hoog wanneer gemeten in de metafase II als in de diakinese/metafase I cellen. Op het moment van bestraling bevonden deze twee cel kategorieën zich hoogst waarschijnlijk in respektievelijk het prometafase I/metafase I en het laat diploteen/vroeg diakinese stadium. Argumenten zijn aangevoerd om de oorzaak van het verschil in chromosomale schade voornamelijk te zoeken in de verschillen in chromosomale processen welke plaatsvinden tijdens het bestralings-fixatie interval en niet in een verschil in stralingsgevoeligheid tussen beide meiose I stadia (3).

III Het meiotisch gedrag in T(1;11.13S),+/+,Rb4Bnr en T70H,+/+,Rb4Bnr mannelijke muizen, ook met betrekking tot het gedrag van de normale bivalenten

Het T(1;11.13S),+/+,Rb4Bnr karyotype is homozygoot voor het metacentrische Rb(11.13)4Bnr chromosoom waarbij de 13 arm van één van de metacentrische chromosomen is betrokken bij een reciproke translokatie met het acrocentrische chromosoom 1. Het T70H,+/+,Rb4Bnr karyotype is dubbel heterozygoot voor de reciproke translokatie T(1;13)70H en het metacentrische Rb(11.13)4Bnr chromosoom. De multivalent gevormd tijdens de meiose I in het laatste karyotype verschilt maar in één opzicht van dat in het vorige karyotype: Het (acrocentrische) chromosoom 11 is hier eenmaal afzonderlijk en eenmaal als één van de twee armen van het metacentrische chromosoom, dus vastzittend aan de 13 arm, betrokken bij de multivalent terwijl in het T(1;11.13S),+/+,Rb4Bnr-multivalent beide chromosomen 11 onderdeel van de metacentrische chromosomen zijn.

- In vergelijking met T70H/+ werd in zowel T(1;11.13S),+/+,Rb4Bnr als T70H,+/+,Rb4Bnr mannelijke muizen chiasma terminalisatie bij

voortschrijdende meiose I voor het korte getransloceerde segment 13_t minder frequent gevonden. Waarschijnlijk was dit een gevolg van de hogere frequentie van degenererende primaire spermatocyten in deze karyotypen - waarin geen terminalisatie meer plaats vond - vergeleken met T70H/+, gekonkludeerd op grond van een relatief grotere frequentie van meiose I cellen met korte, gekontraheerde, bivalenten. In een eerdere studie was gevonden dat dit laatste verschijnsel in hoge mate korreleerde met een afnemende sperma telling (4).

- In 12.5% (N=1000) van de meiose I cellen in T(1;11.13S),+/+, Rb4Bnr werd geen chiasma gevonden in het lange interstitiële segment 13_i, vergelegen met 2.7% (N=1000) voor T70H,+/+,Rb4Bnr. Een verklaring van dit verschil kon niet worden gegeven - het verschijnsel kon niet worden toegeschreven aan een vroegtijdige "verdwijning" van het 13_i chiasma - en lijkt een effekt te zijn van het metacentrische chromosoom, aanwezig in homozygote toestand in het eerste karyotype (4).

- In vergelijking met T70H/+ (3.5%) werd een signifikante toename van de 3:1 segregatie van de multivalent chromosomen na anafase I waargenomen voor T(1;11.13S),+/+,Rb4Bnr (10.9%), maar het meest signifikant voor T70H,+/+,Rb4Bnr (22.6%). In het laatste karyotype duidt "3:1" het segregatie gedrag aan van de vier chromosomen welke deel uitmaakten van het door de T70H translokatie veroorzaakte deel van de multivalent. In T70H,+/+,Rb4Bnr segregeerde het kleine "marker" chromosoom 1^{13} bij voorkeur samen met het metacentrische Rb4Bnr chromosoom en dit kon worden bevestigd door de hoge frequentie van Ts(1^{13})70H tertiaire trisomen welke werd gevonden onder het nageslacht van mannelijke muizen van dit karyotype. De resultaten suggereren dat factoren welke de 3:1 segregatie bevorderen mogelijk samengaan met de verhoogde levensverwachting van het hieruit voortkomende trisome produkt onder het nageslacht (4).

