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H U M A N M A L E M E I O S I S

Thesis

submitted to the

UNIVERSITY OF GLASGOW

for the Degree of

DOCTOR OF PHILOSOPHY

in the

FACULTY OF SCIENCE

by

BRENDA M. PAGE

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CONTENTS

		Page
1.	INTRODUCTION	1
1.1	HISTORICAL	1
1.2	TECHNIQUES	4
1.3	DURATION OF SPERMATOGENESIS	9
1.4	SPERMATOGONIA	9
1.5	MEIOSIS	10
1.6	POLYPLOIDY	19
1.7	POLYMORPHISMS	19
1.8	NONDISJUNCTION	20
1.9	INFERTILITY	22
1.10	TRANSLOCATION HETEROZYGOTES	25
1.11	RADIATION DAMAGE AND HUMAN SPERMATOGENESIS	27
1.12	THE PROBLEM IN PERSPECTIVE	29
2.	AIMS	30
3.	MATERIALS	34
3.1	HUMAN MALE	34
3.2	MATERIAL OTHER THAN HUMAN MALE	35
4.	METHODS	37
4.1	MITOTIC ANALYSIS	37
4.2	MEIOTIC ANALYSIS	44
5.	RESULTS	56
5.1	HUMAN MALE	56
5.2	MATERIAL OTHER THAN HUMAN MALE	121

		Page
6.	CONCLUSIONS AND DISCUSSION	140
6.1	MITOTIC METAPHASE	140
6.2	TESTIS TISSUE	150
6.3	TECHNIQUES	153
6.4	TESTIS HISTOLOGY	157
6.5	SPERMATOGONIAL CELLS	157
6.6	INITIATION OF MEIOSIS	161
6.7	PAIRING OF HOMOLOGUES	161
6.8	ORDER WITHIN THE NUCLEUS	163
6.9	PACHYTENE	165
6.10	DIFFUSE STAGE	179
6.11	DIPLOTENE	179
6.12	DIAKINESIS	180
6.13	METAPHASE II	182
6.14	SPERMIOGENESIS	184
6.15	PRE- AND POST-REDUCTION OF THE SEX CHROMOSOMES	185
6.16	POLYPLOIDY	185
6.17	UNSTAINED REGIONS	188
6.18	CHIASMA ANALYSIS AND CONJUNCTION OF THE SEX CHROMOSOMES AT DIAKINESIS	189
6.19	MATURATION ARREST	201
6.20	MEIOSIS AND TRANSLOCATION HETEROZYGOTES	204
6.21	RADIATION-DAMAGED CASE	207
6.22	TIMING OF THE STAGES OF MEIOSIS	208
6.23	ENVIRONMENTAL AND EXPERIMENTAL FACTORS AFFECTING MEIOSIS	209
6.24	NONDISJUNCTION	212
6.25	SELECTION IN GERM CELLS	215
6.26	ENVOIE	219

Page

7. SUMMARY 220 .

8. REFERENCES 223

9. ACKNOWLEDGEMENTS 249

10. APPENDIX 250

Table	Page
1	57
2	58
3	58
4	60
5	62
6	63
7	64
8	69
9	77
10	78
11	78
12	80
13	81
14	87
15	88
16	93
17	94-95
18	98
19	99
20	105
21	105
22	106
23	106
24	108
25	109
26	111
27	112
28	113
29	113
30	115
31	116
32	119
33	119
34	119
35	125
36	125
37	130
38	135
39	139

Figure	Page
1	41
2	59
3	71
4	86
5	90
6	91
7	92
8	100
9	101
10	102
11	103
12	110
13	117
14	120
15	123
16	126
17	131
18	132
19	136

Plates 1 - 96, see Volume II

1. INTRODUCTION

1.1 HISTORICAL

The germ cells of animals have been popular objects of study with cytologists from the very beginning of "modern" Biology (for example: Jensen 1887, von Ebner 1888, and von Lenhossék 1898).

Hertwig (1876) realised the significance of fertilisation. Van Beneden (1883), demonstrated in Ascaris equorum Goeze, that the egg and sperm nucleus each contained two chromosomes, whereas at mitosis in the fertilised egg, four chromosomes were visible. All cells of the offspring were subsequently derived from the zygote by normal mitotic division and had each chromosome set represented twice. So, the gametes each contributed the haploid number of chromosomes to the zygote, which contained the diploid number in its nucleus. Weismann (1887) invoked the necessity of reduction division in his germ plasm theory. He predicted that a special nuclear division must be repeated in every generation in germ cells of plants and animals, at which the chromosome number is reduced again to half the number contained in the parent nucleus. Both Van Beneden and Weismann rather assumed that the division of chromosomes was not preceded by replication.

It was already noted that abnormal divisions were found to occur in quick succession and Flemming (1887) called them "heterotype" and "homotype" since the first division was the more abnormal of the two. Strasburger (1888) observed in the pollen mother cells and embryo sac mother cells of various plants, that, at late prophase of the first of these divisions when the chromosomes first become clearly visible, the haploid number was present. Even if these observations were based on the wrong stage, the idea was sound and the scene set for a true understanding of the process resulting in a reduction division suggested in, for example,

papers by Korschelt (1895) on Ophryotrocha puerilis Clap. and Mecz. and Schaffner (1897) on Typha latifolia L.

So, by the time Mendel's genetic discoveries were given true recognition (De Vries 1900, Correns 1900, and von Tschermak 1900) meiosis had essentially been discovered, the relation of meiosis to gamete formation in animals was appreciated, and haploidy versus diploidy during the life cycle was made clear.

Montgomery (1901) in studies from 42 species of hemipterous insects concluded that the chromosomes which associate in pairs at prophase I were of paternal and maternal origins. Sutton (1902) stated that identical chromosomes associated, since he was able to positively identify each pair in the grasshopper Brachystola magna Scudder. Sutton drew attention to the similarity between chromosomal behaviour in mitosis and meiosis.

Farmer and Moore (1903) with full understanding of the meaning of the two successive nuclear divisions, described the process in a preliminary communication, in a variety of animals and plants. Grégoire (1904) suggested that the two divisions should be distinguished by Roman numerals I and II, and Farmer and Moore in 1905 coined the term "maiosis" meaning "to lessen". Later, it was considered more correct to call it "meiosis".

TERMS USED FOR THE STAGES OF FIRST MEIOSIS.

The ovary of the rabbit provided the material by which the early parts of the meiotic process were named (von Winiwarter 1900). It was ideal material since meiosis extended over a long time, up to a month from birth. The half day old rabbit demonstrated chromosomes in a 'slender ribbon' configuration termed "leptotene". A chromomeric or 'beads on a string' picture came later, with some parts paired and others unpaired, termed "synaptene". "Zygotene" was substituted for this later (Grégoire 1907). By 4 to 5 days, 'thick ribbon' or "pachytene" chromosomes were apparent and by 10 to 12 days a few shorter and thicker "diplotene" chromosomes

were present, most reaching this stage by 28 days. All stages, mature and immature, were present through the ovary.

Häcker (1897) had subdivided prophase of the first division of meiosis into two parts. For the second of these when thick loops or cross shapes were apparent, he coined "diakinesis", meaning "moving apart". (It pre-dates von Winiwarter's "tene" suffix.) Janssens (1909) gave the name "chiasma", meaning "a cross", to the nodes between the arms or loops of diplotene or diakinetic configurations. The suffix "neme" or "nema" is sometimes used instead of "tene" (Rhoades 1950), but choice appears to be a matter of opinion. In this study, the older terms were used where it was necessary to name stages of an essentially continuous process, because, since the discovery of the synaptinemal complex (Moses 1956, Fawcett 1956) "tene" meaning "ribbon" was considered a superior description to "neme", meaning "thread". The important point was to establish whether all authors used the same name in describing a particular stage and whether the correct position in the meiotic sequence was appreciated.

HUMAN CHROMOSOME NUMBER AS ASCERTAINED FROM TESTIS MATERIAL.

Von Winiwarter (1912) was the first to attempt human testis preparations. Using sections, he proposed a 47 chromosome complement with spermatogonial metaphase, together with XO/XY sex determination, which was contrary to Wilson's (1909) generalised view of XX/XY sex determination. Von Winiwarter appreciated the nature of the chromatin body in spermatocytes, but thought it contained a single sex chromosome.

Also using testis sections, but first metaphase rather than spermatogonial metaphase, Painter (1923, 1924) recorded the human number as 48, with an XX/XY sex determining mechanism, males having dissimilar chromosomes. Incidentally, in his preliminary communication (Painter 1921), he reported his best preparations as showing 46 chromosomes. He considered the discovery of the Y the most important observation, and achieved the

figure 48, by adding one to von Winiwarter's count of 47. Painter certainly agreed with von Winiwarter that the number was between 45 and 48.

Von Winiwarter and Oguma (1930) and others, retained their belief in XO sex determination and counted 47 chromosomes in spermatogonial metaphase. Koller (1937) solved the sex determination problem by firmly establishing the presence of a Y chromosome.

For over 30 years, the reports were consistently of 48, with very occasional counts of 46 being noted (Evans and Swezy 1929, Shivago and Andres 1932). Ford and Hamerton (1956) using hypotonic pretreatment and squash preparations finally confirmed, in meiosis, the correct number, 46, as had been found in fibroblast cultures from foetal lung by Tjio and Levan (1956). Ford and Hamerton showed 23 bivalents at first metaphase in normal meiosis.

Gradually 46 became the rule with only the occasional report of other values in mitotic and meiotic analysis. For example, Kodani (1957, 1958) using material from 21 patients with epididymitis or cancer of the prostate, found four cases with 46, one with 47, and sixteen with 48 chromosomes.

The present study began with the firm knowledge that the normal human chromosome complement was 46.

1.2 TECHNIQUES

LIGHT MICROSCOPY.

Initial work was performed using histological sections. The "break-through" studies of the 1950's can be attributed to the use of the "squash" technique of Heitz (1936) as in Sachs (1952), and the discovery of hypotonic pretreatment of cells (Makino and Nishimura 1952, Hughes 1952, Hsu 1952 and Hsu and Pomerat 1953). The "squash" technique proved popular for the next decade, often with some modification. For example, Gardner and

Punnett (1964) employed softening and concentration of cells and mounting in Hoyer's medium and Bŏŏk and Kjessler (1964) fixed after hypotonic treatment in 5 parts glacial acetic acid: 1 part N HCl: 4 parts distilled water and squashed in one drop of 2% aceto-orcein.

It was the application of the "air-drying" procedure used by Rothfels and Siminovitch (1958) for mammalian cells in culture, that proved another breakthrough for meiotic preparations. Evans, Breckon and Ford (1964) and Ferguson-Smith (1964a) applied air-drying and hypotonic pretreatment to meiotic material, and Ferguson-Smith (1964a) used air-drying without hypotonic pre-treatment, for preservation of nucleoli.

Hypotonic pretreatment and air-drying were therefore available for the present study. For the associated mitotic work there was, in addition, the discovery of phytohaemagglutinin as a mitotic stimulator (Nowell 1960) and used in a technique by Moorhead, Nowell, Mellman, Battips and Hungerford (1960) for peripheral blood preparations, with colcemid (N-Desacetyl-N-methyl colchicine), the less toxic derivative of colchicine, for harvesting.

Annerén, Berggren, Stahl and Kjessler (1970) attempted to improve spread as well as morphology of chromomere pattern at pachytene using various pretreatments, osmotic pressures, time and concentration of enzyme treatments, fixatives, fixation times, stains and staining times, but their results did not compare with those of Hungerford (1971) who, following experiments with various hypotonic pretreatments, described an air-dried technique involving incubation in 0.125M KCl for an hour at 37°C. This gave excellent yield, preservation and spreading of chromosomes in the pachytene stage of meiosis, thus setting the scene for an exploitation of this stage. The technique has been used in the identification of the supernumerary chromosome in Down's syndrome as the smaller G (Hungerford, Mellman, Balabam, LaBadie, Messatzzia and Haller 1970).

ELECTRON MICROSCOPY.

The most important discovery from the application of electron microscopy to meiotic material was that of the synaptinemal complex (frequently termed

synaptonemal complex) observed independently by Moses (1956) in crayfish spermatocytes, and Fawcett (1956) from work on pigeons, cats and humans. Over the years there has been some difficulty in interpreting its structure and function (Wolstenhome and Meyer 1964, Moses 1968, Moens 1968b and Jaworska and Lima de Faria 1969). Initially it was closely associated with crossing-over since it was not found during meiosis in male Drosophila (Moses and Coleman 1964) which classically does not have chiasmata (Bodmer and Parsons 1962) although the female has both.

Synaptinemal complexes are not found in prokaryotes (Moses 1968). They are occasionally found where not expected, for example, in the achiasmate spermatogenesis of the Orthopteran Mantid, Bolbe nigra Giglos-Tos (Gassner 1969) and in asynaptic wheat (La Cour and Wells 1970). Roth (1966) described polycomplexes of synaptinemal complexes in oocytes and nurse cells in the mosquito. Sotelo and Trujillo-Cenóz (1960) found multiple core complexes resembling stacked synaptinemal complexes in haploid spermatids of Gryllus. They are therefore associated with haploids, diploids and triploids (Moens 1969b).

Moses (1969) then put forward the more cautious statement that the synaptinemal complex is necessary but not sufficient for chiasma formation, possibly just providing a framework in which crossing over can occur. It may be regarded as evidence of a mechanism for pulling chromosomes together, not even being necessary for initiation of pairing (Comings and Okada 1970c, 1971b).

Moens (1969a) in locust, found the synaptinemal complex to include the entire length of the paired homologues and to be attached to the nuclear membrane, and Woollam, Millen and Ford (1967) presented evidence using mouse, that each pair of homologues had its own.

The synaptinemal complex is completed during zygotene in Lilium (Moens 1968a) and Fritillaria (La Cour and Wells 1970). It extends the length of the chromosome. However, a number of people have reported

that effective synapsis occurs at a limited number of sites, suggesting that pairing is discontinuous at the time of crossing over (for example Holliday 1964 in fungi, and Roberts 1965 in Drosophila). Even though there is disagreement within the field, there remains the strong belief that the synaptinemal complex is associated in some way with crossing over.

The centre to centre distance separating lateral elements of the tripartite ribbon is generally given as between 0.10 and 0.15μ (Moses 1968). This is, unfortunately, just below the limit of the light microscope resolution (0.2μ).

The application of the Kleinschmidt water spreading technique originally used for the fine structure of the bacterial and viral genophore, has been applied to human meiotic material for low power electron microscopy (Comings and Okada 1971a). Apart from synaptinemal complex detail, fragments of nuclear membrane were seen embedded in the outer fringes of the chromatin fibres suggesting they had been attached to the membrane at that site. This technique is at an early stage, but it is possible that it might enable detailed observations on the mechanism of chromosome pairing in translocations, inversions and deletions.

STAINING.

In 1941 La Cour first described Acetic Orcein as a stain for chromosomes. It was the main one used in this study.

Feulgen (Feulgen and Rossenbeck 1924) is generally accepted as being specific for DNA (Darlington and La Cour 1969). It was especially popular used with the "squash technique" since the hydrolysis step softened the material (see, for example, Ferguson-Smith 1964a and Eberle 1963).

Carbol Fuchsin is particularly well known in the Ziehl-Neilson technique for bacterial staining. It has been used for sex chromatin (Eskelund 1956, and Klinger and Ludwig 1957). Carr and Walker (1961) used it for human mitotic metaphase chromosomes with a squash technique as did Robinson and Puck (1967), who modified the method of Klinger and Ludwig (1957).

Toluidine Blue, a basic dye, was used by Tarkowski (1966) with hydrolysis, for chromosome preparations from eggs.

Giemsa was used by Chen and Falek (1969) with male meiotic air-dried preparations. They claimed centromeres were made particularly obvious at late diplotene. Sasaki and Makino (1965) noted only the G chromosome centromeres when using this stain. Giemsa has recently become very popular in association with the banding techniques resulting from denaturation and re-annealing (Pardue and Gall 1969, Yunis, Roldan, Yasmineh and Lee 1971, and Sumner, Evans and Buckland 1971). With both Toluidine Blue and Giemsa, stains can be removed and the same preparations used afterwards for fluorescence microscopy (Polani and Mutton 1971). This cannot be achieved with permanent preparations stained with orcein.

All these stains were tested on air-dried preparations of meiotic cells. Quinacrine fluorescence (Zech 1969) has been exploited extensively in mitotic analysis, for example, Caspersson, Lomakka and Zech (1971) but success with meiotic preparations has been comparatively limited. Pearson and Bobrow (1970b) found a fluorescing Y in fewer than 5% of spermatogonial metaphases (80 - 90% of lymphocyte metaphases show a fluorescing Y). From meiotic prophase onwards Y bodies could be seen in the vast majority of cells. They reported 1.4% double Y-bodies in mature sperm. As for the autosomes, polymorphism of a fluorescent spot was sometimes demonstrated. Caspersson, Hultén, Lindsten and Zech (1971) applied quinacrine mustard fluorescence to the identification of meiotic bivalents with some success. A fluorescence study was not attempted here.

1.3 DURATION OF SPERMATOGENESIS

The fact that DNA synthesis takes place in the interphase preceding mitosis and meiosis allows one to estimate the duration of meiosis and the whole of spermatogenesis.

Heller and Clermont (1963) injected tritiated thymidine into the scrotal sacs of some inmates from a State Penitentiary, and after an hour found that spermatocytes and some spermatogonia were labelled. Label appeared in pachytene after 12 to 14 days and was present in mid pachytene cells by 16 days. An estimate of the duration of the whole of spermatogenesis was 64 days since free spermatozoa showed label after 32 to 64 days. Each cycle of the seminiferous epithelium, i.e. from spermatogonia stem cell to pre-meiotic interphase, was estimated as lasting 16 days. They confirmed this in 1964 and re-estimated the whole of spermatogenesis to extend over 4.6 cycles giving 74 days $\pm$ 4 to 5 days.

Lima de Faria, German, Chatnekar, McGovern and Anderson (1966 in a preliminary communication, and in 1968) kept human meiotic and other testicular cells in culture extending for 16 days. DNA synthesis was shown to occur at the interphase preceding meiosis and also at the early stages in leptotene and zygotene. (The latter may be attributed to crossing over.) They suggested the stage pachytene was reached after a period of 14 days.

1.4 SPERMATOGONIA

Heller and Clermont (1964) classified spermatogonia from sections of human testis on the basis of staining properties, including glycogen content and also shape of nucleus. They described "Dark" and "Pale" type "A" spermatogonia (the stem cells) and type "B" spermatogonia. Although spermatogonial metaphase was used in the early attempts to assess the human chromosome number (von Winiwarter 1912, von Winiwarter and Oguma

1930, and Koller 1937) it has largely been ignored more recently. Reasons for this include the fact that stretching of secondary constrictions makes an analysis difficult (Hultén and Lindsten 1970).

Pre-meiotic Pairing of Homologous Chromosomes

Wilson (1912) using mainly Hemiptera, first suggested that synapsis may be initiated as a rule in the anaphase or telophase of the last pre-meiotic division. Smith (1942) agreed, and suggested it terminated at late pachytene. Maguire (1967a) found pre-meiotic pairing of homologues in maize and McDermott (1970) observed it in spermatogonia of the human male.

1.5 MEIOSIS

FIRST MEIOTIC DIVISION.

Prophase I

This is a long stage and is usually divided into leptotene, zygotene, pachytene, diplotene and diakinesis. Onset of meiotic prophase is heralded by a marked increase in nuclear volume (Rhoades 1961).

Leptotene. Leptotene is usually described as the appearance of the diploid number of apparently single chromosomes, although there is auto-radiographic and microdensitometric evidence which suggests DNA synthesis has already occurred (see discussion by Riley 1966, and by Hamerton 1971). Wilson (1912) was the first to suggest order, in leptotene confusion, which was indicative of the synapsis which was to follow.

Van Beneden and Julin (1884) described synapsis in *Ascaris*, Eisen (1900) described it in *Batrachoseps*, van Hoof (1912) in various small mammals and Wenrich (1916) in *Phrynotettix*. Ends of the chromosomes tend to lie towards a region at the surface of the nucleus and the chromosomes are then forced to loop round into a configuration described

as a "bouquet". Gelei (1921) in Dendrocoelum lacteum Müller, confirming the bouquet, pointed out that proximity to the nuclear membrane tends to lead to precocious development. The "bouquet" stage seemed accepted for animals, but not established for humans. Severinghaus (1942) mentioned a bouquet stage occurring post-synaptically in a human.

Zygotene. This is considered the stage where homologous synapsis occurs (Rhoades 1961).

Pachytene. Completely paired, partially contracted chromosomes, often exhibiting chromomeres, are described as being in pachytene. Nucleoli are frequently present. Von Winiwarter (1912) observed a chromatin body in human spermatocytes. This has been confirmed and attributed to the sex chromosomes, on numerous occasions (for example: Sachs 1954). It was not established whether the human sex vesicle supported a nucleolus in its own right (Sasaki and Makino 1965).

Schultz and St. Lawrence (1949) attempted chromosome maps of pachytene bivalents. An aceto-carmine squash technique was used. They claimed cytological mapping by chromomere pattern of an autosomal bivalent. This bivalent supported a nucleolus, at its centre, between prominent dense chromomeres. Kodani (1954) claimed to map a second autosomal nucleolar chromosome and Yerganian (1957) isolated and described 9 additional autosomal bivalents using chromomere number, size and sequence. He noticed elements with terminal nucleoli calling them "broken" nucleolus forming ones (the cells were disrupted with a Waring blender). Disruption meant exact chromosome number could not be seen, but differential centrifugation suggested 28 arbitrary groups! Gardner and Punnett (1964) believed they could identify 22 autosomal bivalents, but the XY bivalent was recorded as straightened, not being wholly in the sex vesicle.

Ferguson-Smith and Handmaker (1961) suggested that the bivalent with the median nucleolus might represent two bivalents corresponding to

the satellited mitotic chromosomes, while Shaw (1961) suggested it might be chromosome No. 1 which has a paracentric secondary constriction. Yerganian (1963) reassessed his former findings and agreed with Ferguson-Smith and Handmaker (1961). Eberle (1963, 1966) attempted pachytene mapping and noted that four satellited chromosomes (claimed to be 13, 17 or 18, 21 and22) associated among themselves, with the sex vesicle and the nucleolus. He did not think there was evidence of homologous regions on the X and Y chromosomes from an examination of the sex vesicle, and assumed condensation before crossing-over had occurred.

Using material from 5 young adults with normal histology or with hypospermatogenesis, Ferguson-Smith (1964a) found the principal nucleoli were associated with the "terminal chromomeres" of 3 long and 2 short bivalents equivalent to the satellited mitotic chromosomes. He noted that, of the 2 small G bivalents, the larger had at least 6 distinct chromomeres and the smaller only 4. Ford, Cacheiro, Norby and Heller (1968) using 5 cells claimed to identify the normal human karyotype in pachytene chromosomes and in 1969 they concluded that the size relationships relative to mitotic chromosomes were retained.

Hungerford, La Badie and Balaban (1971) published provisional maps of the 2 smallest autosomes at pachytene, the longer containing 10 prominent chromomeres (3 major) the shorter 6 (2 major). Both possessed a large terminal chromomere in which they suggested the centromere was located. Bordjadze and Prokofieva-Belgovskaya (1971) used 11 subjects, 28-39 years, 3 from accidents and 8 oligospermics. Studying the 5 acrocentrics, they described chromomeric patterns which generally supported those described by Ferguson-Smith (1964a). They did not consider that the centromere was located in the terminal chromomere.

Diplotene. There is repulsion between the homologues of a bivalent except at those places where crossing over has occurred. There is no

relevant data on this stage in the human.

Diakinesis. Further condensation from diplotene results in the diakinesis configuration. Chiasma analysis is usually performed on this stage.

Bridges (1916) with cytogenetic evidence from Drosophila found that two of the four strands in a diakinesis bivalent were involved in crossing over.

Janssens (1909, 1924) assumed that each chiasma represented one genetic crossover. Sax (1932), Darlington (1935b), and Matsuura (1950), associated chiasmata with the morphological expression of crossing-over. Genetic evidence demonstrates that an absence of recombination in male Drosophila is associated with the absence of chiasmata (Bauer 1946). Whether every chiasma represents one crossover was questioned by Cooper (1949) on the basis of observations on male Drosophila of mitotic and meiotic 'chiasmata' not accompanied by crossing-over, and Steinitz-Sears and Sears (1953) found a poor correlation between chiasmata and exchange frequency in wheat. It is generally accepted, however, that chiasmata and cross-overs do correspond (Brown and Zohary 1955). Peacock (1968) considered the most direct evidence of a 1:1 relationship between a chiasma and a cross-over, was that provided by a comparison, using heteromorphic bivalents in the Orthopteran Acridiid Goniaea, between metaphase chiasma frequency and the mode of anaphase segregation of chromosome arms.

Mather (1938) has reviewed chiasma frequency. Chiasma analysis is important, since from it the map length can be calculated by the frequency with which a chromosome segment has at least one chiasma (Sybenga 1970). If 1 chiasma = 50 map units (centimorgans) and C = average number of chiasmata per cell, then the map length = C x 50. In many organisms chiasmata slip towards the ends of the bivalents in preparation for first anaphase, a process called "terminalisation" by Darlington (1937). A "coefficient of terminalisation" is given as the ratio of terminal to total

chiasmata at the stage in which the cell has been fixed (Slizynski 1955).

Ford and Hamerton (1956) used 23 cells from 3 middle-aged to elderly men and found the average chiasma frequency to be 55.9 chiasmata per cell with a range of 50 to 63. Sasaki and Makino (1965) observed bivalents with 1 to 6 chiasmata in material from 16 Japanese men in hospital for various reasons. Böök and Kjessler (1964) gave means of 52.7, 50.9, 52.9, 51.0, 53.3 and 47.0 for 6 cases. McIlree, Tulloch, Newsman and Barclay (1966) with 14 subjects found the mean cell chiasma count to range between 49.8 and 58.3. Kjessler (1966) using a population of 135 infertile males established the mean chiasma frequency to be between 47.0 and 54.9, average 52.7 per cell, no autosomal univalents being found. Eberle (1966) found the range 35 to 50 with a mean of 44. Luciani (1968) found the mean chiasma count ranging from 41 to 58 with the mean around 48.

Falek and Chiarelli (1968) using a 42 year old male with a hydrocoele and studying in detail 8 cells, suggested classification of bivalents according to dimensions, general characteristics and chiasma frequency. They found the chiasma number per bivalent ranged from 3.6 to 1.0, the first 3 chromosomes having a mean greater than 3.0, chromosomes 4 to 16, 2.1 to 2.9 and chromosomes 17 to 20, 2 or less.

Hultén, Eliasson and Tillinger (1970) found a low chiasma count and other meiotic "irregularities" in 2 infertile 46,XY men with spermatogenic arrest. Chiasmata were terminalised and varied from 18 to 29 per cell with a mode of 24 and mean 23.6 (SD 1.3).

Pearson, Ellis and Evans (1970) in a man with virtual azoospermia and with an average number of chiasmata of only 32 to 43 per cell, showed that occasionally homologues, apparently without chiasmata, lay adjacent. Pearson (1970) using "controls" undergoing scrotal surgery other than for infertility tried to relate population age structure with chiasma frequency and location, and attempted to identify the bivalents at diakinesis. He concluded there were certain similarities between infertile males and

aged normal individuals.

Caspersson et al. (1971) attempted identification of bivalents at diakinesis using fluorescence microscopy. They analysed 500 primary spermatocytes, 50 of them being karyotyped, and claimed to identify chromosomes 1, 2, 3, 9, 19, 20, 21, 22, X and Y, placing the rest in groups.

Sex Chromosomes at Diakinesis.

Haldane (1936) discussed the possibility of partial sex linkage in man. This would require homologous segments on the X and Y chromosomes, with chiasma formation between these chromosomes at meiosis. Huskins (1937) first introduced the term "homeologous" to denote partial homology, but there is little genetic evidence for the existence of partial sex linkage in man (Stern 1957, and Fraser Roberts 1963).

Koller (1937) noting end to end pairing of the X and Y chromosomes believed that chiasmata between the pairing segments did occur. Sachs (1954) finding no indication of the existence of pairing segments, considered the terminal connection observed at diakinesis and metaphase I as a remnant of the structure of the sex vesicle rather than a real chiasma. Ford and Hamerton (1956) observed one cell with a possible subterminal chiasma and considered that the terminal association could be consistent with partial linkage. Bøøk and Kjessler (1964) found no evidence for a chiasma between the X and Y in 10 normal men aged 20 to 82 with hydrocoele or inguinal hernia, or in 6 infertiles. Sasaki and Makino (1965), Kjessler (1966) and Eberle (1966) also did not support the existence of a true chiasma.

Sasaki and Makino (1965) suggested the short arms of the X and Y were involved in the association, but Hultén, Lindsten, Pen-Ming and Fraccaro (1966) believed the short arm of the X paired with the long of the Y, agreeing with Ford (1963). Chiarelli and Falek (1967) thought

it was the long arm of the Y, but Chen and Falek (1969) observing the respective centromeres, demonstrated that it was indeed the short arms. Pearson and Bobrow (1970a) from a fluorescence study, confirmed the short arm of the Y associating with the X. Reitalu (1970) using an unusually complex method, believed the medium part of the long arm of the Y to be associated with the distal part of the short arm of the X suggesting homology between these two regions.

There are many different reported values for the percentage of separate X/Y's, for example: Koller (1937) 3 to 4%, Sachs (1954) 50% - the highest figure, Ford and Hamerton (1956) 14%, Kodani (1958) 40%, Sasaki and Makino (1965) 27%, Mikkelsen (1966) 7.78%, Eberle (1966) 11.6%, Kjessler (1966) 4%, Luciani (1968) 6% and Hultén and Lindsten (1970) 16, 32, 18 and 24% for controls aged 23, 46, 52 and 72 years respectively, 4% for a t(D,G) mongol and 0% for a t(D,G) heterozygote.

Interlocked Bivalents.

Mather (1933) suggested that an interlocking of bivalents at diakinesis demonstrated the occurrence of genetical crossing over during chiasma formation. McDermott (1966) found interlocked bivalents repeatedly in a normal male.

Metaphase I

Chromosomes co-orientate on the metaphase plate.

Anaphase I

Terminalisation is completed and homologous centromeres move to opposite poles.

Telophase I and Interphase

Whether there is a telophase and interphase, at this stage, in human is not certain (Hamerton 1971).

SECOND MEIOTIC DIVISION.

Prophase II

Contraction of the chromosomes occurs in all organisms in which an interphase intervenes.

Metaphase II

Chromatid repulsion occurs. Bøsk and Kjessler (1964) did not demonstrate positive heteropycnosis of the X and Y at metaphase II. Hultén and Lindsten (1970) found difficulty in studying metaphase II spreads due to the attentuation of constrictions and Hultén and Pearson (1971) found difficulty, using fluorescence microscopy, because they were too "fuzzy". Sasaki and Makino (1965) did not find any abnormal metaphase II spreads in those few they analysed.

Anaphase II

Centromeres separate and the chromosomes move to opposite poles.

SPERMIOGENESIS

Spermiogenesis consists of the maturation of the products of meiosis to give spermatozoa. Sperm chromatin is 43% DNA and 57% a protein rich in arginine (Leuchtenberger, Schrader, Weir and Gentile 1953). As, for example, rat hepatocyte nuclei have values of 20% DNA and 80% protein (Chauveau 1952), there must be a considerable loss of material other than DNA in maturation. The differentiation process is little understood. The structure of the mammalian spermatozoon has been reviewed by Fawcett (1958) and Beatty (1970).

Shettles (1960) observed a difference between the human X-and Y-bearing sperm. They were rounded and elongated respectively. Rothschild (1960, 1962) reviewed the situation with respect to the size and surface properties of the two classes. Mostly, it is reported that no structural differentiation of the Y can be demonstrated within the sperm head (Ånberg 1957, Bedford 1967, and Pedersen 1969). However, fluorescence microscopy now enables the Y-bearing sperm to be differentiated since the distal part of the long arm of the Y shows as a "Y body" in the sperm head (Pearson and Bobrow 1970b).

Sex Chromosome Disjunction

At first meiotic anaphase homologous centromeres of the autosomes disjoin to opposite poles. The sex chromosomes are largely non-homologous. Chromosomes have doubled and so there are two alternatives available to the sex chromosomes.

a) Pre-reduction.

The double X and Y separate to opposite poles at 1st anaphase presenting a metaphase II containing either an X or a Y. The two chromatids then move to opposite poles at 2nd anaphase.

b) Post-reduction.

At 1st anaphase one chromatid of the X and one of the Y move together to opposite poles, presenting an X and Y consisting of single chromatids at metaphase II. At 2nd anaphase the X and Y move to opposite poles.

With a) or b) the final result is the same - four spermatids, two containing an X and two a Y.

Koller (1937) suggested 90% pre-reduction and 10% post-reduction of the XY bivalent in man. Sachs (1954) noted 100% pre-reduction and the latter has been favoured since.

1.6 POLYPLOIDY

Polyploidy can be defined as multiples of the basic haploid (n) chromosome number higher than the diploid (2n): triploid (3n), tetraploid (4n) etc. (Levan and Müntzing 1963).

Miller, Mittwoch and Penrose (1960) considered polyploid cells in mongol spermatogenesis in relation to the condition, and Darlington and Haque (1962) with 3 biopsies and Feulgen squashes described polyploidy with chromosome breakage, one slide from one biopsy having 17 out of 29 polyploid first metaphases.

Sasaki (1964) presented notes on polyploidy in the human male, and Sasaki and Makino (1965) found mean percentage polyploidy in spermatogonia and primary and secondary spermatocytes to be 6.8, 2.6 and 6.4 respectively in a younger group of males, and 7.1, 2.5, and 6.9 in an older group.

McIlree, Tulloch et al. (1966) noted polyploid cells in meiotic chromosomes and Pearson (1970) tried to relate the frequency of polyploidy to the population age structure.

It is difficult in most preparations to distinguish polyploidy from associations between cells at the same stage of the cell cycle. Additional evidence is required, for example, as to whether multivalents really exist at diakinesis. This would prove polyploidy at an earlier stage (Ford and Evans 1971). Dutrillaux and Guéguen (1971) did report multivalents at diakinesis in a sterile male with increased "polyploidy".

1.7 POLYMORPHISMS

Chromosome polymorphism in human populations appears as a natural variation, segregating normally. Routine mitotic investigation has shown a wide variation in chromosome morphology (Lubs and Ruddle 1970).

Some chromosomes have secondary constrictions adjacent to the centromere, for example: 1, 9 and 16 (Moorhead and Defendi 1958). Elongation of this region in chromosome 16 has been described by Jennings

and Turner (1961), Hungerford (1964), Jacobs, Brunton and Court Brown (1964) and Therkelsen, Lamm and Henningsen (1967). Crawford, Punnet and Carpenter (1967) explained heteromorphism of mitotic 16's by a deletion in one of them, while Crippa, Schwartz and German (1969) reported no increase of DNA content in the variant 16 chromosome. Heteromorphism in chromosome 9 was reported by Ferguson-Smith, Ferguson-Smith, Ellis and Dickson (1962).

A most variable human chromosome is the Y. Court Brown, Jacobs and Brunton (1965) estimated that 3% of the normal population carry unusual Y's. It can vary from a small metacentric chromosome to one the size of a D without producing any known effect. For a review of this see Gripenberg (1964).

There are no reports on polymorphism in human chromosomes at meiosis.

1.8 NONDISJUNCTION

Bridges (1913) introduced the term nondisjunction for a process occurring during the maturation of oocytes in Drosophila melanogaster Meigen where both X chromosomes were retained in the definitive egg nucleus (inferred from genetic evidence). It may be defined as the failure of two chromosomes or sister chromatids at mitosis or meiosis to pass to opposite poles of the spindle. Nondisjunction at various stages is discussed by Hamerton (1971).

Evidence from a variety of sources suggests that mistakes do occur at spermatogenesis. Most evidence in human comes from the sex chromosome aberration material, but the evidence is indirect. For example, from Xg blood grouping (Mann, Cahan, Gelb, Fisher, Hamper, Tippett, Sanger and Race 1962) it is shown generally that both Xs in 47,XXY Klinefelter's syndrome come from the mother, presumably in oogenesis, although error at the first cleavage division cannot be ruled out (Ferguson-Smith, Mack, Ellis, Dickson, Sanger and Race 1964, and Race and Sanger 1969).

There is an increase of mean maternal age at birth for Klinefelter's (Ferguson-Smith et al. 1964). From colour vision studies it may be shown that either the maternal or paternal X chromosome can be lost in Turner's syndrome and that maternal nondisjunction can account for Klinefelter's syndrome (Polani 1961,and Pickford 1964). De la Chapelle, Hortling, Sanger and Race (1964) claimed evidence in an XXYY for successive nondisjunction at the first and second meiotic division of spermatogenesis using chromosomes and Xg. It involved an XYY sperm fertilising a normal ovum.

Other evidence for nondisjunction has come from fluorescence microscopy. Pearson and Bobrow (1970b) reported a YY bivalent in a poor preparation at diakinesis, possible double Y's at metaphase II and 1.4% double fluorescent bodies in spermatozoa in a "normal" seminal specimen.

Estimates of chromosome abnormalities in spontaneous human abortions varies between 15 and 27% (Sumner 1971) and up to 50% has been reported (Boué and Boué 1969). The proportion of the chromosomally abnormal at birth is also high. Turner and Wald (1970) in a newborn survey of 1,000 found 13 aberrations involving the autosomes and 20 aberrations involving sex chromosomes, giving a total of 3.3%.

Hultén, Karlman, Lindsten and Tiepolo (1970) found 15 apparently aneuploid second metaphases out of 392 in the Chinese hamster. Fourteen were hypohaploid and the technique was suggested as responsible for this. One spread had 12 chromosomes instead of 11 and included a heteropycnotic X and a less heteropycnotic Y. This is the only firm evidence for nondisjunction of the sex chromosomes in a mammalian germ cell.

1.9 INFERTILITY

MALE INFERTILITY SURVEYS.

Of the 15% of married couples who are sterile, defects of the reproductive system of the male are responsible in 40-50% of the cases (Goldman and Padeh 1969). Ferguson-Smith, Lennox, Mack and Stewart (1957) found 1 to 3% of subfertile males to be sex chromatin positive, the frequency increasing to 11% in those with a sperm count of less than a million per ml. Kjessler (1966) gave a figure of 16% for the latter.

Nowakowski and Lenz (1961) reviewed the genetic aspects of male hypogonadism showing that apart from major chromosomal defects, some types of hypogonadism have a genetic origin, since behaviour typical of a recessive factor is shown.

Van Wijk, Tijdink and Stolte (1962) among 20 chromatin negative oligospermic and azoospermic males found 3 patients with unusual sized Y's: one had a larger, one a smaller, and one a mixture of large and small Y's. Tabuch, Kadotani, Ohama and Nakayama (1968) in a mitotic survey of 80 couples with primary sterility found chromosomally "abnormal" cases, one female with an assymetrical chromosome 1, another with an assymetrical 3, two males with long Y's and two 47,XXY males.

Kjessler (1966) performed a survey on 135 males attending an infertility clinic, using mitotic, meiotic and seminal analysis. He found 12 of the 135 had numerical or structural variations in mitotic chromosomes. McIlree, Price, Court Brown, Selby, Tulloch, Newsam and MacLean (1966) studied a series of 50 subfertile men with a sperm count of less than 20 million per ml on two occasions. Abnormalities included a ring 21/22 a 45,XO/46,X dicentric Y, a 45,XO/46,X pericentric Y and two patients with multivalents at diakinesis. Similarly, Luciani (1968) studied buccal smears, semen, tissue sections, mitosis and meiosis in 20 patients between 21 and 54 years and with wide infertility problems. Szarvas, Scultéty and Kovács

(1968) surveyed material from 107 biopsies, but had a 60% technical failure rate. Goldman and Padeh (1969) investigated 120 males and examined 38 testicular biopsies from azoospermics or severe oligospermics. They found an agreement between the number of dividing cells seen in cell dispersion preparations and those in tissue sections, but found no chromosomal reason for sterility, in the cells examined. Chandley (1970) surveyed 45 infertiles who were sex chromatin negative, had a sperm count of less than 20 million per ml and had no physical deformity. Cases with a sperm count higher than 20 million per ml were also taken if more than 50% were abnormal.

More specific details from the various surveys will be discussed later with results from the present Infertility Survey.

SPERMATOZOA AND INFERTILITY.

Williams (1950) found a mitochondrial defect affecting the entire sperm population in a man causing absolute sterility. There was prolongation of the midpiece, the tail occasionally doubling back on itself and protruding from the neck region. This affected less than 0.5% of his infertility cases.

Ross, Christie and Kerr (1971) using electron-microscopy found a tail abnormality in spermatozoa from a 38 year old subfertile man with a sperm count of 40 to 50 million per ml, with only 1 to 2% sperm motility. Mitotic and meiotic chromosomes were apparently normal. Ninety-seven % of sperm were structurally abnormal with an elongation of the mid-piece and an absence of the outer sheath on the main piece and extra axial fibres. The overall length, however, was the normal 50µ. A large elongated cytoplasmic droplet was present in 40 to 45% and 10% had amorphous heads.

Gledhill (1970) reported differences in amount of DNA in ejaculated sperm from fertile and infertile males.

MATURATION ARREST.

The term "maturation arrest" is a histological description referring to the gross morphology of the germinal epithelium. Meiosis breaks down at certain stages, and the resulting conditions can be described as "spermatogonial", "spermatocyte" or "spermatid" maturation arrests.

Meiotic chromosome abnormalities as a general feature of spermatogenic arrest were suggested by earlier histological reports (Nelson 1950, Charney, Conston and Meranze 1953, Tillinger 1957, Girgis, Etriby, Ibrahim and Kahil, 1969).

Sasaki (1965) described a male with Down's syndrome as having small testes and a severe maturation arrest with no sperm in the tubules. Miller et al. (1960) found spermatogenic arrest in four mongols; Hultén and Lindsten (1970) found it in one. Maturation arrest, sometimes severe, is occasionally reported in 47,XYY cases (Skakkebaek, Philip, Mikkelsen, Hammen, Nielsen and de Perbøll 1970, and Tettenborn, Gropp, Murken, Tinnefeld, Fuhrmann and Schwinger 1970).

McIlree, Price et al. (1966) reported degenerative changes in a case with a ring 21/22, another case with a reduced short arm 13/15 and a case with a dicentric Y. Chandley (1970) observed maturation arrest in an individual with a ring Y and also in two brothers (at least one of whom was oligospermic - 7 and 3 million) with an extra,small metacentric. Arrest was between the 1st and 2nd meiotic divisions. Kjessler (1966) reported arrest at the spermatid level in an oligospermic t(D,D) heterozygote. Chandley (1970) in a t(D,D) heterozygote, with a sperm count of 17 million per ml, found, at all stages, reduced activity and in t(C,E or F) and t(C,G) heterozygotes, arrest after diakinesis, both having no mature sperm.

Maturation arrest has also been described in males with low chiasma counts (Hultén, Eliasson and Tillinger 1970, and Pearson et al. 1970).

MUSCULAR DYSTROPHY.

Hypogonadism is associated with muscular dystrophy in the human (Clarke, Shapiro and Monroe 1956), the myotonic dystrophic lesions being similar to germinal aplasia after orchitis caused by mumps, or in adults of cryptorchidism (Nadler, Steiger, Troncelleti and Durant 1950). There are many genetically-determined muscular diseases in animals including mouse, chicken, hamster and lamb (Blaxter 1969).

Muldal and Ockey (1961) reported muscular dystrophy and a mild degree of hypospadias and associated it with a deleted Y. Eberle and Becker (1964) performed mitotic analysis on 10 patients with muscular dystrophy and found any additional chromosomes were random. Mutton and Gross (1965) also found aneuploidy involving different chromosomes and attributed it to technique. With 4 males and 4 females, they found one female with a Gp- another with a Dp-, both considered natural variations, one possible XO/XY mosaic female and two males with a higher frequency of breaks and gaps than expected.

1.10 TRANSLOCATION HETEROZYGOTES

"Translocation" was introduced into genetics by Bridges (1923) to connote the transference of a chromosome segment from its normal position to a position in a different chromosome. However, it is usually a reciprocal exchange that is involved (see discussion by Ford and Clegg 1969).

Translocations may be overlooked in an examination of the mitotic karyotype, if the amount of material exchanged is equal, or if small exchanges occur between large chromosomes. It is common for the involvement of one of the chromosomes not to be obvious.

Court Brown (1967) estimated that 5 cases per thousand carry a detectable balanced translocation, and for newborn population surveys, 3.68 (Smith and Jacobs 1970) and 1.7 (Ratcliffe, Stewart, Melville, Jacobs and

Keay 1970). Hamerton (1971) gave an overall estimate of 1.64 per thousand.

Translocation heterozygotes are usually detected by the production of abnormal offspring with characteristics typical of cases with defective chromosomes. The presence of a translocation may be confirmed by meiotic studies. Homologous regions pair at pachytene regardless of which chromosomes are involved. This produces a cross-shaped configuration and has been shown at pachytene in plants (McClintock 1930, Rhoades 1950) and mouse (Ford and Evans 1971), but not in man.

Crossing over within homologous material is likely to produce odd diakinesis configurations. With reciprocal translocations, 22 elements instead of 23 are usually shown at diakinesis, one of which is a quadrivalent.

Meiotic studies have only been performed on male translocation heterozygotes. The first case studied was not a simple translocation heterozygote; it was a child with Down's syndrome and a t(GqGq) with a minute centric fragment that was assumed to be a product of the reciprocal exchange (Miller et al. 1960). At diakinesis, a quadrivalent was formed in some cells, but mostly a trivalent plus minute univalent.

Hamerton, Cowie, Giannelli, Briggs and Polani (1961) reported 93% trivalents at diakinesis in a t(DqGq) translocation heterozygote, while one cell had an unequal bivalent and univalent, and one with 3 univalents. The X/Y value was 23.81%. Lindsten, Fraccaro, Klinger, and Zetterqvist (1965) showed a multivalent of varying structure, (chains more frequently than rings) at diakinesis from a t(Cq-Cp+) heterozygote ascertained through a severely affected daughter. Polani (1964) mentioned a ring-shaped multivalent in a t(Cp-Eq+), with a very few cells with a chain quadrivalent. Two oligospermic cases from a series of 50 infertile males demonstrated multivalents at diakinesis (McIlree, Price et al. 1966). Kjessler (1964) studied an infertile male with a t(DqDq) with all primary spermatocytes showing a trivalent and a reduced number of chromosome bodies.

Hultén, Lindsten, Pen-Ming, Fraccaro, Mannini, Tiepolo, Robson,

Heiken and Tillinger (1966) showed a quadrivalent at diakinesis in a t(2p-?C?+) heterozygote ascertained through recurrent abortions and an abnormal child,and a quadrivalent at diakinesis from a t(2,C) heterozygote was shown by Bender, Reinwein, Gorman and Wolf (1969). De la Chapelle, Kjessler, Johansson, Saari and Kauste (1967) performed mitotic and meiotic studies in a family with repeated miscarriages and two children with trisomy G. A t(E,C) translocation heterozygote was studied by Fraccaro, Hultén and Lindsten (1968) and another by Ferguson-Smith, Boyd and Ferguson-Smith (1968), further analysis of meiotic preparations from this patient being performed in the present study. Hultén and Lindsten (1970) reported two t(D,G) translocation heterozygotes, two oligospermic t(D,G) translocation mongols and two t(D,D) translocation heterozygotes, giving diakinesis configurations. Chandley (1970) reported several translocation carriers, a t(D,D), t(C,E or F) and t(C,G) mostly with maturation arrests at the various levels, the t(C,G) demonstrating, as well as quadrivalent figures, a trivalent and univalent at diakinesis.

The first direct cytological proof of a chromosome rearrangement as a cause of Down's syndrome was performed by Hecht, Delay, Seely and Stoddart (1970). Meiotic evidence ruled out a t(21,22) and so a t(21,21) or isochromosome was present.

1.11 RADIATION DAMAGE AND HUMAN SPERMATOGENESIS

There is little information on the effects of radiation on human meiosis. Mitotic chromosome damage, including dicentrics, fragments, ring chromosomes and breaks, persist for a long time after irradiation (Dolphin, Bolton, Humphreys, Speight and Stradling 1970, and Okladnikova 1970).

Hultén and Lindsten (1970) reported no meiotic disturbance in a man who had received an X-ray dose because of ankylosing spondylitis, but the dose was comparatively low.

Lyon, Phillips and Glenister (1970) found a linear dose response for the yield of translocations in mouse spermatogonia. After repeated small radiation doses there was a reduced effect, a linear response was still obtained, but the scale moved. Léonard and Deknudt (1970) showed persistence of chromosome rearrangements induced in male mice by 600r X-irradiation (300 kV) of pre-meiotic germ cells. There were 8.4% multivalents at 60 days, up to 12.6% at 100 - 200 days and then a decrease to 450 days. At 500 - 600 days a small increase occurred again.

Translocations often result in sterility or semi-sterility, which can be measured in offspring of irradiated animals (Snell 1935). Griffen and Bunker (1967) showed that two stages can be demonstrated after 350 - 700 rads (90 kV) X-irradiation. There is a post-irradiation fertile period (from the spermatocytes, spermatids and spermatozoa present at irradiation) giving 20% semi-steriles. An infertile period follows and after 6 weeks 5% semi-steriles are produced from products of repopulation of the gonad from type A spermatogonia. The radiation eliminates the type B spermatogonia and some of the type A. Lyon and Meredith (1966) found irradiated male mice gave rise, in the pre-sterile period, to 46 semi-sterile daughters out of 148.

Léonard and Deknudt (1968) X-irradiated 70 adult male mice with 300 rads (300 kV) then mated them to virgin females over 9 weeks. The testes and spermatocytes of the F1 male mice were examined. The average number of living embryos was lowest in the 2nd and 3rd week and the average litter size lowest in the 7th week. Chromosome rearrangements were shown in 41 of the males and 6 showed histological abnormalities, but no chromosomal rearrangements. Aberrations were 51.0% in the 1st week, 10.4% in the 2nd, 21.7% in the 3rd, 2.2% in the 4th, 6.3% in the 5th and none at 6-9 weeks. Controls were normal.

Schröder (1969) showed that 100r doses of X-rays in the guppy, Lebistes, increased the exchange frequency between the X and Y chromosomes, in

offspring, compared with that from unirradiated controls, if they were born 161 - 283 days after irradiation. Offspring produced 59 - 145 days after irradiation showed no significant increase in chiasma frequency.

1.12 THE PROBLEM IN PERSPECTIVE

A recent estimate for the number of genes in the human complement was 3×10^9 or less if the "master/slave" hypothesis (Callan 1967) is accepted (Edwards p.c. 1971).

In an analysis of the kind presented here, only gross features are detectable, those involving whole chromosomes or large chromosome segments, and representing vast amounts of genetic material. At the level of the structural gene, repeated or single, the light microscope alone can show nothing.

However, study of meiotic chromosomes, at present, offers richer returns by comparison with mitotic analysis, especially when superior modern techniques are employed. Not only are the chromosomes bigger at certain stages suitable for analysis (Dupraw 1970) but the behaviour of each chromosome can be compared at each stage. Owing to homologous pairing, translocations not detectable at mitosis, because of involvement of small or equal amounts of material, are shown at meiosis, and the chromomeric characteristics of pachytene chromosomes offer a whole field of study.

Studying meiosis may therefore enable the clinician to identify hitherto unrecognised genetic diseases, the cytologist to learn many new facts about the process of cell division, and the geneticist to learn more about the way in which genetic information is arranged in that highest of organisms, man.

2. AIMS

1. To identify the individual chromosomes. Consistent characteristics shown by chromosomes at spermatogonial metaphase and throughout meiosis are sought.

2. To investigate the appearance and behaviour of polymorphisms throughout meiosis. That the chromosomes differ vastly in length, could pose problems in the mechanics of pairing and cell division. An enquiry is made to see whether meiosis is disturbed in any way.

3. To obtain information on the variation in total cell chiasma count at diakinesis. A comparison is made between different cells in the same individual, between individuals and between controls and infertiles. An investigation is made to determine whether chiasma count varies with age or other parameters, for example, the stage of diakinesis used. It is intended to estimate the genetic map length in the human male.

4. To study the pairing relationships and behaviour of the XY bivalent throughout meiosis. A survey is made to find the variation between individuals of the percentage of cells with the X and Y present as univalents, to see if there is any correlation of values with age, chiasma count, stage of diakinesis or any other parameter and to establish whether crossing over occurs between the X and Y and what chromosome arms are involved in the association.

5. To study the behaviour of meiotic chromosomes from translocation heterozygotes. Four known heterozygotes (ascertained through abnormal offspring) and "controls", are compared with respect to homologous chromosome pairing and chiasma count. An estimate of the proportion of unbalanced complements at metaphase II is sought. Also, information from somatic mitotic metaphase, pachytene and diakinesis analysis is sought on the incidence

of translocation heterozygotes in an infertile population and the frequency of random translocation in germ cells of normal persons.

6. To determine the frequency of chromosomal aberrations in the aetiology of male infertility.

7. Miscellaneous

(a) To acquire comparative skill in observation. Before the examination of the human male meiotic material, meiosis is studied in Locust, Syrian Hamster and Mouse to assess the likely configurations of chromosomes through meiotic division, establish criteria and learn about meiosis in general, in order to treat the human male material in as uniform a manner and with as little subjective error as possible.

(b) To perfect modern cytological techniques. Alternatives to aceto-orcein are sought to give greater specificity and for differential staining of the centromere regions and other parts of chromosomes, to facilitate chromosome identification.

(c) To improve the description of the human meiotic cycle. Particular attention is paid to the early prophase stages, to elucidate the correct sequence of meiosis and the mechanism of homologous pairing. The frequency of various stages between normal individuals is compared to investigate the duration of the different stages of meiosis.

(d) To assess the frequency of nondisjunction. Cytological evidence of nondisjunction is sought by counting and karyotyping various stages of meiosis.

(e) To compare spermatogonial metaphase with somatic mitotic metaphase.

(f) To differentiate between various types of spermatogonia. Identificatory characteristics between stem cell spermatogonia and those about to enter meiosis are sought.

(g) To ascertain whether spermatogonial chromosomes show any evidence of homologous pairing.

(h) To study the appearance of centromere regions throughout meiosis. Information is sought on whether centromere regions are more heteropycnotic at any particular stage of meiosis.

(i) To investigate non-random arrangement of chromosomes in the various stages of meiosis. Associations and the behaviour of chromosomes with respect to nucleoli are examined.

(j) To investigate the pairing behaviour of homologous chromosomes at pachytene. Observations are made on whether abnormal pairing or failure to pair is common.

(k) To obtain information on the chiasma count per bivalent at diakinesis. An assessment is made of the variation in chiasmata per bivalent, in what chromosomes a change occurs when there is a change in total cell chiasma count, and whether univalents are ever present.

(l) To assess the occurrence of interlocked bivalents as demonstrated at diakinesis. The question of whether all bivalents are equally prone to interlocking is investigated.

(m) To compare metaphase II spreads with somatic mitotic metaphase.

(n) To obtain information on the mechanism of spermiogenesis and to see if there is any morphological difference between X-bearing and Y-bearing spermatids.

(o) To assess "real" polyploidy as opposed to "apparent" polyploidy in cells throughout meiosis.

(p) To investigate the effect on spermatogenesis of a massive dose of radiation (given 10 months previously). The chromosome damage is assessed at all stages of meiosis, together with the effect on chiasma count. A prognosis is made of the chances of abnormal offspring as a result of any chromosome damage.

(q) To obtain information on spermatogenic maturation arrest. The mechanism of degeneration is studied, together with the stages at which it can occur and its possible association with any specific chromosomal defect.

(r) To investigate the effect of muscular dystrophy on meiosis. The effect is examined in the mouse (Mus musculus L., Strain 129 Re).

3. MATERIALS

3.1 HUMAN MALE

Blood, testis tissue and, in addition, some skin biopsies and buccal smears were taken. Material was obtained from 88 cases which could be classified into several groups:

(a) Infertiles. 66 cases.

These were azoospermic or oligospermic patients with a sperm count of less than 20 million per ml. They often showed the associated conditions of a varicocoele, a hydrocoele, a current or previous history of undescended or ectopic gonads, or an inflammation or repair of hernia. Much of the material was abnormal, but 22 of the infertiles had normal histology and of these 13 were taken as "controls" since they were found, at operation, to have congenital bilateral absence of the vas deferens, or a blockage at the epididymus.

(b) Controls

These were a mixed collection, some more suitable as controls than others and included:

(i) A case, with a large familial variant of chromosome 16, who volunteered a biopsy for the present investigation.

(ii) One patient having an orchidectomy owing to persistent cremaster spasm.

(iii) Two fathers (of congenitally abnormal offspring) suspected, but not found to be translocation heterozygotes. One exhibited heteromorphic number sixteen chromosomes and the other a small Y.

(iv) One patient of normal fertility undergoing repair of a hydrocoele.

(v) Two patients, having orchidectomies in treatment of cancer of the prostate, who had had no previous hormone therapy.

(vi) Two patients having orchidectomies for severe orchitis and epididymitis.

(vii) "Controls" from (a) the infertile population.

(c) Miscellaneous

(i) Four balanced translocation heterozygotes (ascertained through abnormal offspring) who had at least one of the translocation chromosomes apparent in leucocyte analysis. Case H..... was father of case 8 in Leisti (1971) and RW680009 a case reported by Ferguson-Smith, Boyd and Ferguson-Smith (1968).

(ii) One oligospermic (0.6 and 0.5 million sperm per ml) radiation-damaged patient (70-2,000 rads from radioactive Iridium 10 months previously), who was concerned about his fertility.

(iii) One 15% sex chromatin positive trans-sexual having a sex change operation.

(iv) Six young people with ectopic or undescended gonads (as defined by Mack, Scott, Ferguson-Smith and Lennox 1961) undergoing operation to place the testes in the scrotum.

(v) A patient having an orchidectomy for cancer of the prostate and with a previous history of stilboestrol treatment.

3.2 MATERIAL OTHER THAN HUMAN MALE

(A) LOCUST Schistocerca gregaria Forsk. (Acrididae, Orthoptera).

Testes from two specimens (a) and (b) killed by decapitation.

(B) SYRIAN HAMSTER Mesocricetus auratus Waterh. (Cricetidae, Rodentia).

Heart blood, bone marrow and one testis from a specimen (12 weeks old) killed with ether.

(C) MOUSE Mus musculus L. (Muridae, Rodentia)

Testes from 7 specimens all killed by cervical dislocation. Eyes were sometimes taken for corneal mitotic preparations. The mice were:

(a) a C57 Black/A (5 weeks), (b) an A 2G/Tb (6 weeks), (c) an A 2G/Tb (6 weeks), (d) an A 2G/Tb (5 weeks), (e) a homozygous affected mouse (5 months), from strain 129 Re, in which the recessive form of muscular dystrophy segregates (a sickly specimen). (f) As in (e), but younger ($6\frac{1}{2}$ weeks, and less sickly). It clearly showed some phenotypic characteristics of the affected homozygote such as abnormal position of the limbs and behavioural anomalies. (g) Control litter mate of (f), not showing the characteristics and therefore either a heterozygote or a homozygous normal (which are, as yet, indistinguishable).

4. METHODS

4.1 MITOTIC ANALYSIS

HUMAN.

Twenty ml of blood were withdrawn from each patient by venepuncture, usually before the anaesthetic was given, but in about 20% of cases up to 30 minutes after anaesthetic. Ten ml were placed in a sterile Pularin brand Heparin B.P. tube (Evans Medical Ltd.) containing 100 I.U. heparin in a dried film. This was rolled in the hand to prevent clotting. Five ml were placed in a bijou bottle containing 1 ml. acid citrate dextrose with thiomersal (standard B.P. from the Regional Transfusion Service - 3% dextrose, 2% sodium acid citrate) and the rest placed in a dry bijou. Both 5 ml samples were "separated" and stored to be used for blood grouping, if necessary.

Standard Procedures for Cleaning Glassware

All glassware was steeped in Haemo-sol solution (1 oz per gallon of water) at 12°C, for one hour, rinsed six times in tap water, twice in distilled water, drained and dried in an oven. Silicone rubber liners and metal caps were rinsed well, boiled for approximately 30 minutes, cooled, rinsed in distilled water and dried. Universals, silicone liners, etc. were sterilised by dry heat (160°C for 60 minutes) or by autoclaving at 15 lb for 30 minutes.

Preparation of Microscope Slides

Microscope slides (3" x 1", 0.6 - 0.8 mm thick) were placed in chromic acid, made by dissolving 100 g ground potassium dichromate in 900 ml water, slowly adding 100 ml. conc. sulphuric acid, keeping the flask cool. Slides were taken from this when needed, washed individually and left in running tap water for about 3 hours. Alternatively, they were washed, stored in

70% alcohol and washed again for one hour prior to use.

Culture Medium

Medium containing 10% calf serum was made as follows: To 73 ml of Hank's Balanced Salt Solution, 13 ml of Waymouth's stock solution (Paul 1961) was added, then 10 ml inactivated calf serum, 2.5 ml of a 1.4% stock solution of glutamine in Hank's B.S.S. and 0.4 ml of a stock solution containing 500,000 units of sodium-penicillin and 0.5 g of streptomycin in 10 ml of Hank's B.S.S. The final mixture was brought to pH 7.1 with Sodium bicarbonate, as shown by the colour of the phenol red in the medium.

Culture Technique

A "whole blood" culture technique, after the method of Moorhead et al. (1960) was used. The tube containing the heparinised blood was rolled in the hand and, using sterile technique, a 0.5 ml aliquot was placed in 4.5 ml of tissue culture medium containing 0.1 ml of a tested stock solution of phytohaemagglutinin, made by adding 5 ml of sterile distilled water to a freeze-dried extract of Phaseolus vulgaris L. (Borroughs Wellcome Co. Ltd.). Four cultures were made. The tubes were gently mixed and either incubated immediately or placed in a 4°C refrigerator overnight before incubation for 72 hours at 37°C. The next day 0.20 or 0.25 ml of an 80 µg/ml solution of Colcemid (Ciba) in Hank's Balanced Salt Solution was added. The cultures were rolled and incubation was continued for a further 2½ hours. Cultures were then poured into pre-warmed centrifuge tubes and centrifuged at 500 - 700 r.p.m. for 10 minutes. A Mark III, Super-Multex Centrifuge (radius 16.25 cm) was used throughout.

The supernatant was discarded, the pellet dispersed and the cells resuspended in 10 ml of pre-warmed 0.075 M potassium chloride solution (Hungerford 1965). The tubes were placed in a 37°C water bath for 4½ minutes,

with occasional pipetting. Harshaw pipettes were used as they had a uniform bore and consequently gave control over pipetting and also drop size. The tubes were centrifuged for 7 minutes at 600 r.p.m. and as much supernatant as possible discarded. The pellet was dispersed and the cells fixed in about 1 ml. of freshly made cold 3:1 industrial ethanol:glacial acetic acid using gentle pipetting. More fixative was added to a total of 10 ml , and mixed thoroughly. The tubes were covered and placed in a 4°C refrigerator for a minimum of 15 minutes, and a maximum of 2 hours.

The cells were then centrifuged for 10 minutes at 600 r.p.m. and 5 ml fresh fixative added to the pellet, spun down again and four drops of fresh fixative added to the dispersed pellet and pipetted gently to give a milky suspension. Three drops of this suspension were then dropped from a height of 12" onto cold, wet (but drained) microscope slides which were then drained again. Air-drying of the preparations was completed by placing the slides on a 60°C hotplate. The slides were examined under a phase 2 (X16) neofluar lens on a Standard WL Zeiss Microscope using a 546 mμ green filter. Slides with the best quality preparations were chosen for staining, number depending on the density of mitoses. Any slides not needed were stored, unstained, for fluorescence and other studies.

Staining

Slides were stained in 2% orcein in 60% acetic acid for 1 to 1½ hours depending on the quality of the natural orcein (R. A. Lamb) available at the time.

Slides were then passed through 50% acetic acid for 30 seconds, two changes of 95% ethyl alcohol for a total of 3 minutes, absolute ethyl alcohol for 30 seconds and into xylol for at least 15 minutes. The slides were permanently mounted in DePeX mounting medium (G. T. Gurr Ltd.), which was slightly diluted with xylol, to achieve the thin depth of mountant essential with high powered objectives. Coverslips, Chance No. I 22 x 64 mm

were polished from 70% alcohol before use.

Mitotic Analysis

When the mountant was dry, at least 24 hours after staining, mitotic analysis was performed. Mitotic metaphase preparations were located using bright field low power X 10 objective and oculars, and optovar 1.25. Well spread cells were examined in more detail using high power X100 planapochromatic oil immersion lens. When the stain was weak X16 phase 2 and X100 oil immersion phase 3 was used for location and analysis respectively.

Twenty cells were chosen with well spread, extended, chromosomes. The total number of chromosomes was counted twice, starting from different sides of the spread. The D group chromosomes were then counted, G group, F, E, A, B and C, in that order. All those chromosomes larger than Ds and all those smaller were then counted, as a cross check, and then chromosomes 1, 2, 3, 6, 9, 16, 17, 18 and the Y were individually distinguished. Any irregularities, either numerical or structural, for example: breaks, quadriradials dicentrics, additions or deletions and also polymorphisms, for example: odd satellites, unusual size of the Y and pronounced secondary constrictions, were also noted.

At least 5 cells were photographed including at least one "typical" for that patient but especially any cells that were abnormal.

Prints were made X 4,000 and karyotyped according to the Chicago Conference, Standardisation in Human Cytogenetics (1966) incorporating the Denver report (1960) and according to Ferguson-Smith, Ferguson-Smith, Ellis and Dickson (1962). See Figure 1.

Photographic Technique.

Photography was performed using an Exacta camera placed on the microscope column, and loaded with Recordak 35 mm Micro-File film. Before taking a photograph, dust was removed from all exposed surfaces.

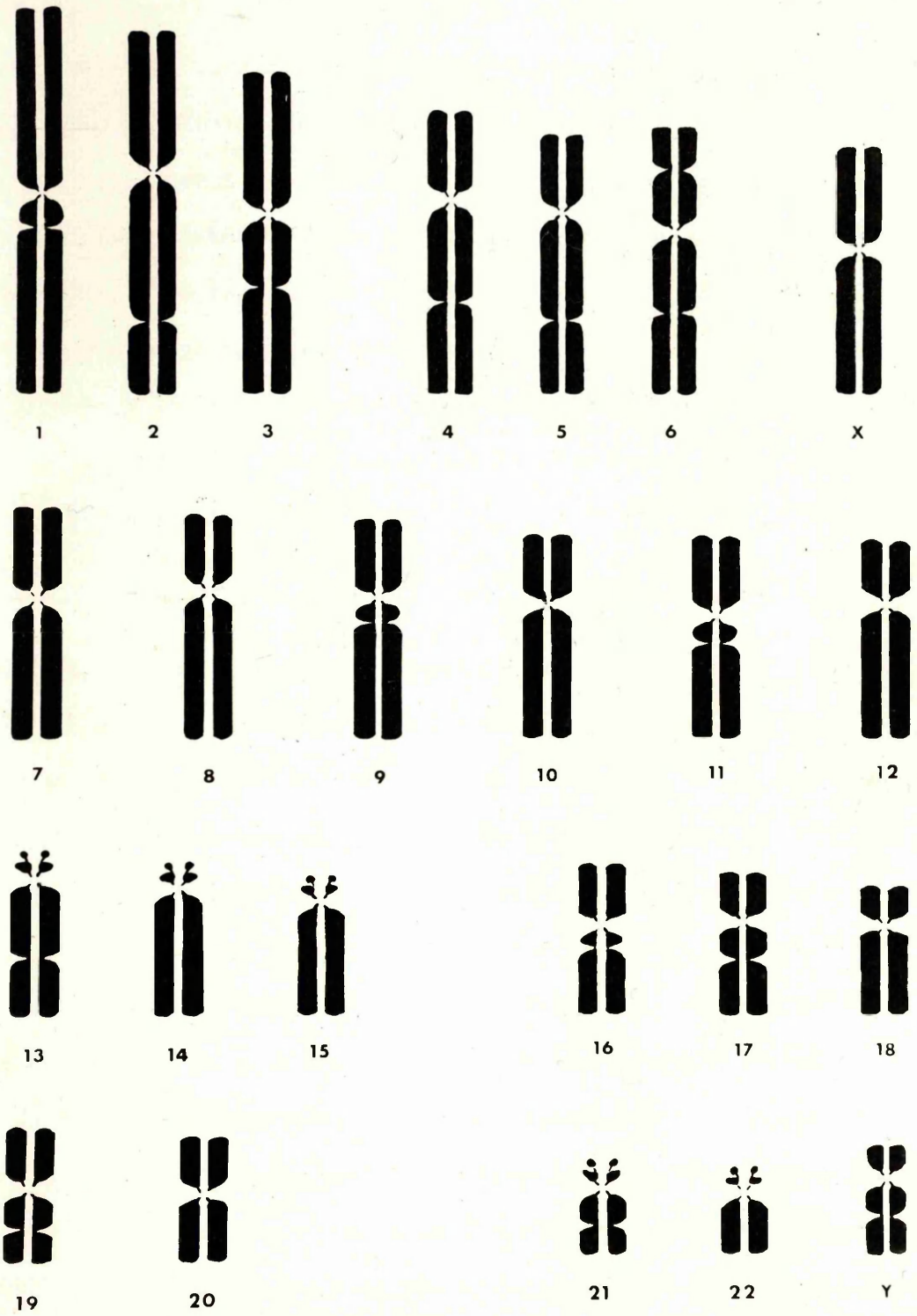


Figure 1. Diagram showing the secondary constriction positions and other criteria used in the identification of both mitotic and meiotic chromosomes in this study (according to: Ferguson-Smith et al. 1962).

The image was focussed with the oil immersion lens, iris closed and condenser focussed, aperture set to 5 and field stop to 10 for bright field microscopy. The reflecting image was switched to the microscope column and was focussed on the silvered mirror of the camera. Light intensity was measured using a Brinkman lightmeter and adjusted to a value determined by a test strip to give the correct exposure. The image was accurately refocussed and the photograph taken by means of an automatic release with a 12 second time-lapse. The exposure was for six seconds. A long exposure was necessary when using phase contrast, with the green filter in place and using the 6v 15w lamp (compared with the 12v 60w of the Zeiss photomicroscope). Longer exposure also minimised effect of vibrations. An antivibration rubber mat was also used.

Developing Film.

The film was wound onto a reel in complete darkness; this was placed in a clean, dry, developing tank and the light-tight lid fitted. Kodak D163 developer, 1 part : 3 parts of tap water at 20°C was poured through the lid. The tank was agitated for the first and every other 30 seconds for a total of 4½ minutes. Developer was then replaced by a 3% acetic acid stop bath at 20°C, with agitation for 30 seconds. This was replaced by Amfix fixative (May and Baker Ltd.), 1 part : 3 parts tap water at 20°C, with agitation for the first few seconds and thereafter, intermittently, for 5 - 8 minutes. The lid was removed, the fixative poured away, and the film rinsed with fast flowing tap water for at least 30 minutes. One drop of Photoflo (Kodak) was added to the tank and, after a few minutes, the film was removed from the reel, surplus water shaken off and the film hung to dry.

Printing.

A Simmon Omega Variable Condenser Enlarger was used. Length of exposure, light intensity and paper were varied. Kodak amber yellow Safelight Filters Wratten Series OB, were used. Most negatives required printing on Kodak Bromide paper WSG 2S using 5 seconds exposure. Exposed prints were immersed in Kodak Universal Developer 1 part:7parts tap water at 20°C for 1½ - 2 minutes. Prints were rinsed in running tap water at 20°C and placed, face down, in Amfix fixative 1 part:5 parts tap water at 20°C for 2 - 5 minutes. They were then washed in running tap water for 30 minutes. A drop of Photoflo was added to a final rinse before the prints were drained and dried on a rotary glazer.

RODENT.

Syrian Hamster Blood

A method similar to that described for human blood culture was tried. 0.1 to 0.2 ml of blood was taken from the heart into a syringe wetted with 5,000 I.U./ml heparin and incubated with 4.5 ml Waymouth's tissue culture medium + 10% calf serum + 0.2 ml heparin. The cultures were incubated for 51 hours (Buckton and Nettesheim 1968) and preparations made as previously described.

Syrian Hamster and Mouse Bone Marrow

The technique of Lee (1969) was modified. The mitotic inhibitor vincaleukoblastine solution was not used for fear of damage to meiosis. Immediately after death, the long bones were removed, stripped of muscle and chopped with thick sterile scissors in a sterile petri dish in 10 ml Waymouth's tissue culture medium containing 0.2 ml of 5,000 I.U./ml heparin and 1 ml 80 µg/ml colchicine (Ciba). Tissue and medium were mixed by pipetting, then incubated at 37°C for 3 hours. The largest lumps

were removed and the rest centrifuged at 700 r.p.m. for 10 minutes. 10 ml. pre-warmed 1.12% sodium citrate were added to the dispersed pellet and left for 10 minutes. Centrifugation was repeated and the pellet fixed in 3:1 absolute ethanol:glacial acetic acid. Larger clumps were allowed to settle out, the supernatant was pipetted off. After 15 minutes the fixative was changed twice, the tube spun again and the pellet suspended in a few drops of fresh fixative. Air-dried preparations were made and stained with aceto-orcein for 1¼ hours.

Mouse Cornea

Fredga's (1964) method was used. The mice were killed by cervical dislocation and an eye dissected out, leaving the optic nerve as a stalk for manipulation. The whole eye was placed in warmed 0.04% colchicine in distilled water and incubated at 37°C for 35 minutes. It was then fixed in a mixture of 9 parts 50% acetic acid:1 part 1N HCl for 5 minutes and stained in 2% orcein in 60% acetic acid for 2 - 6 minutes. The eye was then wiped over a slide in a drop of stain, a coverslip applied and the preparation squashed with pressure from the fingers. Observations were either made directly or on permanent preparations.

4.2 MEIOTIC ANALYSIS

HUMAN MALE.

Light general anaesthetic was given to all patients except HC690328 and JS690329 where a local anaesthetic was used.

At operation, various characteristics of each patient were noted. These included rough height, body proportions, hair distribution, fat distribution, penis size and position of urethral opening.

If a sex chromosome anomaly was suspected, dermatoglyphics were analysed. Either a rough assessment of finger whorls, loops and arches

was made or a detailed assessment (directly or using hand prints) of total ridge count, T.R.C. (Penrose 1963, 1967) and a/b distance with a/b ridge count. The mean ridge breadth (Penrose 1969) could then be calculated.

Testicular Biopsy

This was obtained by the method of Charney (1940) and Nordlander (1948). Usually the gonad was completely exposed to enable more accurate measurements to be obtained (to the nearest mm) of length, breadth and width. With these measurements the testis volume could be calculated and used, with histology, to assess Leydig cell volume (Ahmad, Lennox and Mack 1969). Details including presence or absence of the vas, whether a varicocoele and the state of epididymus were also noted at this stage. An incision was made in the testis, about 0.5 cm. long and with slight pressure on the gonad, a bulge of testis material protruded and was cut away with sharp scissors. Size of biopsy obtained varied from 3 mm. to 8 mm. diameter. Immediately after removal from the patient, the biopsy was halved. One piece was placed in Davidson's fixative (Moore, Graham and Barr 1953). This consists of 4 parts 95% alcohol, 2 parts 40% formaldehyde, 1 part glacial acetic acid and 3 parts distilled water, and was found to be excellent for testis histopathology as well as for the original use which was for nuclear sexing. Histological sections were made for routine assessment of spermatogenesis. The remainder of the biopsy was halved and one half placed in hypotonic 1.12% sodium citrate solution in a small petri dish for technique "C"; $\frac{2}{3}$ of the rest was placed in Waymouth's Tissue Culture Medium + 10% calf serum for technique "M", and $\frac{1}{3}$ was placed in 10 ml. of cold 3:1 absolute ethanol:glacial acetic with 1 drop of chloroform, for technique "F".

The whole testis was available in those patients from categories (b) (ii) (v) (vi) and (c) (iii) and (v), but since it was technically only possible for one person to deal with a limited amount of material,

only slightly more was taken, for the standard techniques, although extra material was used for experimental purposes.

Main Techniques Used

Technique "C". This was similar to that of Ferguson-Smith (1964a) and Evans, Breckon and Ford (1964) but modified for better preservation of all stages of meiosis. The method was primarily used for cells at diakinesis, and was attempted on every specimen.

The biopsy was teased thoroughly in about 5 ml of 1.12% sodium citrate solution, volume depending on the size of the specimen. The sodium citrate was ideally 3 - 4 days old. Younger and older solutions yielded reasonable results, but the best preparations were obtained with those 3 - 4 days old. (This was confirmed by controlled mouse trials, Chandley p.c. 1970). Using fine, sharp, curved scissors, the tubules were chopped. The resulting cell suspension was removed to a centrifuge tube. A few more ml of sodium citrate were added and the process repeated twice. This resulted in about 10 ml of cell suspension. The tubular fragments were left in the petri dish. The cells were left in the solution for 40 minutes. Longer than this gave fewer but better spread diakinesis figures, but loss in number and detail of other stages. Less than 40 minutes gave poorer spreading at diakinesis, although preservation of other stages was better. Forty minutes was therefore considered a convenient compromise. The suspension was centrifuged for 10 mins. at 800 r.p.m. and to the dispersed pellet was gently added 3 ml of freshly made cold 3:1 industrial ethanol:glacial acetic acid, with a drop of chloroform. The suspension was pipetted slowly. About 7 ml of fixative was then added according to the size of the pellet. After pipetting, the tube was placed at 4°C for at least 15 minutes. After this time, the tube was spun and the dispersed pellet suspended in about 10 drops of freshly made, cold fixative, and the suspension pipetted.

Technique "F". This was based on that of Ferguson-Smith (1964a) and used primarily for preservation of cells at pachytene since chromomeres and nucleoli are well preserved. It was also found satisfactory for some early prophase stages and was attempted with most patients, using a small piece of tissue.

The specimen was teased in a few ml of the same fixative used in technique "C". The tubules in the fixative were then placed in a universal container. Later they were removed from the container and finely chopped in a few ml of fresh fixative. The resulting suspension was centrifuged and the pellet resuspended in a few drops of fresh fixative.

Technique "M". This was based on that of Hungerford (1971) and used primarily for detailed analysis of pachytene. It was only used on the final 30 patients.

The specimen was teased in a few ml from 10 ml of tissue culture medium in a universal container, to which 0.2 ml. of Heparin (1,000 I.U./ml Pularin B.P., Evans Medical Ltd.) had been added. The medium was preferably, but not necessarily, warmed to 37°C. After teasing with fine needles the contents of the petri dish were replaced in the universal container. Later the tubules were removed to a petri dish together with a few ml of the medium and chopped finely. The cell suspension was placed in a centrifuge tube and the process was repeated using more medium. The tube was then centrifuged for 10 minutes at 800 r.p.m. and the dispersed pellet suspended in 1 ml of 0.125 M KCl at 37°C from 10 ml to which 200 I.U. of Heparin had been added. After pipetting, more KCl was added to a final volume depending on the size of the pellet. The centrifuge tube was covered and incubated in a 37°C water bath for 1 - 1½ hours with occasional pipetting. The suspension was then centrifuged for 10 min . at 800 r.p.m., and the dispersed pellet fixed in 3:1 methanol:glacial acetic acid, first with a few drops, then to a total volume of 10 ml, with pipetting.

Fixation was for 10 - 15 minutes. The tube was spun again and the dispersed pellet resuspended in about 10 drops of fresh fixative.

Chromosome Preparations from "C", "F" and "M" Techniques.

Three drops of each of the suspensions were dropped from a height of about 12 inches onto cold, wet (but drained) microscope slides. The slides were drained and air-dried high above a bunsen flame, or on a 60°C hot plate. Quality of fixation and density of cells were observed using low power phase contrast and if necessary suspensions diluted with fixative.

Aceto-Orcein Staining.

This was light (from 3 to 5 minutes depending on the quality of the stain). Permanent preparations were made as described for mitotic preparations but the slides were passed rapidly through the dehydration stages to avoid unnecessary leaching of the lightly stained preparations. About half the slides were stained, the rest were stored for fluorescence and other studies.

Meiotic preparations were located using a phase contrast 2 (X16) lens and analysed using X 100 oil immersion phase contrast planapochromatic lens. Optovar 1.25 was used for most of the photography. Prints were magnified X 2,500.

Other Techniques Used.

Meredith's Method. This technique (Meredith 1969) was tested on case JT700346, in a modified form, with view to use with small biopsy specimens. A few tubules were teased in 1.12% sodium citrate and left for 30 minutes. They were then fixed in 3:1 ethanol:glacial acetic acid for 15 minutes and removed to a watch glass containing a small quantity of 45% acetic acid. The tubules contracted and the contents were expelled. The cell

suspension was then transferred to a slide on a 60°C hotplate and pipetted until evaporation had occurred.

Other Staining Techniques Tested on Air-Dried Preparations.

Other techniques originally used on sections or squashes were attempted, often with modification, for air-dried preparations. Of interest included:

Carbol Fuchsin. Two techniques, both essentially from Klinger and Ludwig (1957), that of Carr and Walker (1961) and Robinson and Puck (1967) were modified to produce the technique here. The staining solution was made as follows: Stock solution A was 3.0 g of basic fuchsin in 100 ml of 70% ethyl alcohol. Stock solution B was 10 ml of A in 90 ml of 5% phenol in distilled water. Carbol Fuchsin solution was 45 ml of B, 6 ml glacial acetic acid and 6 ml formaldehyde.

The schedule was as follows: (i) Absolute ethanol 5 min. (ii) Equal parts ethanol and ether 10 sec . (iii) Air-drying. (iv) 70% ethanol 5 min. (v) Distilled water 5 min. (twice). (vi) Carbol Fuchsin solution 4 min . (vii) Absolute ethyl alcohol 1 min. (twice). (viii) Xylene 15 min . (ix) Mount DePeX.

Toluidine Blue. The schedule, from Ford p.c. (1971) was as follows: (i) 5 N HCl 2 min . (ii) 50% ethanol, rinse. (iii) 95% ethanol, rinse. (iv) 3:1 absolute ethanol:glacial acetic acid, rinse. (v) Dry over lamp. (vi) Place four small drops of Toluidine Blue stain (G. T. Gurr) along slide, apply coverslip.

Feulgen. The Feulgen procedure from Gurr (1962) was used. Stages 1, 2 and 11 were omitted and the periods of hydrolysis and staining were varied from 30 sec . to 10 min . and from 30 min . to 1 hr. respectively. Solution A was obtained in three ways:

1. Using the method of Gurr (1962).

2. Using the following method:

3g of basic fuchsin were dissolved in 600 ml of distilled water at 100°C. The flask was cooled to 50°C and the solution filtered. Sulphur dioxide was bubbled through, slowly, at room temperature, until the solution turned reddish. The flask was stoppered and left overnight. The next day, 1g of activated charcoal was added, the mixture agitated for 10 min., the solution filtered and stored, stoppered at 4°C.

3. Feulgen Stain obtained from manufacturers, G. T. Gurr Ltd.

Giemsa. Two methods were used:

1. The method of Yunis (1965). The staining solution was 10 ml of Giemsa (G. T. Gurr) in 90 ml of distilled water with 3.5 ml 0.15 M NH_4OH (pH 7.2). The schedule was: Stain 5 - 6 min., dehydrate in 2 dishes of acetone, 1 dish of ½ acetone : ½ xylol, and 1 dish of xylol. Mount in DePeX.

2. The schedule was as follows. Rinse in methanol and allow to dry. Stain 8 min. in Giemsa, 1 part stain : 4 parts phosphate buffer pH 6.8 (freshly made). Rinse in tap water. Differentiate in phosphate buffer ½ - 1 min. Dry, then place in acetone, then ½ acetone : ½ xylol, rinse in xylol and mount in DePeX.

Meiotic Analysis

In those patients with meiosis, cells at all stages were examined in great detail. Relative frequency of cells at spermatogonial metaphase, diakinesis and metaphase II and numbers of cells in a state of degeneration (measured as a percentage in relation to total metaphase cells) were scored, for evidence of "maturation arrest" of meiosis.

Diakinesis Analysis.

Only the very best cells in mid-diakinesis and with chromosomes well spread were chosen for detailed analysis. The total number of chromosome bodies was noted and also the state of the X and Y, adjacent (XY) or separate as univalents (X/Y). Using at least 8 cells, figures for the mean chiasma count per cell in the autosomes and also proportion of cells with the X and Y separate, were obtained. This was also done in "control" material for 60 cells at a stage slightly earlier than the one used for detailed analysis and for 60 cells at a later, more contracted stage. This information was used to assess the effect of stage of diakinesis on chiasma count and conjunction of the X and Y chromosomes. Each bivalent configuration was assessed as having the minimum number of chiasmata necessary for formation of such a configuration (Huskins and Hearne 1936). Any asymmetry, abnormal pairing or interlocking, etc. were also noted. Chiasma counts were plotted in a histogram for each individual, to show the cell population structure. Graphs were plotted using mean figures from those cases with a sufficient number of diakinesis cells of suitable quality. Graphs were:

1. Mean chiasma count per cell plotted with age.
2. Percentage of cells with separated X/Y plotted with age.
3. Percentage of cells with separated X/Y plotted with mean chiasma count per cell.

In 40 individuals that showed at least one X/Y separate cell, the mean chiasma count per cell was calculated for those spreads where the X and Y were separate and compared with the mean for those where they were attached. It was noted whether the mean was higher or lower in the former compared with the latter.

Chiasmata per Bivalent.

At least 5 diakinesis cells from each case were photographed and karyotyped. Figures for mean chiasma count in each bivalent were obtained. In those patients with major absence of germ cells or severe maturation arrest, 5 cells

of suitable quality were frequently not available. Figures from these cases were not used in analysis for fear of even more bias with few numbers.

Figures obtained were meaningful only if identity of each bivalent was accurate. Karyotyping was performed using essentially the same criteria as for mitotic analysis: relative size, position of centromere and secondary constrictions. Centromere regions were shown as double heteropycnotic bodies in each homologue and secondary constrictions as discontinuities, usually more exaggerated than at mitotic metaphase. Centromere positions were not obvious in every diakinesis spread. Only those informative in this way were used. Similarly, every bivalent did not always show them, but by elimination and use of criteria such as asymmetry caused by polymorphism and variation in depth of heteropycnosis, identification was possible. Number and position of chiasmata were not used in karyotyping. Apart from allowing for apparent increase in bivalent length with increase in number and allowing for illusion affecting size according to position, chiasmata were largely ignored in karyotyping.

Translocation cases received special attention with respect to diakinesis. The quadrivalent was identified by karyotyping the bivalents. Close scrutiny of the quadrivalent was needed in order to ascertain from which chromosome, material was demonstrating chiasmata. This could usually be performed by examining the asymmetry. Mean chiasmata figures for both translocation chromosomes could then be given.

Occasionally, possible quadrivalent configurations were observed in patients not suspected of carrying translocations. These cells were karyotyped and the chromosomes involved identified and compared with other cells from the patient. If no more were seen involving those particular chromosomes, abutting was assumed. For those patients where adequate figures for number of chiasmata per bivalent were obtained, graphs were plotted of chiasmata per bivalent with size of chromosome, taken from

mitotic chromosome length estimated by Ferguson-Smith et al. (1962). For patient HK710139 the figures for chromosome length from Gilbert and Muldal (1971) were also used to compare values.

Analysis at Other Stages.

Counts were also made for chromosome number on good quality spermatogonial metaphase, pachytene and second metaphase spreads. Karyotyping was performed on at least two cells at each stage.

In the case of patients with heteromorphism of secondary constriction regions, metaphase II configurations were scored for evidence of crossing-over between the secondary constriction region and the centromere, i.e. LL LS and SS, long/long, long/short and short/short long arms respectively.

Statistical Tests Used. (Probability figures were obtained from Fisher and Yates 1948).

Chi-Square Method:

$$\chi^2 = \Sigma\left[\frac{(X_{OBS} - X_{EXP})^2}{X_{EXP}}\right]$$

Two-sample t - test:

Observations $x_1, \ldots, x_m \quad y_1, \ldots, y_m$

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{(\Sigma x_i^2 - m\bar{x}^2 + \Sigma y_i^2 - n\bar{y}^2)}{m + n - 2}\left(\frac{1}{m} + \frac{1}{n}\right)}}$$

Test again t with m + n - 2 degrees of freedom.

One-sample t - test:

$$t = \frac{\Sigma z_i/n}{\sqrt{\frac{\Sigma z_i^2 - n\bar{z}^2}{n(n-1)}}}$$

where $\{z_1, \ldots, z_n\}$ are differences.

Test again t with n - 1 degrees of freedom.

Sign Test:

If r negative out of n

then $Z = \dfrac{r - n/2}{\sqrt{n\left(\frac{r}{n}\right)\left(1 - \frac{r}{n}\right)}}$ should be standard normal.

i.e. if > 1.96 or < -1.96, significant at 5%.

Standard Deviation:

Observations $x_1, \ldots, x_n$

Mean $\bar{x} = \dfrac{\Sigma x_i}{n}$

$$\text{Variance} = \frac{\Sigma (x_i^2) - n\bar{x}^2}{n - 1 \text{ (n for n over 30)}}$$

$$\text{S.D.} = \sqrt{\text{Variance}}$$

Correlation Coefficient:

The product-moment correlation coefficient of a set of bivariate data $\{(x_i y_i)\ i = 1, \ldots, n\}$ calculated as

$$r_{xy} = \frac{\Sigma xy - n\bar{x}\bar{y}}{\sqrt{(\Sigma x^2 - n\bar{x}^2)(\Sigma y^2 - n\bar{y}^2)}}$$

For three variables x, y and z the partial correlation coefficient of x and y (for constant z) calculated as:

$$r_{xy.z} = \frac{r_{xy} - r_{xz} r_{yz}}{\sqrt{(1 - r_{xz}^2)(1 - r_{yz}^2)}}$$

MEIOSIS OTHER THAN IN THE HUMAN MALE.

Analysis was as already described for the human male and included the karyotyping of spermatogonial metaphase, diakinesis and metaphase II.

Male Locust

Techniques "C" and "F" were used. Preparations were stained in 2% orcein in 60% acetic acid for 4 min . Chromosome length was estimated from (a) a photograph of a cell at diakinesis and (b) from one at metaphase II.

Male Syrian Hamster

Two-thirds of a testis were used for technique "C" and the remainder for technique "F". Preparations were made as for the human male. Chromosome length was estimated from a photograph of a cell at diakinesis.

Male Mouse

One testis was used for technique "C" and the other for technique "F" in mice (a) to (e). In (a) chromosome length was estimated from a photograph of a cell at diakinesis.

In mice (f) and (g) affected and unaffected with muscular dystrophy, technique "C" preparations only were made, using one gonad from each animal, at the same time and under the same conditions. Carcasses (without eyes or gonads) were weighed. Slides were analysed "blind". All stages of meiotic division were analysed. At diakinesis, all stages from early to late were examined to eliminate some subjective error. Number of chromosome bodies and total chiasmata were counted.

The sex chromosomes were scored "XY"- paired normally; "X/Y" - separated as univalents; "X^Y" - separated but connected by a thread (or threads) over a distance, or sometimes appearing aligned, pointing towards one another; and "XY gap" - the less severe condition of the previous state.

5. RESULTS

5.1 HUMAN MALE

LEUCOCYTE MITOTIC METAPHASE.

Mitotic analysis was possible in every one of 79 cases where leucocyte culture was attempted. However, for patient IT690271, only 10 cells suitable for analysis were found. This might therefore be considered a failure for the 20-cell survey. Results are shown in Tables 1 and 2 and, in detail, in the Appendix.

Certain impressions were obtained during the course of the study. These included: that there was no detectable effect of anaesthetic on quality or quantity of metaphases obtained; metaphases were improved in number by standing the cultures overnight at $4^{\circ}C$; and less contraction of chromosome arms occurred after using 0.20ml of 80µg/ml Colcemid compared with 0.25 ml, as in Moorhead et al. (1960).

Hypodiploidy

Ignoring counts from "confirmed" mosaic cases (those with more than one cell with the same chromosomes "extra" or "missing") and also those with abnormal complements, random loss of chromosomes was found to be 0.28% (202 chromosomes lost from 1,571 cells of which 393 were karyotyped). Table 3 and Figure 2 show that loss was according to chromosome size.

Hyperdiploidy

Only 1 cell out of 1,571 showed evidence of hyperdiploidy which could not be attributed to mosaicism or fragmentation. The cell carried an extra B group chromosome contracted to the same degree as the remainder of the karyotype.

Polymorphisms

Some distinctive examples from 79 cases are shown in Table 4. The

Table 1. Mitotic results from the Infertile Survey.
Of 66 cases 10 had abnormal while 56 had normal chromosome complements.

TOTAL NUMBER	ABNORMALITY	CASE
3	47,XXY Klinefelter's syndrome	AM680160 MF680208 KMK710321
1	46,XY/47,XXY mosaic "confirmed"	BC690104
1	46,XX male	AP690095
1	46,XYq- (The small metacentric was thought to be a Y since it did not take part in "satellite association" and was usually positioned at the periphery of the spread. The Y of a brother was normal).	WC680183 (Plate 9a and b)
4	45,XO/46,XY mosaics "confirmed"	HC690328 DG690405 SML700425 LC710097
	"Unconfirmed" mosaicism	
4	45,XO/46,XY	GS690074 JP690168 TB700653 JW700429

Table 2. Mitotic results from material other than infertile cases. Of 13 cases, 1 had abnormal, while 12 had normal chromosome complements.

TOTAL NUMBER	ABNORMALITY	CASE
1	47,XXY Klinefelter's syndrome	DK700082 (Plate 6a)
	"Unconfirmed" Mosaicism	
1	46,XY/47,XXY CONTROL	CMC700362
1	46,XY with possibly 3 populations of Y, 6 small, 8 medium and 6 large CONTROL	JT700346
1	45,XO/46,XY CONTROL	WMD700011

Table 3. Mitotic Hypodiploidy.

