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SOME ASPECTS OF MEIOSIS IN NORMAL AND ROBERTSONIAN
TRANSLOCATION-CARRYING RAMS

A thesis presented in partial
fulfilment of the requirements for the degree
of Doctor of Philosophy in
Veterinary Science at
Massey University

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July 1974

ABSTRACT

SOME ASPECTS OF MEIOSIS IN NORMAL AND ROBERTSONIAN TRANSLOCATION-CARRYING RAMS

A study was made of the meiotic chromosomes in air-dried preparations from 35 genitally sound rams (*Ovis aries*). The quantity and quality of dividing cells were best when testicular material was obtained by castration under local anaesthesia from rams during their breeding season. Sodium tri-citrate solution was used as hypotonic treatment and the cells were fixed in suspension with glacial acetic acid and absolute alcohol plus a trace of chloroform. Slides were stained either with aceto-orcein or with Giemsa for a "C-banding" technique. Fourteen of the rams had a normal mitotic karyotype (54,xy) and the remainder were heterozygous or homozygous carriers of a Massey I, Massey II or Massey III Robertsonian translocation and had the following mitotic karyotypes: 53,xy,t(5q26q); 52,xy,t(5q26q) + (5q26q); 53,xy,t(8q11q); 53,xy,t(7q25q); 52,xy,t(7q25q) + (7q25q). One ram was heterozygous for both the Massey I and Massey III Robertsonian translocations.

Analysis of primary spermatocyte cells showed that a modal number of 27 chromosomal elements was present in normal rams, while in both heterozygous and homozygous Robertsonian translocation-carriers, a modal number of 26 chromosomal elements was recorded. Heterozygous carriers of the three types of Robertsonian translocations, which involved non-homologous chromosomes, were characterized by the presence of a trivalent in cells at the diplotene, diakinesis and metaphase I stages. The modal number of chromosomal elements was recorded in over 80 per cent of the cells at diakinesis and metaphase I in the heterozygous and normal rams while over 77 per cent of the cells in the Massey I homozygote and 90 per cent of the cells in the Massey III homozygote had modal counts. An association between the sex bivalent and a small autosomal bivalent was recorded in 7.5 per cent of the diakinesis-metaphase I cells from normal rams and in between 3.4 and 4.7 per cent of the cells from the translocation-carrying rams. Separation of the sex chromosomes was observed in 0.5 to 1.5 per cent of the diakinesis-metaphase I cells in both Robertsonian translocation-carrying and normal rams. However no evidence at metaphase II of the

sex chromosome aneuploidy expected if the two univalents disjoined at random at first anaphase was observed.

Eighty seven metaphase II figures from normal rams and 1,145 metaphase II figures from Robertsonian translocation-carrying rams were recorded. Detailed analysis of 1,131 cells showed that over 80 per cent of the non-polyploid metaphase II figures from the normal and homozygous rams had euploid chromosome arm counts. In contrast between 54 and 67 per cent of the metaphase II figures from the heterozygous rams were euploid. No hypermodal cells were recorded at metaphase II in either the normal or homozygous rams but from 4.5 to 9.2 per cent of the metaphase II cells in the three types of heterozygous ram were hypermodal. The results obtained in this thesis showed that the proportion of cells with chromosome arm counts of 29 was higher in the heterozygotes than in the normal or homozygous Robertsonian translocation-carrying rams. There were significantly greater numbers of cells with 29 chromosome arms than with 31 chromosome arms in the three heterozygote classes which suggested that chromosome loss due to lagging at first anaphase or technical manipulation, must have occurred in addition to non-disjunction. Statistically significant differences in chromosome arm distributions were shown to exist between rams which were heterozygous for a particular translocation and rams which were homozygous for the same translocation or normal rams. In addition to differences between individual rams, a significant difference between the Massey II Robertsonian translocation and the Massey I and III Robertsonian translocations occurred. Fewer balanced translocation X-bearing metaphase II cells were recorded than expected in three of the four Massey II Robertsonian translocation heterozygotes. Non-translocation bearing X and Y cells predominated at metaphase II in the three translocation types when total metaphase II counts were considered, and greater than expected numbers of X-bearing cells were found in both the euploid and aneuploid classes in the Massey III Robertsonian translocation heterozygotes.

Since the majority of normal ewes mated with Robertsonian translocation heterozygous rams conceived to their first service, and because no lambs with unbalanced karyotypes associated with a Robertsonian translocation have been recorded, it is suggested that only

chromosomally balanced (euploid) spermatozoa are involved in fertilization. It is further suggested that the absence of unbalanced karyotypes in progeny is explicable on the basis of a degeneration of aneuploid spermatocytes occurring prior to their maturation.

The three translocations in the homozygous state behaved as normal autosomal bivalents at meiosis with regular segregation at anaphase I.

It is suggested that the lowered fertility seen in matings involving heterozygous rams and heterozygous ewes cannot be attributed to any deficiencies in the spermatogenic function of the ram.

ACKNOWLEDGEMENTS

I wish to thank my supervisor Dr. A.N. Bruère for giving me the opportunity to undertake this study, and for his interest, enthusiasm and continuous encouragement throughout it. He has spent many hours in discussion and constructive criticism of my work and for this I am extremely grateful.

My thanks go to Professor E.D. Fielden for his criticism of the manuscript and for the use of the facilities in his department, as well as to the staff of the Veterinary Clinical Sciences Department for their co-operation and help at all times.

I have received technical assistance from Mr. C.D. Barkwith, Miss R.J. Morris and Miss P.M. Jaine and I wish to thank these people for their willing help.

The staff of the Veterinary Photographic Unit allowed me to use their facilities, and Mrs. J. Lang and her staff prepared the histological sections; my appreciation goes to these people.

I wish to thank the staff of the Massey University library, especially Miss E.M. Green and the staff of the Interloan Department, for their help.

My thanks also go to Mr. P.H. Whitehead who provided grazing for the rams used in my research.

I wish to thank Mrs. C.E. McSporran, Mrs. S. Beaumont and Miss S. Fauchelle for the help they have so willingly given on many occasions. Mrs. S. Beaumont helped prepare many of the figures whilst the French papers were translated by Miss S. Fauchelle.

Some of the tables were prepared in the Massey Printery and I am grateful to Mr. P. Herbert and his staff for the care they took with these.

I wish, also, to thank Professor R.E. Munford for his help with the statistics.

Drafts of this thesis were typed by Mrs. S.J. West, Mrs. J.M. Trass and Mrs. J.B. Pearce; my thanks are due to them. Mrs. J.B. Pearce also typed the final copy. I am very grateful to her for undertaking this arduous task and for her patience and help at all times.

The work for this thesis was carried out while the author was a recipient of a grant from the Veterinary Services Council.

Finally, I wish to express my thanks to my Parents, Bruce, and other friends without whose encouragement and support this work would never have been completed.

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CHAPTER I

INTRODUCTION

The term "meiosis" is given to the process by which four haploid cells are produced from a single diploid cell by means of two successive cell divisions with only one reduplication of the chromosomes. The genetic diversity of the gamete end-products is assured through the independent assortment of members of different pairs of chromosomes, as well as through the recombination due to exchange of chromatid segments between maternally derived and paternally derived homologues (Rhodes, 1961).