- In T70H,+/+,Rb4Bnr mannelijke muizen konden de "alternate" en "adjacent 1" segregatie produkten in het meiose II stadium worden

onderscheiden en beiden werden bijna even frequent gevonden. Onder het levend geboren nageslacht echter, werden karyotypen die het produkt van adjacent 1 segregatie waren signifikant vaker waargenomen. De meest waarschijnlijke verklaring voor dit verschil moet worden gezocht in het beperkte aantal dieren welke werd gekaryotypeerd onder het nageslacht (4).

- In vergelijking met +/+ en T70H/+, was in zowel T(1;11.13S),+/+, Rb4Bnr als T70H,+/+,Rb4Bnr de non-disjunctie frequentie tijdens de anafase I voor de normale bivalenten sterk verhoogd. In T(1;11.13S),+/+,Rb4Bnr werd bovendien een korrelatie gevonden tussen non-disjunctie voor de normale bivalenten en adjacent 2 segregatie voor de multivalent chromosomen. Dit suggereert een interaktie tussen beide verschijnselen, of hun coincidentie is mogelijk het gevolg van de algemene (slechtere) fysiologische toestand waarin de cellen in kwestie verkeerden. Een scherp onderscheid tussen beide alternatieven kan niet worden gemaakt. De indikatie voor "spermatocyt I degeneratie" (zie eerste punt in sektie III) in beide karyotypen onderstreept het laatste alternatief, welke een interaktie veronderstelt tussen de slechtere fysiologische toestand van de cel en adjacent 2/normale bivalent non-disjunctie (4).

Het in dit proefschrift beschreven onderzoek aan de meiose van mannelijke muizen, heterozygoot voor typen translokaties welke ook bij de mens voorkomen, is bedoeld geweest om na te gaan in hoeverre de muis bruikbaar is als testsysteem voor de situatie bij de mens. Voor wat betreft het "spontane" non-disjunctie nivo, dus wanneer er geen translokaties aanwezig zijn, was namelijk bekend dat de mens een duidelijk hoger nivo dan de muis vertoonde. Omdat in de heterozygoot voor een translokatie van het "meta/acro type" is gebleken dat niet alleen de chromosomen betrokken bij de translokatie multivalent, maar ook de normale bivalenten aanzienlijk hebben bijgedragen tot het non-disjunctie nivo, lijkt dit materiaal bruikbaarder als experimenteel substituut voor de mens dan oorspronkelijk was verwacht.

CURRICULUM VITAE

De auteur werd geboren op 15 januari 1947 te Enschede en behaalde in 1964 het HBS-B diploma aan de Rijks HBS te Tiel.

In datzelfde jaar werd begonnen met de studie biochemie aan de Gemeentelijke Universiteit te Amsterdam. Het doctoraal examen Scheikunde (uitgebreid) werd in september 1975 afgelegd, met als hoofdvak biochemie, als bijvak neurofysiologie en als speciale richting microbiologie.

Van 1 februari 1976 tot en met maart 1978 en van april 1979 tot en met januari 1980 werd gewerkt binnen het kader van een promotieonderzoek aan de afdeling Erfelijkheidsleer van de Landbouwhogeschool. Dit projekt werd van april 1978 tot april 1979 onderbroken voor een onderzoek, in opdracht van de Europese Commissie (Directorat-General for Research, Science and Education) aan de Landbouwhogeschool, welk onderzoek eveneens bij de afdeling Erfelijkheidsleer werd verricht.

Vanaf 1 februari 1981 is hij opnieuw, in het kader van een onderzoeksopdracht van de Europese Commissie, werkzaam bij de afdeling Erfelijkheidsleer van de Landbouwhogeschool, welke werkzaamheden nog voortduren.