CHROMOSOME GROUP	NO. OF CHROMOSOMES IN GROUP (x)	NO. OF "MISSING" CHROMOSOMES IN EACH GROUP (y)	LOSS RATIO (y/x)
A	6	8	1.33
B	4	5	1.25
C	15	53	3.53
D	6	25	4.17
E	6	30	5.00
F	4	25	6.25
G	5	56	11.20

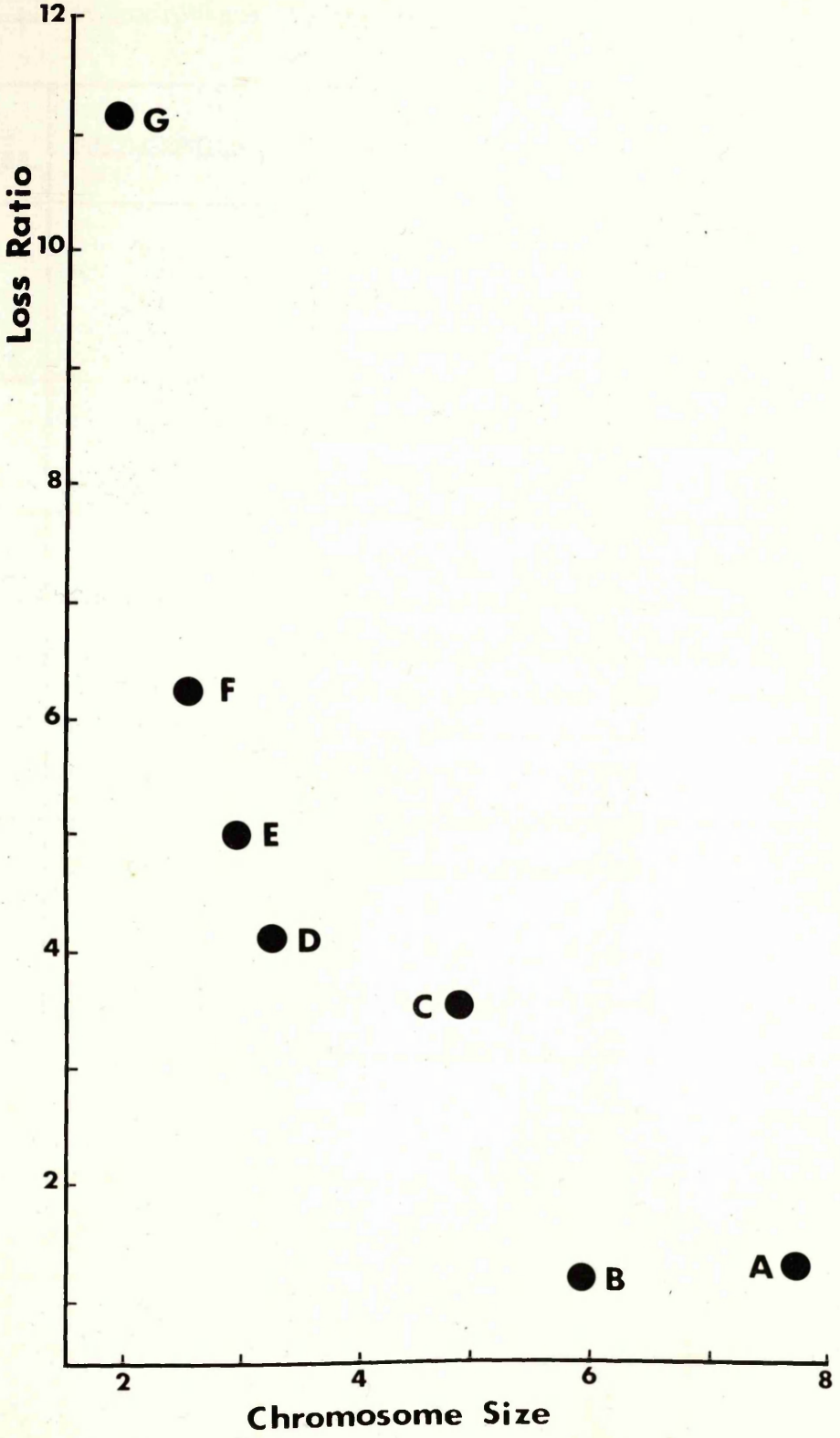


Figure 2. Loss ratio (see Table 3) plotted against the mean chromosome length for each group (calculated from figures given by Ferguson-Smith et al. 1962).

Table 4. Polymorphisms (79 individuals).

TOTAL NUMBER	POLYMORPHISM	GENERAL INFORMATION
15	Dp+	Proximal short arms, satellites, or tandem satellites, caused elongation in different instances.
12	Gp+	Elongation was due to short arms or satellites.
4	Satellited 17's	
7	Yq+	Variants only reaching to the size of the E group chromosomes were recorded
2	Yq-	
13	Prominent paracentric constriction regions	1 involved chromosome 1 10 " " 9 1 " " 11 1 " " 16

most extreme polymorphisms were drawn from the control population, for example, control case CMC700362 had heteromorphic chromosomes 9 (Plate 10a).

Anomalies

Some anomalies found during a 20-cell survey are shown in Table 5.

TESTIS CELL PREPARATIONS.

Air-dried preparations included germ cells in the various stages of meiosis, cells undergoing the pre-meiotic "mitotic" divisions, and somatic cells, including Sertoli cells, Leydig cells, fibroblasts and white blood cells.

The generally accepted rule that the length of time spent at a particular stage is reflected in relative frequency in air dried preparations needs reassessing in the light of the particular technique used. For example, technique "M" (page 47) harvests many cells in pachytene and prophase stages but only perhaps three cells in diakinesis, per slide. Certain stages are more sensitive to technique than others and are presumably lysed and lost to the supernatant.

Material

Histological sections, from each of the biopsies also used to make air-dried preparations, demonstrated an enormous variation in the quality of material available for study. Table 7 shows the classification of this material. Most of the best air-dried meiotic preparations were obtained from gonads found to have normal meiosis. A few deviations from this rule were found:

1. Gonads with normal spermatogenesis, which also had the tubules occluded by cells in division (for example Plate 4b), gave fewer suitable metaphase preparations than gonads with normal spermatogenesis and tubules with an open lumen (for example Plate 1a).

Table 5. Mitotic anomalies found in a 20-cell survey (79 cases).

No. of CELLS	A N O M A L Y
3	Quadriradials (Plate 11a)
5	"Endomitotic Reduplication" figures
3	Colchicine Anaphases
2	Cell Translocations - a t(17,17) and a t(Dq-4q+)
1	Deleted D
2	Dicentrics (Plate 11b)
2	Ring G's
5	Cells with characteristics of viral shattering
10	Cells with fragments (Plate 11b)
23	Breaks, both chromatid and isochromatid.
11	Small, attached bodies, paired or single
3	Acrocentric satellite anomalies, for example: satellite present but short arm material absent

Table 6. Dermatoglyphic results.

CASE	LEFT HAND					Total Left	RIGHT HAND					Total right	TRC	a - b						TRANS-VERSE PALMAR CREASE	SIMIAN CREASE V DIGIT	DISTAL AXIAL TRI-RADIUS
	V	IV	III	II	I		I	II	III	IV	V			DISTANCE		COUNT		MEAN RIDGE WIDTH				
														L	R	L	R	L μm	R μm	Mean μm		
WC680180 46XYq-	15UL	9UL	10UL	7UL	15UL	56	16UL	6UL	10UL	8UL	10UL	50	106	1.90	1.85	31	24	612.9	770.8	691.85		No	About 42°
AP690095 46,XX male	12W	16W	13W	16W	20DL	77	13DL	20W	14W	12W	9W	68	145										
JMF690231 46,XY (Gonads not found)	UL	A	A	A	UL		UL	A	A	A	UL		22										
DK700082 47,XXY Klinefelter	3UL	6UL	3UL	2UL	9UL	23	13UL	5UL	4UL	2UL	8UL	32	55	2.54	2.68	44	47	534.1	570.2	552.15	No		

UL = ulnar loop, DL = double loop, W = whorl, A = arch, TRC = total ridge count, L = left, R = right

Table 7. Histological classification of material.

NUMBER	CATEGORY	DESCRIPTION
31	"Normals"	This category includes defects that may be found in any gonad of proved fertility, and allows for up to 10% "infertile" tubules. (Plates 1 and 4b)
16	Absence of germ cells	Germ cells are completely absent. (Plates 2, 6b and 8)
10	Major absence of germ cells	Over 90% of tubules have no germ cells. (Plate 7)
7	Partial absence of germ cells	Between 10% and 80% of tubules have active spermatogenesis.
13	"Maturation Arrests"	Stem cells are present and a breakdown of spermatogenesis occurs, apparently at certain levels (Plate 3)
8	Maturation Arrest with partial absence of germ cells.	(Plate 4a)

2. Older gonads (for example Plate 5a) gave fewer stages at diakinesis and metaphase II, and more stages at spermatogonial metaphase and at the early prophase stages of first meiosis, by comparison with gonads from younger men.

3. There appears to be an optimum size of sample used in each technique. Taking into account the fact that smaller biopsies tended to be taken from smaller gonads (which are also likely to have abnormal spermatogenesis) the fixation of the cells tended to be poor. Yet where the whole gonad was available, the use of extra material for each technique frequently resulted in a similar loss of quality. Cell density in the later stages of processing was not critical because dilution or concentration prior to slide preparation altered cell density, but not the quality of fixation.

Techniques

Technique "C" (page 46) was found reliable and satisfactory as a general technique for all stages of meiosis. It was the best technique for preservation of cells in diakinesis.

Technique "F" (page 47) was not so reliable. When fixation was good, fine chromosome detail could be observed, although without the hypotonic pretreatment, spreading was poor.

Technique "M" (page 47) was particularly convenient since it needed little immediate attention. Excellent results were achieved with cells in the early prophase stages of first meiosis. Chromosomes were well enough spread for pachytene karyotyping to be performed.

Staining.

Preparations stained and permanently mounted immediately, were superior to those made using slides that had been stored.

Aceto-Orcein was found satisfactory especially when staining was brief and phase-contrast employed in observation. The slides did not fade during a period of 3 years. The quality of stain obtained from the manufacturer was unreliable; careful testing before use was essential. Carbol Fuchsin was good for all stages of meiosis, although staining was not intense and phase-contrast was occasionally needed. Centromere regions were no more distinct than when light orcein staining and phase-contrast were employed. Carbol Fuchsin had the serious disadvantage that fading occurred after 3 to 4 days.

Toluidine Blue was adequate for both mitotic and meiotic preparations, but the hydrolysis step tended to detach the cells from the slide.

Feulgen processing also tended to detach the cells. The technique, using the different methods of obtaining solution "A", was not successful in staining air-dried chromosome preparations. Control slides, buccal smears from a female with normal chromosomes, showed good staining of the sex chromatin in each case. Eight minutes hydrolysis and one hour in solution "A" was satisfactory for sex chromatin.

Giemsa staining was successful for mitotic and meiotic preparations, but centromere regions at diakinesis were again not shown any more distinctly than when light orcein staining and phase-contrast were employed.

Interpretation of the Stages of Meiosis

The cells at spermatogonial metaphase diakinesis and metaphase II were distinctive. The prophase stages of all divisions were less so. The only clues to identification were cell size and the number of heteropycnotic elements.

Allowing for some error in interpretation, the sequence of spermatogenesis is illustrated by Plates 13 to 60. Stages that could be mutually confused are not included.

In each cell, only chromosome material was stained to any degree. Nucleoli were very lightly stained but most of these were destroyed by the hypotonic pre-treatment.

Spermatogonial Cell Division

Interphase spermatogonia could not be subclassed. They were round, had granular chromatin, no prominent nucleoli and were larger than Leydig and Sertoli cells. At prophase, the diploid number of chromosomes condensed.

At metaphase, coiling of the chromosomes was usually evident (Plate 13). This can be observed, although rarely, at mitotic metaphase and cannot be considered characteristic for somatic cells. Occasionally, meiotic metaphases which resembled those of somatic cells were observed. There was no coiling and chromatids were distinct (Plate 12b). These metaphases could have been either spermatogonial or somatic. The fact that they were found in testis cell preparations (Plate 12a) with a complete absence of germ cells (shown by histological section) strongly suggests they are actually somatic. No mitotic inhibitor was used to "harvest" the metaphases and so the rarity of this stage is explained.

Coiling frequently obscured the centromere position, so karyotyping was more difficult than when using leucocyte metaphases (Plate 14). Paracentric constriction regions were, however, more prominent. They did not contract as much as the rest of the chromosome and were the most exaggerated in cells showing maximal chromosome contraction. The length of the secondary constriction reflected the degree of polymorphism shown by mitotic karyotypes (Plates 15 and 16). Very contracted spermatogonial metaphases were not used when chromosomes were counted for evidence of nondisjunction. This was because the constriction regions tended to stain poorly. There was no fear of erroneously counting cells as hyperdiploid in the less contracted spreads, especially when using phase contrast.

There was no evidence of nondisjunction in any of the spermatogonial

metaphase counted or karyotyped. The exact number of cells analysed at spermatogonial metaphase is not available, but it was at least 450. A few spreads were hypodiploid, but the cells were "broken". It was difficult to establish whether any rearrangements were present because of the coiling which obscured the centromere position. None was suggested.

The sex chromosomes could be identified by their lengths and arm ratios and also by the fact that they were more compactly coiled than autosomes. This often gave the illusion of their being slightly more heteropycnotic.

Pre-meiotic Pairing of Homologous Chromosomes.

Evidence of pre-meiotic pairing in the prophase or metaphase stages of spermatogonial cell division was rare. There were only five spreads, where homologous "affinity" was in any way convincing (for example Plate 17). Statistical tests were not performed. Spermatogonial anaphase was never seen.

Prophase of First Meiosis

Leptotene and Zygotene.

The diploid number of elements appeared in leptotene. This prophase could be distinguished from that at the early prophase of spermatogonial division because the homologues, as they condensed, paired from telomeres situated to one side giving a typical bouquet configuration (Plates 18 and 19). Pairing in progress was observed in only 32 spreads in the whole investigation, but it was clearly shown to commence from both telomeres (Plate 19a). Centromere regions were only slightly more heteropycnotic so chromosome identification was difficult at this stage. Centromeres were not involved in the initiation of pairing. The sex chromosomes could not be identified and there was nothing which resembled the sex vesicle as seen at pachytene. Towards the end of pairing the chromosomes were condensed and their chromomeric nature manifest.

Pachytene.

By this stage, the bouquet configurations had largely disappeared. A relic occasionally remained in individual bivalents (Plate 22a). Chromomeres were present along each chromosome. Homologous chromomeres usually (but not always) matched (Plate 20a).

In early pachytene it was difficult to identify the centromere region as it had the appearance of another large chromomere. Nucleoli were prominent and acrocentric chromosomes were frequently associated with them (Plate 28).

Centromeric chromomeres of all five acrocentric chromosomes (13, 14, 15, 21 and 22) were occasionally in association (Plate 27). These also associated firstly with the sex vesicle (Plate 22a) and secondly with parts of chromosomes thought to correspond to secondary constrictions (Table 8). The behaviour of the acrocentrics made them easy to identify.

Table 8. Pachytene associations observed in 46 cells from JMA700635.

	ACROCENTRICS WITH SEX VESICLE	PARACENTRIC REGION OF 9 WITH ACROCENTRICS	PARACENTRIC REGION OF 9 WITH SEX VESICLE	OTHER ASSOCIATIONS
NUMBERS OF ASSOCIATIONS	32	5	6	15
PERCENTAGES	55	9	10	26

The sex vesicle was usually pear-shaped and any association was by the "thinner end" (Plate 26b). Within the vesicle the sex chromosomes could sometimes be seen looped back on themselves (Plates 32a and 37). This implies that the association occurred where one telomere from each sex chromosome abutted at the vesicular envelope. Occasionally, material which stained like the nucleoli was seen around the sex vesicle, even when other

chromosomes were not apparently vesicle-associated.

Tiny nucleoli (Plate 28 a and c) as well as larger ones (Plate 31) were sometimes present at telomeres. Telomeric participation in pachytene associations is frequent especially in relation to the sex vesicle (Plate 22a, 26a and 28b). The long-arm telomere of an acrocentric, already associated centromerically with a nucleolus, may bend round to give a second association with the same nucleolus. Heteropycnotic bodies apparently attached to bivalents were sometimes seen within nucleoli (Plate 30).

At every stage of meiosis, cells could be observed closer together than would be expected considering the method of preparation. Pachytene was no exception. Telomeres of pachytene bivalents were frequently orientated towards a smaller nucleus with granular chromatin (Plate 33).

Very few anomalies of pachytene pairing were observed. There was no "looping out" in one homologue to indicate a deficiency, duplication or inversion. Occasionally there was failure of pairing (Plates 34 and 35), as when a fully paired bivalent separated two otherwise paired homologous chromosomes (Plates 23b and 36).

By later pachytene, the chromosomes were more diffuse, leaving the centromere-region heteropycnotic. At this stage identification and karyotyping of bivalents became possible (Plates 22a, 24 and 25). Heteropycnotic bodies at centromere regions of different chromosomes were not all of the same size (Figure 3), but appeared characteristic within any one person as the variation between cells from the same individual was small.

Chromosome Nine.

One very distinctive configuration became even more apparent as the chromosomes relaxed. This was a cluster of heteropycnotic chromomeres each about 0.5 µm in diameter (Plate 21b), paracentric to a bivalent

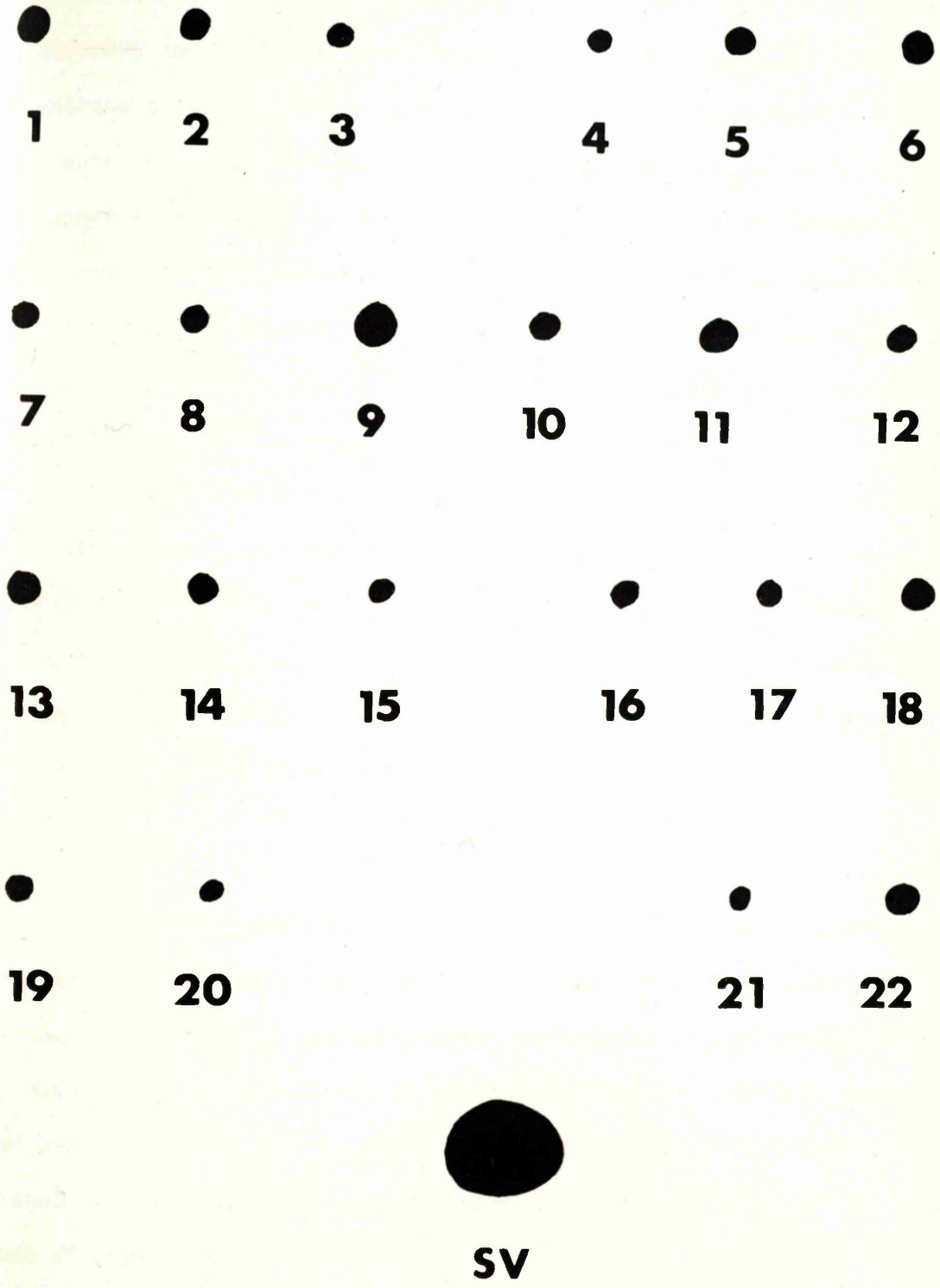


Figure 3. Heteropycnotic centromere regions in pachytene, X5,000.
(Based on tracings of micrographs of 5 cells from GM710110).

(Plate 21a) which karyotyping has shown to be a C and probably a 9 (Plates 22a and 25). The cluster, frequently "showered" to one side, was observed in every individual with meiosis. The degree of showering depended largely on the pre-treatment, fixation and the stage of pachytene. In JB690075 and CMC700362 there was an additional correlation between an extreme heteromorphism of mitotic nines and the size of a bulge below the centromere region of this bivalent at pachytene (Plate 21a). Such chromomeres were often in pairs and packets of four and occasionally intervened in regions of dislocation where the bivalent was stretched across the cell, with the long arms separated from the short arms and the centromere (Plate 21a). Four tenuous strands from the chromomeres appeared to bridge the gap.

The centromere and adjacent region behaved like that of the acrocentric chromosomes: it associated (a) with the sex vesicle (Plate 29b) as frequently as did any <u>one</u> acrocentric (Table 8), (b) with secondary constriction regions of other chromosomes, such as the paracentric constriction region of chromosome 16 (Plate 23); and (c) with (Plate 27b), or showered through (Plate 29a), nucleoli.

Chromosome 16.

HK710139 had extremely heteromorphic chromosomes 16 in mitotic metaphases (Plate 106). In meiosis, pachytene pairing was normal. Karyotyping demonstrated an unusually large centromeric heterochromatin mass in bivalent 16 (Plates 22 and 23). Its size in other individuals varied and was correlated with the degree of heteromorphism of mitotic 16's (compare Plates 22, 24 and 25). The paracentric region never "showered" as did that of chromosome 9.

Diffuse Stage.

Bivalents became even more diffuse, excepting the centromere regions, the paracentric chromomeres of number nine and the sex vesicle. These remained deeply heteropycnotic through most of the diffuse stage (Plate 38). In autosomal bivalents the centromere regions became clearly double (Plate 39), then moved apart by various small distances.

The X and Y chromosomes paired at a single terminal region. The bivalent at different stages showed a transition from being tightly looped within the sex vesicle to being fully extended. Sex chromosome centromere regions were relatively more heteropycnotic and the short arms appeared to lie adjacent.

Nucleoli had largely disappeared by this stage.

Diplotene.

Chromosomes recondensed (Plate 40b). The centromere regions of each homologue were observed as double heteropycnotic bodies while secondary constriction regions were pale (Plate 41b). In the diffuse stage, homologues had moved apart, except at chiasmata.

The diplotene stage was less frequently observed in man (hence was of shorter duration) than in the locust or the Syrian hamster. This was unfortunate because a maximum number of chiasmata becomes visible at this stage. For example, in Plate 41b, the bivalent to the right is probably number 2 and it appears that six chiasmata are present. The mean number for this bivalent at diakinesis was 3.90 (Table 18). However some of the "crossover points" are possibly twists and not real chiasmata and a total analysis is not possible since bivalents are invariably intertwined. Plate 41b also shows crossing over in the secondary constriction region of the short arm of an acrocentric D. This was probably not a twist since arm rotation should have been complete in a terminal region by this stage.

At diplotene and early diakinesis, homologous centromere regions

were occasionally interconnected by fine threads seemingly under tension (Plate 41b).

Diakinesis.

The chromosomes contracted further, resulting in an increased depth of heteropycnosis: the centromere regions became less distinct, but they remained visible in some bivalents of many spreads as double heteropycnotic bodies in each homologue (Plate 42a, 46 and 48). Secondary constriction regions appeared as discontinuities, sometimes extended, and were more exaggerated than was generally seen in mitotic metaphases (Plate 46a and 47a).

Most bivalents at diakinesis could be identified by their relative size, centromere position and the behaviour of secondary constriction regions. It was unusual for the full range of characteristics to be manifest by all bivalents of any one spread. Eliminating first those bivalents that could be positively identified the remainder could be assigned largely by size.

Characteristics of Individual Diakinesis Bivalents.

Number 1 had in the distal portion of the long arms a darkly staining region (Plate 48).

Number 2 often appeared the largest bivalent (Plate 48).

Number 3, although metacentric, was always smaller than 1 and sometimes appeared smaller than 4 and 5.

Numbers 4 and 5 were very alike, but could be distinguished on the basis of centromere position and by size. The distal region of the long arm of 5 was more heteropycnotic than that of 4 (Plate 48).

Number 6 showed the secondary constriction in the short arm more distinctly than that in the long arm. It was clearly larger than 7 and 8, but centromere regions were sometimes indistinct.

Numbers 7 and 8 were difficult to distinguish. Centromere regions had to be obvious in both for arm length to serve as a reliable identificatory criterion. Bivalent 7 was generally less heteropycnotic than was 8.
Number 9 was the easiest autosomal bivalent to identify: the paracentric constriction region appeared as a pale extended region (Plate 47a). This could cause the bivalent to appear as large as a 6.

Most individuals have heteromorphic mitotic 9's (Plate 10a). The vertical columns in Plate 47a are arranged in order of increasing heteromorphism of mitotic 9's. There is a good correlation between the asymmetry of the meiotic bivalent and the degree of mitotic heteromorphism. On three occasions a configuration, resembling the paracentric chromomeres of 9 at pachytene, was observed unilaterally in a diakinesis bivalent, shown, by karyotyping, to be a 9 (Plate 48). In each case, the marked mitotic heteromorphism was represented in the diakinesis bivalent by the chromomeric mass at one side.

Centromere regions were usually apparent but were not essential for the identification of this bivalent.
Number 10 was uniformly condensed with centromere regions consequently less often apparent. It could be distinguished from 12 by centromere position, and the fact that the latter had a smaller heteropycnotic centromere-region.
Number 11, although it has a paracentric constriction region, did not resemble 9 at diakinesis. The centromere region was in a more metacentric position than in any of the other C group bivalents (Plate 48).
Number 12 had a more heteropycnotic region in the distal part of the long arm.
Numbers 13, 14 and 15 were distinctive as a group owing to their size, heteropycnotic centromere regions and satellites. Thirteen was larger than 14 and 15 (Plate 48) which were more alike. In addition, 13 had

a discontinuity in the long arm and fifteen tended to be more heteropycnotic by comparison with 14.

Number 16 was the least heteropycnotic of the E group, had indistinct centromere regions and reflected the heteromorphism shown by mitotic 16's (Plate 49).

Number 17 was the most heteropycnotic of its group (Plate 43a) and tended to become compressed when there were only two chiasmata.

Number 18 was the most symmetrical of the E group (Plate 48).

Numbers 19 and 20 were distinguished from the E and G groups by size and centromere position, and from each other by the discontinuity in the long arm of 19.

Numbers 21 and 22 were the smallest bivalents and had heteropycnotic centromere regions and satellites, as did the D's. A discontinuity in the long arm of 21 distinguished the two.

The Sex Bivalent was distinctive. It usually stretched across the cell, the X and Y chromosomes paired end to end (Plate 48). The centromere regions were demonstrated less frequently than in the autosomes, but it was clearly shown that the short arms were usually involved in pairing. The Y tended to curl, but, in about 1% of the cells lay straight and had a heteropycnotic region at the unpaired end (Plate 45a). This might represent long arm pairing. In only thirteen spreads were X and Y chromosomes separated by an "apparent gap" (Plate 44b).

A G-bivalent frequently abutted onto one sex-chromosome telomere (Plate 45b). This was interpreted as a sign of a non-random arrangement of bivalents at this stage.

Univalent sex chromosomes (X/Y) were characteristic and unlikely to be confused with autosomal bivalents (Plate 43b). The Y tended to lie straight and the X tended to curl.

Occasionally, sex chromosomes showed a configuration, at their junction, that might be interpreted as a chiasma. It was usually in the

form of double dots but Plate 44a shows it as a tiny cross. This can be observed in autosomal bivalents with a single subterminal chiasma. Arms distal to the chiasma rotate forming a cross. A region of deeper heteropycnosis lay on each side of the cross in the sex bivalent in Plate 44a.

Details of chiasma analysis and conjunction of the sex chromosomes are given elsewhere (pages 85 to 103).

Anomalies found at diakinesis and likely to lead to unbalanced gametes are shown in Table 9 and Plate 51.

Table 9. Anomalies found in 1,182 diakinesis cells.

ANOMALY	CELLS AT DIAKINESIS	
	NO.	PERCENTAGE
XO primary spermatocytes	4	0.34
YO " " (Plate 51b)	1	0.09
G's as univalents (Plate 51a)	3	0.25
Extra element (Not X/Y or G's as univalents)	1	0.09
1 element missing - apparent random loss	14	1.19
Detectable translocation	1	0.09
2 elements missing - apparent random loss	7	0.59
More than 2 elements missing	2	0.17
TOTAL	33	2.81

Interlocked Autosomal Bivalents, and the Sex Bivalent Looped Through an Autosomal Bivalent.

With close examination and careful focussing many cases of suspected "interlocking" and "looping through " (Plate 50a) were found to be caused by simple overlapping. However, apparently genuine instances of 21 of the former and 20 cases of the latter were found.

Karyotyping revealed the bivalents likely to be involved (Tables 10 and 11).

Table 10. Autosomal bivalents involved in "interlocking".

BIVALENTS	A			B		C						D			E			F		G	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
AS LARGER	2		1	2	1	2	1	1	2	3	2	1		1	1						1	
AS SMALLER				1	1	1		2	1	1	3	1	1			3	1	1			1	3
TOTAL	2		1	2	2	3	2	1	4	4	3	4	1	2	1	3	1	1			2	3

(= 42 Bivalents, 21 instances)

Table 11. Autosomal bivalents looped through by the sex bivalent.

END NEAREST TO THE AUTOSOMAL BIVALENT			AUTOSOMAL BIVALENT																			
			A			B		C						D			E			F		G	
X	Y	?	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
5	12	3	1	1	1	1		1	1	2	1	2						1			1	4	1	2

(= 20 autosomal bivalents)

Almost all bivalents seemed capable of participating and there was no correlation between these phenomena and chromosome size or other characteristics. No conclusions can be drawn from the non-involvement of any chromosome,

since small numbers of cells were analysed. In sex-chromosome looping, the autosomal bivalent was more frequently nearer the "Y end".

Metaphase I

Bivalents were never seen aligned in a metaphase plate. In this study metaphase I was usually taken to be the most contracted form of diakinesis, a stage that cannot be used for chiasma analysis.

Plate 53a shows homologues (and indeed chromatids) separated. This was possibly in anticipation of first meiotic anaphase, but this was an exceptional spread and interpretation was difficult.

Metaphase II

Chromosomes contract into metaphase II (Plate 53b) so some kind of interphase must have occurred.

No evidence of nondisjunction was obtained from an analysis of at least 450 spreads. Any "abnormalities" found could be explained by anomalous coiling.

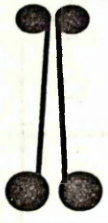
It was possible to karyotype some metaphase II spreads using the usual criteria, size and position of the centromere and extended secondary constriction regions (Plates 54a, 57 and 58) but it was difficult to detect small chromosomal rearrangements because of longitudinal contraction and coiling. Centromere regions sometimes moved apart, but in that case the chromosome arms remained aligned (Plate 54b). The long and short arms frequently rotated (Plate 54a) but this proved no problem with respect to identification.

Chromosome 9 could again be identified by the behaviour of its paracentric constriction region (Plate 56). Chromosome 9 tended to lie towards the periphery of the spread with its arms conspicuously condensed. It was easy to identify, even when the rest of the complement was obscure

(Plate 56d). As for spermatogonial metaphase and diakinesis, the configuration closely followed the type of polymorphism of mitotic chromosomes 9. Their behaviour at metaphase II was best illustrated in CMC700362 and JB690075 although there were fewer metaphase II spreads in these patients, since CMC had severe orchitis possibly caused by brucellosis and JB a mild maturation arrest.

Three different populations of metaphase II could be demonstrated in each patient (Table 12). One population had the anomalous chromosome 9 showing both long-arms long (LL - Plate 56a), one with both long-arms short (SS - Plate 56c) and a third with one long and one short long-arm (LS - Plate 56b). The three populations were quite distinct. JB had more LS cells, 6 out of 24, then CMC with one out of 20.

Table 12. Crossing over in chromosome 9 as demonstrated by an analysis of metaphase II in 2 individuals with heteromorphic mitotic 9's.

SUBJECT	 LL	 LS	 SS	TOTAL
CMC700362	8	1	11	20
JB690075	8	6	10	24

Referring to their diakinesis analysis (Tables 14 and 15) CMC had a significantly lower mean total-chiasma-count, 54.82, compared with that of JB, 57.86 (t-test, $P < 0.001$). A large difference in mean count is shown for their nines (Tables 18 and 19 and Figure 10), 2.21 and 2.79

respectively. The difference in means was much larger than that for the C group as a whole, values being 2.67 and 2.63 respectively. JB must have had more cells with a third chiasma in bivalent 9 (Plate 47a). A positive correlation is therefore shown between chiasma analysis at diakinesis and crossing over as demonstrated by an analysis of metaphase II.

HK710139 had slightly heteromorphic 9's as well as extremely heteromorphic 16's (Plate 10b). A similar analysis was attempted for 60 metaphase II spreads, from this case. Of the 22 spreads, informative for both 9 and 16 (Plates 57 and 58) one long/short 16 and two long/short 9's were found (Table 13). Scoring for number 9 was less accurate because the degree of heteromorphism was small.

In all scorings, SS and LL chromosomes segregated in a 1 : 1 manner.

Table 13. Crossing over in chromosomes 9 and 16 as demonstrated by an analysis of metaphase II from HK710139.

NINES			SIXTEENS		
SS	LS	LL	SS	LS	LL
8	2	12	11	1	10

Sex Chromosomes and Metaphase II.

Spreads could be scored as "X-bearing" or "Y-bearing" (Plate 54). As for spermatogonial metaphase, the sex chromsomes (especially the X) sometimes appeared slightly more heteropycnotic (Plate 55).

Of those spreads photographed for metaphase II karyotyping, 59 cells (64%) carried a Y and only 33 (36%) carried an X. This is significantly different from the expected (50%). (χ^2, $P < 0.01\%$). It is not suggested that different numbers are produced. An alternative

explanation, such as that Y-bearing metaphase II cells are preserved well-spread and with more distinct chromosomes than the X-bearing ones, seems more likely.

Spermiogenesis

In the spermatid, condensation of the chromatin began at what was to be the acrosomal head then extended back uniformly (Plate 59). The tail was formed and the cytoplasm passed down the tail as a droplet (Plate 60). The presence of sex chromosomes in spermatids could not be conclusively demonstrated and there did not appear to be any morphological difference between the X and Y-bearing sperm although refraction dots could be mistaken for sex chromosomes (Plate 60c).

Sex Chromosome Reduction

The segregation of the sex chromosomes was 100% pre-reductional, i.e. before the separation of the sister chromatids. Evidence included:

1. The X and Y appeared to have 2 chromatids present in univalents at diakinesis (Plate 43b), never as two single paired XY elements or 4 separate elements.
2. Some diakinesis sex-chromosomes were separated by a gap (Plate 44b) and had tightly paired chromatids.
3. Karyotyping always showed metaphase II spreads with <u>either</u> an X <u>or</u> a Y. Two chromatids were always present (Plate 54).

Polyploidy

No cells, at any stage, were convincingly polyploid. Karyotyping "apparently polyploid" cells, from two sides, revealed full sets of chromosomes. Adjacent cells in synchrony were therefore responsible.

No multivalents (which might be indicative of polyploidy at an earlier stage) were found at diakinesis.

Summary of Observations on the Human Pre-Meiotic and Meiotic Cycles

Much of the material used was selected from an infertile population but there is no reason to believe that the behaviour of chromosomes was abnormal, since an identical picture was presented in a series of controls.

The meiotic sequence was established by the examination of a large number of intermediate stages.

It was observed that:

1. Cells at spermatogonial metaphase could be identified by the presence of a diploid number of chromosomes and by a coiling characteristic of this stage. Chromosomes could be identified using their relative sizes and positions of extended paracentric constriction regions. Centromere positions were often obscured by the coiling and by the fact that chromatids were not usually distinct.

   No evidence of nondisjunction was obtained in over 450 cells.

   There was slight evidence for pre-meiotic pairing of homologous chromosomes.

2. In the early prophase of first meiosis, chromosomes condensed and paired from the telomeres which all lay to one side in a "bouquet". Not even the sex chromosomes could be identified at this stage.

3. At pachytene most chromosomes could be positively identified firstly by their relative size and secondly by the position of heteropycnotic centromere regions, which also varied in size between chromosomes and between the same chromosomes in different individuals. In chromosome 16, for example, their size reflected the degree of mitotic heteromorphism. Chromosome 9 could be identified by its paracentric constriction region which exhibited a shower of chromomeres about 0.5 μm in diameter. Behaviour in non-random association was also useful for chromosome identification at this stage.

4. A diffuse stage was present after pachytene and before diplotene. Only the centromere regions, the sex vesicle and the paracentric chromomeres of number 9 retained their previous morphology and staining characteristics.

Homologous centromere regions moved apart and the sex vesicle unravelled to leave an extended sex bivalent. The chromosomes then recondensed into the stage called diplotene.

5. Diplotene was infrequently observed. When it was, bivalents could rarely be identified as they were invariably intertwined.

6. Diakinesis, reached after further chromosome condensation, was the stage at which chiasma analysis could be performed. Bivalents could be identified by their relative size, the position of heteropycnotic centromere regions (showing as double bodies in each homologue), and by the position of secondary constriction regions (either appearing extended or as discontinuities). Mitotic heteromorphism in chromosomes 9 and 16 was usually reflected by the asymmetry of corresponding bivalents at diakinesis.

When the sex chromosomes paired, they generally did so by their short arms, where there was some cytological evidence of a chiasma.

Most autosomes seemed capable of participating in apparent interlocking and looping with the sex bivalent.

Of 1,182 cells, 2.8% showed anomalies that could have led to unbalanced gametes.

7. A form of interphase is likely to exist between metaphase I and metaphase II.

8. Cells at metaphase II could be identified by the presence of the haploid number of chromosomes. These displayed a coiling characteristic of this stage. Chromosomes could be distinguished by their relative size, the position of the centromere (visible as a constriction) and some by extended paracentric regions. The paracentric constriction

regions in chromosomes 9 and 16 reflected mitotic polymorphism. Crossing over in these two pairs of chromosomes, at a point between the secondary constriction region and the centromere, could be demonstrated by an analysis at metaphase II.

No evidence of nondisjunction was obtained in over 450 cells. Sex chromosomes could be identified by their size, slightly deeper staining and the fact that their arms tended to align more closely, than in the autosomes. Segregation of the sex chromosomes was always pre-reductional.

9. The identity of sex chromosomes in spermatids or sperm was not established by the methods employed.

Analysis of Chiasmata and Conjunction of the Sex Chromosomes at Diakinesis

Total Chiasma Count per Cell.

A population of 1,152 cells was analysed at mid diakinesis (Plate 42b). Chiasmata in the autosomes were scored. It was not assumed that a chiasma existed between the X and Y chromosomes when they were adjacent, but the percentage of cells in which they were present as univalents (Plate 43b) was calculated.

Plotting the total cell chiasma counts for each individual showed a normal distribution about a mean (for example Figure 4). Means in 45 individuals varied from 44.62 to 63.80 (Tables 14 and 15). Standard deviations (SD = a figure including about two-thirds of the observations) also varied between 2.06 and 6.46, with a mean of 4.28.

No case was found with cells all showing a consistently low chiasma count. There were indeed only 11 cells throughout the entire study which showed a chiasma count of under 30 (Plate 52), and these probably represented extremes of the normal distribution.

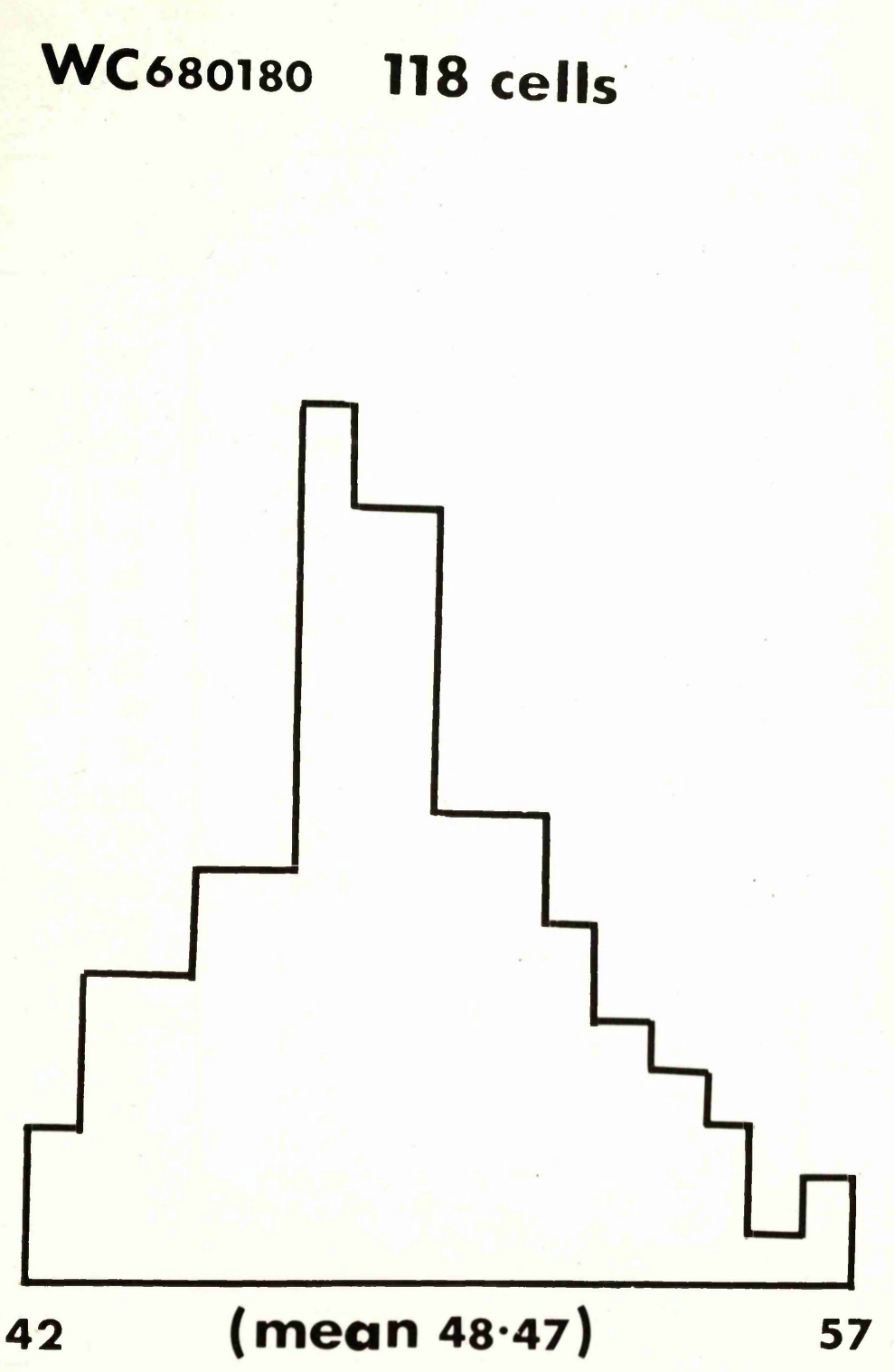


Figure 4. Variation in total chiasma count in cells from WC680180.

Table 14. Mean total-chiasma-count and conjunction of the sex chromosomes in 23 "controls".

CASE	AGE	MEAN TOTAL-CHIASMA-COUNT	STANDARD DEVIATION	NO. OF CELLS (Total 849)	% X/Y
AS680045	31	51.23	4.34	22	18.18
PMC680170	39	55.25	4.99	8	20.00
WC680180	33	48.47	3.37	118	1.60
WT690067	28	53.09	4.77	11	9.09
RC690124	27	45.28	2.82	25	8.00
JG690141	27	55.12	3.22	26	15.38
JP690168	30	50.08	3.63	12	41.67
DD690194	34	53.97	3.92	63	13.85
JB690219	31	49.22	6.46	14	33.33
AB690334	32	51.44	3.85	16	25.00
TS690371	49	51.50	5.00	12	16.67
JT700005	71	47.42	3.58	12	50.00
WMD700011	67	44.62	2.66	13	45.15
WF700071	25	50.50	5.39	12	16.67
AG700098	49	53.23	6.17	13	15.38
JS700110	65	49.15	4.68	20	45.00
JT700346	29	52.61	4.03	36	13.89
CMC700362	53	54.82	3.57	57	29.82
PML700552	30	49.00	4.98	36	25.00
RMC700596	34	53.50	4.26	48	4.17
JMA700635	39	54.53	5.77	15	0.00
HK710139	50	50.18	5.33	44	25.71
RW710174	37	51.28	4.57	18	27.78
MEANS	39.57	51.11 SD 2.97	4.41	36.91	21.84 SD 14.16

Table 15. Mean total-chiasma-count and conjunction of the sex chromosomes in 22 infertiles.

CASE	AGE	MEAN TOTAL-CHIASMA-COUNT	STANDARD DEVIATION	NO. OF CELLS (Total 303)	% X/Y
RMA680117	28	54.44	5.90	41	12.20
KMI680120	30	52.55	4.69	11	9.09
JC690026	37	55.92	5.14	12	16.67
JD690068	32	55.29	4.61	8	0.00
GS690074	31	55.13	4.21	15	6.67
JB690075	23	57.86	2.87	22	4.35
BC690104	37	59.50	4.93	14	14.29
JMG690142	37	50.64	5.16	14	0.00
RC690167	28	55.13	4.41	14	0.00
RME690178	28	46.57	3.55	8	57.14
DMC690184	24	53.82	6.42	11	0.00
RW690226	42	56.00	3.92	11	18.18
TJ700101	37	51.09	3.42	11	9.09
DW700153	24	51.00	2.20	14	0.00
WG700237	25	54.13	4.60	16	6.25
JW700429	23	53.45	6.70	20	25.00
AC700506	35	63.80	5.22	8	0.00
JG700577	30	52.40	4.39	8	0.00
TB700653	31	56.00	5.66	8	0.00
AB710009	35	50.50	2.06	8	0.00
GM710110	28	53.88	3.12	17	29.41
DH710125	28	53.92	4.75	12	50.00
MEANS	30.59	54.23 SD 3.50	4.45	13.77	11.74 SD 16.11

There was no correlation between mean total-chiasma-count and age (correlation coefficient - 0.29, which is not significant)(Figure 5). One feature, however, was that the "control" population (albeit a mixed collection and with an age mean significantly higher, 39.57 compared with the infertiles' 30.59) had a significantly lower mean total-chiasma-count 51.11 SD 2.97 compared with infertiles 54.23 SD 3.50 (t-test, P = 0.01 - 0.001). This is also indicated by the clumping of points in Figure 5.

The percentage of cells with the X and Y as univalents (X/Y) also varied enormously from case to case (Tables 14 and 15) from 0%, or a figure too small to be detected with few counts, to 57.14%.

The "controls" had a higher mean X/Y%, 21.84 SD 14.16 by comparison with "infertiles", 11.74 SD 16.11. There was a positive correlation of disjunction and advancing age in controls (correlation coefficient 0.63 $P < 0.01$) (Table 14 and Figure 6), and a negative correlation of percent separate X/Y with mean total-chiasma-count (correlation coefficient -0.45, $P < 0.05$) (Figure 7).

For each individual, the mean chiasma count per cell was calculated independently for cells with the X and Y together (XY) and those where they were separated (X/Y) (Table 16). In 12 out of 40 cases the mean chiasma count per cell was higher (+) for XY as compared with X/Y and in 28, lower (-), a significant difference (χ^2, P = 0.02 to 0.01; sign test, $P < 0.01$; and t-test, $P \simeq 0.01$). The difference was at least one chiasma, since adding one to each X/Y mean changed the minus value to plus in those cells marked with an asterisk in Table 16 giving 17+ and 23-, figures which were not significantly different (P = 0.5 to 0.3). Scoring diakinesis cells from controls, 60 at a stage slightly earlier and 60 at a stage slightly later, than that used for the analysis, and recording the state of the X and Y (Table 17) showed that, in the former, 9 cells (15.00%), and in the latter, 11 cells (22.45%), were X/Y separate, not

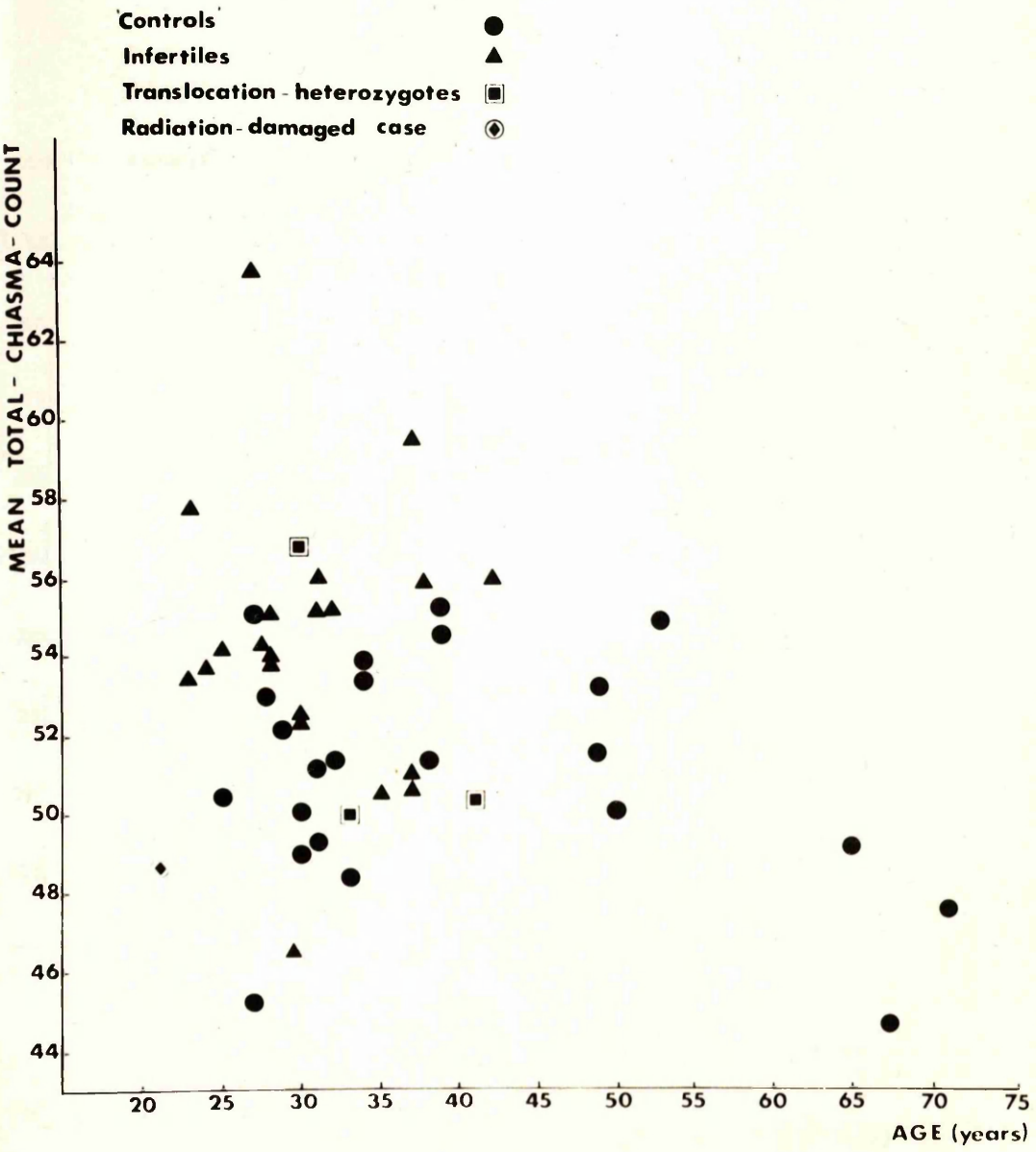


Figure 5. Mean total-chiasma-count plotted against age.

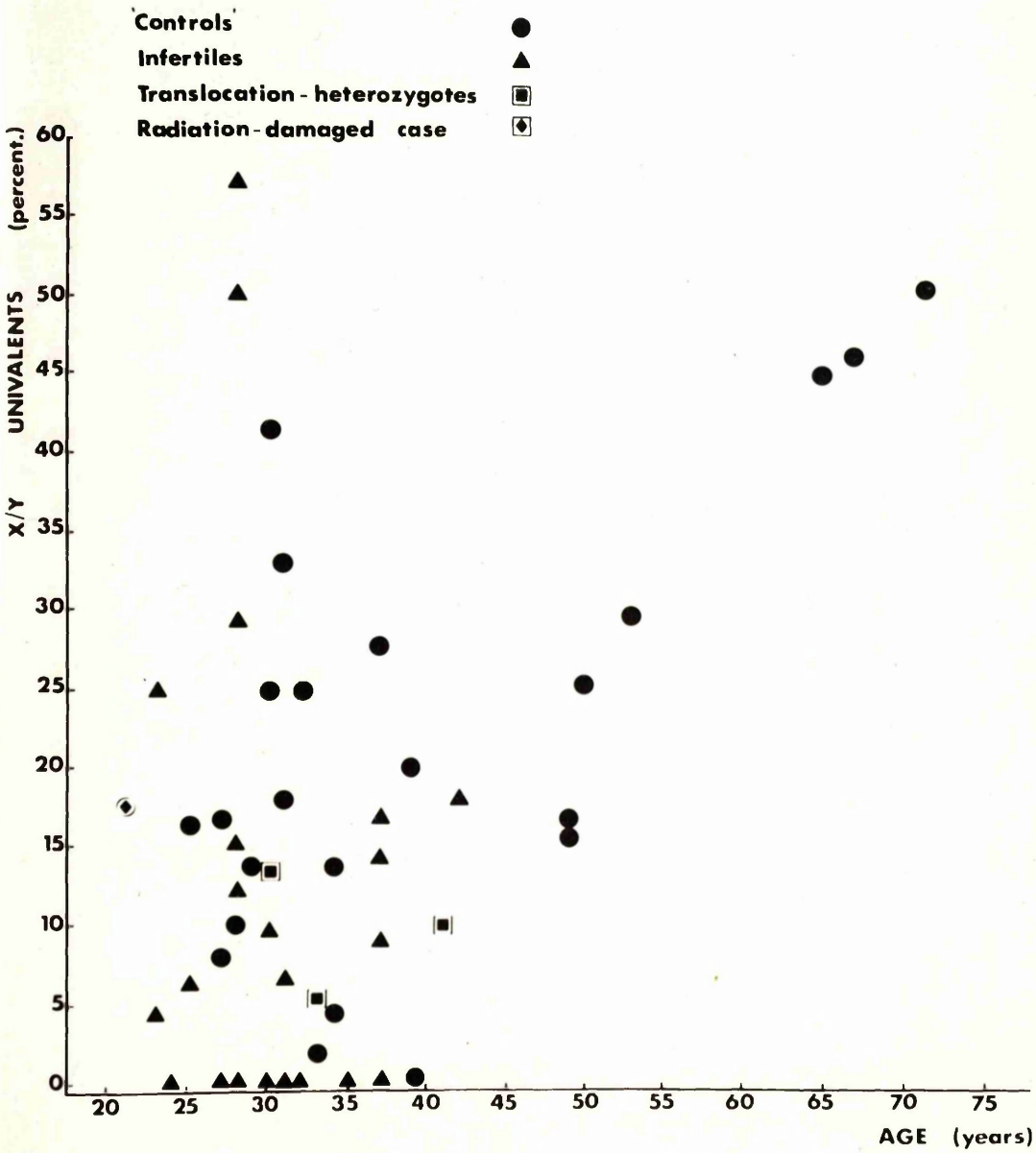


Figure 6. Percentage of separate X/Y plotted against age.

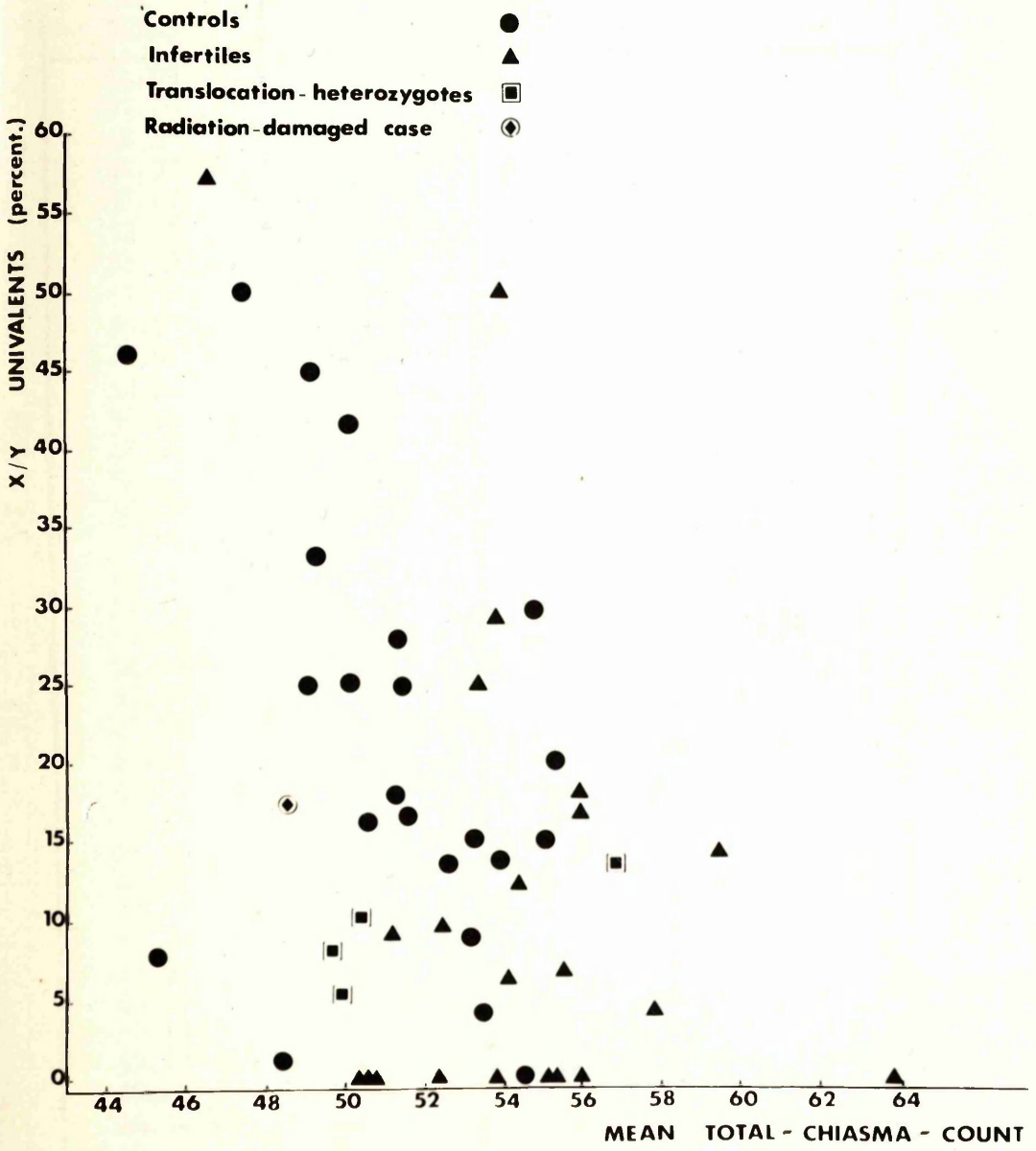


Figure 7. Percentage of separate X/Y plotted against mean total-chiasma-count.

Table 16. Mean total-chiasma-count of cells, with the X and Y together, and separate, for each individual with at least one X/Y separate cell.

MEAN TOTAL-CHIASMA-COUNT		DIFFERENCE X/Y HIGHER (+) OR LOWER (-)
XY CELLS	X/Y CELLS	
54.69	52.40	-
57.00	51.00	-
48.46	49.00	+
51.37	50.33	-
37.00	50.50	-
52.70	57.00	+
55.07	56.00	+
57.90	57.00	-*
59.92	57.00	-
45.04	48.00	+
52.54	57.33	+
50.14	50.00	-*
48.67	45.00	-
54.35	51.38	-
50.00	49.50	-*
48.29	52.50	+
45.14	44.00	-
51.11	51.00	-*
50.66	47.33	-
54.53	48.00	-
52.97	50.40	-
48.00	50.71	+
55.03	54.35	-*
49.79	48.67	-
55.13	48.40	-
57.27	54.25	-
49.48	47.56	-
53.43	55.00	+
54.42	52.60	-
53.67	54.17	+
49.99	51.11	+
52.15	49.00	-
53.72	51.00	-
50.40	51.00	+
48.50	46.33	-
50.60	56.00	+
53.08	46.50	-
57.11	51.00	-
50.17	47.33	-
52.90	49.00	-
TOTAL = 40		12+ 28-
*denotes change of - to + when one is added to X/Y value giving:		17+ 23-

Table 17. Total chiasma analysis at early and late diakinesis in "Controls".

60 Early Diakinesis Spreads		60 Late Diakinesis Spreads	
Chiasma count	X/Y	Chiasma count	X/Y
53	-	49	+
62	-	49	-
54	-	54	-
52	-	46	-
54	-	56	-
52	-	56	-
57	-	50	-
47	-	52	-
51	-	47	+
40	-	57	-
66	-	53	-
56	-	63	-
56	-	47	-
54	-	54	-
54	-	60	-
52	-	47	-
54	-	56	-
56	-	47	+
55	-	54	-
53	-	52	-
53	-	47	+
54	+	48	-
55	+	47	-
56	-	56	+
55	-	47	-
63	+	47	-
63	-	48	+
50	+	55	-
47	-	50	+
53	-	47	+

(contd.)

Table 17 contd.

60 Early Diakinesis Spreads		60 Late Diakinesis Spreads	
Chiasma count	X/Y	Chiasma count	X/Y
50	-	49	-
58	-	47	+
43	-	56	-
51	-	48	-
54	-	55	-
51	-	52	-
43	-	43	-
54	-	48	-
50	-	43	-
47	-	47	-
55	-	52	-
49	-	48	-
47	-	50	-
56	+	53	-
39	-	44	-
46	-	48	-
58	-	46	-
58	-	49	-
57	-	54	-
55	-	51	-
63	+	55	-
56	-	45	+
62	-	51	-
62	+	66	-
52	-	59	-
54	-	51	+
57	+	67	-
54	-	55	-
46	+	50	-
44	-	50	-
Mean = 53.30 SD 5.57	Total 9+ 51- (15%) (85%)	Mean = 51.22 SD 5.09	Total 11+ 49- (22%) (78%)

a significant difference (χ^2, P = 0.70 to 0.50). Therefore, there was little evidence of precocious disjunction of the sex chromosomes over this range of diakinesis, and therefore even less for those used for detailed chiasma analysis.

The mean total-chiasma-count for those cells in early and late diakinesis was 53.30 SD 5.57 and 51.22 SD 5.09 respectively, a difference just significant (t-test, P = 0.05 to 0.02), a slightly higher count in "early" compared with "late" diakinesis.

Chiasma Count per Bivalent.

Any estimate of the mean number of chiasmata per bivalent is only meaningful if the bivalents can be identified. Only those cells (a total of 441) showing a full range of characteristics necessary for identification, were used in this study.

Two bivalents, 7 and 8, were difficult to distinguish. This was not as serious as might be expected, since they usually carried the same number of chiasmata. The largest number of chiasmata recorded for any bivalent was 8 (in chromosome 2). In only 3 cells were autosomes present as univalents and these were G's.

The mean number of chiasmata for each bivalent in 37 individuals (Tables 18 and 19) was plotted against mitotic chromosome length (Figures 8, 9 and 10). The number of chiasmata per bivalent increased according to chromosome size. Smaller chromosomes had more, and larger ones had fewer, chiasmata than would be expected if the number was in direct proportion to size. In a case with a lower chiasma count overall, the decrease in chiasmata per bivalent was in proportion to size (Figure 9). Some bivalents repeatedly showed more chiasmata than expected, for example 10, 17 and 20, and others fewer, for example, 6, 9 and the G's (Figure 11).

The summed mean value per bivalent was calculated for 21 "controls" and for 16 infertiles (Tables 18 and 19). The values for the two groups

are shown, plotted with chromosome size in Figure 11. It was confirmed that infertiles had a higher count which was reflected proportionally throughout the complement.

Table 18.

98

Table 18. Number of chiasmata per bivalent as shown at diakinesis in 21 "controls"

CASE NUMBER	AGE	CHIASMATA PER BIVALENT																						Mean	No. of Cells Keryotyped
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		
AS680045	31	3.71	3.07	3.00	2.86	2.50	2.57	2.57	2.29	2.21	2.43	2.29	2.43	2.07	2.21	1.93	2.21	2.29	1.86	1.64	1.86	1.43	1.21	2.30	14
WC680180	33	3.85	3.22	2.83	2.72	2.54	2.50	2.54	2.50	2.24	2.22	2.24	2.19	1.76	1.81	1.81	2.09	1.83	1.94	1.63	1.61	1.11	1.09	2.20	54
WT690067	28	3.83	4.00	3.17	2.50	2.83	3.17	2.83	2.67	2.50	2.83	2.67	2.50	2.17	2.33	1.67	2.00	2.00	2.00	2.00	1.67	1.67	1.33	2.48	6
RG690124	27	3.38	3.00	2.38	2.38	2.38	2.25	2.25	2.25	2.13	2.25	2.13	2.13	1.75	1.63	1.75	2.00	2.00	1.75	1.63	1.63	1.25	1.25	2.07	8
JG690141	27	4.10	4.20	3.80	2.90	3.40	3.10	2.80	2.80	2.60	2.90	2.70	2.60	2.30	2.40	2.30	2.00	2.60	2.20	1.70	2.00	1.40	1.10	2.63	10
JP690168	30	3.60	4.20	2.60	3.20	2.40	2.40	2.20	2.60	2.20	2.00	2.00	2.00	2.20	1.80	2.00	2.00	2.00	1.80	1.80	2.00	1.40	1.40	2.26	5
DD690194	34	4.39	4.44	3.44	3.22	3.22	2.72	2.67	2.67	2.56	2.44	2.39	2.44	2.06	1.78	1.72	2.06	2.17	1.67	1.94	2.11	1.67	1.28	2.50	18
JB690219	31	4.22	3.78	3.56	3.33	3.11	2.56	2.44	2.67	2.44	2.22	2.00	2.22	2.00	2.00	1.89	2.11	1.89	2.11	1.67	1.67	1.11	1.33	2.38	9
AB690334	32	4.70	4.20	3.60	3.10	2.60	2.90	2.50	2.40	2.20	2.40	2.10	2.50	2.00	1.90	1.80	2.00	2.00	1.90	1.90	1.90	1.20	1.00	2.40	10
TS690371	49	3.80	4.20	3.80	2.80	2.80	2.60	2.40	2.60	2.40	2.60	2.00	2.20	2.00	2.00	2.00	2.00	2.00	1.80	1.80	2.00	1.20	1.00	2.36	5
JT700005	71	3.86	3.29	2.71	2.86	2.57	2.43	2.43	2.14	2.14	2.43	2.14	2.00	1.57	1.71	2.00	2.00	2.00	1.86	2.14	1.86	1.14	1.00	2.19	7
WF700071	25	3.83	3.17	2.67	2.83	2.50	2.17	2.17	2.33	2.50	1.83	2.17	1.67	2.00	1.83	1.67	2.00	2.00	2.00	1.83	2.00	1.67	1.33	2.19	6
AG700098	49	4.71	4.43	3.43	3.71	2.57	2.57	2.43	2.43	2.86	2.57	2.43	2.43	2.14	2.14	1.71	2.00	2.00	2.00	1.86	2.00	1.29	1.14	2.49	7
JS700110	65	3.60	4.10	2.90	3.10	2.90	2.30	2.30	2.10	2.40	2.30	2.00	2.30	1.70	1.90	1.60	2.00	2.00	1.70	1.70	2.00	1.10	0.90	2.22	10
JT700346	29	4.26	3.87	3.26	3.13	2.91	2.78	2.78	2.57	2.48	2.52	2.26	2.43	2.22	1.87	1.96	2.17	2.26	2.00	1.91	1.87	1.30	1.17	2.45	23
CMC700362	53	4.05	4.37	3.68	3.37	2.79	2.89	2.74	2.79	2.21	2.74	2.79	2.26	2.21	2.16	2.11	1.95	2.32	1.74	1.84	1.53	1.37	1.32	2.51	19
PML700552	30	3.64	3.76	2.84	2.72	2.92	2.48	2.44	2.44	2.28	2.20	2.44	2.16	1.96	1.68	1.72	2.04	2.00	2.00	1.84	1.80	1.16	1.04	2.25	25
RMC7005.6	34	4.24	4.00	3.00	3.06	2.82	2.65	2.65	2.59	2.47	2.41	2.24	2.41	1.94	2.00	1.88	2.06	2.18	1.94	1.76	1.82	1.24	1.00	2.38	17
JMA700635	39	4.70	4.70	3.20	3.20	3.70	3.10	2.50	2.60	2.40	2.50	2.60	2.30	2.20	2.20	2.10	2.00	2.10	2.10	1.70	2.00	1.40	1.30	2.57	10
HK710139	50	3.63	3.75	2.83	3.04	2.88	2.71	2.21	2.50	2.20	2.25	2.13	2.36	1.92	1.75	1.92	2.08	2.00	1.96	1.88	1.75	1.25	1.25	2.28	24
RW710174	37	4.20	4.10	2.80	3.20	3.30	2.80	2.70	2.30	2.50	2.60	2.30	2.30	2.20	1.90	1.80	2.00	2.00	1.90	2.00	1.70	1.20	1.10	2.40	10
Total		84.30	81.85	65.50	63.23	59.64	55.65	52.55	52.24	49.92	50.64	48.02	47.83	42.37	41.00	39.34	42.77	43.64	40.23	38.17	38.78	27.56	24.54		297
Mean	38.29	4.01	3.90	3.12	3.01	2.84	2.65	2.50	2.49	2.38	2.41	2.29	2.28	2.02	1.95	1.87	2.04	2.08	1.92	1.82	1.85	1.31	1.17	2.36	
	SD 10.44	SD 0.39	SD 0.49	SD 0.45	SD 0.31	SD 0.35	SD 0.28	SD 0.20	SD 0.20	SD 0.18	SD 0.26	SD 0.24	SD 0.21	SD 0.19	SD 0.28	SD 0.17	SD 0.06	SD 0.17	SD 0.14	SD 0.14	SD 0.16	SD 0.18	SD 0.14		
% of 51.91 Total Cell Mean		7.72	7.51	6.01	5.80	5.47	5.10	4.82	4.80	4.58	4.64	4.41	4.39	3.89	3.76	3.60	3.93	4.01	3.70	3.51	3.56	2.52	2.25		

Table 19.

99

Table 19. Number of Chiasmata per bivalent as shown at diakinesis in 16 infertiles

CASE NUMBER	AGE	CHIASMATA PER BIVALENT																						Mean	No. of cells Karyotyped
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		
RMA680117	28	4.47	3.89	3.32	3.05	3.05	2.95	2.95	2.63	2.79	2.63	2.79	2.47	2.37	2.26	2.00	2.05	2.11	1.84	1.84	1.74	1.26	1.16	2.53	19
KMI680120	30	4.11	3.56	3.33	2.78	3.00	2.78	2.89	2.89	2.78	2.56	2.56	2.11	2.00	1.78	2.11	2.11	2.22	2.00	1.78	1.22	1.56	1.00	2.41	9
JC690026	37	3.89	3.78	3.67	3.22	3.11	3.33	3.00	2.89	2.67	3.00	2.44	2.78	2.33	2.44	1.89	2.11	2.67	2.11	2.22	1.89	1.56	1.22	2.65	9
JD690068	32	4.60	3.60	3.60	3.20	3.40	2.80	2.80	2.60	2.40	2.80	2.80	2.80	2.00	2.20	2.00	2.00	2.40	2.00	1.80	1.80	1.40	1.20	2.55	5
GS690074	31	4.38	3.85	3.31	2.92	2.85	3.08	2.69	2.77	2.38	2.69	2.54	2.38	2.00	2.08	2.08	2.31	2.46	2.08	2.08	1.85	1.38	1.31	2.52	13
JB690075	23	4.38	4.13	3.63	3.25	3.50	3.25	3.25	3.13	2.75	2.63	2.50	2.63	2.13	2.13	2.00	2.13	2.50	2.13	1.88	1.75	1.25	1.13	2.64	8
BC790104	37	4.71	4.14	3.57	3.57	3.29	3.14	3.29	3.14	2.57	2.86	3.00	2.71	2.43	2.00	1.86	2.14	2.71	1.86	2.00	1.71	1.57	1.57	2.72	7
JW690142	37	4.17	3.83	3.00	3.17	3.00	3.17	2.83	2.83	2.33	2.33	2.67	2.17	2.00	1.67	1.67	2.00	2.67	2.33	1.67	1.33	1.67	1.67	2.44	6
RG690167	28	4.25	4.63	3.75	2.50	3.13	2.88	3.25	2.63	2.75	2.88	2.50	2.25	2.25	2.25	2.00	2.00	2.25	2.00	1.63	1.63	1.00	1.25	2.57	8
RME690178	28	3.00	3.80	2.20	2.60	2.20	2.20	2.40	2.60	2.40	2.40	2.20	2.00	2.20	1.40	2.00	2.00	2.00	1.60	2.00	1.80	1.40	1.00	2.15	5
DM069[illegible]184	24	4.17	4.33	3.33	3.83	4.00	3.17	2.83	2.67	2.33	2.50	2.83	2.67	1.83	2.17	2.67	2.17	2.17	2.00	2.00	2.00	1.67	1.33	2.64	6
KM690226	42	4.25	4.75	4.25	4.25	3.00	3.25	2.75	3.50	3.00	2.25	2.00	2.25	2.00	1.75	2.25	2.00	2.00	2.00	2.00	2.00	1.25	1.25	2.64	8
WG700237	25	4.00	3.69	3.23	3.23	2.85	2.69	2.69	3.08	3.08	2.62	2.31	2.23	2.00	2.23	2.31	2.00	2.31	2.00	1.69	1.69	1.12	1.31	2.35	13
JW700429	23	4.38	4.75	3.75	3.25	2.88	3.38	3.13	3.13	2.38	2.50	2.88	2.63	2.50	2.00	2.00	2.25	2.13	2.00	1.88	2.00	1.13	1.38	2.65	8
GM710110	28	4.20	4.50	3.00	3.10	3.00	2.70	2.50	2.50	2.50	2.60	2.40	2.70	2.10	2.00	1.90	2.10	2.00	1.90	2.00	1.90	1.50	1.20	2.47	10
DH710125	28	4.00	4.60	3.80	3.10	3.10	2.60	2.20	2.40	2.70	2.10	2.10	2.20	2.30	1.80	2.00	2.00	2.00	2.10	1.90	1.70	1.50	1.20	2.43	10
TOTAL		66.96	65.83	54.74	50.95	49.36	47.37	45.45	45.39	41.11	41.35	40.52	38.98	34.44	32.16	32.74	33.37	36.60	31.95	30.37	27.94	22.22	20.18		144
Mean	30.06	4.19	4.11	3.42	3.18	3.09	2.96	2.84	2.84	2.57	2.58	2.53	2.44	2.15	2.01	2.05	2.09	2.29	2.00	1.90	1.75	1.39	1.26	2.53	
	SD 5.62	SD 0.40	SD 0.42	SD 0.52	SD 0.43	SD 0.38	SD 0.32	SD 0.31	SD 0.29	SD 0.21	SD 0.24	SD 0.32	SD 0.27	SD 0.22	SD 0.27	SD 0.12	SD 0.08	SD 0.25	SD 0.18	SD 0.16	SD 0.23	SD 0.20	SD 0.17			
% of 55.64 Total Cell Mean		7.53	7.39	6.15	5.72	5.55	5.32	5.10	5.10	4.62	4.64	4.55	4.39	3.86	3.61	3.68	3.76	4.12	3.59	3.41	3.15	2.50	2.26		

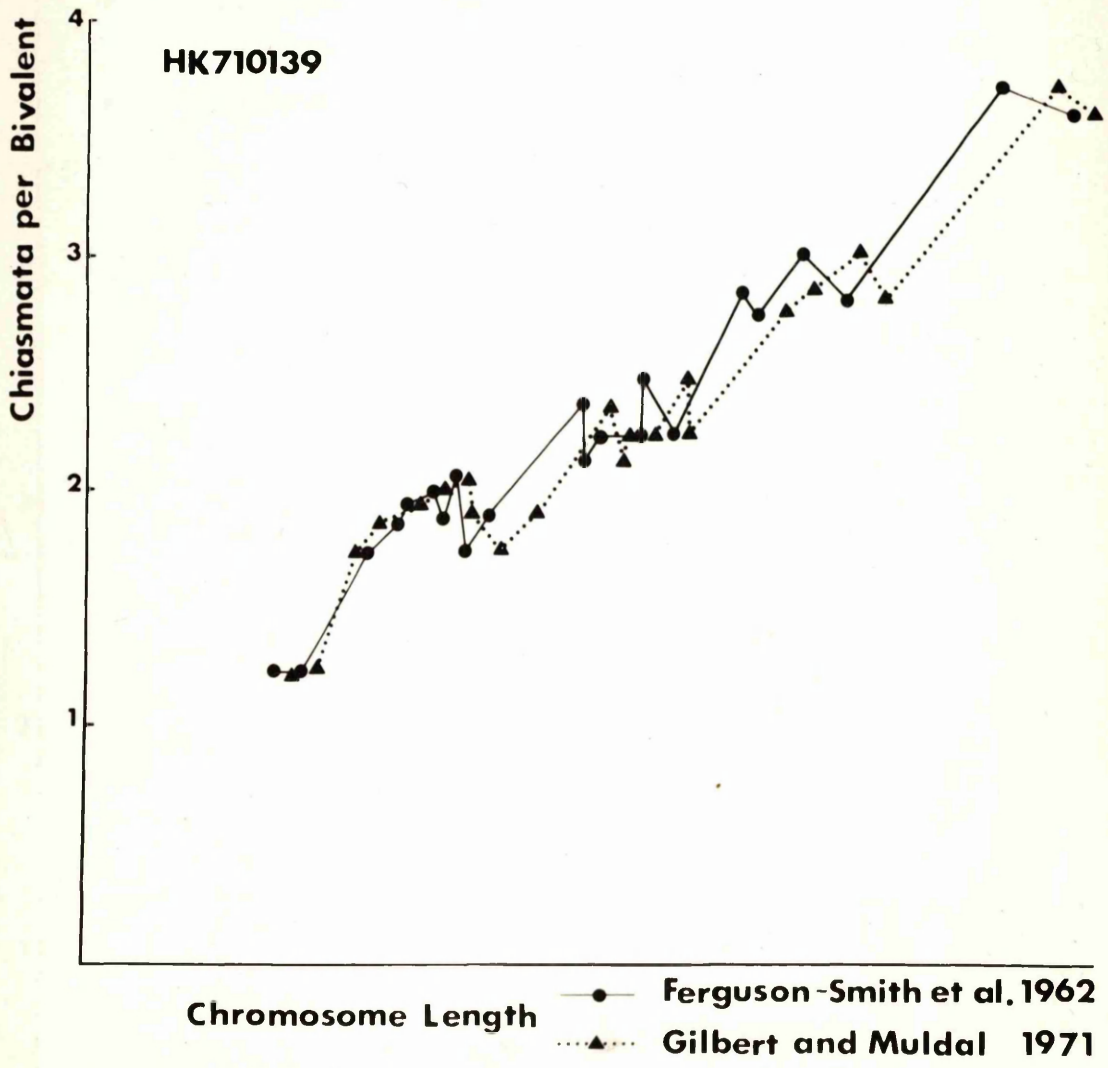


Figure 8. Mean number of chiasmata per bivalent plotted with two different estimates of mitotic chromosome length.

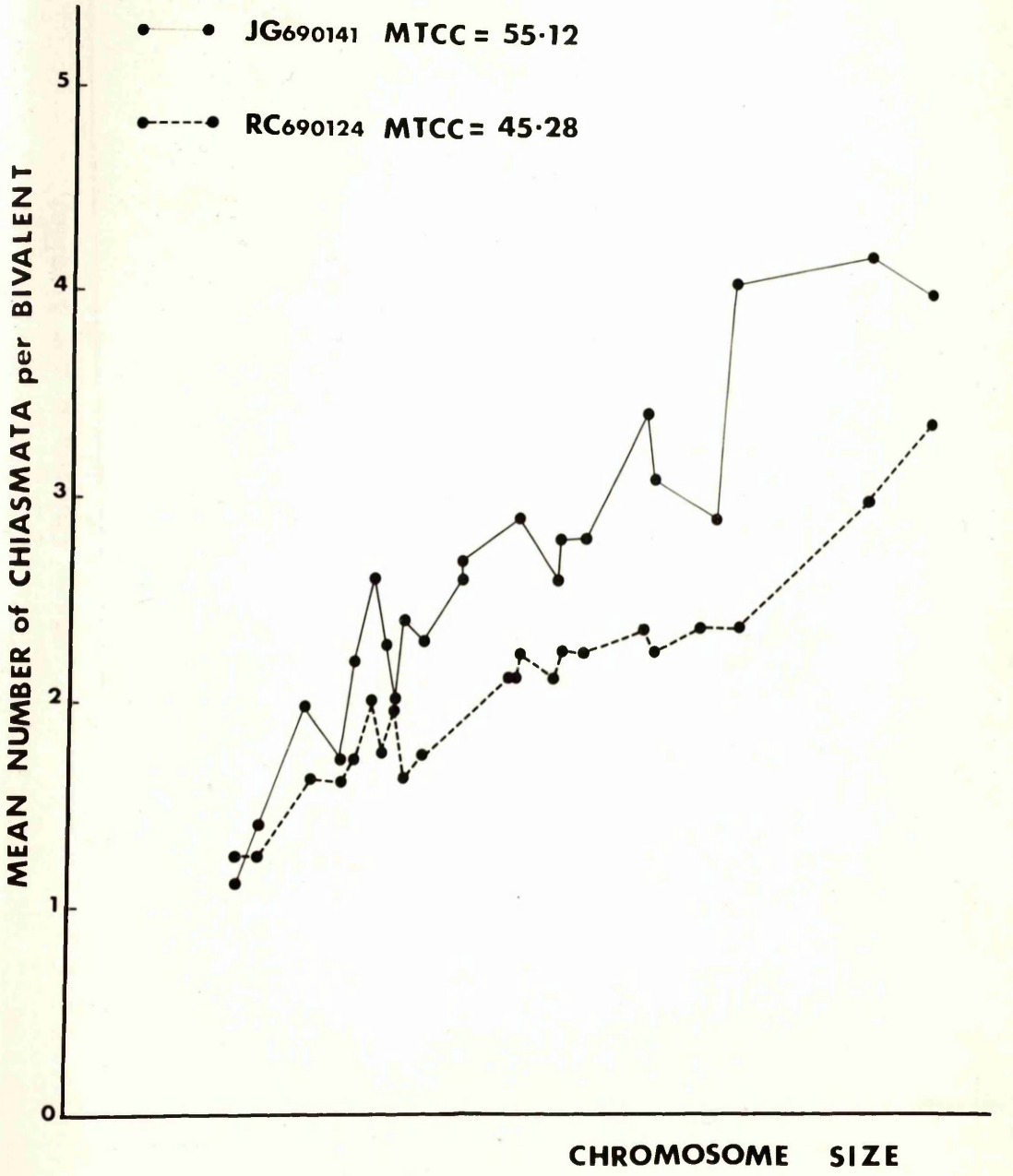


Figure 9. Mean number of chiasmata per bivalent in 2 cases, one with a high and one a low chiasma count, plotted with chromosome length.

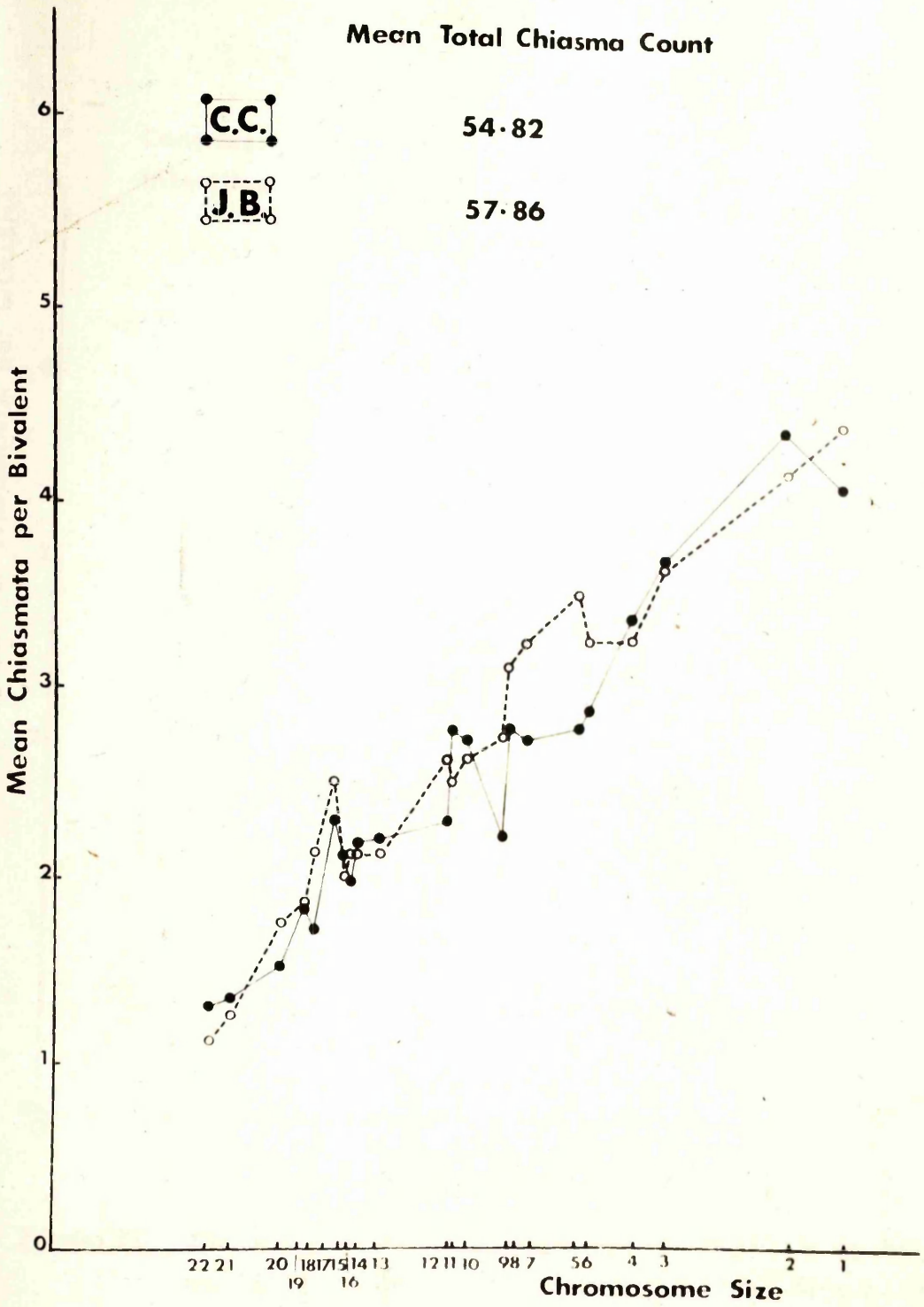


Figure 10. Mean number of chiasmata per bivalent in CNC700362 compared with that from JB690075.

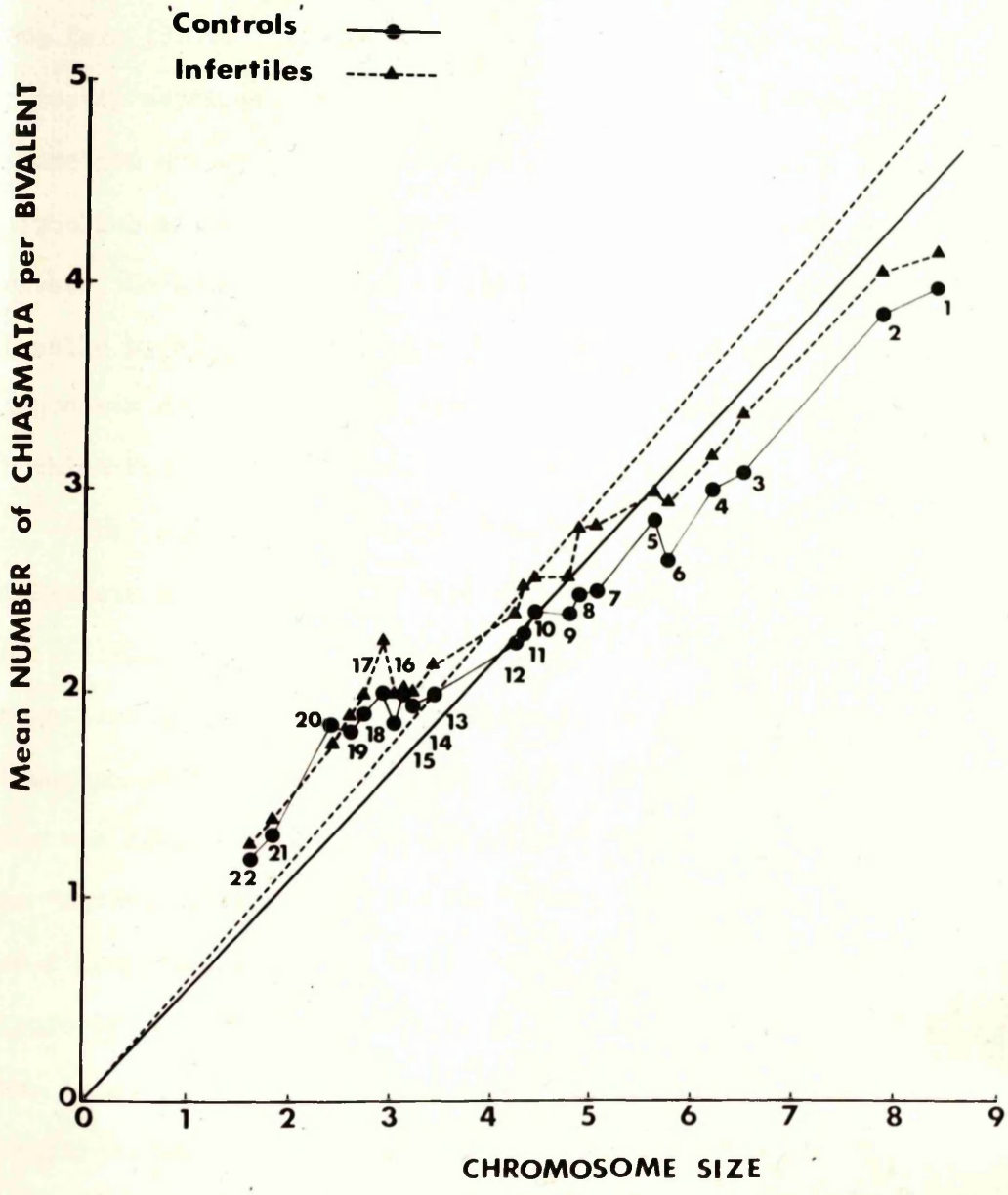


Figure 11. The mean values for chiasmata per bivalent from 21 "controls" and 16 infertiles plotted against chromosome length. (The straight lines through the origin assume a zero chiasma count for a chromosome of zero length, and extrapolate to points of mean chiasma count and overall mean chromosome size.)

Maturation Arrest

Meiotic analysis, even in normal material revealed a few degenerating cells. In these cells the chromatin clumped and there was loss of chromosome form (Plate 61). In those patients with "maturation arrest" evident in testis sections, degeneration was exaggerated. Classification of maturation arrest is based on the gross appearance of the germinal epithelium and is described as: spermatogonial, spermatocyte or spermatid arrest. However, air-dried chromosome preparations showed degeneration of cells in all stages of meiosis. Absence of degeneration at later stages was apparent only in cases with a severe degeneration (Plate 3a) in which few cells survived the earlier stages.

The relative proportions of cells at spermatogonial metaphase, diakinesis and metaphase II were scored in preparations from each of 32 individuals (Tables 20, 21 and 22). One might suppose a disproportionately large percentage of cells at spermatogonial metaphase, with fewer cells at metaphase II, should be present in a "maturation arrest" condition. This was clearly so in some cases, for example, in Table 20, AC700506 has "maturation arrest". However, JW700429 has the same condition and had a large proportion of metaphase II cells (Table 20). Comparison of figures from cases supposed to have "maturation arrest" (Table 20), those from "controls" (Table 21) and those from infertiles without arrest (Table 22) emphasises the wide variation and also the poor reliability of the spermatogonial metaphase: diakinesis: metaphase II scoring method to demonstrate maturation arrest.

A comparison of the number of cells (at all stages of spermatogenesis) showing signs of degeneration, with the number of "metaphase stages" and expressed as a percentage might prove a more reliable guide (see Table 23).

Table 20. Metaphase scoring in cases classified histologically as "maturation arrests".

CASE NUMBER	PERCENTAGES		
	SPERMATOGONIAL METAPHASE	DIAKINESIS	METAPHASE II
JMG690142	17	66	17
RME690178	29	54	17
RW690226	26	57	15
WG700237	45	50	5
CHC700362	34	58	8
JW700429	39	40	22
AC700506	94	6	0

Table 21. Metaphase scoring in "controls".