Meiosis has been well described in plants and insects (Darlington, 1931; Rhodes, 1961; John and Lewis, 1965), but only recently has it been possible to study this process in detail in higher mammals. Testicular tissue was the classical source of material for chromosome studies in animals and meiotic studies were initially done to determine or confirm the diploid chromosome number.

Improved cytological techniques have resulted in the rapid accumulation of new information on mammalian chromosomes, particularly those of man. These methods including the use of tissue culture techniques (Moorhead, Nowell, Mellman, Battips and Hungerford, 1960), autoradiography (Miller, 1970) and recently the techniques of fluorochrome staining (Caspersson, Farber, Foley, Kudynowski, Modest, Simonsson, Wagh and Zech, 1968) and Giemsa banding (Sumner, Evans and Buckland, 1971) have been applied to both mitotic and meiotic material. In man, the development of these techniques has helped to define a whole series of structural and developmental abnormalities which are directly related to chromosomal anomalies.

Although relatively few workers have studied the chromosomes of domestic animals many anomalies similar to those seen in man have been described (Fechheimer, 1971). Most of these studies have been on mitotic chromosomes and only in the last year or two have the more sophisticated techniques now used routinely in human studies, been applied to animal chromosomes. It is probable that this will produce an increase in the range and understanding of the chromosomal aberrations

found in animals.

The examination of mammalian meiotic chromosomes has enabled the detection of changes which cannot be recognized in mitotic chromosomes. These include some pericentric inversions, paracentric inversions, and certain types of reciprocal translocations, all of which are recognizable by their unusual pairing configurations at meiosis. In addition, it is also possible to study the segregation products of the first meiotic division; a prerequisite to the understanding of zygote imbalance which is such an important factor in the study of embryonic loss.

Meiotic chromosome studies have aided the diagnosis and understanding of clinical infertility in man, and examination of the early meiotic stages has provided data such as that on chiasma frequencies and pachytene chromomeric patterns.

Robertsonian translocations are probably the most commonly described chromosomal change in man (Court Brown, 1967), and have been described in domestic animals at an even higher incidence (Bruère, 1969; Bruère and Mills, 1971; Bruère, Chapman and Wyllie, 1972; Bruère, 1973; 1974). Since 1967 investigations at Massey University (Bruère, 1974) have been carried out in an effort to understand the effects of these translocations on fertility, particularly in sheep and cattle. A large flock of sheep bearing single, double (homozygous) and double (heterozygous) translocations of three different types have been bred. This thesis forms an important part of this study being a detailed examination of the meiotic chromosomes of normal rams as well as rams from the special flocks of translocation-carrying sheep.

The thesis has three aims:

1. To describe the method developed for the preparation of meiotic chromosomes from rams.
2. To describe the meiotic "karyogram" of the normal ram.
3. To describe in detail the behaviour in primary and secondary spermatocytes of the various translocation chromosomes of this unique flock of sheep.

CHAPTER II

CYTOGENETIC STUDIES IN SHEEP - LITERATURE REVIEW

II. 1. ESTABLISHMENT OF THE DIPLOID CHROMOSOME NUMBER AND MORPHOLOGY OF SHEEP CHROMOSOMES

Early work on sheep cytogenetics was concerned mainly with determining the diploid chromosome number, the sex chromosome complement and chromosome morphology. The historical findings on the chromosomes of sheep are summarized in Table II. 1.

Wodsdalek (1922; 1929) made the first studies on sheep chromosomes. Varying estimates of the diploid chromosome number and comments on the sex chromosome complement and chromosome morphology were made later by Shivago (1930), Krallinger (1931), Bruce (1935), Butarin (1933-1934; 1935), Novikov (1935) and Pchakadze (1936).

Berry (1938; 1941) found a diploid chromosome number of 54 which was similar to that found earlier by Shivago (1930) and later confirmed by Ahmed (1940), Makino (1943,a), Melander (1959), Nakanishi and Mizutani (1959), Bomsel-Helmreich (1959), Gimenez-Martin and Lopez-Saez (1962,a; 1962,b; 1966), Lopez-Saez and Gimenez-Martin (1963), Borland (1964), and Makino, Shimba, Sofuni and Ikeuchi (1967).

Ahmed (1940), Berry (1941), Makino (1943,a), Melander (1959) and Borland (1964) all attempted to classify according to their size, the chromosomes of the sheep. Uniformity in the arrangements was not achieved because there was disagreement among authors as to the morphology of the sex chromosomes.

The techniques for chromosome study available to the early workers yielded preparations greatly inferior to those produced by leucocyte culture techniques. It is therefore not surprising that considerable disagreement as to chromosome number as well as morphology occurred. Krallinger (1931), Bruce (1935), Butarin (1935) and Novikov (1935) for example, who reported 60 as the diploid number, probably counted the two arms of one long metacentric chromosome as two chromosomes lying close to one another (Berry, 1941).

In 1960, a method for the in vitro growth of leucocytes and the preparation of air-dried chromosome spreads from such cultures was

TABLE II.1
SUMMARY OF HISTORICAL FINDINGS ON
THE CHROMOSOMES OF DOMESTIC SHEEP

Investigator	Year	Breeds examined	Number of Animals	Material used	Number of Cells	Chromosome Number		Sex Chromosome Complement	Chromosome Morphology	
						Diploid (2n)	Haploid (n)		Autosomes	Sex Chromosomes
WODEDALEK, J.E.	1922	Not stated		Spermatogonial cells Oogonia		33 34	17	xo xx		
	1929	Southdown Lincoln Dorset Horn Rambouillet						xy xx		y Chromosome larger in horned than polled breeds. Suggested variation in y chromosome associated with + or - horns
SHIVAGO, P.I.	1930	Merino		Amnion Cells		54		xy		
KRALLINGER, H.F.	1931	Merino		Spermatogonial cells		50 to 60	30	xy		
BRUCE, H.P.	1935	Merino		Spermatogonial cells		60		xy	Rod-like	
BUTARIN, N.S.	1933-1934	Arkhar Fat-rumped sheep	4 10	Spermatogonial cells		54 to 56 52 to 54	x	xy xy	Majority rod-shaped	x is V-shaped y one of the smallest
	1935	Arkhar Kurdichny ram Hybrids of the above		Spermatogonial cells		60		xy		
NOVIKOV, I.I.	1935	European mouflon x Merino		Spermatogonial cells	60	60		Not identified	Elongated rods and v-shaped chromosomes	
PCHAKADZE, G.M.	1936	Georgian fat-tailed		Primary Spermatocyte cells			30	—	—	
BERRY, R.O.	1938	Rambouillet	3	Amnion cells		54				
	1941	Rambouillet		Spermatogonial cells	>65	54	27	xy	3 pairs large v-shaped 42 rod-shaped 4 bent rods	x - medium sized rod y - smallest & spherical
AHMEO, I.A.	1940	Leicester		Spermatogonial cells		54	27	xy	3 large v-shaped chromosomes one of which is x which has a submedian centromere	y - small rod-shaped subterminal centromere
MAKINO, S.	1943	Merino Karakul Corriedale		Testicular material		54	27	xy	3 pairs v-shaped 23 pairs telomitic rod-shaped	x larger than largest telomitic y minute size
MELANDER, R.Y.	1959	Karakul	2	Lung tissue from embryos	118	54		xy	3 pairs large chromosomes with submedian centromeres Rest telocentric	x - not identified y - median centromere smallest chromosome
BOMSEL-HELMREICH, O.	1959	Ile-de-France Limousin Southdown Merino d'Arles	7 1 1 3	Spermatogonial tissue	567	46 to 60 mode of 54		xy	6 v-shaped Rest rod-shaped	x - probably medium-sized y - round & smallest of complement
NAKANISHI, Y.H. MIZUTANI, M.	1959	Lamb (<i>Ovis aries</i>)	1	Lung tissue culture	50	54		xy	6 v-shaped metacentrics 40-44 J shaped subterminal centromeres. 2-6 rod-shaped chromosomes with terminal centromeres.	x - J shaped with a subterminal centromere. In length it lies between autosomes number 6 and 7. Y - rod shaped with a terminal centromere smallest of the complement.
GIMENEZ-MARTIN, G.	1962a	?				54		Couldn't differentiate.		
LOPEZ-SAEZ, J.F.	1962b					54		xy	3 pairs metacentric rest telocentric	x large telocentric y smallest telocentric
LOPEZ-SAEZ, J.F. GIMENEZ-MARTIN, G.	1966 1963	Merino ?		Bone-marrow		54 54		xx xy		
BORLAND, R.	1964	Merino Southdown Ryeland Dorset Horn Border Leicester Romney Marsh Cheviot	26 2 2 4 1 2 1	Direct examination of bone marrow following parenteral colcemid injection	1927	54		xy	3 pairs large metacentrics 23 pairs acrocentrics	x - small acrocentric y - 'dot-like'
McFEE, A.F. BANNER, M.W. MURPHREE, R.L.	1965	Suffolk & Crosses	8	Leucocyte culture	100	54		xy xx	3 pairs large submetacentric or metacentric 23 pairs acrocentric	y - small submetacentric x - large acrocentric
BUTTLE, H.L. HANCOCK, J.L.	1966			Embryo material	9	54			3 large metacentrics	
BRUÈRE, A.N.	1966	Scottish Black Face Gray Face Suffolk Border Leicester Soay Clun Forest Welsh Mountain Cheviot	22	Leucocyte culture	1831	54		xy xx	6 metacentrics 46 acrocentrics	y submetacentric x - largest of the acrocentric chromosomes. Has small short arms
GERNEKE, W.H.	1967	Merino Karakul Afrikaner		Bone marrow		54			6 large submetacentrics 46 acrocentrics	y - Submetacentric x - acrocentric and not easily distinguished from autosomes
MAKINO, S. SHIMBA, H. SOFUNI, T. IKEUCHI, T.	1967	Corriedale	2	Leucocyte culture	39	54			6 large submetacentrics 46 acrocentrics	y - smallest metacentric x - largest acrocentric
DAIN, A.R.	1970 1972	Clun Forest ?	26	Leucocyte culture Leucocyte culture	61	54 54		xy xx		x - largest acrocentric y - submetacentric
BRUÈRE, A.N.	(Pers. Com.) 1973	Drysdale Romney Cheviot Perendale Suffolk Southdown Tasmanian Merino	2030	Leucocyte culture	Several thousand	52-54 (See Table V.2).	26-27	xy xx	6 large metacentrics 46 acrocentrics	x - largest of acrocentrics y - submetacentric

published by Moorhead et al.. This technique was developed as a result of the discovery by Nowell (1960,a; 1960,b) that peripheral blood lymphocytes, when placed in suitable culture media, could be stimulated to divide repeatedly under the influence of a mitogenic agent named bactophytohaemagglutinin, a natural extract of red bean. Basrur and Gilman (1964) gave the first description of a whole blood culture method applicable to sheep and cattle. Their technique produced good quality well spread metaphase chromosomes and because of its simplicity was suitable for chromosome examination of large numbers of animals.

Using the technique of Basrur and Gilman (1964), McFee, Banner and Murphree (1965) attempted to determine the requirements for in vitro growth of sheep leucocytes and the chromosomal characteristics of such cells. They studied 100 metaphases from Suffolk sheep and their crosses and again found a modal number of 54 chromosomes. Photographic karyotypes of representative male and female cells were produced. The interpretation of the X chromosome made by McFee et al. (1965) did not agree with that of Borland (1964). In contrast, McFee et al. (1965) identified the X as the largest of the acrocentric group and the Y as a very small submetacentric chromosome. They did describe however three pairs of submetacentric or metacentric autosomes and 23 pairs of acrocentric autosomes which was in agreement with Borland (1964).

An extensive study of the chromosomes of sheep was carried out by Bruère (1966) using a modification of Basrur and Gilman's (1964) leucocyte culture method. Homologous sheep serum (10 per cent) was used in place of calf serum, and the cells were treated for 20 minutes (including centrifugation) with hypotonic; initially 1/10th Hanks balanced salt solution, and in later preparations, 0.7 per cent sodium citrate. Chromosomes were fixed with glacial acetic acid and ethyl alcohol 3 : 1, stained with aceto-orcein, and mounted in D.P.X.. Bruère (1966) examined and counted a total of 1831 metaphases from 12 rams and 10 ewes, all less than two years of age, as well as 1449 metaphases from six older intersex sheep. All hypermodal cells were analysed from photomicrographs as well as by direct microscopy. Altogether 333 karyotypes of the clearest metaphases were prepared. The chromosomes in each karyotype were arranged in descending order of size with centromeres aligned. A basic idiogram was constructed from ten of the best photographic karyotypes (five rams and five ewes). Finally a sketch

idiogram was prepared for each karyotype and chromosomes with secondary constrictions marked on this. Recorded data for each chromosome was sorted, and lines drawn to scale and measured from the sketch idiogram were added to the basic idiogram. Bruère (1956) used the term "karyotype" to describe the "systematised array of chromosomes of a cell, prepared either by drawing or photography, with the extension in meaning that the chromosomes of a single cell can typify the chromosomes of an individual or even of a species". The term "idiogram" was reserved for the "diagrammatic representation of a karyotype based on measurements of individual chromosomes in several or many cells". These terms were defined by the Denver Report (1960).

The centromere, the chromosomal site of attachment of the spindle fibre is referred to as the "primary constriction" (Swanson, 1958). Additional constriction regions "secondary constrictions" sometimes occur in certain somatic metaphase chromosomes. When found in the distal portion of an arm, they constrict the terminal segment (a "satellite") which remains attached to the main body of the chromosome by a thread of chromatin (Swanson, 1958). Secondary constrictions have usually arisen as the result of nucleolar formation (Heitz, 1931). The nucleolus decreases in volume during prophase until finally it detaches from the chromosome and disappears into the cytoplasm, leaving an uncondensed or lightly staining area in the space it formerly occupied. The nucleolus forms in the same area during telophase.