CASE NUMBER	PERCENTAGES		
	SPERMATOGONIAL METAPHASE	DIAKINESIS	METAPHASE II
JP690168	48	41	10
DD690194	10	60	30
JB690219	22	50	28
AB690334	25	59	16
TS690371	20	49	31
JT700005	25	44	31
WAD700011	26	59	15
AG700098	18	49	33
WF700071	5	50	45
JS700110	14	60	26
JT700346	7	56	37
PHL700552	18	51	31
RHC700596	11	44	45
JMA700635	23	38	40
HK710139	31	38	31
RW710174	11	46	43

Table 22. Metaphase scoring in infertiles not classified as having "maturation arrest".

CASE NUMBER	PERCENTAGES		
	SPERMATOGONIAL METAPHASE	DIAKINESIS	METAPHASE II
RC690167	6	76	18
DMC690184	28	47	25
TJ700101	29	64	7
DW700153	40	40	20
JG700577	6	75	19
TB700653	54	38	8
AB710009	50	21	29
GW710110	18	47	35
DH710125	37	33	31

Table 23. Proportions of cells in degeneration.

CASE NUMBER	CATEGORY	PERCENTAGES			
		DEGENERATED	SPERMATOGONIAL METAPHASE	DIAKINESIS	METAPHASE II
JW700429	Infertile with maturation arrest	24	39	40	22
DH710125	Infertile with partial absence of germ cells	2	37	33	31
HK710139	Control	1	31	38	31

Normal Variant Small Y from DD690194

Plate 62a shows the small Y at leucocyte mitotic metaphase. Sections of testis and chromosomes in air-dried preparations from this patient were found to be normal and the results obtained have been included in the "control" series (Tables 14, 18 and 21).

At spermatogonial metaphase (Plate 62b) the Y was identified by its peripheral position (usual for the Y) and by its denser staining.

At pachytene (Plate 63a) the sex vesicle appeared slightly smaller but otherwise was normal.

At diakinesis the small size of the Y was demonstrated (Plate 63b). The proportion of cells with separated X/Y (Plate 63c), 13.85% (Table 14), was unremarkable ("control" mean was 21.84% SD 14.16). Two abnormal diakinesis cells were observed. One cell included two bivalents, possibly numbers 5 and 9 largely of pachytene appearance (Plate 64a). The other cell included a configuration that could be interpreted as a duplication of most of one chromosome 1 (Plate 64b). However, neither of these abnormalities could be shown to have any connection with the small Y.

At metaphase II the Y could again be identified, as its chromatids remained closely aligned (Plate 64c).

It was confirmed that the small Y was a normal variant; its small size but normal behaviour was observed throughout meiosis.

Translocation Heterozygotes

Testis cell preparations, of material from four translocation heterozygotes, using techniques "C" and "F" (pages 46 and 47, respectively) were analysed in great detail, at every stage of meiosis. Stages suitable for analysis were karyotyped. In each heterozygote, at least one of the translocation chromosomes was apparent at mitotic metaphase (Table 24).

Table 24. Chromosomes involved in translocation.

CASE NUMBER	CHROMOSOMES WITH OVERALL CHANGE OF MATERIAL			
	MITOTIC ANALYSIS		MEIOTIC ANALYSIS	
	LOSS	GAIN	LOSS	GAIN
H.....	18p-	?	18p-	5q+
RW680009	5p-	8q+	5p-	8q+
JD690204	14q-	?	14q-	7p+
EA690217	11q-	15q+	11q-	15q+

1. Heterozygote H....., t(5q+ 18p-).

The mitotic karyotype (Plate 65a) shows that two-thirds of the short arm of chromosome 18 is apparently missing. In spite of the small amount of material involved, the exchange seems to have been asymmetrically reciprocal, since every diakinesis cell showed 22 elements (23 if the X and Y were separate), including a quadrivalent (Plate 65b). Crossing over between homologous material must have occurred. Configurations of the quadrivalent are roughly classed in Figure 12. Karyotyping the bivalents revealed that the other chromosome involved in the translocation was number 5. Close examination of the quadrivalent showed that chromosome 18 material must have been on the long arm (Table 24).

Results of chiasma analysis are shown in Tables 25, 30 and 31 and in Figure 13; conjunction of the sex chromosomes in Table 30.

Table 25. H....., t(5q+ 18p-). Quadrivalent chiasma analysis on 37 of a total of 42 diakinesis cells.

TOTAL QUADRIVALENT CHIASMA COUNT	4		5		6	
NO. OF CHIASMATA IN E CHROMOSOME MATERIAL	1	2	1	2	1	2
NO. OF CHIASMATA IN B CHROMOSOME MATERIAL	3	2	4	3	5	4
NO. OF CELLS WITH THE CONFIGURATION INDICATED BY THE CHIASMA NUMBERS SHOWN ABOVE	14	5	4	9	2	3
MEAN CHIASMA COUNT PER CELL	50.68		48.92		49.40	

2. Heterozygote RW680009, t(5p- 8q+).

The mitotic karyotype (Plate 66a) shows an apparent deletion of two-thirds of the short arm of chromosome 5, and possibly a longer long arm of chromosome 8. The relative numbers of cells in the different metaphases of spermatogenesis are shown in Table 29.

Again, every spread showed a quadrivalent (Plates 66b and 67). Proportions of quadrivalent configurations can be assessed from Table 26. Karyotyping the bivalents confirmed the other chromosome involved in the translocation to be number 8 (long arm) (Table 24). Results of chiasma analysis are shown in Tables 26, 30 and 31 and in Figure 13; conjunction of the sex chromosomes in Table 30.

Metaphase II analysis frequently demonstrated cells with a short chromosome 5 (Plate 68) but numbers of cells analysed were small. There was no cytological evidence of crossing over, such as would be indicated at this stage by chromosomes with unequal arms.

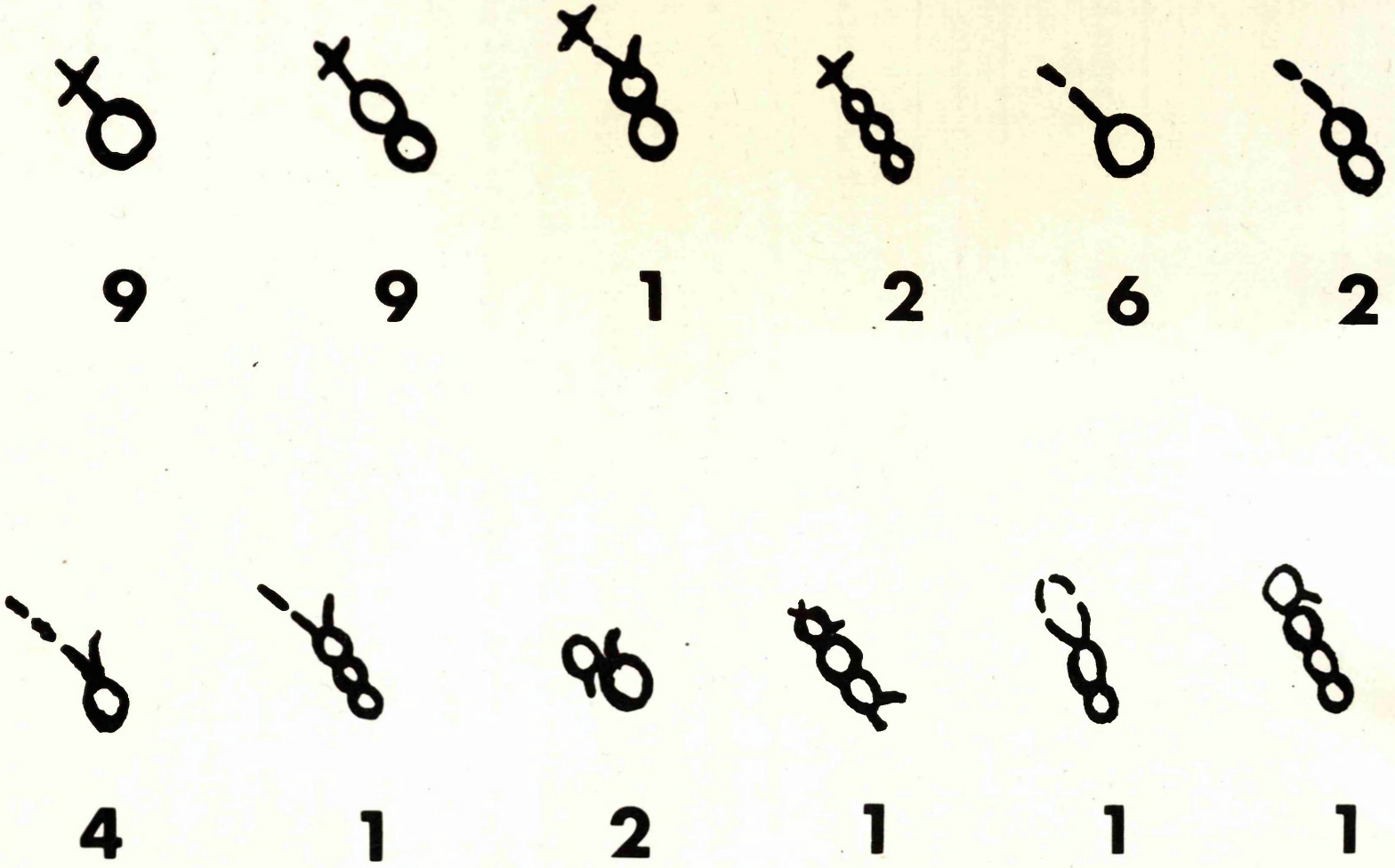


Figure 12. Configurations of quadrivalents at diakinesis in the heterozygote H....., t(5q+ 18p-). Numbers of each type are shown.

Table 26. RW680009, t(5p- 8q+). Quadrivalent chiasma analysis on 51 of a total of 59 diakinesis cells.

TOTAL QUADRIVALENT CHIASMA COUNT	4		5	6		7		8		9	
NO. OF CHIASMATA IN C CHROMOSOME MATERIAL	1	2	2	2	3	3	4	3	4	4	3
NO. OF CHIASMATA IN B CHROMOSOME MATERIAL	3	2	3	4	3	4	3	5	4	5	6
NO. OF CELLS WITH THE CONFIGURATION INDICATED BY THE CHIASMA NUMBERS SHOWN ABOVE	1	3	4	1	18	14	1	2	5	1	1
MEAN CHIASMA COUNT PER CELL	50.50		55.25	56.42		57.93		60.14		64.0	

3. Heterozygote JD690204, t(7p+ 14q-).

The mitotic karyotype (Plate 69a) shows a large deletion of one-third of the long arm of a D chromosome, suggested by autoradiography to be a number 14. The piece of chromosome involved was larger than in the previous two cases. Metaphase population structure is shown in Table 29.

One or two cells at pachytene could possibly be interpreted as showing evidence of the translocation, as such cells had one quadrivalent at this stage (Plate 69b).

At diakinesis every cell showed a quadrivalent (Plates 70 and 71). Proportions of configurations can be assessed from Table 27. Karyotyping the bivalents revealed the other chromosome involved in the translocation to be number 7 (long arm) (Table 24). Results of chiasma analysis are shown in Tables 27, 30 and 31 and in Figure 13; conjunction of the sex chromosomes in Table 30.

Table 27. JD690204, t(7p+ 14q-). Quadrivalent chiasma analysis on 27 of a total of 38 diakinesis cells.

TOTAL QUADRIVALENT CHIASMA COUNT	4	5		6		7
NO. OF CHIASMATA IN D CHROMOSOME MATERIAL	2	2	1	3	2	3
NO. OF CHIASMATA IN C CHROMOSOME MATERIAL	2	3	4	3	4	4
NO. OF CELLS WITH THE CONFIGURATION INDICATED BY THE CHIASMA NUMBERS SHOWN ABOVE.	5	11	1	5	4	1
MEAN CHIASMA COUNT PER CELL	47.20	50.58		48.22		56.00

4. Heterozygote EA690217, t(11q- 15q+).

The mitotic karyotype (Plate 72a) shows a large deletion of the long arm of chromosome 11 and extra material on chromosome 15 (identified by autoradiography). Metaphase population structure is shown in Table 29. There was little evidence of "maturation arrest" in sections of testis but there were large numbers (14%) of degenerating cells in the air-dried preparations. Again, every diakinesis cell showed a quadrivalent. This is considered as confirming a reciprocal exchange and indicating the chromosomes involved (Table 24). Proportions of quadrivalent configurations can be assessed from Table 28. Results of chiasma analysis are shown in Tables 28, 30 and 31 and Figure 13.

Metaphase II analysis showed both balanced and unbalanced complements. Plate 73 may show a chromosome 15 with a long and a short long arm (and hence a previous crossover), but coiling at metaphase II makes this stage difficult to interpret.

Table 28. EA690217, t(11q- 15q+). Quadrivalent chiasma analysis on 34 of a total of 40 diakinesis cells.

TOTAL QUADRIVALENT CHIASMA COUNT	3	4	5	
NO. OF CHIASMATA IN C CHROMOSOME MATERIAL	2	2	2	3
NO. OF CHIASMATA IN D CHROMOSOME MATERIAL	1	2	3	2
NO. OF CELLS WITH THE CONFIGURATION INDICATED BY THE CHIASMA NUMBERS SHOWN ABOVE	2	25	4	3
MEAN CHIASMA COUNT PER CELL	47.20	49.36	52.71	

Table 29. Metaphase population structure in translocation heterozygotes.

CASE NUMBER	PERCENTAGES		
	SPERMATOGONIAL METAPHASE	DIAKINESIS	METAPHASE II
RW680009	13	65	24
JD690204	16	66	19
EA690217	9	55	36

Comparison of Cells from Translocation Heterozygotes

Karyotyping of the spermatogonial metaphases revealed the chromosomes in translocation only where the change in chromosome size was large. Coiling obscured small differences.

Few pachytene configurations were sufficiently well spread to ascertain convincingly the quadrivalent formed by homologous pairing, although certain

chromosomes could be positively identified and excluded (Plate 69b). In no case was any failure of pairing detectable at pachytene.

Diakinesis analysis gave more positive information. In every case a quadrivalent was present giving 22 elements in the spread rather than 23 (23 only in the X/Y separated cells). A quadrivalent could be identified in the majority of spreads, and its presence suggested that all the translocations studied were reciprocal. Karyotyping using criteria previously described gave positive determinations for chromosomes not involved in the quadrivalent, and identified the "missing" chromosomes by difference. Centromere regions in the quadrivalent were obvious in most of the diakinesis spreads (Plate 66b) and showed whether short or long arms were involved in the translocation.

Total Chiasma Count per Cell in Translocation Heterozygotes

The overall mean chiasma count per cell was 51.72 SD 3.44 (Table 30), which was not significantly different from that from the "control" population, 51.11 SD 2.97 (t_s = 0.23, P = 0.90 to 0.80) (Table 14 and Figures 5 and 7). Mean proportion of cells with separated X/Y was 8.99% SD 3.62 (Table 30) which was again unremarkable (Figures 6 and 7).

Chiasma Count per Bivalent in Translocation Heterozygotes

Calculation of the number of chiasmata per bivalent, including an estimate for the translocation chromosomes indicated figures for the translocation heterozygotes (Table 31) closely similar to those for controls (Table 18). Deciding which chromosome the material including a chiasma belonged to was fairly difficult, especially in H....., but was usually possible from a consideration of the position of the centromeres and from the asymmetry of the quadrivalent (chiasmata were therefore scored as though the translocated segment was still on its original chromosome).

Translocation had little effect on the chiasma count in the chromosomes

involved (Figure 13). A slight increase above the count due to the proportional change in size appeared to be present in the larger chromosome in H..... and JD690204, and in the smaller chromosome in RW680009 and JD690204. There was never a decrease in chiasma count in the larger chromosome but there was a decrease in the smaller chromosome in H.....

The number of chiasmata in the quadrivalent in 3 of the cases increased with total chiasma count per cell (Tables 26, 27 and 28), but this trend was not so obvious in H..... (Table 25).

Metaphase II cells occasionally showed which chromosomes had translocations (Plates 68 and 73) but there were insufficient spreads to obtain information on the frequency of balanced and unbalanced complements, caused by crossing over. When small amounts of material were involved, the metaphase II cells did not appear abnormal.

Table 30. Mean chiasma count per cell and conjunction of the sex chromosomes in 4 translocation heterozygotes.

CASE NUMBER	AGE	MEAN CHIASMA COUNT PER CELL	STANDARD DEVIATION	NUMBER OF CELLS	X/Y%
H.....	?	49.71	3.74	42	7.14
RW680009	30	56.86	5.07	60	13.56
JD690204	33	49.97	4.09	38	5.26
EA690217	41	50.32	4.14	40	10.00
MEANS	34.67	51.72 SD 3.44	4.26	45	8.99 SD 3.62

Table 31.

Table 31. Number of chiasmata per bivalent as shown at diakinesis in four translocation heterozygotes.

CASE NUMBER	AGE	CHROMOSOME NUMBER																					MEAN	NUMBER OF CELLS KARYOTYPED
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
H.....	?	3.39	3.29	2.96	2.68	(3.11)	2.50	2.46	2.29	2.29	2.32	2.36	2.14	1.96	2.07	1.79	1.86	2.11	(1.61)	1.93	1.86	1.46	1.11	2.25	28
RW680009	30	4.30	4.58	3.73	3.42	(3.42)	3.03	2.85	(2.97)	2.45	2.61	2.52	2.45	2.15	2.06	2.03	2.06	2.21	1.97	1.91	1.88	1.33	1.21	2.59	33
JD690204	33	3.41	3.88	3.18	2.94	2.53	2.35	(2.71)	2.24	2.29	2.35	2.23	2.18	1.88	(2.29)	1.82	2.00	2.00	1.88	2.00	1.71	1.12	1.12	2.18	17
EA690217	41	3.58	3.79	2.83	3.13	2.88	2.38	2.46	2.38	2.21	2.42	(2.08)	2.21	2.08	1.83	(2.08)	2.00	1.88	1.75	1.83	1.58	1.08	1.04	2.25	24
TOTAL		14.68	15.54	12.70	12.17	11.94	10.26	10.48	9.88	9.24	9.70	9.19	8.98	8.07	8.25	7.72	7.92	8.20	7.21	7.67	7.03	4.99	4.48	51.61/22	102
MEAN	34.67	3.67	3.89	3.18	3.04	2.99	2.57	2.62	2.47	2.31	2.43	2.30	2.25	2.02	2.06	1.93	1.98	2.05	1.80	1.92	1.76	1.25	1.12	2.35	25.5

Circles denote translocation chromosomes

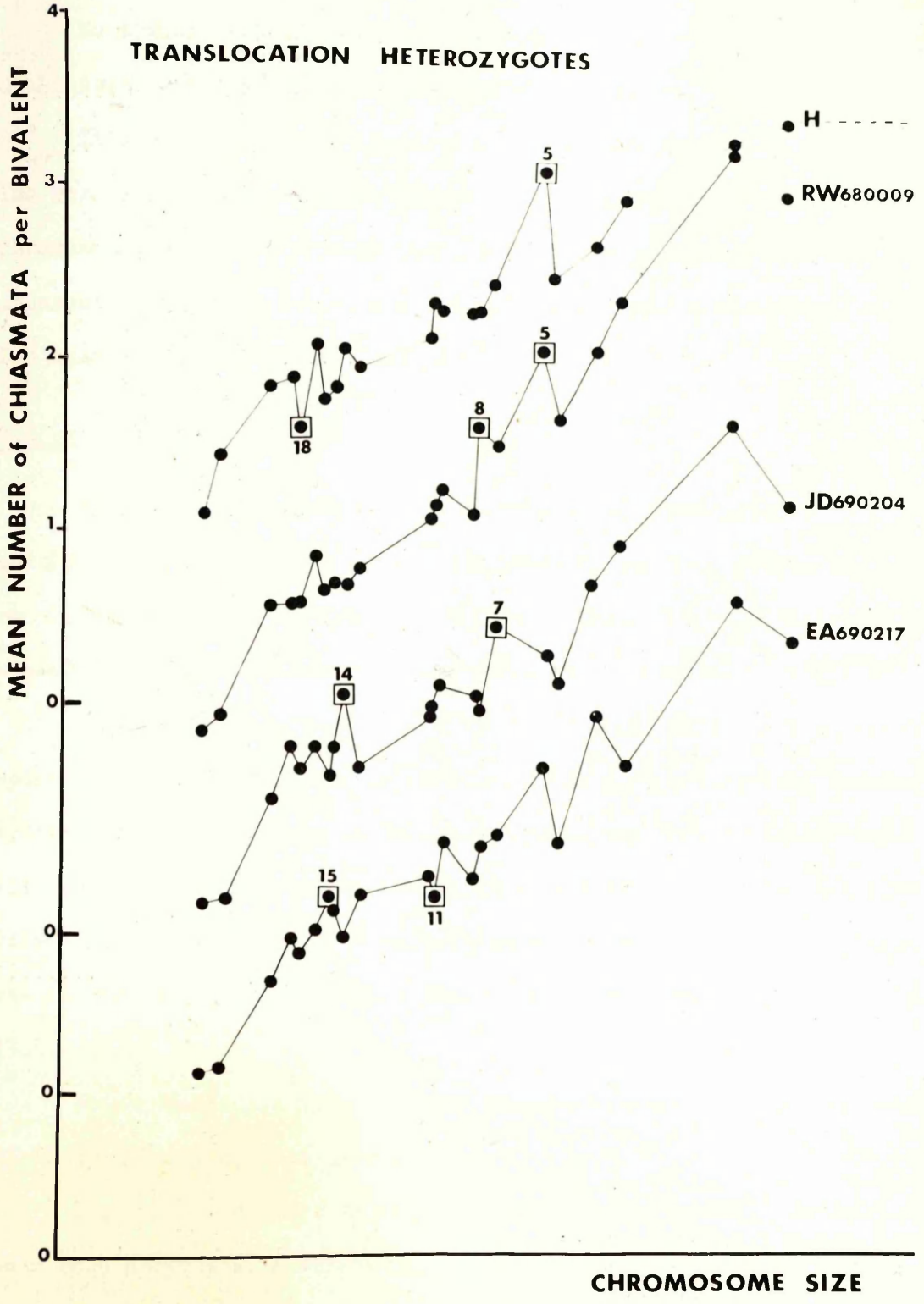


Figure 13. Number of chiasmata in chromosomes involved in reciprocal translocations, compared with the figures for the other chromosomes of each complement.

Translocations in Cells of Other Individuals

No translocations, undetected in somatic metaphases were found by diakinesis analysis in either controls or infertiles.

In some cells of some individuals there appeared to be quadrivalents. The chromosomes involved were identified by karyotyping. As different chromosomes had associated in each cell, it was concluded that the apparent pairing was due to a non-specific juxtaposition and not to, for example, translocation mosaicism.

Radiation-Damaged BS700349

Mitotic leucocyte cultures had revealed 8% abnormalities nine months after the accident. These included: dicentrics, fragments, ring chromosomes and breaks. Scrotal fibroblasts (20-cell analysis) showed no evidence of radiation damage after ten months.

Seminal analysis gave figures of 0.6 and 0.5 million of non-motile sperm per ml. Testis sections revealed fewer germ cells, very reduced spermatogenesis and signs of severe damage to the tubules (Plate 74a). The condition was not unlike a maturation arrest. In the meiotic air-dried preparations, signs of cell degeneration were present, but there was no indication of maturation arrest in the metaphase population figures (Table 32).

No abnormalities were observed at spermatogonial metaphase, prophase of first meiosis or metaphase II.

Most of the diakinesis figures also appeared normal. However, there were more spreads with "apparently" 22 elements at diakinesis than were usually observed in a study of this kind. Of 5 cells, from a total of 41, one showed a definite quadrivalent as evidence of a translocation (Plate 74b) and two others showed probable quadrivalents (Plate 74c). On the evidence obtained from karyotyping the bivalents, the chromosomes involved in the quadrivalents were suggested to be 14 and 11 in one cell

and 8 and 14 in two cells but some centromere regions were not distinctive in these particular cells. There was also one probable X/Y/Y cell.

The mean chiasma count per cell was a little low at 48.54 SD 4.75 (Table 33) but within "normal" limits (Figures 5 and 7). The proportion of cells showing separated X/Y was also normal at 17.5% (Figures 6 and 7) as were figures for chiasmata per bivalent (Table 34) except perhaps for an unusually low value for number 15 (Figure 14).

Table 32. Metaphase population structure in radiation-damaged BS700349.

CASE NUMBER	PERCENTAGES		
	SPERMATOGONIAL METAPHASE	DIAKINESIS	METAPHASE II
BS700349	24	53	23

Table 33. Mean chiasma count per cell and conjunction of the sex chromosomes in radiation-damaged BS700349.

CASE NUMBER	AGE	MEAN TOTAL-CHIASMA-COUNT	STANDARD DEVIATION	NUMBER OF CELLS	X/Y%
BS700349	21	48.54	4.75	35	17.50

Table 34. Number of chiasmata per bivalent as shown at diakinesis in radi

CASE NUMBER	CHROMOSOME NUMBER														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BS700349 BIVALENT MEAN	3.92	3.67	3.08	3.00	2.58	2.92	2.50	2.50	2.42	2.25	2.17	2.08	1.83	1.92	1.5

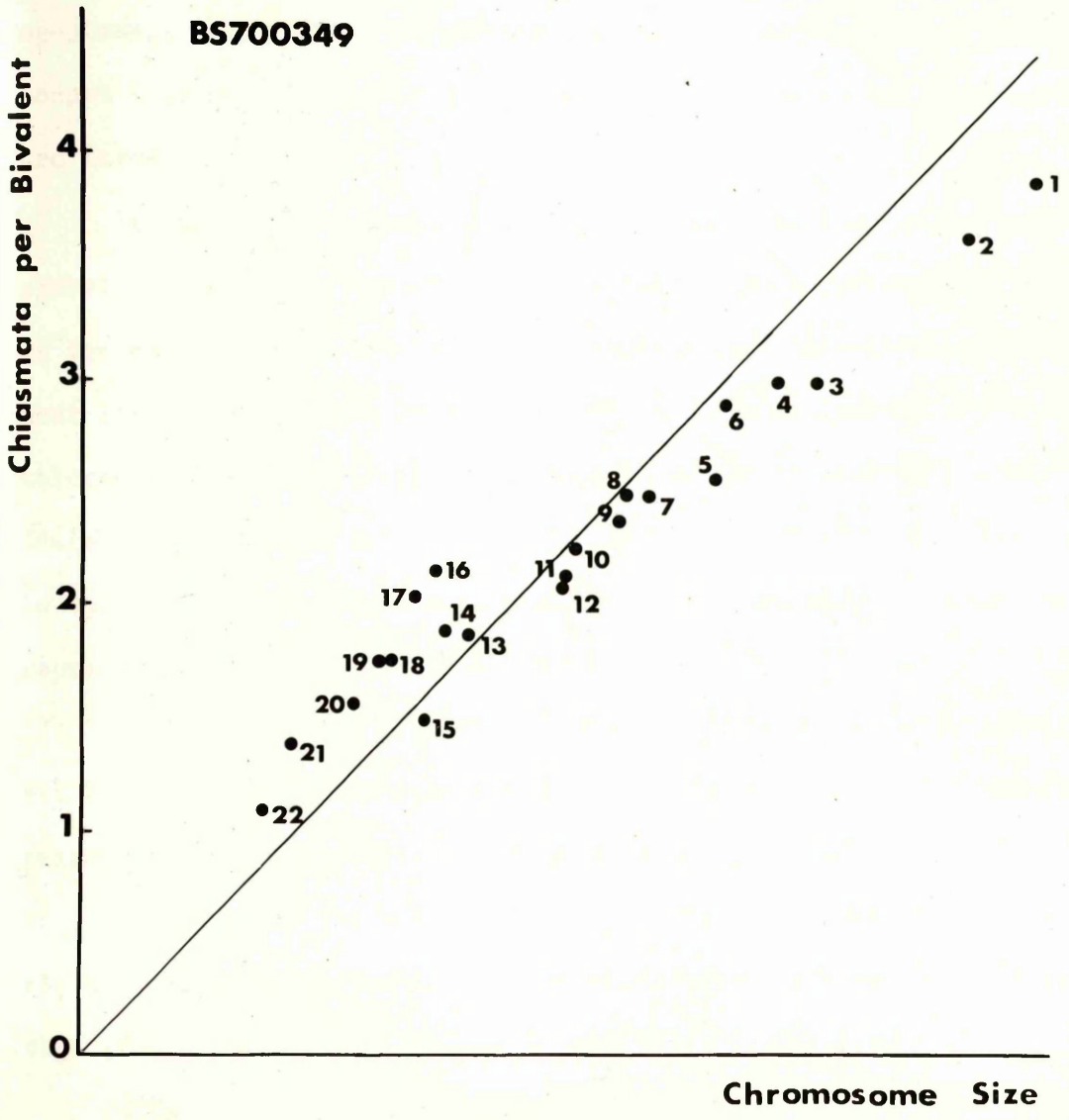


Figure 14. Mean number of chiasmata per bivalent in BS700349, plotted with chromosome size.

5.2 MATERIAL OTHER THAN HUMAN MALE

Locust

Preparations from locust (a) were unsuccessful, in that germ cells were present but spermatogenesis absent. This suggested an immature specimen. Good meiotic preparations were obtained from locust (b). Locust testis is ideal for the study of meiosis, since the few chromosomes are large.

In the few spermatogonial metaphases seen, the chromosomes appeared spiral, 23 in number the odd one being the single X (XO sex determination in the male locust). The centromere regions were heteropycnotic and near a telomere in each chromosome. All chromosomes were therefore telocentric. Possible pairing of homologous centromere regions was demonstrated in some cells. The cell shown in Plate 75a may, however, be an X-bearing metaphase II cell at pre-anaphase, since a single element representing the X cannot be distinguished.

At leptotene the diploid number of chromosomes began to appear and condense from heteropycnotic telomeres at one side, and homologues paired from telomeres (Plate 75b) backwards into a bouquet (Plate 76b). The sex chromosome was heteropycnotic at this stage (unlike the situation at this stage in the human male) and appeared to possess radially projecting tags (Plate 76a).

A fairly classical pachytene occurred with a deeply stained sex vesicle and centromere regions. Bivalents showed chromomeres and nucleoli were present (Plate 77). With further study it would be possible to karyotype this stage.

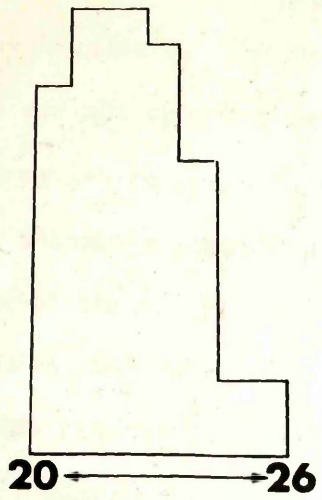
After pachytene, a diffuse stage was present. The sex chromosome unrolled to give a rod formed of two chromatids. The centromere regions were heteropycnotic and sometimes showed as a double body (Plate 78).

Re-condensation led to diplotene (Plate 79); the centromere regions remained heteropycnotic. At diakinesis, (by contrast to man) the four-strandedness of the bivalent could be demonstrated, including double centromere regions in each homologue (Plate 80). More chiasmata are shown at diplotene by comparison with diakinesis. The largest bivalent in Plate 79b shows at least 6 chiasmata whereas the mean in this bivalent at diakinesis was 3.70 (Table 35). Chiasmata could not be counted at all at late diakinesis (Plate 81a).

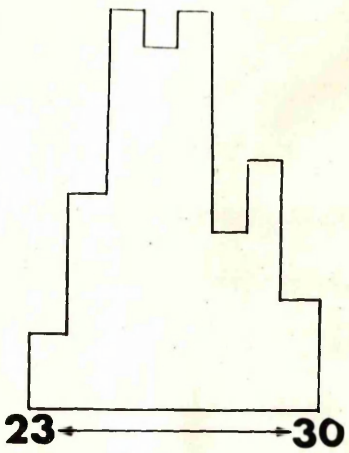
The more frequently observed "mid diakinesis" was used for chiasma analysis. A problem in observing chiasmata arose when the 4 strands of the bivalent did not separate, and lay parallel over varying lengths (Plate 80a). This was especially common in the large bivalents where it could be present more than once. In such cases careful focussing could not elucidate the true number of chiasmata present, so, in this study they were scored as a nominal two chiasmata, the minimum necessary to hold the configuration. Terminalisation occurred in locust mainly towards the non-centromeric end, since homologous centromeres showed strong repulsion. Bivalents were never observed to interlock. In most of the diakinesis spreads there was one large bivalent that appeared to have a gap, usually in only one of its homologues (Plate 80b). With careful focussing, material could be seen to traverse the gap. At pachytene, a large bivalent supported a nucleolus at this point (Plate 77a) and so the "gap" was attributed to a secondary constriction or nucleolar organiser region.

Fifty-seven mid-diakinesis cells, with well spread chromosomes, were chosen for analysis. All showed 11 bivalents, with the X as a univalent. A histogram for the chiasma count per cell showed a narrow distribution over a range 20 to 26 (Figure 15). The mean chiasma count per cell was 22.16 SD 1.59.

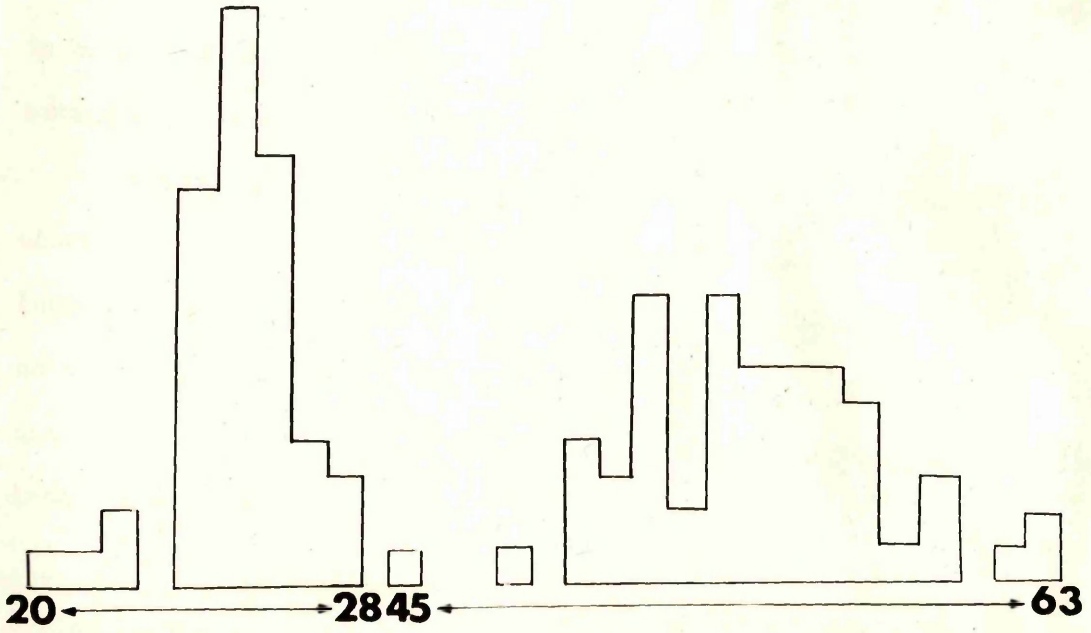
Twenty cells were karyotyped using size and individual characteristics.



a. LOCUST 57cells



b. SYRIAN HAMSTER
55 cells



c. MOUSE 50cells

d. MAN 57cells

CMC700362

Figure 15. Histograms of chiasma counts per cell in a locust, Syrian hamster, mouse and man.

The contraction of the X univalent was not a good indication of contraction in the bivalents. The bivalent with the gap was the third largest. When a gap was not apparent in this bivalent, there were two darker bands at an equivalent position.

Chiasmata seemed not to occur in the heteropycnotic centromere region of the larger bivalents. This was not the case, however, for the the three smallest chromosomes. These always had a single chiasma. One of three alternatives was scored for each cell: chiasma nearest the centromere region; nearest the middle, and nearest the region away from the centromere (Table 36). It was shown that significantly more were near the centromeric end (χ^2, P = 0.01).

Plotting the number of chiasmata per bivalent against chromosome length shows an increase with size above a minimum value of one chiasma (Figure 16). The slightly lower value for chromosome III may be attributed to an over-estimation of its size, possibly caused by the presence of the extended secondary constriction region.

By first anaphase (Plate 81b) repulsion of the chromatids has occurred; centromere regions collected round an unstained area with the long arms pointing outwards (Plate 82a). The X segregated to one pole, as shown by metaphase II spreads (Plate 82b and c). An interphase between anaphase I and metaphase II was not observed. Spermatids then differentiated into spermatozoa (Plate 83).

Syrian hamster

Somatic Tissue.

The blood cultures failed. There were no cells in division. Clotting may have been responsible.

The bone marrow preparations also failed.

Table 35. Chiasmata per bivalent in the locust.

CHROMOSOME NUMBER	MEAN NO. OF CHIASMATA PER BIVALENT	RELATIVE LENGTH (mm) IN X2,500 PRINTS OF CELLS. (Absolute length µm in parenthesis)	
		DIAKINESIS	METAPHASE II
I	3.70	55 (22.0)	65 (26.0)
II	3.45	52 (20.8)	60 (24.0)
III	2.45	45 (18.0)	50 (20.0)
IV	2.45	42 (16.8)	45 (18.0)
V	2.15	39 (15.6)	40 (16.0)
VI	1.95	30 (12.0)	38 (15.2)
VII	1.95	25 (10.0)	33 (13.2)
VIII	1.55	23 (9.4)	25 (10.0)
IX	1.00	17 (6.8)	22 (8.8)
X	1.00	16 (6.4)	19 (7.6)
XI	1.00	14 (5.6)	17 (6.8)

Table 36. Chiasma analysis in the 3 smallest chromosomes in locust.

CHROMOSOME NUMBER	NEAREST CENTROMERE	NEAREST MIDDLE	NEAREST END OPPOSITE TO CENTROMERE
IX	12	8	0
X	10	7	3
XI	8	3	9
Observed	30	18	12
Expected	21	-	21

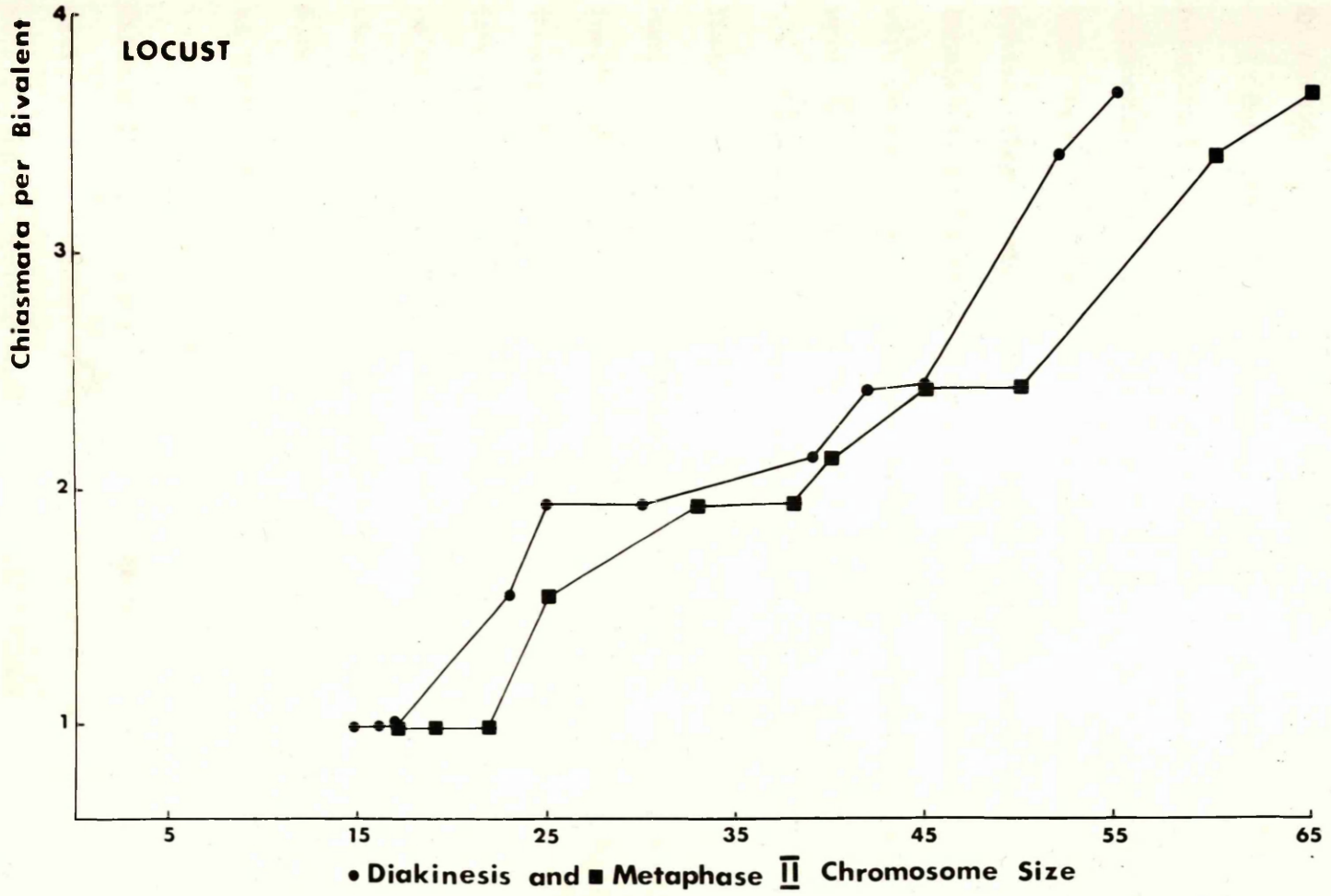


Figure 16. Number of chiasmata per bivalent plotted with chromosome length, in the locust.

Testis Tissue.

There was a lower proportion of these stages suitable for analysis - spermatogonial metaphase, diakinesis and metaphase II - by comparison with intermediate stages. Pachytene was more frequently observed than in locust.

Spermatogonia in interphase were large and distinct. Spermatogonial metaphases were less often observed and never with pairing of homologous chromosomes. The diploid number of chromosomes, 44, was confirmed using this stage. The sex chromosomes stained like the autosomes (Plate 84a). Condensation of chromosomes was again shown to occur from the telomeres bunched at one side of the nucleus. No good examples of homologous pairing were observed and neither was the bouquet configuration, but few slides were analysed.

Pachytene chromosomes tended to be slightly diffuse (Plate 84b). Twenty-one elements and the sex vesicle were apparent at this stage. Most centromere regions were heteropycnotic; the deeper staining sometimes including the whole of the short arm. These heteropycnotic regions tended to associate. The sex vesicle varied greatly in appearance. There were two parts: a larger, deeply staining and which possibly represented the pairing sections of the X and Y chromosomes, and a smaller, lightly stained, and which possibly represented the long arm of the X chromosome. Some spreads at pachytene showed double the usual number of elements as though polyploid, but pairing was strictly "two by two".

Chromosomes, except for heteropycnotic regions, then became more diffuse (Plates 85a and b). The sex chromosomes unravelled from the sex vesicle and chromosomes recondensed into the stage termed diplotene, heteropycnotic regions remaining distinct (Plate 85c). Chiasma counts at diplotene did not differ from those at diakinesis. This was unlike the situation in locust.

The four strands of the diakinesis bivalents were not as distinct

as those in locust but were more distinct than those in human (Plate 86). The sex chromosomes were present in one of three alternative configurations: as a sex bivalent with the long arm of the Y paired with the short arm of the X (XY, Plate 86b); as a bivalent, but with a substantial gap between (X Y); or as univalents (X/Y, Plate 87a). Apart from an association, there was no evidence of a chiasma between the sex chromosomes. Cells with separated X/Y were found in some preparations even when hypotonic treatment was not used (Plate 87a). Bivalents apparently joined by their telomeres were commonly observed. Regions remaining parallel, commonly seen in locust were rarely seen in hamster.

Fifty-five well spread cells at mid-diakinesis were analysed for chiasmata and conjunction of the X and Y chromosomes. Diakinesis cells containing the "haploid" number of bivalents were used. Apparent polyploidy was common. In many of the seemingly tetraploid cells ("diploid" number of bivalents), karyotyping from opposite edges revealed two full haploid complements with little overlap in the centre of the spread, showing the presence of two cells in synchrony.

A histogram of chiasma counts per cell (Figure 15) showed a narrow range between 23 to 30, but not quite as narrow as in locust. The mean chiasma count was 26.45 SD 1.83 and the proportion of cells with separated X/Y was 10.90%.

Twenty good diakinesis cells were karyotyped. Centromere regions were obvious only when the whole of the short arm was heteropycnotic. Variation in the amounts of heterochromatin present in different chromosomes was useful in karyotyping. Chiasmata tended not to occur in regions of deeper heteropycnosis. The smallest bivalent was usually more heteropycnotic than the others, especially at early diakinesis (Plate 86a). The next smallest was also distinctive, but because of its size. The four smallest bivalents each had only one chiasma (Table 37). A graph for the hamster material did not show the clear proportionate increase in chiasma number

per bivalent with increasing chromosome size (Figure 17), such as was seen in the locust.

An interphase was probably present between metaphase I and metaphase II, since the haploid number of chromosomes was seen to condense into metaphase II from an extended prophase configuration. The short arm of the X was the first to show deep staining. Later, centromere regions and the whole of the short arms of some autosomes became heteropycnotic and these short arms tended to lie closer together. Coiling was prominent at metaphase II. Cells could be scored as "X-present" or "X-absent" and as the X always had two chromatids, this showed pre-reduction of the sex chromosomes (Plate 88a and b).

Metaphase II cells were as frequently seen as were those at diakinesis. Seven were karyotyped, using first size then other individual chromosome characteristics. The Y, in those cells that did not have an X, appeared to have more compact chromosome arms than the autosomes (Plate 88b). Some chromosomes showed extended regions in one or both arms. The definition and spreading of the chromosomes at metaphase II proved adequate for the construction of an idiogram arranged by size and the heteropycnosis of chromosome regions (Figure 18).

Most apparently polyploid metaphase II cells carried 44 chromosomes, shown by karyotyping to be two full sets of homologous chromosomes including both an X and a Y. Closer inspection showed that the two sets of homologues were arranged with one set on the "X-side", the other on the "Y-side" of the spread and with only a slight overlap in the middle. This strongly suggested that adjacent, overlapped cells were present (Plate 87b) as the synchronous division products of the same diakinesis cell.

Maturing spermatozoa showed a distinctive acrosome and middle piece (Plate 88c).

Table 37. Chiasmata per diakinesis bivalent in the Syrian hamster.

POSITION	RELATIVE LENGTH (mm) IN X2,500 PRINT OF A CELL AT METAPHASE II (Absolute length, μm, in parenthesis)	MEAN NO. OF CHIASMATA PER BIVALENT
I	25.0 (10.0)	1.70
II	24.0 (9.6)	1.50
III	23.0 (9.2)	1.30
IV	21.0 (8.4)	1.60
V	19.5 (7.8)	1.50
VI	19.0 (7.6)	1.65
VII	18.5 (7.4)	1.70
VIII	18.0 (7.2)	1.40
IX	17.0 (6.8)	1.35
X	16.0 (6.4)	1.15
XI	15.0 (6.0)	1.25
XII	14.0 (5.6)	1.20
XIII	13.0 (5.2)	1.20
XIV	11.5 (4.6)	1.05
XV	11.0 (4.4)	1.25
XVI	10.5 (4.2)	1.10
XVII	10.0 (4.0)	1.20
XVIII	9.0 (3.6)	1.00
XIX	7.5 (3.0)	1.00
XX	7.0 (2.8)	1.00
XXI	4.0 (1.6)	1.00

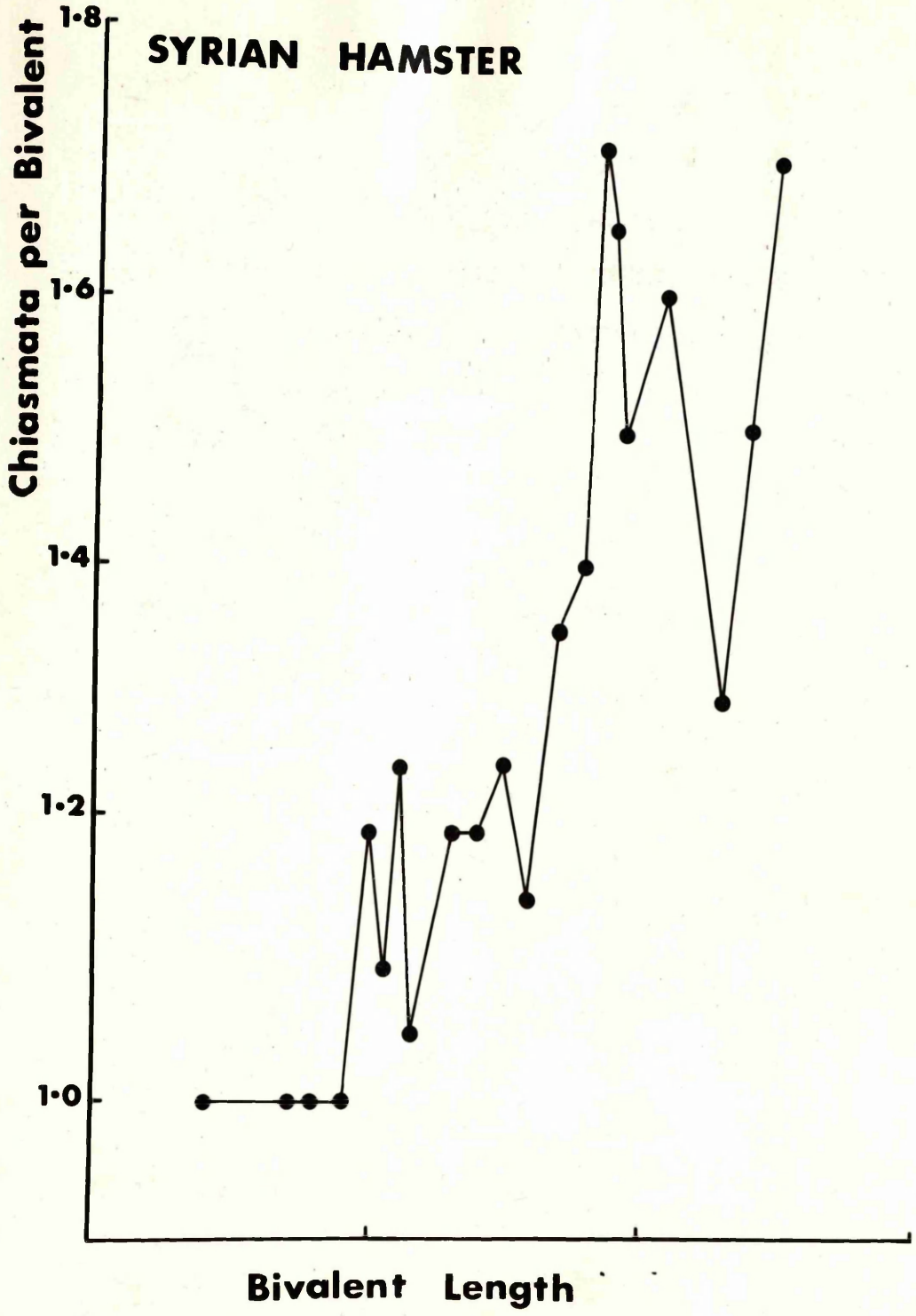


Figure 17. Number of chiasmata per bivalent plotted with chromosome length, in the Syrian hamster.

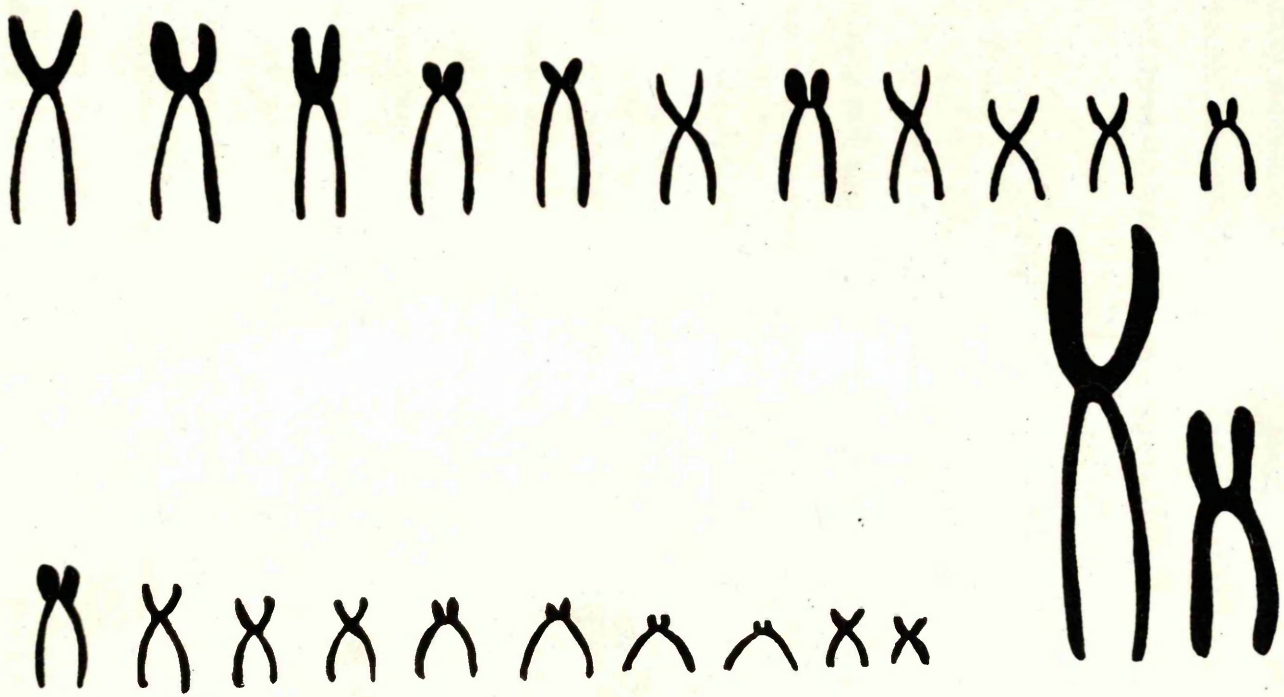


Figure 18. Syrian hamster idiogram constructed from metaphase II analysis.
(X and Y shown together, bottom right)

Mouse

Somatic Tissue.

Blood culture and bone marrow preparations failed. Corneal preparations gave good metaphases. About 20 mitoses from each eye, with some 10 of a quality suitable for detailed analysis, were regularly obtained. Using this technique, the normal 2n = 40 acrocentric complement of Mus musculus was confirmed in mice (a) (5 karyotypes), (e)(6 karyotypes), and (f)(2 karyotypes).

Testis Tissue.

In the mouse, the tubules were packed tightly into the capsule in a more ordered manner than in humans. The teased tubules showed sharp angular twists. Waves of meiosis could be observed through the tubule walls, using low power microscopy (Plate 89a).

Chromosomes more closely resembled one another than did those from the hamster or the locust and were consequently more difficult to study.

Spermatogonial metaphases (Plate 89b) were less frequently observed but occasionally showed spiralling. The diploid 2n = 40 chromosome complement was confirmed at this stage. There was little evidence of homologous pairing at spermatogonial metaphase (Plate 89b), but few preparations were examined.

Relics of a bouquet configuration were occasionally observed at the prophase of first meiosis. Pachytene was infrequently seen but centromere regions were heteropycnotic and there were 20 elements which included a sex vesicle (Plate 90a). A pachytene cell sufficiently well spread for karyotyping was not found. A diffuse stage followed pachytene. Centromere regions were heteropycnotic and appeared either single or double (Plate 90b). The sex chromosomes unravelled from their looped position in the sex vesicle to reveal end to end pairing. The chromosomes then recondensed into diplotene (Plate 91).

Further chromosome condensation preceded diakinesis by which stage the centromere regions tended to be obscured. There were usually only one or two chiasmata in each bivalent (Plate 92), with no evidence, apart from adjacent positioning, of a chiasma between the sex chromosomes. One late diakinesis cell showed precocious disjunction of some of the chromosomes and the long arms of the Y (beginning to separate) seemed paired with the long arms of the X (Plate 93a). Separated X/Y cells were occasionally present and more rarely X^Y, with the sex chromosomes aligned, but separated by a substantial gap. Autosomes were occasionally observed with homologues apparently separated but lying side by side. Careful focussing revealed connections which suggested that terminal chiasmata were present, or that terminalisation had already occurred.

Fifty well-spread cells at mid-diakinesis were examined for the number of elements, chiasmata and the conjunction of the X and Y. All cells showed 19 autosomal bivalents, while the X and Y chromosomes could be either together or separate. The mean chiasma count per cell was 25.06 SD 1.58 and the proportion of cells with separated X/Y was 4.00%. An attempt to karyotype 20 cells at diakinesis was made, but chromosome identifications were fairly arbitrary as they were based only on size. The number of chiasmata per bivalent was estimated (Table 38).

Unlike the smaller chromosomes of the hamster, the smaller chromosomes of the mouse occasionally had two chiasmata. The number of chiasmata per bivalent increased proportionately with increasing bivalent length (Figure 19).

At metaphase II the chromosomes tended to be spiral and the sex chromosomes were heteropycnotic (Plate 93b). Cells could be scored as "X-bearing" (Plate 94a) or "Y-bearing" (Plate 94b). There was 100% pre-reduction of the sex chromosomes. Six metaphase II spreads were karyotyped using chromosome size as the principal criterion. The sperm head showed a marked differential staining in spermiogenesis (Plate 94c).

Table 38. Chiasmata per diakinesis bivalent in the mouse.

CHROMOSOME POSITION	RELATIVE LENGTH (mm) IN X2,500 PRINT OF A CELL AT DIAKINESIS. (Absolute length, μm, in parenthesis)	MEAN NUMBER OF CHIASMATA PER BIVALENT
I	20.0 (8.0)	1.95
II	18.0 (7.2)	1.60
III	17.0 (6.8)	1.60
IV	16.0 (6.4)	1.60
V	14.0 (5.6)	1.45
VI	13.0 (5.2)	1.30
VII	12.5 (5.0)	1.35
VIII	12.0 (4.8)	1.40
IX	11.5 (4.6)	1.35
X	11.0 (4.4)	1.25
XI	10.5 (4.2)	1.15
XII	10.0 (4.0)	1.15
XIII	9.5 (3.8)	1.25
XIV	9.0 (3.6)	1.20
XV	8.5 (3.4)	1.15
XVI	8.0 (3.2)	1.10
XVII	7.5 (3.0)	1.00
XVIII	6.0 (2.4)	1.05
XIX	5.0 (2.0)	1.05

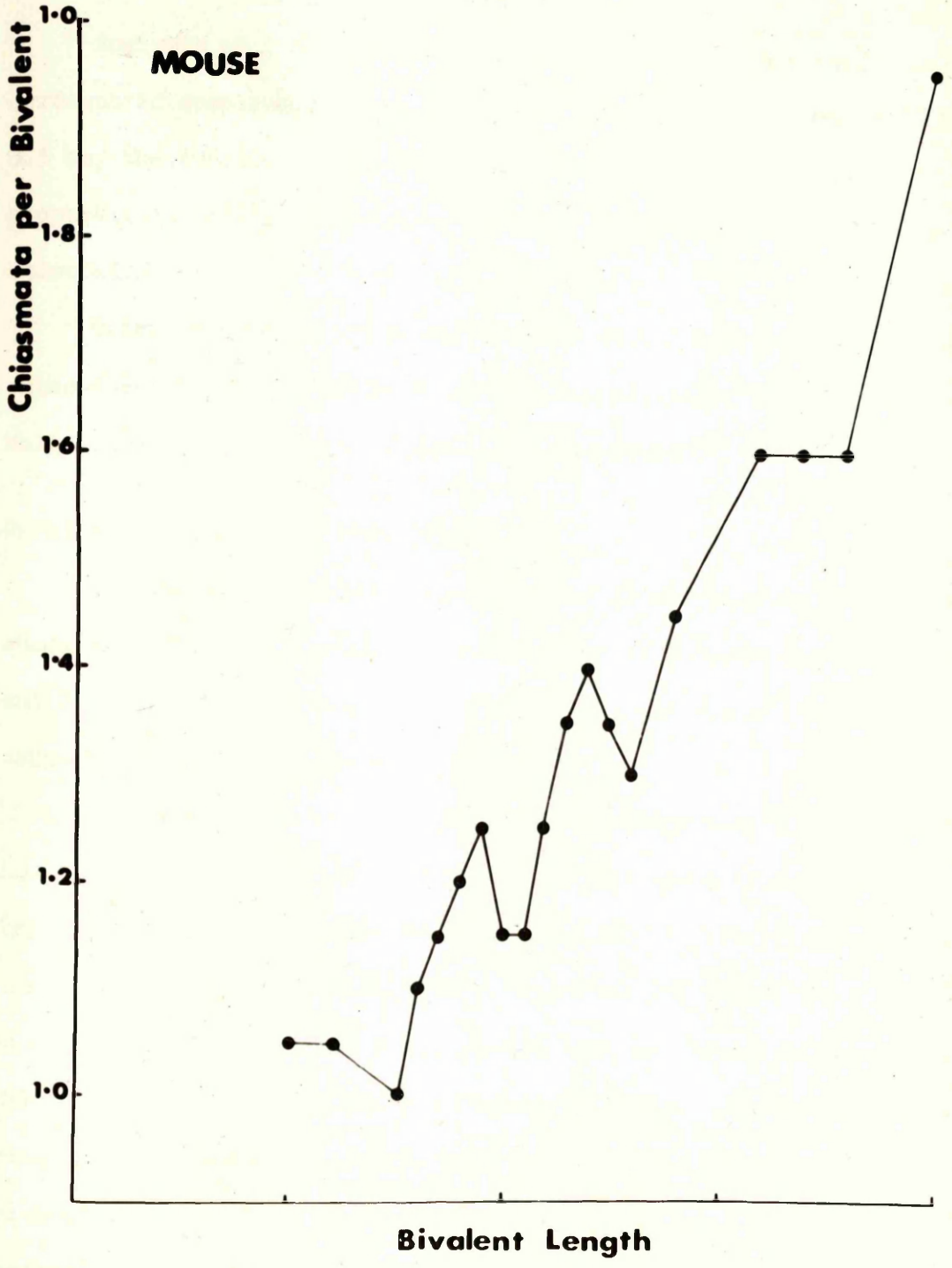


Figure 19. Number of chiasmata per bivalent plotted with chromosome length, in the mouse.

Mouse with Muscular Dystrophy

Preliminary Investigation, Mouse (e).

Analysis of 4 spermatogonial metaphases confirmed a normal 2n = 40 acrocentric complement of Mus musculus. The testes were small 6.5 x 4.5 x 3.5 mm, the tubules thin. Stages suitable for analysis in the testis preparations were scarce, but no specific disturbance of meiosis was observed.

Detailed analysis of 20 cells at mid diakinesis revealed a mean chiasma count per cell of 25.75 SD 1.86 and separated X/Y in 20% of the cells (4% X/Y was found in a non-dystrophic mouse).

Detailed Investigation, Mice (f) and (g).

In both an unaffected mouse (specimen g) and another affected with muscular dystrophy (specimen f), examination of 4 spermatogonial metaphases and 2 metaphase II cells confirmed the normal acrocentric chromosome complement of 2n = 40.

Testis sections indicated a normal histology for (g) (Plate 95b); in (f) there were fewer of both germ cells and sperm (Plate 95a). Mouse (g) was almost twice the weight of (f) (Table 39). This table also shows the smaller proportion of cells with separated X/Y (Plate 96d) in the unaffected mouse (g) (20%) compared with that in the affected mouse (f) (36%). The figures were significantly different (χ^2, P = 0.05 - 0.02). A wider range of diakinesis was used in this investigation (see Page 55) so precocious disjunction may explain why the value for X/Y in the unaffected mouse (g) was higher than that for the previous control mouse (4%). The proportion of XY gap (Plate 96b) and X⌒Y cells (Plate 96c) was also larger in the affected mouse (f) compared with the unaffected (g).

In chiasma analysis, the mean chiasma count per cell for the unaffected (g) was 24.95 SD 1.88 and for the affected (f) 25.78 SD 2.27 the larger standard deviation here indicating a wider spread of values.

For the unaffected (♂) closer analysis showed the X/Y spreads had a lower mean chiasma count (23.36) than other X⌢Y or XY gap cells (23.50 and 23.86, respectively), which in turn had a lower count than the XY ones (25.60). Such a trend might be expected since the chromosomes contract progressively as diakinesis proceeds. This contraction tends to obscure chiasmata and the later (more contracted) stages are those most likely to show a precocious disjunction of the X and Y. For the affected mouse, the X/Y spreads had a higher mean chiasma count (26.46), compared with X⌢Y and XY gap (26.25 and 25.32 respectively), which in turn had a higher count than XY (25.27). The trend is thus shown to be in the <u>opposite</u> direction and unlikely to be caused by precocious disjunction of the sex chromosomes.

Table 39. Chiasma analysis and conjunction of the sex chromosomes in mice with and without muscular dystrophy.

	UNAFFECTED MOUSE (g)		MOUSE (f) AFFECTED WITH MUSCULAR DYSTROPHY	
Weight of mouse in g	22.5		12.0	
Testis size mm	7.0 x 4.5 x 3.5		5.0 x 3.5 x 2.5	
	No.	%	No.	%
Total no. of diakinesis cells	115	100	115	100
Sex Chromosomes				
XY paired (XY + XY gap + X^Y)	92	80	74	64.35
X/Y not paired	23	20	41	35.65
X^Y	6	5.22	9	7.83
XY gap	8	6.96	19	16.52
Y extra	-	-	1	0.87
Y missing (XO)	-	-	1	0.87
X broken	-	-	1	0.87
Y broken	-	-	1	0.87
Gap in X (like secondary constriction)	1	0.87	-	-
Autosomes				
Cells not complete	3	2.61	2	1.74
Whole cells counted	112	100	113	100
Of these No. with 2 univalents	5	4.46	9	7.96
Autosomal extra fragment	1	0.89	-	-
CHIASMA ANALYSIS				
MEAN CHIASMA COUNT PER CELL	24.95		25.78	
MEAN CHIASMA COUNT PER CELL for X/Y	23.36		26.46	
MEAN CHIASMA COUNT PER CELL for XY + XY gap + X^Y	25.33		25.39	
MEAN CHIASMA COUNT PER CELL for XY	25.60		25.27	
MEAN CHIASMA COUNT PER CELL for XY gap	23.86		25.32	
MEAN CHIASMA COUNT PER CELL for X^Y	23.50		26.25	

6. CONCLUSIONS AND DISCUSSION

The sequence of subjects discussed follows in broad outline their appearance in Chapter 5. An attempt is made to assess the methods used as well as the results obtained and to bring both into perspective with the work of others on meiosis, especially human meiosis.

A section on mitotic results used as an essential background for the meiotic study and infertility survey is discussed first. This is followed by a discussion on human spermatogenesis, noting particularly the occurrence of a diffuse stage which was discovered during the course of the work. The observations are compared, where relevant, with those made from studying other material. Sections on autosome chiasma analysis and conjunction of the sex chromosomes, maturation arrest, translocation heterozygotes and a radiation-damaged patient then precede a general discussion on the timing of the stages of meiosis, environmental factors affecting reduction division, nondisjunction, and selection in gametogenesis.

6.1 MITOTIC METAPHASE

The leukocyte culturing technique was very reliable. However, blood is not always considered an ideal somatic tissue for detecting chromosome mosaicism in man, since less than six cells are said to give rise to the haemopoietic tissue of the body (Gandini, Gartler, Angioni, Argiolas and Del'Aqua 1968).

Blood is certainly very convenient for mitotic analysis. Ferrier, Ferrier and Kelley (1970) concluded that few instances of sex chromosome mosaicism would be overlooked if blood alone was examined. However, using the 20-cell survey method, it is possible that some minor lines of cells could have been missed or attributed to random loss. Nor was the method

of microscope analysis ideal. However, successive backchecks were made by counting first the cell totals, then numbers for each of the chromosome groups and individual chromosomes (see Chapter 4). It is recognised that scoring chromosomes by quadrants may be better. The defects of the method actually used were probably overcome to a large extent by the fact that a quarter of the cells were karyotyped from photomicrographs.

Random Loss of Mitotic Chromosomes

The smaller the chromosomes, the greater the chance of random chromosome loss (Figure 2). Figures for losses of G chromosomes may have been even higher due to the inclusion of "unconfirmed" XO/XY mosaics in the total. An unusually high loss of G's has been observed previously. Hypodiploidy especially due to Y chromosome loss is considered to increase with age (Neurath, De Remer, Bell, Jarvick and Kato 1970), though not all reports confirm this. Zang and Zankl (1970) maintained that acrocentrics were seldom lost, as they apparently possess "stickiness to nucleolar material". Figure 2 also shows that more C group chromosomes are missing than might be expected from the general trend of loss according to chromosome size. This could have been due to 45,OY/46,XY mosaicism, but any karyotyped C- cells never did suggest an absent X.

Random loss seemed unlikely to be due to nondisjunction since, apart from sex-chromosome mosaics, only one hyperdiploid cell was found. This cell had an extra B group chromosome which genuinely appeared to belong to the remainder of the chromosome complement. Since the frequency of cells with missing chromosomes did not appear to be balanced by cells bearing corresponding additional chromosomes, apparent losses may result from the process of preparing the chromosome spreads.

POLYMORPHISM.

Natural variation in human mitotic chromosomes is fairly well documented (Lubs and Ruddle 1970); Benirschke (1969) demonstrated some degree of chromosome polymorphism in a study of 120 lower primates. The polymorphisms found in the present study were not exceptional (Table 4). They were present in controls and infertiles. The most obviously variable autosome was number 9 (Plate 10a).

The study also confirmed the wide variability of the Y (c.f. Gripenberg 1964). As Y deletions can lead to infertility (as in patient WC680183) a correct assessment as to whether an unusual Y is abnormal, or a polymorphism, is critical. Abnormalities caused by "abnormal" Y chromosomes, have been claimed in many reports. Makino, Kikuchi, Sasaki and Yoshida (1962) mentioned a long Y associated with oligospermia or aspermia, Makino, Takagi, Makita and Hikita (1964) a large Y in one of three individuals displaying hypospadias and Kadotani, Ohama, Nakayama and Tabuchi (1970) two long Y's in an infertility survey of 88 couples. In the present study, unusual Y's, both large and small, were frequently present in cases with the Del Castillo syndrome. Named by Del Castillo, Trabucco and de la Balze (1947) this was described as absence of germ cells without impairment of Sertoli or Leydig cells. There is sex-chromatin negativity, hypogonadism, no apparent Leydig cell hyperplasia and increased gonadotrophins (Ferguson-Smith, Mack, Ellis and Dickson 1963). However, the chromosomes are normal 46,XY (Ferguson-Smith, Lennox, Stewart and Mack 1960, Solari, Videla and del Castillo 1963).

The size of the Y varies from cell to cell, in any individual, largely depending on the general contraction of the chromosomes (Van Wijk et al. 1962, Bender and Gooch 1961). Allowing for this, it seemed that control JT700346 had three populations of cells with small, medium and large Y chromosomes. The differences were subtle and might have been ignored but for the fact that Reitalu, Bergman and Ekwall (1970) found large and small

Y populations together, and postulated somatic crossing-over early in embryogenesis.

Mitotic Anomalies

Anomalous cells are usual in any mitotic study. Table 5 shows those found in the present investigation. Quadriradial chromosome configurations are sometimes taken to represent somatic crossing over and, for example, used to explain genetic differences in homozygous twins, mosaicism of blood type or antigens in neoplasms (German 1964), and even the structural and genetic properties of the immunoglobulins (Gally and Edelman 1970).

Paired structures protruding from chromatids are difficult to interpret. A variety of morphological peculiarities were also observed at meiosis, not only in man, but also in the other material studied.

INFERTILITY SURVEY.

The survey gave a figure of 15% abnormal chromosome complements (complements other than normal 46,XY, including mosaicism confirmed by the presence of more than one cell of the minor line). Court Brown and Smith (1969) stated that 0.3% of the general population were chromosomally abnormal.

47,XXY Klinefelter's Syndrome

This is perhaps the most common form of trisomy. Moore (1959) believed the frequency of the syndrome to be as high as 1 in 400 male births (0.25%) and for the newborn Ratcliffe et al. (1970) stated 0.09%. A buccal smear survey of high-school children found only 3 out of 4,481 males to be chromatin positive. By mitotic analysis these were shown to be 47,XXY (Omori, Morrow, Ishimaru, Johnson, Maeda, Shibukawa and Takaki 1969).

Three XXY's (4.55%) with no evidence of mosaicism, were found in the present infertility survey (66 cases). None of the three had morphological

characteristics typical of Klinefelter's syndrome other than hypogonadism. The number approximates to what others have found in similar surveys. McIlree, Price et al. (1966) found 6% Klinefelter's (a sperm count of less than 20 million) and Kjessler (1966) 16%. Kadotani et al. (1970) found 1.14% and Philip, Skakkebaek, Hammen, Johnson and Rebbe (1970) found 6.12% chromatin positive Klinefelter's.

In this study, two 47,XXY cases had major absence of germ cells (one had a few cells maturing to free sperm and the other had some spermatogonial mitoses) and the third was most likely to have complete absence of germ cells, since no sperm were present in the epididymal tubules which were accidentally biopsied instead of the gonad. Klinefelter's syndrome with small areas of spermatogenesis has been described: without a supporting chromosome study, by Grumbach, Blanc and Engle (1957) and Ferguson-Smith and Munro (1958); with accompanying chromosome studies, by Steinberger, Smith and Perloff (1965) and Skakkebaek et al. (1969). Miller et al. (1960) described the usual condition: absence of spermatogenesis, in 3 Klinefelters and a Klinefelter mongol.

Outside the infertility survey, the sex-chromatin positive transsexual DK700082 was found to have 47,XXY Klinefelter's syndrome, with no evidence of mosaicism in the 20 cells analysed in detail. The Y, distinctive in this case, was present in every one of a further 100 cells analysed. Gynaecomastia was present (in claimed absence of hormone treatment) and clinical features were those typical of a Klinefelter. Sections of testis showed a severe condition with only 0.2% of the biopy occupied by tubules. Leydig cell volume was 1.0 ml in the right gonad and 0.6 ml in the left. Normal volume is considered 0.5 to 1.2 ml (Ahmed, Lennox and Mack 1969). Dermatoglyphic analysis showed ridge width of the right hand a/b (Table 6) was normal for a Klinefelter (Penrose 1967), the value for the left hand a little lower. The total ridge count of 55 also suggested the presence of at least one extra sex chromosome. Only the previously established figure

of 15% sex chromatin positivity hinted at mosaicism. Other laboratories found sex chromosome mosaicism in this patient's bone marrow (Edwards pc. 1970).

Abnormal psychosexual behaviour is often associated with Klinefelter's syndrome or found in men with Klinefelter characteristics(Bishun 1968).

47,XXY/46XY mosaics

Kjessler (1966) found two XXY/XY mosaics with absence of germ cells, and one with normal spermatogenesis from a series of 135 cases. Here a possible XXY/XY mosaic from the infertility survey had a partial absence of germ cells and maturation arrest. An unconfirmed mosaic, a control, had very slight maturation arrest, but this was probably caused by an inflammation which raised the temperature of the gonad.

46,XX Male

The incidence of XX males in a newborn survey was given as 0.03% (Ratcliffe et al. 1970). The case presented here had the hypogonadism, testis histology and the high total gonadotrophin excretion characteristic of Klinefelter's syndrome, but had the intelligence and body proportions of a normal male. Dermatoglyphic total ridge count was 145.0, the figure given as typical for a normal male (Penrose 1967).

De la Chapelle, Hortling, Niemi and Wennström (1964) reported the first case of an XX male. Therkelsen (1964) described a 46,XX sterile, hypogonad male with a slightly hypoplastic penis and Sertoli cells chromatin negative. Other reported XX males include: one by Strauch, Engel, Taft, Atkins and Forbes (1965); three by Lindsten, Bergstrand, Tillinger Schwarzacher, Tiepolo, Muldal and Häkfelt (1966), while Bishun (1968) found four XX males in a series of 30 patients, three with ambiguous genitalia and one like a Klinefelter. Hayato, Makino and Suton (1969) reviewed the field and reported another XX male with hypospadias and testes like those of a Klinefelter.

Brøgger and Aagenaes (1964), Hecht, Antonius, Maguire and Hale (1966) and Lindsten et al. (1966) claimed that Y chromosomes are sometimes found in XX males. Fluorescence analysis of blood and buccal smear in the case presented here, did not show any Y bodies, so undetected mosaicism was unlikely. Sebaoun, Fourmier, Drayfin and Nettier (1969) favoured Y to X translocation. Autoradiography and fluorescence analysis in this case and others (Wennström and de la Chapelle 1967, Caspersson, de la Chapelle, Lindsten, Schröder and Zech 1971) has failed to demonstrate Y material on one of the two XX's. However, Bergman, Nowakowski and Reitalu (1970) using autoradiography claimed that most of the long arm of the Y was present, and demonstrated its own replicative behaviour, on the distal part of the short arm of the X. Incidentally, they emphasised the normal IQ of their case, as did Boczkowski, Janczewski, Philip and Mikkelsen (1969). On the whole, XX males are less severely affected than are those with Klinefelter's syndrome.

46,X deleted Y

Absence of germ cells of WC680183 was attributed to the deleted Y, since the Y of his brother was of normal size. There was no evidence, from blood or fibroblast cultures or fluorescence of a buccal smear, of a cell line with a normal Y chromosome. The Y was so small (Plate 9) that it could have been an isochromosome for the short arm of the Y, a Y with most of the long arm deleted, or even a remnant of an X. Its peripheral position (Ockey 1969) suggested a Y. It did not fluoresce using quinacrine fluorescence, but this is usual for a normal small Y (Borgaonkar and Hollander 1971). Autoradiography showed it to be unlabelled by thymidine administered late in S. Craig and Shaw (1971) found the Y completed its replication with the first 10 chromosomes. In this case the Y was probably too small to draw firm conclusions from autoradiography.

Dermatoglyphics (Table 6) again proved interesting. Total ridge count was 106, near to that estimated for 48,XXYY, certainly far from that for 46,XY (145) or 45,XO (179). Mean ridge width was more than that suggested for 48,XXYY (Penrose 1967).

The presence of a small chromosome with a sex chromosome "missing" has been associated with Turner's syndrome (Jacobs 1969). When phenotypic development was in the male direction, the small chromosome was assumed to be derived from a Y. (Conen, Bailey, Allemang, Thompson and Ezrin 1961, Fraccaro, Bott, Salzano, Russell, Ross and Cranston 1962, Surana, Forbath and Conen 1971). Muldal and Ockey (1962) found a deleted Y chromosome in a family with muscular dystrophy and hypospadias. Kjessler (1966) found a case with a deleted Y in an infertility survey. There was a childless uncle and the paternal grandfather was the fully fertile male carrier. Another of his cases had hypospadias possibly associated with a deleted Y. Melnyk, Derencsenyi, Vanasek, Rucci and Thompson (1969) found a 46,XYq- in 200 tall antisocial men who were mentally ill and criminally insane. A chromosome like that described here is sometimes found as a supernumerary in somatic and germ line cells, for example, as shown in an oligospermic male reported by Hultén, Lindsten, Fraccaro, Mannini and Tiepolo (1966).

45,XO/46,XY Mosaics

Confirmed XO/XY mosaics included 2 cases with an absence of germ cells, one case with major absence of germ cells and another with partial absence of germ cells and with maturation arrest. "Unconfirmed" mosaics (one XO cell) included 2 cases with maturation arrest, one case with unilateral absence of the gonad (histology probably normal in the other), one "normal" infertile, and a control (cancer of the prostate) case. Kjessler (1966) found two XO/XY cases with normal histology.

Total gonadotrophin results were available for 3 of the 4

confirmed mosaic cases in this study. They were all high at 21, 27 and 83 HMG units/24 hours (normal range 5 to 23 units). Apart from this and abnormal testes, three of the four were apparently normal males and had no stigmata of Turner's syndrome. Such stigmata have been found in a phenotypic male with XO/XY mosaicism (Ross, Holland, Kiser and Douglas 1965). The fourth case was abnormal in having a bifid scrotum and epispadias. Epispadias is a rare condition present in one in every 300 to 1,000 males (Hasche-Klünder 1963), while one case occurs for every 100-150 cases of hypospadias.

The cases here represent the far end of a spectrum ranging from individuals with features typical of Turner's syndrome, to males with normally descended testes (Ferguson-Smith, Alexander, Bowen, Goodman, Kaufman, Jones and Heller 1964, Desjeux, Gagnon, Leboeuf, St.-Rome and Ducharme 1970).

Abnormal Complements not Found in the Infertility Survey.

A male survey gave the incidence of 47,XYY at birth as 0.14% (Ratcliffe 1970). The XYY condition is expressed in varying degree from hypogonad individuals to normals (Court Brown 1968) and it has often been associated with infertility (Stenchever and MacIntyre 1969, Philip et al. 1970). The association, however, remains far from clear. None was found in this study.

Translocation heterozygotes were even more likely to be found by chance in a series of 66 cases. The birth incidence of detectable translocation heterozygotes in a normal population was estimated as 0.37% (Smith and Jacobs 1970), and 0.17% (Ratcliffe et al. 1970). Not all balanced translocations can be detected at mitotic metaphase but a quadrivalent would have been shown at diakinesis. In meiotic studies, Kjessler (1966) found one t(D,D) heterozygote in 135 cases and Chandley (1970) one t(C,E) heterozygote in 45 cases.

RODENT.

Failure to obtain chromosome preparations from cultured rodent blood could have been caused by clotting, in spite of the use of a vast dose of heparin, or by an insensitivity to colchicine. Willard, Hoppe and Nettesheim (1965) commented on the ineffectiveness of the use of bean phytohaemagglutinin and the standard human technique for mouse. Buckton and Nettesheim (1968) succeeded with a complicated procedure and which included the use of pokeweed as a mitotic agent.

The bone marrow technique failed probably owing to the omission of vincaleukoblastine (Lee 1969) so as not to damage the meiotic chromosomes in the gonads. Lee's technique was originally adopted for use on animals in the field and was known to be unreliable for animals kept in captivity.

Corneal preparations (Fredga 1964) yielded good results, but contrary to this author's claims, preparations were not obtained from a Syrian hamster killed with ether, rather than by cervical dislocation.

In this study, failure to obtain mitotic metaphases was not serious, since, in the study of normal meiosis, excellent spermatogonial metaphase preparations were adequate to check the chromosome complement. All mice showed the 2n = 40,XY chromosome complement of Mus musculus L., described by Levan, Hsu and Stich (1962) and Hsu and Benirschke (1967). The Syrian hamster had the normal 2n = 44,XY, typical of Mesocricetus auratus Waterh., described by Sachs (1952), Lehman, Macpherson and Moorhead (1963) and Galton and Holt (1964).

A smaller proportion of animals with abnormal chromosome complements has been found, than is usual for man (Ford 1970). Russell and Chu (1961) reported a probable 41,XXY Klinefelter mouse and Cattanach (1964) described a sterile (but otherwise phenotypically normal) male mouse, with 41 chromosomes, claimed to be an autosomal trisomic. Ford (1970) reviewed the population cytogenetics of other mammalian species. In this study,

mitotic chromosomes, from the mice of strain Re/129 affected with muscular dystrophy, were apparently normal.

6.2 TESTIS TISSUE

Up to now, the study of human germ cell material has been more misleading, with respect to human chromosome number, than has the study of any other tissue. This may be attributed to the anomalous behaviour of certain chromosomes at various stages of meiosis.

The study of meiosis using human male material has some advantages as well as drawbacks. A definite advantage is that spermatogenesis is not a seasonal phenomenon and can therefore be studied all the year round. Unlike the salamander, which has a caudocephalic wave of meiosis through the gonad allowing the sequence to be followed by longitudinal section (Kezer 1970, Kezer and Macgregor 1971), sections of human male testis fail to reveal any information on sequence. It is claimed that certain cellular associations are predictable (Clermont 1963) but there are no waves of meiosis such as may be seen in testis sections of the mouse or rat to give a key to the sequence of events (Heller and Clermont 1964). Even the boar shows greater meiotic regularity as Henricson and Bäckström (1963) commented on the rarity of finding metaphases of both meiotic divisions together in the same tubular section.

By contrast, it is fortunate that the mitotic chromosomes of man have been very well characterised in the last 15 years both with respect to abnormality and polymorphism. Great advances have also been made in employing biochemistry in the study of human genetics (for example, Xg blood grouping in detecting the origin of aneuploidy, Race 1970). Knowledge of human biochemistry is probably in advance of what is known of other organisms, and as meiotic recombination provides the mainspring of genetic and biochemical diversity, studying meiosis in man may well provide most important data for the understanding of the genetic process as a whole. Unlike

mitosis, elements of the entire genome are probably active in spermatogenesis (Ford 1970) including some in the Y. Benirschke (1969) likewise recognised that an analysis of meiosis is more likely to yield information on karyotype evolution in primates than analysis of mitotic metaphase.

The air-dried technique, using a cell suspension (Rothfels and Siminovitch 1958) might be expected to break up cellular associations. But the frequent synchronies between neighbouring cells seen in testis sections were in fact also found in the air dried preparations. Not only were cells at certain stages found together, but cells in immediate proximity were closely synchronised, without there being any reason to believe any were polyploid.

Human testis tissue is not easily available. Post-mortem material has been found unsatisfactory for the study of meiosis, especially with regard to diakinesis, and metaphase II (Edwards and Guli 1963). Woollam and Ford (1964) and Kjessler (1966) stated that material must be used within one hour and four hours after biopsy, respectively. In this study, any delay resulted in poor spreading and reduced definition. For prophase stages of the first division, freshness of tissue seemed less important. Edwards and Guli (1963) reported stages up to "zygotene" were well preserved if material was used up to 3 days after death (provided the corpse was adequately refrigerated), Hungerford (1971) stated that autopsy must be performed within an hour. The present study always employed fresh material, but some of the biopsy was left in tissue culture medium, sometimes for a couple of hours, before technique "M" was commenced, when there was excellent preservation of early prophase stages.

"Living" material therefore seems essential. One of the pioneer studies (Painter 1921) was performed on material from 3 subjects in a Texas State Institution, punitively castrated (using local anaesthetic) for "self abuse". There is also the occasional report of material being taken when the patient is undergoing surgery "other than for testicular pathology"

(for example Comings and Okada 1971a). In the present study material came mainly from testicular biopsies and blood samples taken as a service to individuals with an infertility problem. Cytogenetic (mitotic and meiotic) as well as testis section data proved invaluable for more accurate diagnosis.

An infertile population may not be thought optimal for the study of "normal meiosis", but individuals found at operation to have bilateral absence of the vas deferens or a blockage of the epididymus need not be considered as having meiosis in any way abnormal. There has been a report of bilateral absence of the vas deferens associated with 46,XY/47,XXY mosaicism in skin and testis tissue, but not blood (Leiba, Ber, Joshua, Lazebnik and Ben-Basset 1969); the gonad, however, appeared normal.

Meiotic screening for possible chromosome translocation was essential for advice with respect to future pregnancies and was a further source of controls, since translocations were not always present (for example in DD690194).

There are many clinical reasons where orchidectomy of normal gonads is necessary, for example, in the treatment of cancer of the prostate. Pogosianz and Brujako (1971) did not consider such material suitable, at least when polyploidy was being considered.

The fact that it is rarely possible to obtain a second biopsy from the same patient is a serious disadvantage, and also that a general anaesthetic is considered preferable. Only small amounts of tissue should be removed by biopsy as a much larger area of gonad might be rendered ineffective due to the coiling of the tubules (an important consideration with the condition of oligospermia). Experimentation with technique can therefore be attempted only when whole gonads are available or when cytogenic analysis is less important for diagnosis.

6.3 TECHNIQUES

Prefixation treatments

Technique "C" was considered generally adequate and reliable for all stages of meiosis, but was especially good for diakinesis. Few pachytene cells remained well preserved. No modification was necessary when used with the mouse, Syrian hamster or locust, although there is probably an optimal period of pre-treatment for each, which would improve the yield at certain stages. Notably fewer diakinesis figures were observed in a Syrian hamster which was killed with ether. Either the gas was responsible, or, more likely, diakinesis is a shorter stage in the hamster, than in the mouse or man.

The volume of hypotonic fluid used could be responsible for an observed correlation between the size of the biopsy used and the quality of the various preparations. Welshons, Gibson and Scandlyn (1962) appreciated that the volume of citrate, and hence cell concentrations could be important: work on Chinese hamster meiosis suggested a 15:1 ratio of fluid to tissue was ideal. Meredith (1969) considered a 20:1 ratio to be better

Technique "M" was good for preparation of the early prophase stages, especially cells at pachytene. Potassium chloride pre-treatment was favoured by Brooke, Jenkins, Lawson and Osgood (1962) who suggested that 0.01M KCl caused uncoiling and lengthening of chromosomes. Woollam and Ford (1964) attributed to uncoiling the fact that the centromere regions became more obvious.

Technique "F" was less reliable but did preserve chromomeres at pachytene and also nucleoli, particularly well. Bøök and Kjessler (1964), Eberle (1963), Ferguson-Smith (1964a) and Annéren et al. (1970) agreed that better spreading was obtained with hypotonic pre-treatment but gave poor preservation of pachytene chromosome structure independent of the fixative used.

It is comforting to note that an identical picture of meiosis was presented by all techniques, with differences only in spreading and the relative numbers of the different stages. For example, technique "F", without any pre-treatment, occasionally produced well spread diakinesis cells. Bödk and Kjessler (1964) noted that isotonic saline was better for the early stages of meiosis and hypotonic for the later. Technique "M" rather contradicted this. Corin-Frederic (1968) using a technique with a Ca++ and Mg++-free isotonic salt solution buffered with phosphatase, claimed to show the X and Y in end to end association in the sex vesicle, but the illustrations were unimpressive.

A technique based on that of Meredith (1969) was rather disappointing since chromosome spreading proved inadequate and the proportion of stages suitable for analysis, present on each slide, was small. It is that method most likely to conserve cells when only small amounts of tissue are available, but the technique used was not a true test of that of Meredith since so many modifications were made.

Rothfels and Siminovitch (1958) suggested slow fixation was essential. In this study, fixative was always added down the side of the tube but fixation could not be described as slow.

As shown by testis sections of normal gonads, tubules packed to the centre with cells in division (Plate 4b) were considered to give fewer meiotic metaphases in air-dried preparations, than those with an open lumen. Explanations might include: that the cells were not easily released during hypotonic pre-treatment. Edwards and Guli (1963) advised squeezing the tubes to release their contents. Here only fine chopping was used. Fibrous material in older gonads might be responsible for failure of release of cells at diakinesis. This does not, however, explain the abundance of cells in early first prophase which should theoretically lie deeper in the tubule wall.

Staining

Light staining with orcein, used with phase contrast, was entirely satisfactory if a good batch of stain was obtained from the manufacturer. Permanent preparations were preferred, in spite of the loss of some chromosome detail, since it was frequently necessary to check such details as chiasma counts, when the number of chiasmata per bivalent was being assessed. A considerable disadvantage with orcein was that it could not be removed, to use the same preparation subsequently for fluorescence analysis. This is possible with Toluidine blue or Giemsa (Polani and Mutton 1971) but these stains did not carry any other advantage. Carbol fuchsin faded after a few days. This was considered a serious disadvantage.

The centromere region could be identified by its deeper staining at most stages, but a reliable method showing centromere regions at every stage and in every chromosome would be ideal. Using Benda stain on sections of Opisthocanthus, Wilson (1931) described the centromere as being clear violet against a brown chromosome background. It is likely that a technique like that described by Arrighi and Hsu (1971) for mitotic chromosomes, will provide the same kind of information and facilitate chromosome identification at meiosis. Both centromeric heterochromatin and banding techniques (for example, Summer et al. 1971 and Yunis et al. 1971), so successfully applied to human chromosome identification, will only be useful if chromosome morphology is retained.

Feulgen failed on air-dried preparations but was satisfactory for staining a sex-chromatin positive smear from buccal mucosa. Brown and Ris (1959) queried the reliability of Feulgen specificity in experiments with amphibian oocyte nucleoli and Gledhill (1970) commented on the unreliability of Feulgen tests on ejaculated sperm since widely differing results were found. It is suggested that the failure of Feulgen, in this study, was either due to a reduction of DNA concentration inevitable with the superior spreading of an air-dried technique, or to the denaturation

of DNA either by fixation or air-drying.

Rafalko (1946) noted that Feulgen positive results were not usually obtained by the conventional method of smear preparation of small amoebae, oocyte prophases of a parasitic wasp and yeasts, with tiny amounts of DNA. He successfully revised the technique using direct charging of the sulphurous acid bath with SO_2 gas instead of using HCl with sulphites. Swift (1950), using sections, found Feulgen intensity varied directly with the thickness of the absorbing layer. Air-dried preparations are much thinner than sections. Associated with this, Lessler (1953) commented on the inability of the human eye to distinguish between small differences in colour intensity of Feulgen, which might explain the difference between densitometry and visual data. Gaillard, van Duijn and Schaberg (1968) thought they prevented loss of DNA by avoiding contact with acetic acid-alcohol mixtures and then successfully used photographic colorimetry with Feulgen stain. Fixation may therefore have been responsible for a failure in this study. The successfully stained buccal smear was fixed in 50% ethanol and 50% ether - not acetic alcohol. Von Borstel, Prescott, and Bollum (1966) suggested that acid fixation could indeed denature DNA. Amenta (1961) found Feulgen did not stain when the chromosomes were ultra violet irradiated, probably because of alterations in the DNA.

Any kind of hydrolysis on air-dried preparations, including that in the Feulgen or in the Toluidine blue technique, was inclined to detach the air-dried cells from the slide.

All routine cytological techniques were performed very carefully to standardise the results obtained. However, this meant that the scope for improvement was limited. Some of the most important "break-throughs" have been discovered by accident. For example, Hsu (1952), by mistake, washed foetal fibroblast cells in hypotonic Tyrode solution before fixation, instead of isotonic saline and discovered the advantage of

hypotonic pre-treatment.