Secondary constrictions have been reported in the cat (Chu, Thuline and Norby, 1964), pig (Harvey, 1969) and the sheep (Bruère, 1966). The latter author used criteria similar to those of Ferguson-Smith, Ferguson-Smith, Ellis and Dickson, (1962), and Oppenheim and Fishbein (1965), for his analysis of secondary constrictions. An obvious constricted region distinct from the centromere in both chromatids, or a region which appeared as a sharply demarcated area of negative heteropyknosis, was taken as a "secondary constriction". Non-alignment of the chromatids at a euchromatic gap was assumed due to breakage, unless clear strands of chromatin could be seen bridging the gap (Ferguson-Smith et al., 1962; Oppenheim and Fishbein, 1965).

Bruère (1966) in contrast to previous workers, attempted to identify individual chromosomes of the sheep's complement. He concluded that clearly measurable differences existed between chromosome lengths

in the sheep, but that these differences were very small. The accuracy of visual estimation of chromosome length and position, as shown in karyotypes, proved to be low at positions below chromosome 7. There was frequently dissimilarity in length between homologues, especially in chromosome 1.

Gimenez-Martin and Lopez-Saez (1966) found that the largest chromosome had a submedian centromere, the second chromosome of almost the same length a submedian or median centromere and the third, with a median centromere, differed in size by a greater amount than the difference which existed between the first two chromosomes. Bruère and McLaren (1967) and Roca and Rodero (1971) however, found that the size of chromosome 2 was midway between that of chromosome 1 and 3. Dain (1972) claimed that the long arms of the first and second pairs of metacentric chromosomes and the fourth pair which are acrocentric, were longer in rams than in ewes, and discussed her theory that these observations are consistent with the presence of male-determining genes on the long arms of the first two pairs of autosomes. Dain's observations were not in agreement with, and no reference was made by her to, the work of Bruère (1966) or Bruère and McLaren (1967).

There was some variation both in position and morphology of secondary constrictions. The majority were found on autosome pairs 1, 2, 3 and 4 and the X chromosome. Half of the recorded secondary constrictions occurred on the three pairs of metacentric chromosomes. It was suggested (Bruère, 1966) that these may be the main nucleolar organizer chromosomes in the sheep. Subsequent evidence from autoradiographic and Giemsa banding studies has confirmed this suggestion (Zartman and Bruère, 1974). The various constrictions recorded on the remainder of the acrocentric chromosomes did not suggest any means of differentiating these chromosomes. Bruère and McLaren (1967) noted an increase in the incidence of secondary constrictions when hypotonic sodium citrate solution was used instead of Hanks balanced salt solution and suggested that this could be associated with removal of calcium from the medium during the hypotonic treatment.

The centromere position was never seen very clearly in either the metacentric or acrocentric chromosomes, although it appeared to be more central in chromosome 3 than in 1 or 2 (Bruère and McLaren, 1967).

Bruère and McLaren (1967) stressed two morphological features

in the karyotype of the sheep not emphasized by previous authors; firstly, the presence of clear, short arms on the X chromosome, and secondly the submetacentric nature of the Y chromosome. The X chromosome which was the largest of the acrocentric chromosomes had much more clearly defined short arms than those occasionally seen on the other acrocentric chromosomes.

Bruère (1966) found that the modal diploid number of chromosomes (54) was present in 87.44 per cent of cells counted from a total of 1831. Later, in a comparison of chromosome counts of 22 normal sheep with five freemartin sheep, Bruère (1967) reported a significant increase in both hypomodal and hypermodal cells with increasing age. He suggested that similar biological mechanisms to those which contribute to age aneuploidy in man (Jacobs, Court Brown and Doll, 1961) could also apply to the sheep.

Gerneke (1967) found a diploid number of 54 chromosomes in the Merino, Karakul and Afrikaner breeds. He was unable to decide which of the acrocentric chromosomes was the X, but his description of the Y chromosome was in agreement with that of Bruère (1966).

Although no features comparable to the satellites seen in human acrocentric chromosomes (Ferguson-Smith et al., 1962; Ferguson-Smith and Handmaker, 1961; 1963) were recorded by Bruère (1966), what he interpreted as associations between acrocentric chromosomes were seen. Associations between acrocentric and metacentric chromosomes were also recorded by Bruère (1966) but he could not draw any correlations between these associations and secondary constrictions. Ferguson-Smith and Handmaker (1963) found that excessive treatment with hypotonic solution appeared to reduce the frequency of chromosome associations. Likewise in the sheep, Bruère (1966) suggested that the satellite associations in the sheep might be more complex and delicate than in man and might be disrupted by technical manipulation.

The diploid chromosome number, sex chromosome complement and chromosome morphology of the domestic sheep (Ovis aries) have been established and are independent of the breed studied. Using conventional methods, identification of the individual chromosomes in the karyotype is difficult as many are morphologically similar. It is now possible to identify every chromosome in the sheep's complement using Quinacrine fluorescence techniques (Evans, Buckland and Sumner,

1973; Hansen, 1973,a) and Giemsa banding techniques (Evans et al., 1973; Zartman and Bruère, 1974). These technical advances should aid the detection of chromosomal abnormalities in sheep. This suggestion is discussed further in Chapter V.

II. 2 TAXONOMIC AND EVOLUTIONARY INVESTIGATIONS IN SHEEP

Chromosome studies have recently been applied to taxonomic and evolutionary investigations in sheep (Wurster and Benirschke, 1968; Dain, 1970; Nadler, 1971; Nadler, Lay and Hassinger, 1971; Evans et al., 1973; Nadler, Hoffmann and Woolf, 1973). Different types of wild and domestic sheep have been shown to be characterized by different diploid chromosome numbers ($2n = 54, 55, 56, 57$ and 58) with in all cases a fundamental number or the number of chromosome arms (Matthey, 1945) of 60 (Nadler et al., 1971; 1973). Examination of two other species belonging to the family Bovidae, the goat (Capra hircus) and the ox (Bos taurus), as well as the sheep has shown that although the chromosome number is variable, the fundamental number (N.F.) is remarkably constant, varying between 58 and 62 in the majority of species, (Wurster and Benirschke, 1968). This has been regarded as good evidence that the chief mechanism of karyotype evolution in the Bovidae is by Robertsonian translocation (Wurster and Benirschke, 1968). This view is supported by Evans et al. (1973) who used modern banding techniques to study chromosome homologies as well as centromeric heterochromatin in the ox, goat and sheep. These authors consider however that banding techniques are not able to give an unequivocal indication of the direction of evolution (Evans et al., 1973).

Nadler et al. (1973) suggested that although Robertsonian changes due to centric fusions between acrocentric chromosomes might be responsible for changes in karyotype in sheep from $2n = 58$ to $2n = 54$, in certain populations centric fission could have occurred to raise the chromosome number.