Phase contrast is considered by some to be unsatisfactory for the examination of chromosomes. Ford (1969) commented on some areas of chromosomes appearing thicker than others as a consequence of phase distortion. However, in this study, it was considered imperative for the observation of fine detail, without overstaining coarse structures at the same time. The chromosomes at the secondary constriction region of bivalent number 9 at pachytene were here estimated to be 0.5 μm in diameter whereas an estimate by Hungerford et al. (1971) gave 0.3 μm. The difference might be attributed to the use of phase contrast.

6.4 TESTIS HISTOLOGY

The classification shown here (Table 7 and Appendix) was a simple one. There are more complex versions (for example, that in Tillinger 1957). There was a continuous spectrum of types of gonad and it was impossible to extract all the possible detail from the collection. As expected, there was a good correlation of numbers of dividing cells in air-dried preparations with the amount of spermatogenesis shown by sections.

6.5 SPERMATOGONIAL CELLS

Preparations for the analysis of somatic mitotic metaphase are selected for showing well spread chromosomes, with extended arms. A similar selection among spermatogonial metaphase cells is essential. Where chromosomes are extended, material could be observed spanning the secondary constriction region and (allowing for exaggeration of chromosome size) the presence of these regions in the paracentric position could then be used to identify certain chromosomes, for example 1, 9 and 16 and, by elimination, others. The behaviour of these regions is less likely to cause error in estimating

chromosome number than was suggested by Hultén and Lindsten (1970). In fact Koller (1937) showed an illustration of spermatogonial metaphase with 46 chromosomes even though he believed the human diploid number to be 48. It is possible that the behaviour of secondary constriction regions at spermatogonial metaphase was responsible for the original false estimates of the human chromosome number (von Winiwarter 1912, von Winiwarter and Oguma 1930).

Abnormalities in spermatogonial stem cells would tend to continuously propagate abnormal gametes, were it not for strong selection against the various maturation products. Although it was not possible to distinguish the different spermatogonial metaphases, among more than 450 cells, no abnormalities, certainly none of chromosome number, were detected.

The sex chromosomes seemed more compactly coiled at spermatogonial metaphase. This gave the impression of more extreme heteropycnosis. Coiling of chromosomes at spermatogonial metaphase hindered their identification, but seemed characteristic of germ cells in mitosis. It was first observed in 1880 by Barenetsky in pollen mother cells of Tradescantia. Coiling does also occur occasionally in somatic mitotic metaphase and can be experimentally demonstrated by a solution containing KCl, KSCN, $NaNO_3$ and sodium acetate (Ohnuki 1968). Secondary constriction regions can also be experimentally enhanced to give extended regions like those seen in spermatogonial metaphase, for example by treatment with BUDR (Kaback, Saksela and Mellman 1964) and in permanent human cell lines by treatment with herpes simplex 2 virus (Miles and O'Neill 1969). Miles, O'Neill, Armstrong, Clarkson and Keane (1965) reported accentuation of secondary constrictions in cell lines particularly with respect to 1 and/or 16. It was believed that some cells with chromosomes that did not demonstrate the coiling and which appeared like leukocyte mitotic metaphases (Plate 12) were indeed somatic cells, since one was found in a case with complete absence of germ cells, as diagnosed by testis sections. It remains possible (but unlikely) that the piece of the

biopsy used for the air-dried preparations did contain some spermatogenesis.

Homologous Pairing at Spermatogonial Metaphase

Very little evidence was found, here, of pairing of homologous chromosomes in spermatogonial prophase or metaphase in humans, although, with some reservations about the stage observed, there was some evidence in the locust. No statistical analysis of pairing of homologues was performed. This could probably be done for the number nine chromosomes which were the easiest to identify because of the distinctive paracentric secondary constriction (Plate 17a). A fluorescence study might also be useful in a statistical analysis. Unfortunately only 5% of spermatogonial metaphases fluoresce, as compared to 80-90% of leukocyte metaphases, and even then with a lesser intensity (Pearson and Bobrow 1970b).

There is a plethora of literature on somatic pairing of homologous chromosomes both with or without discussion on its implications with respect to meiosis: initiation; preventing of interlocking of bivalents, and maintenance of order (Wilson 1912, Metz 1916 and Smith 1942). Sonnenblick (1950) showed that, in Diptera, homologous pairing was the rule, homologous reassociation occurring at every mitotic anaphase. (This is difficult to correlate with males being achiasmate.) Boss (1955) showed pairing, in the newt, at anaphase; White (1965) described it in African Eumastacid grasshoppers, and Feldman, Mello-Sampayo and Sears (1966) in Triticum aestivum L. Chauhan and Abel (1968) showed association of homologues during pre-meiotic stages in Impatiens and Salva, suggesting pairing up to at least pre-meiotic interphase. Stack and Brown (1969) discussed somatic pairing in Diptera, Yucca and other plants, pre-meiotic pairing in spermatogonia of various animals and somatic recombination in fungi. Dubuc and McGinnis (1970) identified homologous pairing by studying homologous and non-homologous distances in Avena sativa L. root-tips, and Gibson (1970) did so, in one cell, among thousands analysed of the Tasmanian rat kangaroo (2n = 12),

where 11 chromosomes paired and one appeared to be missing. Schneiderman and Smith (1962) demonstrated, likewise, that homologous chromosomes tended to lie closer to each other on the metaphase plate than would be expected by chance, by measuring centromere distances in human metaphase cells. True somatic pairing was claimed by Gropp and Odunjo (1963) and Hungerford (1964).

There is other evidence for somatic pairing, Evans (1961) induced interchanges in Vicia faba L. by gamma irradiation and of the interchanges 88% were between homologues. Rao and Natarajan (1967) induced mitotic recombination by mitomycin C and alkylating agents in Vicia. There were more homologous chromatid exchanges than expected which is suggestive of association of homologues throughout the life cycle. In human material, Shaw and Cohen (1965) using mitomycin C found 50% of exchanges involving real or apparent homologues, almost always at corresponding sites. Specificity of the association between number 9 chromosomes using mitomycin C was beautifully demonstrated by Kistenmacher and Punnet (1970). Homologous chromosomes do associate in somatic cells and quadriradial configurations have been compared with diakinesis figures at meiosis (German 1964) especially since homologues are so often involved. Pontecorvo (1958), discussing somatic crossing over, suggested it was frequent in primitive conditions and had been reduced in the course of evolution. Westergaard (1964) elaborated this argument.

In the light of the vast amount of information of somatic homologous pairing, throughout the animal and plant kingdoms, it would be surprising if homologous pairing was not shown in spermatogonia.

6.6 INITIATION OF MEIOSIS

Nothing is known of the stimulus that initiates meiosis and it is rare that hypotheses are put forward. There must be some kind of induced capacity after a certain number of spermatogonial divisions. An explanation, rather like that needed to explain why subculture of human diploid cells is only possible 50 or 60 times, seems required (Hayflick and Moorhead 1961).

6.7 PAIRING OF HOMOLOGUES

The present investigation clearly demonstrated that pairing began from both telomeres at one side of the nucleus, then continued back into a typical bouquet configuration (Gelei 1921). There was no positive evidence of centromere regions playing any role in the initiation of homologous pairing in humans. The bouquet was most likely present in the hamster and the mouse and was definitely present in the locust.

There have been a few suggestions, usually in theoretical papers, that the centromeres were responsible for beginning pairing. For example, Novitski (1955) discussed a controlling element in the heterochromatin near the centromere that might have a recognising and attracting property and Ratnayake (1968) considered the theory for meiosis. Pairing in Fritillaria is described as initiating at the centromere irrespective of the number of chiasmata formed (Darlington 1937) while Wagenaar (1969) confirmed this in a study of several other plants.

In much of the material studied, the chromosomes were telocentric. Behaviour of a telomere may have been attributed to that of a centromere. It is interesting to note that the possible evidence of homologous pairing in locust in this study (but not in the human material), was by attraction of centromere ends of the chromosomes (Plate 75a) possibly a point indicating that the cell was in early anaphase of second meiosis rather than at spermatogonial metaphase. Synapsis must be a relatively fast process since

there is so little cytological evidence: less than a dozen cells in the whole study showed the process in action. It is probably even faster in other species. For example, apart from a transient leptotene to zygotene, Henricson and Bäckström (1963) found only two pachytenes in their preparations from the boar.

Eisen (1900) described the bouquet in Batrachoseps and Janssens (1905) observed that synapsis proceeded inwards in the salamander, but did not observe the process as occurring simultaneously from both ends. Davis (1908) pointed out that centres of polarisation were close to the nuclear membrane in Acrididae and Locustidae spermatogenesis, and Ribbands (1941) showed that proximity to the nuclear membrane tends to lead to precocious development. Snook and Long (1914) in Aneides and Scudder (1942) in Desmognothus fuscus Raf. both appreciated that synapsis was from telomeres back. Kasha and Burnam (1965) showed that meiotic pairing was initiated at or near the ends of the chromosomes in barley and Sved (1966) further suggested that pairing was initiated at the ends in higher plants because homologous telomeres are attached close to each other at the nuclear membrane. Beçak, Beçak and Rabello (1967) demonstrated that pairing proceeded back from the ends in polyploid amphibians. For maize, Tabata (1962, 1963) with trivalent configurations visualised the necessity of alignment of homologues prior to meiotic prophase and also for the initiation of pairing at the telomeres. Clutterbuck (1970) suggested that a variegated position effect in Aspergillus might be explained by meiotic synapsis starting at the ends of the chromosomes and working towards the centromere. Kezer (1970) and Kezer and Macgregor (1971) using sections of Batrachoseps and superb squashed from various salamanders including Batrachoseps and Plethodon c. cinereus Green clearly demonstrated that chromosomes are directed to one side and synapsis proceeds from telomeres backwards into a bouquet, exactly as presented here. Each organism will probably have its own peculiarities of prophase. For example, in the mosquito, there is no traditional leptotene or zygotene, since in

the pre-meiotic mitoses there is somatic pairing of homologues and full pairing occurs at the onset of meiosis (Akstein 1962). In Lilium longiflorum Croft, chromosomes are separate entities at pre-leptotene with no evidence of pre-meiotic pairing (Walters 1970).

Other ideas are that either telomeres or centromeres may be responsible for pairing, rather than by short sequences all along the chromosome (Pritchard 1960, von Wettstein 1971). In view of this, it is certainly interesting that Moens (1969a) described at zygotene in the locust, short stretches of two homologous chromosomes joined by a complete piece of synaptinemal complex, while centromeres and telomeres were not joined.

The bouquet stage had not been described in detail in the human male, although Koller (1937) showed a bouquet without naming it. Severinghaus (1942) mentioned the bouquet as being post-synaptic rather than synaptic. An unusual feature suggested by this study, although by no means conclusive, was that the sex chromosomes were not heteropycnotic at the bouquet stage and could not be identified in any way (Plate 19). The locust bouquet, however, shows clearly a heteropycnotic X (Plate 76), and in Koller's (1937) illustration there appears to be a sex vesicle at the top. Sasaki and Makino (1965) noted two separate heteropycnotic bodies at early leptotene and Solari (1969) the same in a mouse. John and Lewis (1965) described chromosome ends at the nuclear membrane near the centriolar system, with heteropycnotic centromeres raised to the top of the bouquet and the heteropycnotic Y at the base.

6.8 ORDER WITHIN THE NUCLEUS

Both the pairing of homologues and the complexities of cell division are less difficult to understand if strict order within the interphase nucleus is assumed. Meiosis with 168 chromosome in the lamprey Petromyzon marinus L., the highest chromosome number recorded in vertebrates (Potter and Rothwell 1970), is intriguing. The observation that

no extensive chromosome movement occurs in interphase was made as long ago as 1909 by Boveri and more recently, Belar (1929) and Rhoades (1961). Maguire (1961) commented on little movement at pachytene. Miller, Breg, Mukherjee, Gamble and Christakos (1963) commented on larger chromosomes 1 and 2 being near the middle and smaller ones Y, 21, 17-18 and 13 near the periphery, which suggested order in human mitotic metaphase. In the sperm of the rat, Bianchi and de Bianchi (1969) commented on a constant position of the Y.

To explain any order, classical cytologists observed fine connections between metaphase chromosomes during mitosis in spermatogonia of hemipteran insects (Wilson 1925). Order is sometimes attributed to the nuclear membrane (Lane 1967); but it is considered that the chromosomes take an active part in the formation of the nuclear membrane (Barer, Joseph and Meek 1959). Woollam and Ford (1964) reported the synaptinemal complex as being attached to the nuclear membrane by basal knobs. Comings (1968), in an electron microscopy and autoradiography study of human amnion, establishe attachment of chromatin to the nuclear membrane and, also, that at least some of the attachment sites corresponded to the points of initiation of DNA synthesis in each replicon. Ockey (1969) related time of DNA synthesis in human mitotic chromosomes to proximity to the nuclear membrane. Using human fibroblasts, Comings and Okada (1970b) showed that metaphase chromosomes carried fragments of nuclear membrane material up and down their surfaces. They also showed that chromatin fibres with annuli of nuclear membrane, were present in Chinese hamster, quail and human interphases (Comings and Okada 1970a). They suggested that the annuli may serve as attachment to the new nuclear membrane.

Other explanations of synapsis in addition to, or instead of, close proximity have been suggested: Delbrück (1941) postulated resonance - bonding to explain synapsis; Fabergé (1942) suggested Guyot - Bjerknes hydrodynamic forces; Hinton (1945) an antigen/antibody reaction while Rhoades (1961)

suggested Van der Waals forces, but all these hypotheses are essentially untestable. Thomas (1966) suggested that the molecular pairing of DNA molecules in bacteria and bacteriophage can probably occur simply by random motion and recognition of short segments of complementary base sequences. Ratnayake (1968) also supported a similar molecular mechanism, and Hoyer, McCarthy and Bolton (1964) pointed out that the concentration of homologous DNA seen at or near the region around the synaptinemal complex would be many times that necessary to produce efficient hybridisation in vitro. Perhaps one of the least testable hypotheses is that of Comings and Okada (1970) who suggested that the most mysterious part of somatic and meiotic pairing may take place immediately after fertilization and may be related to the topology of the pronuclear fusion process.

6.9 PACHYTENE

Abnormal homologous pairing observed here included: the failure to pair in parts of chromosomes (Plates 34 and 35a) or in a whole chromosome (Plate 35b), and failure due to a physical impossibility, where one bivalent passed between the homologues of another (Plate 36). Abnormal pachytene configurations had not previously been reported in normal humans. It is easy to conclude that any deficiencies, inversions or duplications must be very small, since no hairpin configurations, like those seen in maize (Rhoades 1968), were observed. Specificity of pairing has not been doubted since Wenrich (1916) observed corresponding chromomeres closely associated at zygotene and pachytene in Phrynotettix; but Maguire (1960) noted that pachytene chromomere patterns do not always coincide in synapsed homologues.

Hultén, Eliasson and Tillinger (1970) reported normal pachytene pairing in two males with a low chiasma count, as did Pearson et al. (1970) in one. Hungerford et al. (1971) reported no significant structural difference between paired homologues of either of the bivalents 21 and 22, among the patients studied. Non-pairing in the short arm region of the

trivalent formed in the smaller G, in cells from a mosaic mongol, has been described (Hungerford et al. 1970), but no explanation for it,was given.

Pachytene Chromosome Analysis

Pachytene analysis has been made possible by the advent of modern techniques, such as that of Hungerford (1971). The potential offered seems vast.

It has been shown that heteromorphic homologues show no difficulty in pairing. Here were investigated polymorphisms in two chromosomes, 9 and 16. Both were secondary constriction region variants (Plate 10). Pachytene cells showed tight condensation at these regions, which consequently became as heteropycnotic as the centromere bodies. In chromosome 9 there was a series of paracentric chromomeres (Plate 21) and in 16, heteromorphism was reflected in a larger size of centromeric heterochromatin (Plates 22 and 23). When such large heteromorphisms have little effect on homologous pairing, it is possible that small deletions or duplications might be missed. Of course, the argument remains that these types of heteromorphism are not caused by extra material (Starkman and Shaw 1967, Hsu, Brinkley and Arrighi 1967, Crippa, Schwartz and German 1969). However, it would seem reasonable to assume that extra material is involved with a variant as large as that shown in Plate 10b. At pachytene,"despiralisation" of the secondary constriction regions is not a marked feature (as it is in these regions at spermatogonial metaphase) and so differences reflecting degree of heteromorphism (Plates 22, 24 and 25) supports that different amounts of chromosome material is involved.

A detailed chromomere map of each bivalent at pachytene is needed, before any variations can be studied. It is possible that a cytological map equivalent to the genetic map, as shown in giant salivary gland chromosomes (Dupraw 1970) could be made, but it seems unlikely that an identical picture of chromomeres will be demonstrated all through the

pachytene stage which can last some 10 days (Lima de Faria et al. 1968). The frequency with which a pachytene chromosome is mappable is a function of its length (Hungerford 1971). Pachytene mapping has been attempted for the D and G bivalents (Ferguson-Smith 1964a, Bordjadze and Prokofieva-Belgovskaya 1971, Hungerford et al. 1971). Hungerford et al. (1970) used the chromomere pattern of Hungerford et al. (1971) to identify the G chromosome involved in Down's syndrome. Using a mosaic mongol they found a trivalent was sometimes formed in the shorter of the two chromosomes. No trivalent was observed by Finch, Böök, Findley, Findley and Tucker (1966).

Mitotic karyotyping is broadly based on positioning according to size, although there are minor discrepancies, for example, chromosome 6 was found to be slightly larger than 5 in measurements by Ferguson-Smith et al. (1962). This study has shown that chromosomes at pachytene can be "karyotyped" according to those rules used in mitotic analysis. There is certainly a size difference in the G's (Plate 22a) shown particularly well at pachytene, since the overall increase in length of chromosomes at this stage exaggerates small differences. Eberle (1963) estimated the increase in size over mitotic metaphase was six times. It is suggested that the trisomy traditionally called "21" should now be properly termed "trisomy 22", on the basis of the evidence at pachytene by Hungerford et al. (1971) which was particularly convincing owing to the mosaicism shown in the case.

Centromere Regions

The most extremely heteropycnotic regions in pachytene chromosomes were here shown to be centromere regions, in human, locust and mouse. The bodies now described as centromeric heterochromatin have been described by others (Eisen 1900, Janssens 1905, Snook and Long 1914). The point at which the spindle microtubules attach has been called the "kinetochore" by various authors (for example, Luyukx 1965, Bajer and Mole-Bajer 1969).

Woollam and Ford (1964) mentioned an enhancement of contracted bodies of heterochromatin at centromere positions by 0.1 molar KCl treatment; this was confirmed by this study using technique "M". Kezer (1970) reported that centromeres were heteropycnotic in salamanders.

Here, material resembling knobbed satellites was occasionally seen protruding from the centromere region of the acrocentric chromosomes (Plate 30a) This was also shown by Hungerford et al. (1971). As the heteropycnosis must extend some way each side of the centromere, it would be expected that the short arm of the acrocentric chromosomes would not be represented, although the presence of a chromosome satellite is not surprising. However this made others believe that absence of the short arms meant the heteropycnotic bodies on the D's and G's were not centromere regions and, as a consequence, the heteropycnotic bodies on all the other pachytene chromosomes were largely ignored.

Ferguson-Smith (1964a) termed the acrocentric centromere regions "nucleolar chromomeres" and Eberle (1966) "satellited chromomeres". Gardner and Punnett (1964) ignored the heteropycnotic regions and consequently placed the D chromosomes upside down. Ford et al. (1968) and Ford (1969), with the whole pachytene complement, termed centromeres as detectable pale areas with deep staining on either side and dismissed what are shown in this study to be the centromere regions as equivalent to the "knobs" of maize. Datta (1969), with meiosis in the 9-banded armadillo, could not explain "heteropycnotic dots". Bordjadze and Prokovieva-Belgovskaya (1971) suggested that the centromere was located in the acrocentrics between chromomeres I (representing the whole of the short arm) and II, the next small chromomere down. Disagreement over something as fundamental as centromere position makes the idea of chromosome mapping seem rather ridiculous.

The amount of centromeric heterochromatin at pachytene varied greatly between bivalents (Figure 3) and also between the same bivalents in different

individuals. The studies presented have confirmed results obtained with reannealing experiments on mitotic metaphase cells, that chromosomes known to have prominent paracentric secondary constrictions (for example 1, 9 and 16) also have the largest amount of centromeric heterochromatin (Chen and Ruddle 1971, Arrighi and Hsu 1971). Rowley and Bodmer (1971) commented that metaphase chromosomes could be distinguished on the basis of different amounts of centromeric heterochromatin. Here, superimposed on this, the variation according to polymorphism is emphasised.

In attempts to elucidate the pachytene complement, Hungerford et al. (1971) described a C bivalent, present in every individual with spermatogenesis, having a cluster of small dots that were orcein-positive and RNase resistant. Figure 70 of Luciani (1968) may show the same configuration but the dots have been cut off and the chromosome is placed as a 6 in karyotyping. The description by Hungerford et al. (1971) is identical with that shown to be number 9 in this study (Plate 21a) from criteria of: size, centromere position of this chromosome, size of the paracentric bulge closely following the degree of heteromorphism in mitotic nines, and by a persistence of the pachytene chromomeres into diakinesis (Plate 48a). The chromomeres were about 0.5 μm in diameter. It is to be noted that Saksela and Moorhead (1962) enhanced secondary constriction regions by treatment with 1 : 1 methanol : glacial acetic acid. The number nine in their illustration was, in the paracentric region, puffy and almost "chromomeric".

The four strands of chromomeres shown in the stretched bivalents (Plate 21a) probably represent the four strands of the pachytene bivalent. The chromomeres were sometimes in two's or fours and it is tempting to again suggest that the four strands of each pachytene bivalent are represented. It is probable that variation in number, rather than size, of chromomeres copes with the problem of heteromorphism. Heteromorphism in number 16 was not solved in the same way. A dense block of heterochromatin was formed

instead, and which must be functionally equivalent since there was normal homologous pairing in the rest of the bivalent.

NUCLEOLI.

Heitz (1931) first associated nucleolar organising loci and the nucleoli, which disappeared during mitosis and reappeared at telophase. McClintock (1933) showed that nucleoli were associated with particular regions of chromosomes for which she coined the term "secondary constrictions". When the nucleolus was absent at the region containing the nucleolar organiser, no secondary constriction was formed either and when the nucleolar organising body was broken, nucleoli developed from each of two segments (McClintock 1934). Vanderlyn (1949), in a wide variety of plants, observed nucleoli associated with chromocentres. Ritossa and Spiegelmann (1965) reported on the location of DNA complementary to ribosomal RNA in the mammalian nucleolar organiser region.

This study confirmed the original observation of Ferguson-Smith (1964a) that all five acrocentric bivalents are capable of "association" (Plate 27). This was also shown by Hungerford (1971) and Bordjadze and Prokofieva-Belgovskaya (1971) using chromomeric identification. It reflects "satellite association", in cultured human somatic cells, of specific parts of chromosomes believed to be nucleolar organising regions (Ferguson-Smith and Handmaker 1961). This was confirmed by large scale statistical studies (Cohen and Shaw 1967, Zang and Back 1968). Huberman and Attardi (1967) found that at least 50% of the sites to which rRNA annealed, were associated with the smaller chromosomes, confirming cytological observations on the nucleolar organisers. The nucleoli produce rRNA only (Perry 1965).

Sex Chromosomes and Nucleoli

In this study, the sex vesicle was observed to support a nucleolus when no other chromosome was in the vicinity. However, this by itself was not proof of the nucleolar organising capacity of the sex chromosomes. Such an appearance could still originate from acrocentric chromosomes, associated with it, being sheared away from the sex vesicle during flattening of the cell. Acrocentric chromosomes do associate with the sex vesicle (Table 8, see also Eberle 1966, Luciani 1968), as well as with each other and with secondary constriction regions of other chromosomes, for example the paracentric regions of 16 and 9. This property is a great help in identifying such chromosomes. Acrocentric association with the sex vesicle is considered evidence for a nucleolar organising capacity of at least one of the sex chromosomes. Bobrow, Pearson and Collacott (1971) reported the para-nucleolar position of the human Y chromosome in interphase nuclei, shown by fluorescence analysis, and Gripenberg (1964) described satellite association of the Y.

Koller (1938) has reported nucleoli associated with the sex chromosomes in the golden hamster. Sotelo and Wettstein (1964) claimed the sex vesicle in Gryllus supported a nucleolus in its own right. Sachs (1954) reported material rich in RNA filling the sex vesicle and surrounding the sex chromosomes, but Monesi (1965) claimed a lack of RNA synthesis in the meiotic sex chromosomes of the mouse. Urena and Solari (1970) found no histologically detected RNA on the X-Y pair of Rattus but described a small number of cells having nucleoli near the sex pair, but with no continuity with it. Ferguson-Smith (1964a) believed that nucleoli were independent of the human sex vesicle. This was confirmed by Solari and Tres (1967) and Solari and Tres (1970) using evidence from electron microscopy. They suggested that the heteropycnotic basal knobs (shown here to be the centromere regions of the acrocentric chromosomes) were responsible for the nucleoli.

Reitalu (1970) claimed that "as pachytene progressed the weakly staining portion of the sex vesicle took on a true nucleolar staining". Miles (1970) found that tritiated actinomycin D, which binds to DNA and at low concentrations is thought to bind preferentially to ribosomal cistrons, did not label the human mitotic X.

Inclusions Within the Nucleoli

As has been described, heteropycnotic bodies have been observed projecting into the nucleolus. Some were attributed to chromosome material distal to the secondary constriction region of the short arms of the acrocentric chromosomes. However, other heteropycnotic elements were frequently observed within the nucleolar material. There are many fluffy loops and blocks extending from pachytene chromosomes (Plate 26b) and some dots in nucleoli might be caused by chance fixation of the nucleoli over such structures. The anomalous paracentric constriction region of bivalent nine was one of the regions that occasionally showered through a nucleolus (Plate 29a). It is not assumed, in this instance, that it was a chance occurrence.

There have been various reports for and against the presence of DNA in the nucleolus. Many were based on results obtained with the Feulgen reaction, which was found, in this study, to be unsuitable for air-dried preparations. Reports favouring the presence of DNA in nucleoli include: Painter and Taylor (1942) for toad's egg; Ohno, Weiler and Stenius (1961) for guinea pig germ cells, and Barr and Plaut (1966) for Drosophila salivary gland cells. Miller and Beatty (1969) demonstrated that the extra chromosomal nucleolar DNA loops of amphibian oocytes were actively transcribing rRNA precursors or molecules visible in the electron microscope. The disposition of the DNA and RNA was elegantly demonstrated by the use of RNase and DNase treatments of different preparations. Evidence against the presence of DNA in the nucleolus includes the data of:

Caspersson and Schultze (1940) on Drosophila salivary gland cells and sea urchin oocytes; Swift (1953) who considered contamination gave spurious results and Brown and Ris (1959) who blamed the unreliability of the Feulgen method.

Telomeres

It is a well known fact that telomeres of non-homologous chromosomes have a close affinity for one another (Warters and Griffen 1950, Wagenaar 1969). There is strong affinity shown by the acrocentric chromosomes for the free telomeres of the conjoined sex chromosomes. Griffen (1955) with a Sudan Black B squash technique and oblique illumination, constructed a late pachytene map in the male mouse and showed a large single nucleolus at the end of the XY bivalent, where there was a short section of chromomeric bands. This study has shown that bodies like little nucleoli may sometimes be observed at the telomeres of chromosomes at pachytene (Plate 28a). Wenrich (1916) described little fluffy polar tips of chromosomes in Phrynotettix. Observations of nucleoli in association with telomeres, as well as with secondary constriction regions of mitotic chromosomes, have been made by Hsu, Arrighi, Klevecz and Brinkley (1965) Ohno, Trujillo, Kaplan, Kinosita and Stenuis (1961) and Heenan and Nichols (1966).

Miles (1970) found peaks of tritiated actinomycin D concentrated at the ends of human chromosomes and at the centromere, which may mean that ribosomal cistrons are present at the telomeres. The telomeres of chromosomes take part in association with the sex vesicle or with the nucleolus (Plates 22a, 26a and 28b). It could of course be argued, and cannot be disproved, that this feature is a remnant of the bouquet configuration. If telomeres did have a nucleolar organising property, which is what "association" is assumed to represent, this would mean that a lot of anomalous chromosome behaviour would be explained, including "stickiness", not only

described at pachytene, but at diplotene and diakinesis. Lima de Faria and Bose (1962) described telomeres being last to separate at anaphase, because of an attracting property. Non-specific association of telomeric heterochromatin, persisting to dictyotene, was described by Ohno, Christian and Stenius (1963) in mouse oogenesis. There is, in addition, a telomere on the short arms of acrocentric chromosomes and this might reinforce any property usually attributed to either the secondary constriction region or the centromere.

Repeated Sequences in Chromosomes

Rapidly annealing DNA (sDNA) is associated with centromere regions (Walker, McLaren and Flamm 1969, Pardue and Gall 1970, Jones 1970). Reannealing experiments tend to enhance the centromere regions at mitotic metaphase (Yunis et al. 1971). This reflects what is observed at pachytene, when no special treatment is used. An interesting question is whether nucleolar organiser sequences are found in the satellite fraction. Reports of nucleoli being associated with the satellite fraction are not few (Schildkraut and Maio 1968, and Smith 1970, both in the mouse). Mattoccia and Comings (1971), reported the satellite DNA most intimately associated with the fraction of DNA which is closely bound to nucleoli, figures being 10% of DNA of whole nuclei, 16% in the heterochromatin + nucleoli fraction, 12% in the heterochromatin rich supernatant, 25% in purified nucleoli and 41% in nucleoli treated with 2M NaCl. Macgregor and Kezer (1971) showed in the salamander Plethodon c. cinereus Green that the sDNA was not related to the nucleolar organiser and does not include ribosomal cistrons, but confirmed association with centromere regions on all chromosomes.

Theoretical arguments, as to the function of repetitive DNA, are interesting. Novitski (1952) described centromeres as being classified as having different strengths on the basis of behaviour in double first anaphase bridges. A strong centromere would pull a weak one attached by

the bridge over to its pole. Lindsley and Novitski (1958) attributed this to the amount and kind of heterochromatin. Walker (1971) suggested there might be an advantage conferred on the chromosomes possessing more repetitive sequences, as they might be able to survive the mechanical rigors of meiosis better. There might be some kind of selecting factor operating against loss in the polar body since the Y chromosome happens to be the only one not having satellite sequences in mouse (Pardue and Gall 1970). There is weak but observable linkage between genes in different, non-homologous, chromosomes in mice (Michie 1953) and in Drosophila (Peacock 1965). Mongols resembling their mothers rather than their fathers over several unlinked markers, remains unexplained (Penrose 1957). Jones and Robertson (1970) comment on reiterated nucleotide sequences in Drosophila and mouse as being near the centromere, and which could promote close physical approximation of homologous and non-homologous chromosomal regions, to facilitate regulation of function. Telomeres of the A and C chromosomes in Rhynchosciara have been shown to have repetitive sequences (Eckhardt and Gall 1971). Repetitive sequences on telomeres might help to explain much of the chromosome behaviour shown in this study.

MORPHOLOGY OF THE SEX VESICLE.

Eliasson, Gustavsson, Hultén and Lindsten (1967) reported the absence of a sex vesicle in pachytene of the dog, but one bivalent was perhaps slightly more heteropycnotic. In all the material studied here, a sex vesicle was always present at pachytene. The morphology of the sex chromosomes in the sex vesicle was difficult to establish, but there was no evidence suggesting that they were not paired at one end and then looped (Plate 32c). Reitalu (1970) described two bodies representing the X and Y in the sex vesicle - something never observed in this study. Egozcue (1968) showed pachytene evidence suggesting that crossing over occurred within the X chromosome in almost 100% of pachytenes in the male squirrel

monkey and 70% in the rhesus monkey, dwarf galago and tarsier. The remainder of cells apparently showed the darkly stained sex vesicle. In this study some cells were observed with configurations identical to those described by Egozcue (1962) (Plate 37). Here, however, twisting of the sex chromosome loop was believed responsible for the appearance. In the male locust the single X was shown to behave in an odd manner in the prophase of meiosis and might be interpreted as folding back upon itself, a configuration possibly maintained into diakinesis (John and Lewis 1965). Khush and Rick (1968) stated that it was not unusual to observe synapsis between a single unpaired arm that folds back to pair with itself.

Gardner and Punnett (1964) misinterpreted an acrocentric association with the sex vesicle and recorded that the sex chromosomes in humans lay straightened out or "not wholly in the sex vesicle". Solari and Tres (1967) said, in a fine structure study, that one pole of the sex vesicle protruded and stained heavily. This could have been an acrocentric centromere in association.

6.10 DIFFUSE STAGE

The only way that individual cells observed in this study, could be fitted into any kind of sequence in prophase of first meiosis, in the human male, was to introduce a "diffuse stage" after pachytene and before diplotene (Plates 38 to 40). It was, that enough of the less frequently observed intermediate stages were obtained, the behaviour of the sex chromosomes unravelling from the sex vesicle, the nucleoli regressing, and the persistent chromomeres of the paracentric region of bivalent nine, that confirmed the interpretation. There was also evidence for the presence of a diffuse stage in the locust, Syrian hamster and mouse.

The fact that this stage had not been previously observed in humans, possibly means that work in a variety of fields must be reassessed. Erroneous

interpretation of zygotene as a more advanced stage has been noted by others (Maguire 1960, Moens 1964). For example, McDermott (1970) claimed evidence for pre-meiotic pairing of homologous centromere regions. With the interpretation presented here, he could have been describing cells in the middle of the diffuse stage (Plate 40a). Reitalu (1970), with human male meiosis, could not have interpreted the sequence as described here. Hungerford (1971) suggested cells at "late pachytene" good for mapping chromomeres "or prediplotene", but obviously he is not referring to cells with diffuse bivalents, which have little differential staining except for the centromere regions. In describing the cellular association of the seminal epithelium in man, Clermont (1963) did not mention the diffuse stage although the diagram in Heller and Clermont (1963) suggests its presence. Autoradiography has not demonstrated it in man (Lima de Faria et al. 1968) or mouse (Kofman-Alfaro and Chandley 1970). Yerganian (1957) emphasised the absence of lampbrushing in pachytene chromosomes. Solari and Tres (1967) mentioned that as pachytene progressed, the sex vesicle became less condensed, which might possibly be a symptom of the diffuse stage.

Singleton (1953) hinted at a diffuse diplotene stage in Neurospora and Barry (1966) confirmed its existence in the same mould because the ascus continually enlarges and can be used as an index for maturity. Barry (1969) reaffirmed the position of the diffuse stage between pachytene and diplotene. Lu (1967) reported a diffuse stage in Gelasinospora and Lu and Raju (1970) in Coprinus. Nagl (1969) measured nuclear volume and other criteria in Beta and found a zygotene - like stage in early diplotene. Henderson (1961) noted the presence of diffuse diplotene stages in British Tetrigidae (Orthoptera). Conclusive evidence of a diffuse stage between pachytene and diplotene was shown by Kezer (1970) and Kezer and Macgregor (1971) in the salamander. Histological section of Batrachoseps showed a diffuse stage between pachytene and diplotene, position in sequence being

known because of the presence of a caudocephalic wave of meiosis. The picture presented by histological sections was matched by air dried preparations and these were exactly as described here for the human male. Nebel and Coulon (1962), using electron microscopy, found a diffuse diplotene stage in pigeon spermatocytes, and commented on fuzziness at pachytene and first meiotic metaphase. Sachs (1953) described fluffiness at diplotene in male Microtus agrestis L. and Zenzes and Wolf (1971) showed and mentioned a diffuse stage in Microtus which they attributed to diplotene. A diffuse stage was described in the Djungarian hamster by Pogosianz (1970), from the same kind of evidence as presented in this study.

In the female, "dictyotene" is classically described as a relaxed diplotene (see Roth 1966 for mosquito). It follows diplotene in human oogenesis (Ohno, Klinger and Atkin 1962). If this is so, the human male diffuse stage is not equivalent, since it occurs before diplotene and merges into it. Slizynski (1961) with mammalian oocytes hinted at the diffuse stage, dictyotene, following pachytene, without referring to it as relaxed diplotene.

Dictyotene in oocytes has been attributed to a stage necessary for vast synthetic activity and lampbrushing is probably present in the oocytes of all animals (Callan 1963) and plants, for example in Rosa ruguso Thunb. (Klášterská 1971). This might also be true of the "diffuse stage" shown here. Synthetic activity must be essential for: the survival of the cell (for another 50 or so days in human); for division, and possibly for supporting other cells at later stages of meiosis. The close association between cells shown in this study, especially with respect to pachytene (Plate 33) was very apparent, and there could well be a "nurse cell" role for some cells. There are probably several ways of achieving synthesis for storage. One is reminded of the tailed frog Ascaphus where, in the last oogonial mitoses, all daughter nuclei remain in the same cell and each show the full lampbrush configuration (352 lampbrush chromosomes, all

synthesising RNA). Seven of the nuclei disappear in late oogenesis (Macgregor and Kezer 1970). Ribbert and Bier (1969) showed that the nurse cells in the blowfly Calliphora have a functional similarity to the oocyte nucleus. Feulgen and fluorescence staining with N,N'diethylpseudoisocyanin chloride showed gene amplification beyond the level of polyploidisation. However, Barry (1969) suggested that the diffuse stage did not represent a stage of intense synthetic activity in Neurospora since he considered that metabolic activity was not a feature of the ascus at this stage. Lu (1967), by contrast, did think it represented increased gene activity for ascus growth in Gelasinospora. The Y chromosome of Drosophila forms evanescent lampbrush-like structures, active in RNA synthesis, in nuclei of spermatocytes (Meyer, Hess and Beerman 1961). Deficiencies of the Y cause sterility by sperm being non-functional (Hess 1970). This perhaps correlates loss of RNA synthetic activity with later differentiation of spermatids.

It was shown here, that chromosomes are quite condensed, when they pair at the beginning of prophase (Plate 19a). This was also noted for Neurospora, by McClintock (1945) Singleton (1953) and Barry (1966). Knowing the specificity in crossing over, it seems difficult to envisage that recombination occurs at this time. Barry (1966) thought that crossing over was likely to occur in the diffuse stage as also did Rossen and Westergaard (1966) for Neotilla rutilans Dennis. But Lu (1967) considered crossing over had already taken place in Gelasinospora since paired homologues were separating in the diffuse stage except at certain points, which suggested that chiasma formation had already occurred.

6.11 DIPLOTENE

Diplotene was infrequently observed. Centromere regions were heteropycnotic and secondary constriction regions pale. Chiasma analysis was considered unreliable owing to the twisting and overlapping of the bivalents.

It has been stated that chiasmata do not occur in the short arms of acrocentric chromosomes (Kjessler 1966). Plate 41b shows crossing over in the short arms of a D bivalent. Crossing over in acrocentric short arms was also observed at diakinesis.

Heteropycnotic centromere regions apparently held by mutual attraction have been described by Kezer and Macgregor (1971) in the salamander Plethodon c. cinereus Green. They interpret this as a mechanism to prevent precocious parting. The phenomenon was occasionally observed in the present study, especially at diplotene, and care was taken not to attribute it to a chiasma.

6.12 DIAKINESIS

Bivalents at diakinesis could be identified by their relative size, centromere and secondary constriction positions, and also by other characteristics such as asymmetry caused by heteromorphism and variation in degree of heteropycnosis.

Centromere regions were observed as two bodies in each homologue, at diplotene and diakinesis. Macgregor and Kezer (1971) noted this in Plethodon and interpreted it as evidence for centromeres having replicated in pre-meiotic S. However, here, the presence of two bodies could be interpreted as representing a heteropycnotic patch above and below the centromere region, and which give an appearance of being side-by-side when the chromosome contracts.

In their illustration, Falek and Chiarelli (1968) included bivalent 9 as a 4 and 16 (positioned on its side) as a 14. Luciani (1968) in his "figure 73" had 9 as a B (upside down). Centromere regions were ignored by Sasaki and Makino (1965) in their karyotype; most of the bivalents below the size of a D and some C's were rotated 45 degrees and nine was placed in their "plate A" as an 8. Groupings were, in general, as would

be shown here, but many bivalents were inverted. Caspersson et al. (1971) using fluorescence microscopy to karyotype diakinesis bivalents mostly placed them as might be shown here, but there were still some bivalents swivelled 45 degrees.

The repeated misplacing of bivalent 9 to the position of a larger bivalent, by some authors, demonstrates the weakness in using relative size as the major criterion for karyotyping. The attenuated constriction regions cause the bivalent to appear much larger. Sometimes no material could be observed at these points and it is possible that the bivalent could be mistaken for two smaller ones. This phenomenon might have been responsible for errors in some of the earlier estimates of the human diploid number of chromosomes. Falek and Back (1967) and Falek and Chiarelli (1968) suggested that the G bivalents can be distinguished by chiasma position, but no evidence of this was shown here. The G bivalents occasionally abutted on to one telomere of the sex bivalent. This was interpreted as reflecting association at pachytene. In one of the locust bivalents an apparent gap, that was attributed to a nucleolar organiser, was described by John and Henderson (1962), who also found it usually present in only one homologue.

In humans, 2.8% of diakinesis cells were possibly abnormal. This was a maximum estimate since, for example, some of the cells counted as containing 22 elements could have had two bivalents overlapped, appearing as one.

Interlocked Bivalents

It must be emphasised that the identified bivalents which appeared to interlock and loop with the sex bivalent, could not be conclusively shown to do so, from an examination of flattened air dried preparations. As all chromosomes seemed capable of such involvements, the phenomenon could perhaps be merely an illusion, as it would be expected that the larger

bivalents would be involved most often.

There was little evidence of interlocking at the previous stage, pachytene.

Sex Chromosomes at Diakinesis

The present study showed that it was generally the short arms of the sex chromosomes that paired. It was not excluded that the long arm of the Y could appear to be involved, but only in a small minority of cells. Such occurrences need to be confirmed using fluorescence microscopy on a large number of cells. So far, fluorescence studies have only shown pairing in the short arm of the Y (Pearson and Bobrow 1970a). Reitalu (1970) reported that the distal short arm of the X associated with the middle of the long arm of the Y.

In the Syrian hamster the short arm of the X and the long arm of the Y paired (Plate 86b). This confirmed the observations of Fredga and Santesson (1964). In the mouse the long arms of the X and Y were involved (Plate 93a), also shown by autoradiography by Kofman-Alfaro and Chandley (1970).

6.13 METAPHASE II

Since the chromosomes at early metaphase II are longer than at either the preceding diakinesis, or subsequent late metaphase II stages, it is suggested that an interphase probably intervenes between metaphase I and II. As long ago as 1916, Wenrich described a diffuse stage between the first and second spermatocyte of Phrynotettix.

It was shown that chromosomes at metaphase II can be karyotyped using the standard criteria. Caution is required in interpreting chromosome arm length, as there may be considerably extended paracentric secondary constriction regions or, alternatively, there may be length reductions due to coiling. Arm rotation (Plate 54a) may also be present. Sex chromosomes

in man could be identified by their compact coiling which made them appear heteropycnotic. This was also observed for the Y and the short arm of the X in the Syrian hamster, as well as for mouse sex chromosomes. Ohno (1962) had suggested that the human X and Y chromosomes were positively heteropycnotic at metaphase II. Hamster metaphase II cells were of particularly good quality and a "karyotype" was prepared which differed in detail from that of Galton and Holt (1964), who used foetal mitotic cultures.

Many of the metaphase II chromosomes showed what might be interpreted as a double stranded nature of each chromatid (Plate 54b). Multi-strandedness versus single-strandedness in chromosome structure is too large a subject to discuss here. However, using the light microscope, others that have observed "half-chromatids"include: Nebel (1932) in Tradescantia, Manton (1945) in Todea and Maguire (1968) in maize, the latter author using Nomarski interference microscopy. Trosko and Wolff (1968) found that trypsin digestion studies enhanced the half-chromatids in Vicia faba L. .

It was interesting that, of those cells chosen as suitable for karyotyping (with chromosomes well spread and most distinct), 64% were Y-bearing and 36% X-bearing. It was assumed that the cells bearing Y chromosomes were different in some way, such as having less contracted or less diffuse chromosomes and so were more likely to be photographed, rather than that there was a differential viability. However, there does seem to be an excess of males conceived, and at birth (Robinson, Goad, Puck and Harris 1969). The point could possibly be solved by scoring metaphase II cells of all qualities, using fluorescence microscopy. Even then the results might not be conclusive, since fluorescence on metaphase II cells is unreliable,as the spreads are so pale (Pearson and Bobrow 1970b). There was no evidence of nondisjunction in over 450 metaphase II cells analysed.

A most interesting finding in examination of metaphase II spreads

in man concerned the individuals carrying large polymorphisms of 9 and 16. The population with long and short long arms in the chromosomes concerned, can only be explained as representing cytological evidence of crossing over between the secondary constriction region and the centromere, at an earlier stage. The three populations were quite distinct (a feature only apparent with a large difference between the homologues because of variation due to coiling), showing crossing over had not occurred within the secondary constriction region itself.

6.4 SPERMIOGENESIS

This involved condensation of chromatin from the acrosomal end (Plate 59) and then the excess cytoplasm passed down the tail of the sperm (Plate 60). The latter process has also been described by Beeman (1970) in the anaspidean opisthobranch Phyllaplysia taylori Dall. Condensation of the chromatin has been demonstrated by the DNA becoming progressively more resistant to heat denaturation (Ringertz, Gledhill and Darzynkiewicz 1970). Binding of actinomycin D also decreases with sperm maturation (Gledhill 1970) maybe owing to steric exclusion of actinomycin D by nuclear proteins. Gledhill (1970) described differences in the amount of DNA in ejaculated sperm from fertile and infertile males. Sperm with an abnormal morphology had a greater binding capacity of actinomycin D than normal sperm.

X and Y bearing spermatids or spermatozoa could not be distinguished. In many cases, bodies that might be interpreted as sex chromosomes were shown, but they could possibly be explained by heteropycnotic regions in other chromosomes, or interference patterns (Van Duijn 1960). In locusts 50% of spermatid nuclei demonstrate a heteropycnotic X (Henderson 1964). This was not observed in the present study.

Using the results of seminal analysis associated with this study, the only abnormalities were oligospermia and low motility. Hollander,

Bryan and Gowen (1960) reported abnormal sperm forms causing infertility in the mouse. Donald and Hancock (1953) described a change of sperm morphology in bulls, resulting in a loss of reproductive function, and which was caused by a recessive gene. Blom (1966) reported 40% of sperm tails strongly coiled, folded together or split into fibres in a bull. In addition, normal histological testis sections, but low motility of sperm were reported in two of its male siblings.

6.15 PRE- AND POST-REDUCTION OF THE SEX CHROMOSOMES

No evidence was found of post-reduction of the X and Y in man, hamster or mouse. Wahrman and Ritte (1963) while not observing a chiasma between the X and Y, showed post-reduction at anaphase II in *Apodemus sylvaticus* L. with pre- and post-reduction existing in different cells of the same animal in *Apodemus mystacinus* Danford and Alston.

6.16 POLYPLOIDY

There are several reports of polyploid germ cells in man (see Chapter 1). Indeed, in this study, there were many spreads that appeared polyploid, with chromosomes of the same contraction and appearance. The same was observed for the locust, hamster, and mouse but in a higher proportion of cells. However, karyotyping apparent "tetraploid" spreads, at all stages of meiosis suitable for analysis, showed that usually two complete complements could be distinguished, which were on either side of a central region, where there was sometimes an overlap. This suggested the presence of adjacent synchronous cells and was considered strong evidence against tetraploidy, at least in these cells.

Matthey (1952) described polyploid cells in the hamster. Hultén, Karlman, Lindsten and Tillinger (1970) were more cautious and described, in the male Chinese hamster, "apparently" polyploid spermatogonial metaphase

and also first and second metaphase spreads. They claimed interpretation was difficult. If tetra- and octoploid cells did exist they could be formed by endomitosis, non-reduction or fusion of diploid cells. Hexaploidy could only be explained by fusion or unequal segregation of a highly polyploid cell in a multipolar division, like that described in mitosis of Microtus agrestis L. by Pera and Schwarzacher (1969). It has not been described for germ cells. Pogosianz and Brujako (1969) upheld the existence of polyploid cells in mammalian meiosis in the lemming and hairy-footed (Djungarian) hamsters.

Ford and Evans (1971) claimed they found convincing polyploidy in the mouse because of uniformity of appearance and contraction of the chromosomes. Their main argument against it was that there were never multivalents in such spreads at diakinesis, as would be expected if the spreads originated from polyploid germ cells. Pogosianz and Brujako (1970) also noted that multivalents were not formed in the Djungarian hamster, but interpreted this as fusion necessarily occurring post-synaptically. Multivalents at diakinesis were not seen in the material in the present investigation. Dutrillaux and Guéguen (1971) had claimed multivalents in polyploid metaphase I cells in human material.

Although they were never particularly sought, Ford and Evans (1971) never found tetraploid cells at zygotene or pachytene in their study. They were not observed in the present investigation, in spite of a rigorous investigation of the prophase stages. In real polyploidy homologous pairing would be expected. Homologous pairing in trisomic cells can be observed (Hungerford et al. 1970) and so tetraploidy should be obvious at pachytene. Whitehouse (1963) reported a switch in pairing partners with tetraploids, also beautifully demonstrated by Beçak et al. (1967) in polyploid frogs. Moens (1970) presented electron microscope evidence of switches in axial cores of synaptinemal complexes in autotetraploids of Lilium longiflorum Croft. 4n = 48, at prophase. Therefore there is no ground for assuming that crossing

over in a tetraploid situation would only occur between pairs of chromosomes.

Evidence for real, as opposed to apparent, tetraploidy was presented by White (1970) in the Australian Morabine grasshopper. In this there has been a fusion between the original sex chromosomes and autosomes. A "neo-X" alone usually shows negative heteropycnosis. However, tetraploid spermatogonia show 2 neo-X's and 2 neo Y's, one of the neo-X's being heteropycnotic, as is usual for one of the 2 X's in somatic cells of female mammals. Thus a close interaction is shown, which is possibly only explained by real tetraploidy. Patau (1963) and Sumner, Robinson and Evans (1971) reported diploid spermatozoa in man. Hereditary conditions affecting spermatogenesis was suggested by the discovery of multi nucleated giant cells in the ejaculate of two brothers with maturation arrest, by Weyeneth (1956).

In this study, most mature spermatozoa were lost to the supernatant. However, polyploid spermatozoa would be heavier and should be observed in the air-dried preparations. Only a very few of those observed could possibly have been "diploid sperm", and these were certainly not as frequently seen as were "polyploid" metaphase spreads. Sumner (1971) measured, with Feulgen and densitometry, sperm from four infertiles aged 23-27 and found a range of 0.74% to 1.53% polyploid sperm with a mean of 1.02% but was critical of whether they were actually involved in causing triploidy. This is also much lower than the proportions of "polyploid" spreads demonstrated. Pogosianz and Brujako (1971) attributed polyploid conceptions to polyploid gametes. Triploid zygotes are common in man (Carr 1965). However, Penrose and Delhanty (1961) showed that a triploid abortus had resulted from digyny, since two of three sets of chromosomes resembled the mother's. Schindler and Mikamo (1970) preferred double fertilisation to explain diandry. An increase in the numbers of polyploid embryos following delayed insemination in pigs (Bomsel-Helmreich 1961) also suggests that this is more likely.

If the spreads are not really polyploid, the synchrony of adjacent cells needs to be explained, likewise their clustered occurrence in air-dried preparations. Lima de Faria and Nordqvist (1962) described the disintegration of synchronous cells caused by tritium labelling. Fawcett, Ito and Slautterback (1959) reported clumps of 32 cells labelling synchronously in various species, with intercellular bridges shown in the electron microscope to connect the cells. Heslop-Harrison (1966) described cytoplasmic connections between angiosperm meiocytes. Dyer, Ruby and Skalko (1968), in a fine structure study, described intercellular bridges between developing oocytes from mouse foetal ovaries. Connected oocytes seemed to be at the same stage of development.

In animals, polyploidy is usually associated with a functional role, for example, for massive RNA synthesis in nurse cells (Bier 1965). Autoradiography showed that RNA is transported over cytoplasmic bridges which extend between oocytes and nurse cells.

It is suggested that polyploidy is not as common in germ cells as was described in some studies. Instead, many cases of apparent polyploidy are due simply to the presence of adjacent synchronous cells.

6.17 UNSTAINED REGIONS

Regions of spreads that appeared "white" but with a definite structure were observed at most stages of meiosis throughout the study, both in human and other material (Plates 19c, 34b and 82a).

Since no specific stains were used, it was difficult to say what these areas represented. However, there seemed to be a close association with centromere regions and such patches might well have been spindle proteins. A small halo is sometimes observed at each side of the centromere at mitotic metaphase. Kaufman (1926) observed paired chromonemata surrounded by an achromatic matrix at the centromere region in Tradescantia.

6.18 CHIASMA ANALYSIS AND CONJUNCTION OF THE SEX CHROMOSOMES AT DIAKINESIS

VARIATION IN TOTAL CHIASMA COUNT.

Chiasma counts in cells from each individual showed a normal distribution about a mean. Forty-five individual means varied from 44.62 to 63.80. For "controls" the average figure was 51.11 SD 2.97, providing a genetic map length for the human male of 2,556 centimorgans. Compared the other male material, the spread of cell counts was far greater in man, where the standard deviation (average figure) was 4.43. For a locust, standard deviation was 1.59, a Syrian hamster, 1.83, and for a male mouse 1.58. Therefore, in man, it is important to obtain a mean from several cells for the figure to be relevant.

It is difficult to compare chiasma counts obtained in this study with other reports (see Chapter 1), which have often been performed on few cells, from few individuals, and by different observers, with the additional disadvantage that their preparations were not comparable. Where figures for standard deviation are also given, they are usually smaller than was found here. For example, in the males with a low chiasma count reported by Hultén, Eliasson and Tillinger (1970) the mean was 23.6 with a standard deviation of only 1.3. No cases where most of the cells had low chiasma counts were found here, although an occasional cell could show an unusually low count (Plate 52). With such a large variation of means shown here, in a small population, it might be expected that low chiasmate individuals reported by others would be rare extremes in a spectrum. However, Hultén, Eliasson and Tillinger (1970) found their two cases in a series of only 51 consecutive patients.

On cytological grounds, the human female oocyte preparations generally show a lower chiasma count than do male spermatocyte preparations. Henderson and Edwards (1968) estimated the female total chiasma count to range between

36 and 42; Jagiello, Karnicki and Ryan, established the mean value to be between 46 and 51; Yuncken (1968) estimated the mean to be 52, while Edwards (1970) described 15 oocytes as having counts ranging from 37 to 48. Oocyte preparations are poor by comparison with spermatocyte preparations, and also numbers of cells are necessarily smaller, so data cannot be compared. Linkage studies, however, suggest that there is more crossing over in the female (Cook 1965, Renwick and Schulze 1965).

There was no correlation of total chiasma count with age. Hultén and Lindsten (1970) and Jacobs (1970) reported a slight increase in chiasma count, if anything, with age while Edwards (1970) reported little evidence of an age effect. A decrease in chiasma count and an increase in frequency of univalents has been found in mouse oocytes with increase in maternal age (Henderson and Edwards 1968). The recombination frequency in some linked genes in mouse declines consistently with maternal, but not paternal age (Edwards 1970). However, it must be remembered that positioning of the chiasmata rather than a change in number could explain these results; location could become progressively further towards the ends of the chromosomes with increasing age.

When an individual had a lower total chiasma count the decrease was reflected throughout the complement, according to the size of the chromosome (Figure 9), but with the G's usually showing at least one chiasma. There were only 3 cells, out of 1,152, that showed a G pair as univalents. Kjessler (1966) did not find any autosomal univalents in his study. Rees (1957) found an exceptional male locust with a low chiasma frequency and many univalents. Data plotted with those from its sib showed the condition of asynapsis was not the same for all classes of chromosomes, but was relatively greater for the larger chromosomes, while small chromosomes were unable to become completely asynaptic.

The mean chiasma count in 60 cells from controls was just significantly higher at a diakinesis stage slightly earlier than that usually used for

scoring chiasmata: 53.30 SD 5.57, compared with a slightly later stage: 51.22 SD 5.09. It is surprising that the difference was not more. Obscuring of chiasmata was therefore small over the range actually used. The mean for later diakinesis is, in fact, higher than the overall mean for the analysed stage (51.11). This can be attributed to bias by using smaller numbers of cells and by taking more cells from some patients than others.

"Terminalisation" has been avoided here (for human material) in preference to "contraction causing obscuring" to explain a lower count at later stages of diakinesis. Mather (1940) looked on terminalisation as virtually an "all or nothing" event. However, the term is still very much used (Dupraw 1970) and indeed it seemed likely that the process occurred in the locust, but probably not in the hamster.

Total Chiasma Count in "Controls" and Infertiles

The total chiasma count was significantly lower for "controls" compared with infertiles: 51.11 SD 2.97 and 54.23 SD 3.50, respectively. Luciani (1968) also reported a difference in mean count in controls (47.5) and in infertiles (48.5) but the number of patients he examined was smaller. There are several possible explanations for the results found in the present survey:

1. The infertiles often had fewer diakinesis cells available for analysis compared with controls. Every care was taken to use preparations of comparable quality. However, any failure to do so should result in a lower, not higher count, so the results might reflect an over-compensation.
2. The infertile population had a lower mean age (30.59) compared with controls (39.57), but no correlation of chiasma count with age was shown here and, indeed, others have found an increase with age.
3. Cohen put forward a hypothesis in 1967, which he elaborated in 1969, to explain the vast numbers of sperm that are needed to effect one successful fertilization (he quoted 3.5×10^8 for man). He called

this gametic redundancy, and observed that it increased as the number of chiasmata typical for that species increased. For example, Drosophila, with no crossing over in the male, has half its sperm effective in fertilization. He proposed that inexact chiasma formation usually leads to a minor interpolation or deletion, so only say 2 gametes out of the whole ejaculate could ever be successful in fertilization.

A higher chiasma count associated with infertility would be consistent with this hypothesis. A small increase might be all that is necessary to render ineffective the few good sperm. It could also explain infertility in some cases where there is no apparent reason for it. The hypothesis points to a balance mechanism working against increase in chiasma count and its evolutionary advantages (Maynard Smith 1971).

CHIASMA COUNTS FOR INDIVIDUAL BIVALENTS.

Estimates of the number of chiasmata per bivalent are only meaningful if the identification of the bivalents is accurate. Mistakes are, however, less serious when the bivalents to be distinguished carry the same number of chiasmata. A mean must ideally be taken from several cells to ascertain the small differences in particular bivalents.

Adding the means for each bivalent gave a slightly higher total, 51.91, than that estimated for controls 51.11 and the same for infertiles, 55.64 compared with 54.23. This may be attributed to the smaller numbers of cells used, as much as that the quality of the karyotyped spreads was superior.

Microscope analysis may be considered more efficient for counting chiasmata since it is possible to adjust the focus. However, with chiasmata per bivalent this is not possible, since some of the individual bivalents need to be compared directly with others for the purpose of identification. Obtaining photographs in optimal focus was facilitated with air-dried preparations, since the chromosomes effectively lie in the same plane.

Caspersson, Hultén et al. (1971) stated that, at least in some bivalents chiasmata were not randomly localised. No measurements for position of chiasmata at diakinesis were taken in the human material in this study. Beadle (1932) and Baker (1958) showed that crossing over was reduced towards the centromere position. Here, the presence, at metaphase II, of evidence of crossing over between the paracentric constriction region and the centromere, shows that crossing over can occur adjacent to the latter. White (1942) described how chiasmata seem to occur most frequently at or near the junction of hetero- and euchromatin.

Various difficulties had to be met in performing chiasma analysis, and the estimation of chromosome length, at whatever stage, is also difficult. The plots of two different estimates of mitotic chromosome lengths (Ferguson-Smith et al. 1962, Gilbert and Muldal 1971) against chiasma count per bivalent for the same person, compared well (Figure 8). Pachytene chromosome lengths would be more accurate because they are greater than mitotic lengths (and small differences might become apparent), also secondary constriction regions are not usually extended at pachytene.

The number of chiasmata per bivalent was roughly in proportion to chromosome length. However the graphs showed too many chiasmata in the smaller bivalents and too few in the larger. An apparent deficiency in the larger bivalents due to obscuring of chiasmata is very likely. The estimated number of chiasmata in the smaller chromosomes is probably more accurate because where one or two chiasmata are present, accompanied by a "rotation" of the free arms, there can be little doubt of the total. For this reason chiasma analysis in the mouse is easier in spite of the bivalents being less distinct. Obe (1969) showed the recombination frequencies of human leukocyte chromosomes in chemically induced translocations to be related to the relative lengths of the groups.

Some bivalents repeatedly showed a higher number of chiasmata compared with their neighbours, for example 10 and 20, which share an absence of

secondary constriction (Figure 1). Nine and 16 usually showed a lower number but probably owing to an overestimation of the mitotic length due to the attentuation of the secondary constriction regions.

GENETICS AND CHIASMATA.

Genetic effects which give rise to abnormal chromosome behaviour during meiotic division are known in a range of organisms. The genetic control of synapsis is particularly well known (Wald 1936), but mainly for plants. For example Soost (1950) reported an asynaptic major gene mutant in the tomato and Miller (1963) an asynaptic maize with preferential reduction in the number of chiasmata in the long arms. In plants, supernumerary chromosomes can increase number of chiasmata (Hanson 1969). Feldman (1966) showed that in wheat the long arm of 5B prevents pairing of homologues. In quadruple dose it slightly reduces chiasma frequency, while 6 doses cause considerable asynapsis of homologues, prevent some pairing and induce a high frequency of interlocked bivalents. He concluded that 5B regulates the pre-meiotic association of homologues, and suggested that 6 doses may suppress all pre-meiotic association causing random distribution of the chromosomes in the pre-meiotic nucleus. In hybrid Beta procumbens X B. vulgaris, Nagl (1969) found asynchronous condensation of bivalents and a reduction of chiasma frequency in diplotene and diakinesis, although pachytene pairing had appeared normal.

There are many mutants of Drosophila affecting synapsis and disjunction at meiosis (Sandler, Lindsley, Nicoletti and Trippa 1968). As far as human material is concerned, there is very little evidence for genetic factors playing a part in disorders of human meiosis (Riley 1966). A supposed desynaptic mutant of man was found to have reduced rates of DNA synthesis and a deficient DNA repair system in his mitotic cells (Pearson et al. 1970). Genes affecting synapsis may be mutants affecting the enzyme systems that determine the breakage or union of chromosomes, or the

synthesis of DNA during recombination (Ratnayake 1968, Howell and Stern 1971).

OTHER AGENTS AFFECTING CHIASMATA.

Various physical and chemical agents affect recombination and chiasma formation; treatment is effective during the S phase before leptotene and during meiotic DNA synthesis at zygotene/pachytene. Suzuki (1965) showed that actinomycin D modified crossing over in Drosophila. Heat can affect chiasma formation. Henderson (1962) showed that a heatshock of 40°C caused a reduction in chiasma frequency and asynapsis in Schistocerca gregaria Forsk. Spiralisation was also affected. Maguire (1967b) noted, in maize translocation heterozygotes, departure from control recombination levels induced by heat treatment in pre-meiotic interphase, when DNA synthesis occurs. There is a reduction of chiasmata when the temperature is increased from 26°C to 37°C as a shock in early pachytene to Goniaea spermatocytes (Peacock 1968). Westerman (1967) showed 2 radiosensitive periods in Schistocerca gregaria: a decrease in chiasma frequency at meiotic DNA synthesis, and an increase at leptotene and early zygotene (the small bivalents not responding at either time). Reddi, Reddi and Rao (1965) induced crossing over in the classically achiasmate male Drosophila by treatment with an ovarian extract.

CONJUNCTION OF THE SEX CHROMOSOMES.

This study did not unequivocally demonstrate whether true chiasmata occur between the human X and Y chromosomes. There was certainly one diakinesis cell where there appeared to be a chiasma (Plate 44a), resembling that observed in the autosomes. This cell showed a stretched sex bivalent, which is probably the reason for detail being visible here, and not in other cells. However, the sex bivalent is prone to anomalous behaviour

and although this is the most convincing example to date, it must be interpreted with caution.

In the hamster and mouse also, there was no evidence of sex chromosome crossing over, except for adjacent positioning. Falek and Chiarelli (1968) counted one chiasma when the X and Y were in association. It was probably the feature of the X and Y being found as univalents that was reponsible for Painter (1921, 1923, 1924) to suggest the diploid complement was 48. Twenty four elements, doubled, results in the figure 48.

Despite the apparent variability of sex chromosome morphology, all placental mammals retain an XX/XY sex determining mechanism (Corin-Frederic 1969). The X and Y probably evolved by gradual differentiation from a homologous pair of chromosomes (Ohno 1967). To achieve this differentiation, one of the pair must accumulate the factors governing development of one sex, the other that of the opposite sex or become inert in a sex determining capacity. This will be accomplished only if crossing over is prevented in the heterogametic sex through the entire course of phylogeny, at least in the material responsible for sex determination. There is no reason why crossing over could not occur in a small homologous region. On each side of the putative chiasma shown in Plate 44a, fine focussing showed two heteropycnotic regions. Heterochromatin does not usually exhibit crossing over (Brown 1966) and this might provide a mechanism that would prevent any recombination that would upset sex determination.

Crossing over is common in the sex chromosomes of some animals, especially those in the process of speciation. For example, in Cricetus griseus Milne-Edw. there is presumably a translocation of the sex chromosomes to a homologous pair of autosomes and up to 2 chiasmata are shown in the "sex chromosomes" (Ohno and Weiler 1962).

Wahrman and Ritte (1963) showed chiasmata as well as end to end association figures in Apodemus mystacinus Danford and Alston. The male of the Alticid beatle Cyrsylus volkameriae F. has the only functional sex

quadrivalent known (Virkki 1968) and is assumed to be 2 reciprocal translocations between a largely or totally heteromorphic autosome and both neo-X and neo-Y.

Evidence is strong for crossing over between the sex chromosomes but cannot be proven for man (Ford 1963). Crossing over involving too much material has been put forward to explain the existence of testes in true hermaphrodites (Ferguson-Smith, Johnston and Weinberg 1960, Ferguson-Smith 1965, 1966, 1970). Fluorescence does not reveal any of the fluorescing part of the Y on either X of XX males, which might be expected if the short arms of the X and Y are involved in pairing (Pearson and Bobrow 1970a). Further evidence for homology between the X and Y comes from karyotype-phenotype correlations in patients with structural sex chromosome aberrations (Ferguson-Smith 1965, 1966). Abnormalities of sex chromosomes can provide cytological evidence of a chiasma being involved in sex chromosome association. Chandley (1970) reported, in a case with a ring Y, that the X and Y chromosomes occurred as univalents in those diakinesis cells where the Y was present. McIlree, Price et al. (1966) reported a case with an XO/X dicentric Y, which showed mostly separated X/Y. It was assumed that the short arms were deleted. However an association did occur in low frequency, which possibly demonstrates that long arm pairing is possible. In a case with an XO/X pericentric Y, with short arms presumed present, there was no abnormality in pairing. The pericentric Y was found (without the XO line) in all his male relatives and the father (McIlree, Price et al. 1966).

In the "desynaptic mutants" in man, illustrations frequently show the X and Y paired end to end, yet very few chiasmate in the autosomes (Pearson et al. 1970). This makes the argument, that association is caused by a chiasma, less acceptable unless different rules govern its presence. In male Drosophila, which lacks chiasmata or genetic crossing over, a thread-like connection may be observed between the X and Y chromosomes in

Drosophila spermatocytes (Cooper 1964).

It is assumed that the sex chromosomes remain effectively intact in the heterogametic sex by the precocious condensation of the sex pair during the synaptic stages of meiosis. The sex chromosomes of the human female are isopycnotic throughout meiotic prophase, which occurs late in foetal life (Ohno, Makino, Kaplan and Kinosita 1961).

In the few spreads observed in the early stages of homologous pairing, a prominent sex vesicle or heteropycnotic sex chromosomes were never observed, though this may have been due to chance. Heterochromatin is classically associated with recombinational inactivity and so it is possible that the sex chromosomes undergo any crossing over at this time. At pachytene there was always one sex vesicle. If there was no homologous pairing, it would seem unlikely that the sex chromosomes would associate in a sex vesicle, so this could be taken as circumstantial evidence for at least homologous regions on the X and Y.

The sex vesicle is so dense at pachytene that it is difficult to see the sex chromosomes, and even more difficult to clarify their pairing. Ford (1969) found no evidence for a chiasma at pachytene, in man. The presence of a synaptinemal complex is generally (but not always) associated with crossing over (see Chapter 1). Solari (1969)has found a common synaptinemal complex in the sex vesicle of the mouse at a stage when complexes were developed by the autosomes, and Solari and Tres (1970) from 1000Å serial sections found a minute synaptinemal complex of 0.4 to 0.8 μm length in man, at zygotene to mid pachytene. It became obliterated by late pachytene and was attributed to a homologous region on the X and Y chromosomes.

There are three main hypotheses that might explain the presence of the sex chromosomes as univalents in diakinesis cells:

1. Technique. Hypotonic pretreatment and air-drying might be considered responsible. However, in this study, preparations made using technique

"F" (without hypotonic pre-treatment) showed that among the few well spread diakinesis figures, separated X/Y cells were as frequently observed as in preparations by other methods. Technique did seem to be implicated by finding the X/Y cells in clumps. This also emphasises the necessity to score several cells to make the separated X/Y percentage meaningful. However, such a result might equally be interpreted as the clonal production of a single spermatogonial stem cell. The presence of a chiasma might then be a property inherent to the clone. Since a whole range of values 0% to 57% separated X/Y were obtained over all patients, using as nearly as possible the same technique, this was also considered evidence that preparative artefact was not responsible.

2. Precocious Disjunction. Eliasson et al. (1967) found, in the dog, that the X and Y were separated more often at late metaphase than at diakinesis. Hughes-Schrader (1969) found sex chromosomes could segregate precociously in mantispids. King and Breams (1936) and Ferguson-Smith (1964b) mentioned precocious disjunction of the X and Y in man. This study showed that it was not a feature for humans, at least over the range used here for detailed analysis (Table 17). In the mouse, the situation may be different. Scoring a wide range of diakinesis stages and taking the mean chiasma count of cells with chromosomes as XY, XY gap, X^Y and X/Y (Plate 96), there was a progressive decrease in chiasma count, which might be interpreted as a trend associated with chromosome contraction as diakinesis proceeds. There are many more X^Y and XY gap configurations in mouse by comparison with man.

3. Lower Chiasma Count per Cell. Whether the sex chromosomes are found as univalents might depend on the number of chiasmata shown by the cell. There was a negative correlation of proportion of separated X/Y with increase in mean chiasma count, in "controls". For 40 cases showing

at least one separated X/Y cell the mean chiasma count for separated X/Y cells was compared with that for adjacent XY cells, and scored as to whether values were higher or lower than for XY cells (Table 16). It would be expected, by chance, that in half of the cells the count would be higher. In fact only 12 out of 40 cells had higher counts, a significant difference, so cells with separated X/Y tended to have a lower chiasma count. This was only by about one chiasma, since adding one to each separated X/Y mean adjusted the score to 17 higher and 23 lower, not significantly different.

Variation in Proportion of Separated X/Y Cells

There was such an enormous range in the proportion of separated X/Y cells shown between cases (in "controls" 0% to 50%). Previously reported figures (mainly from squash techniques) all fall within this range (see Chapter 1).

Perhaps figures presented here tend to be higher. This could be attributed to better spreading with the air-dried technique, and therefore more accurate identification of all the chromosomes, to enable the final figure to be a full estimate. There was a positive correlation of separated X/Y cells with increase in age in "controls", although the reason for this is not clear. The age effect rather rules out that variation in the size of the homologous pairing segments on the X and Y chromosomes could have been responsible, but it would be interesting to compare a father and son in this respect, where the Y's, at least, would be common to both. Kodani (1958) reported a high figure of 40% separated X/Y cells. He most likely used older material since "cancer of the prostate" cases were included. Kjessler (1966) did not find the frequency of separated X/Y in infertiles differ ing significantly from controls. In the present study, the "controls" had a higher mean percentage X/Y, 21.84 SD 14.16, compared with infertiles, 11.74 SD 16.11, which could be explained by the age difference, but variation

is large anyway.

The increase in the proportion of separated X/Y in the mouse affected by muscular dystrophy, is difficult to explain. It is unlikely to be caused by precocious disjunction of the sex chromosomes, since there was an overall increase in chiasma frequency XY to X/Y. It is possible that only the sex chromosomes are affected. The normal variation in the proportion of separated X/Y in mouse is large, so perhaps it is not possible to draw any particular conclusions. The unaffected "control" was a sibling and possibly a heterozygous carrier for muscular dystrophy. The analysis should be repeated for several animals, using age-matched controls from the same strain, unequivocally non-carriers of muscular dystrophy. The increase in proportion of separated X/Y cells was the only effect observed on chromosomes. A difference in proportion of separated X/Y cells in mice was found by Schröder, Halkka and Brummer-Korvenkontio (1970): 4% in a mouse strain free from ectromelia infection, 10% in a strain harbouring latent infection. They suggested the difference could be attributed to the strain or the virus. Differences associated with muscular dystrophy have been found, but usually with respect to proteins. Das, Watts and Watts (1971) found an altered erythrocyte acetylcholinesterase in affected mice.

6.19 MATURATION ARREST

The condition termed "maturation arrest" of spermatogenesis was extremely common among infertile cases, as frequently observed as was an absence of germ cells. Where maturation arrest was severe, sometimes only a few cells reached the prophase of first meiosis. The milder forms were seen in histological sections to have fewer sperm than expected, "hypo-spermatogenesis", but air-dried preparations of such material revealed cells in degeneration with loss of nuclear structure and the chromatin clumped into globules.

In "normal" spermatogenesis cells in degeneration are occasionally observed (Beaumont and Mandl 1963, Swierstra and Foote 1963). The meiotic stages of cells in degeneration could frequently be recognised in the air-dried preparations (Plate 61), and it was found that where degeneration occurred, all stages were in fact involved, even though the degeneration had been assigned to a particular stage in histological sections. It was shown that estimating the proportions of cells at spermatogonial metaphase, diakinesis and metaphase II was often misleading in demonstrating maturation arrest and it is difficult to see why, though easy to understand an arrest at the spermatid stage being missed. A possible explanation will be discussed later. The number of degenerated cells in proportion to total metaphase stages was considered more valuable in assessing the condition in air-dried preparations.

There are many reports of abnormal mitotic karyotypes being accompanied by spermatogenic arrest in man. In the present survey, mitotic abnormalities usually resulted in an absence of germ cells rather than a disorder of meiosis. Most of the cases of maturation arrest were associated with a normal 46,XY karyotype, as was found by Raboch, Engelberth and Chrz (1969). Kjessler (1970) interpreted degeneration at early prophase of primary spermatocytes in a 46,XY man, as suggesting a hidden pericentric inversion. Preparations from one of the translocation heterozygotes, EA690217, showed a significant proportion of cells in degeneration. It remains possible that in some of the very early maturation arrests, where no diakinesis analysis could be perfor med, there were translocations not apparent at mitotic metaphase. McIlree, Price et al. (1966), in two patients with multivalents at diakinesis, showed an arrest between diakinesis and metaphase II.

Hultén, Eliasson and Tillinger (1970) found maturation arrest at the spermatocyte level in two males with a low chiasma count and interpreted the asynapsis and the maturation arrest as a genetic factor. They believed

the asymmetrical bivalents, presumptive multivalents, univalents, fragments and despiralisation were connected with the maturation arrest. Pearson et al. (1970) also found maturation arrest in two males with an overall low chiasma count. In this study, there were no cases with a very low chiasma count, and there was no indication that maturation arrest was associated with a lower count. Weyeneth (1956) suggested spermatogenic arrest was mostly of a genetic nature, since he had seen the condition in two brothers. Similarly, genetic factors were suggested as being involved by Tonutti, Weller, Schuchardt and Heinke (1960), and in other organisms by Riley and Law (1965). Sinks (1955) described a guinea pig mutant strain with spermatogenic arrest. Menzies (1957) described, in the African mouse Mastomys, spermatogenic arrest, which was caused by a single pair of genes and associated with pale coat colour.

In the present study, testis sections from the mouse affected with muscular dystrophy, showed a picture rather like a maturation arrest. However, it was unusual in that no degeneration was visible in the air-dried preparations, so the situation was perhaps not equivalent. Gonads in ectopic positions may often be considered as having an "arrest" for example, Severinghaus (1942) described it in a human intersex with testes below the labia. This is probably a secondary effect rather than defective gene action, since arrest may also be found as a consequence of orchitis (increase in scrotal temperature) as in, for example, CMC700362.

Griffen and Bunker (1967) described interrupted meiosis rather like that seen in maturation arrest in the germ cells of irradiated mice. White (1935) described X-ray killed cells exhibiting pycnosis; the effect was apparently much like in maturation arrest, although no illustration was given. Lima de Faria and Nordqvist (1962) reported disintegration of tritium-labelled spermatocytes like in maturation arrest, in Melanoplus. Here, cell degeneration was shown in a radiation-damaged case, although radiation was ten months prior to biopsy.

Hybrids may also appear, at meiosis, to possess characteristics similar to maturation arrest. Crew and Koller (1936) reported a failure of meiosis after a normal first metaphase in a Cairina X Anas hybrid duck. Trujillo, Stenius, Christian and Ohno (1962) stated that meiosis did not extend beyond early meiotic prophase in the mule. Mott, Lockhart and Rigdon (1968) in the hybrid duck Muscovy X White Pekin showed that meiosis went to the first stage, but synapsis did not occur since it was hindered by differences in chromosome morphology, so breakdown followed.

6.20 MEIOSIS AND TRANSLOCATION HETEROZYGOTES

There was no clear demonstration of translocation chromosome homologous pairing, in the form of a cross-shaped pachytene configuration. This was largely owing to the fact that technique "M", the best for pachytene preparations, was not used in the earlier period of the work. The pachytene appearance of translocation heterozygotes has been described in maize (McClintock 1930, Rhoades 1950) and in mouse (Ford and Evans 1971), but not in man. The situation in man would be particularly interesting since it would indicate the exchange points in each of the chromosomes involved.

Balanced and unbalanced complements could be demonstrated at metaphase II (Plates 68 and 73), but counts were too few to assess the proportions of cells at this stage likely to give rise to abnormal gametes. The nature of metaphase II chromosomes made scoring difficult, especially when small changes in chromosome length were involved.

Translocation chromosomes could be identified at diakinesis by karyotyping the bivalents, since in the cases studied, a quadrivalent was formed in every cell. To form a quadrivalent, there must be a minimum of three chiasmata (Ford and Evans 1971), one of which must occur in a translocated segment. It is hard to see why, in all the material, a chiasma always did occur in at least one of the translocated segments giving the quadrivalent configuration, especially where such small amounts of material

appeared to be involved. One might expect homologous pachytene pairing but not the invariable occurrence of a chiasma. Chandley (1970), in a t(C,G) heterozygote, saw at diakinesis a quadrivalent, or a trivalent + univalent, the presence of the latter demonstrating that crossing over occurs in at least one of the translocated segments, in preference to the original chromosome. The case had a lower than usual chiasma count (41.5) and had mitotic chromosomes apparently normal. Chandley (1970) also reported a case with an additional small metacentric which was never paired at diakinesis.

Mosaicism for a germ cell line carrying a translocation might have been overlooked in the infertile population, especially for cases where few cells could be analysed. As abutting bivalents could be scored in error for quadrivalents, a translocation was not counted as such unless observed in particular chromosomes in more than one cell. Edwards (1970) stated that mosaicism was unusual in the structural anomalies of the autosomes and was far less common than the 10% mosaicism reported among trisomics. This should also imply that the translocation occurs during meiosis rather than embryogenesis. Ford (1970) did however report a mouse that was considered mosaic for a reciprocal translocation.

The mean chiasma count for the four translocation heterozygotes (51.72 SD 3.44) was not significantly different from that of controls (51.11 SD 2.97), and the mean proportion of separated X/Y cells was not unusual at 8.99%SD 3.62. Kjessler (1966) found a lower mean count compared with other infertiles in a t(D,D), and Chandley (1970) in a t(C,G) heterozygote.

In the present study, the description of the types of quadrivalent formed was presented in terms of the number of chiasmata in material from each of the translocation chromosomes. This was considered more instructive, since chiasmata are the factors responsible for any particular configuration. This study showed a slight increase, if anything, in the number of chiasmata in the bivalents involved in any translocation. In EA690217, with presumably

the largest amount of material exchanged, the effect on chiasma count was slight, but there was an increase in the smaller of the two chromosome pairs involved. It is submitted that the counts for the whole quadrivalent were more accurate than those for each chromosome pair treated separately, since numbers could be inferred from the type of configuration formed. As was shown in the section on chiasma analysis, there is probably an underestimate of chiasmata especially in the larger bivalents. A possible slight increase in chiasmata in chromosomes with structural rearrangements was reported by Hultén and Lindsten (1970). Here, the number of chiasmata in the quadrivalent appeared to increase with increasing total chiasma count in three cases (Tables 26, 27 and 28). However, H..... seemed to show a reverse trend, which can probably be attributed to the smaller number of cells analysed and the small range of chiasma numbers shown by the quadrivalent (Table 25).

Translocation has been associated with infertility, and oligospermia (Böök, Santesson and Zetterqvist 1961, Bauchinger and Schmid 1970). In a female t(B,C) heterozygote there was a report of primary amenorrhoea (Mann, Valdmanis, Capps and Pinte 1965). Koller and Auerbach (1941) in mice, and Henricson and Bäckström (1964) in the boar, reported semi-sterile translocation heterozygotes, while Lyon and Meredith (1966) described reduced testis size and litter size in mouse translocation heterozygotes. Fertility is not always affected and in the cases studied here, there appeared little reason to suggest that it was. There was some cell degeneration in EA690217 and a previous infertility investigation for RW680009 (incidentally, with a high chiasma count of 56.86); the cases were, however, ascertained through abnormal offspring. The fertility of t(D,G) and t(D,D) heterozygotes is usually close to normal (Penrose 1970).

6.21 RADIATION-DAMAGED CASE

In case BS700349, the mitotic picture 9 months after a radiation accident, showed 8% abnormal cells, including dicentrics, fragments, ring chromosomes and breaks. Such findings resemble previous reports on such accidents (for example, Dolphin et al. 1970). Persistent damage visible in mitotic preparations might be expected since small lymphocytes are long lived in man (Little, Brecher, Bradley and Rose 1962).

Histological sections of testis taken 10 months after the accident showed reduced spermatogenesis, and damaged tubules with few germ cells. This state was in turn reflected by a severe oligospermia. Air-dried preparations showed many cells in degeneration, confirming a type of "maturation arrest" that was also present. As was frequently found in other cases, the maturation arrest was not revealed by the metaphase population figures (Table 32).

In meiotic air-dried preparations, there were no detectably abnormal cells at spermatogonial metaphase, prophase of first meiosis or metaphase II. Most of the diakinesis cells were also normal, but there was a high number of cells, in the 40 analysed, with 22 elements (5). In an analysis of this kind an odd cell with apparently 22 elements may be found and can usually be attributed to two bivalents abutting. Here, however, one cell definitely showed a translocation configuration (Plate 74b) and in two other cells they were suspected. Karyotyping suggested that chromosome 14 was involved in all three cells, once with an 11, twice with an 8. Hecht and Kimberling (1971) showed the preference for 14 to be involved in Robertsonian rearrangements. The two cells believed to have 8/14 translocations may have been products of a single spermatogonial stem cell. Products of any of the translocation configurations could result in abnormal conceptions, if there was no selection at a later stage. The products of germ cell repopulation were being examined, so any abnormal stem cells would always be present, unless there was selective pressure at this stage also.

Total chiasma count was 48.54 SD 4.75 which was lower than the control mean (51.11) but within the range accepted as normal (SD 2.97). The proportion of cells with separated X/Y was also normal at 17.50%. In the measurements of chiasmata per bivalent, a decrease in count for number 15 remained unexplained, but was probably not significant. Mather (1934) scored a significant increase in chiasmata in anthers of Viciafaba L. 24 days post-irradiation, which he considered as showing the sensitivity of pre-meiotic stages.

6.22 TIMING OF THE STAGES OF MEIOSIS

As described here and elsewhere (Reitalu 1970), the pachytene stage is more frequently observed in older gonads. In fact, there are indications that the whole of early prophase is longer in older men. It appears that the length of time occupied by each stage varies widely between individuals and even different cells of the same individual. Causes of variation could be age, genetic constitution or environment; these will be discussed later.

Lima de Faria et al. (1968) found some variation between in vitro cultures of material from 14 patients aged 26 to 79. In one patient pachytene cells had labelled after 10 days rather than the usual 12, a small, but possibly important difference. Younger patients often had labelling at late pachytene and diplotene after 14 days and older patients mid pachytene at 15 and 16 days. These figures were obtained from "in vitro" studies but they are comparable with the figures of Heller and Clermont (1963, 1964) for the "in vivo" situation.

Any variation in timing could explain many unsolved problems in meiosis. Scoring metaphase stages was considered thoroughly unreliable for detecting "maturation arrest" in meiosis; this would be explained if the time spent at each stage varied between individuals. The length of time spent at the stage when crossing over occurs could determine the number of chiasmata in any cell. Any delay in order to cope with the physical

difficulties of pairing in translocation chromosomes from heterozygotes, might explain the increase in count in the quadrivalent.

Slizynski (1961) noted that the times of appearance of leptotene and dictyotene in mouse oocytes depended on genetic constitution. Reader and Solari (1969) showed a longer duration of zygotene-pachytene with Searle's X-autosome translocation in the mouse. Smith and King (1968) found there was relatively less time spent at zygotene or pachytene in the strain C3G Drosophila female homozygote, where the formation of chiasmata is affected. Hultén, Eliasson and Tillinger (1970) suggested premature desynapsis (before crossing over) to explain males with a low chiasma count. Ayonoadu and Rees (1968) showed, in rye, that addition of B chromosomes alters length of S and so the influence on chiasma count could be a timing effect.

6.23 ENVIRONMENTAL AND EXPERIMENTAL FACTORS AFFECTING MEIOSIS

Leblond, Steinberger and Roosen-Runge (1963) described a variety of environmental factors: such as infection, physical and mental strain, malnutrition and drugs,which affected spermatogenesis, giving changes in sperm count and morphology. Tobias (1956) attributed things like sex-hormonal balance, or diurnal periodicity as providing stimuli that regulate spermatogenesis.

Change in temperature is the most obvious variable that might affect, for example, timing or chiasma count. The higher temperature is probably the factor that is reponsible for arrest of meiosis in ectopic testes. Gonial cells of explanted mammalian gonads either fail to enter meiosis, or meiosis is arrested, indicating that completion of meiosis is dependent upon stimuli external to the testis (Martinovitch 1939). Hunter and Manning (1969) measured the subcutaneous scrotal temperature to be about 1°C below that of the central tissue. McLeod and Hotchkiss (1941) estimated scrotal temperature to be 35°C and found that artificially elevating the temperature above 37°C for 45 minutes caused azoospermia in an adult male,

which was followed by a recovery period of reduced sperm count that could last for over two months. Mills (1919) stated that fevers depressed number and motility of sperm. Rathore (1970) found, from mating trials with Merino rams, that a heavy scrotal wool cover was associated with reduced fertility. Henderson (1963) reported reduced chiasma formation in the Desert locust after treatment at 40°C. Church and Wimber (1969) gave grasshoppers a heat treatment of 42°C and four days later the chiasma frequency dropped. It was normal again on the sixth day, then it dropped again. The counts in the larger chromosomes were affected, not those in the smaller chromosomes. Nolte, Dési and Meyers (1969) described a 10°C increase in temperature in the locust as giving an increase in chiasma frequency.

Hormones are other variables that might affect spermatogenesis. Nolte et al. (1969) found a pheromone affecting chiasma count and operating at the 5th nymphal instar in the locust. Any experiments involving the use of hormones are difficult to interpret. MacLeod, Pazianos and Ray (1964) described restoration of spermatogenesis by menopausal gonadotrophins. Participation of gene products in chiasma formation is demonstrated by the induction of crossing over in Drosophila males following treatment with ovarian extract (Reddi et al. 1965). Williams, Runyon and Hagen (1968) perfused canine testis with progesterone, which caused sticky degeneration and improper spreading and clumping of chromosomes. Testosterone produced no change, while with oestrogen, chromosome fragmentation and splitting of bivalents, including the XY, occurred in leptotene and zygotene. Human chorionic gonadotrophin caused elongation of the pachytene bivalents and disappearance of chromosomes. Heller and Clermont (1964) found the duration of constants in the meiotic cycle was not affected by norethandrolone or chorionic gonadotrophin.

Nolte et al. (1969) found CO_2 did not affect chiasmata in the locust. Kahn (1970) showed that male CFI mice kept for eight weeks (prior to mating

with females kept under control conditions) in lower oxygen concentration and increased CO_2 concentration, gave offspring, the females of which showed consistently higher mean haemoglobin concentration than controls. Males did not show any difference. An experimental environment imposed on sires prior to mating with control females influencing sperm in a manner which produced the phenotypic changes described in female offspring, requires an explanation.

Chemicals can experimentally affect spermatogenesis. On the whole chemicals are more inclined to affect mitotic chromosomes than meiotic. Mitomycin C produces breaks and rearrangements and quadriradials in leukocyte chromosomes (German and La Rock 1969). Egozcue and Irvin (1969) tested effect of LSD on mitotic and meiotic chromosomes of mice and monkeys. There was no increase in chromosomal damage in bone marrow and testes, but a significant increase in the number of chromosomal breaks and rearrangements in blood both in vitro and in vivo. However, Skakkebaek and Beatty (1970) treated mice over a 5 week period (twice a week) with LSD, when meiotic preparations two days after the last injection, showed obvious increase in the frequency of structural changes in the chromosomes compared with controls and there was a visual difference in sperm heads. Adler and Röhrborn (1969) found no effect at metaphase I in male mice after chronic caffeine treatment. Klassen, Chang and Eide (1969) showed, in the grasshopper, that apholate damage in spermatogonia persisted and was seen in spermatocytes. Legator, Palmer, Green and Petersen (1969) induced chromosome breaks in rat spermatogonial cells following the injection of low doses 1 mg/kg of cyclamate metabolite cyclohexamine over a 5 day period.

Treatments have to be drastic before meiosis is disrupted, but it is possible that slight environmental fluctuations could be responsible for variation in, for example, chiasma count.

6.24 NONDISJUNCTION

In both mitotic and meiotic analysis, there was very little evidence of nondisjunction as shown by the presence of extra chromosomes. Loss of chromosomes is less satisfactory evidence. Very few cells with abnormal chromosome complements were seen at meiosis. Cells with elements missing were far less frequently observed than at mitotic metaphase.

One diakinesis cell showed an extra Y with all the sex chromosomes separated X/Y/Y, but this was in the radiation-damaged patient. Nondisjunction of the Y's in meiosis was much rarer than that established by fluorescence studies on sperm, suggested to be 1.4% by Pearson and Bobrow (1970b). These authors claimed a YY bivalent at diakinesis in one poor preparation and also in some second meiotic metaphases. Barlow and Vosa (1970) queried some fluorescence scores, since when there were two F bodies there were sometimes two tails. It is possible that some XYY cells at diakinesis may have been missed. It seems that with the 47,XYY constitution, the two Y's usually form a bivalent, with the X present as a univalent (Hultén and Pearson 1971). This gives a cell with 24 elements that could be scored simply as separated X/Y. It seems unlikely that a Y univalent would be confused with a Y bivalent, if the standard of the preparation is high and if the expected size of the Y is known from mitotic analysis. Hultén and Lindsten (1970) never observed separated X/Y/Y so perhaps the cell like this observed here, was rather unusual.

Sumner et al. (1971) presumed that nondisjunction of the autosomes was less frequent than Y nondisjunction, as if it were not so, more than half of all the sperm produced would be aneuploid. Ohno, Kaplan and Kinosita (1959) found no sex chromosome addition or loss out of 2,192 meiotic figures examined in the mouse. Eliasson et al. (1967) did not find aneuploid cells with extra chromosomes in studying meiosis in the dog. The X but not the Y could be distinguished at metaphase II and out of 157 cells

examined at this stage, all had 39 chromosomes except two, in which random loss was assumed. It is possible that cells would be more sensitive to chromosome loss than cells in mitosis, if a greater proportion of the chromosome complement was active in meiosis. Epstein (1969) found all the oocytes from XO mice showed G6P Dehydrogenase activity of one half that of normal XX female mice, so the inactivation of the X does not occur in the oocyte of the mouse.

Aneuploid zygotes have often been used as evidence for nondisjunction of chromosomes at the first and second meiotic division, or both (for example, Greenstein, Harris, Luzzatti and Cann 1970). This field has been reviewed by Frøland, Sanger and Race (1968), who generally supported the idea that mistakes can occur during gametogenesis. Using sex-linked marker genes it can be demonstrated that nondisjunction may indeed occur at the first meiotic division in normal male mice, but is very rare (Russell and Chu 1961).

There are specific genes causing nondisjunction at meiosis in maize and Drosophila (White 1942). In man, this might be detected by familial incidence of nondisjunction. So far this has not been found (Juberg and Davis 1970) but there have been connections proposed between other chromosome anomalies and nondisjunction; for example, in a 47,XYY father and his abnormal progeny (Hauschka, Hasson, Goldstein, Koepf and Sandberg 1962) and a t(D,D) heterozygote and his mongoloid and XXXXY progeny (Atkins, Bartsocas and Porter 1968). Evans (1967) attributed nondisjunction to persistence of the nucleolus preventing separation of bivalents associated with it, but there was no evidence here of acrocentric nondisjunction that might support such a hypothesis. Nondisjunction, and therefore loss of the extra X, may explain the few cells that were undergoing spermatogenesis in two of the Klinefelter cases. In the creeping vole, Microtus oregoni Bachman, XO is a normal female, but XX oogonia may be produced by selective nondisjunction (Ohno, Jainchill and Stenius 1963).

There may be cytological evidence pointing to the origin of extra chromosomes. For example, a trisomy 13 child had two Dp- chromosomes which were shown by tritium labelling to be 13's. The mother carried one 13p-, and the father had normal 13's, so both chromosomes in the child must have come from the mother and the nondisjunction presumably occurred at metaphase II (Ferguson-Smith p.c. 1971). Both Y's in 47,XYY cases must have come from the father. Nondisjunction involving the autosomes is more frequent in the female than in the male (Court-Brown, Law and Smith 1969). If non-disjunction was caused by lagging at cell division, it would be expected that the female would show less, since gamete to gamete involved about 20 divisions compared with that in the male of 40 to 60 divisions, where there would be more chances for nondisjunction to occur (Edwards p.c. 1971). Gametic selection might alter the picture.

Mistakes do not have to occur during gametogenesis, although it is usual to suggest this. A likely place is in the first few divisions of the fertilized egg (Edwards, Bavister and Steptoe 1969, Edwards, Steptoe and Purdy 1970). Reciprocal lines may be lost since only a few cells from the blastocyst go on to form the embryo. There is some support for zygotic error; Russell (1961) favoured loss of paternal sex chromosomes between fertilization and first cleavage, since the frequency of XO offspring in mice can be increased by irradiation shortly after fertilization.

Mather (1938) said that nondisjunction may arise by absence of recombination. The amount of DNA in spermatids has been measured by densitometry in material from males with a low chiasma count and a large variation was found, which possibly indicated unbalanced complements (Hultén, Eliasson and Tillinger 1970, Pearson et al. 1970). Weber (1969) showed that two extra chromosome univalents in Zea mays L. disjoined at random. In the C3G homozygous achiasmate mutant female Drosophila, semi-sterility is caused by meiotic nondisjunction, whether crossing over occurs or not (Smith and King 1968). In the plant Colocasia antiquorum Schott.

the normal female has 95% pollen production and the asynaptic mutant less than 20% (Krishnan, Magoon and Bai 1970). Henderson and Edwards (1968) attributed a decline in fertility and a higher frequency of chromosomally and phenotypically abnormal offspring, to decreasing recombination with increasing age, in the mouse. An increase of nondisjunction in older males was not observed in this study and there was no significant change in chiasma count with age.

6.25 SELECTION IN GERM CELLS

The overwhelming "normality" shown by analysed cells in the present investigation, is probably indicative of the efficient selective forces in operation during gametogenesis.

It is suggested that cell degeneration is associated with selection. Although degeneration was commonly observed in infertile patients with apparently normal karyotypes, it was also found in a high proportion of cells in cases where cells with unbalanced chromosome complements were expected, for example in the t(Dq+Cq-) heterozygote and the radiation-damaged BS700349. Pomerantzeva (1969), with radiation experiments on male mice, suggested that selective elimination of genetic damage occurred not only at the spermatogonial stages, but at later stages of spermatogenesis. Once the spermatogonial population had built up, to produce a sperm concentration in the ejaculate sufficient to achieve fertilization, it is possible that selective forces would have eliminated any abnormalities as a result of the radiation.

Evidence for selection is best demonstrated in 47,XYY meiotic material. It is usual for the extra Y to be eliminated (Thompson, Melnyk and Hecht 1967, Melnyk, Thompson, Rucci, Vanaselc and Hayes 1969). Evans, Ford, Chaganti, Blank and Hunter (1970) observed 47 chromosomes in spermatogonia, but in only 2 out of 237 diakinesis cells was an extra Y suspected; Tettenborn et al. 1970 did not observe an extra Y in meiosis. A patient

previously found to have very few YY bivalents at diakinesis (15%, Hultén 1970), was subsequently found by Hultén and Pearson (1971) using fluorescence microscopy, to have 45% YY bivalents. Since a YY bivalent is formed, with a chiasma usually on the short arms of the Y, it is possible that other studies, where fluorescence was not used, underestimated the frequency of the occurrence of extra Y chromosomes. Evans et al. (1970) gave the frequency of cells with separated X/Y to be 6.54%, a low to normal figure (mean frequency in "controls" here was 21.84%). A higher figure might have been expected had some X/YY's been scored as X/Y. The study by Hultén and Pearson (1971) still demonstrated selection, since 49% of diakinesis cells had normal XY bivalents, 3% X/Y and 3% XO, and the sperm F body count was only slightly higher than normal. Diasio and Glass (1970) found a higher proportion of spermatozoa containing a Y (70%) or double Y (5%) in 1,000 spermatozoa. They favoured selection on the journey to the egg since XYY's do not usually father XYY children (Thompson et al. 1967) or XXY children (which is more likely if it is assumed that the two Y's of a Y bivalent would move to opposite poles). Tsoneva-Maneva, Bosajieva and Petrov (1966) and Sundequist and Hellström (1969) reported XYY fathers and sons, but since the ♂ newborn incidence is 0.14% (Ratcliffe et al. 1970), the occasional report of this nature would be expected. All types of maturation arrest can be present in gonads of XYY's (Tettenborn et al. 1970). This, again, might be associated with any selection.

Skakkebaek, Philip and Hammen (1969) presented some evidence for selection in germ cells of a 47,XXY Klinefelter. Three out of 8 informative diakinesis cells appeared as XY and 2 as XX/Y. Chandley (1970) found, in a case with a ring Y, that the Y was mostly absent at diakinesis. Similarly Lehrnbecher, Lucas, Picciano and Jacobson (1970) reported a supernumerary acrocentric never observed at diakinesis.

Loss of a chromosome present in somatic tissues is not so unusual when the male creeping vole Microtus oregoni Bachman, is considered.

This is a natural gonosomic mosaic with somatic cells XY and germ cells YO, the X being lost in spermatogonia (Ohno, Jainchill and Stenuis 1963).

Another phenomenon that requires the invocation of a selective mechanism concerns translocation heterozygotes. The latter have both a much smaller than expected frequency of unbalanced progeny, and an only slightly higher frequency of spontaneous abortion (21.7%, Ford and Clegg 1969) as compared with the frequency in the general population (15.0%, W.H.O. 1966). Also, male translocation heterozygotes produce a lower frequency of unbalanced progeny than do female heterozygotes. There appears to be a greater gametic selection in the male than in the female, possibly because of the difference between spermatogenesis and oogenesis; with a lesser chance, in the latter, of selection operating once the gamete is formed. With a t(D,G) heterozygote, Hamerton (1970) assessed the risk of unbalanced progeny, when the mother was the carrier, as 10-15% and for the father 0.5%. In this study, a translocation heterozygote showed a large number of degenerating cells, but degeneration was shown to extend over all stages of meiosis, in spite of the fact that it is unlikely that stages pre-anaphase would be expressing many imbalances in the genome.

Cohen's hypothesis (Cohen 1967, 1969) requires a massive selective force, against any marginally abnormal sperm, to be in operation. He assumes that most gametes are defective owing to inexact crossing over, giving minor interpolations or deletions within the gene. Man produces one of the highest numbers of spermatozoa of any animal. Most of these are detectably abnormal, and even among experts there are different opinions as to what a morphologically normal sperm is like (Pedersen 1969). It is shown that large errors involving whole chromosomes can be eliminated, but what about more subtle characteristics?

Differential selection with respect to X and Y sperm seems to occur in a manner associated with the father's age, for the sex ratio gradually alters as the father becomes older (Novitski and Sandler 1956). There is

an excess of female 18- trisomic newborns and also a small excess of female abortions with this trisomy, suggesting that trisomic 18 Y- bearing sperm may be at a similar disadvantage. Matsunaga and Hiraizumi (1962) have presented evidence that sperm competition leading to selective fertilization may be responsible for an excess of O offspring from a father heterozygous for A and O, or B and O alleles. Failure of transmission of Leber's disease, an autosomal dominant, through the male (Penrose 1970) suggests that single genes can also cause highly effective selection against sperm which carry them. Evidence for gametic selection in mammals comes mainly from studies of abnormal segregation ratios at the T locus in mouse (Braden and Weiler 1964, Bennett and Dunn 1967, Beatty 1970).

For Cohen's hypothesis to agree with other data, it is necessary for the defects in the sperm to be expressed in some way for selective elimination to be possible. Sperm are generally believed to possess condensed chromatin and are largely inactive, relying for maintenance on other cells, for example, Sertoli cells, during maturation. Their nucleoli disappear (Yasuzumi, Sawda, Sugihara, Kiryama and Sugioka 1958). Darzynkiewicz, Gledhill and Ringertz (1969) showed that there was a progressive decrease in binding of tritiated actinomycin D as spermatid nuclei matured. However, some gene activity has been claimed in sperm: Monesi (1965) showed RNA synthesis by a haploid chromosome set in mouse; Gullbring(1957)showed human sperm can manifest a phenotype in ABO and Fellous and Dausset (1970) showed it with respect to HL-A antigens. Kulangara and Beatty (1971) showed reflection of genetic differences by antigens of the albino locus in rabbit. It is therefore possible that a range of genes (normal or abnormal) may be expressed, although results might be interpreted as demonstrating sperm contamination by products formed from allelic expression in, for example, "nurse cells".

Cohen (1967) preferred an antibody/antigen type reaction, in the genital tract, to eliminate defective sperm. Cohen (1969) found that

even in inbred female mice that accept their brother's skin as a permanent graft, most of the same brother's sperm becomes coated with antibody; the uncoated sperm are presumably those he would class as the non-defective minority. Jackson, Mann and Schull (1969) showed with Xg^a positive fathers and Xg^a negative mothers the male:female ratio of progeny is 3:2, and it is closer to unity in the other mating categories. For children born after the birth of a first daughter, the former ratio is even greater. Such an observation might accord with an antibody effect. As discussed, the human female chiasma counts cannot be compared with those of the male, but are probably not much different. It would be expected that the count would be lower, since there must be less selection involved than in the male and a larger number of gametes are "functional". Cohen (1969) glosses over this problem by invoking selection before ovulation.

6.26 ENVOIE

The study of meiosis has always been central to an understanding of genetics. Following the period when reduction division was first discovered, it provided a basis for understanding the inheritance of maternal and paternal characters by the next generation. The morphological discovery of chiasmata similarly provided a rationale for the processes underlying recombination. At a time when we are beginning to approach genetic defects in man at the level of molecular biology, the data offered here concerning sequence of meiosis, chromosome morphology, and chiasma frequencies (and hence the genetic map length in man), may serve as further basic material towards a human molecular genetics.

To obtain normal material can be a problem, but the fact that patients present themselves for treatment at clinics does save searching for the abnormal individuals. This is but one factor that makes human genetics such a fruitful field.

7. SUMMARY

1. Modern cytological techniques were adapted and applied to human male meiotic tissue. Material from 88 cases was analysed. It consisted of "controls", infertiles, four translocation heterozygotes and one radiation-damaged case. The study provided unbiased, basic information on human male meiosis.

2. By critically examining all stages of meiosis and sufficient numbers of intermediate stages, several new interpretations had to be invoked. The main one concerned the sequence of prophase of first meiosis where a "diffuse stage" was discovered. This stage occurred after pachytene and before diplotene.

3. It was shown that both autosomes and sex chromosomes could be identified at various stages of meiosis by: (a) Their relative size, (b) The position of centromere regions, which were usually heteropycnotic, and (c) The position and behaviour of secondary constrictions, which were usually pale, extended according to chromosome polymorphism.

4. One chromosome, in particular, was consistently distinguished by its characteristic appearance and behaviour.

Evidence that this was the same chromosome, from stage to stage, included: (a) Paracentric constrictions remained consistently visible, (b) Pachytene chromomeres persisted into diakinesis, and (c) Chiasma analysis at diakinesis was correlated with crossing over, as demonstrated by a morphological analysis of metaphase II, using material from cases with extreme heteromorphism at the paracentric secondary constriction region.

Evidence that this chromosome was number nine included: (a) Correlation of size, centromere position and constriction site with well known mitotic features, (b) Behaviour of the paracentric region as a nucleolar organiser, reflecting, for example, "association" in mitotic metaphase, and (c) Correlation of mitotic heteromorphism of nine and asymmetry of a chromosome

throughout meiosis.

Chromosome 16 was similarly identified, using material from a case who had extremely heteromorphic 16's.

5. There was little cytological evidence supporting the existence of pre-meiotic pairing of homologous chromosomes.

6. Abnormalities at pachytene included: failure of pairing of parts of homologous chromosomes and, in one instance, of a whole chromosome.

7. The existence, at diakinesis, of interlocking, and sex chromosome looping of autosomes, could not be disproved. "Apparent" instances indicated that most chromosomes seemed capable of participation.

8. By analysis of 1,152 well spread, mid-diakinesis cells, karyotyping 441, it was shown that large numbers need to be scored to obtain meaningful estimates of chiasma totals, owing to the large chiasma variation between cells. (a) The chiasma count per cell varied from 44.62 to 55.25 in "controls", each with a normal distribution of counts about the mean. (b) There was no correlation of total chiasma count with age. (c) The total count was significantly lower (51.11) in "controls" compared with infertiles (54.23). For "controls" this would give a genetic map length for the human male of 2,556 centimorgans. (d) The proportion of cells with separated X/Y varied from 0% to 50% in "controls". (e) There was a positive correlation of separated X/Y with increase in age in "controls", and a negative correlation with chiasma count per cell. (f) No evidence was obtained contrary to there being a chiasma between small homologous regions on the X and Y chromosomes. There was some cytological evidence to support the existence of crossing over between the sex chromosomes. (g) The number of chiasmata per diakinesis bivalent increased in proportion to chromosome size. (h) Any change in total chiasma count was reflected in proportion throughout the whole complement. (i) In only 3 cells were autosomes present as univalents.

9. There was little evidence of nondisjunction at any stage of meiosis.

10. There was little evidence of "real" as opposed to "apparent" polyploidy in germ cells.

11. Segregation of the sex chromosomes was always pre-reductional.

12. Meiosis in the translocation heterozygotes showed little disturbance, except for pairing of homologous regions of chromosomes. Chiasma counts and conjunction of the sex chromosomes were unremarkable.

13. The radiation-damaged case showed evidence of spermatogonial stem cell chromosome damage, by the presence of translocation configurations at diakinesis.

14. Abnormal mitotic chromosomes were present in 15% of infertile cases. Abnormal karyotypes were usually associated with an absence of germ cells.

8. REFERENCES

Journal abbreviations are: for 1900-1960, according to the "World List of Scientific Periodicals", 4th Ed. 1(1963) 2(1964) 3(1965) ed. P. Brown and G. B. Stratton; for 1960-1968, according to "British Union - Catalogue of Periodicals" (1971) ed. K. I. Porter and C. J. Koster; both Butterworth, London.

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10. APPENDIX (overleaf)

	CASE	AGE	OCCUPATION	PROBLEM (I=infertility)	PARENTAL AGES AT BIRTH		SIBS (+fertile)		FERTILE	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (E=external)	TUBULE TEASING	UNUSUAL HAIR DISTRIBUTION				HORMONE LEVELS		SEMINAL ANALYSIS					HISTOLOGY															MITOTIC ANALYSIS					
					MAT.	PAT.	♂	♀							Pubic	Chest	Face	Head	TOTAL GONADO-TROPHINS RAG units/24 hrs.	17-KETOSTEROIDS mg/24 hrs.	VOLUME (ml)	COUNT/ml X10⁶	% NORMAL	% MOTILE	VISCOSITY	ABSENCE C.d.	PARTIAL A.S.C. MAJOR	PARTIAL A.S.C. MINOR	P.A.S.C. + MAT. ARREST	MAT. ARREST	HYPOSPERMATO-GENESIS	INFLAMMATION	NORMAL	TESTIS VOLUME (ml)	TESTIS PERCENT	LEYDIG CELLS VOLUME(ml) 0.5-1.2 normal	LEYDIG CELLS PERCENT	GENERAL	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN ♀+ve	43	44	45	46	47	GENERAL
1	SMA680117	28	Postman	I. Old inflammation	29		1				-		R45x25x20 L50x35x30	Poor		Scant			29.0	24.6	0.5	0.2										•											3C- F- P-	17		46,XY D, + sh.arm + sats. Break long of B one cel
2	DO1680120	30	Sheet Metal Worker	I. Hernia repaired 1964			4+ + + +	4+ +		Lean	-		R35x25x20 L35x20x20	Good			Scant		11.0	5.8	5.4	Az.						+						11.5 7.3	60 61	0.77 0.50	6.7 6.8					1E-S-	3E- E- G-	16		46,XY
3	A1680137	28	Plumber	I.	34	36	1+	3+ +			-		30x20x20	Poor		Lots			29.0	16.0	6.5	Az.				•												Del. Cast.						1C-	31		46,XY
4	AM680160	29		I.		37		1+		5'10" 9st.9lb.	-	Large	26x20x20	Poor		Scant	1/week		28.0	9.4	0.6 2.2	0.7 Az.	30			•								5.4		1.22	22.4	Sertoli 16%+ve Ghost 4% C.T. 37%			41			3C-C- G-G- D-C-	16-	17	47,XXY
5	GM1680162	27		I.	31	35	2+				-		55x35x30			Scant	3/week		10.0	11.4	5.1 5.6 5.1 4.8	Az. " " "			Norm. " " "								•						•				20		46,XY Qp+
6	PM0680170	39	Farmer	I.							-		53x35x30	Good					9.0	6.7	4.2	Az.			Hypo.								•							•			20		46,XY Iq+Dp+
7	WO680180	33	Driver	I.	25	26	1+			Well built	-		R50x40x35 L55x40x35	Good							1.0 0.5	Az.			Hyper.								•							•			20		46,XY
8	WO680183	27		I.						5'6" 7st.4lb	-		45x25x20	Poor		Scant	Scant		28.0	12.5	2.9 1.7	Az. "			Hypo. "	•																	20		46,XIq- Brother 46
9	AS680045	31	Teacher	I.	22	22		1+		Ave.	-		55x43x35						8.8	9.1	3.4 4.4 2.4	Az. " "											•							•			26- E-	16		46,XY
10	MF680208	24	Assembler	I. Herniorrhaphy One Orchidectomy Desc. of other.						5'11" 12st.7lb	-		30x20x20	Poor					81.0	12.2	1.2 0.7	Az. "			Hypo. Norm.		•										59.1	L.c.hyperpl. 2.5% Tubules Spgl. mitoses						1E-	18	47,XXY
11	JO690026	37	Metal Fixer	I. Bicycle injury		30	1	1		Ave.	-		45x25x20	Good					11.0	22.7	9.0 6.0	6.6 4.4	65 62 Def. heads	10 9			•															1C-	18	1B+	46,XY No D sats.
12	RM690029	29	Shipping Manager	I. Desc. of gonad	30	30				6'1" Thin	-		44x26x22	Poor					3.0	13.4	4.8 6.0	1.1 2.0	60	40 40	Hypo.			•												B.M. thickened L.c. hyperpl. Sertoli cell inclusions						1E-	21		46,XY Qp+
13	JM690034	26		I.			1+				-		53x30x25	Fair					32.0	30.2	3.0 2.1	1.1 1.3	44 57					+						28.6		1.70	6.1						1F-	19		46,XY

	CASE	AGE	OCCUPATION	PROBLEM (I=infertility)	PARENTAL AGES AT BIRTH MAT.	PAT.	SIBS (+fertile) ♂	♀	FERTILE	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (E=external)	TUBULE TEASING	UNUSUAL HAIR DISTRIBUTION PUBIC	CHEST	FACE	HEAD
14	RM690051	28	Engineer	I.	23	21				Obese	-		48x35x30				Scant	
15	RG690058	31	Quantity Surveyor	I						Sl.obese Long legs	-	Small	38x23x22	Fair	Scant	Scant		
16	WT690067	28		I.	28	27	4	1+			-	Small	40x25x25	Good				
17	JD690068	32	Painter	I.						Obese	-	Small	40x25x25			Scant		
18	DMD690138	14	Student	Desc. of gonad Repair of Hypospadias							-							
19	MS.....	27		I.							-							
20	GS690074	31	Metal Dresser	I.			5+	4+		Thin	-		57x35x32	Good		None		Low hair line
21	JB690075	23	Naval Assistant Steward	I.	29	29				Athletic	-	Large	45x30x25					
22	JV690086	28		I.Bilat. undesc.			2	1		Athletic	-		46x30x24	Fair				
23	AP690095	23	Representative	I.	25	27	1	1			-		23x16x14					
24	BG690104	37		I.	27					Ave.	-		36x26x23	Good				
25	RS690108	30		I. Sl. varicocoele	18			1+			-		39x26x23	Poor (thin)				

	HORMONE LEVELS: TOTAL GONADOTROPHINS mg units/24 hrs.	17-KETOSTEROIDS mg/24 hrs.	SEMINAL ANALYSIS: VOLUME (ml)	COUNT/ml 10^6	% NORMAL	% MOTILE	VISCOSITY	HISTOLOGY: ABSENCE G.C.	PARTIAL A.G.C. MAJOR	MINOR	P.A.G.C. + MAT. ARREST	MAT. ARREST	HYPOSPERMATOGENESIS	INFLAMMATION	NORMAL	TESTIS VOLUME (ml)	PER CENT	LEYDIG CELLS VOLUME(ml) (0.5–1.2 NORMAL)	PER CENT	GENERAL
14	13.0	13.5	0.5 0.6 0.3	 0.7 1.7	81 50 81										+			0.69	2.6	
15	35.0	33.9	4.8 7.8	As. As.			V.hypo. Hypo.	+								10.0		1.29	12.9	Del. Cast.
16	<5.0	20.2	1.1 2.3 2.0 2.8	0.05 0.40 0.05 0.05	45										+			0.52		Centre packed
17	<3.0	11.9	2.8 2.4 2.8	 8.5 1.6	84 91							+						0.49	3.8	Low Leydigs
18	10.0	5.6						+								4.94		0.11	2.24	
19											+									
20	<3.0	24.4	0.1 0.1	As. Olig									+	+				0.45	1.36	Low Leydigs
21	8.0	31.1	2.4 5.2 4.5 2.9	5.7 5.8 4.6 1.5	43 34 44		Norm. Norm. Norm. Hypov.					+						0.63	3.6	
22	130.0 ?	16.9	3.8	As.				+										1.17	6.7	Fibrous
23	30.0	14.6	3.8 3.7	As. "				+										3.80	16	Interstitial fibrosis.
24			1.2 1.8	As. "				+			+							0.55	5.19	
25	<3.0	2.1						+										0.60	5.8	Bil. Cast.

	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN % +ve	MITOTIC ANALYSIS: 43	44	45	46	47	GENERAL
14						3F- C- ?-	16		46,XY
15						2C- A-	18		46,XY Two Dp+
16		+				1G-	19		Poss. 45, XO/46, XY mosaic One cell t(4q-4q+)
17					1E-E-	1G-	18		46,XY
18					1B-C-	1D-	18		46,XY
19									
20						5D- F- Y- E- E-	25		Poss. 45 XO/46 XY mosaic Large Y. Heterom. 9's
21					1G-G-	2E- F-	17		46,XY V.heterom. 9's
22					1D-D-	1E-	18		46,XY Large Y
23			37				25		46,XX Gp+ 16q+ MAT 46,XX Gp+ 1 PAT 46,XY
24					2C-G- D-E-	1C-	23	1C+	Poss. 46,XY/47,X mosaic
25						3C- D- D-	17		46,XY. Mod. Y. Heterom. 9's.

	CASE	AGE	OCCUPATION	PROBLEM (Infertility)	PARENTAL AGES AT BIRTH		SIBS (+fertile)		FEMALE	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (Bexternal)	TUBULE TEASING	UNUSUAL HAIR DISTRIBUTION				HORMONE LEVELS		SEMINAL ANALYSIS					HISTOLOGY																		MITOTIC ANALYSIS					
					MAT.	PAT.	♂	♀							Pubic	Chest	Face	Head	TOTAL GEN. ADENOTROPHINS IRSG units/24 hrs.	17-KETOSTEROIDS mg/24 hrs.	VOLUME (ml)	COUNT/ml $\times 10^6$	% NORMAL	% MOTILE	VISCOSITY	ABSENCE D.C	PARTIAL A.a.C. MAJOR	PARTIAL A.a.C. MINOR	A.a.C. MAT. ARREST	HYPOSPERMATOGENESIS	INFLAMMATION	NORMAL	TESTIS VOLUME(ml)	TESTIS PER CENT	LEYDIG CELLS VOLUME (ml) (0.5-1.2 NORMAL)	LEYDIG CELLS PER CENT	GENERAL	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN % + ve	43	44	45	46	47	GENERAL
26	[illegible]	17		Undesc.							-															+																			
27	MS690123	29	Miner	I. Unilat. Ectopic			1	1+		Sl.obese			L36x19x17								3.1	57.5	89					+										0.20	3.4							20-G-	20		46,XY. Long G(8)
28	RO690124	27	Lecturer	I.				1		Thin	-		37x27x25			Scant			10.0	9.1	0.2 0.5	As. "										+					0.50	3.76		+			14-A-F-		20-E-	17		46, XY
29	RF690140	12	Student	Ectopic							-		10x50x50							5.4						+										High					10-C-	1F-	18		46,XY Heteros. 17's Mod. Y.
30	JG690141	27	Transport Foreman	I. Previous hydrocoele & hernia						Muscular	-		Ave.	Fair		Scant		Low hair line	25.0	16.5		As.										+					1.66	8.3	High Leydigs		+				3B-C-A-	17		46,XY Dp+
31	JM0690142	37		I.			2				-		43x30x30	Poor					8.0	14.0	1.6 2.2	1.0 0.6	45		Hyper. Hyper.					+					0.22	1.12	Low Leydigs Basm. el. thickened					1C-C-		19		46,XY Dp+ short arm Heteros. 9's
32	AG690153	38	Accountant	I.			1			Sl.obese	-	Very small	25x24x19	Fair (thin)					26.0	16.1							+										0.88	14.8						5C-F-G-G-Gp-	15		Poss. 45,XO/ 46,XY mosaic
33	RO690167	28	Insurance Clerk	I. Unilat. undesc.			1			5'9½" 10st.12lb	-		R40x28x20 L35x23x23	Good							1.8 2.5	6.6 3.9	 89	25 27		L+		R+											High Leydigs						4G-G-G-E-	16		46,XY Gp+
34	JF690168	30	Technician Turner	I.	40	40	1+	2+		Ave.	-		45x30x30	Poor (thick but stuck)					11.0	21.6	0.4 0.4	As. As.										+					0.86	4.8	Fibrous	+				1E-G-	3G-G-G-			Poss. 45,XO/ 46,XY mosaic
35	RM690178	28	Joiner	I. Lig. varicocoele			1				-		43x29x26	Fair (thin)					10.0	24.4	2.2 2.2	4.0 1.2	72 40	21 10					+								1.70	10.6						1F-	19		46,XY One cell 3 f. = long arm o.
36	DM0690184	24		I.			1				-	Large	43x25x25	Good (thick)					10.0	19.3	2.6 2.7	As. As.										+									+			1A-	19		46,XY Gp+short arm
37	DD690194	34	P.O. Engineer	Screening for Translocation					+		-		45x30x30	Good																		+														46,XY. Normal variant small Y
38	JD690204	33		Translocation Heterozygote					+	Slim	-		R45x30x28 L40x20x20	Fair (thin)																		+					R 0.45 L 0.24	5.0 2.88	Fewer sperm									46, XY Dq-

	CASE	AGE	OCCUPATION	PROBLEM (I=infertility)	PARENTAL AGES AT BIRTH		SIBS (+fertile)		FAMILY	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (@=external)	TUBULE TRASING	UNUSUAL HAIR DISTRIBUTION				HORMONE LEVELS		SEMINAL ANALYSIS					HISTOLOGY																MITOTIC ANALYSIS					
					MAT.	PAT.	♂	♀							PUBIC	CHEST	FACE	HEAD	TOTAL GON-ADOTROPHINS mIU/ml/24 hrs.	17-KETOSTEROIDS mg/24 hrs.	VOLUME (ml)	COUNT/ml x10⁶	% NORMAL	% MOTILE	VISCOSITY	ABSENCE G.C.	PARTIAL A.G.C. MAJOR	PARTIAL A.G.C. MINOR	P.A.G.C. MAT. ARREST	MAT. ARREST	HYPOSPERMATOGENESIS	INFLAMMATION	NORMAL	TESTIS VOLUME(ml)	TESTIS PER CENT	LEYDIG CELLS VOLUME(ml) (3.5-1.2 NORMAL)	LEYDIG CELLS PER CENT	GENERAL	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN % +ve	43	44	45	46	47	GENE
39	SA690217	41	Plant Operator	Translocation Heterozygote					+		-		51x30x30E	Fair (fibrous)											+								0.53	2.2									46,XY Dq+ Oq-
40	JB690219	31	Catering Manager	I.				1+		Sl.obese	-		42x31x30	Good (medium)					13.0		3.4	Az.									+		0.99	4.5		+	+			10-	19		46,XY Long 4
41	RA690226	42	Police Constable	I.	37	39	1+	1+		Well built	-		47x34x30	Good		None			29.0	19.5	3.0 4.6 3.0 3.7	11.7 8.9 12.3 11.2	43 59 78	<1 30 53	Hypo. Hyper. V.hyper					+											22- G-	18		46,XY
42	AF690227	27		I.							-		50x30x22	Fair							3.4 3.7	23.9 16.8	53 54	8 21					+											17-I-	18-	17	1(break)	46,XY Some cells chromosomal breakage
43	JMF690231	33	Teacher	I. Bil. Orchidopexy							-	Large						Low hair line			2.1	Az.													Anorchia					20- E-	18		46,XY
44	IT690271	18		Gonads not palpable							-																								Anorchia			18-E-		9		46,XY
45	JO690293	26	Joiner	I.			1	1			-		46x29x27	Good					10.0	7.8	1.0 1.3	Az. 0.1					+								Del. Cast. (incomplete)					10- C-	2F- C- D- F-	15		Poss.45,XO/ 46 XY mosai
46	JM690306	14		Gonads not palpable						Obese	-								<3.0	4.6															Anorchia								
47	HO690328	26	Welder	I.			4+ + +	3+ +		V.obese	-		37x20x20						83.0	18.4	4.9 4.4	Az. Az.			Norm. Hyper.	+											High Leydigs					1Y-	19		Poss. 45,XO 46, XY mosaic
48	JS690329	25	Turner Engineer	I.						Athletic	-		37x20x20			Scant			42.0	20.6	1.1 1.9	0.1 Az.							+											3C- D- G-	17		46,XY Sat. 17
49	AB690334	32	Research Economist	I. Inguinal herniorraphy			4	3+		Small Sl.obese	-		42x35x30	Fair (stuck)					10.0	11.2		Az.			Hyper.							+				Centre packed					1D-	19		46,XY
50	TS690371	49	Civil Engineer	I. Unilat. undesc. scrotal hernia			1+	1+		Sl.obese	-		40x35x27			Lots			28.0		2.3 1.6	Az. "			Hypo.							+						+				2C- E-	17		46,XY
51	DO690405	22	Lab. Technician	I. Epispadias Bifid scrotum Ectopic bladder							-		40x26x26								2.3 1.6	Az. Az.							+										+	1G-C-	1F-	18		Poss. 45,XY 46,XY mosai

	CASE	AGE	OCCUPATION	PROBLEM (I=infertility)	PARENTAL AGES AT BIRTH MAT.	PAT.	SIBS (+fertile) ♂	♀	FERTILE	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (E=external)	TUBULE TEASING
52	BMA.....			I.							-			
53	JT700005	71		Ca.prostate Orchidectomy					+		-		Large	Fair
54	WND700011	67		Ca.prostate Orchidectomy					+	Tall Muscular	-		Large	
55	GW700056	53	Joiner	Ca.prostate Orchidectomy (Stilboestrol)					+		+			
56	WF700071	25		I.						Sl.obese	-		50x33x25	
57	DK700082	42		Trans-sexual "Sex-Change"						Tall. Large frame	+		R26x13x13 L21x?x?	V.poor
58	AG700098	49		Epididymitis							-		45x30x25	
59	GF700099	15		Ectopic testis							-		32x20x16	
60	TJ700101	37	Buyer	I. Varicocoele			1+	1+		Sl.obese	-	V.large	R25x20x15 L33x25x23	
61	JM700107	24	Shopkeeper	I. Varicocoele			3+	1+		Muscular	-		55x30x30	Fair (Thin & stuck)
62	JHM700108	34		I. Varicocoele			1			Slender	-	Small	45x30x30	Good
63	J?700110	65	Insulating Engineer	Hydrocoele							-		60x50x50	V.good

	UNUSUAL HAIR DISTRIBUTION Pubic	Chest	Face	Head	HORMONE LEVELS TOTAL GONADOTROPHINS HMG units/24 hrs	17-KETOSTEROIDS mg/24 hrs.	SEMINAL ANALYSIS VOLUME (ml)	COUNT/ml x10^6	% NORMAL	% MOTILE	VISCOSITY
52											
53											
54											
55											
56											
57	♀	None (shaved?)	Shaves occasionally	Low hair line							
58											
59											
60					26.0	4.8	2.3 2.1	0.5 2.7		2	
61		Scant		Low hair line			2.7	7.6	92	54	
62							3.8	51.7	95	Low	Hyper.
63											

	HISTOLOGY ABSENCE G.C.	PARTIAL A.G.C. MAJOR	MINOR	PACH. + MAT. ARREST.	MAT. ARREST	HYPOSPERMATOGENESIS	INFLAMMATION	NORMAL	TESTIS VOLUME(ml)	PER CENT	LEYDIG CELLS VOLUME(ml) (0.5-1.2 NORMAL)	PER CENT	GENERAL	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN % +ve
52			+										High Leydigs			
53								+					Low Leydigs Interstitial fibrosis			
54								+								
55													Only Sertoli cells + 1 spgonium per tubule			
56								+								
57	+								R1.8 L1.2		1.00 0.60	57.7 49.5	< 0.2% occupied by tubules			15 Sertoli -ve
58								+	17.7		0.67	3.8				
59					+								Germ cells → pachytene			
60								+	R3.9 L9.6		0.69 0.30	7.3				
61								+	[illegible]							
62								+								
63								+					Inter-tubular fluid			

	MITOTIC ANALYSIS 43	44	45	46	47	GENERAL
52						
53	1D-G-A-	1E-C-	1C-	17		46,XY
54		3C-C- G-F- F-E-	2F- G-	14	1(frgt)	Poss. 45XO/ 46,XY mosaic
55				20		46,XY Blobs sh. arm of 1
56			3C- G- D-	17		46,XY Dp+ Hetero. 9's
57			1G-	3E- G- C-	16	47,XXY. Gp+ sat. Distinct Y present in furt[illegible] 100 cells.
58			1F-	19		46,XY Cells with blobs sh. arm 1[illegible] long 1, 3 and 1[illegible]
59			2E- D-	18		46,XY
60			4D- A- G- C-	16		46,XY 1 cell ring G.
61			2G- G-	18		46,XY Large Y Sat. 17
62			1E-	19		46,XY Heterom. 9's Blob long arm 4
63		1G-F-	3G- A- C-	16		46,XY Heterom. 9's Viral damage

CASE	AGE	OCCUPATION	PROBLEM (I=infertility)	PARENTAL AGES AT BIRTH: MAT.	PARENTAL AGES AT BIRTH: PAT.	SIBS (+fertile) ♂	SIBS (+fertile) ♀	FERTILE	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (E=external)	TUBULE TEASING	UNUSUAL HAIR DISTRIBUTION: Pubic	Chest	Face	Head
64 DW700153	24		I.						Large Obese	-		40x25x25	Good				
65 WG700237	25	Production Inspector	I.			3	1			-		38x23x23					
66 JT700346	29	Fitters Labourer	Cremaster spasm orchidectomy					+	Obese	-		48x36x33					
67 BS700349	21		Radiation damaged						Ave.	-		Ave.	Fair				
68 CMD700362	53		Orchitis & epididymitis					+		-		47x32x25					
69 R.......			Translocation heterozygote					+		-							
70 SML700425	32		I.			4+ +	2+		Athletic	-		25x15x15					
71 JW700429	23	Painter	I.			3+	2		Slender	-	Large	Ave.			Scant		
72 AC700506	35		I.			2	2+ +			-		Small					
73 JB700530	27		I.							-		40x28x28	Fair (thin)				
74 RW680009	30		Translocation heterozygote							-							

CASE	HORMONE LEVELS: TOTAL GONADOTROPHINS MU units/24 hrs.	17-KETOSTEROIDS mg/24 hrs.	SEMINAL ANALYSIS: VOLUME (ml)	COUNT/mlX10^6	% NORMAL	% MOTILE	VISCOSITY
64 DW700153	11.0	16.5	0.9 2.4	1.7 0.6	8 25		
65 WG700237	12.0		3.7 2.8	0.3 0.1	20 10		
66 JT700346							
67 BS700349				0.6 0.5		0 0	
68 CMD700362							
69 R.......							
70 SML700425	27.0	22.6		Az.			
71 JW700429	11.0		0.1 1.4	0.2		0	Hyper.
72 AC700506	9.0	7.9	3.0	0.1			
73 JB700530							
74 RW680009							

CASE	HISTOLOGY: ABSENCE G.C.	PARTIAL A.G.C.: MAJOR	PARTIAL A.G.C.: MINOR	P.A.G.C. MAT. ARREST	MAT. ARREST	HYPOSPERMATO-GENESIS	INFLAMMATION	NORMAL	TESTIS: VOLUME (ml)	TESTIS: PER CENT	LEYDIG CELLS: VOLUME (ml) (0.5-1.2 NORMAL)	LEYDIG CELLS: PER CENT	GENERAL	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN % +ve
64 DW700153		+							13.9		0.45	3.5	Del. Cast. (incomplete)			
65 WG700237					+				10.50		0.32	3.0				
66 JT700346								+					Interstitial oedema. Scanty free sperm forms.			
67 BS700349					+								Very reduced spermatogenesis			
68 CMD700362						+							Thickened tubules			
69 R.......																
70 SML700425		+									0.70	3.5	B.M. thickened Del. Cast. (incomplete)			
71 JW700429					+						0.32	1.6				
72 AC700506					+							4.4				
73 JB700530	+												Del. Cast.			
74 RW680009																

CASE	MITOTIC ANALYSIS: 43	44	45	46	47	GENERAL
64 DW700153		3C-C- C-C- F-G-	3E- C- F-	14		46,XY Gp+ sats.
65 WG700237		1G-G-	2C- E-	17		46,XY Two Dp+ sat[illegible] Two Gp+ sat[illegible] Mod. Y
66 JT700346				20		46,XX. Poss. small and large [illegible]
67 BS700349				20 20 (scrotal fibrobl.)		46,XY. 8% abnormal dicentrics, fragts, br[illegible] etc.
68 CMD700362			4G- G- F- F-	15	1C+	46,XY V. heterom[illegible]
69 R.......			1G-	9		46,XY Ep-
70 SML700425		1C-C-	5G- G- G- F- F-	14		Poss. 45,X[illegible] mosaic. Fi[illegible] normal.
71 JW700429			3D- D- G-	17		Poss. 45,XO mosaic
72 AC700506			1G-	19		46,XY (One cell [illegible]
73 JB700530			1C-	19		46,XY One cell [illegible] Heterom. 9[illegible]
74 RW680009						46,XY 5p-

	CASE	AGE	OCCUPATION	PROBLEM (I=infertility)	PARENTAL AGES AT BIRTH		SIBS (+fertile)		EUNUCHOID	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (l×external)	TUBULE TRAUMA	UNUSUAL HAIR DISTRIBUTION				HORMONE LEVELS		SEMINAL ANALYSIS					HISTOLOGY																MITOTIC ANALYSIS					
					MAT.	PAT.	♂	♀							Pubic	Chest	Face	Head	TOTAL GON-ADOTROPHINS IRG units/24 hrs.	17-KETOSTEROIDS mg/24 hrs.	VOLUME (ml)	COUNT/mm³	% NORMAL	% MOTILE	VISCOSITY	ABSENCE G.C.	PARTIAL A.G.O. MAJOR	PARTIAL A.G.O. MINOR	P.A.G.C. MAT. ARREST	MAT. ARREST	HYPOSPERMATOGENESIS	INFLAMMATION	TESTIS NORMAL	TESTIS VOLUME(ml)	TESTIS PER CENT	LEYDIG CELLS VOLUME(ml) (0.5-1.2 NORMAL)	LEYDIG CELLS PER CENT	GENERAL	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN %	43	44	45	46	47	GENERAL
75	PML700552	30	Electric Welder	I.			6+ + + +	2+ +		M.built Sl. obese	-		53×35×30					Low hair line	9.0	7.5	0.2 0.2	As. "											+							Some atrophic tubules	+				10-	19		Poss. 45,XO/46,XY mosaic Heterom. 1's Two cells D gaps
76	JU700577	30		I. Varicocoele							-	Large	45×27×25	Good				Low hair line										+																3D- D- E-	17		46,XY One cell 18p- Two Dp+ Two Gp+
77	HNC700596	34		Screening for Translocation							-	Large	50×30×30															+																	10		46,XY. Mod. heterom. 16's
78	JMA700635	39		I.							-		55×35×31	Good														+																3D- D- C-	16	1(frgt)	46,XY one triradial and fragts.
79	LJ700636	22		I. Bil. ectopic							-	Large	30×20×20						65.0	19.2						+								6.3		0.72	11.4	Del. Gest						19	1(fgt)	46,XY Mod. X.
80	TB700653	31	Lorry Driver	I. One gonad not palp.						Athletic	-										1.2 2.5	3.3 15.6	75	22 30												1.33							10-	19		Poss. 45,XO/ 46,XY mosaic
81	AH710009	35		I.						Small	-	Large	37×24×22	Good								As.				+																	3D- C- E-	17		46,XY
82	LG710097	27		I.			0	0			-		35×22×20	Fair (thin & stuck)					21.0	5.7	2.0	As.			Hyper.	+							8.1		0.92	11.4	S.m. sl. thickened				2X-C- C-C-	3D- D- C-	15		Poss. 45,XO/ 46,XY mosaic Sat. 18.
83	GH710110	28		I. Varicocoele							-		42×27×25														+																	20		46,XY. One cell sult. quadriradials. Heterom. 11's. Two cells gap B
84	DH710125	28	Cost Clerk	I.							-		33×20×19½	Good (some stuck)		None	Scant		8.0	10.7	1.0	8 dead			Sl. Hypo.		+					+	5.6		0.63					1C-G-	1D-	18		46,XY. Mod. Y. One cell ri One cell dicentr + frgts.
85	HK710139	50		Volunteer							-		49×28×25½	Fair (thin)														+																	10		46,XY 16q+

	CASE	AGE	OCCUPATION	PROBLEM (I=infertility)	PARENTAL AGES AT BIRTH MAT.	PAT.	SIBS (+fertile) ♂	♀	FERTILE	BUILD	GYNAECOMASTIA	UNUSUAL PENIS SIZE	TESTIS DIMENSIONS (mm) (E=external)	TUBULE TEASING	UNUSUAL HAIR DISTRIBUTION Pubic	Chest	Face	Head	HORMONE LEVELS TOTAL GONADOTROPHINS BMG units/24 hrs.	17-KETOSTEROIDS mg/24 hrs.
86	JS710173	33	Clerk	I. Bil. ectopic			1	2		Slender	-		42x20x20				Scant			
87	RW710174	37		I. Small varicocoele			1+			Sl.obese	-		R50x30x15 L60x30x30							
88	KMK710321	34		I.			1	1+		Slender	-		30x20x20E	Poor						

CASE	SEMINAL ANALYSIS VOLUME (ml)	COUNT/mlx10^6	% NORMAL	% MOTILE	VISCOSITY	ABSENCE G.C.	PARTIAL A.G.C. MAJOR	MINOR	HISTOLOGY P.A.G.C. + MAT. ARREST	MAT. ARREST	HYPOSPERMATOGENESIS	INFLAMMATION	NORMAL	TESTIS VOLUME (ml)	PER CENT	LEYDIG CELLS VOLUME (ml) (0.5-1.2 NORMAL)	PER CENT	GENERAL	ABSENT VAS	EPIDIDYMAL BLOCK	SEX CHROMATIN % +ve	MITOTIC ANALYSIS 43	44	45	46	47	GENE
86 JS710173	1.2	As.					+							8.8		2.60	29.0	High Leydigs							20		46,XY
87 RW710174	1.7 1.5	Few As.											+	28.0		0.45	1.6			+			1C-G-	3E- E- G-	16		46,XY
88 KMK710321	1.5	As.				+																		1C-C-		19	47,XXY

H U M A N M A L E M E I O S I S

ILLUSTRATIONS

for the

Thesis

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CONTENTS

		Plates
A.	HUMAN MALE MATERIAL	1-74
	Testis sections and mitotic chromosomes.	1-12
	Sequence in spermatogenesis.	13-60
	Cells in degeneration.	61
	Cells from a case with a small Y chromosome.	62-64
	Cells from translocation heterozygotes.	65-73
	Material from radiation-damaged case.	74
B.	MALE MATERIAL OTHER THAN HUMAN	75-96
	Locust cells.	75-83
	Syrian hamster cells.	84-88
	Mouse cells.	89-96

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Unless otherwise stated, all photomicrographs are magnified X 2,500.

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Abbreviations are at the rear of the volume.

A. HUMAN MALE MATERIAL

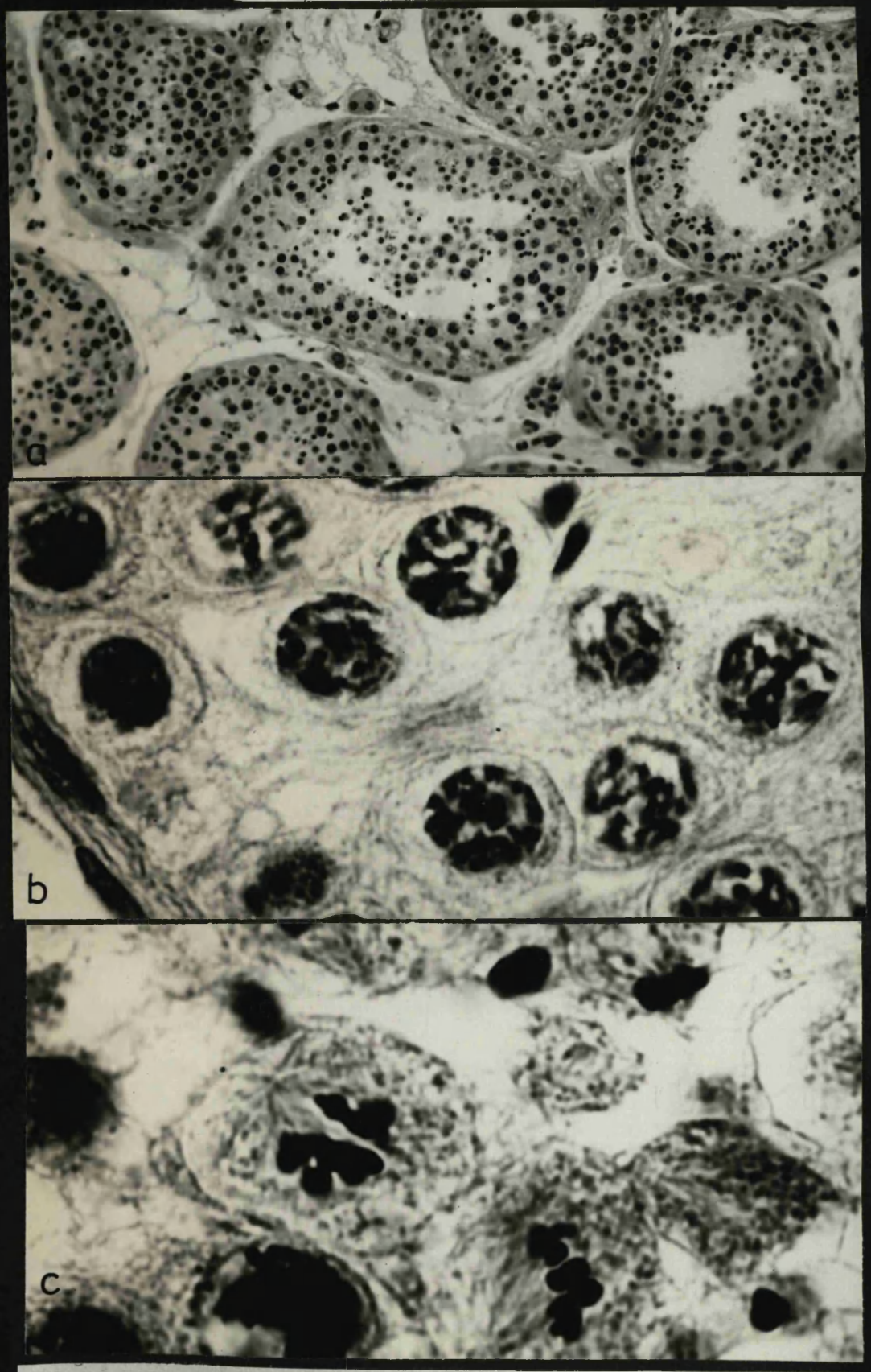


Plate 1. Sections of testis. All show normal spermatogenesis.
(a) x 250

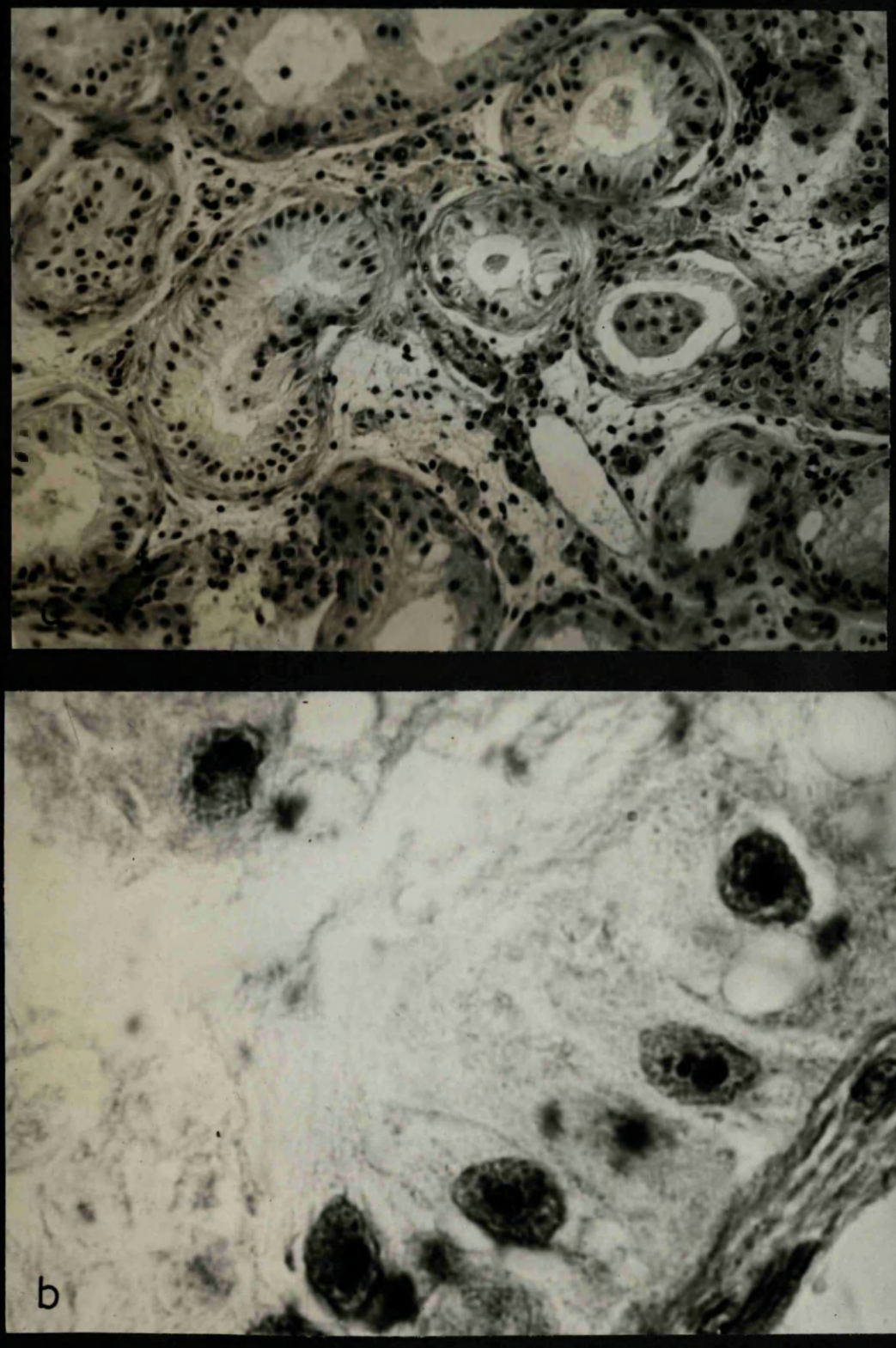


Plate 2. Sections of testis showing an absence of germ cells.
(a) x 250.

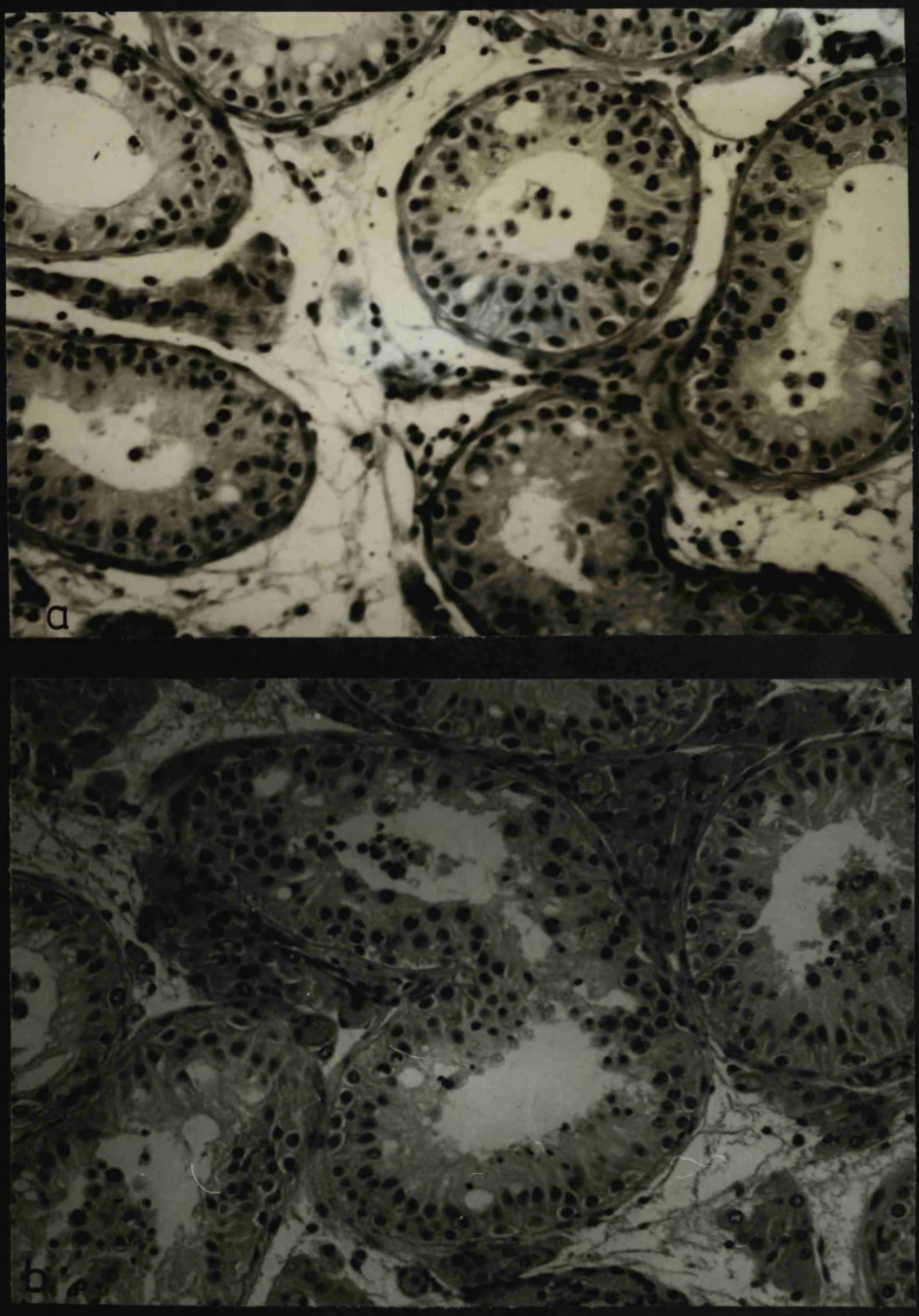
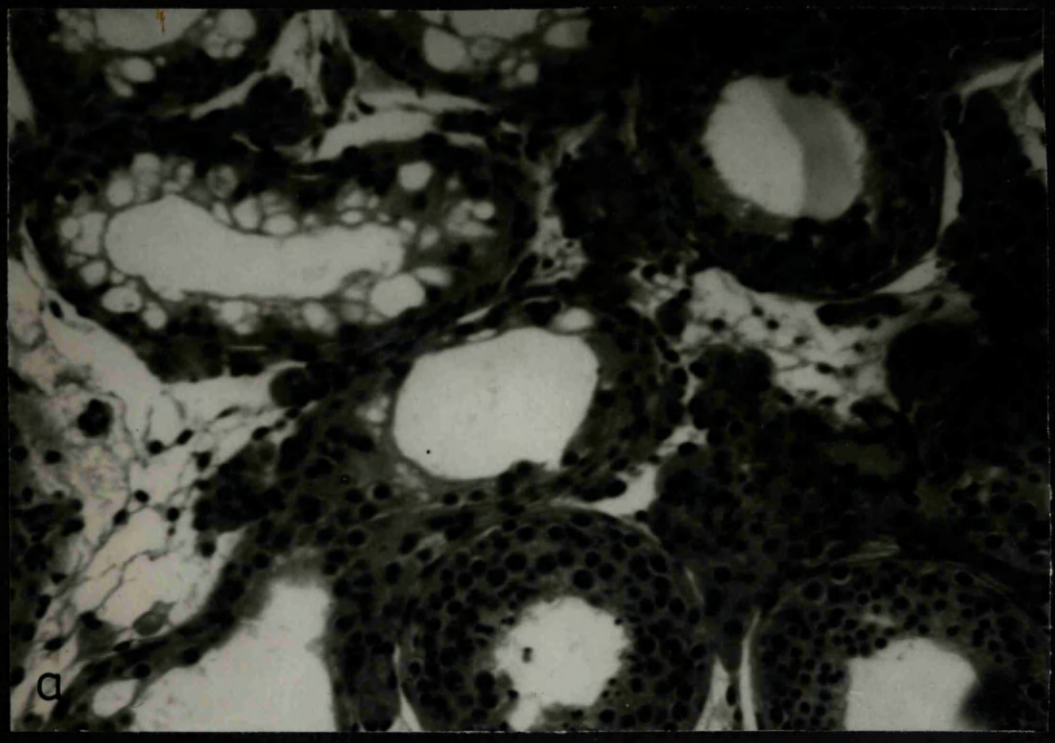


Plate 3. Sections of testis, x 250, showing "maturation arrest" in spermatogenesis.

(a) No mature sperm are present.

(b) Less severe condition. A few mature sperm are present.



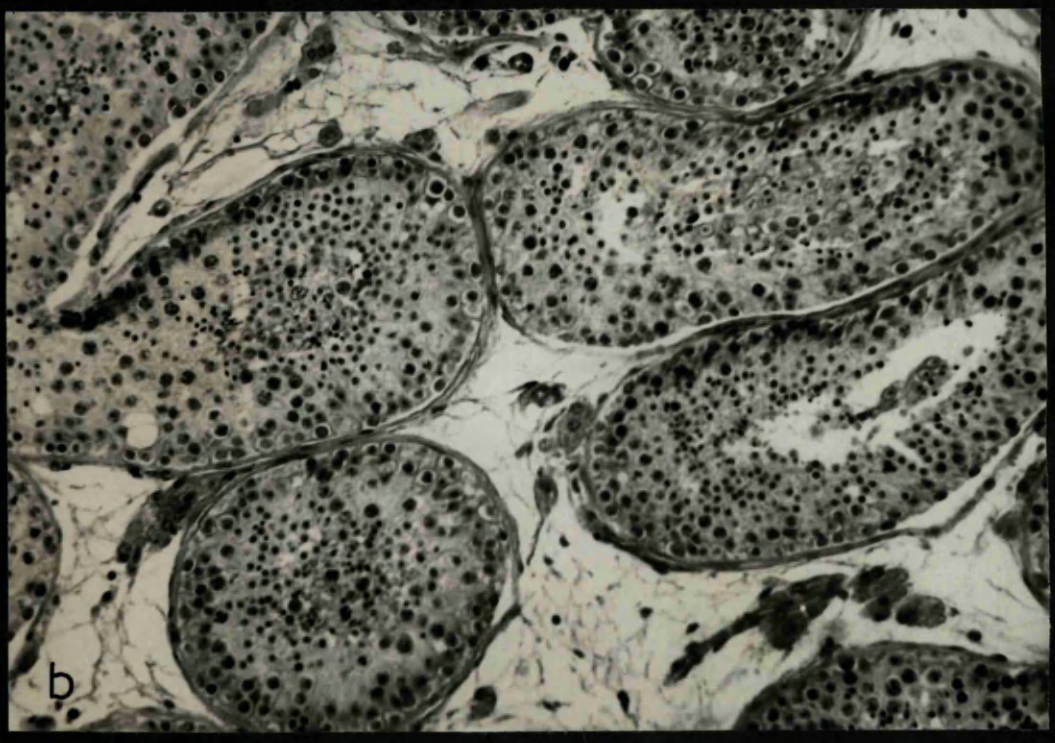
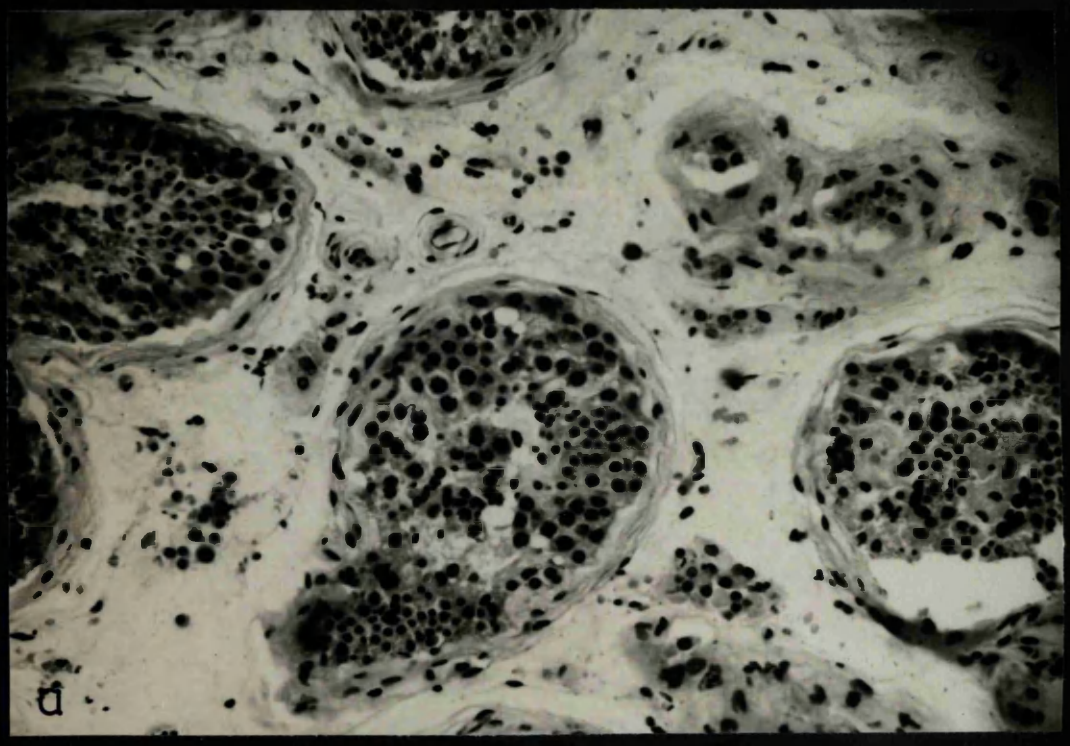


Plate 4. Sections of testis, x 250.

(a) Shows partial absence of germ cells with maturation arrest in those tubules with spermatogenesis.

(b) Shows normal spermatogenesis. The lumen of most tubules is blocked with cells in division. This type of histological picture seems to be correlated with fewer meiotic metaphases in air-dried preparations.



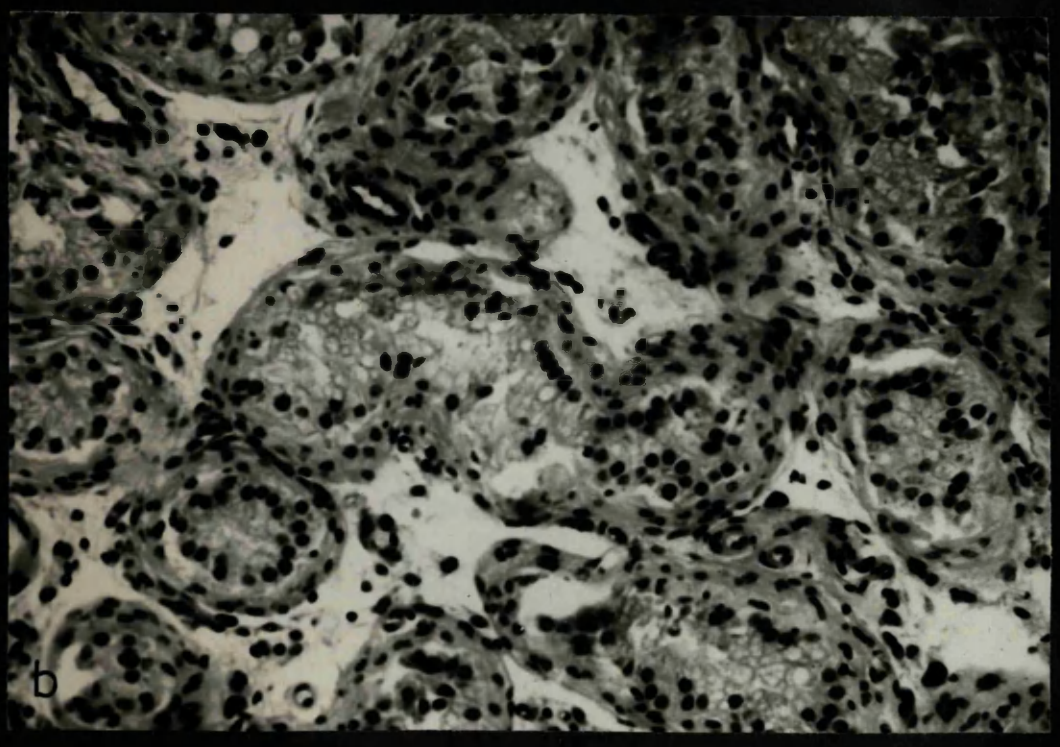
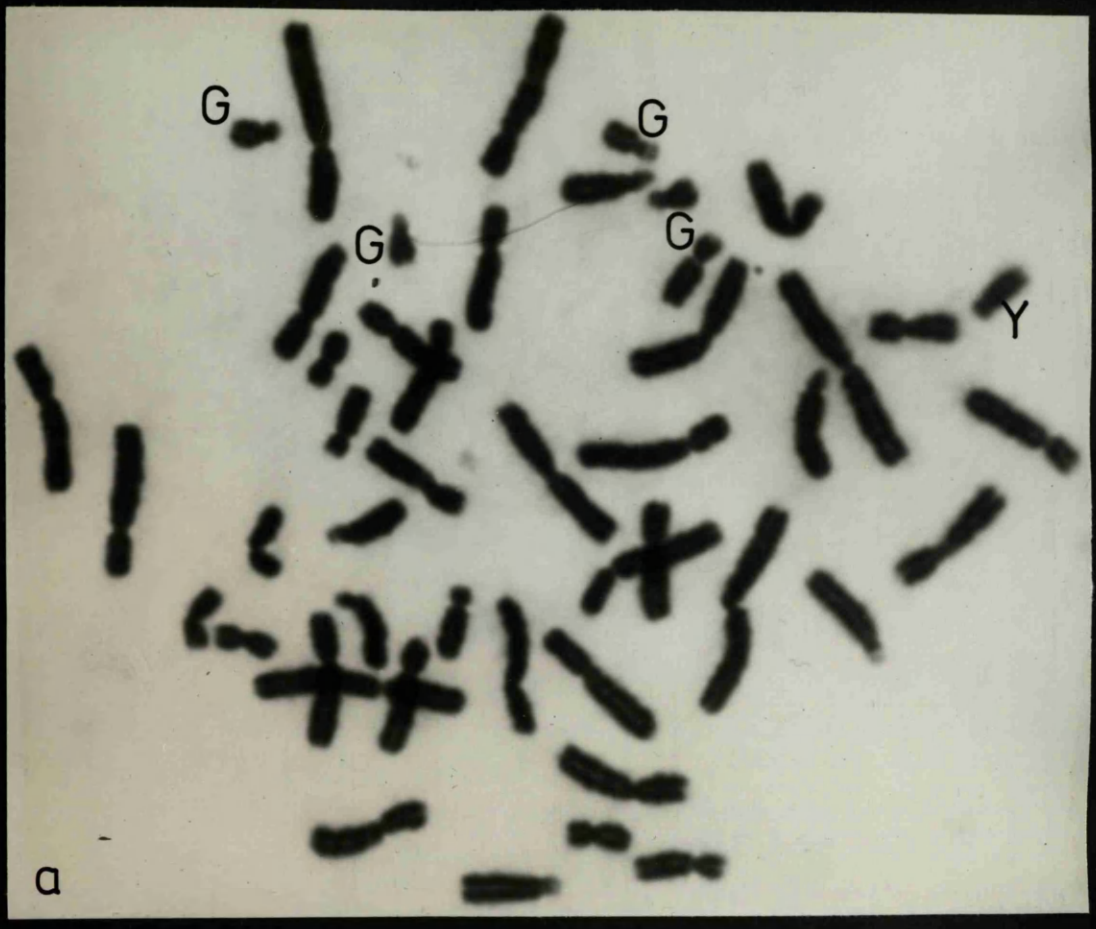


Plate 5. Sections of testis, x 250.

(a) From JT700005, aged 71. Extensive fibrous material surrounds the tubules. Spermatogenesis is active.

(b) From GW700056, aged 53, who had been given stilboestrol in the treatment of cancer of the prostate. Spermatogenesis is absent.



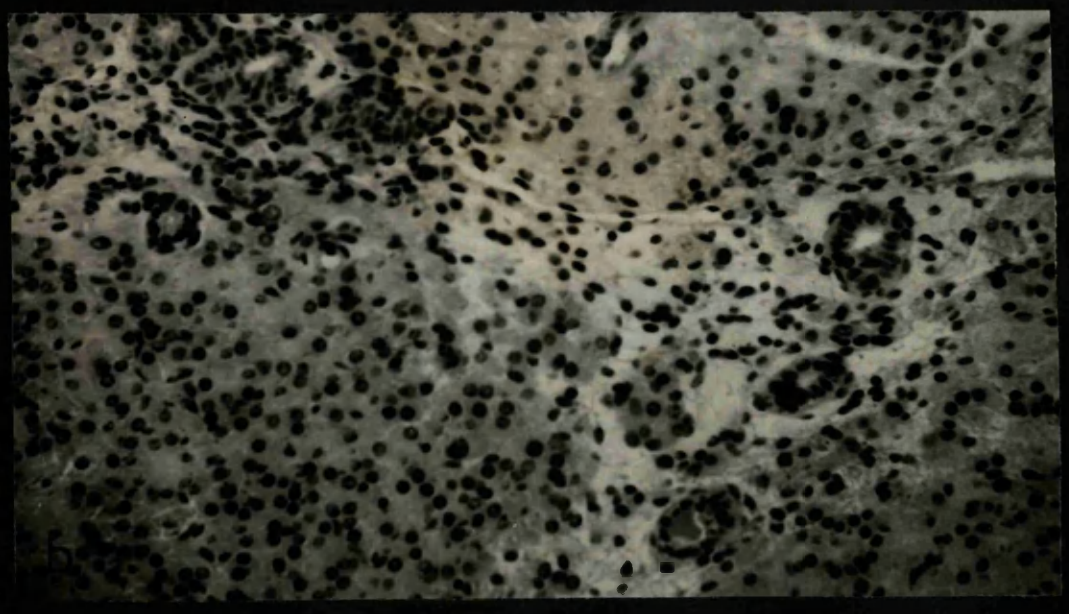
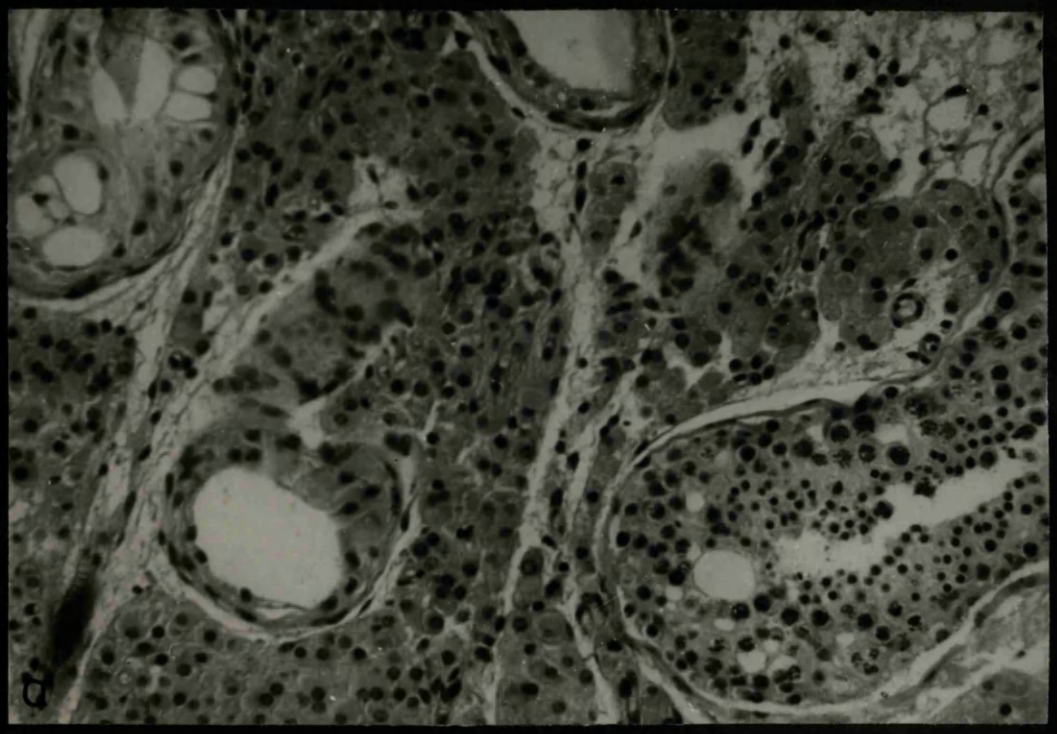


Plate 6. Material from DK700082 with 47,XXY Klinefelter's syndrome.

(a) Mitotic metaphase which shows the distinctive Y chromosome. All G- cells could therefore be accurately scored for the presence or absence of the Y.

(b) Section of testis, x 250. It shows mainly Leydig cells and very few tubules. The condition is an extreme manifestation of the Klinefelter syndrome.



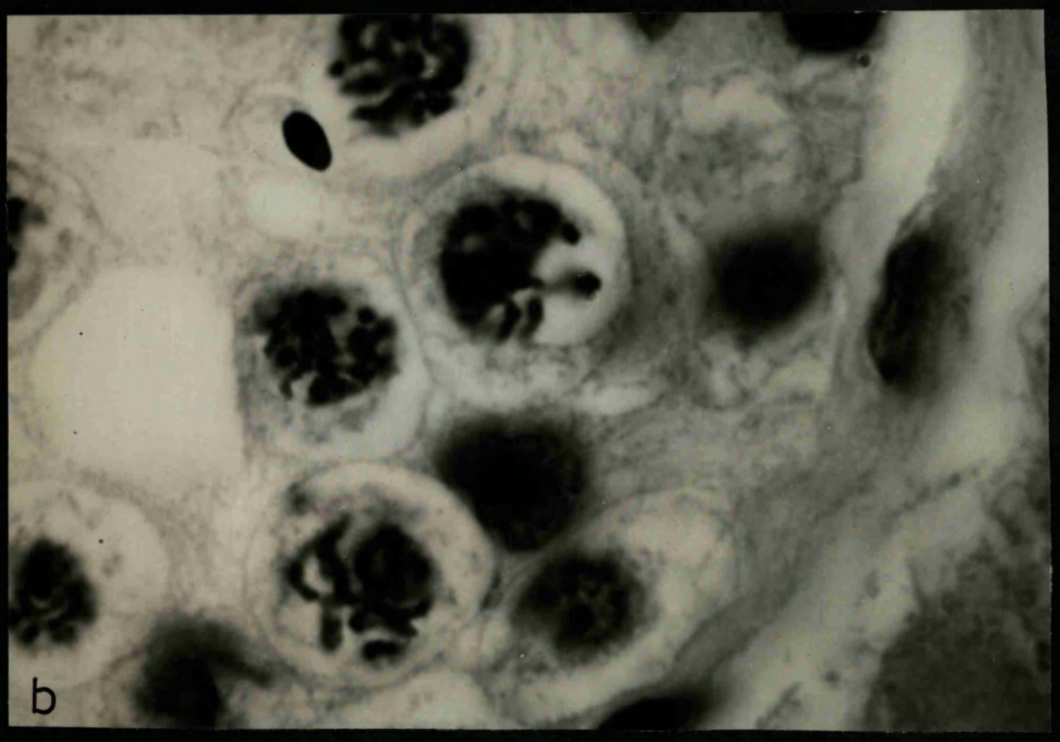


Plate 7. Testis sections from 47,XXY Klinefelter AM680180.

(a) x 250. One tubule shows spermatogenesis.

(b) Area with spermatogenesis. A single sperm is present.

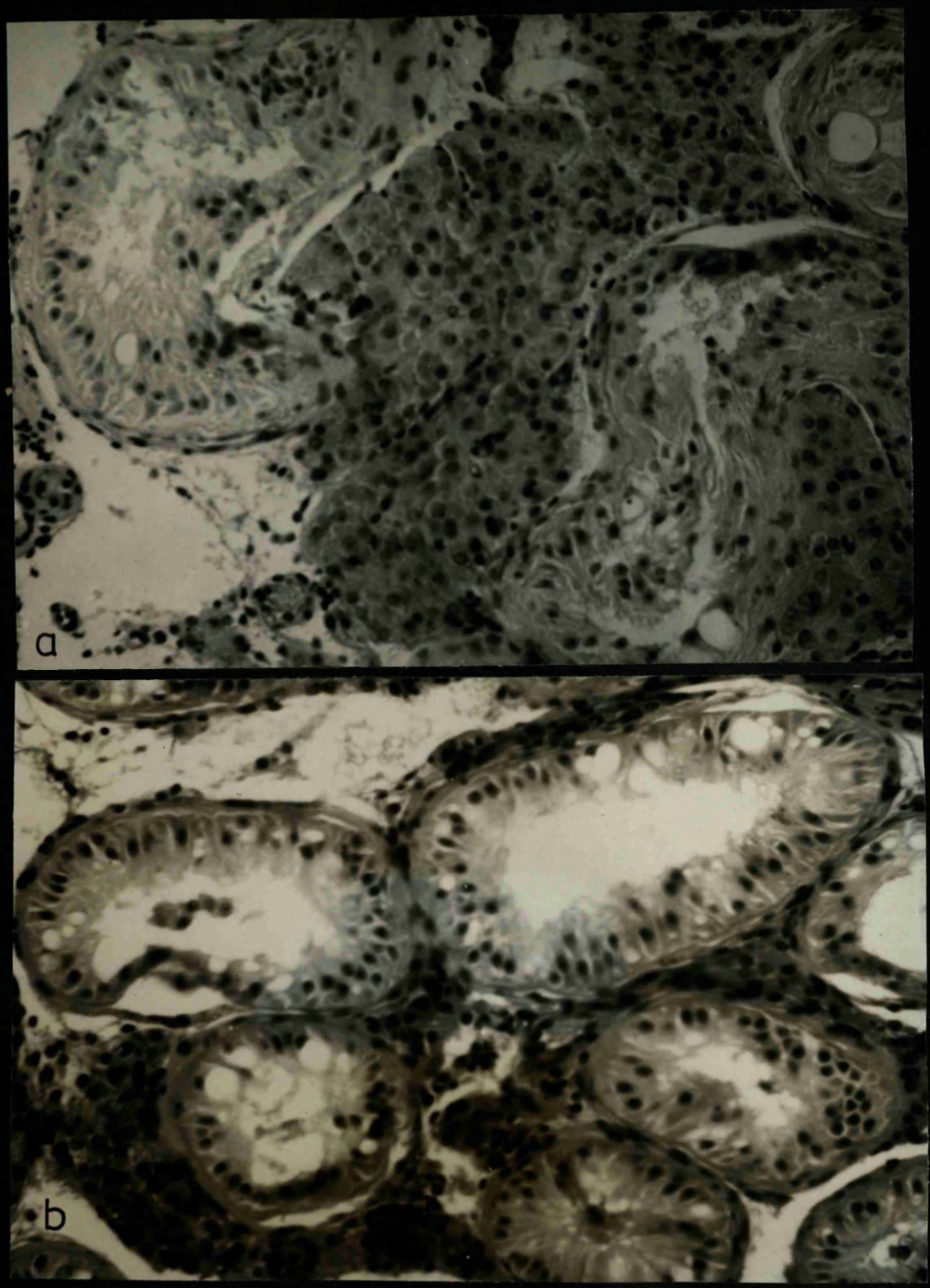
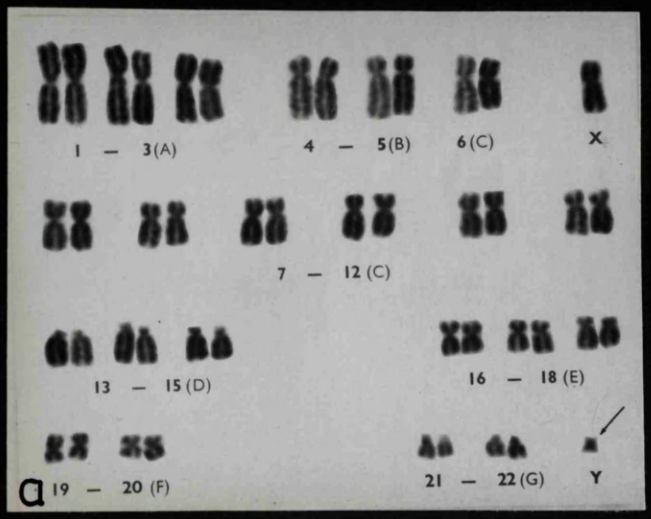


Plate 8. Sections of testis, x 250, showing an absence of germ cells.

(a) Apparent Leydig cell hyperplasia. This is a condition usually associated with 47,XXY Klinefelter's syndrome and is from AP690095, a 46,XX male.

(b) From HC690328 with 45,XO/46,XY mosaicism.



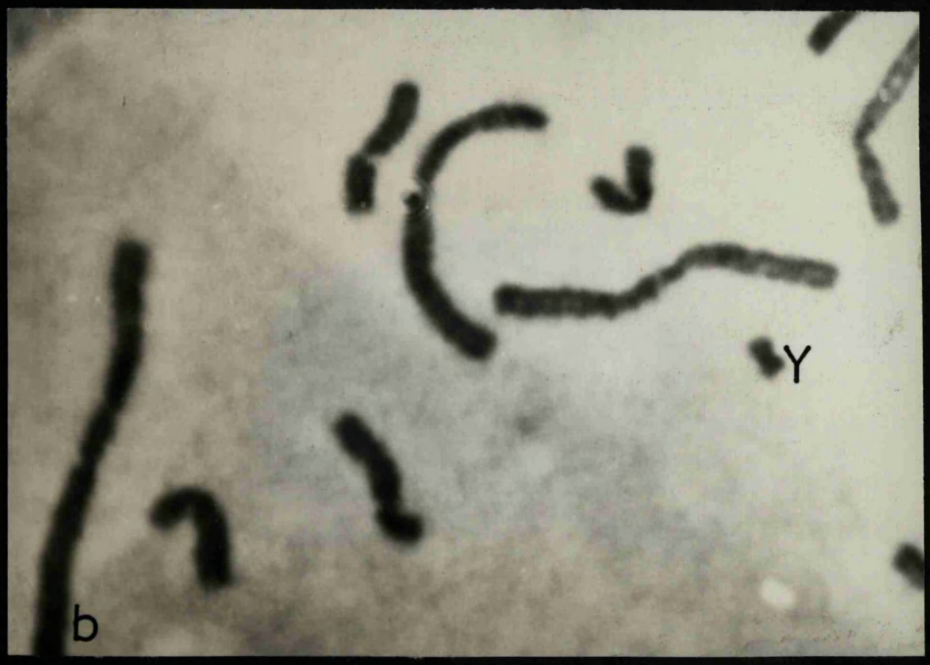
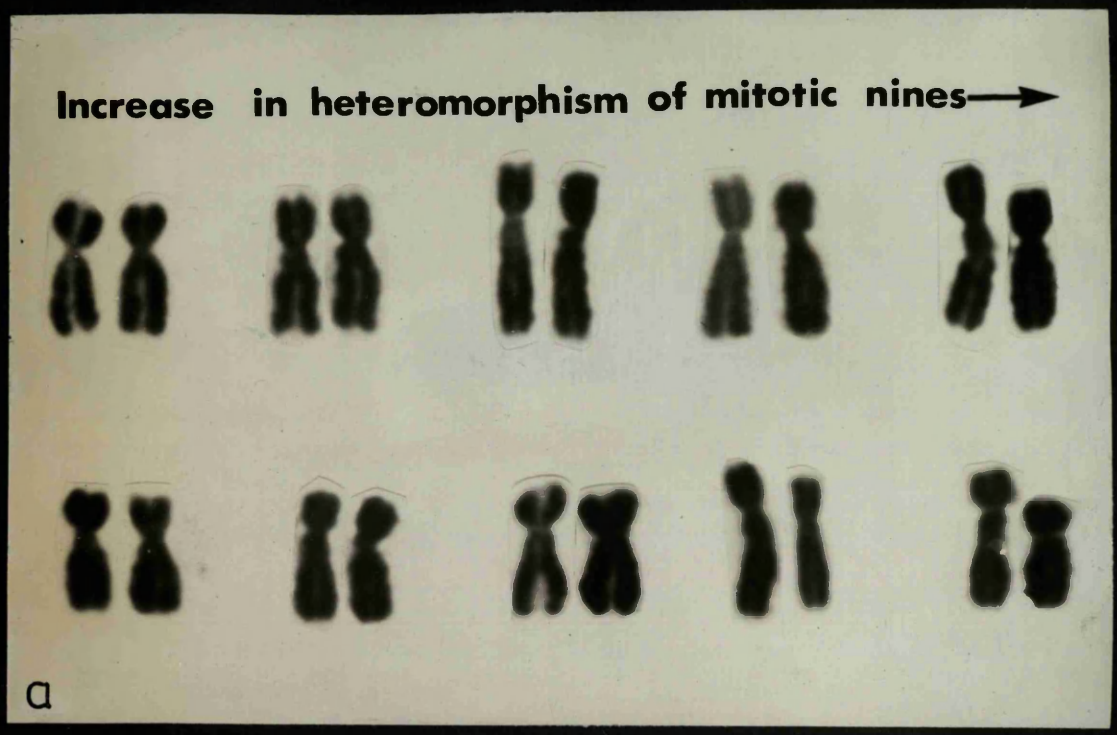


Plate 9. Leucocyte mitotic chromosomes from WC680180 with a large deletion of the Y chromosome.

(a) x 1,750. Karyotyped.

(b) Early metaphase, x 4,500. The Y is clearly shown to be a small metacentric.



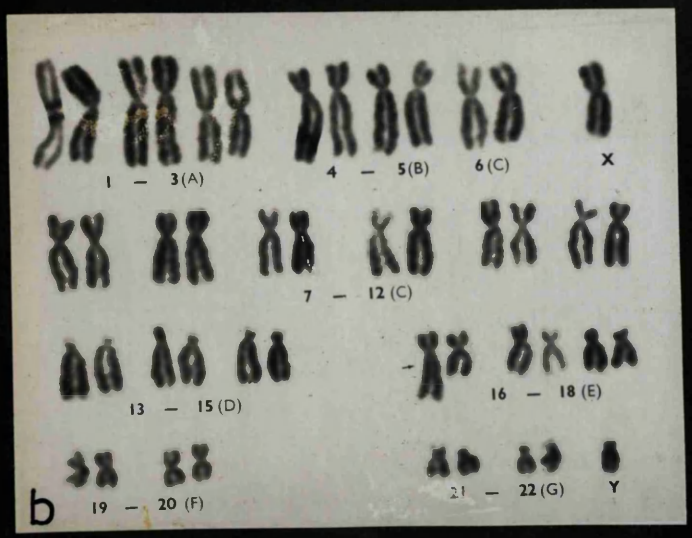
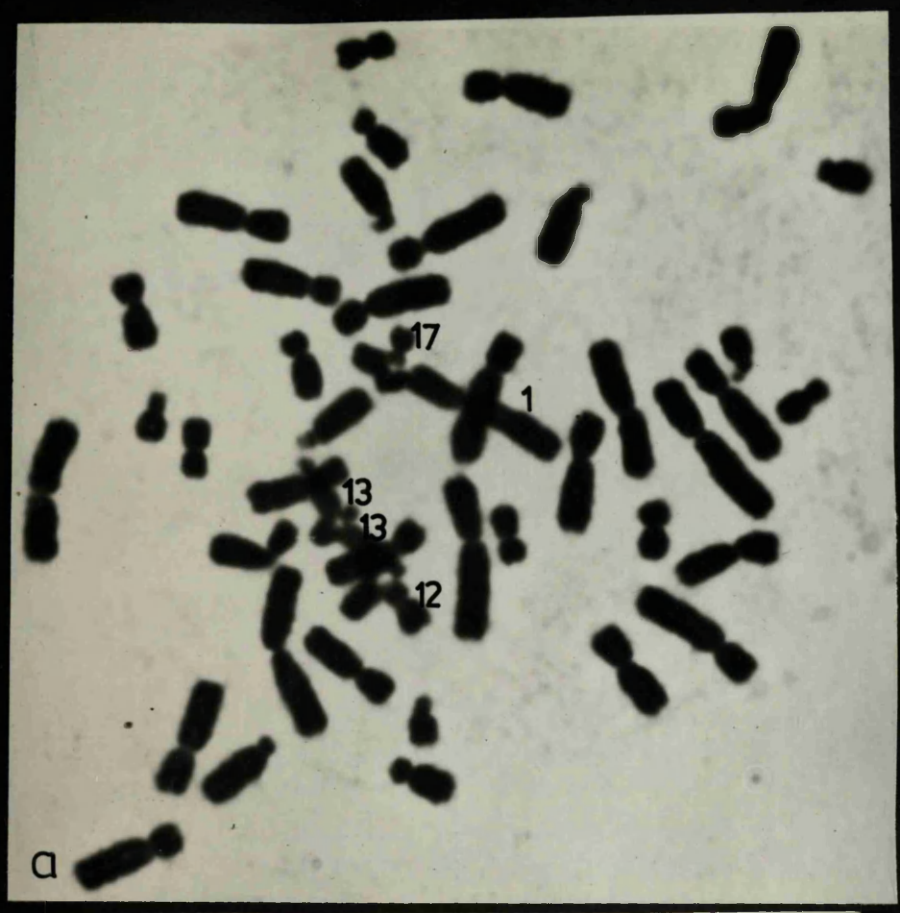


Plate 10. Heteromorphism in mitotic chromosomes.

(a) x 4,250. Shows the range in heteromorphism of chromosome 9 as demonstrated by different individuals. At the extreme right are homologues from CMC700362, and second from the right those from JB690075.

(b) x 1,750. Karyotype from control HK700139, showing extreme heteromorphism of chromosomes 16.



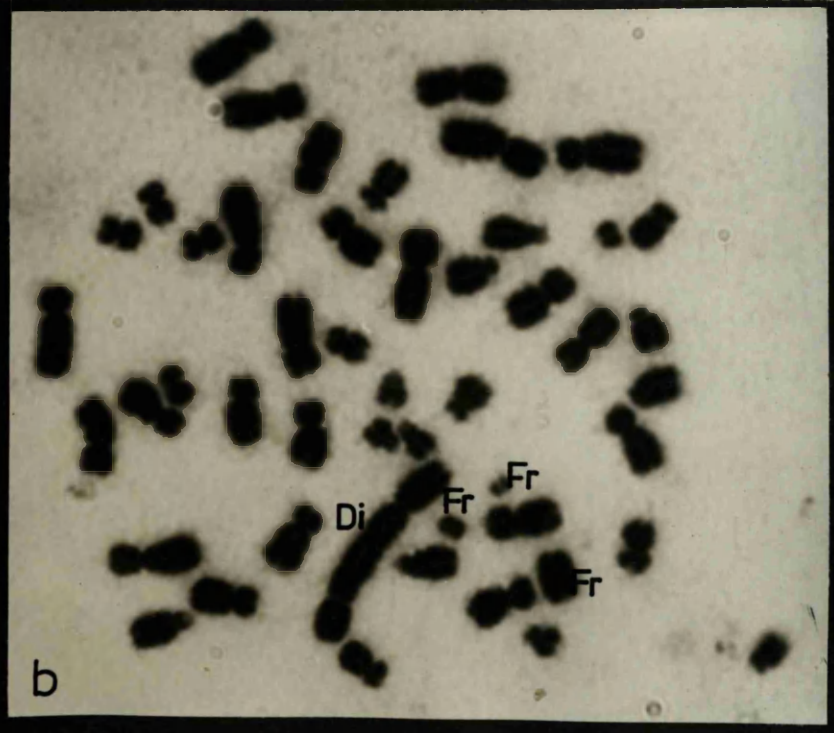


Plate 11. Anomalies at mitotic metaphase.

(a) Multiple quadriradials. Secondary constriction regions are possibly involved in some of the "points of crossing over".

(b) A dicentric and fragments. The case, DH710125, had otherwise normal chromosomes.