II. 3 SHEEP x GOAT HYBRIDS

The problem of hybridisation of sheep and goats has interested many workers in the fields of animal breeding and reproductive physiology (Hancock, McGovern and Stamp, 1968; McGovern, 1969). Inability to

produce viable hybrids led to investigations into the cytological constitution of hybrid embryos in an effort to determine whether abnormalities of chromosome number or structure contributed to embryonic death in female goats inseminated with ram semen (Berry, 1938; Buttle and Hancock, 1966; Hancock and Jacobs, 1966; Ilbery, Alexander and Williams, 1967; Roca and Rodero, 1971). The cells of the hybrid were found to contain 57 chromosomes (Berry, 1938; Buttle and Hancock, 1966; Hancock and Jacobs, 1966; Ilbery et al., 1967; Roca and Rodero, 1971), a number half way between 54 in the sheep (Berry, 1938; Makino, 1943,a; Melander, 1959) and 60 in the goat (Makino, 1943,a; Buttle and Hancock, 1966).

Berry (1938) thought that the sire contributed two large metacentric chromosomes to the hybrid. In contrast, Hancock and Jacobs (1966), Buttle and Hancock (1966), Ilbery et al. (1967) and Roca and Rodero (1971) agreed that hybrid cells contained three metacentric chromosomes, all contributed by the sire. These findings are useful as they show that goat x sheep hybrids can be distinguished from either of the parent species by examination of the karyotype (McGovern, 1969).

There is at least one report of 57 chromosomes being found in the cells of a viable chabin (sheep x goat hybrid) although the authors do not state whether this diploid number was present in all of the 35 metaphases examined (Roca and Rodero, 1971). When mated to a ram, the chabin aborted at about four months gestation (Roca and Rodero, 1971).

Buttle and Hancock (1966) did not reject the possibility that hybrid death was due to cytological anomalies because they could not determine whether the variation in chromosome number observed in the cells they examined was a feature of the cultured tissues or of the original cells. In a later publication however, Hancock and Jacobs (1966) showed that there was no evidence of any chromosomal abnormalities which could explain the embryonic mortality. Similar views were expressed by Roca and Rodero (1971), Berry (1938) and Ilbery et al. (1967). Other possible causes of death are discussed by Berry (1938), Hancock et al. (1968) and McGovern (1969; 1973,a; 1973,b).

II. 4 CYTOGENETICAL INVESTIGATIONS ON MALFORMED SHEEP

Three chromosomally distinct types of intersexes have been studied in sheep. Because of their anatomical appearance, two of them are usually referred to as male pseudohermaphrodites. This term includes male hypospadias which have XY sex chromosomes in all tissues (Bruère, McDonald and Marshall, 1969,a), and male-type freemartins (Gerneke, 1965; Bruère and Macnab, 1968). The latter have ambiguous external genitalia and testicular gonads but always XX sex chromosomes in tissues other than blood and bone marrow, which show XX/XY sex chromosome mosaicism. This mosaicism is the result of intra-uterine transplacental exchange of blood between opposite sexed fetuses during pregnancy.

A third sheep intersex is typified by rams with normal phenotype except for testicular hypoplasia, but with an XXY sex-chromosome complement similar to that seen in chromatin-positive Klinefelter's syndrome in man (Bruère, Marshall and Ward, 1969,b). Metaphases from these rams contain 55 chromosomes. Bruère et al. (1969,b) suggested that non-disjunction of the X chromosomes during spermatogenesis and subsequent fusion of the resultant X and Y bearing spermatozoon with an ovum bearing normal chromosomes or vice versa occurred, a situation analogous to the origin of Klinefelter's syndrome in man (Jacobs and Strong, 1959; Ferguson-Smith, 1961). The extra X chromosome may inhibit testicular development and spermatogenesis more in the ram than in man (Bruère et al., 1969,b). Limited data on the ram indicates that in contrast to the situation in man, "mental" abilities of Klinefelter rams do not appear very different from those of normal sheep (Kilgour and Bruère, 1970).

Rams which were heterozygous for Robertsonian chromosome translocations (Chapter V) and with deformed testes have been reported on several occasions (Bruère, 1969; Bruère and Mills, 1971; Bruère et al., 1972). The relationship between these phenotypic anomalies and the chromosome translocations is uncertain.

A possible chromosomal aberration in an acephalic-acardiac monster born as one of a set of triplets, was suggested by Dunn and Roberts (1972). These authors did not however examine sufficient cells and tissues to enable the type of aberration to be determined conclusively.

During an investigation of congenital craniofacial malformations in sheep, Henry, Ingalls and Binns (1966) reported one deformed lamb with a deletion of the short arms of one submetacentric chromosome. This defect was seen in every metaphase plate. In addition, cultures from four other abnormal lambs showed the presence in 37 out of 1218 metaphases, of chromosomes which had apparently split longitudinally through the centromere. Statistically this was a significantly higher frequency than was seen in cultures from control animals. Such "ski-pair" arrangements are usually interpreted as artefacts due to treatment of the cultures with colchicine (Henry et al., 1966). Studies on plates cultured from the blood of both normal and malformed lambs in which colchicine treatment was omitted however, also showed a higher proportion of chromosomes split at the centromere in malformed animals. The significance of these findings remains speculative.

II. 5 SEX CHROMATIN IN SHEEP

The sex chromatin body first observed in the neurones of the female cat by Barr and Bertram (1949), is clearly demonstrable in Purkinje cells from the cerebellum and ventral horn cells of the spinal cord in the ewe (Schmidtke, 1957; Bruère, 1966; Bruère and Macnab, 1968).

Because information on sex chromatin in sheep is especially useful as a diagnostic aid in studies on intersexual animals (Moore, 1966; Bruère and Macnab, 1968; Bruère et al., 1969,b), it is unfortunate that the sex chromatin body cannot be reliably detected in tissues which may have application for screening tests (Struck, 1961; Bruère, 1966; Moore, 1966).

II. 6 CHROMOSOMAL POLYMORPHISMS IN DOMESTIC SHEEP

Bruère, in 1969, discovered an autosomal translocation involving the centric fusion of two acrocentric chromosomes in five closely related Romney sheep. This was the first report of the occurrence of this type of translocation in domestic sheep. Subsequently, following extensive cytogenetic studies, two further autosomal translocations were reported, a second type in the Romney breed (Bruère and Mills, 1971) and a third

type in the Drysdale breed (Bruère et al., 1972; Bruère, 1973). The incidence, identification, behaviour at meiosis, segregational consequences and implications of these translocations are discussed in Chapter V of this thesis.

II. 7 THE CHROMOSOMES OF SHEEP AT MEIOSIS

Testicular material was commonly used by the pioneer workers in their attempts to elucidate the number and morphology of sheep chromosomes (Wodsedalek, 1922; Krallinger, 1931; Bruce, 1935; Butarin, 1933-34, 1935; Novikov, 1935; Pchakadze, 1936; Ahmed, 1940; Berry, 1941; Makino, 1943,a; Bomsel-Helmreich, 1959). In general these workers examined spermatogonial cells only, but Pchakadze (1936) gave his estimate of chromosome number from a study of primary spermatocytes, and Wodsedalek (1922), Krallinger (1931), Berry (1941) and Bomsel-Helmreich (1959) all confirmed their counts with spermatocyte material.

There is very little information on the meiotic chromosomes of sheep. Ahmed (1940), Makino (1943,a), Bruère (1969), Loir (1971) and Nadler et al. (1973) have described various stages of meiosis in the ram, and Edwards (1965) has described the maturation in vitro of ewe oocytes, but none of these authors has examined a large number of cells, nor studied in detail the secondary spermatocytes.

CHAPTER III

MATERIALS AND METHODS

In order to avoid repetition, all materials and methods used in this study of meiosis in the ram, are recorded and discussed in this chapter.

III. 1 EXPERIMENTAL ANIMALS(i) SELECTION

The rams used in this study were from flocks of sheep carrying the three recognized Robertsonian translocations, which have been established by Dr. A.N. Bruere at Massey University.

Meiosis was studied in preparations from a total of 35 rams, 14 of which had a normal karyotype and the remainder were translocation bearing animals (Table III. 1).

TABLE III. 1

<u>NUMBERS AND KARYOTYPES OF RAMS</u>			
<u>EXAMINED</u>			
<u>Ram Type as Determined by Leucocyte Culture</u>		<u>Number of Rams</u>	<u>Number of Meiotic Preparations</u>
Normal	(54xy)	14	24
Massey I heterozygote	(53xy)	9	14
Massey II heterozygote	(53xy)	4	6
Massey III heterozygote	(53xy)	5	8
Massey I homozygote	(52xy)	1	1
Massey III homozygote	(52xy)	1	1
"Double" Translocation heterozygote (MI & MIII)	(52xy)	1	1
Total		35	55

Selection of individual rams for meiotic investigation was based on four criteria - history, genital examination, semen analysis and mitotic karyotype.

(A) History

"Normal" rams were healthy, genitally sound (Chapter III, section 1 (i) (C) (D)) and had a karyotype of $2n = 54,xy$.

All the Massey I heterozygous rams were related to a propositus Romney ram (M61/66). This ram was born as a twin to a ewe (M62/66) and was ascertained from his testicular hypoplasia. M61/66 had small, abnormally shaped testicles and was azoospermic, although he showed normal libido in the presence of ewes on heat. Leucocyte culture revealed that M61/66 had a Robertsonian translocation between two acrocentric chromosomes and a resultant number of 53 instead of the usual 54 chromosomes (Bruère, 1969).

One homozygous Massey I translocation ram was studied. This ram was produced as a result of mating a heterozygous ram (B236/67) to a heterozygous ewe. B236/67 was one of the heterozygotes included in the study.

The Massey II translocation heterozygous rams were offspring from the mating of normal ewes to B131/71, a Massey II heterozygous ram which was the son of ewe A102, one of the first metacentric (Massey II) translocation animals found.

Two of the Drysdale (Massey III) translocation heterozygotes came from the Utuwai flock, a pedigree breeding flock. The other three were offspring of matings of homozygous rams A134 and D12/70 to normal ewes.

The "double translocation" ram (B9/72) which was the first recorded sheep of this type (Bruère and Chapman, 1974), was the offspring of a Massey III homozygous ram (Dry 12/70) and a Massey I heterozygous ewe (M283/69). B9/72 had a karyotype of $2n = 52,xy$, having received one Robertsonian translocation chromosome from each parent.

Individual rams are discussed in greater detail in later sections of this thesis.

(B) Management

Experimental rams were grazed rotationally on perennial ryegrass pasture. During the winter, their diet was supplemented daily with meadow hay and approximately four ounces of Moose pure linseed oilcake nuts per ram. Fresh water was always available. The rams were drenched regularly with a broad spectrum anthelmintic and vaccinated twice against footrot. Late in October, and again in April, all rams were shorn, special care being taken of the scrotal area.

Initially the rams were brought indoors, housed in concrete pens, bedded down on straw and fed hay and nuts for up to one week prior to castration. Latterly, they were brought from, and returned to, the paddock on the same day as they were castrated.

(C) Genital Examination

Rams were examined and found to be genitally sound (Bruère, 1970). Testicular size and tone were assessed by manual palpation. The testes were classed as small, medium or large and their tone as soft and dull, firm and elastic, or firm and dull.

It is generally accepted that testis size is a reasonable guide to spermatogenesis in the ram and that increases in size are due mainly to increased activity of the seminiferous tubules (Watson, Sapsford and McCance, 1956; Voglmayr and Mattner, 1968). However, there is little evidence that testis size is closely related to fertility (Bruère, 1970).

In relation to testicular tone, it is generally accepted that firm and elastic testes produce better quality semen than soft and flabby testes (Dun, 1956).

(D) Semen Analysis

a Collection

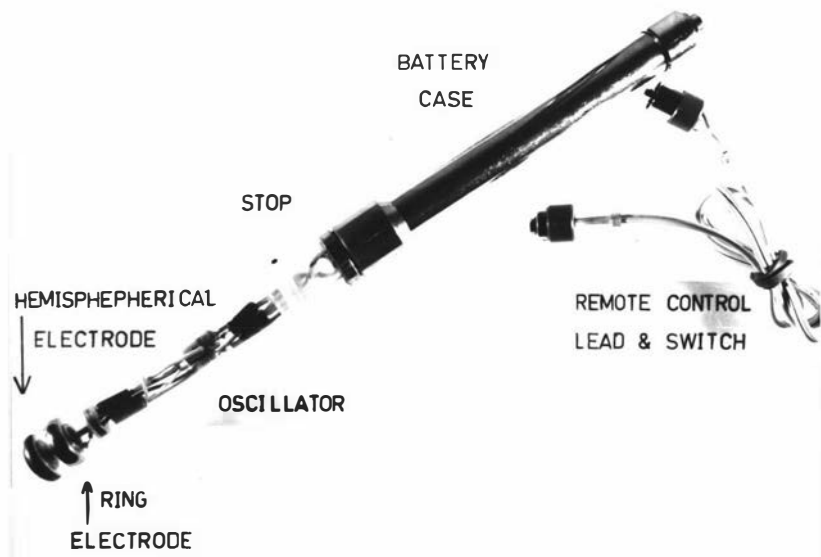
The ram was restrained in a Begg's foot trimming cradle (Appendix I) (Plate III. 1). The penis was gently extruded from its sheath, held with gauze and the urethral process directed into the collection tube. A transistorized probe (Appendix II), (Plate III. 2) was inserted into the ram's rectum, and operated as suggested by Nichols and Edgar (1964).

Plate III. 1: Semen collection (by electro-ejaculation) from a ram restrained in a Begg's foot trimming cradle.

Plate III. 2: Transistorized rectal probe for ejaculating rams.



1



2

After collection, the semen was put into an incubator at 35°C .