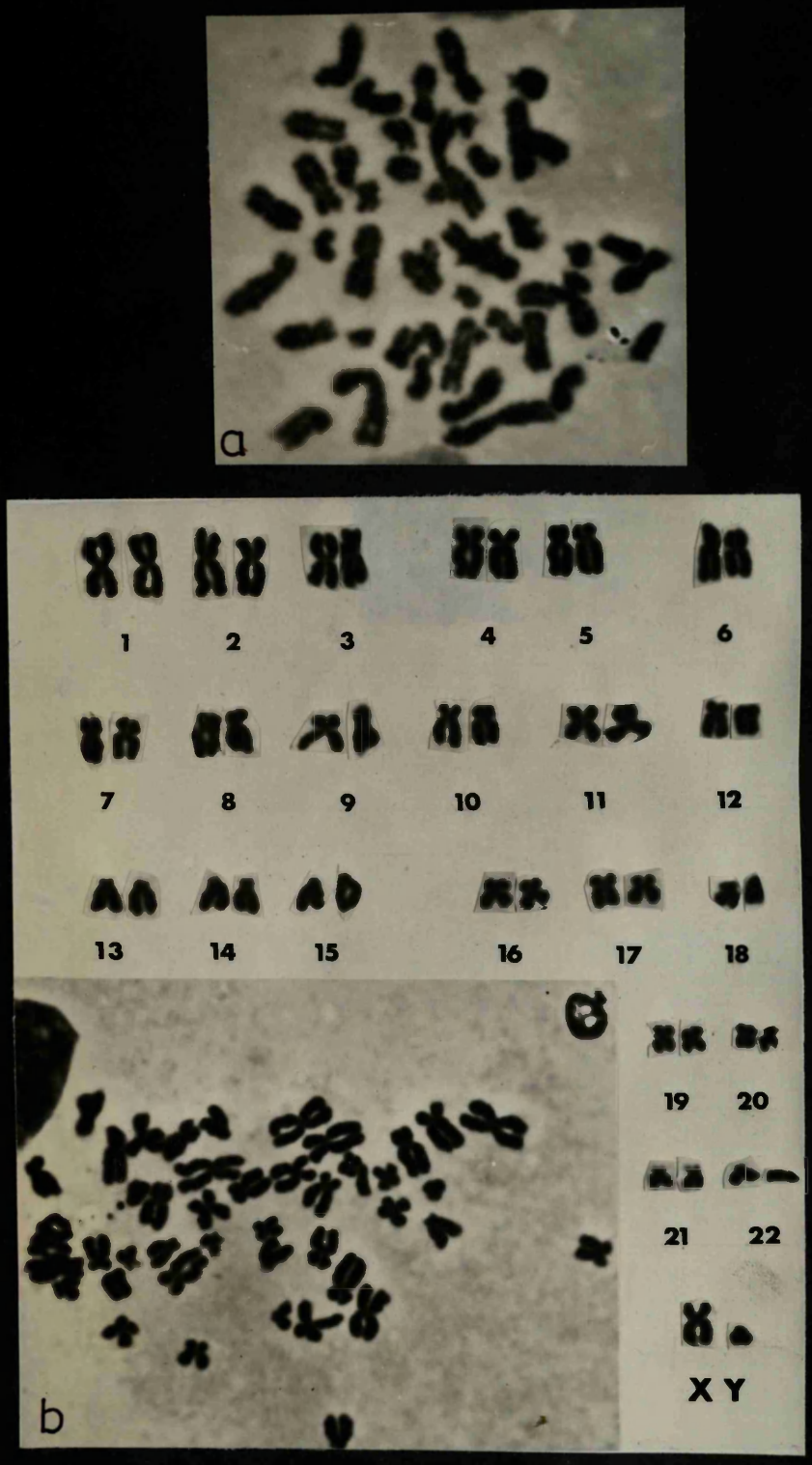
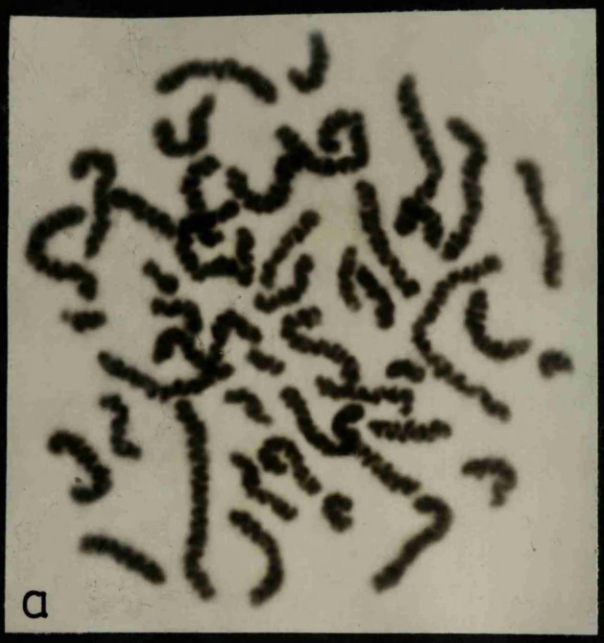


Plate 12. Testis tissue metaphases.

(a) From H0690328 who showed a complete absence of germ cells in testis sections. The spread was therefore considered to be a somatic mitotic metaphase.

(b) x 2,000. From a case with germ cells. This could also be a somatic metaphase rather than a spermatogonial metaphase.



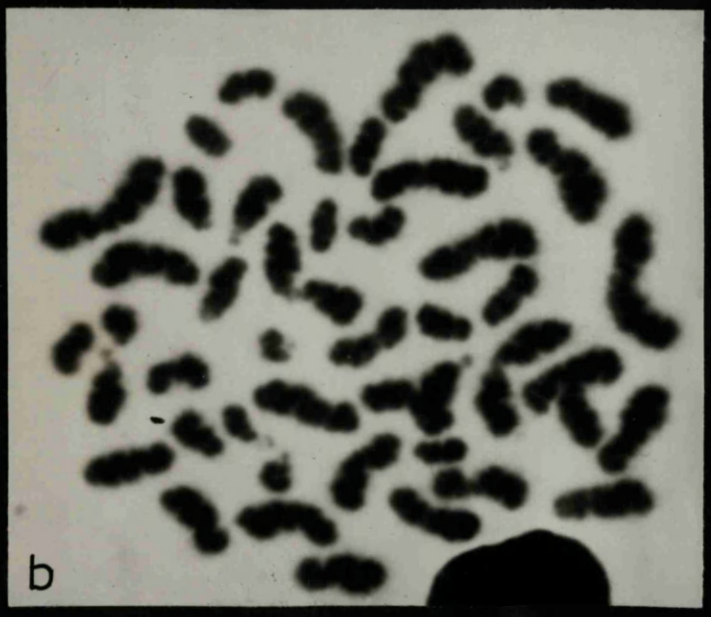


Plate 13. Spermatogonial metaphases showing characteristic coiling. The two chromatids of each chromosome are obscured.

(a) Shows chromosomes less contracted.

(b) Shows satellites on the acrocentric chromosomes.

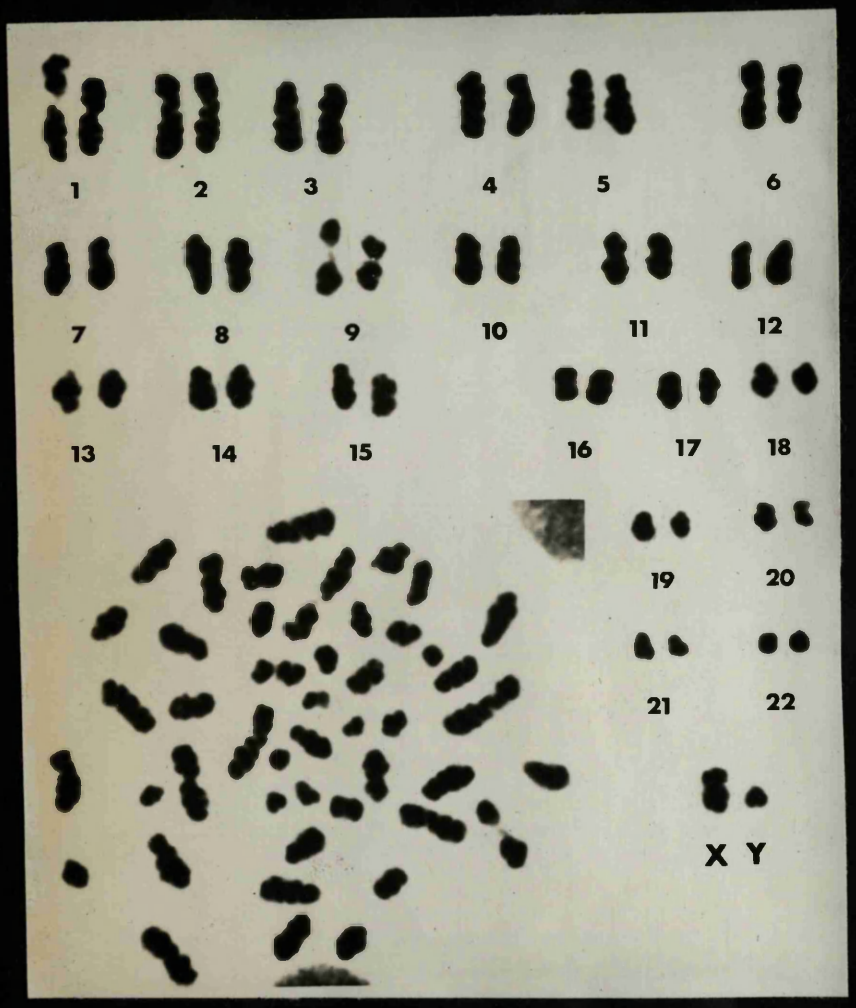
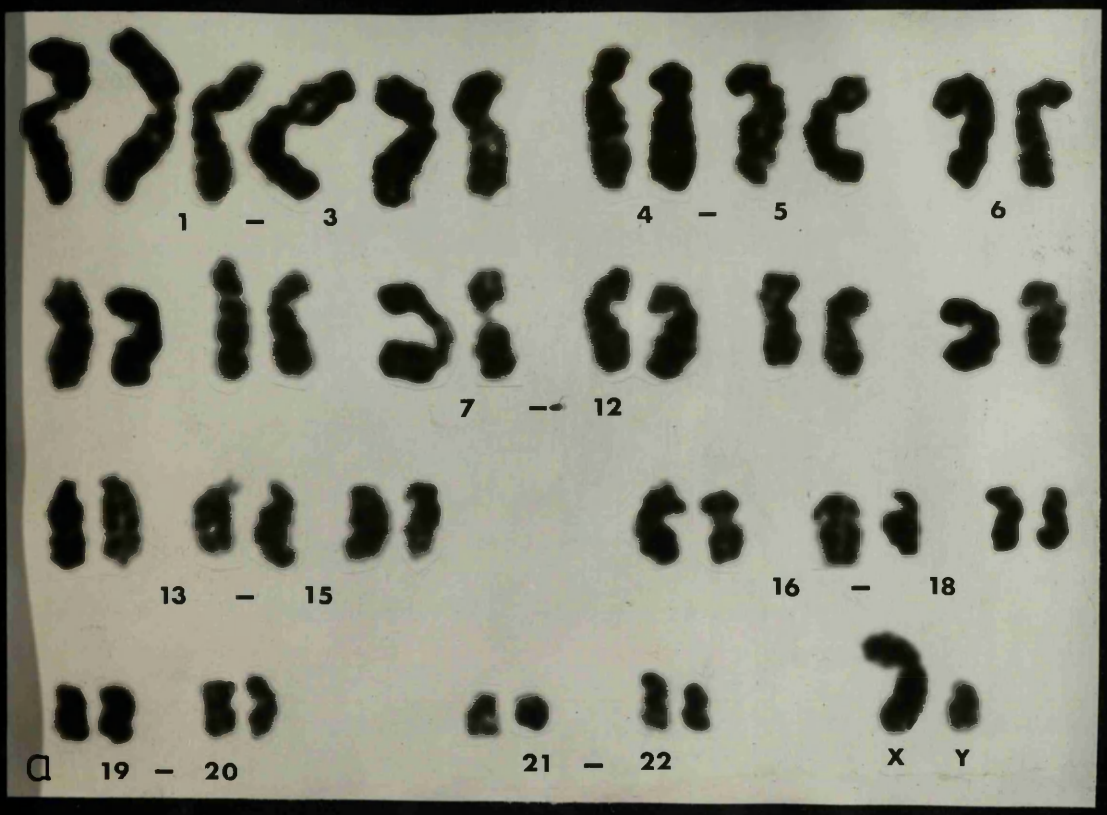
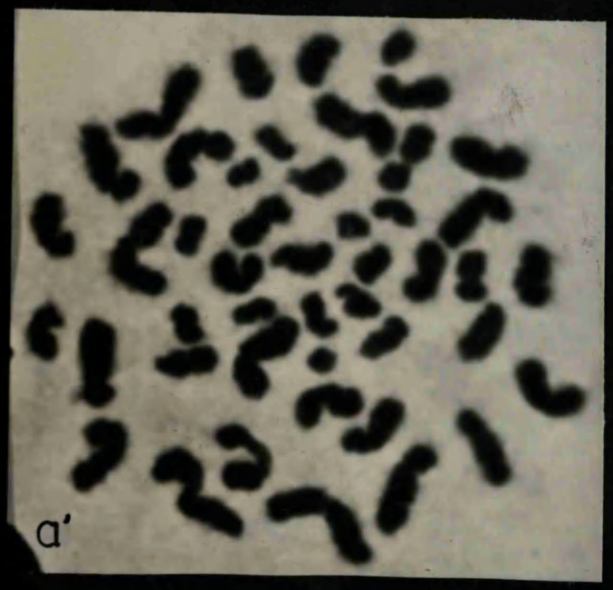


Plate 14. Karyotyped spermatogonial metaphase, x 2,000. The paracentric secondary constriction in one member of chromosome pairs 1 and 9 is elongated, exaggerating the overall length.





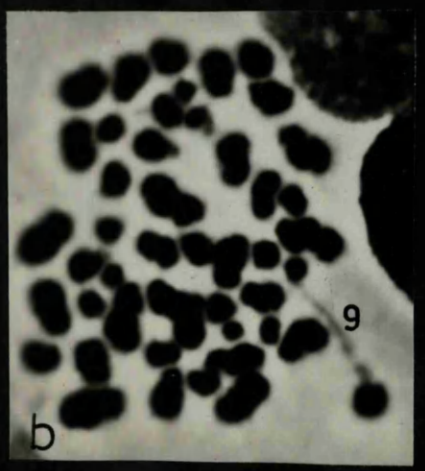


Plate 15. Spermatogonial metaphases from CMC690369 with heteromorphic number 9 chromosomes (Plate 10a).

(a) x 3,250. Karyotyped. Heteromorphism is reflected in the elongation of a paracentric region in a chromosome.

(b) At a later stage. The chromosomes are more contracted and the length of the paracentric region is even more exaggerated.

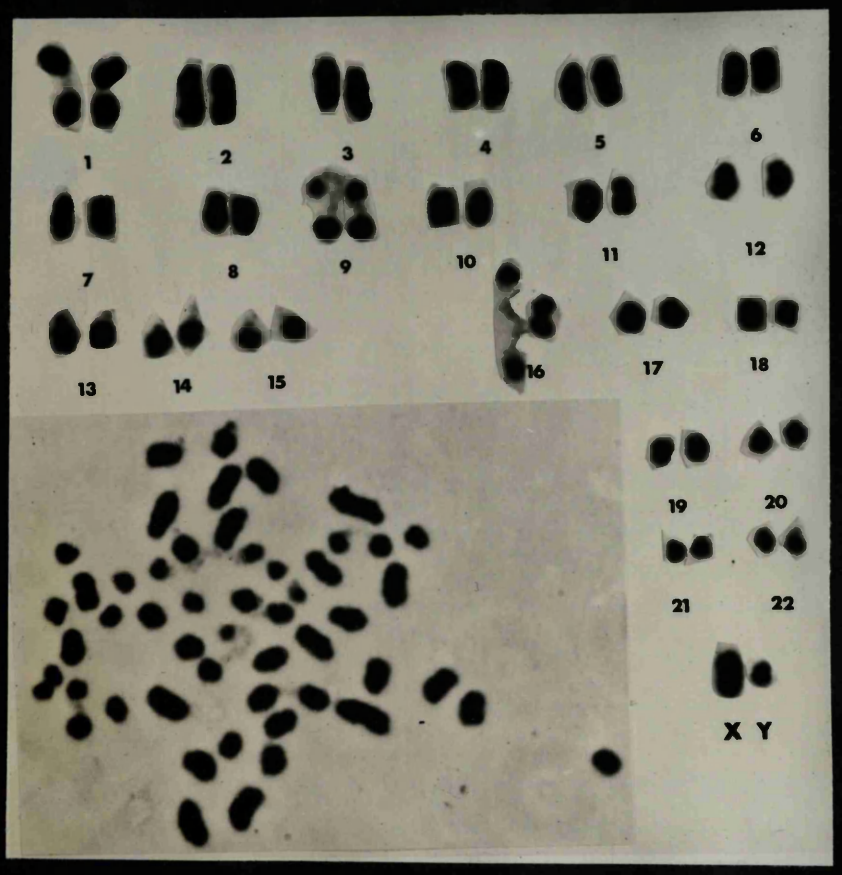
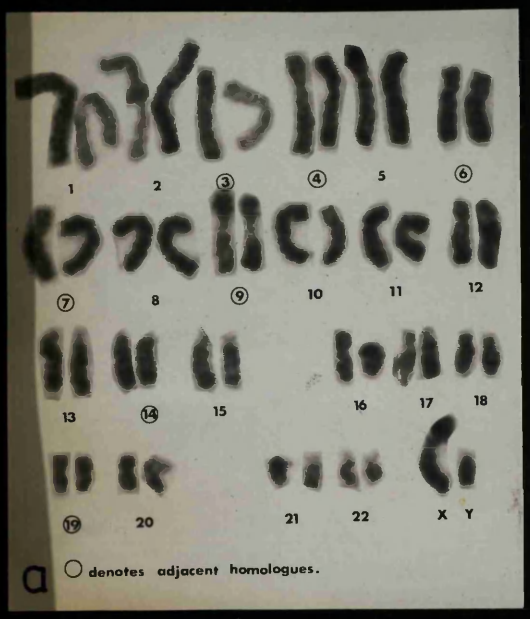
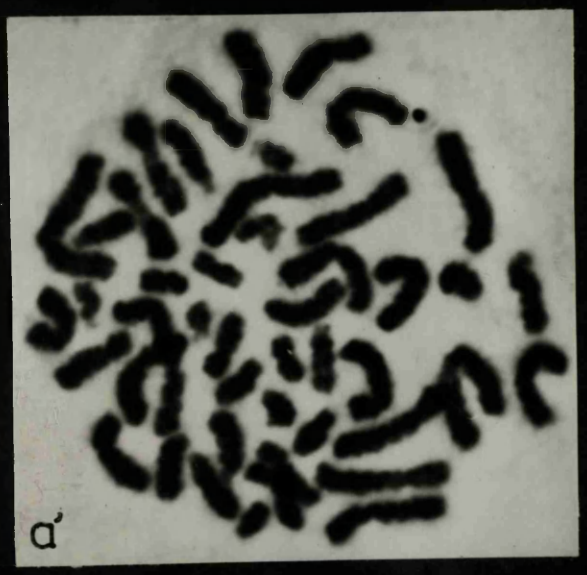


Plate 16. Late spermatogonial metaphase, x 1,500, from HK710139 with extremely heteromorphic number 16 chromosomes and with slightly heteromorphic number 9 chromosomes (Plate 10b). Lengths of the paracentric constriction regions at this stage are grossly exaggerated.





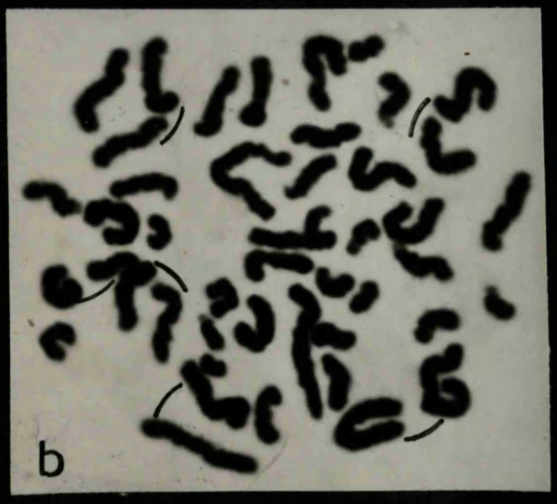
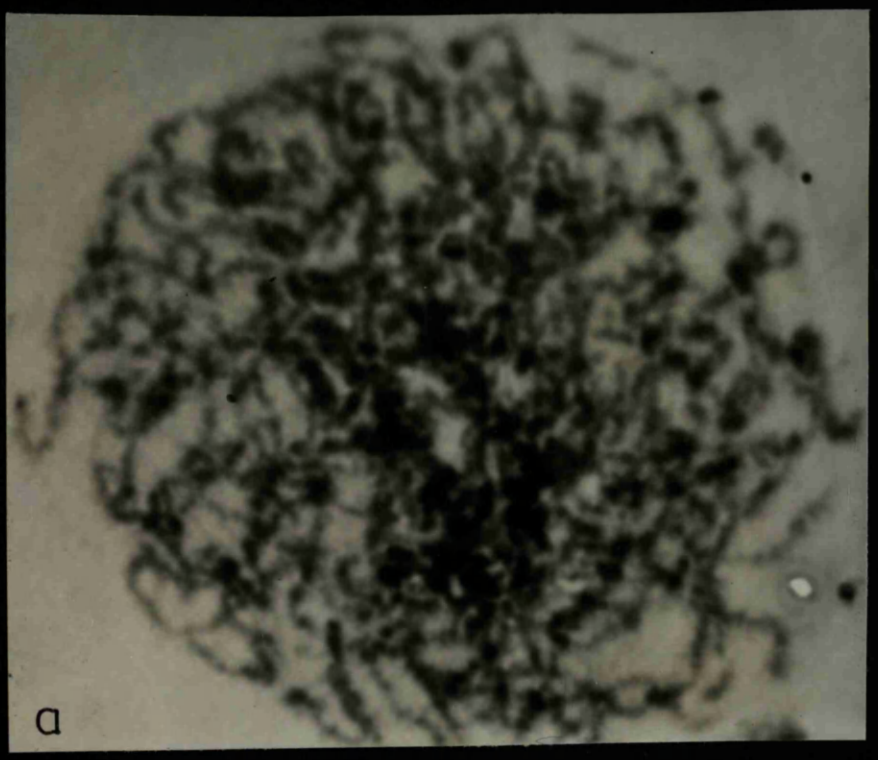


Plate 17. Exceptional spermatogonial metaphases showing possible evidence of premeiotic pairing in homologous chromosomes.

(a) x 1,500. Karyotyped. Several chromosomes, which may be homologues, are adjacent. See, for example, the number 9 chromosomes.

(b) Chromosomes are difficult to identify, but the affinity of those of equal size is clearly shown.



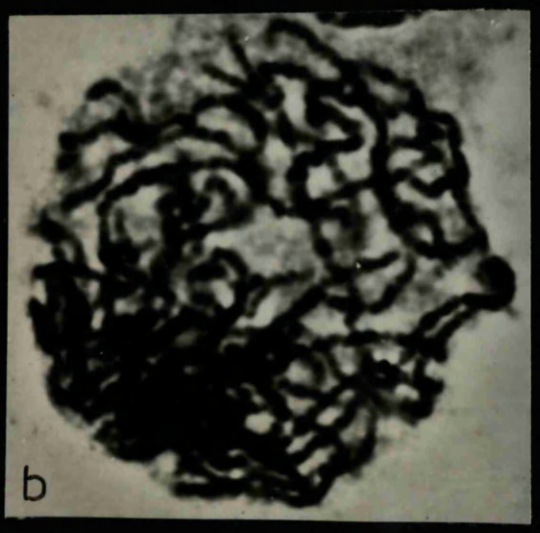


Plate 18. Leptotene. The diploid number of chromosomes appears; condensation then proceeds from the telomeres which are to one side.

(a) Early leptotene.

(b) Late leptotene.

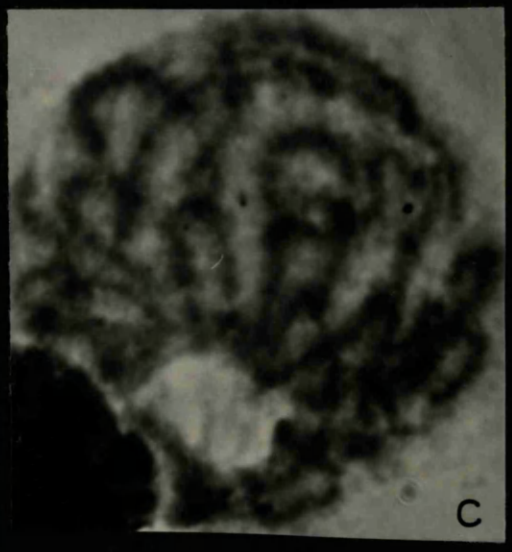
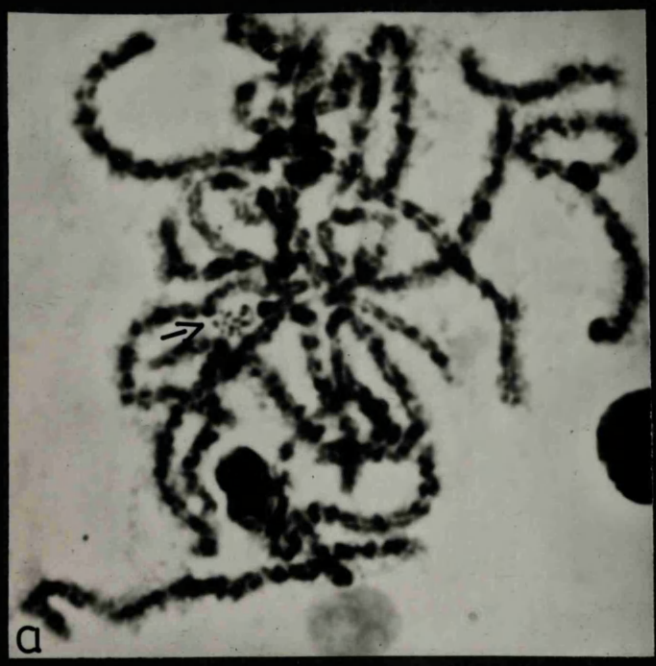


Plate 19. "Bouquet stage". Homologous chromosomes pair. There is no obvious sex vesicle; sex chromosomes cannot be distinguished. Centromere regions are only slightly more heteropycnotic.

(a) Shows the pairing is from both telomeres backwards along the homologous chromosomes.

(b) Shows pairing in homologues is almost complete.

(c) Shows that bouquet telomere orientation is towards an area that does not stain.



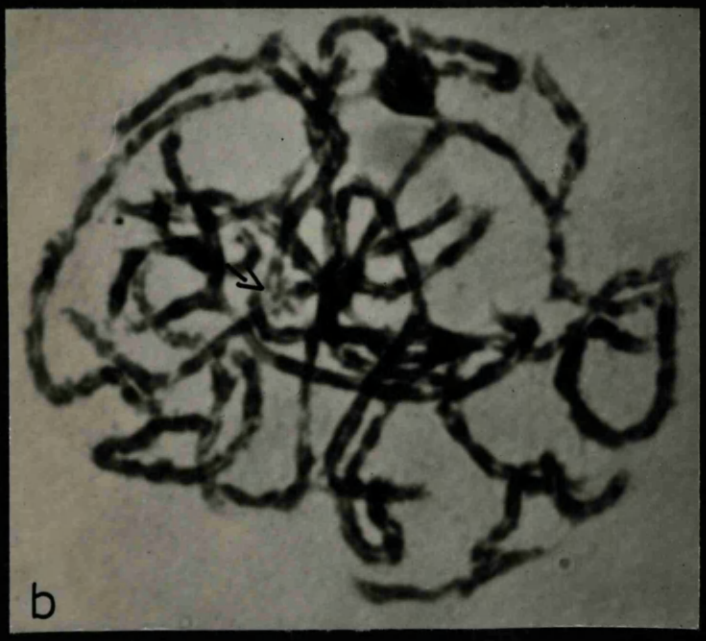
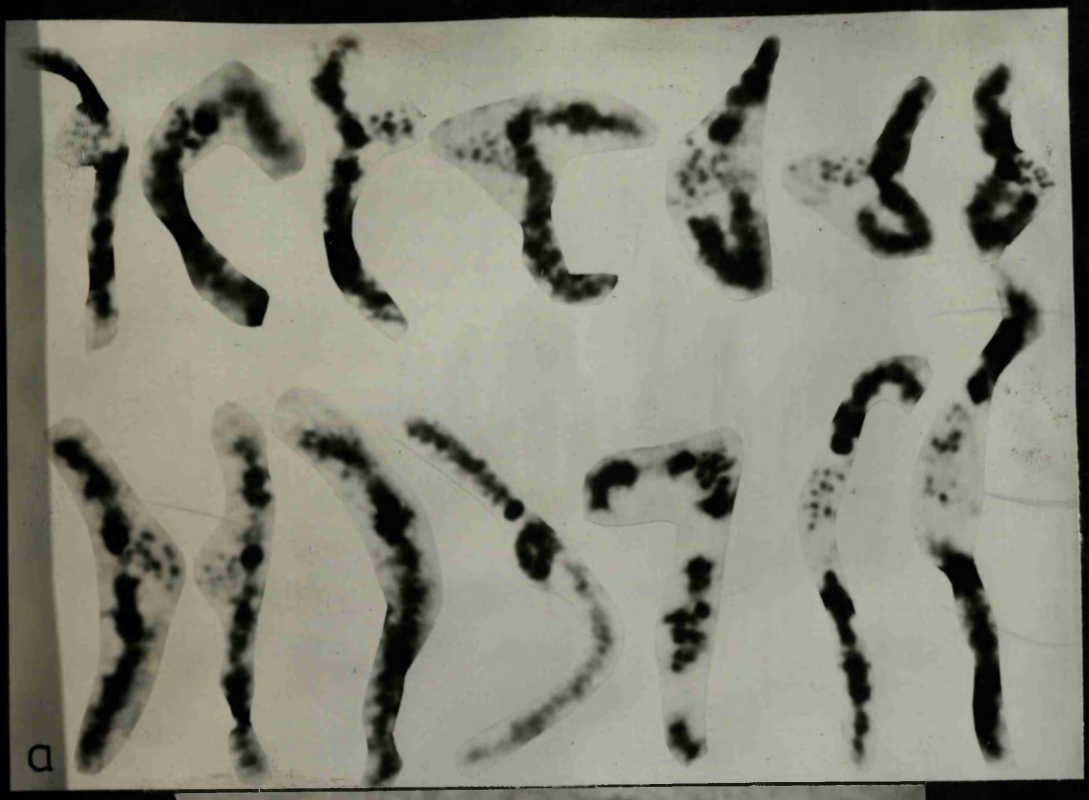


Plate 20. Cells at a stage of pachytene where chromomeres are distinct. Homologous chromomeres are usually, but not always, equal in size. The tiny chromomeres typical of the paracentric region of chromosome 9 (see Plate 21a) are shown.



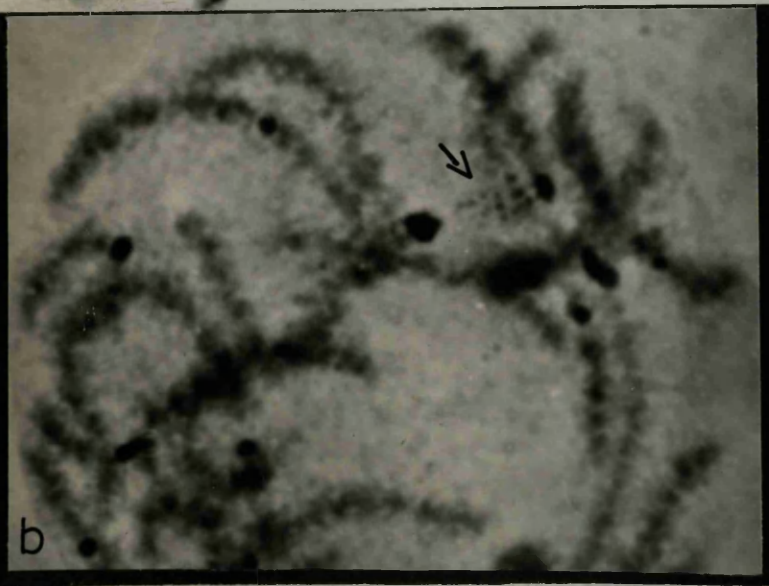


Plate 21. Chromosome 9 at pachytene.

(a) x, 3,000. A range of typical configurations. Below the heteropycnotic centromere region, the paracentric constriction region exhibits a shower of chromomeres.
Upper row: The bivalent at the extreme right shows the chromomeres in two's and fours.
Lower row: The two bivalents to the right show the long and short arms separated by a gap bridged by four rows of chromomeres.
The middle bivalent is from CM.C700362 known to have extreme mitotic heteromorphism in chromosome 9 (Plate 10a). The paracentric region is enlarged.

(b) Shows the paracentric chromomeres of bivalent 9 showering towards another heteropycnotic region.

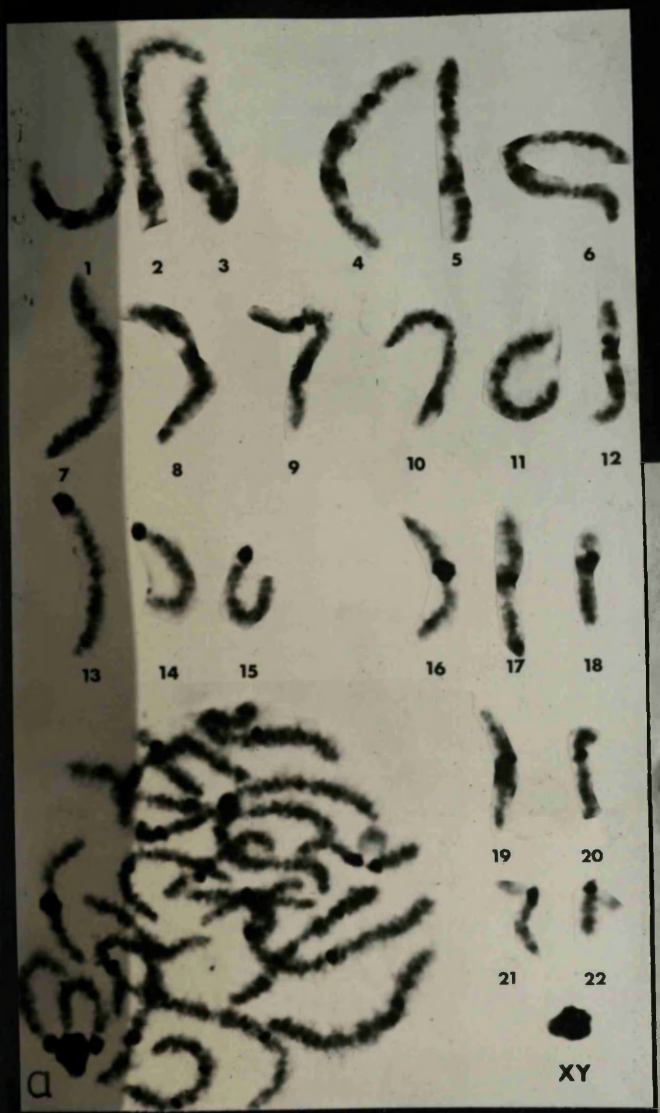
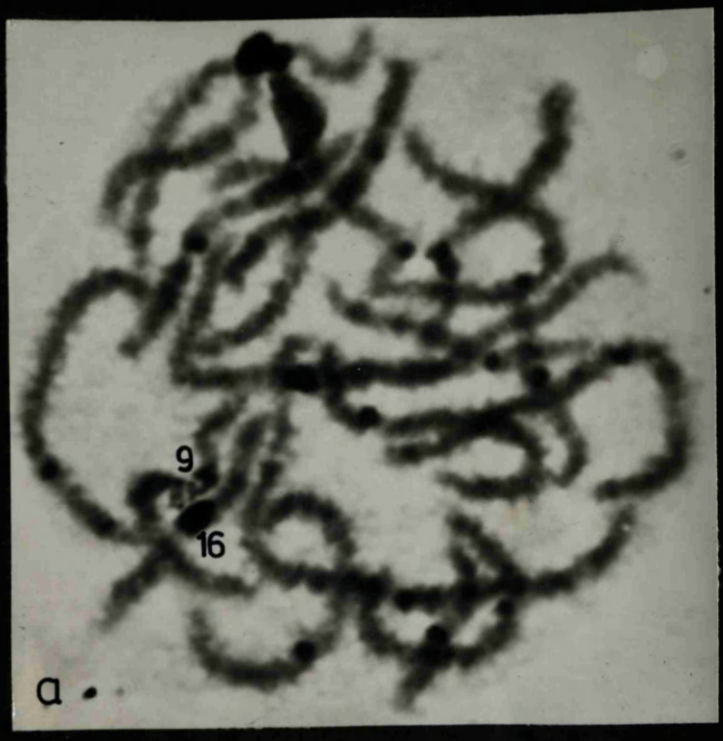


Plate 22. Pachytene cells from HK710139 who had heteromorphic chromosomes 16 (see Plate 10b). Centromeric heterochromatin is greatly enlarged in this bivalent compared with that in cases with moderately heteromorphic and homomorphic number 16 chromosomes (Plates 24 and 25 respectively). The bivalent is in its usual position, which is towards the periphery of the spread.

(a) x 1,500. Karyotyped. Shows chromosomes 14 and 15 associating with the sex vesicle. The telomere of 15 is looped round and also appears to associate. This is possibly a relic of the bouquet. The size differential in the G's is demonstrated. Here they share a nucleolus.



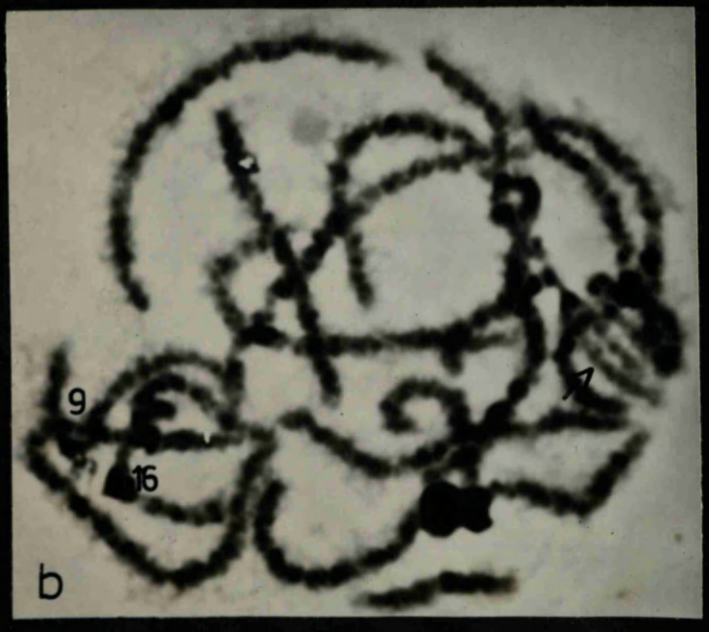


Plate 23. Pachytene cells from HK710139 who had heteromorphic chromosomes 16 (Plate 10b). The enlarged centromere region is in association with the paracentric chromomere region of bivalent 9. This behaviour is common.

(b) Shows a bivalent with homologues partly unpaired. This is probably a result of another bivalent passing between the homologues (see also Plate 36).

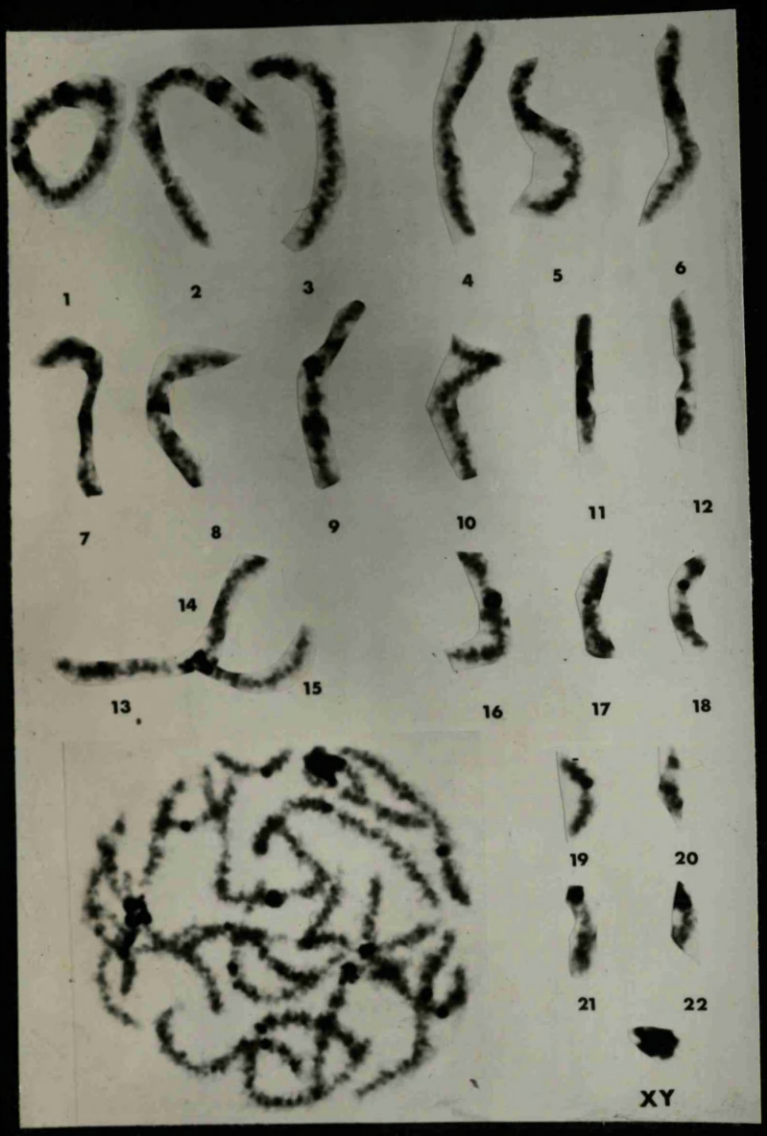


Plate 24. Karyotyped pachytene cell, x 1,500, from a case with mildly heteromorphic chromosomes 16. The centromeric heterochromatin of bivalent 16 is slightly enlarged. Four of the 5 acrocentric chromosomes are in association.

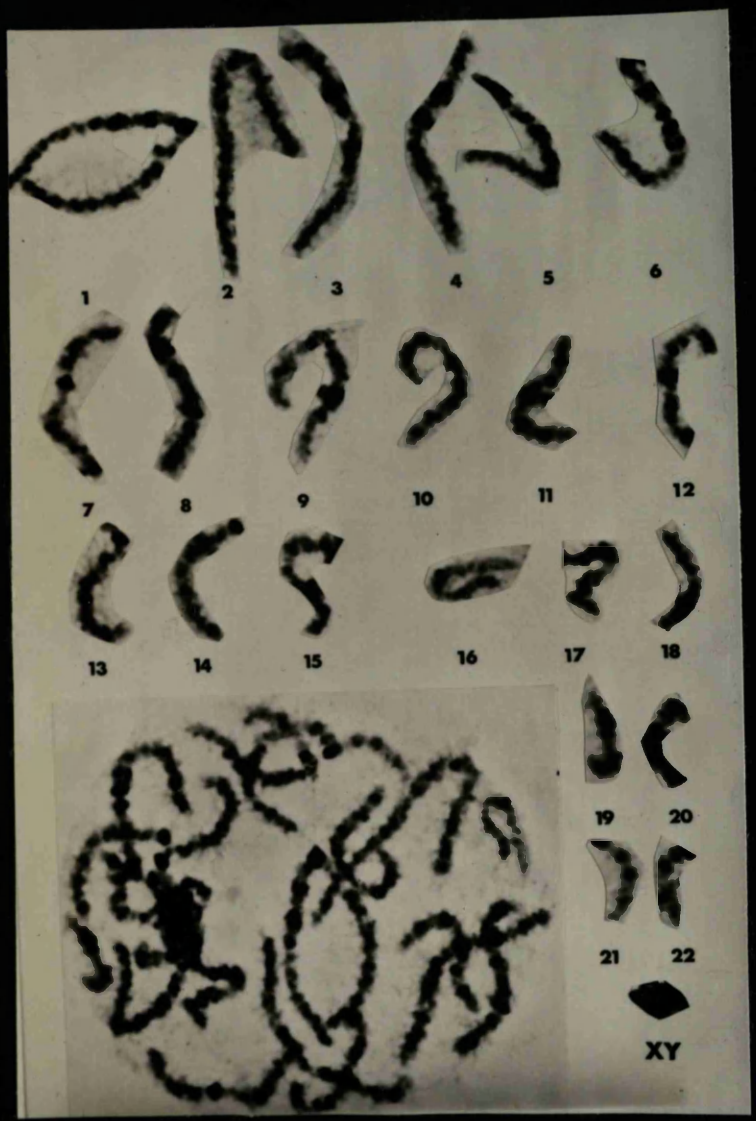
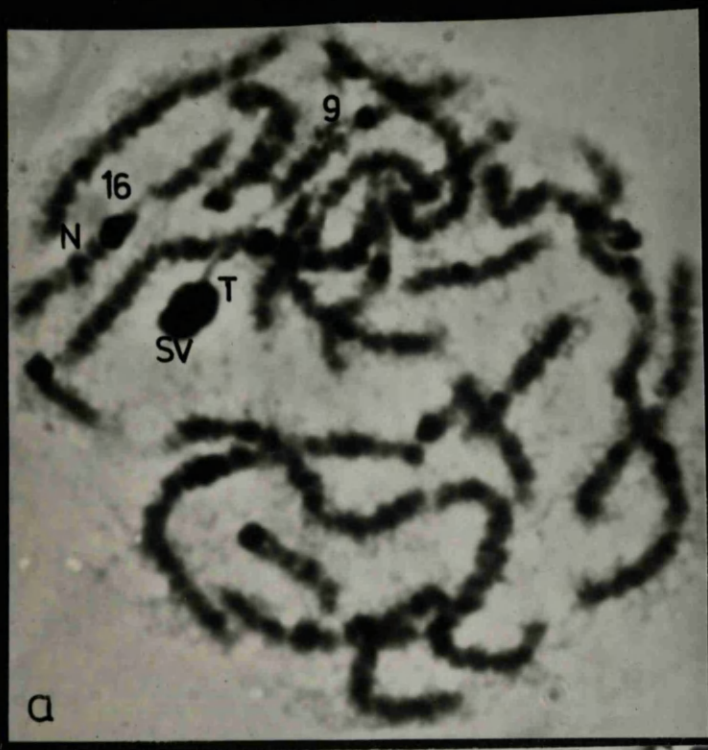


Plate 25. Karyotyped pachytene cell, x 1,500 from a case with homomorphic chromosomes 16. The centromeric heterochromatin of bivalent 16 is of normal size.



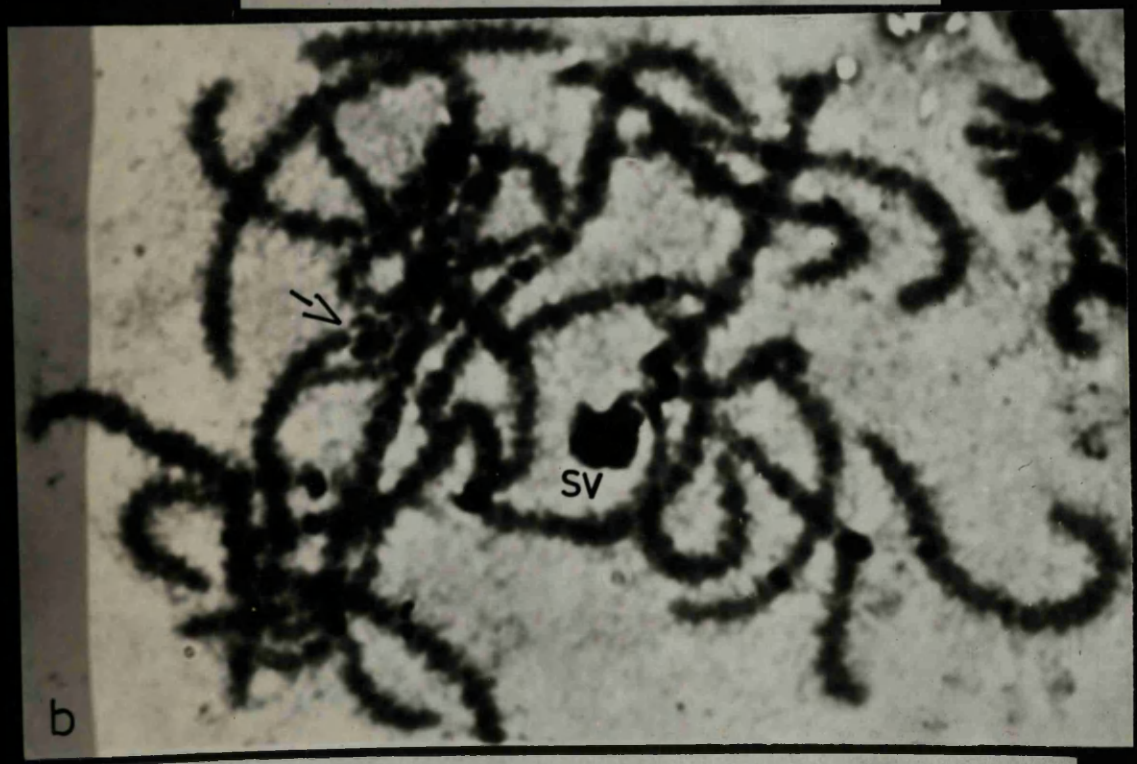
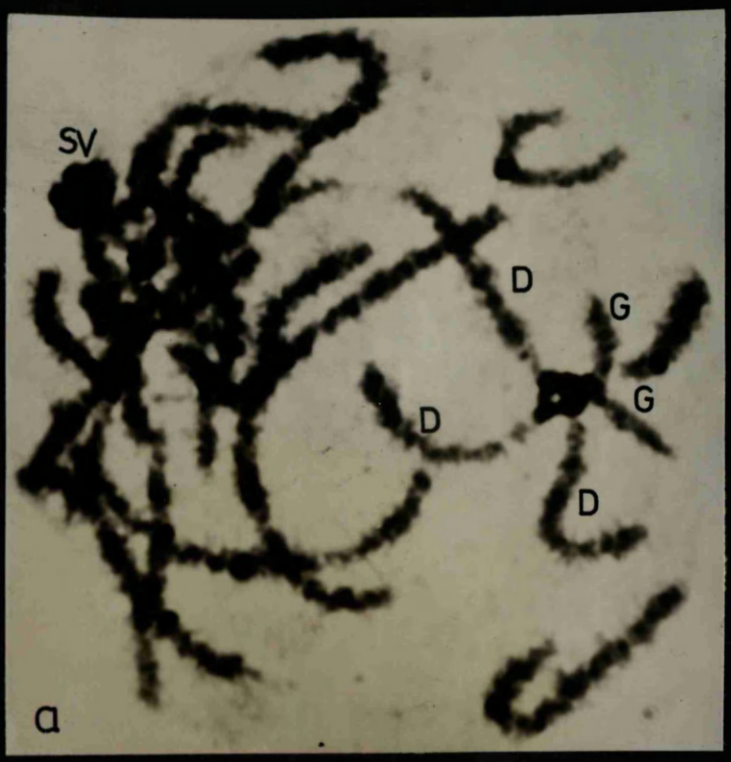


Plate 26. Pachytene cells.

(a) Telomere region of bivalent 9 is extended and is in association with the sex vesicle. The cell is from HK710139 with distinctive centromeric heterochromatin in bivalent 16, which is here associated with a nucleolus.

(b) Acrocentric chromosome centromere regions are in association with the "thinner" end of the pear-shaped sex vesicle. The bunching of the paracentric chromomeres on chromosome 9 is also shown.



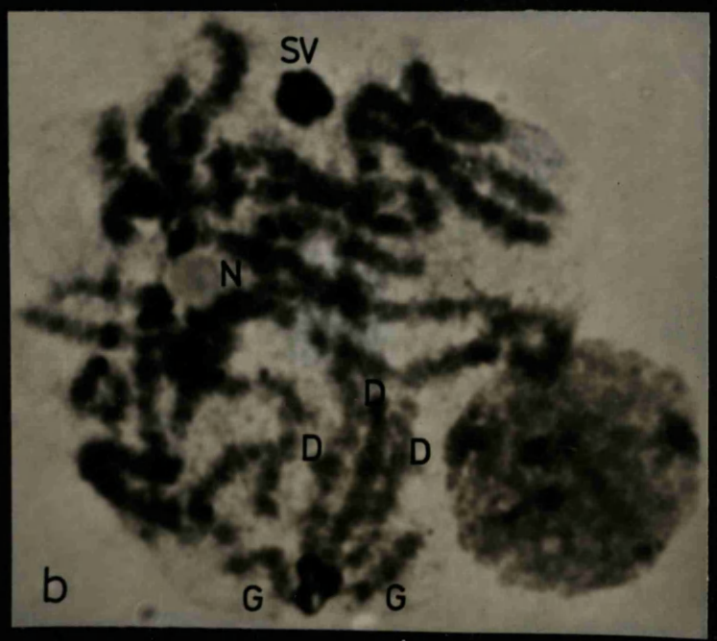


Plate 27. Pachytene cells showing all 5 acrocentric chromosomes in "satellite association". The sex vesicle is not involved.

(b) A nucleolus is in association with regions of chromosomes which include the paracentric chromomeres of 9.

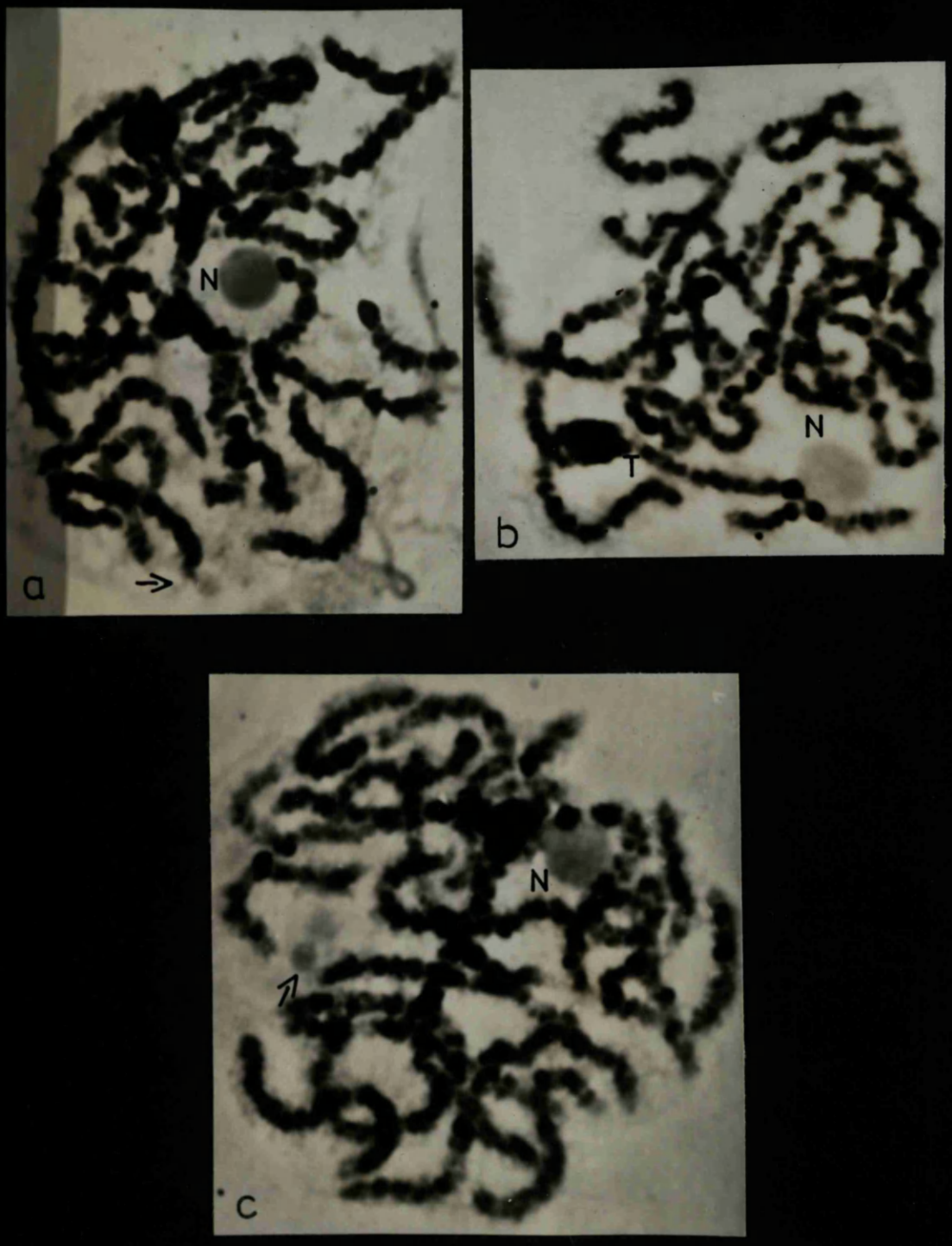
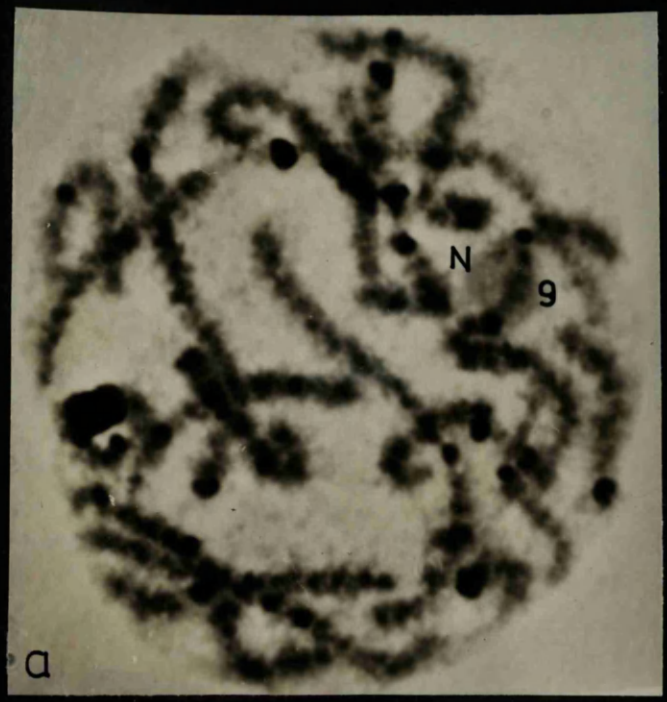


Plate 28. Centromeric heterochromatin of the acrocentric chromosomes associating with nucleoli.

(a) Other bodies are present which stain as nucleoli. Some are at the telomeres of bivalents.

(b) Long arm telomere of a D bivalent is in association with the sex vesicle.



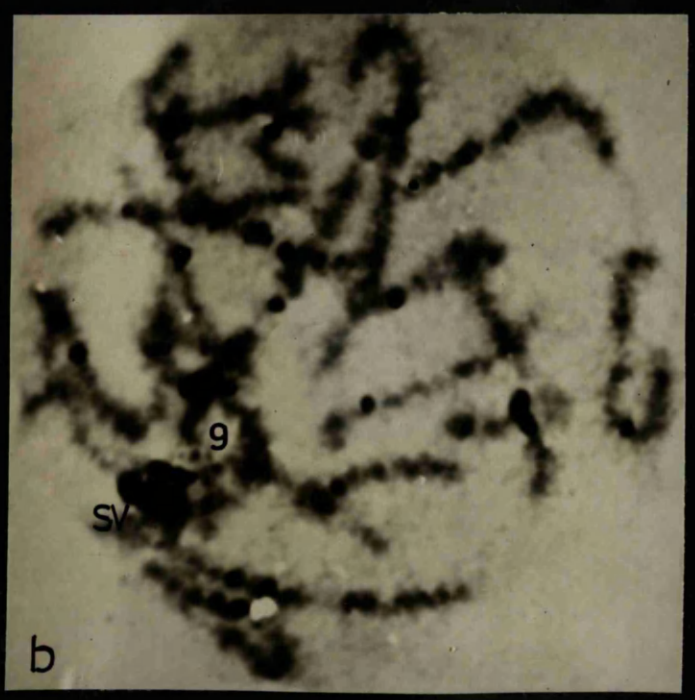
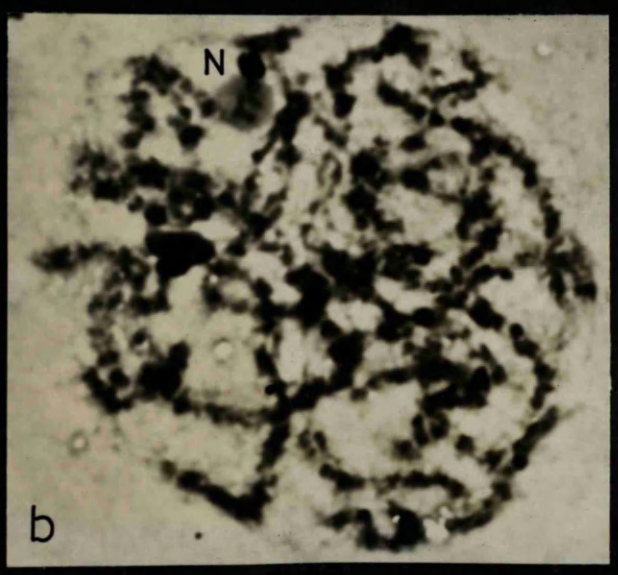


Plate 29. The paracentric region of chromosome 9 at pachytene.
(a) Chromomeres "showered" through a nucleolus.
(b) In association with the sex vesicle.



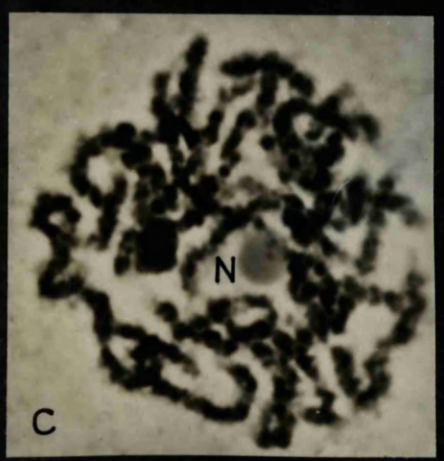


Plate 30. Heteropycnotic bodies within nucleoli.
(a) "Satellites" of an acrocentric chromosome.

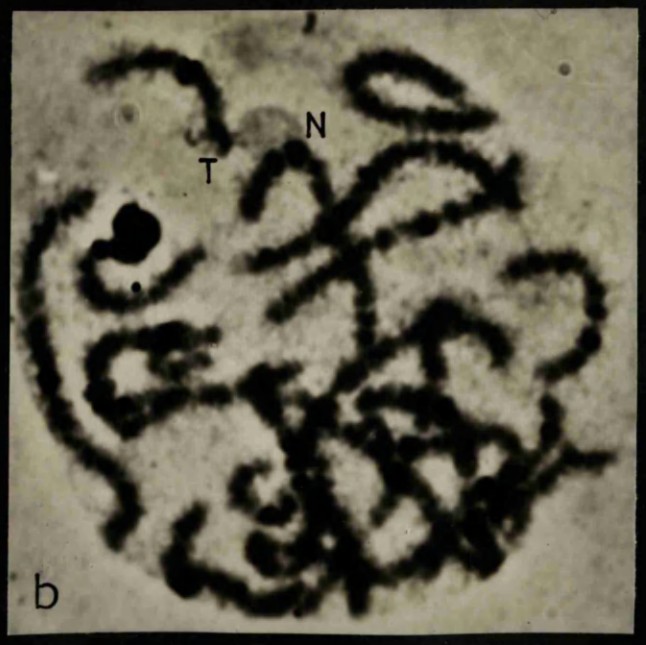
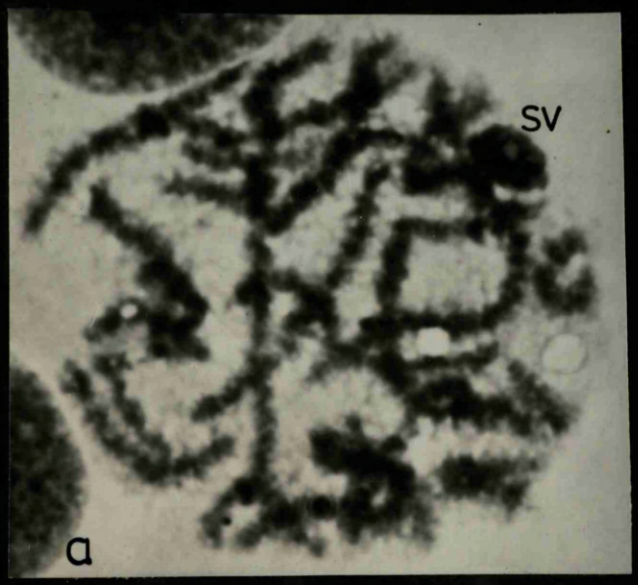


Plate 31. Telomere association with nucleoli at pachytene.

(a) Nucleolus is associated with part of the long arm of a D bivalent. Homologues are separated at that point.

(b) The associating telomere is from bivalent 16.



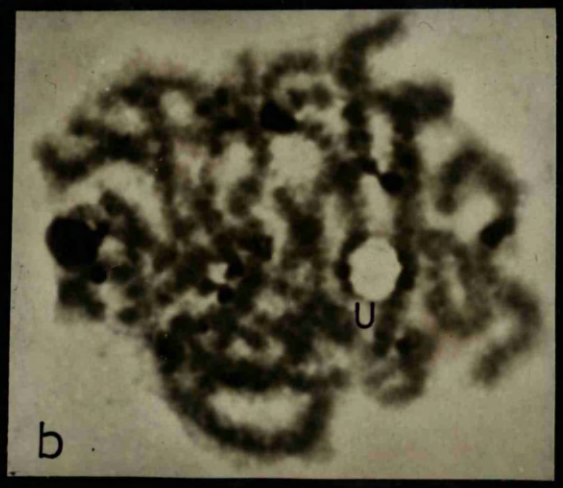


Plate 32. Cells at pachytene.

(a) Sex chromosomes are shown curled in the sex vesicle.

(b) Unstained patches are associated with centromere regions.

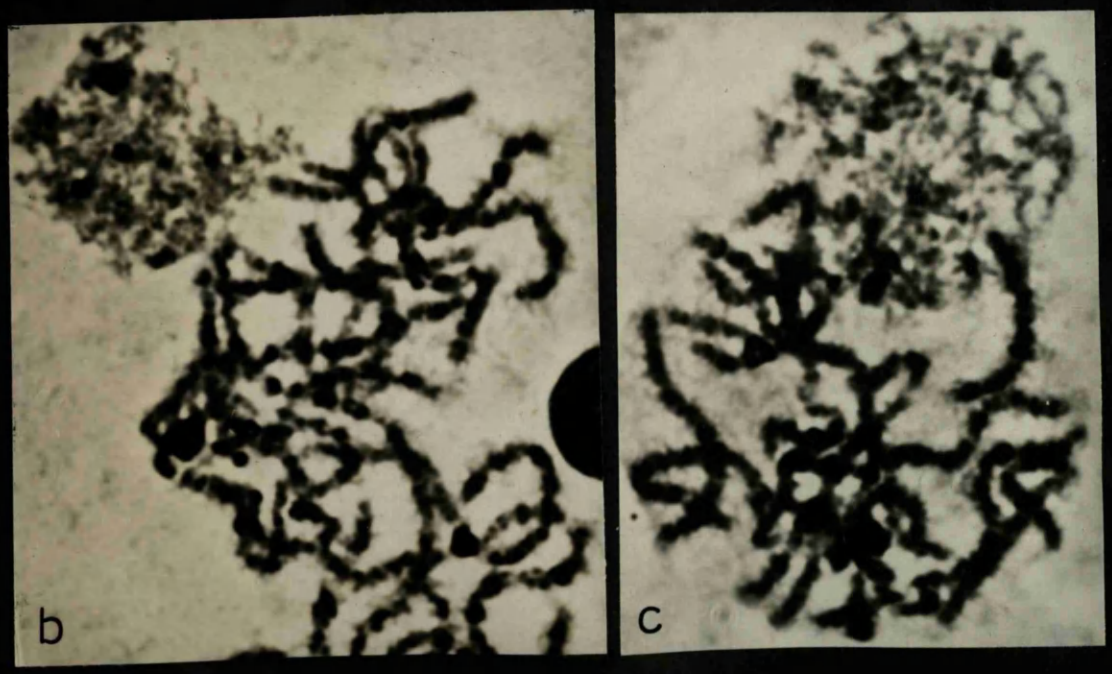
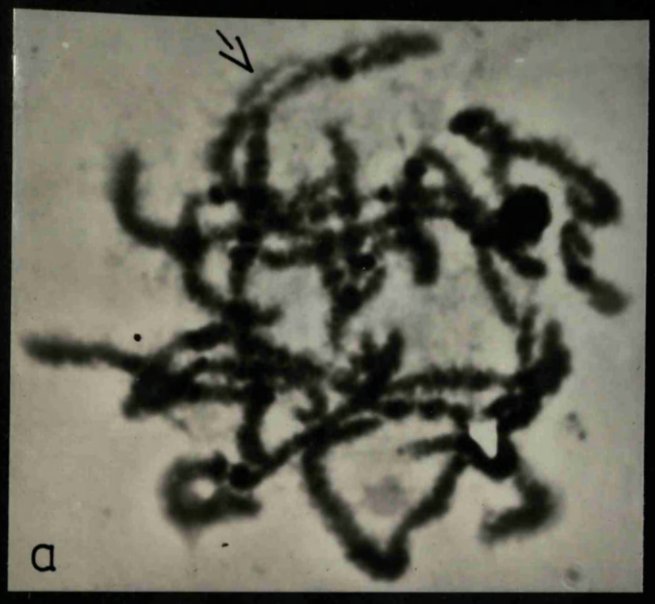


Plate 33. Close association between pachytene cells and other nuclei towards which telomeres of some bivalents are orientated.



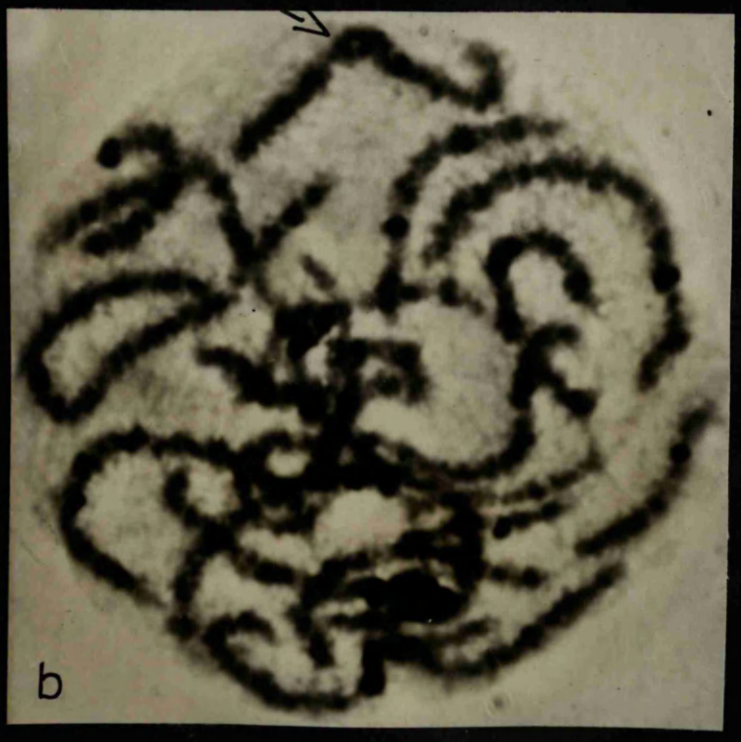
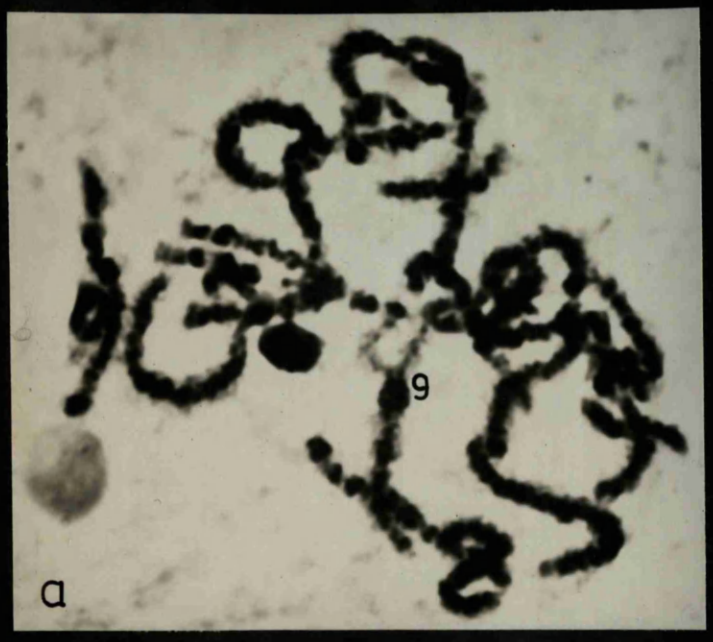


Plate 34. Abnormal pairing at pachytene.

(a) Intermittent pairing in a bivalent.

(b) Small region of a homologue unpaired. This was not observed in other cells of the same individual and so a small inversion is unlikely.



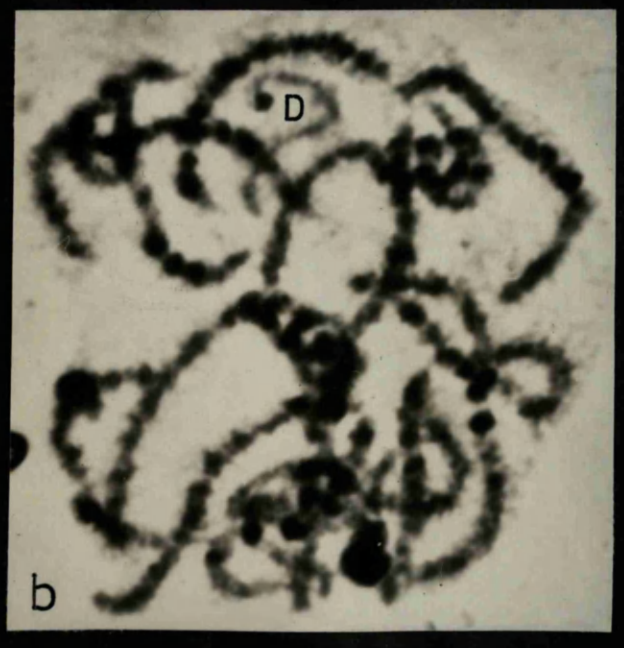
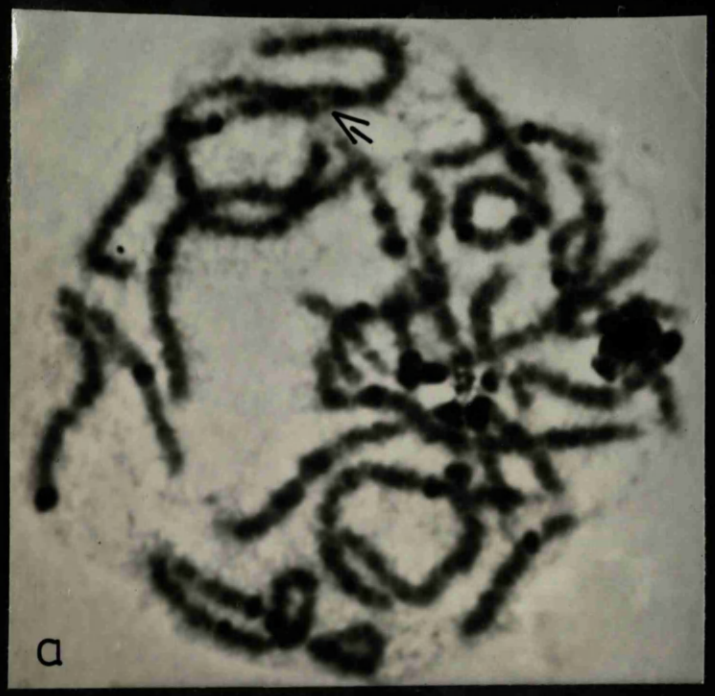


Plate 35. Abnormal pairing at pachytene.
(a) Short arms of chromosome 9 unpaired.
(b) An entire D chromosome unpaired.



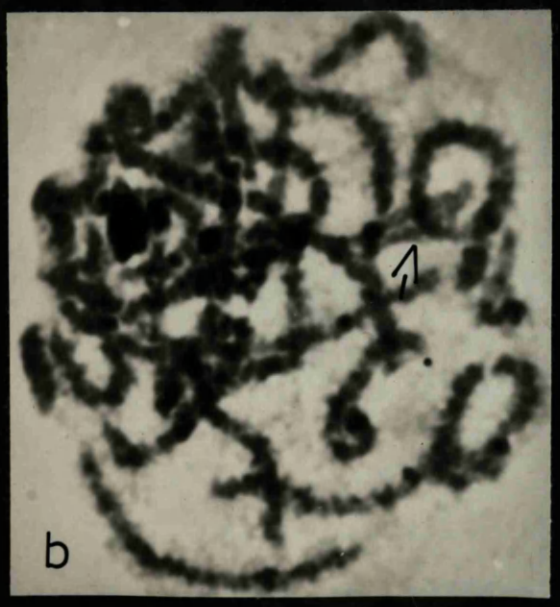
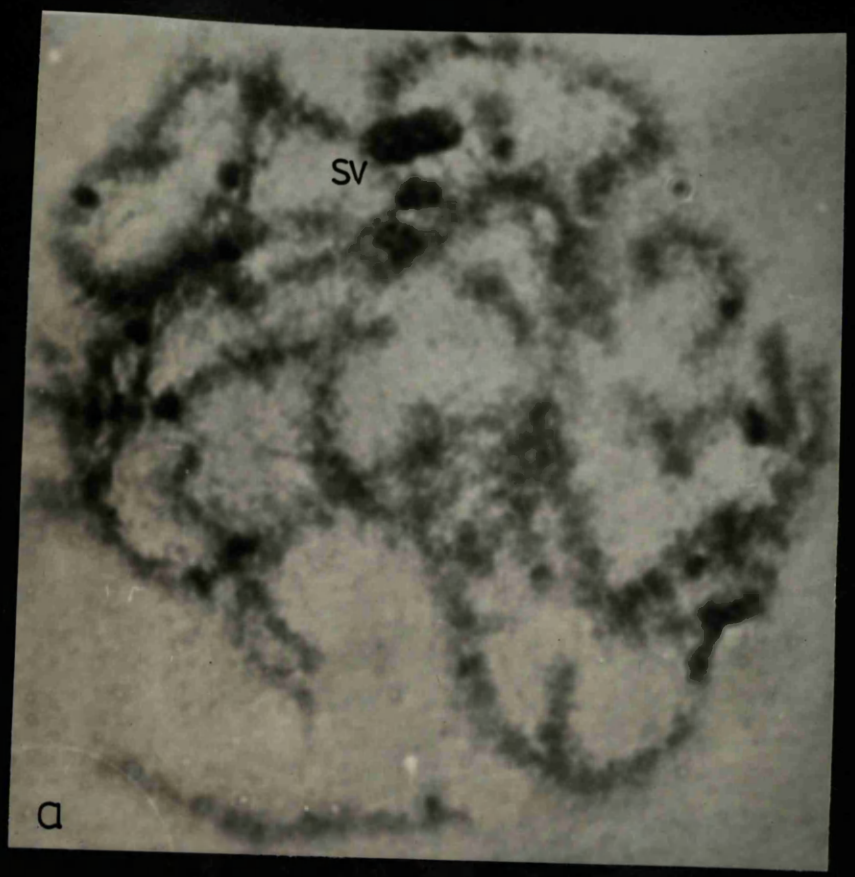
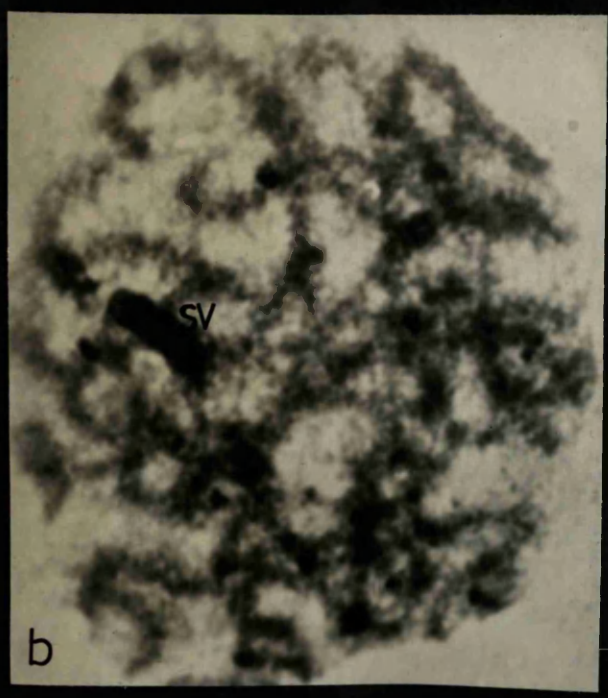


Plate 36. Pachytene cells showing one bivalent passing between the homologues of another.





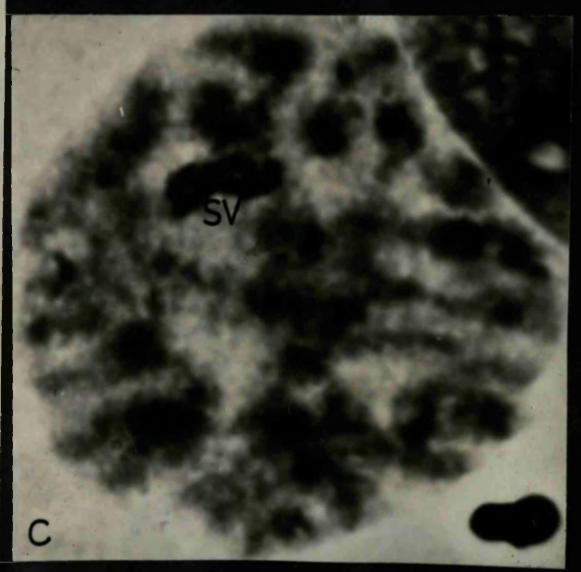


Plate 37. Cells entering the diffuse stage. The odd configuration in the sex vesicle is probably caused by a twisting of the sex chromosome loop.

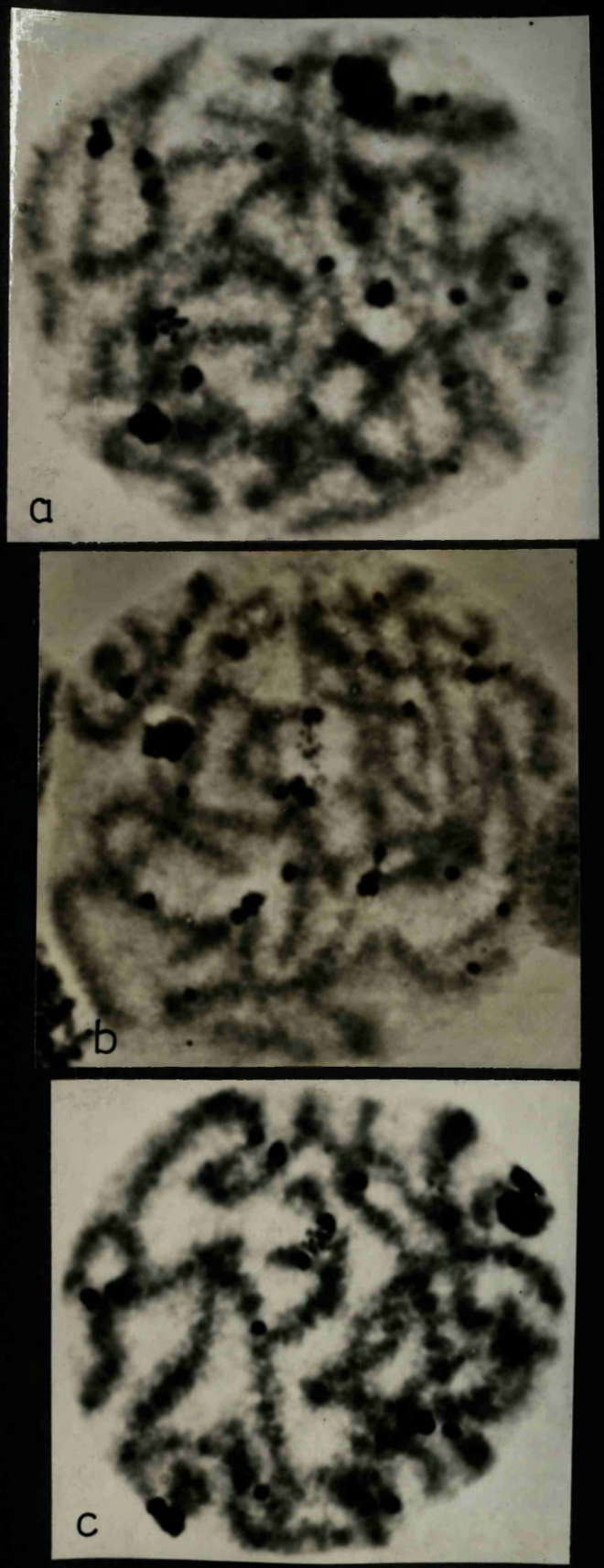
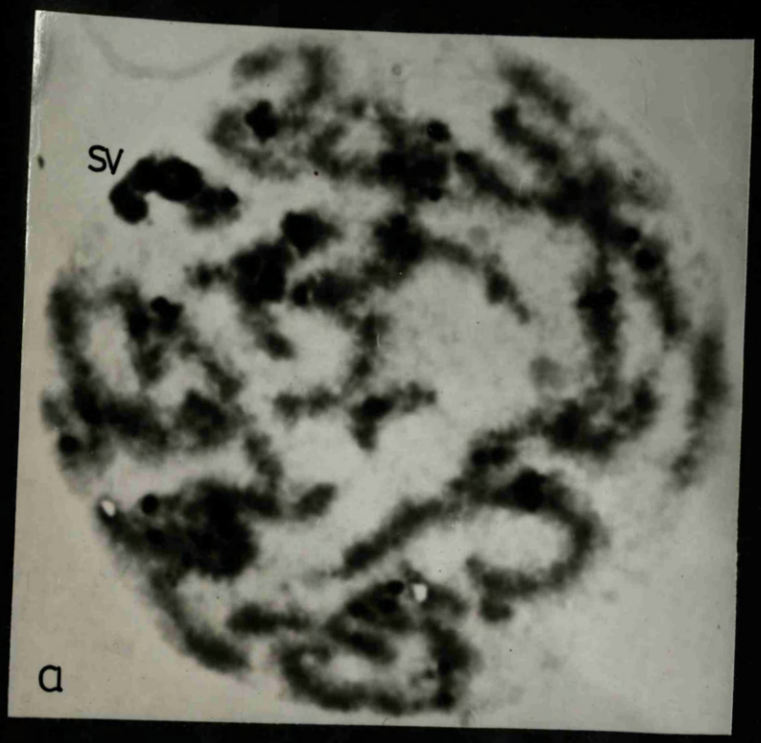


Plate 38. Cells entering the diffuse stage. Centromere regions, sex vesicle and the paracentric chromomeres of number 9 remain heteropycnotic.



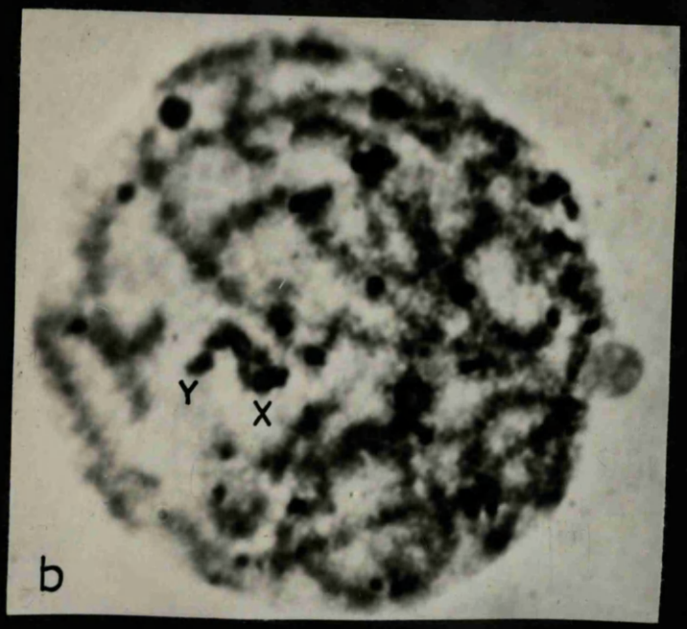
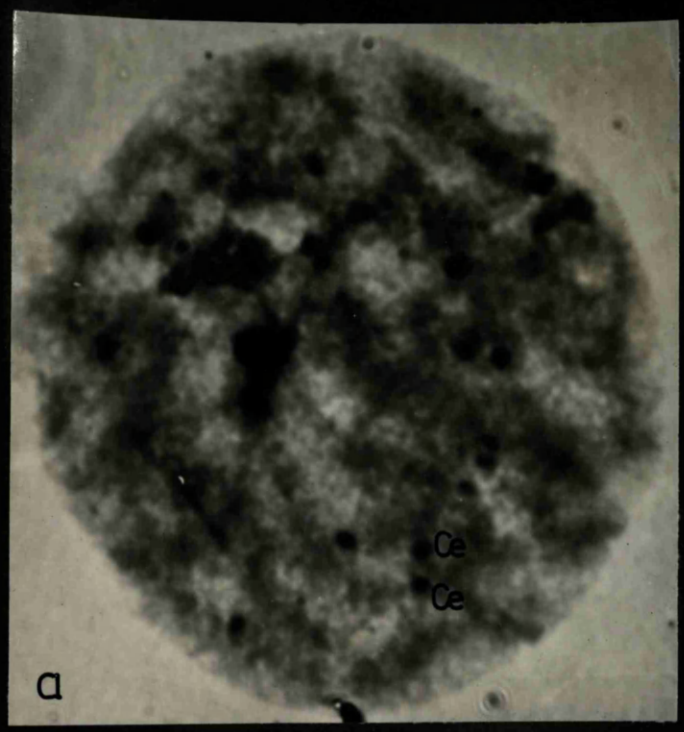


Plate 39. Diffuse stage. Some homologous centromeres are separate and the sex bivalent is beginning to uncurl from the looped form, that was characteristic of pachytene.



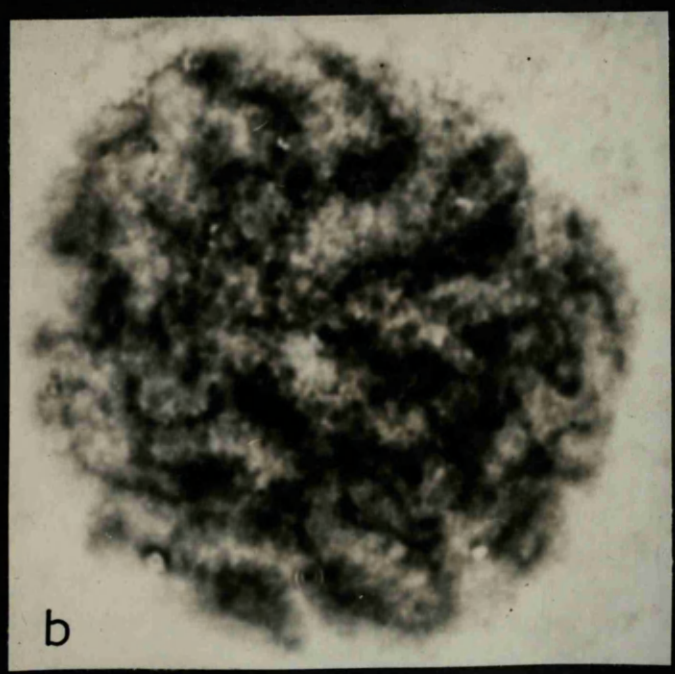
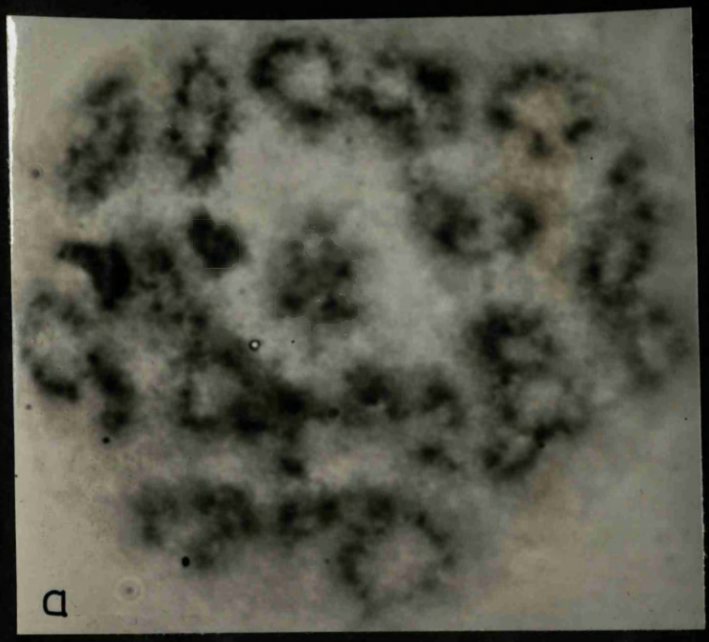


Plate 40. Diffuse stage.

(a) Chromosome shape is lost and some homologous centromeres are separated by distances which vary in size.

(b) Pre-diplotene diffuse stage. Chromosomes have started to condense.