The concentration of semen is less and its volume greater with collection by electro-ejaculation than in collections made following natural service (Mattner and Voglmayr, 1962). The usefulness of the parameters of volume and concentration may thus be limited in an analysis of semen collected by electro-ejaculation. It is probable however, that spermatozoa obtained by this technique reflect the state of spermatogenesis as accurately as sperm collected by an artificial vagina (Rhodes, 1971).

b Volume

Immediately after collection, the volume of semen was noted, the sperm concentration estimated visually and classified according to the criteria used by Gunn, Sanders and Granger (1942):-

creamy	(approximately	20×10^8	spermatozoa/ml)
milky	("	5×10^8	" ")
cloudy	("	1×10^8	" ")
clear	(less than	1×10^8	" ")

c Motility

A drop of semen was diluted with pre-warmed normal saline on a warmed microscope slide and a drop of this mixture transferred, using a warm pasteur pipette, to a standard depth slide and covered with a cover slip. (Saline was used to dilute the semen so that individual spermatozoa could be examined more easily (Salisbury and Van Demark, 1961)). The slide was placed on a warm stage (Appendix IIIa), maintained at $35^{\circ} \pm 0.5^{\circ}\text{C}$ and the motility assessed at a magnification of $\times 100$ (Appendix IIIb).

Both the percentage of motile spermatozoa and their rate of progression were assessed:

0 -	20%	motile spermatozoa	
20 -	40%	"	"
40 -	60%	"	"
60 -	80%	"	"
80 -	100%	"	"

- 1 oscillating movement only
- 2 slow progressive movement
- 3 moderately rapid progressive movement
- 4 rapid progressive movement
- 5 very rapid progressive movement

d Proportion of live spermatozoa

A small drop of semen was gently mixed with a few drops of Nigrosin Eosin stain (Hancock, 1951) which had been pre-warmed to 35°C. After staining for two minutes, a thin smear was prepared. The slide was allowed to dry in the incubator, labelled and mounted with D.P.X. before being examined under oil immersion at x 500 (Appendix IV). Dead spermatozoa stain a pink or red colour with Eosin and live spermatozoa remain colourless. Nigrosin provides a blue-black background stain. In many smears partially stained spermatozoa were observed. If these took up the stain with similar intensity to fully stained spermatozoa, they were classified as dead, as suggested by Mayer, Squiers, Bogart and Oloufa (1951) and Salisbury and Van Demark (1961).

The numbers of live and dead spermatozoa in several randomly chosen fields were counted until 100 spermatozoa had been evaluated. If present, morphologically abnormal spermatozoa were included in this count.

e Morphological examination

Routine morphological examinations were made on 100 randomly chosen spermatozoa using the Nigrosin Eosin smears prepared for live/dead spermatozoal counts. This number was based on work done by Salisbury and Mercier (1945).

Abnormal spermatozoa were classified once only according to either the most severe or the most obvious defect seen. These defects were categorised (Roberts, 1971) according to the part of the spermatozoon affected:-

- Head abnormalities including - detached or free, small, large double, elongated, pear-shaped, irregular shaped.
- Neck abnormalities including - kinked neck, broken neck, abaxial attachment of neck to head.
- Midpiece abnormalities including - bent, broken, coiled, double.
- Tail abnormalities including - returned, broken, tightly coiled, double.
- Cytoplasmic droplets - proximal and distal.

f Correlation between semen quality and fertility

Hulet and Ercanbrack's (1962) semen standards for rams with satisfactory to very good fertility were used as a guide in this study:- 60 to 90 per cent motile sperm cells with a moderate degree of motility and progressive movement, 70 to 90 per cent live normal spermatozoa, and sperm cell concentrations of 10 to 18 x 10⁸/ml (milky or creamy semen).

Semen quality has been shown to be of limited value in predicting the reproductive potential of clinically normal rams. There is probably no single criterion apart from actual conception rates which can be used as an accurate overall measure of sperm quality (Roberts, 1971; Clarke, O'Neill, Hewetson and Thompson, 1973). For this reason, semen examination was used as an adjunct to genital examination in the choice of rams with "normal" spermatogenic function for meiotic studies.

(E) Mitotic Karyotype

The mitotic karyotype of each of the rams used in this study was determined in the course of a survey carried out by Dr. Bruère, (Plates IV. 1 and V. 1-6).

Leucocyte cultures were prepared by a whole blood technique. Two 10 ml samples of blood were collected aseptically from the jugular vein into a 10 ml Venoject tube containing 143 I.U. sodium heparin. The tube was sealed in an envelope on which the sheep's tag number was written.

The leucocyte culture technique for sheep is shown in the flow-diagram Figure III. 1. Details of the materials used are given in Appendix V.

Each slide was labelled with the slide number, year and culture number. When dry, the slides were dipped in absolute alcohol stained with two per cent Giemsa for ten minutes, rinsed under tap water and dried on the hotplate.

A small amount of D.P.X. was placed on a cover slip which had been cleaned with absolute alcohol. Slides which had been soaking in Xylol were inverted onto the cover slip, placed right side up and all air bubbles gently removed.