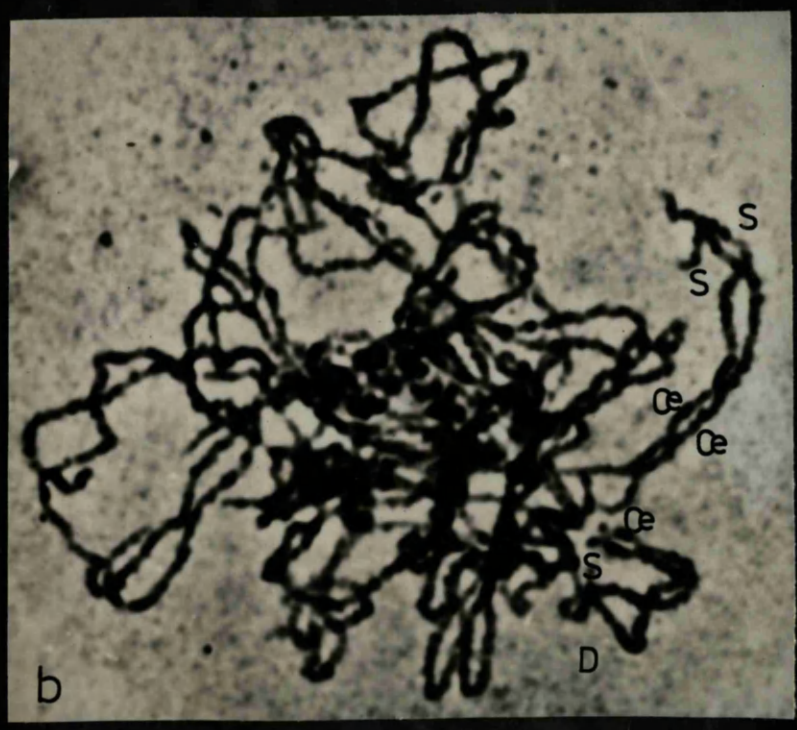
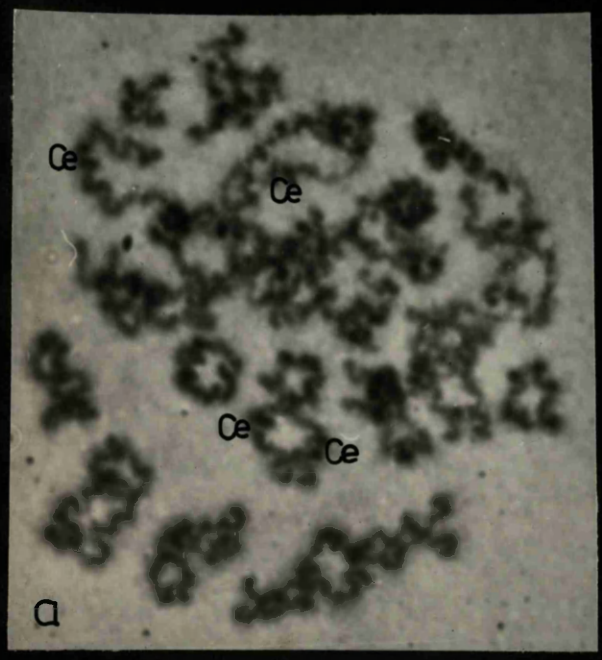


Plate 41. Diplotene.

(a) Early diplotene. Chromosomes are very diffuse.

(b) The bivalent to the right is probably a number 2 and below this a D. Centromere regions are shown as double heteropycnotic bodies in each homologue. In bivalent 2, there are fine connections between the homologous centromere regions. Secondary constriction regions are euchromatic. Places where homologues cross do not necessarily represent chiasmata since bivalents are probably twisted. A cross is shown in the secondary constriction region of the D. This is probably a true chiasma since "rotation" of arms would have occurred by this stage.



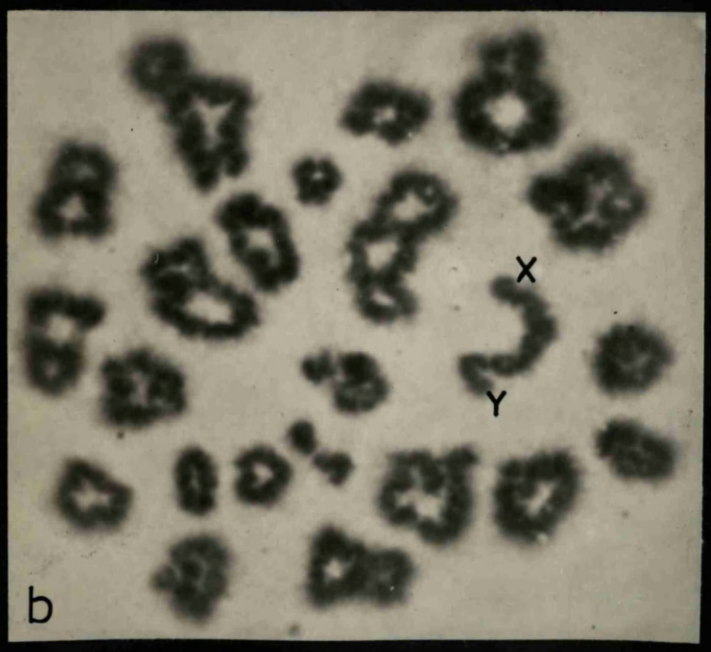
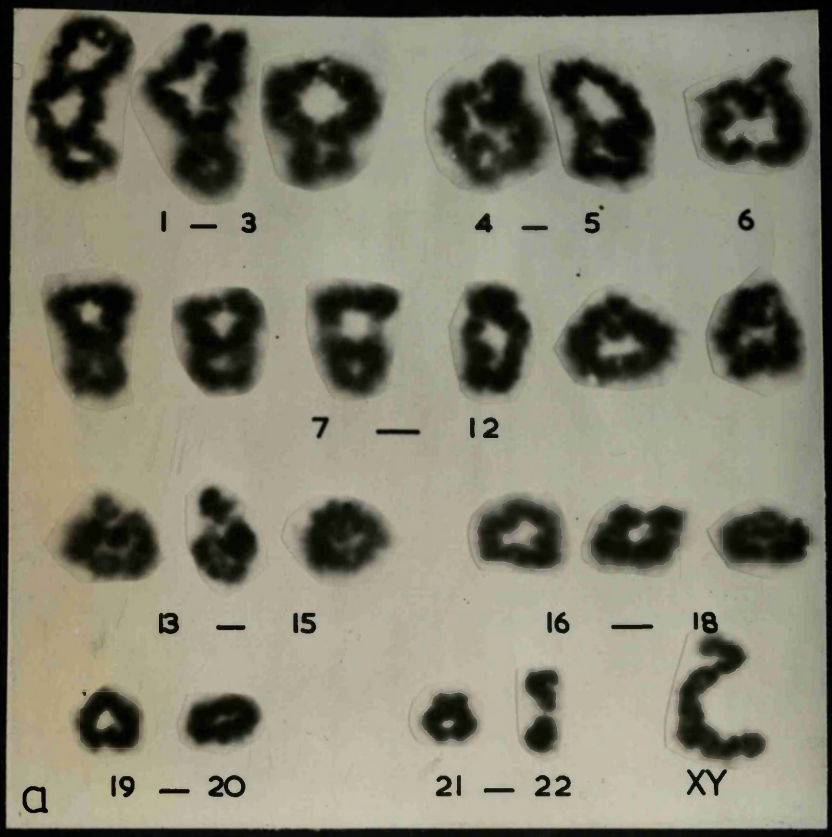


Plate 42. Diakinesis.

(a) Early diakinesis. Prominent double heteropycnotic centromere regions are shown.

(b) Mid diakinesis. This stage was used for chromosome counts and chiasma analysis.



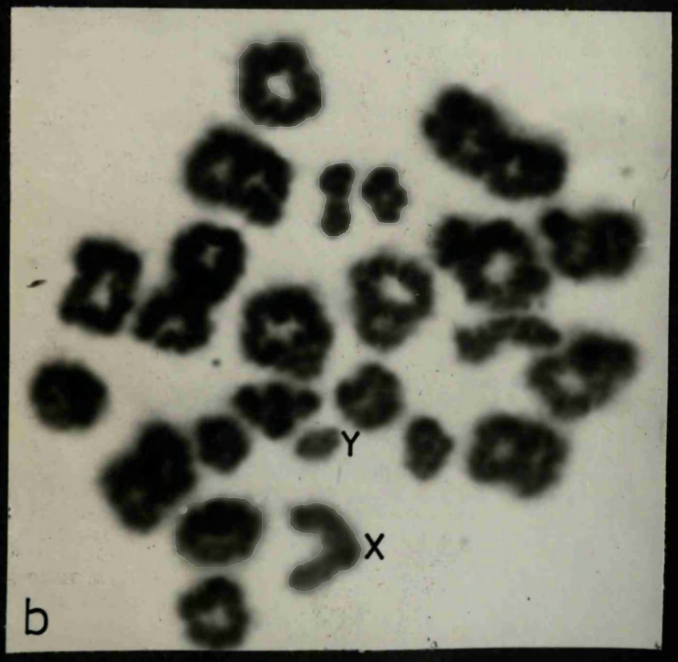
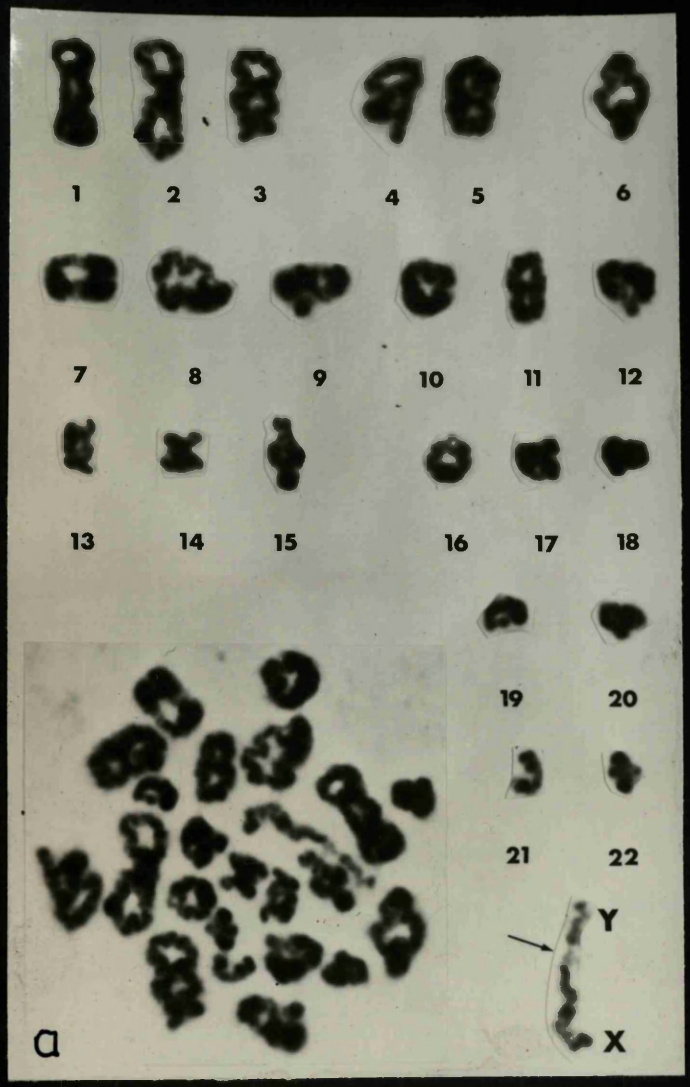


Plate 43. Diakinesis.

(a) Karyotype of the cell shown in Plate 42b, x 3,250. The total cell chiasma count is 53. Bivalent 9 shows three chiasmata and its paracentric secondary constriction region is euchromatic.

(b) Shows the sex chromosomes separated (X/Y).



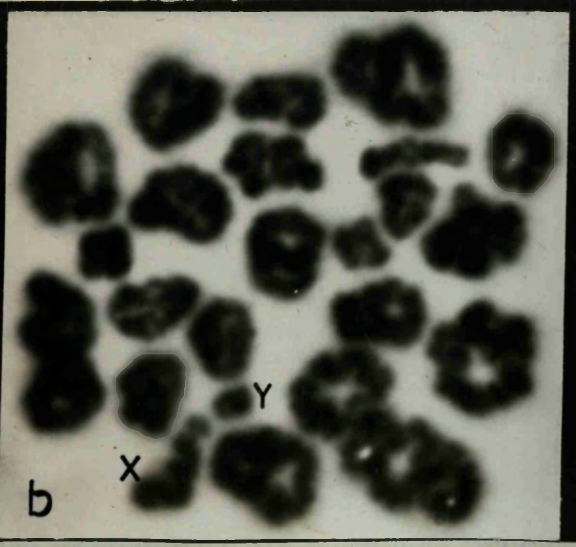
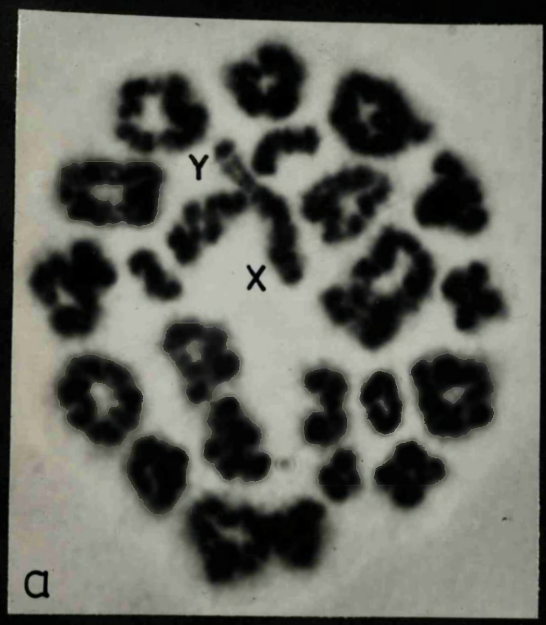


Plate 44. Sex chromosomes at diakinesis.

(a) x 1,750. Between the X and Y chromosomes is a configuration that might be interpreted as a chiasma.

(b) The sex chromosomes lie adjacent. Careful focussing reveals material spanning the apparent gap.



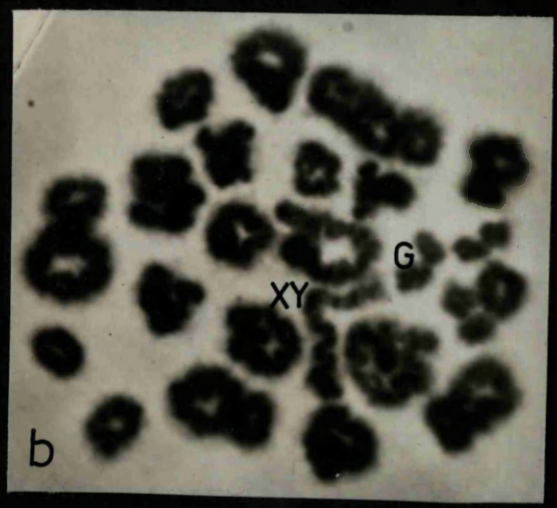
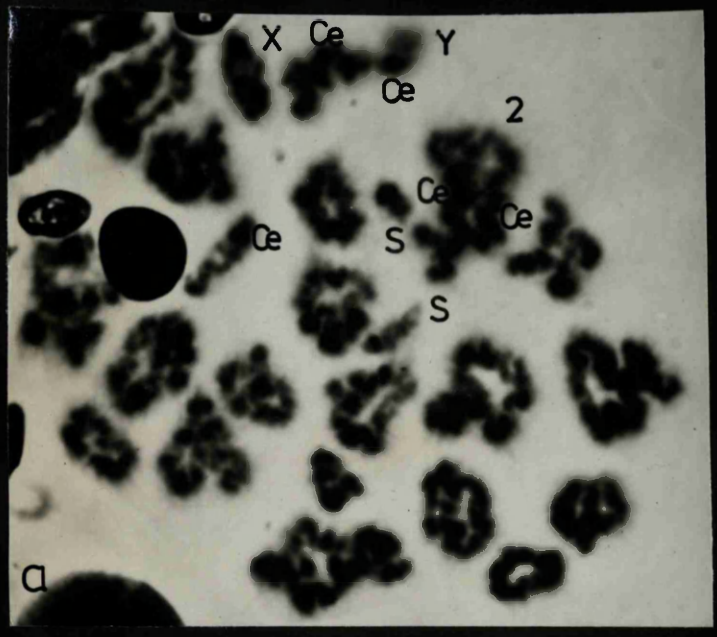


Plate 45. Diakinesis.

(a) Cell might be interpreted as showing the pairing of the long arm of the Y with the X.

(b) A G-bivalent is in a very characteristic position which is with the centromere of one homologue in association with a telomere of the sex bivalent.



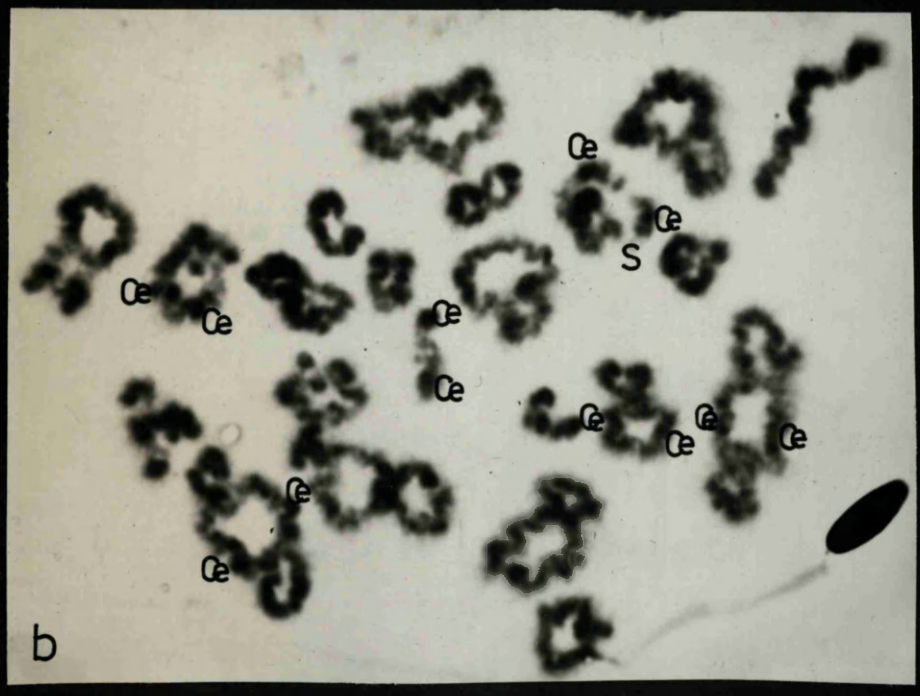
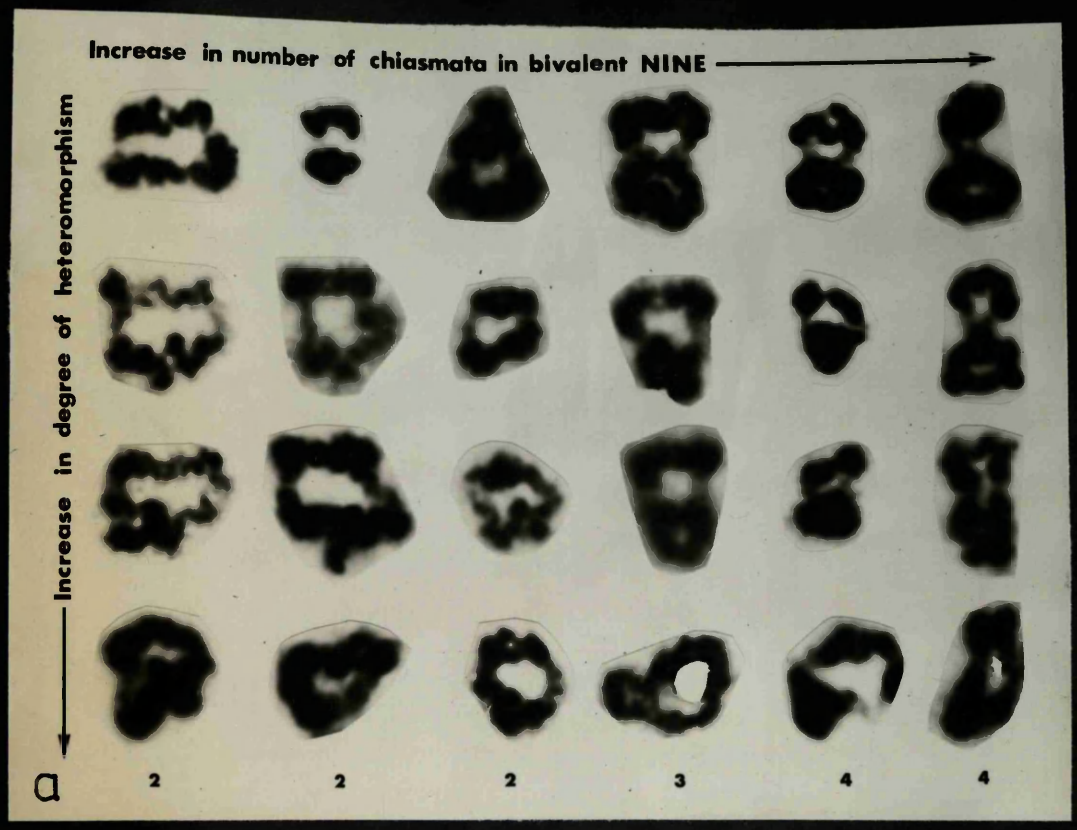


Plate 46. Diakinesis cells suitable for bivalent identification. Centromere regions are heteropycnotic.

(a) The secondary constrictions on the long arms of chromosome 2, which are infrequently observed at mitotic metaphase, are here exaggerated.



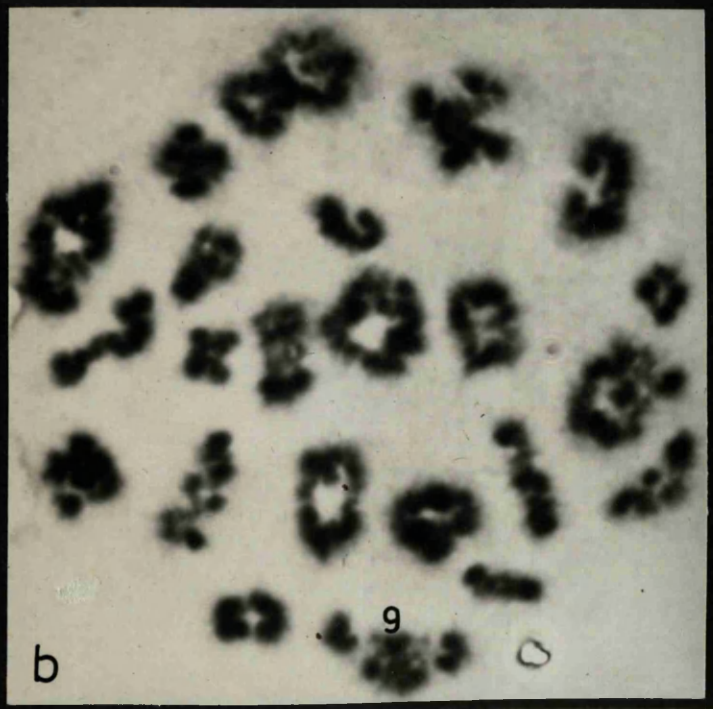
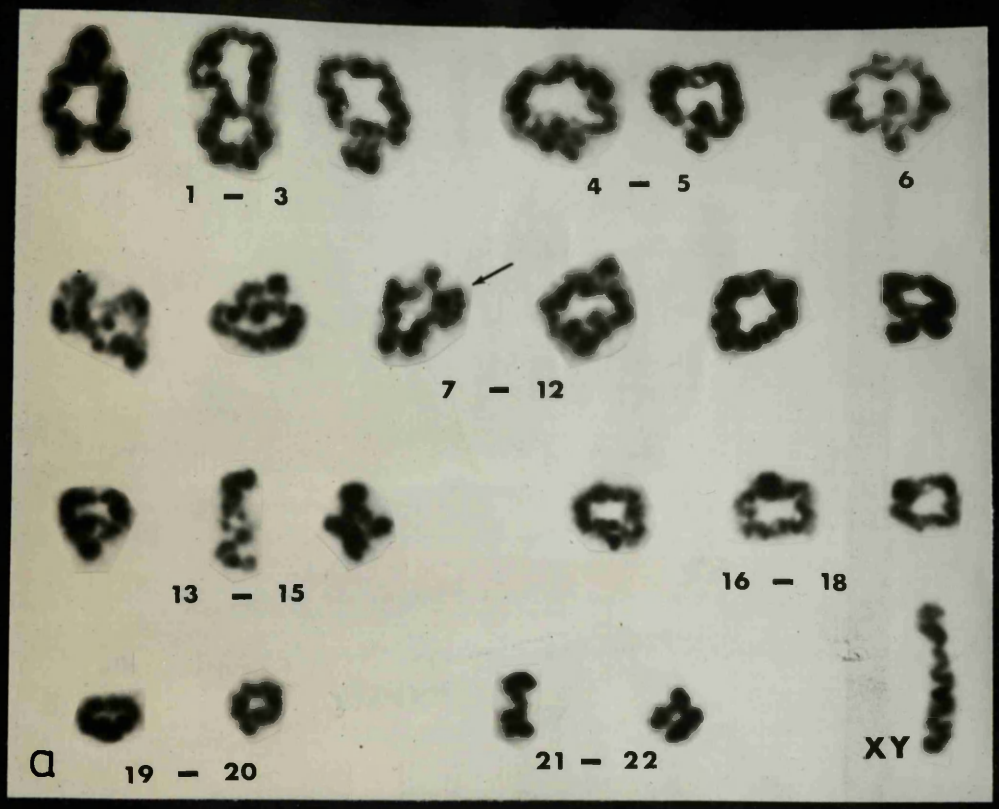


Plate 47. Bivalent 9 at diakinesis. There were always 2, 3 or 4 chiasmata.

(a) x 3,500. From various individuals. The bivalents are placed short arms uppermost. Secondary constriction regions are euchromatic. Degree of asymmetry of the bivalent reflects degree of heteromorphism of mitotic nines.
Top row: Extreme right shows a chiasma between the secondary constriction region and the centromere.

(b) There are no chiasmata in the short arm, but two in the long arm.



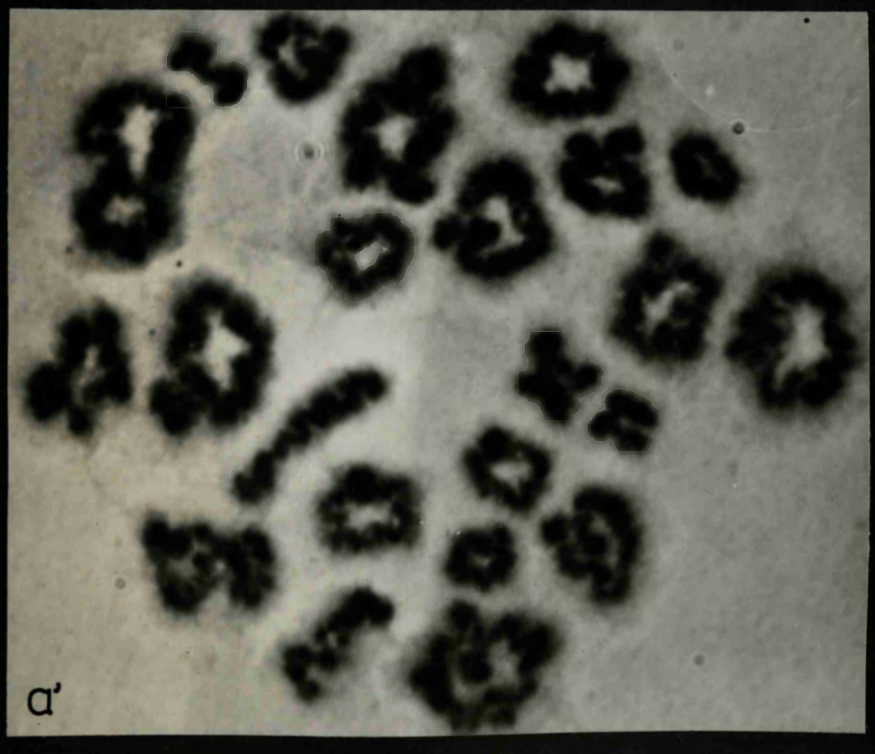
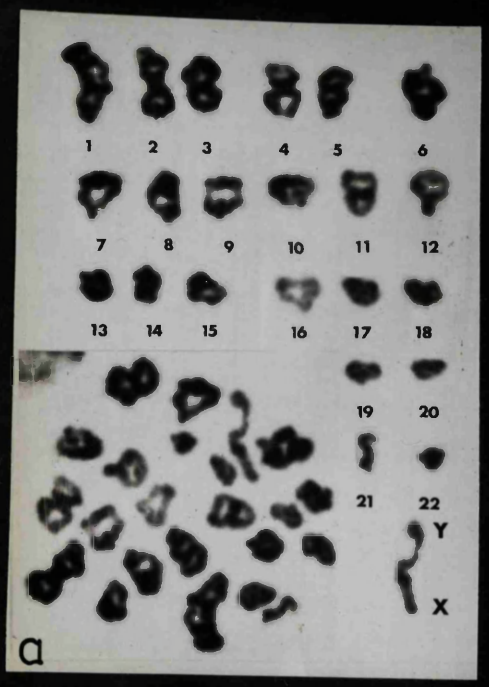


Plate 48. x 2,000. Diakinesis cell karyotyped using size and centromere position. From the degree of heteromorphism of mitotic number 9 chromosomes it would be predicted that this bivalent at diakinesis would be asymmetrical. In this cell asymmetry is reflected in a bulge of chromomeres below the centromere in one of the homologues. These are reminiscent of the paracentric chromomeres at pachytene.



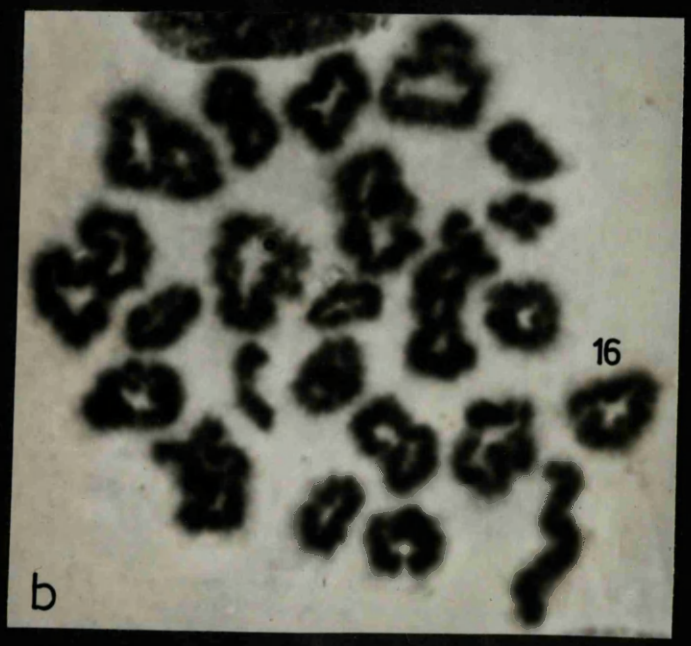
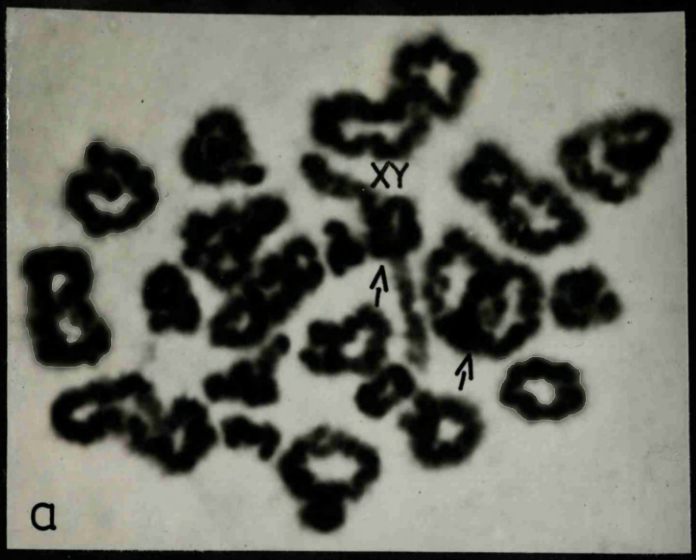


Plate 49. Diakinesis cells from HK710139, with heteromorphic chromosomes 16 (Plate 10b). Heteromorphism is reflected in asymmetry of bivalent 16. Degree of asymmetry is not as exaggerated as would be shown by a number nines with the same heteromorphism (Plate 48a bottom row).

(a) x 1,500. Karyotyped.



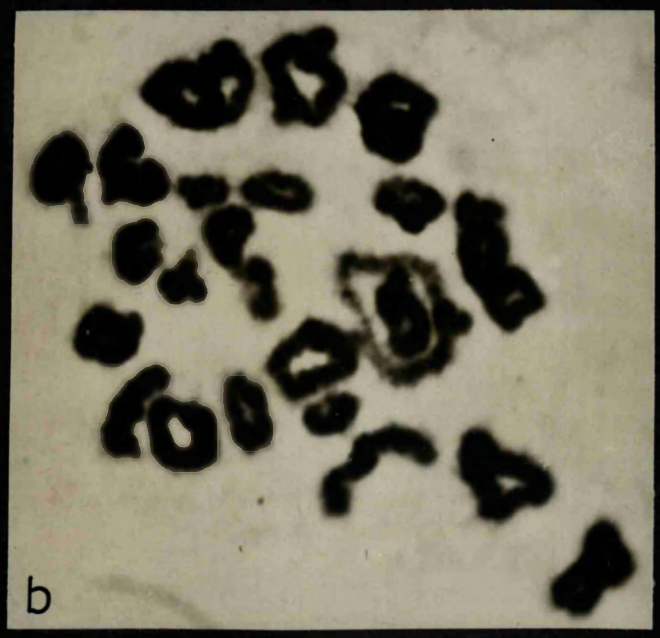
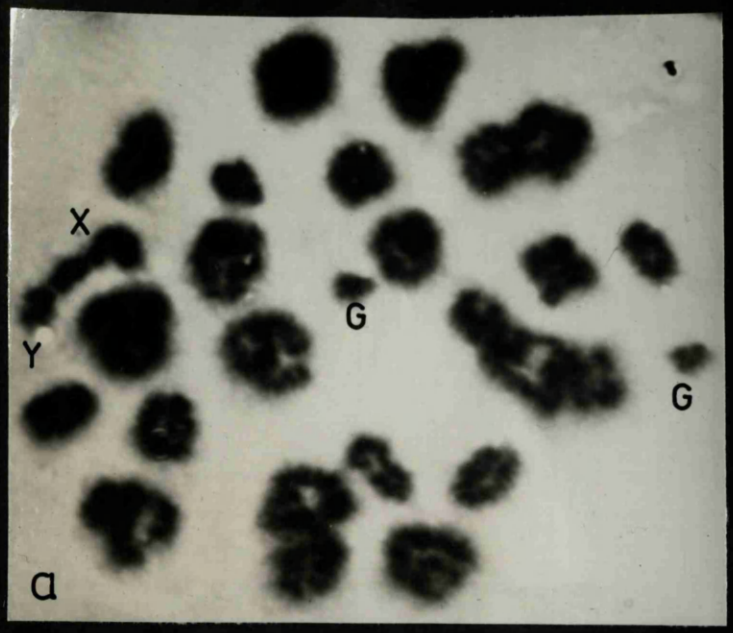


Plate 50. Diakinesis.

(a) Possible interlocked bivalents and the XY bivalent looped through an autosomal bivalent are shown in the same cell.

(b) Part of one bivalent is stretched round another.



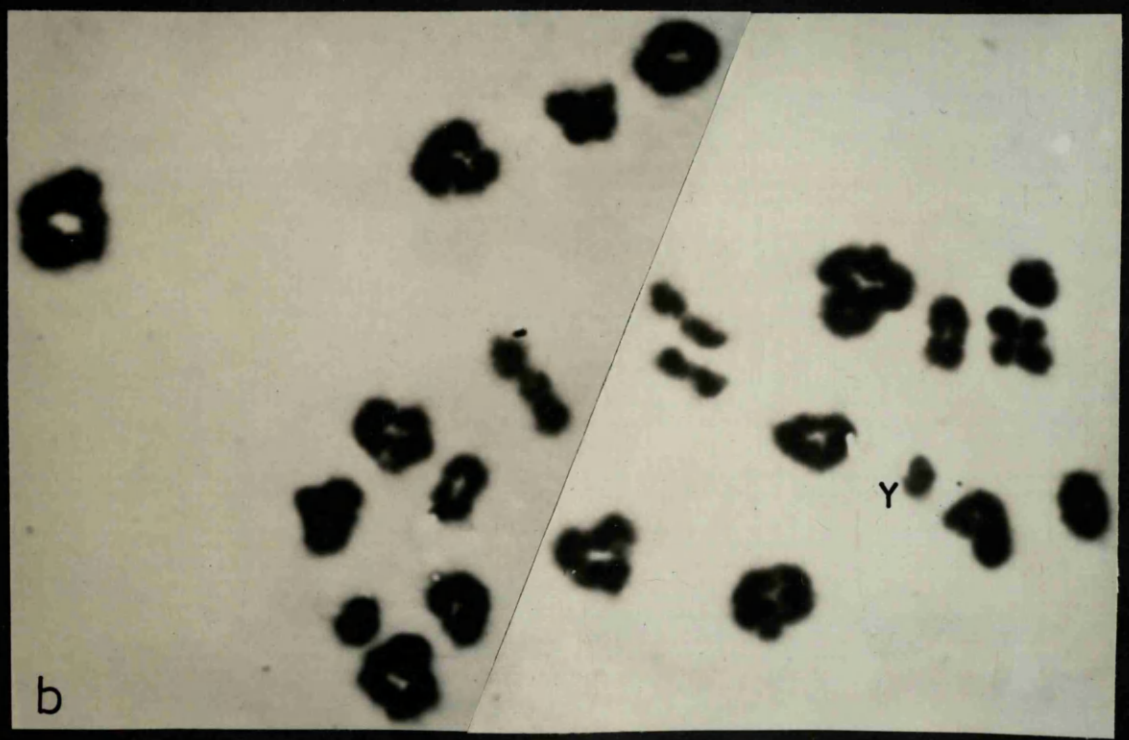
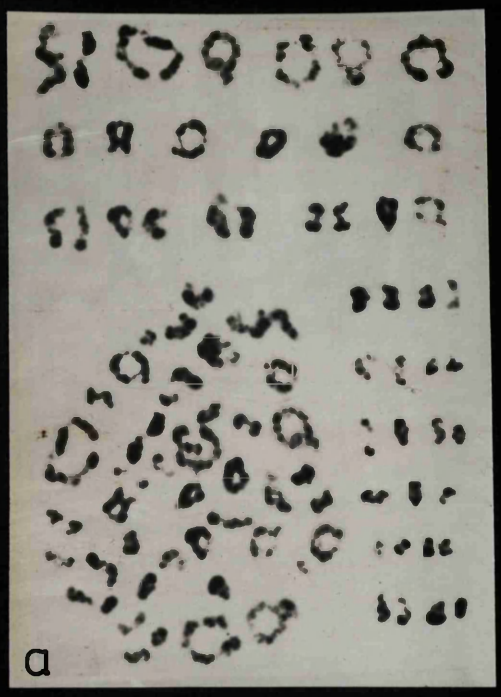


Plate 51. Abnormal diakinesis cells.

(a) Shows G univalents, 22 other elements and the X and Y adjacent.

(b) Shows a cell apparently without an X chromosome.



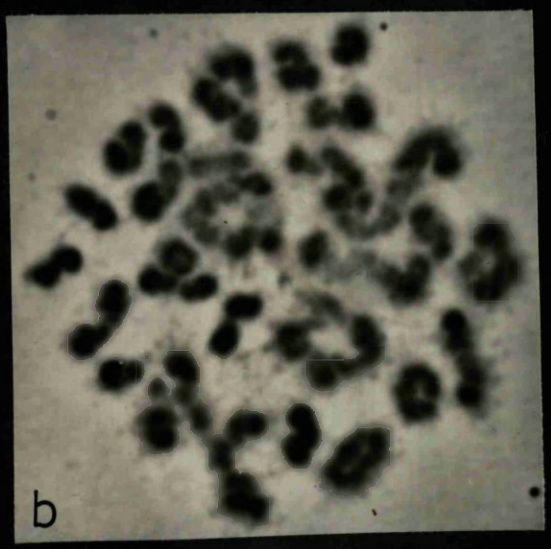


Plate 52. Cells at diakinesis with a low chiasma count.

(a) x 1,000. Appears aneuploid with too many smaller elements. Attenuation of constriction regions could be responsible for this.

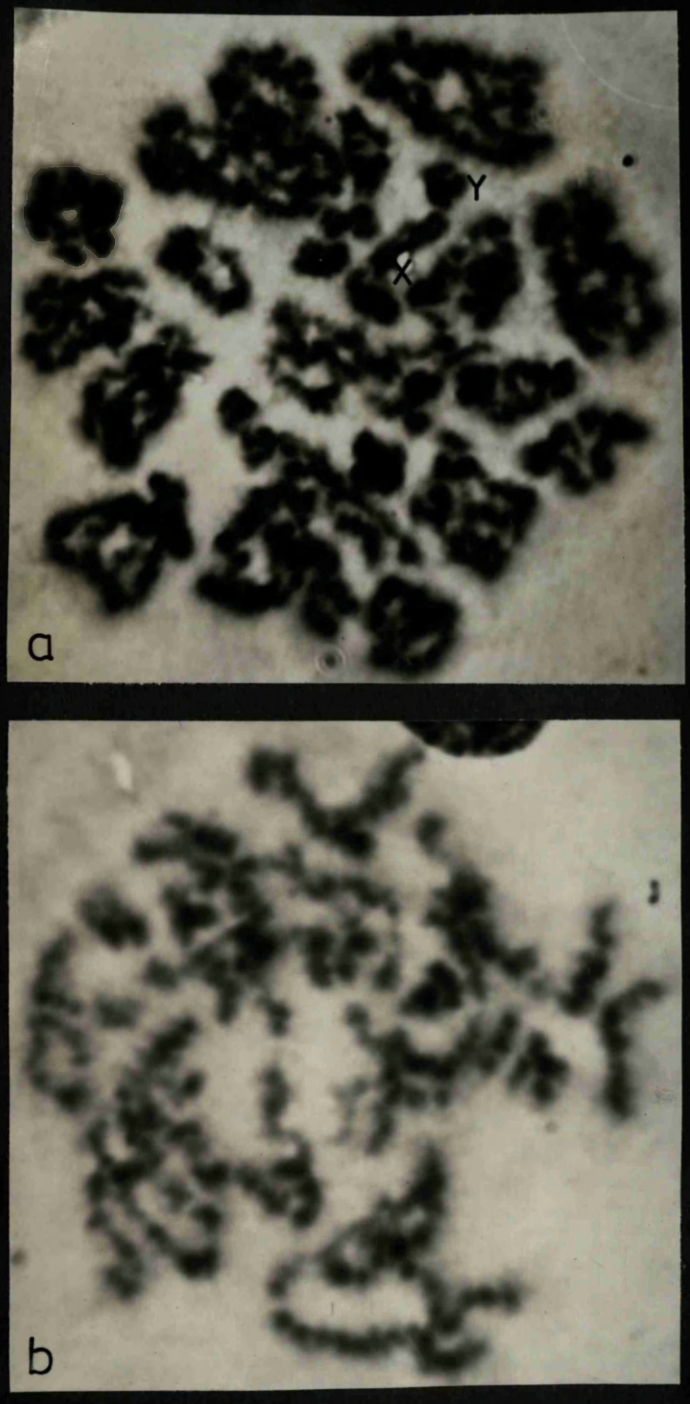
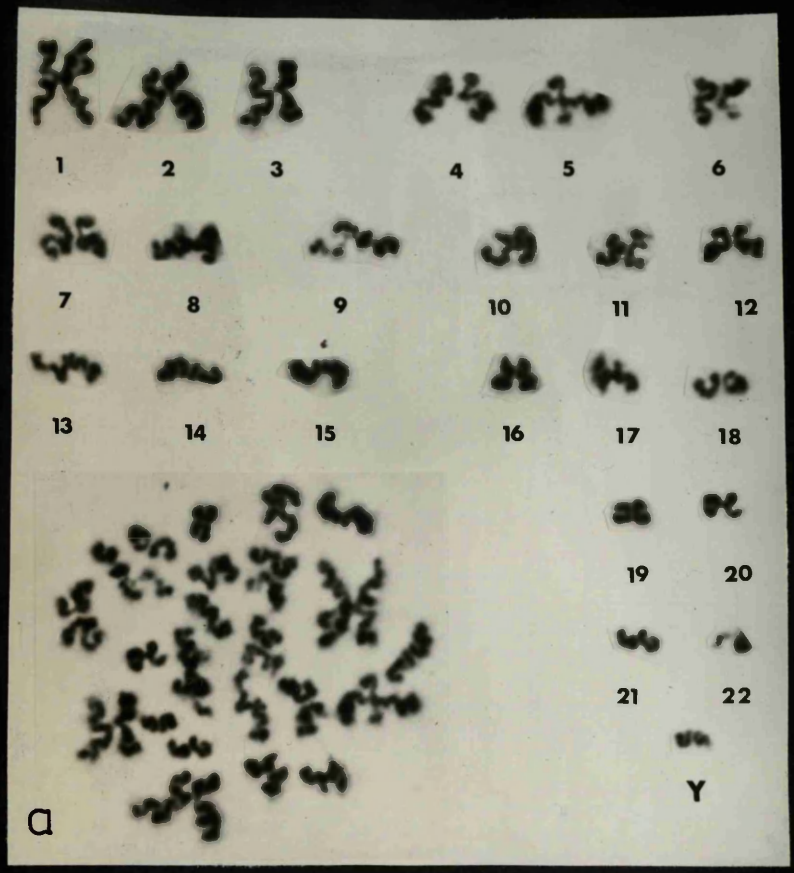


Plate 53. (a) Diakinesis cell showing the four strands of bivalents and the crossover points. The chromatids of the X and Y are separated and the short arm pairing of the Y with the X is shown. This is possibly just prior to first anaphase.

(b) Early metaphase II. Chromosome arms are extended and pale. This suggests that an interphase is likely to exist between anaphase I and metaphase II.



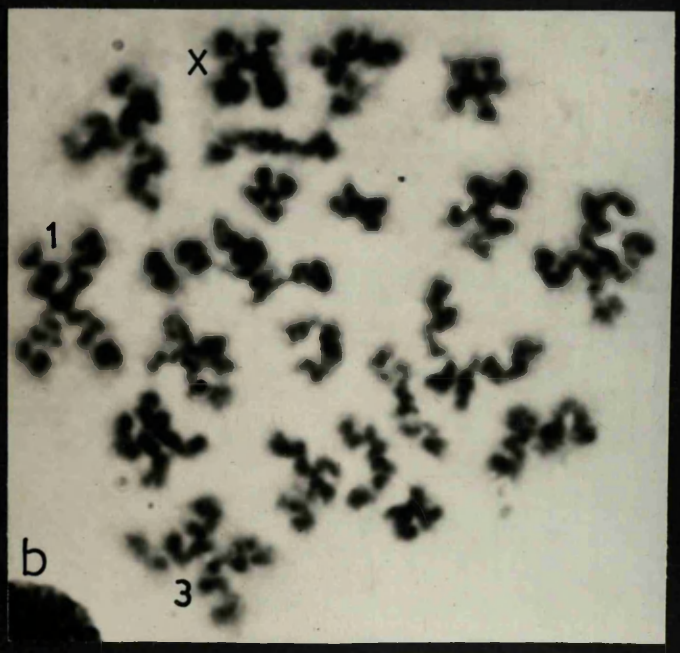


Plate 54. Metaphase II. Chromosomes exhibit coiling, but can be identified.

(a) x 1,750. Y-bearing. Chromosome 5 shows rotation of arms.

(b) X-bearing. Each chromatid may appear double at this stage (see chromosome 1). Homologous centromeres occasionally move a small distance apart (see chromosome 3).

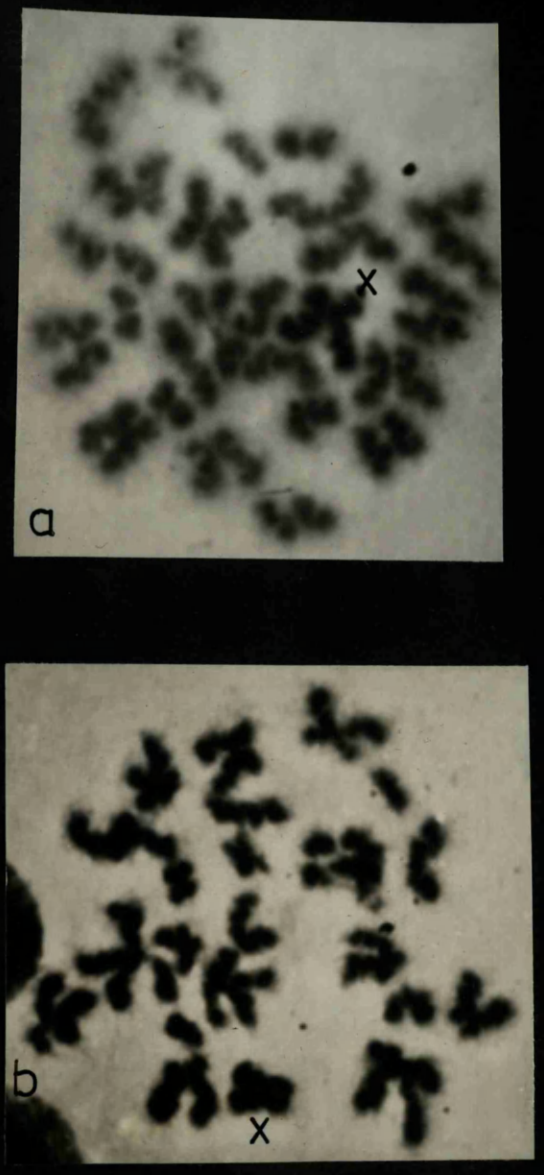
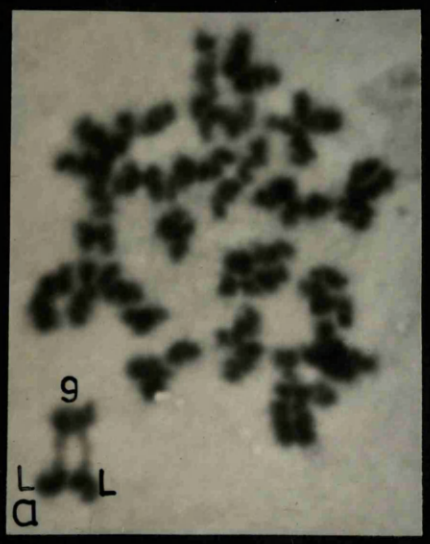
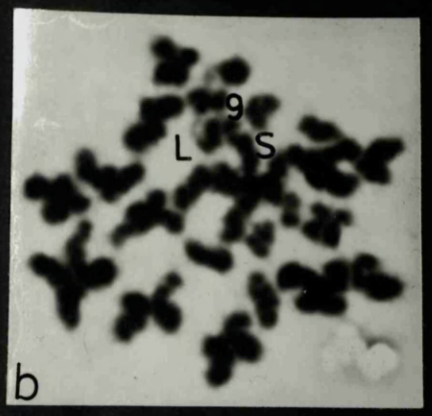


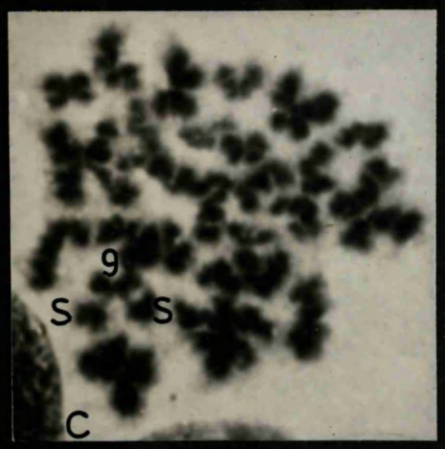
Plate 55. Differential appearance of the X chromosome at metaphase II.

(a) Shows the X heteropycnotic.

(b) Shows the chromatids of the X lying closer together than those of the autosomes.







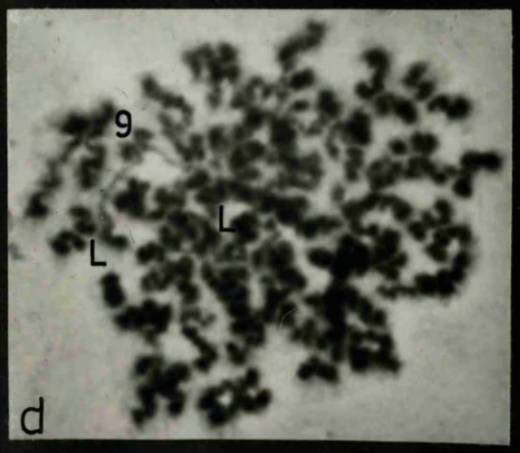


Plate 56. Chromosome 9 at metaphase II in JB690075. This chromosome is easy to identify because the paracentric secondary constriction region is pale and extended in proportion to any polymorphism of this region. The rest of the chromosome is packed more uniformly than in other autosomes

(a) Shows chromosome 9 with two "long", long arms.
(b) Shows chromosome 9 with one "long" and one "short" long arm. This is attributed to crossing over between the secondary constriction region and the centromere at an earlier stage of meiosis.
(c) Shows chromosome 9 with two "short", long arms.
(d) Shows the ease of scoring LL when the rest of the complement is obscure.

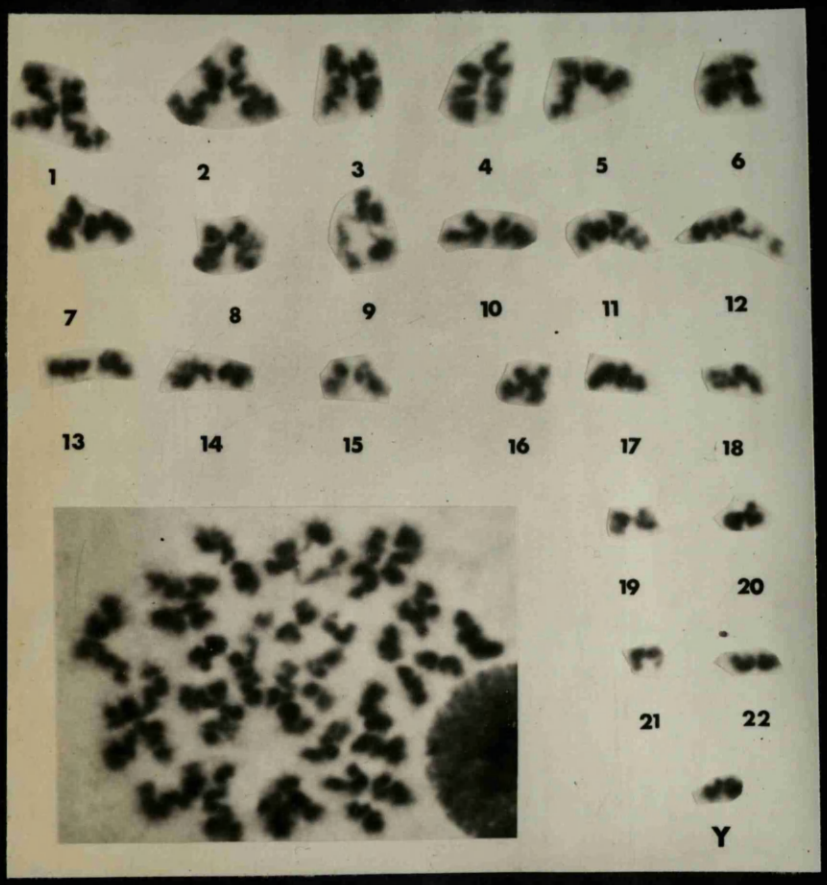


Plate 57. Karyotyped metaphase II, x 1,750, from HK700139 with heteromorphic chromosomes 16 (Plate 10b). Here chromosome 16 shows "short" long arms. Nines were less heteromorphic and are here probably LL.

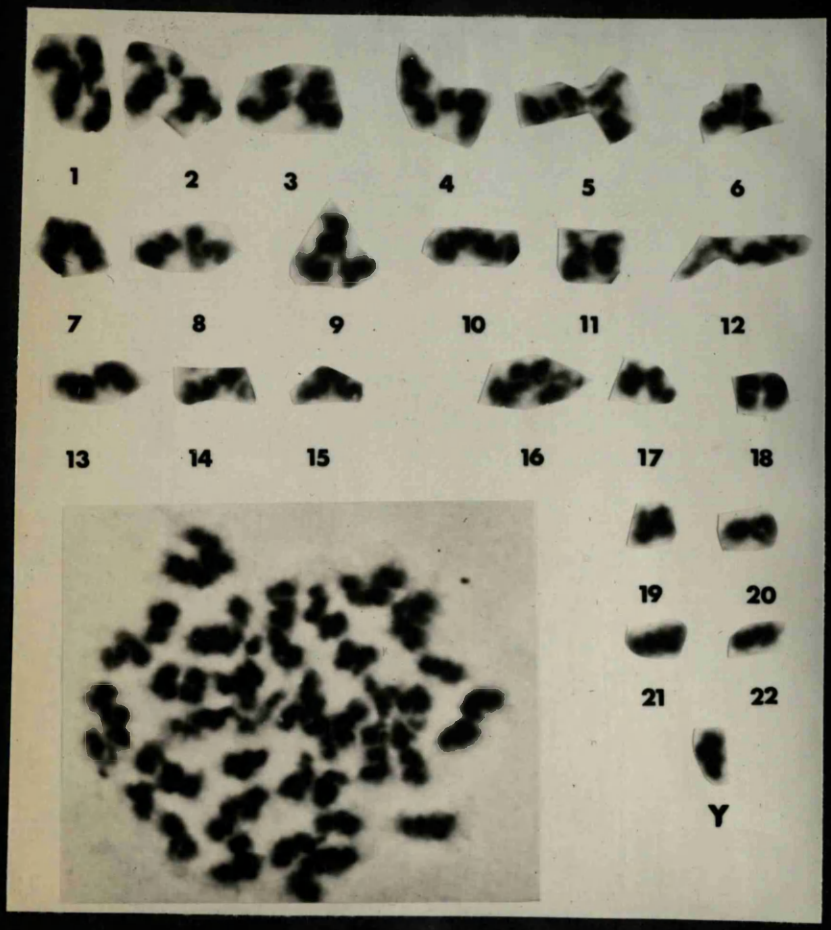
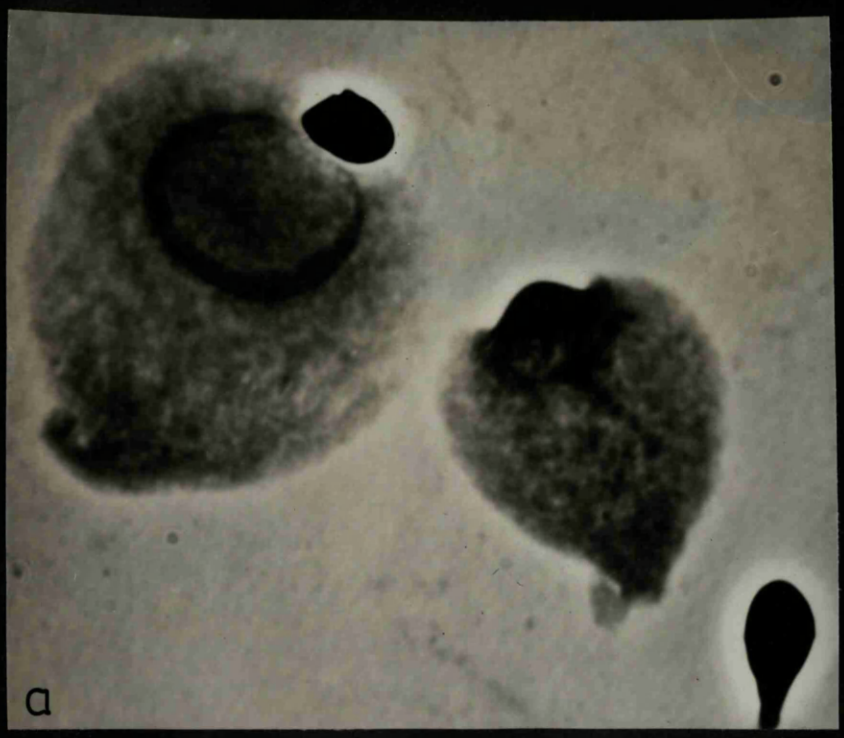


Plate 58. Karyotyped metaphase II, x 1,750, from HK700139 with heteromorphic chromosomes 16 (Plate 10b). Here chromosome 16 shows "long" long arms. Nines were less heteromorphic and are here probably LL.



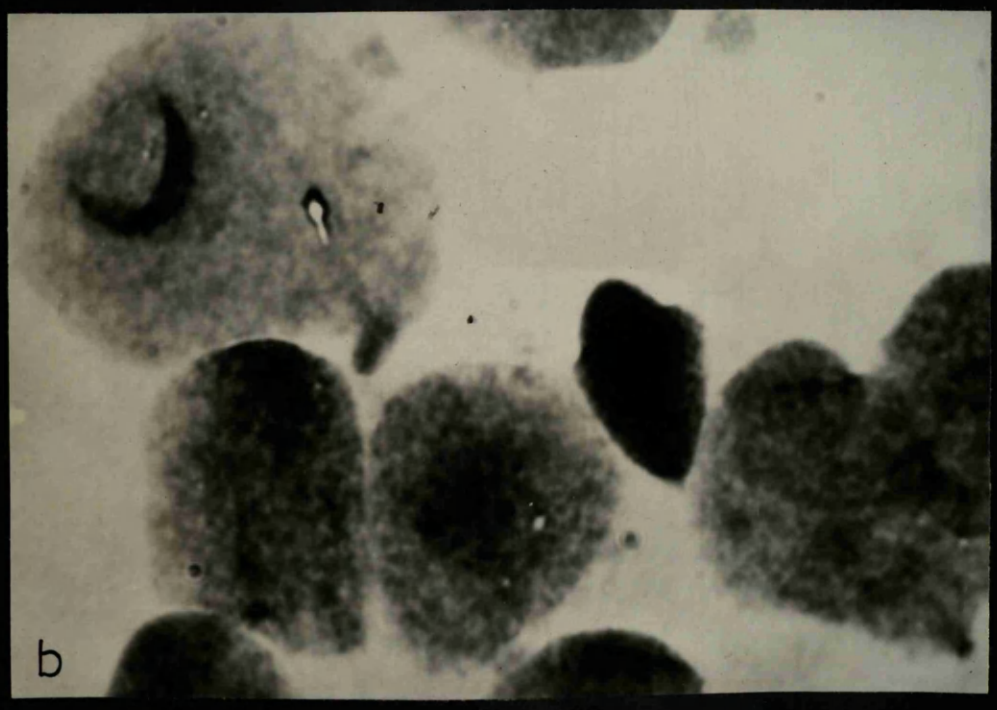
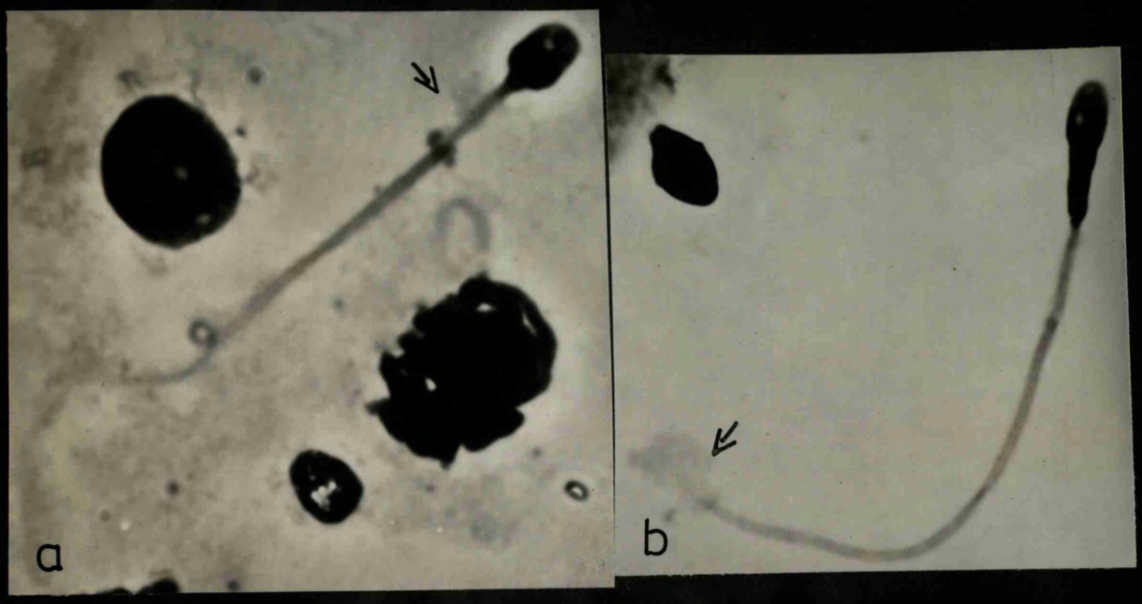


Plate 59. Spermiogenesis. Condensation of chromatin occurs from the acrosomal head.



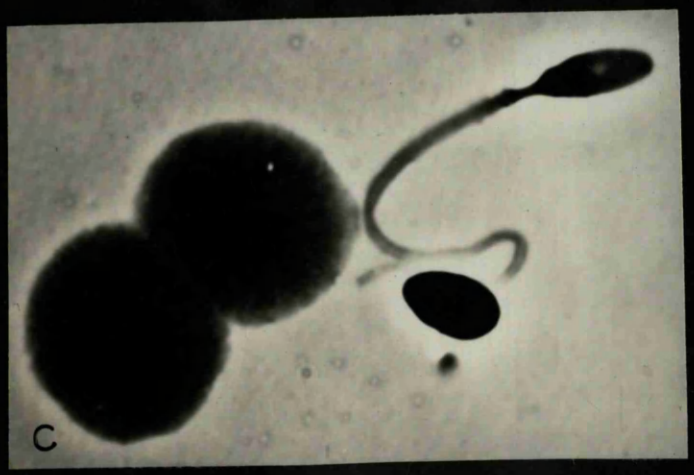


Plate 60. Sperm maturation.

(a) and (b) Cytoplasm falls off the tail.

(c) Four spermatids in various stages of maturation. No significance is attached to the pale spot in the most mature. This is probably an artefact caused by the optical system.

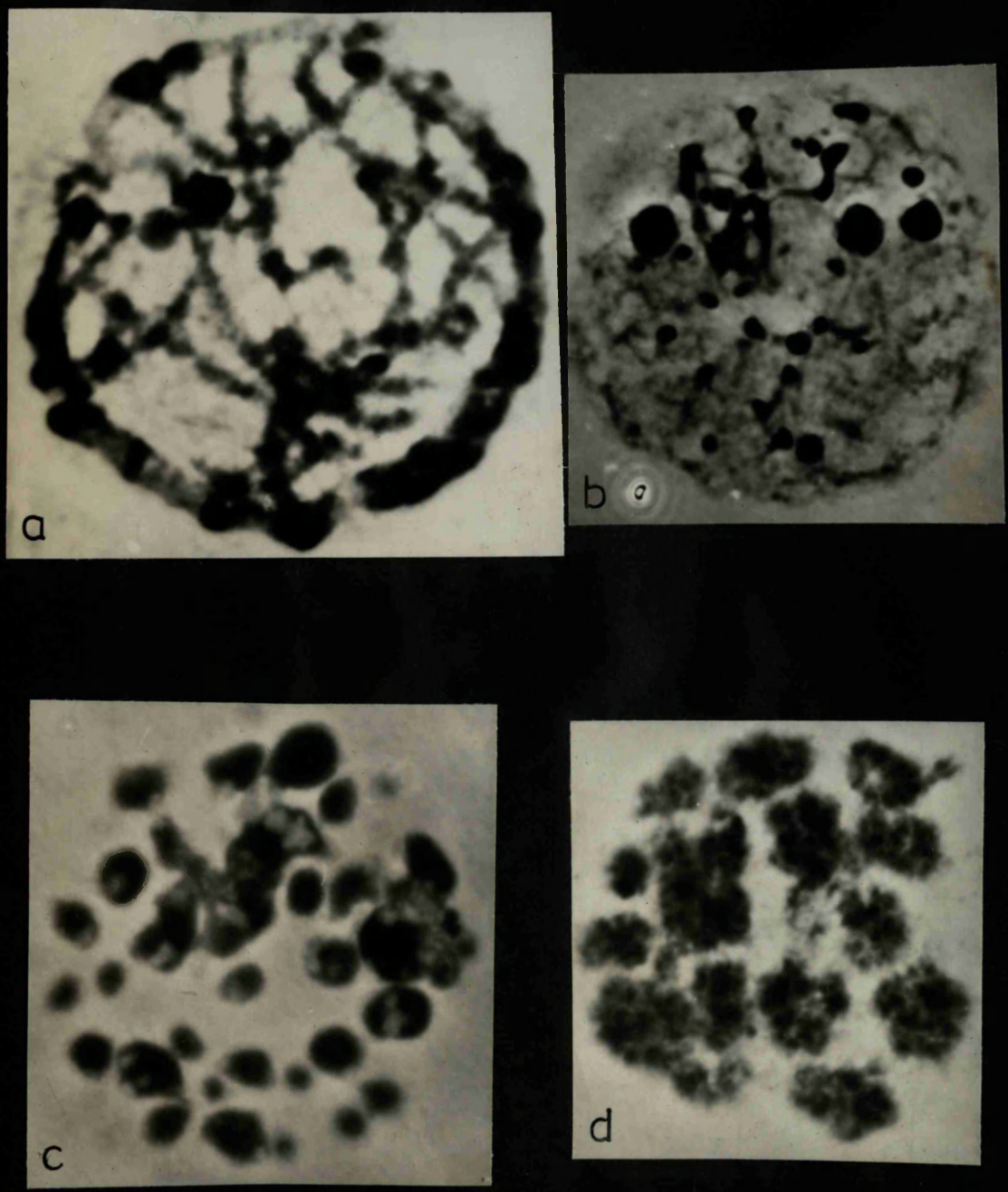
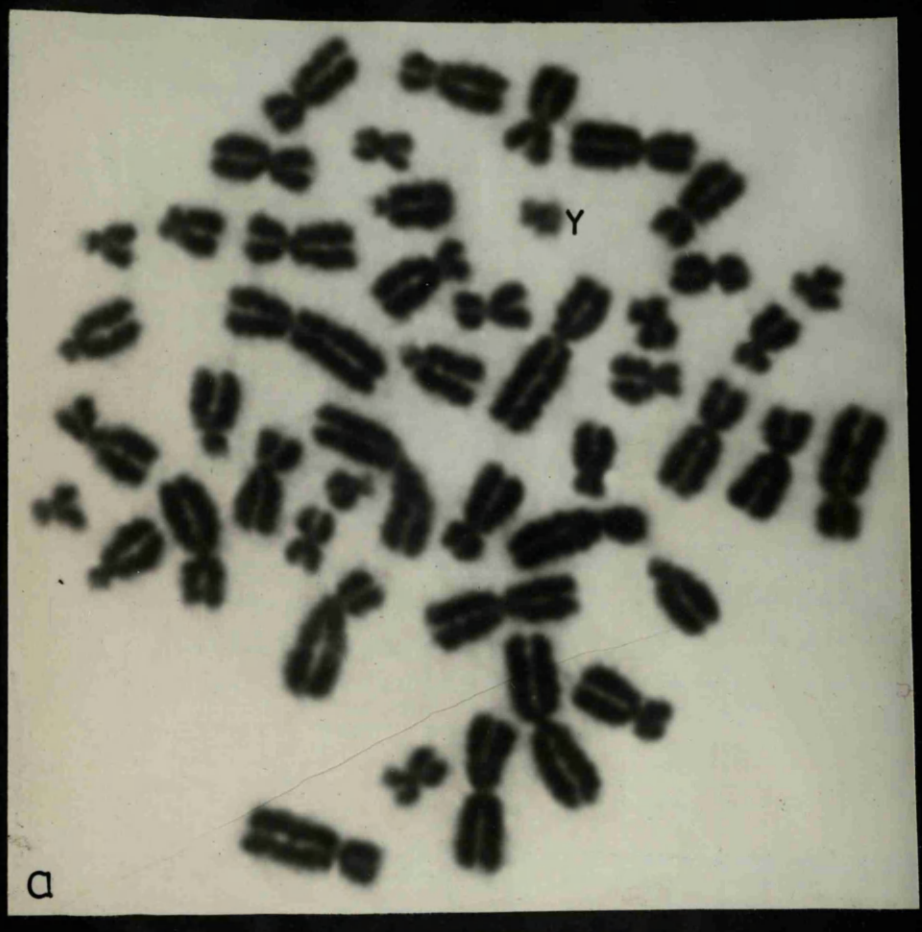


Plate 61. Cells in degeneration.
(a) At pachytene.
(b) Possibly at the "diffuse" stage.
(c) and (d) At diakinesis.



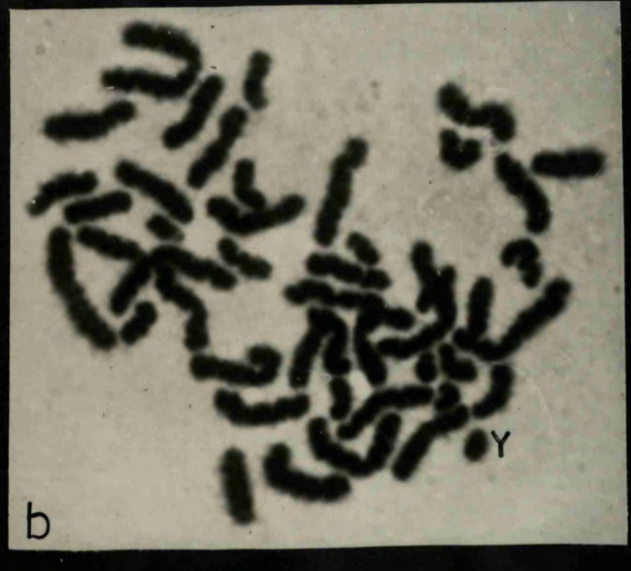


Plate 62. Cells from DD690194 who has a small Y chromosome.
(a) Leucocyte mitotic metaphase, x 3,500.
(b) Spermatogonial metaphase.

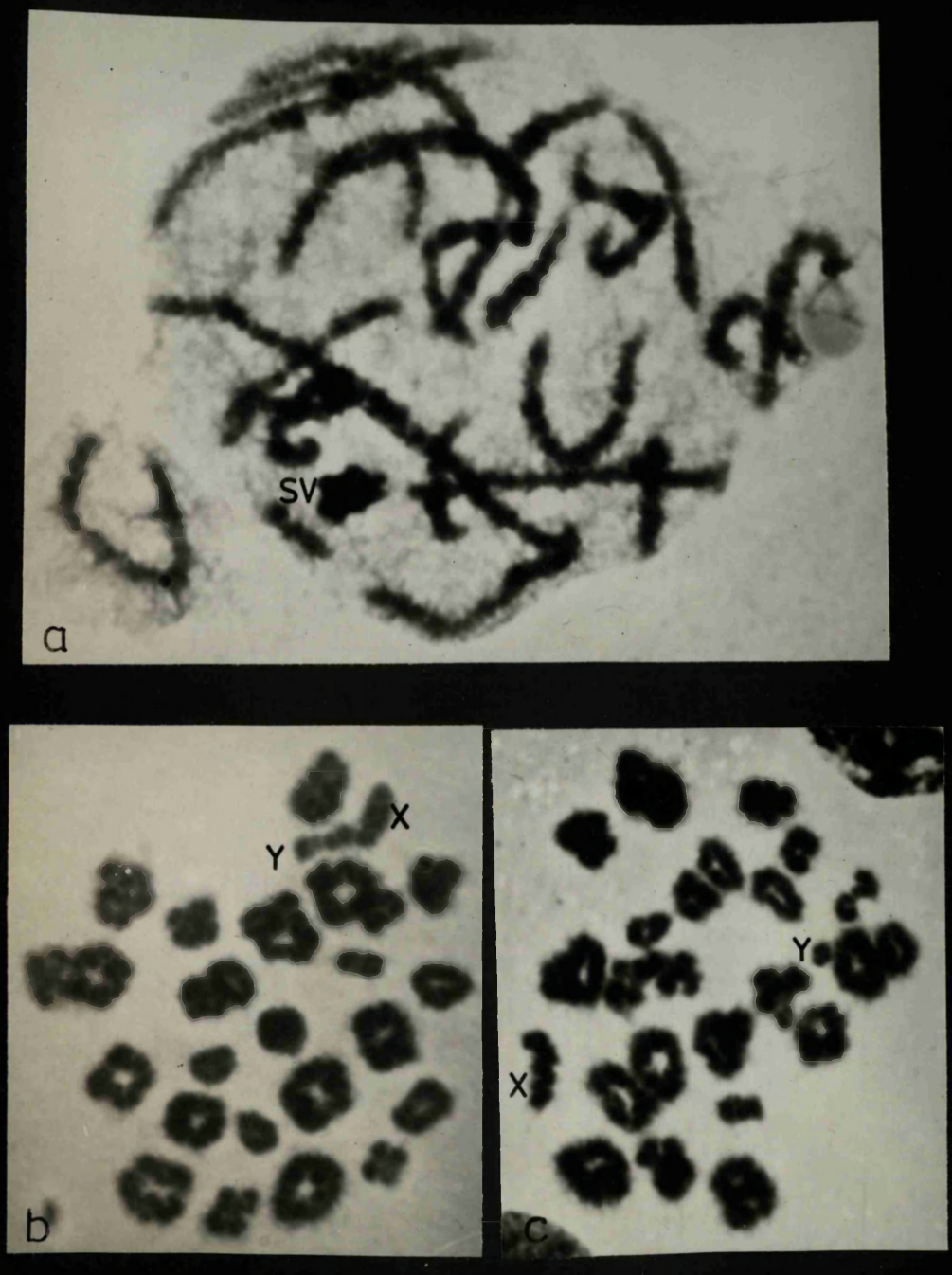
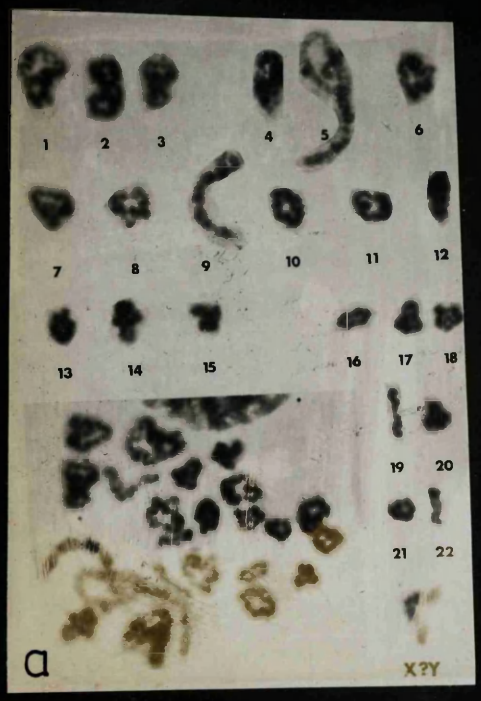


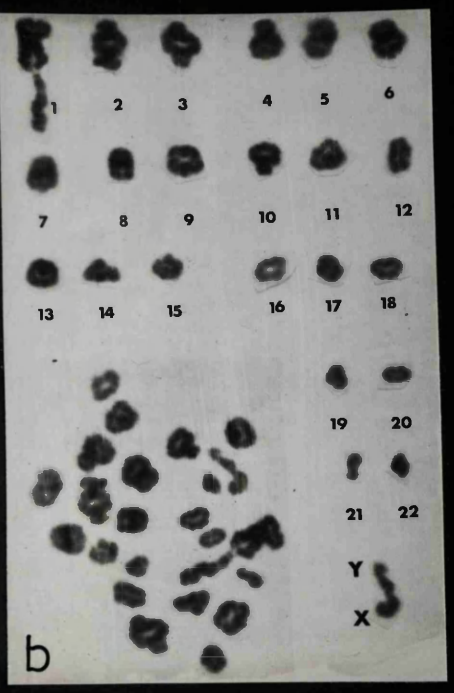
Plate 63. Cells from DD690194 who has a small Y chromosome. The sex chromosomes behave normally.

(a) Pachytene. The sex vesicle is small, but otherwise normal.

(b) Diakinesis. XY adjacent.

(c) Diakinesis. X/Y separate.





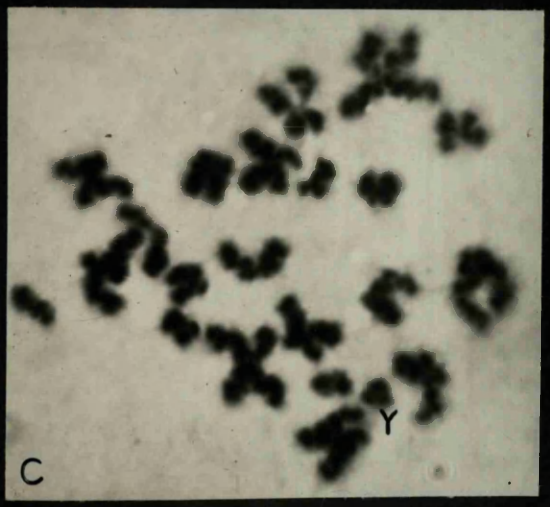
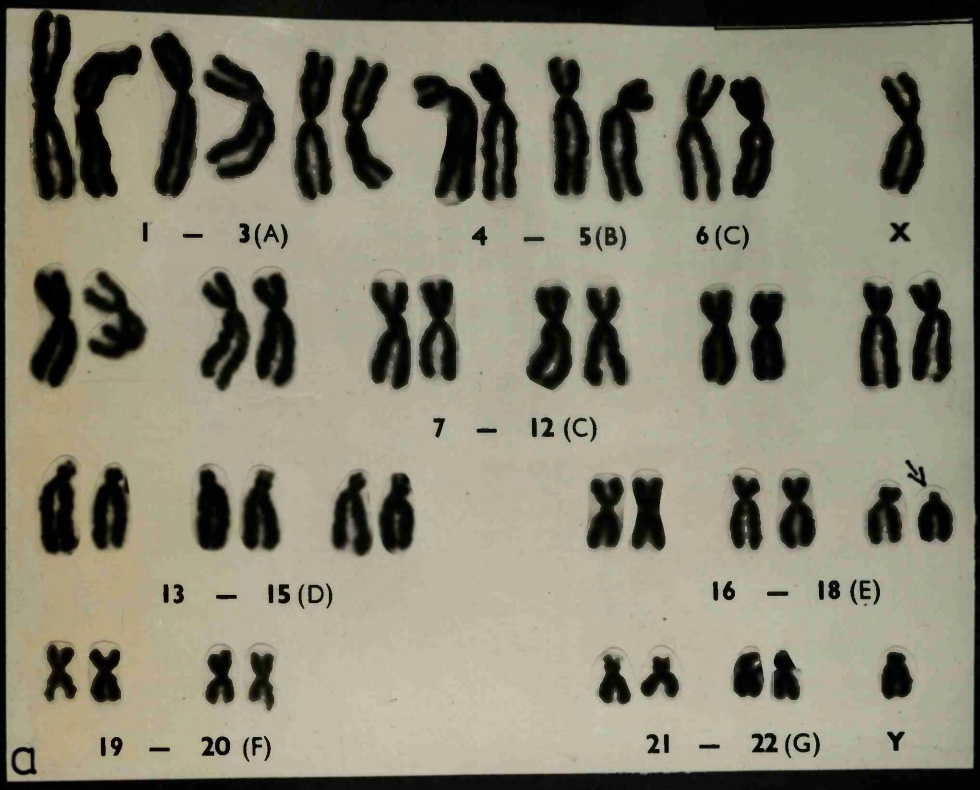


Plate 64. Cells from DD690194 who has a small Y chromosome.

(a) Diakinesis, x 1,500. Two bivalents have a pachytene appearance; in one of these, homologues are not fully paired.

(b) Diakinesis, x 1,500. There may be a duplication of most of chromosome number 1, excepting the long arm telomere. Twenty-two other elements are present, including the X and Y which are adjacent.

(c) Y-bearing metaphase II.



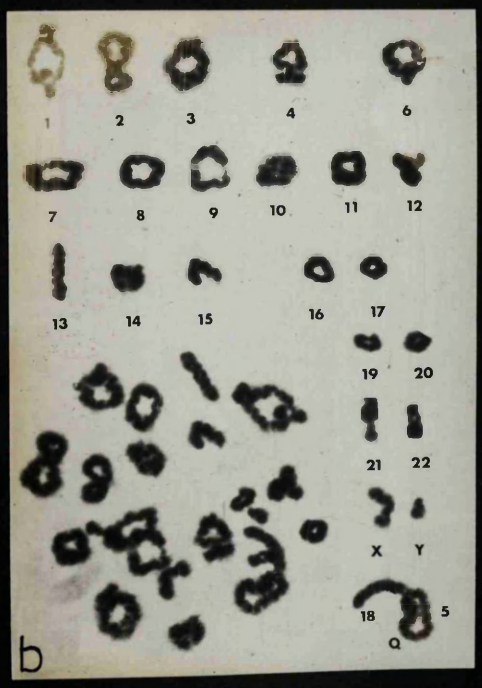
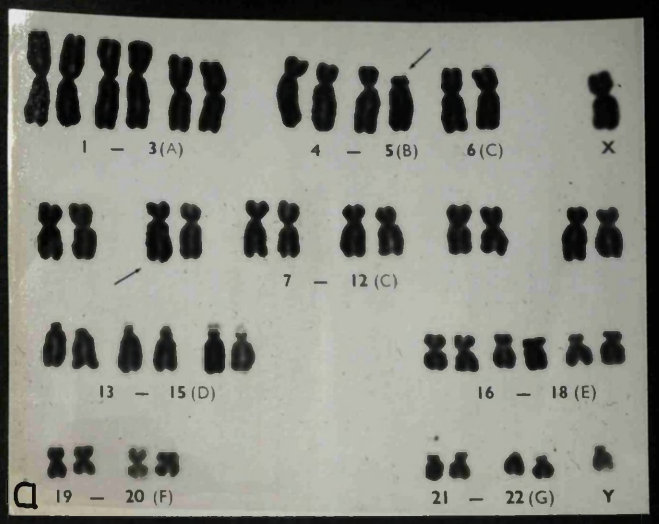


Plate 65. Cells from H....., a t(5q+18p-) heterozygote.

(a) Leucocyte mitotic metaphase, x 2,750, karyotyped.

(b) Karyotyped diakinesis, x 1,500, showing a ring quadrivalent and two elements "missing".



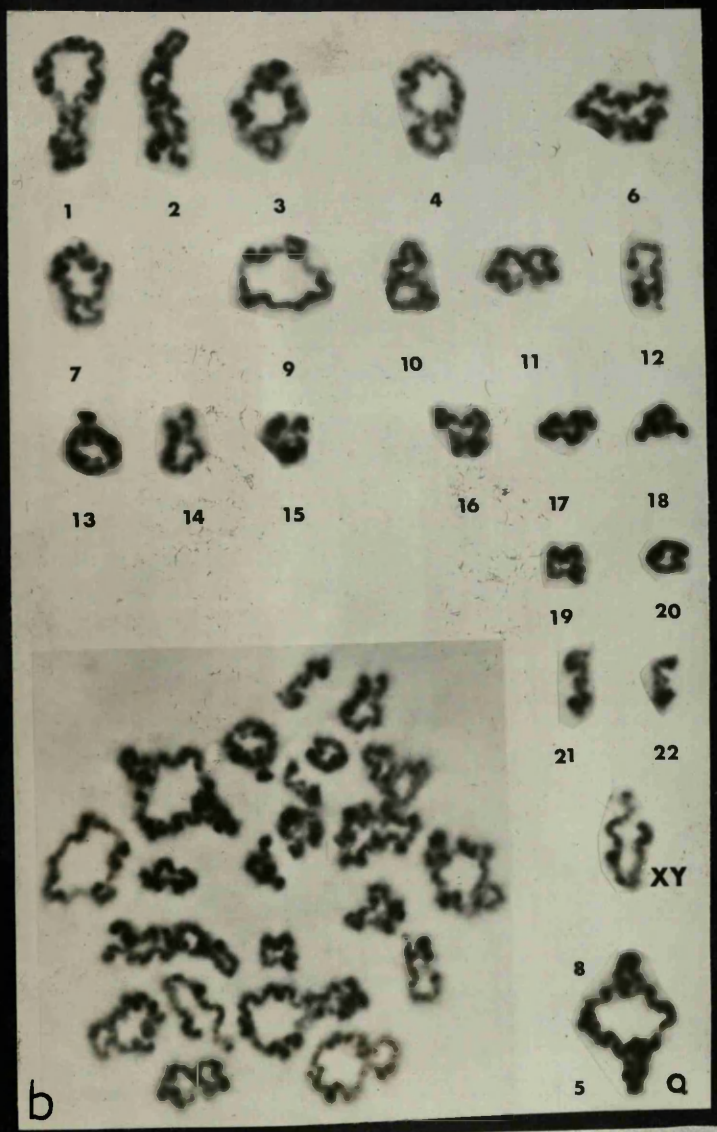
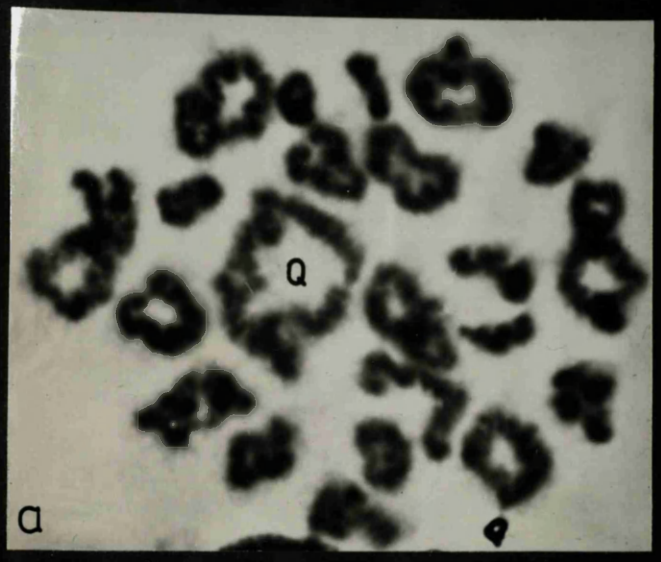
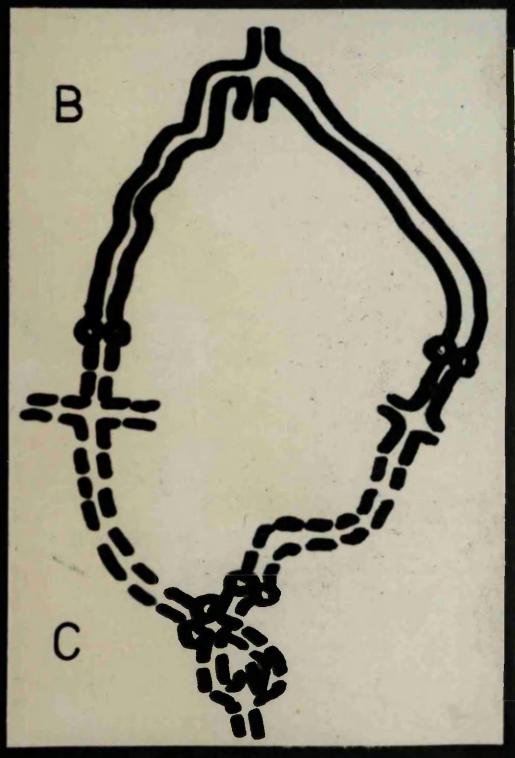


Plate 66. Cells from RW680009 a t(5p-8q+) heterozygote.

(a) Leucocyte mitotic metaphase, x 1,750, karyotyped.

(b) Karyotyped diakinesis, x 1,500, showing a ring quadrivalent and two elements "missing". Centromere regions are particularly clear in this cell.





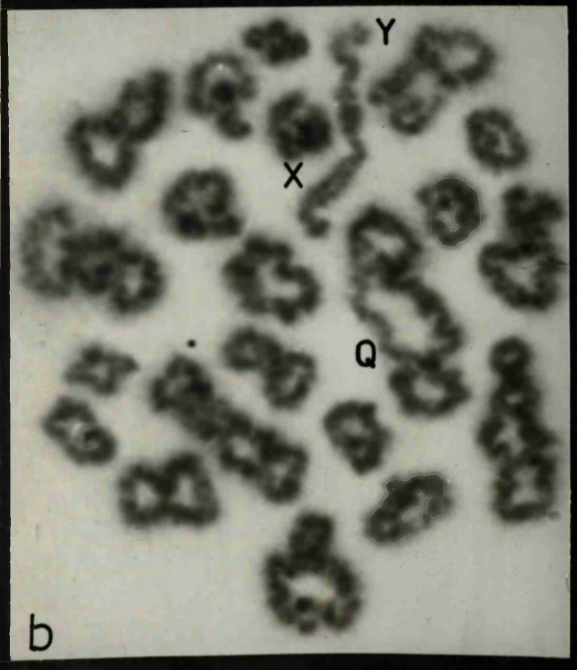


Plate 67. Diakinesis cells from MW680009, a t(5p-8q+) heterozygote showing a quadrivalent.

(a) With a diagram of the configuration.

(b) The sex bivalent morphology is particularly clear.

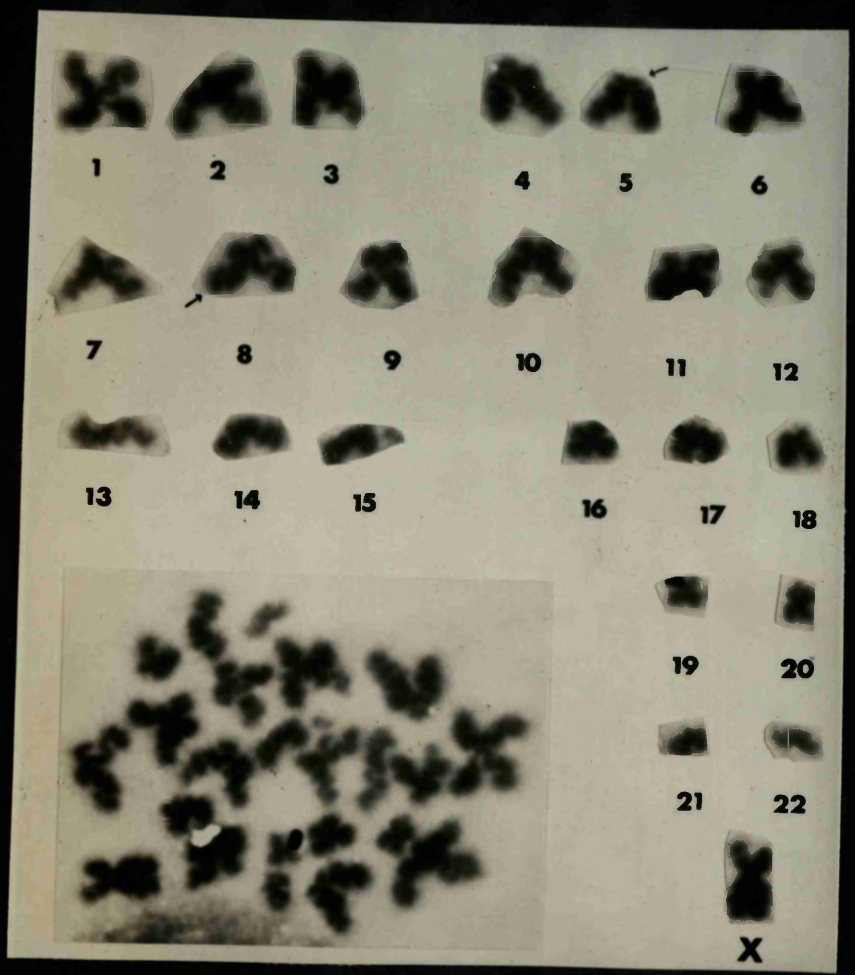
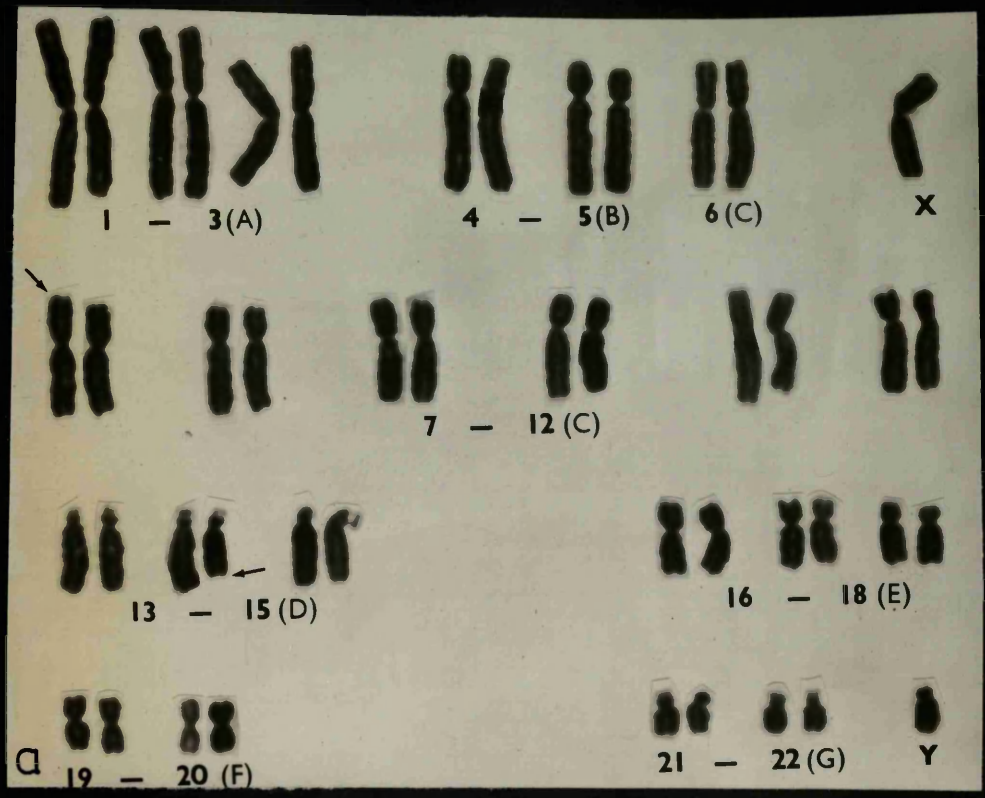
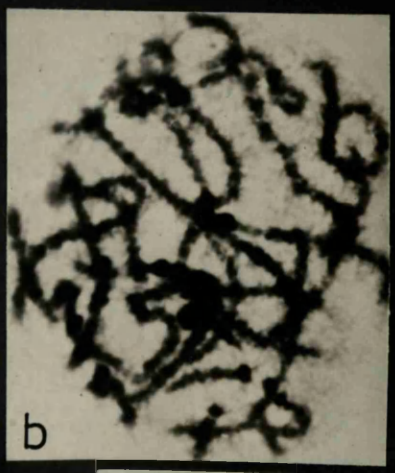


Plate 68. Karyotyped metaphase II, x 2,000, from RW680009, a t(5p-8q+) heterozygote. The balanced complement of a short 5 and long 8 is shown.





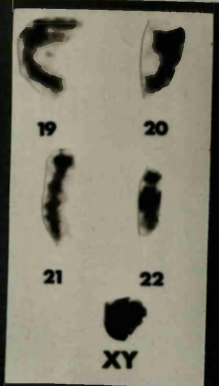


Plate 69. Cells from JD690204, a t(7p+14q-) heterozygote.

(a) Leucocyte mitotic metaphase, x 2,750, karyotyped.

(b) Rather poor pachytene, x 1,500 karyotyped (technique "M" not used). This may represent homologous pairing at pachytene.

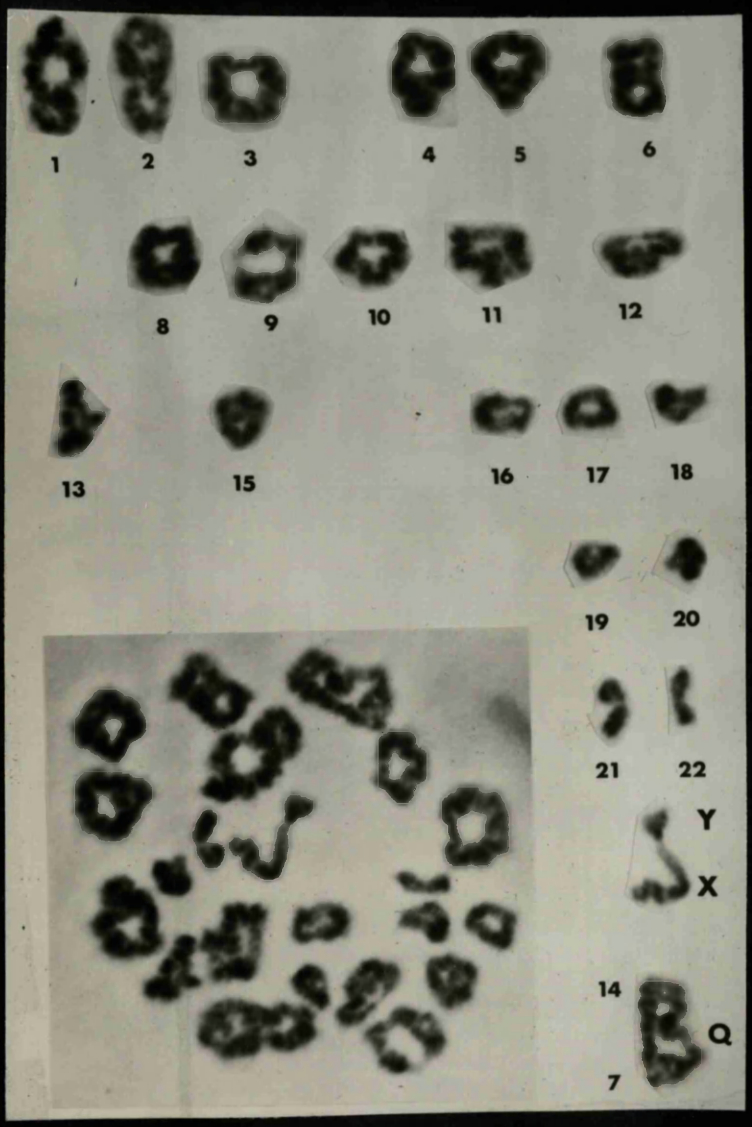
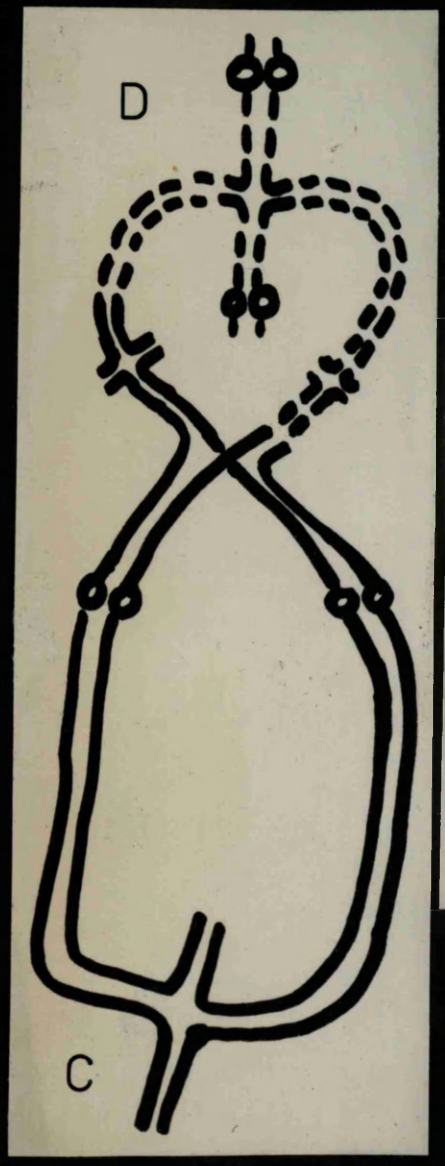


Plate 70. Diakinesis cell, x 1,750, from JD690204, a t(7p+14q-) heterozygote. There is a ring quadrivalent and two elements "missing".



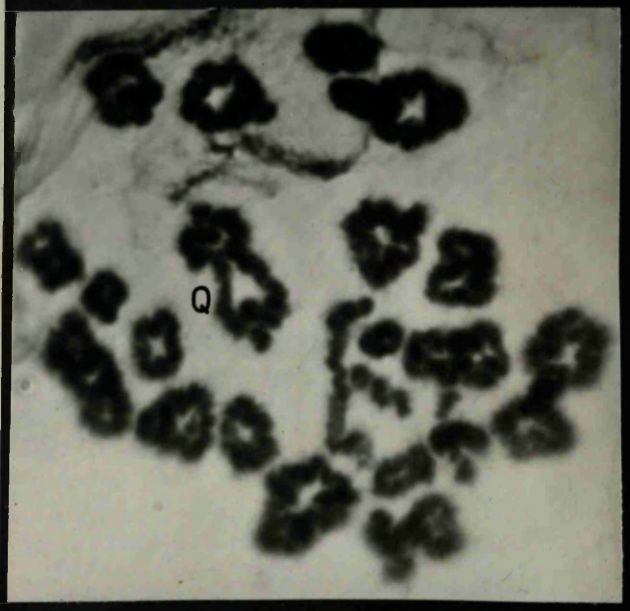
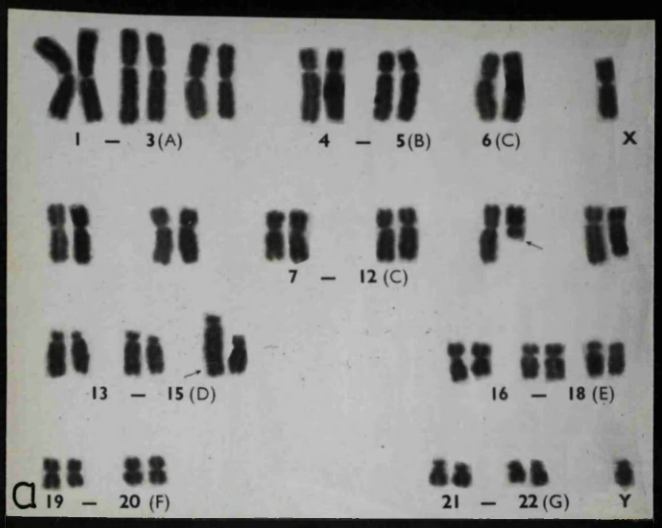


Plate 71. Diakinesis cell from JD690204 a t(7p+14q-) heterozygote. A diagram shows the quadrivalent configuration.



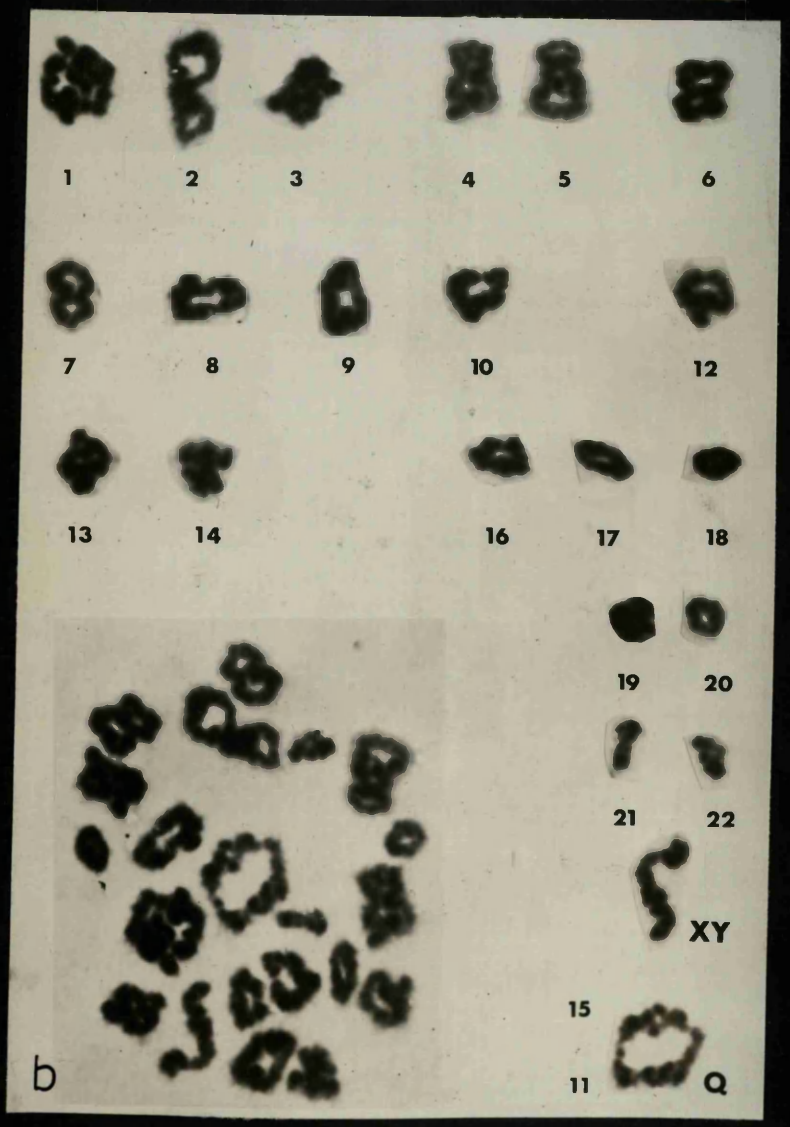


Plate 72. Cells from EA690217, a t(11q-15q+) heterozygote.

(a) Leucocyte mitotic karyotype, x 1,750.

(b) Diakinesis cell, x 1,750 showing a ring quadrivalent and two elements "missing".

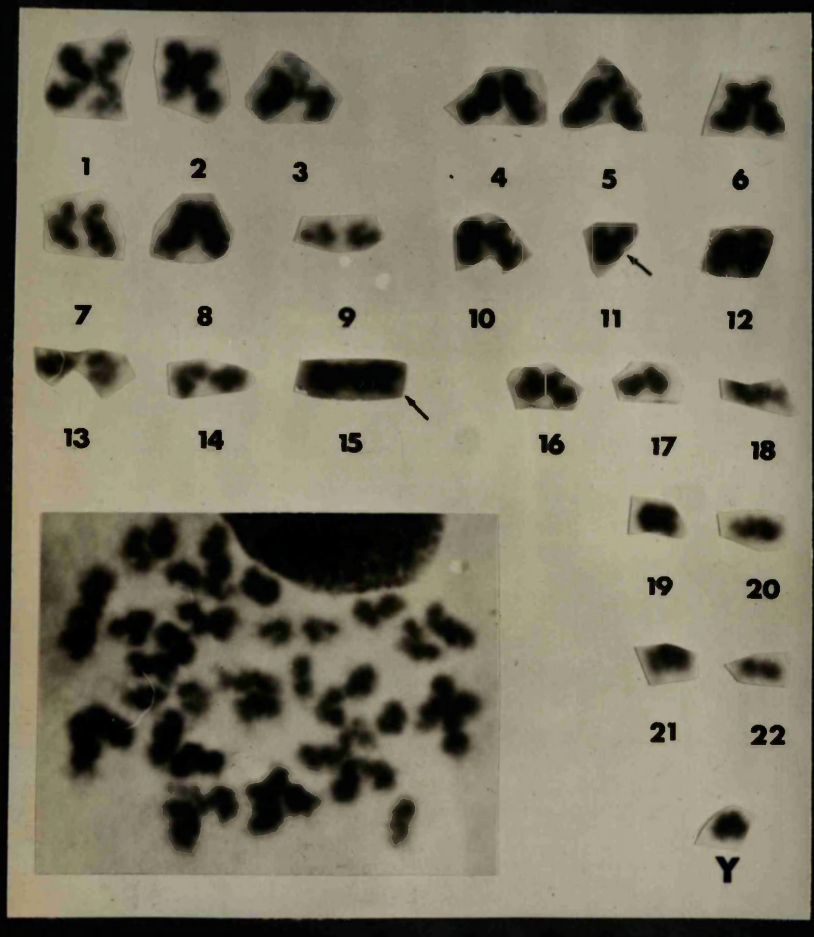
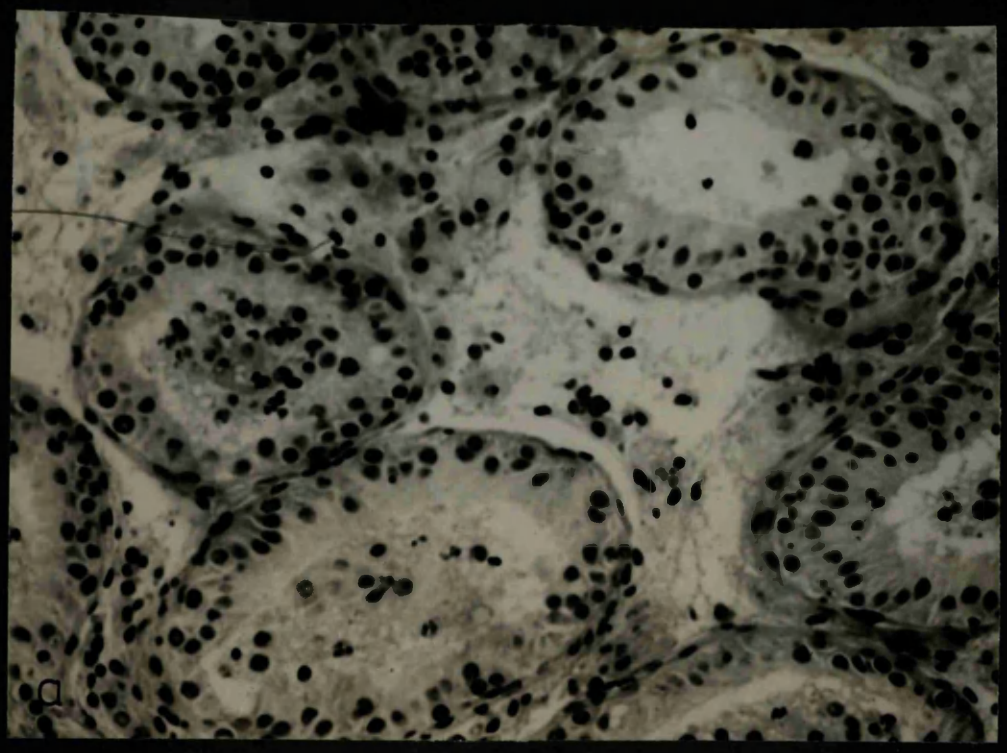
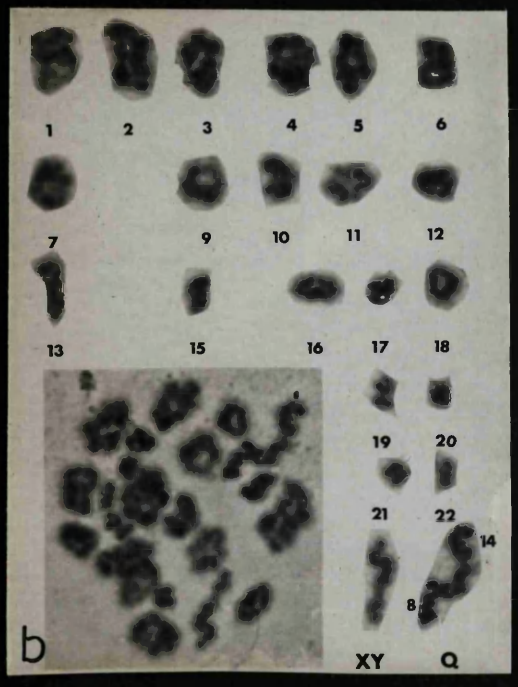


Plate 73. Metaphase II karyotype, x 2,000, from EA690217, a t(11q-15q+) heterozygote. There is a short number 11 chromosome and a number 15 with possibly one "long" and one "short" long arm which would represent cytological evidence of crossing over.





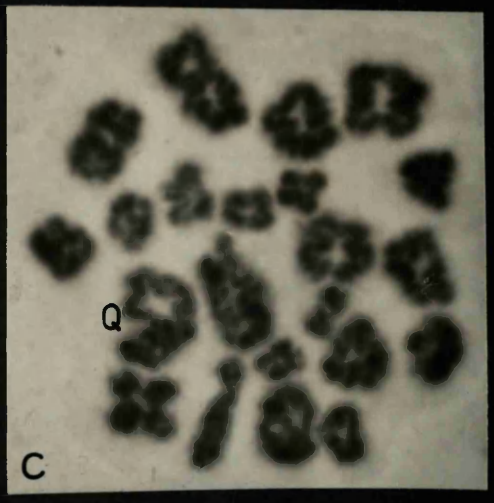


Plate 74. Material from BS700349 who had radiation damage.

(a) Section of testis, x 250.

(b) Abnormal diakinesis cell karyotyped. A quadrivalent demonstrated the presence of a reciprocal translocation in this cell. Chromosomes 8 and 14 may be involved.

(c) Another diakinesis cell with 22 elements including a possible quadrivalent.

B. MALE MATERIAL OTHER THAN HUMAN.

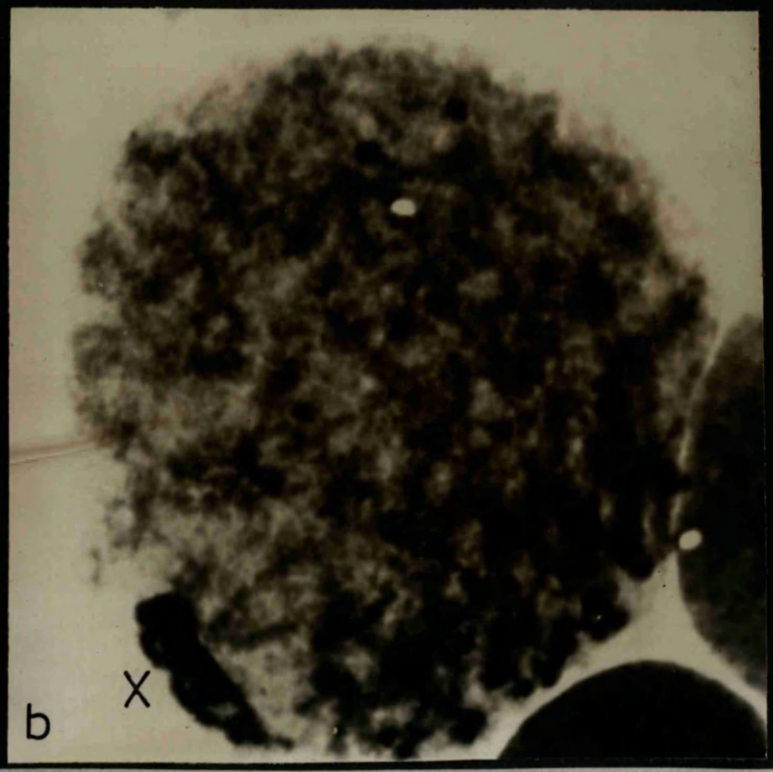
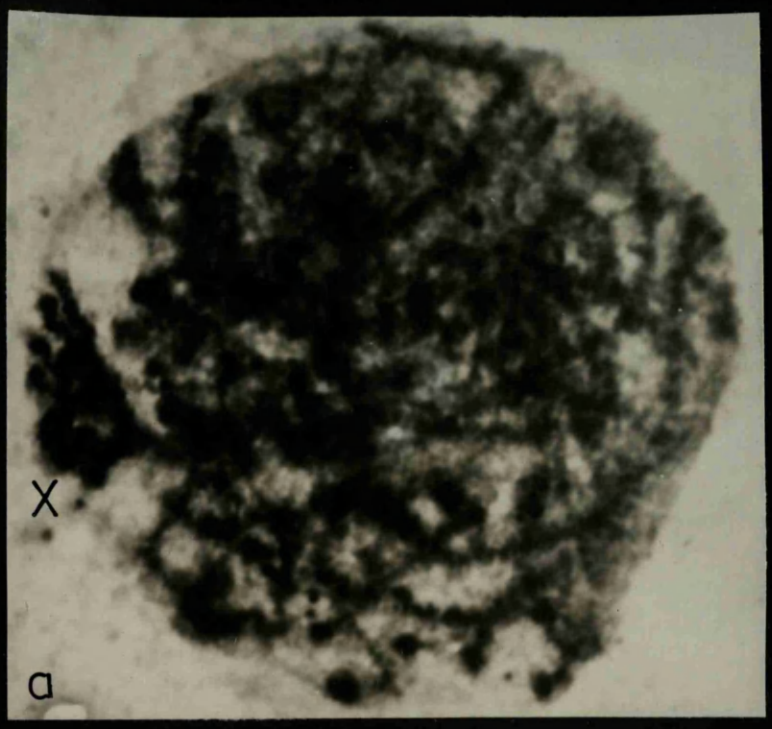


Plate 75. Locust cells.

(a) Possibly a spermatogonial metaphase showing pre-meiotic pairing of homologues at centromere regions. (This could be a first anaphase and sex chromosome complement does not clarify the point.)

(b) Leptotene. Chromosomes are condensing and pairing from telomeres situated at one side. The X chromosome is heteropycnotic.



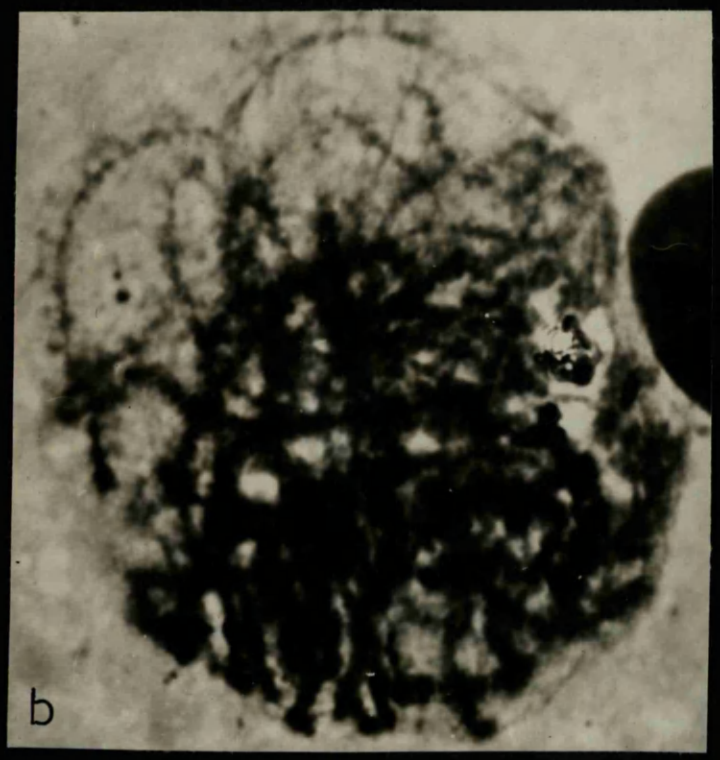
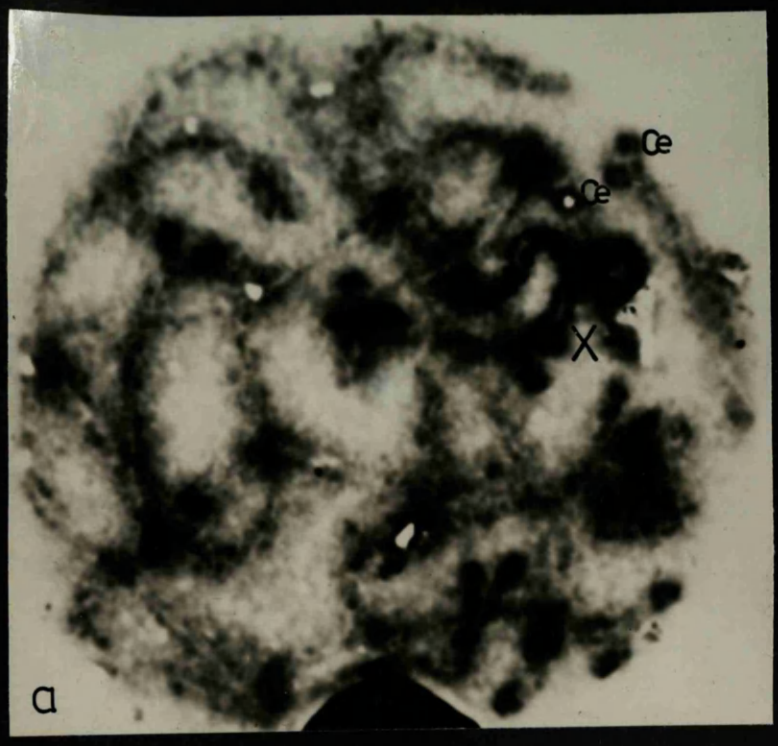


Plate 76. Locust. Further pairing of homologues gives a "bouquet" configuration.

(a) X chromosome exhibits an odd appearance and has side processes.

Plate 77. Locust pachytene cells. The sex vesicle is heteropycnotic and nucleoli are present.

(b) Centromere regions of homologues seem to repel one another.



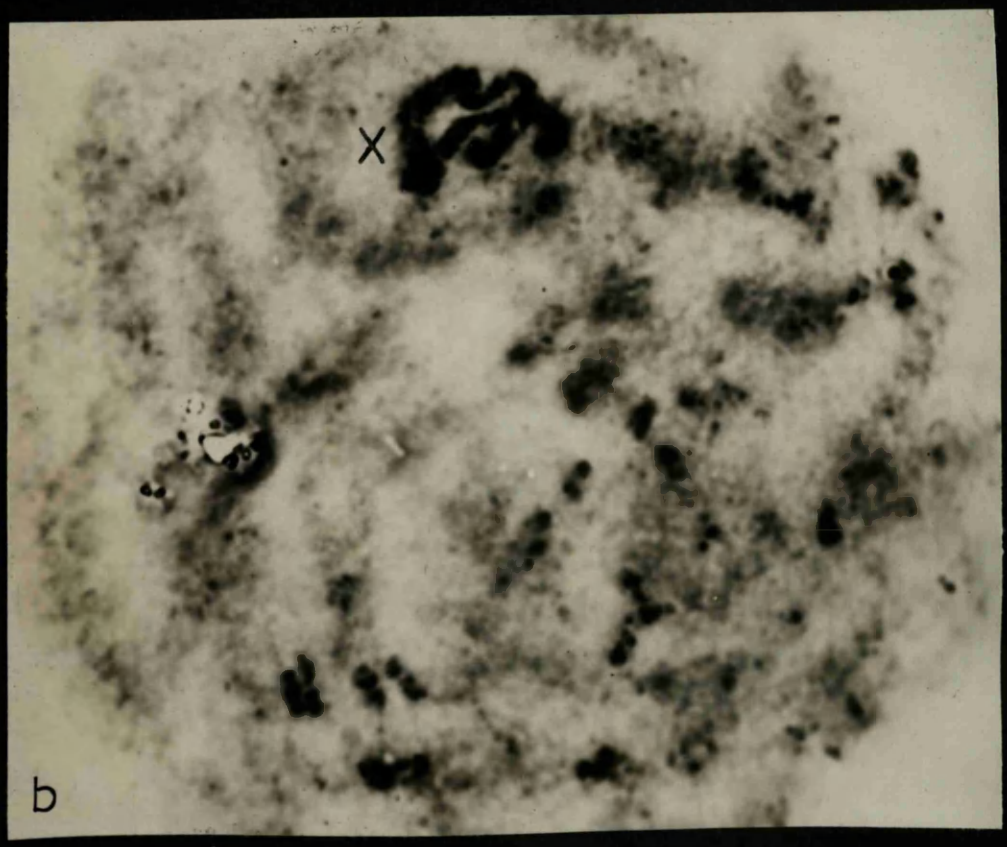
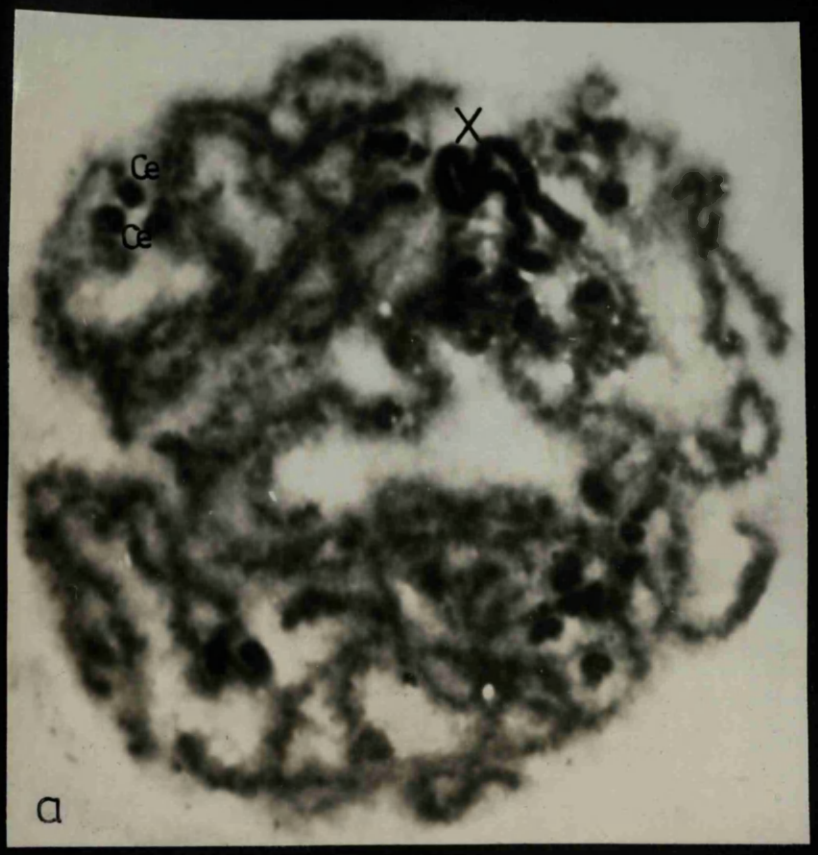


Plate 78. Locust, diffuse stage.

(a) Heteropycnotic sex chromosome begins to unravel, and the centromere regions remain heteropycnotic.



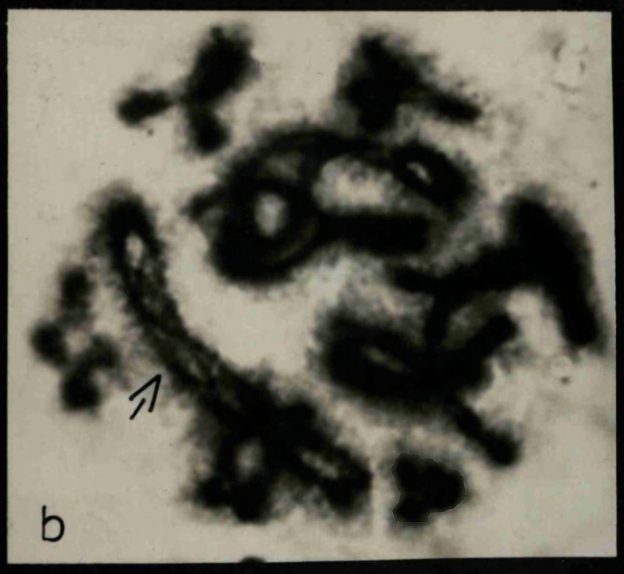
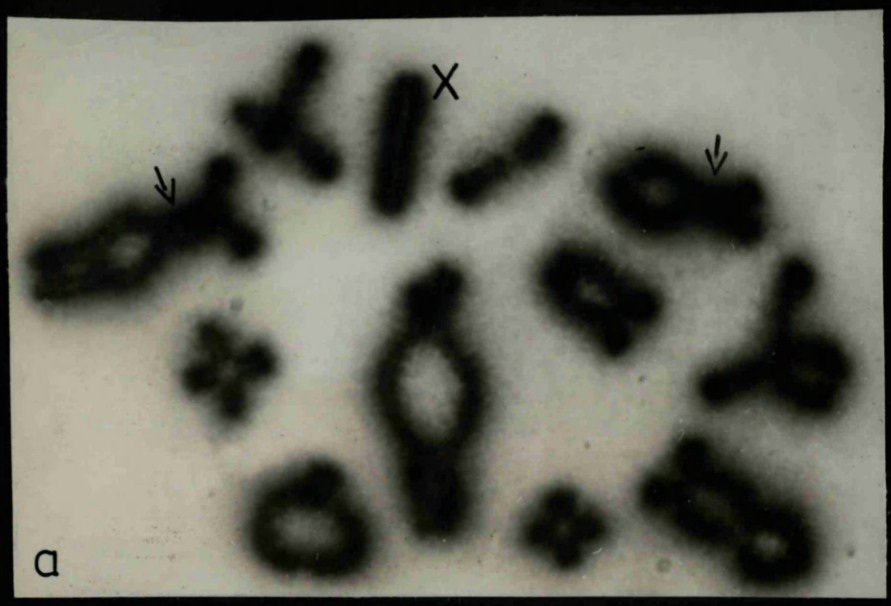


Plate 79. Locust diplotene cells.

(a) Sex chromosome and also centromere regions are heteropycnotic.

(b) The largest bivalent shows at least 6 chiasmata.



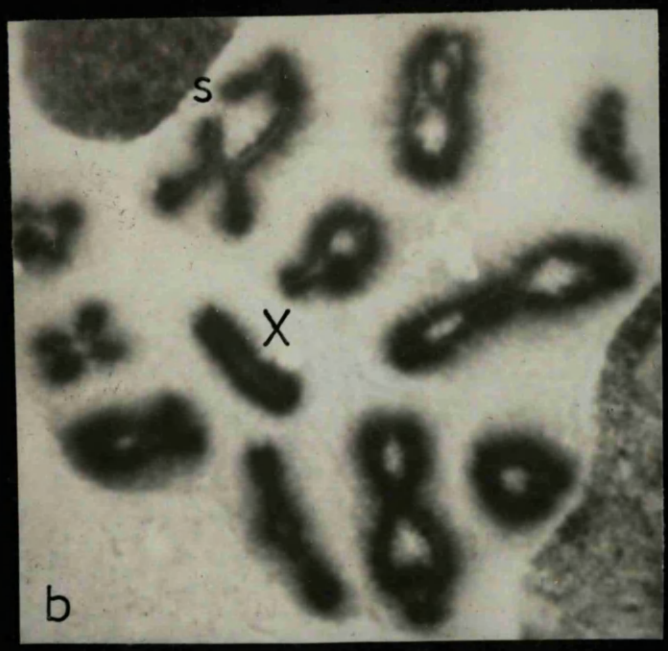
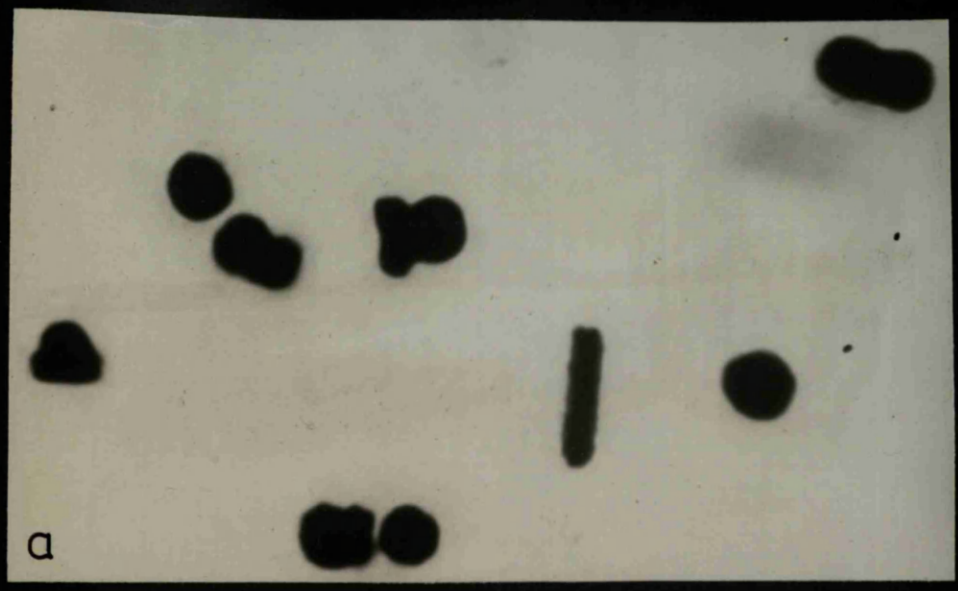


Plate 80. Locust diakinesis cells. The X is an unpaired rod and is more deeply stained.

(a) All four strands in each bivalent are visible. There are straight regions where homologues have failed to separate.

(b) There is an apparent gap in one homologue of a large bivalent.



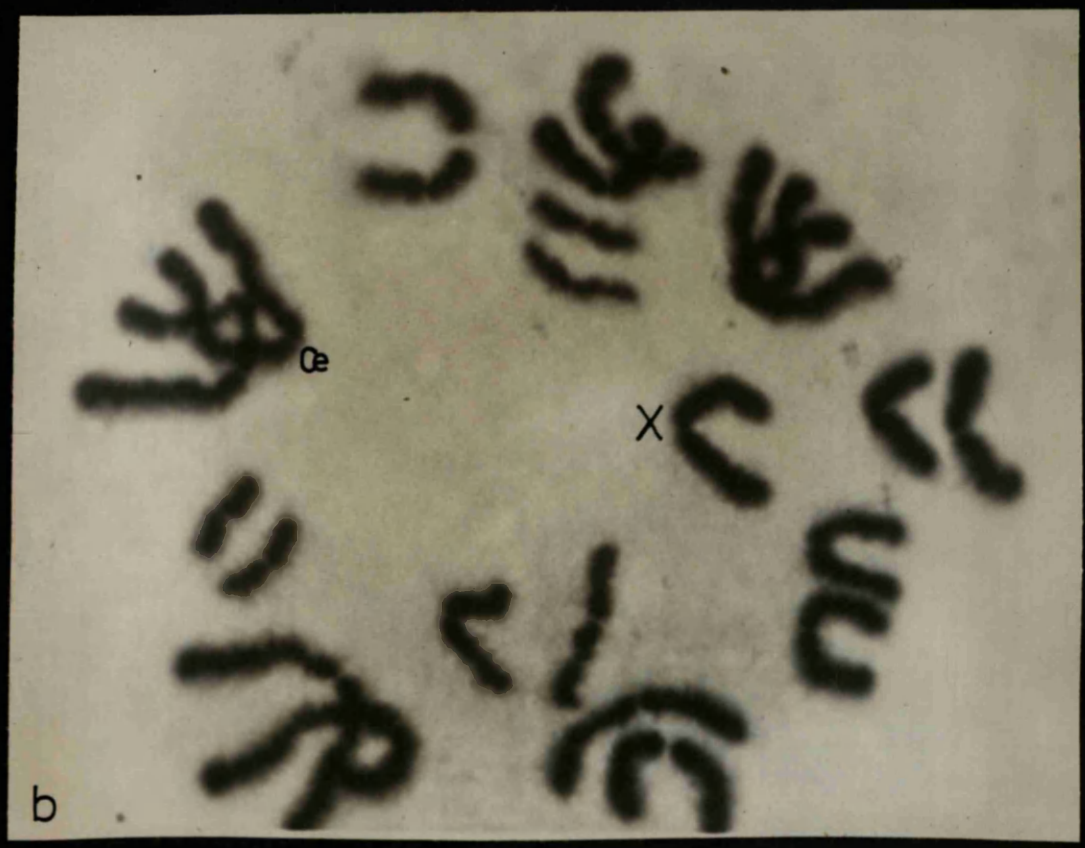


Plate 81. Locust cells.

(a) Late diakinesis. Bivalents are very contracted.

(b) First anaphase. The X segregates to one pole. Chromatids have already separated except at centromere regions.

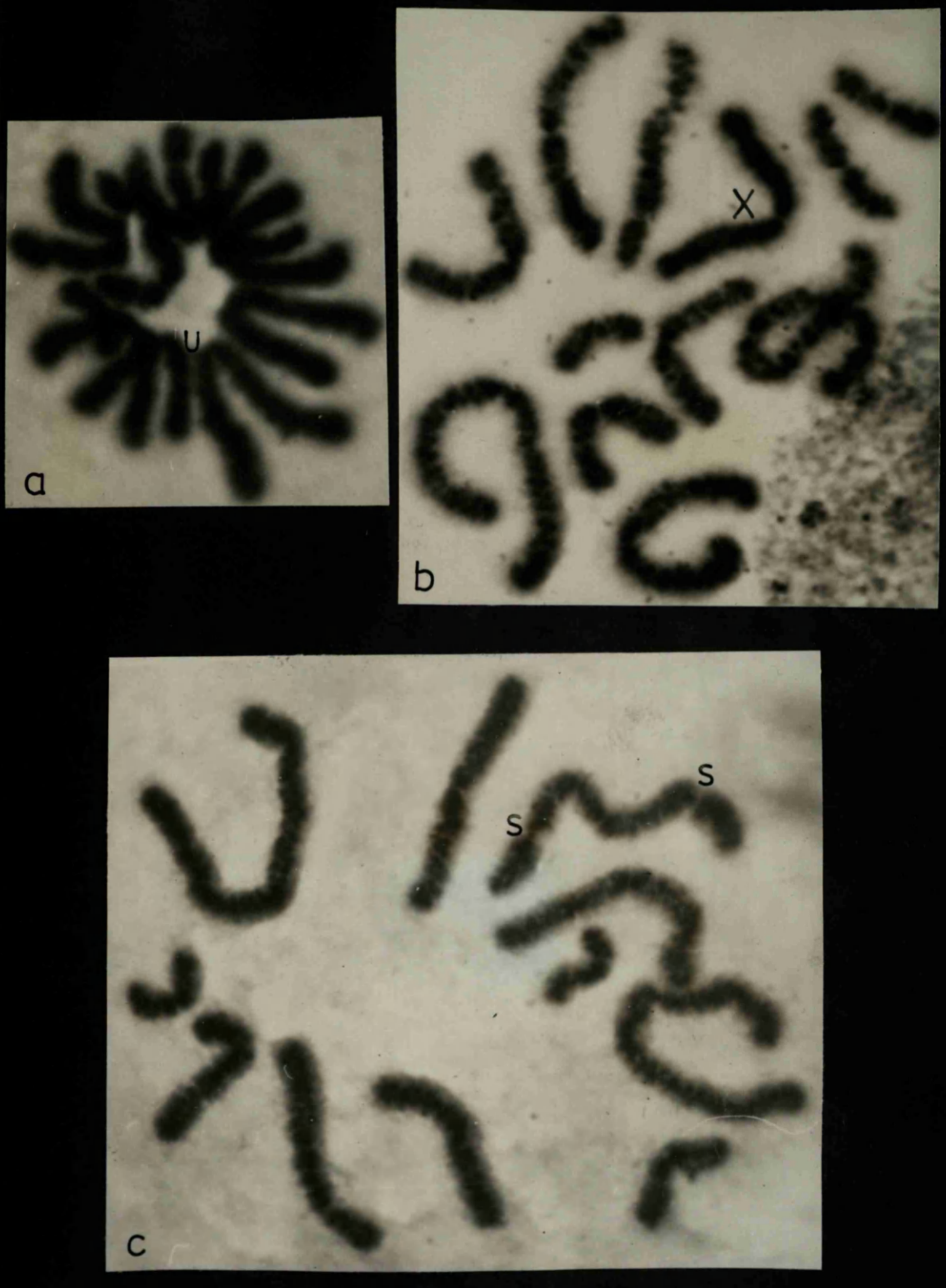


Plate 82. Locust cells.

(a) First anaphase. There is a non-staining area at the focal point of the centromeres.

(b) Metaphase II carrying the more heteropycnotic X.

(c) Metaphase II not carrying the X. Secondary constriction regions are shown as discontinuities.

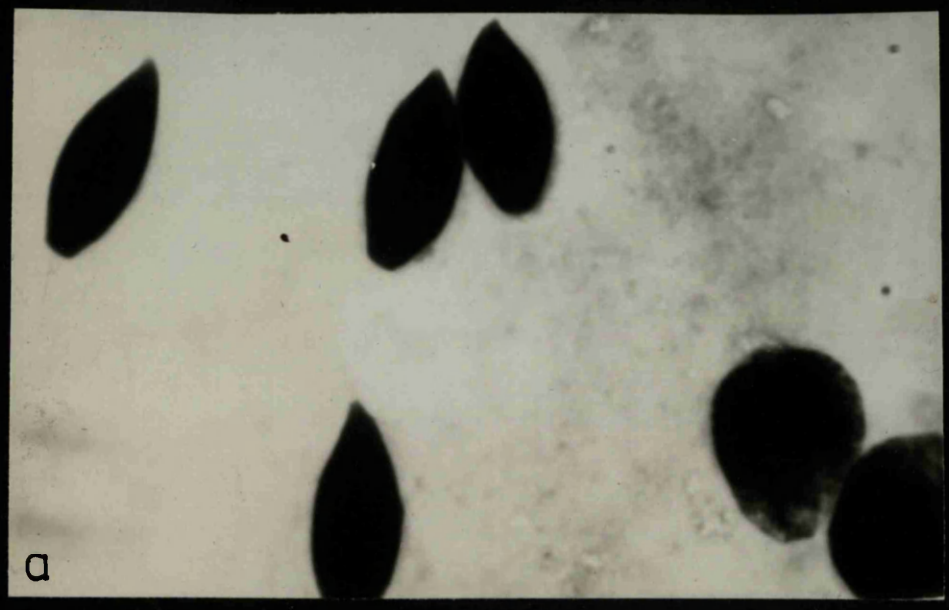


Plate 83. Locust spermiogenesis.

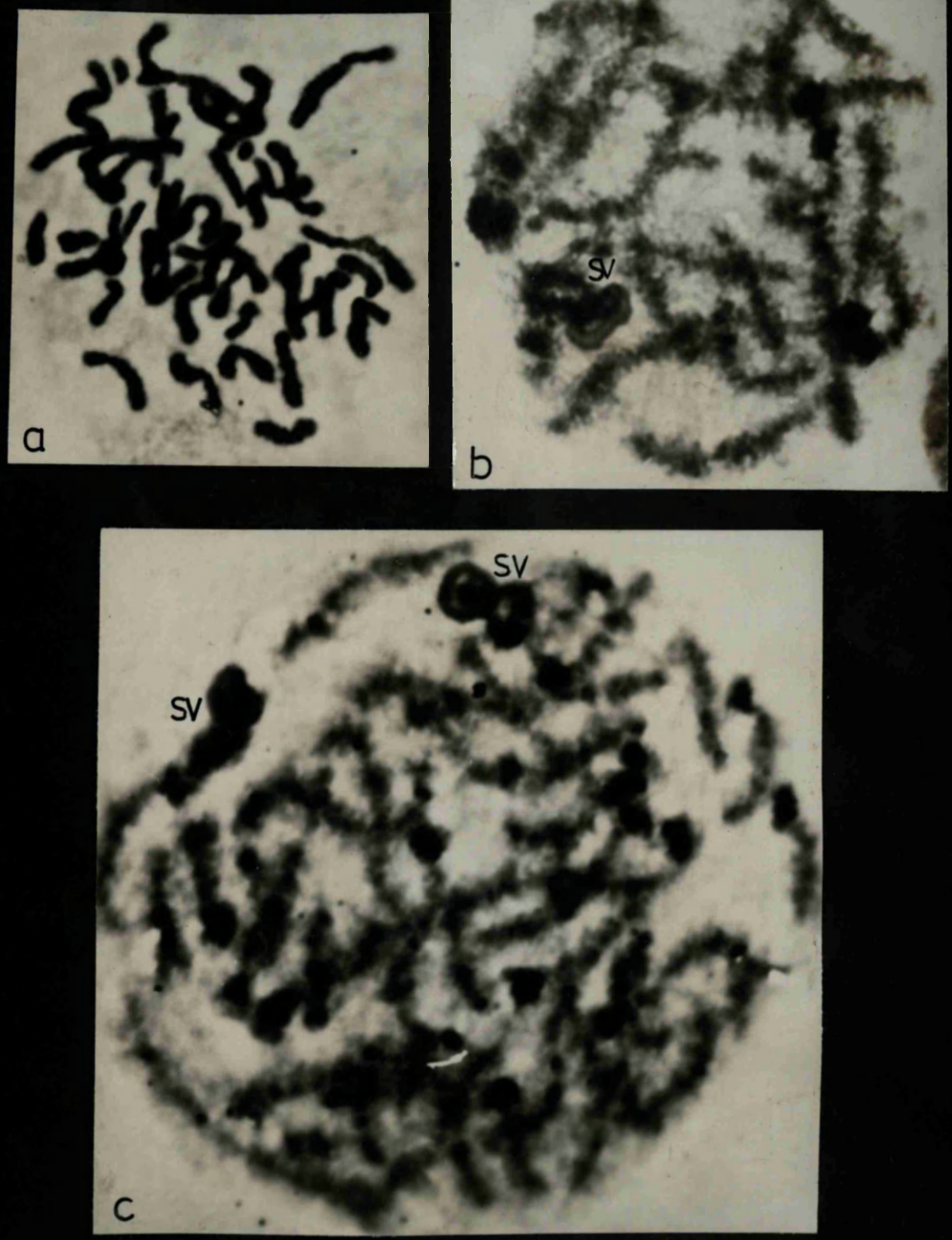


Plate 84. Syrian hamster cells.

(a) Spermatogonial metaphase.

(b) Pachytene. The sex vesicle shows the two regions which stain differently. Other heteropycnotic regions tend to associate.

(c) Pachytene. There are double the number of elements; two sex vesicles are present.

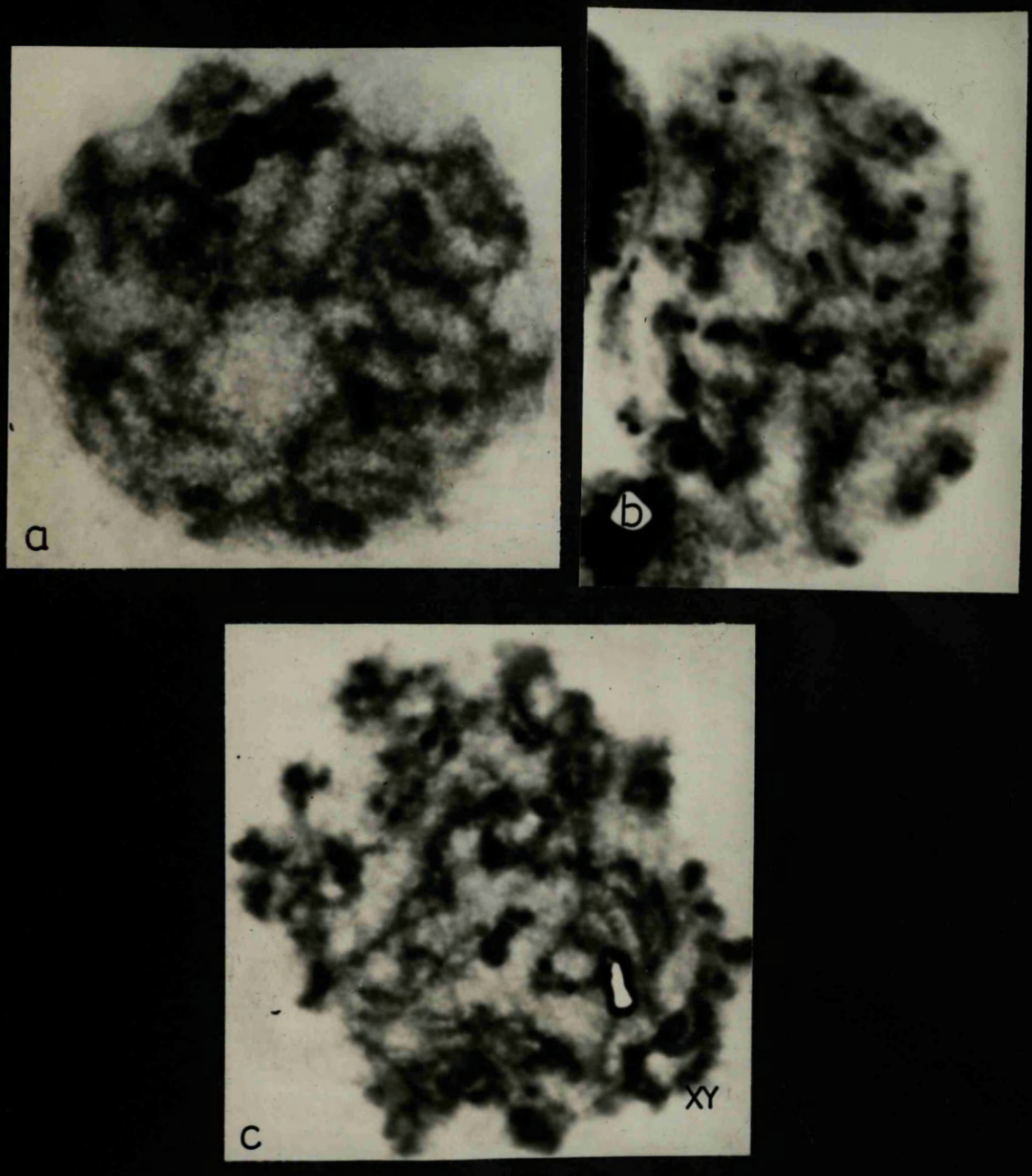
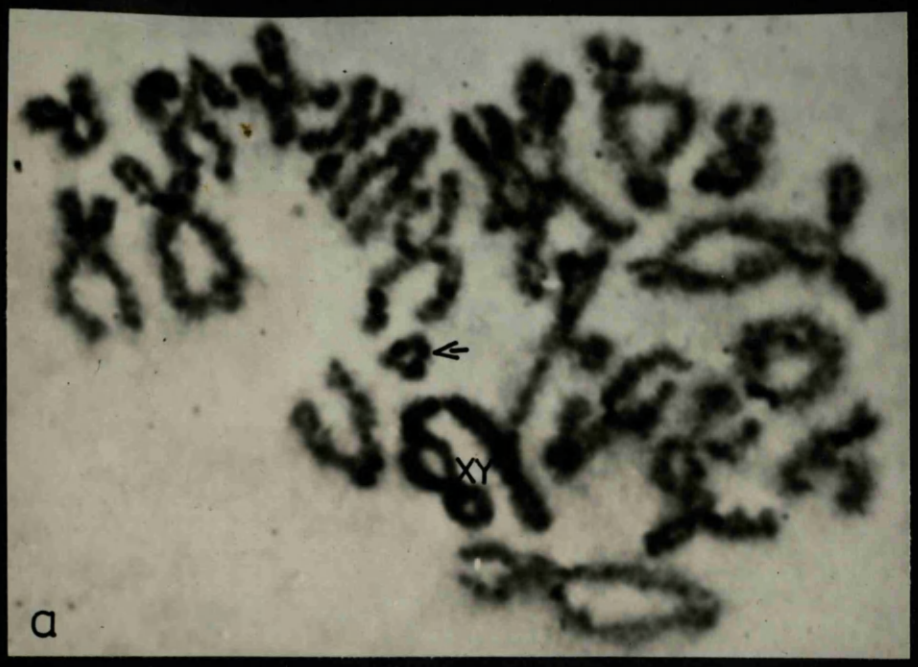


Plate 85. Syrian hamster cells in the diffuse stage.

(a) Pachytene cell entering the diffuse stage.

(b) Diffuse stage. Centromere regions appear as double heteropycnotic bodies and homologues are moving apart.

(c) Diplotene. The sex bivalent has unravelled from the sex vesicle.



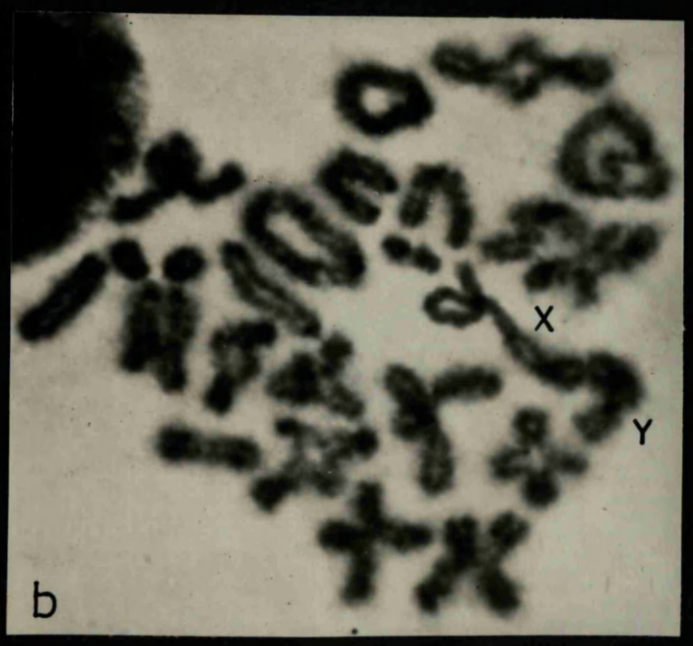


Plate 86. Syrian hamster cells in diakinesis.

(a) Early diakinesis. The sex chromosomes and some of the short arms and the smallest bivalent are heteropycnotic.

(b) The sex chromosomes are paired, the short arm of the X with the long arm of the Y. The long arm of the X is extended.

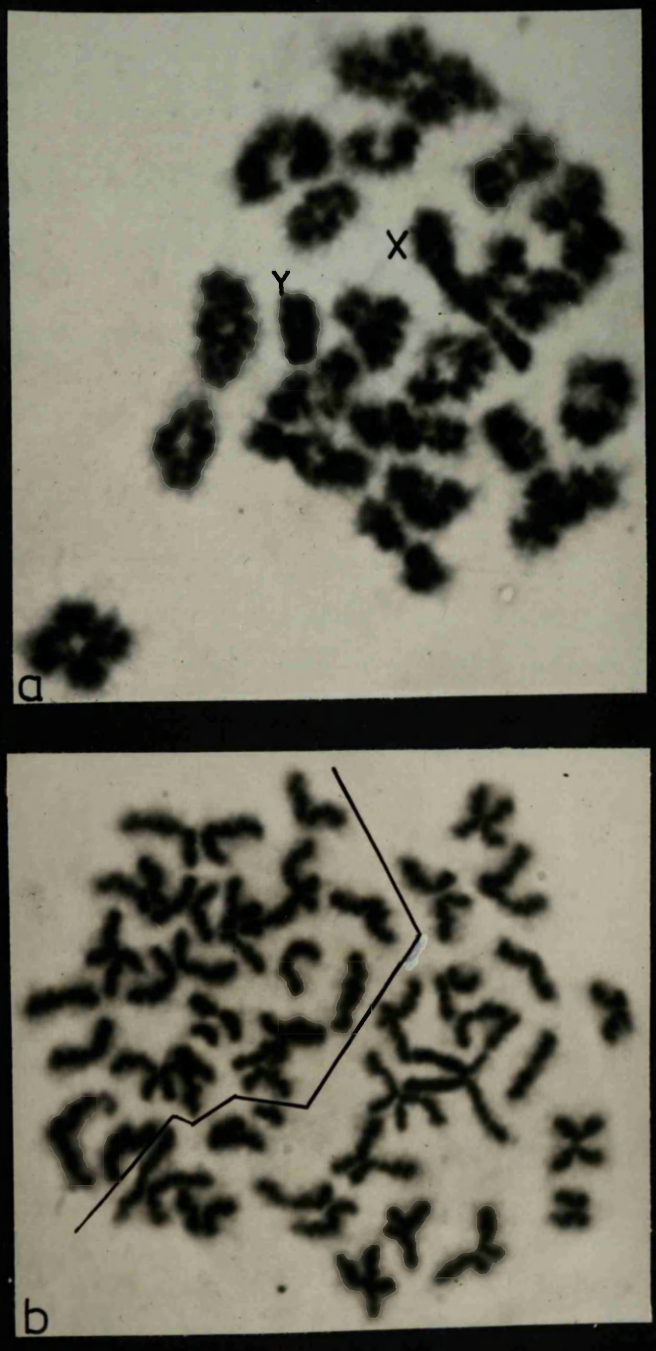


Plate 87. Syrian hamster cells.

(a) Diakinesis cell with the sex chromosomes separated (X/Y). (No hypotonic treatment was used in preparation of this cell.)

(b) Metaphase II with apparently twice the number of chromosomes. Karyotyping suggests that adjacent cells are present.

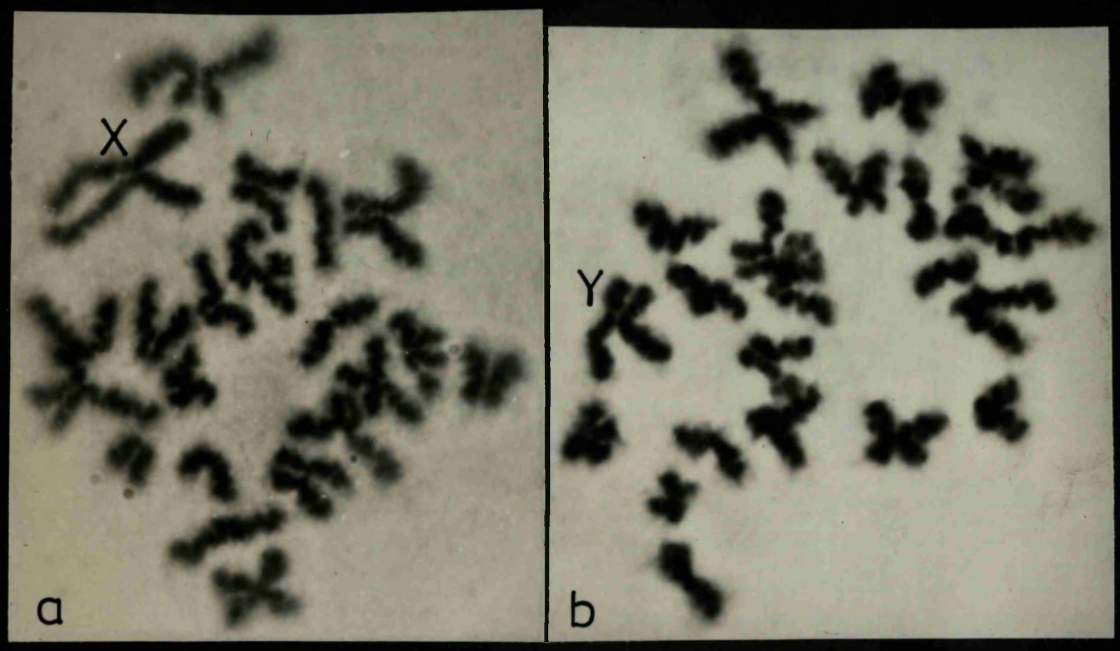
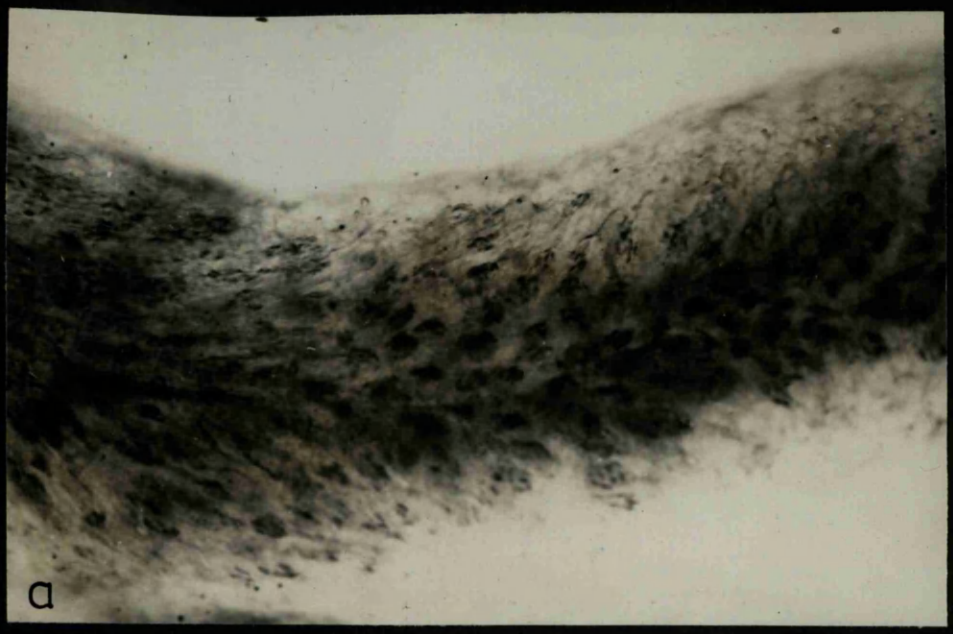


Plate 88. Syrian hamster cells.
(a) X-bearing metaphase II.
(b) Y-bearing metaphase II.
(c) Maturing spermatozoa.



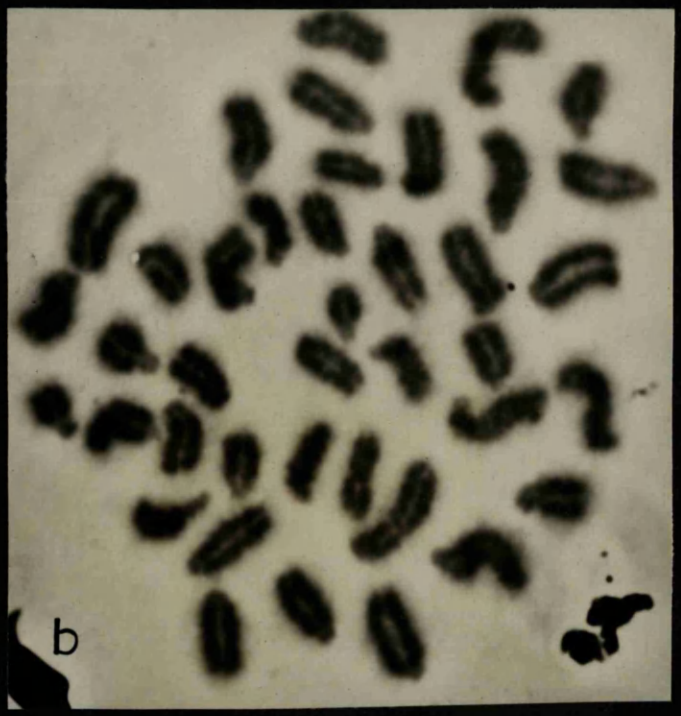
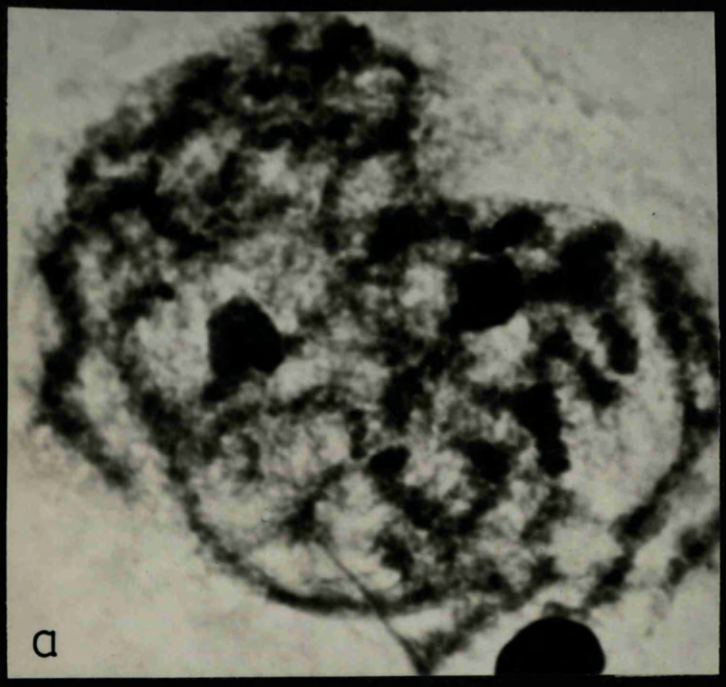


Plate 89. Mouse material.

(a) Whole testis tubule, x 250 (no staining). Active spermatogenesis is seen through the walls.

(b) Spermatogonial metaphase.



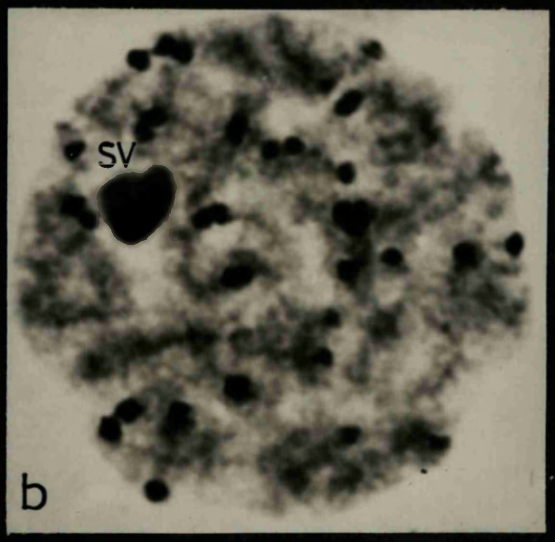
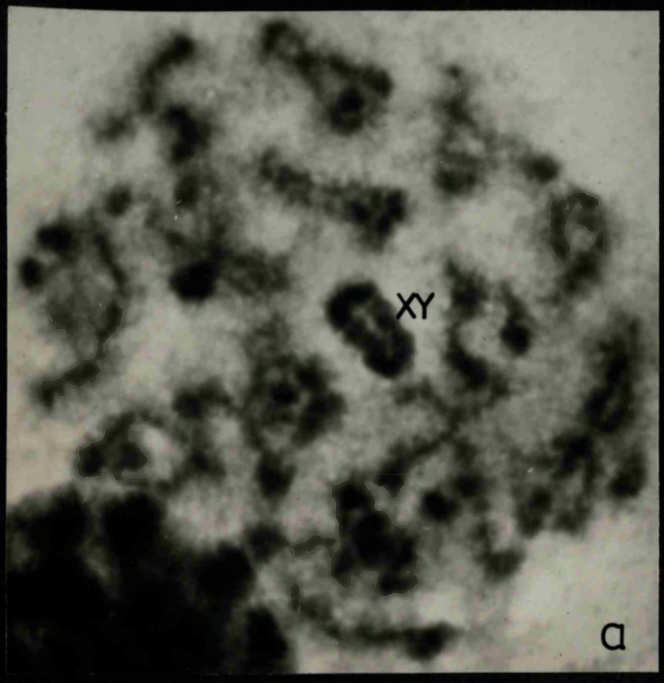


Plate 90. Mouse cells.

(a) Pachytene. Two adjacent cells.

(b) Diffuse stage. Centromere regions appear double and, as the sex vesicle, are heteropycnotic.



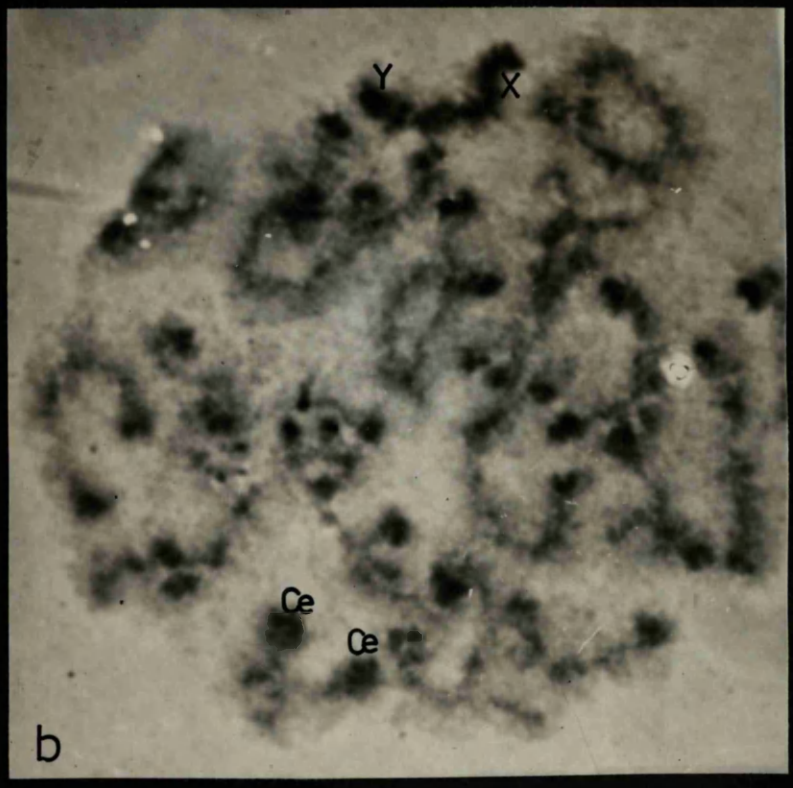
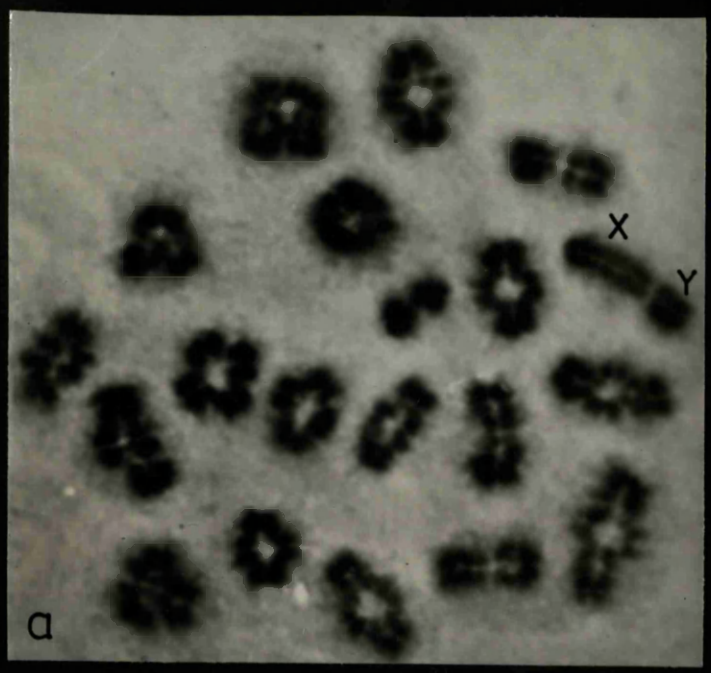


Plate 91. Mouse late diffuse stage or pre-diplotene. Centromere regions are heteropycnotic.

(a) Sex bivalent still rolled.

(b) Sex bivalent unrolled.



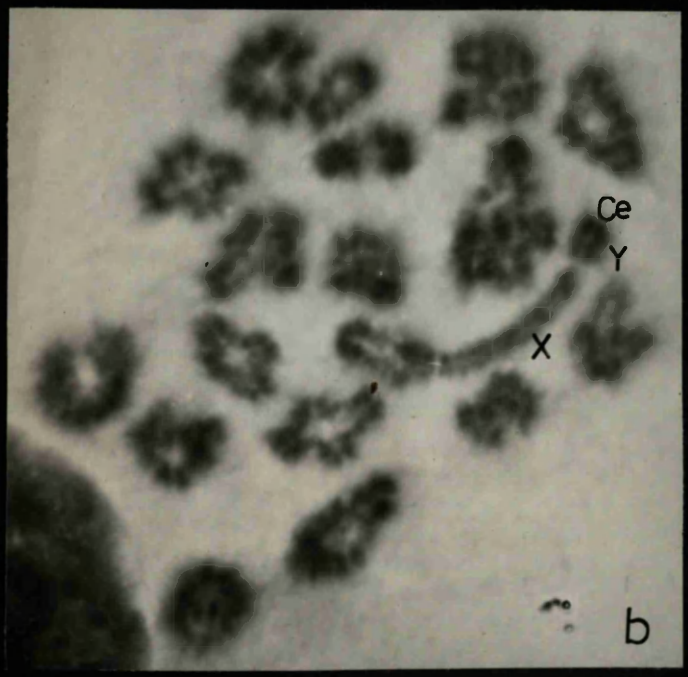


Plate 92. Mouse diakinesis cells, X and Y adjacent.

(a) Bivalents show one or two chiasmata.

(b) Heteropycnotic centromere regions demonstrate that the long arm of the Y pairs with the X. An autosomal bivalent is associated with the other telomere of the X.

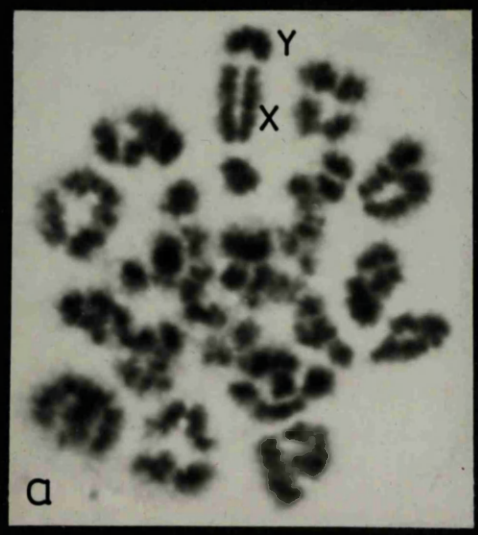
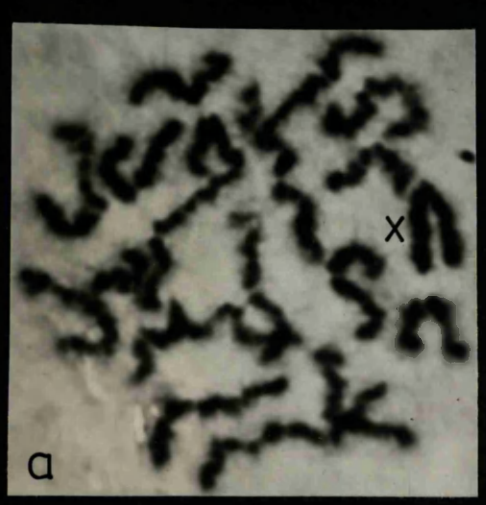


Plate 93. Mouse cells.

(a) Metaphase I. Chromatid separation shows that it is the long arm of the Y which pairs with that of the X.

(b) Adjacent metaphase II cells, probably products of one diakinesis cell. Sex chromosomes are heteropycnotic.



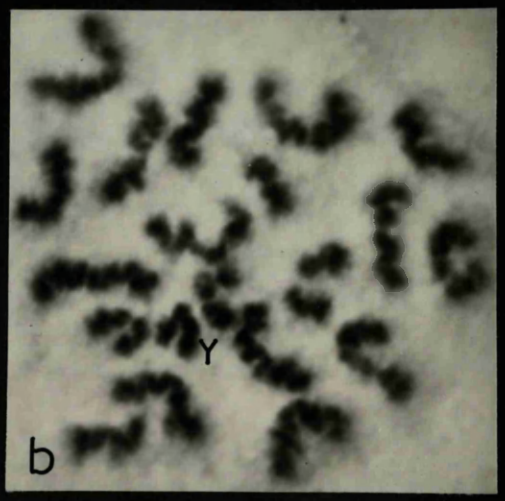
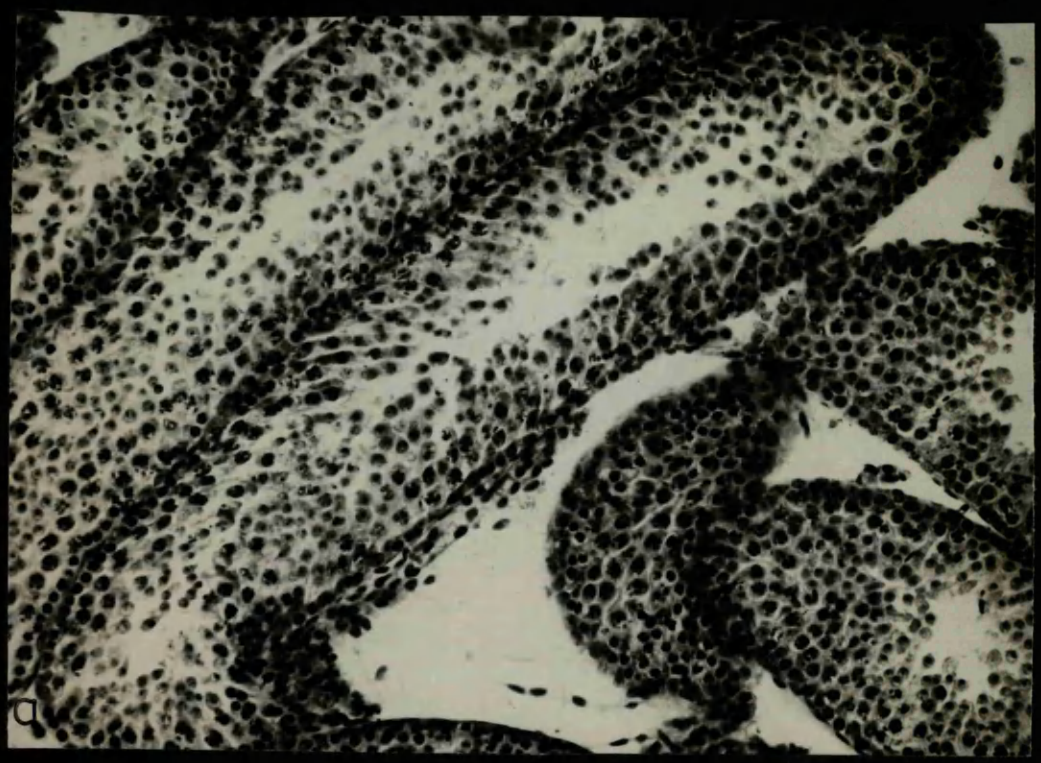


Plate 94. Mouse cells.

(a) X-bearing metaphase II.

(b) Y-bearing metaphase II.

(c) Maturing spermatozoa showing the differential staining of the sperm head.



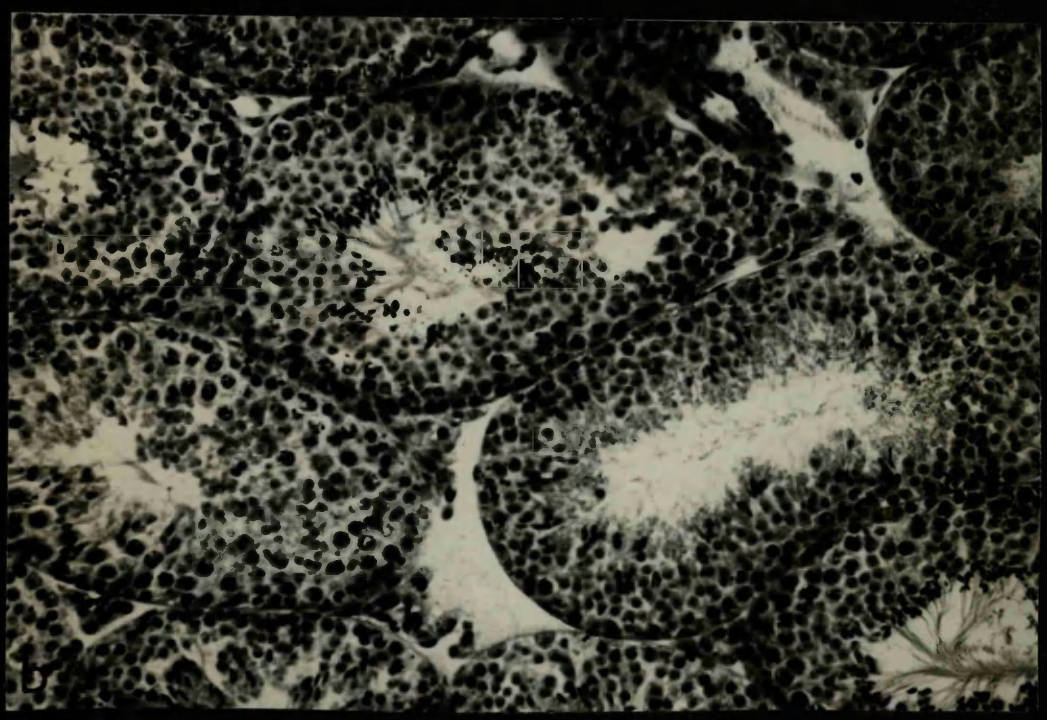


Plate 95. Sections of mouse testis, x 250.
(a) From a mouse with muscular dystrophy.
(b) From unaffected sibling.

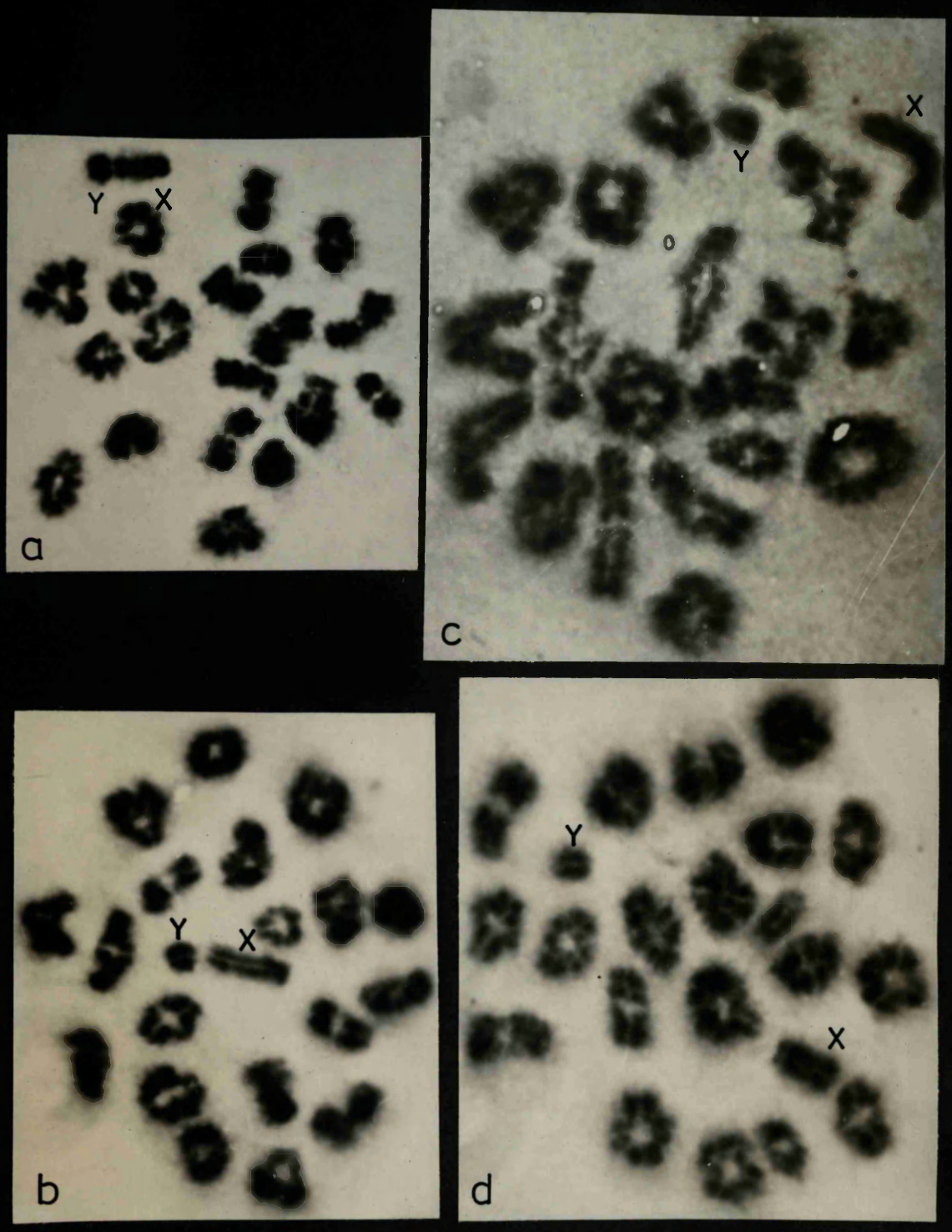


Plate 96. Mouse with muscular dystrophy. Categories of diakinesis cells are scored according to the behaviour of the sex chromosomes.

(a) XY

(b) XY gap

(c) X Y

(d) X/Y

ABBREVIATIONS

Ce	=	centromere region
Di	=	dicentric
Fr	=	fragment
N	=	nucleolus
S	=	secondary constriction region
SV	=	sex vesicle
T	=	telomere
Q	=	quadrivalent
U	=	unstained area
LL	=	chromosome with two "long" long arms
LS	=	chromosome with one "long" and one "short" long arm
SS	=	chromosome with two "short" long arms
X and Y	=	sex chromosomes
1 - 22	=	chromosome or bivalent numbers
A	=	chromosomes 1 - 3
B	=	chromosomes 4 - 5
C	=	chromosomes 6 - 12 (including the X)
D	=	chromosomes 13 - 15
E	=	chromosomes 16 - 18
F	=	chromosomes 19 - 20
G	=	chromosomes 21 - 22 (including the Y)