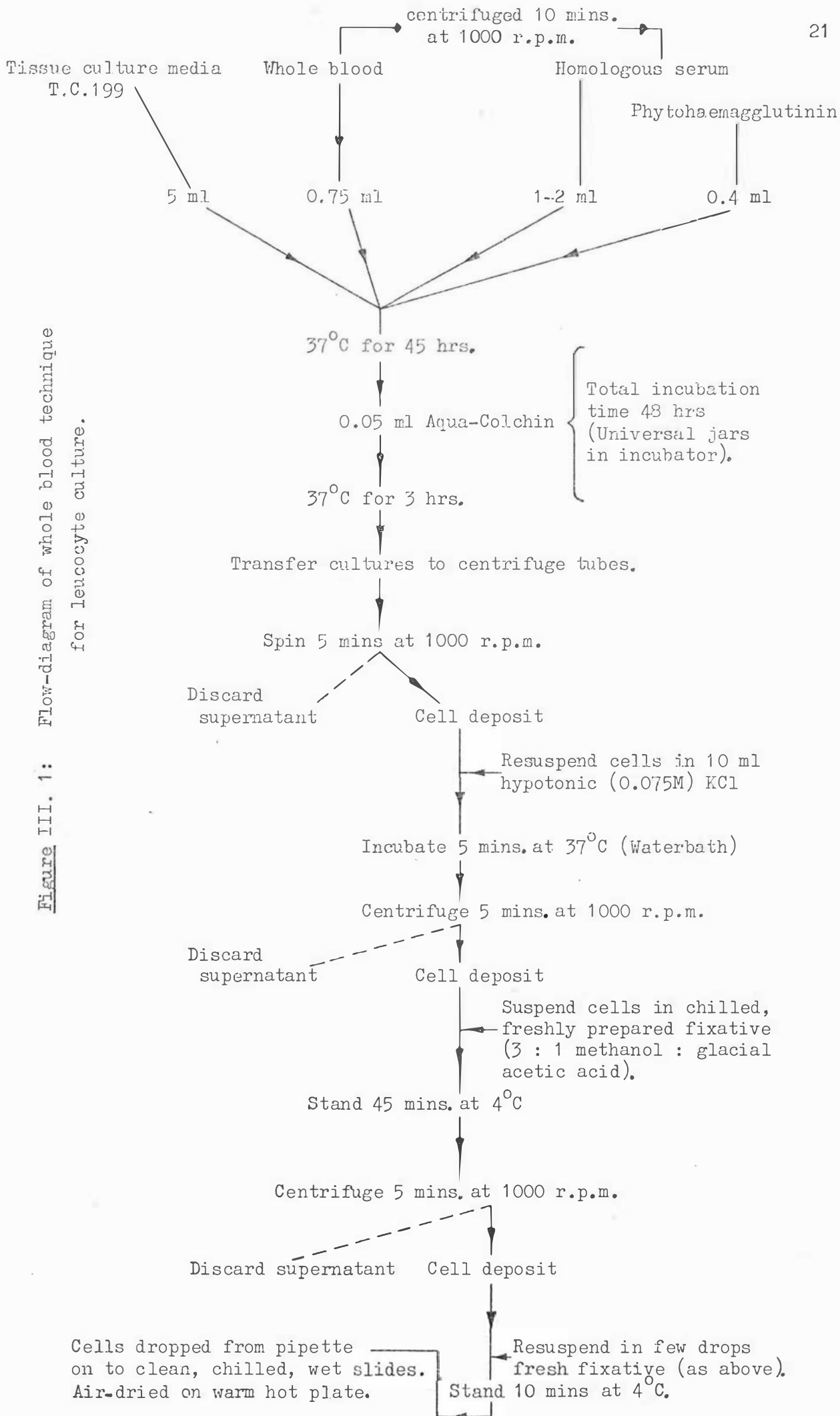


Figure III. 1: Flow-diagram of whole blood technique for leucocyte culture.

(ii) METHOD FOR OBTAINING TESTICULAR MATERIAL

Two methods, namely testicular biopsy and castration are available for obtaining testicular material from the living animal.

(A) Testicular Biopsy

Charny (1940) first described the technique of testicular biopsy in man. Since that time there have been a number of papers describing the method in both man (Charny, 1963; Rowley and Heller, 1966; Hendricks, Lambird and Murphy, 1969) and the domestic animals (Sykes, Wrenn, Moore, Underwood and Sweetman, 1949; Byers, 1953; Knudsen, 1960; McDonald and Hudson, 1960; Galina, 1971).

Authors differ in their evaluation of testicular biopsy in farm animals (Galina, 1971). Decrease in sperm concentration, morphological changes in tubules and other detrimental effects such as coagulation necrosis near the biopsy site, have been reported in the bull (Sykes et al., 1949; Gassner and Hill, 1955; Věžník, 1962; Gordon, Barr, Herrigel and Paulsen, 1965).

Conversely, Byers (1953), McDonald (1960) and Knudsen (1960) considered that the technique did not appreciably alter semen quality or testicular histology. Stipančević (1966) suggested that surgical trauma produced by biopsy in the ram caused inflammatory responses of the testicle, resulting in spermatogenic depression which was slow to resolve. Similarly in the bull, protracted recovery occurred following biopsy. In one study, four to eight months elapsed before an acceptable degree of improvement in semen quality was seen (Hill and Gassner, 1955; Gassner and Hill, 1955).

Biopsy is an accepted investigational technique in human medicine providing material for both histological and cytogenetical analysis, but the distribution of the testicular blood vessels in the ram (Harrison, 1949) makes this technique difficult (Galina, 1971). The spermatic artery forms an accessory arterial plexus in the ventral part of the testicle and haemorrhage is a common sequel to biopsy (Galina, 1971). In man, the spermatic artery does not ramify in the ventral part of the testis and the pampiniform plexus is less well developed than in the ram (Harrison, 1949). Galina (1971) has overcome the anatomical handicap described above and reported an improved biopsy method suitable for rams, boars, bulls and stallions. Satisfactory samples were obtained and the method caused neither testicular damage nor interfered with normal spermatogenesis, at least in the ram. This

method could therefore be used if small samples only are required for analysis.

(B) Castration

A second method for obtaining testicular material and the one used in this study, was that of unilateral castration. This method ensured adequate material for both histological and meiotic studies.

Samples from both testicles were utilized in the majority of rams. The second testis was removed at any time between three and 416 days after the first. It was observed that in two of the five cases of bilateral castration where the second testis was removed within ten days of the first, its weight was less than that of the first. This did not affect the result although there is at least one report suggesting that surgical interference may adversely affect the spermatogenic function of the contralateral testis (Gassner and Hill, 1955). In all other cases where weights were recorded for both testicles, the second testis was found to have increased in weight (Table III. 2) due to compensatory hypertrophy, an effect similar to that observed by Voglmayr and Mattner (1968).

Rams which were to be given a general anaesthetic, were starved overnight prior to castration. The scrotum was clipped and if soiled, cleansed with warm water containing detergent.

Pentobarbitone sodium, 60 mg/ml, was given intravenously to effect. This method was used for the first 38 operations. Subsequently, all surgery was done under local anaesthesia. For this, the ram was pre-medicated with 4 mg acetyl promazine intramuscularly, and 1.8 ml of Xylocaine was injected subcutaneously along the line of the intended incision (Appendix VI). Galina (1971) used a similar anaesthetic technique for testicular biopsy.

For castration under general anaesthesia, the ram was restrained on its side, while for local anaesthesia it was held firmly in an upright position on its hindquarters. Before surgery was commenced, the scrotum was first disinfected with aqueous hibitane and then with hibitane tincture. Sterile drapes were placed around the surgical site and the ram unilaterally castrated by the "open" method.

TABLE III. 2

EFFECT OF UNILATERAL CASTRATION ON TESTIS WEIGHT IN THE RAM

Ram Identification	Weight (g) first testis removed	Weight (g) second testis removed	Difference in weight (g)	Time interval days
X435/69	287 ^L	298 ^R	(+) 11	12
X117/69	228 ^L	413 ^R	(+) 185	128
X214/69	210 ^R	279 ^L	(+) 69	127
R244/69	242 ^R	337 ^L	(+) 95	119
B68/69	225 ^L	259 ^R	(+) 34	23
B12/69	219 ^R	244 ^L	(+) 25	5
B53/69	238 ^R	257 ^L	(+) 19	416
A182	231 ^L	207 ^R	(-) 24	6
B25/72	207 ^L	205 ^R	(-) 2	10
B66/72	206 ^L	245 ^R	(+) 39	120
HML99/58	343 ^L	374 ^R	(+) 31	3
B116/71	211 ^L	213 ^R	(+) 2	6
B76/72	187 ^L	251 ^R	(+) 64	216
B8/72	179 ^L	217 ^R	(+) 38	235

R = Right testis

L = Left testis

An incision was made down to the tunica albuginea, care being taken not to cut into the substance of the testicle, emasculators were applied across the spermatic cord and the testicle removed. The blood vessels were tied off and the incision sutured.

Immediately after removal from the body, the testicle was taken to the laboratory where it was weighed. Pieces were fixed for histological examination and other sections used in the meiotic preparations.

III. 2 METHOD FOR OBTAINING MEIOTIC CELLS

(i) PRIMARY AND SECONDARY SPERMATOCYTES

Three main techniques are available for the preparation of chromosomes from testicular material.

(A) Histological Sections

In the past, much of the work on animal chromosomes was done on sectioned material preserved with various fixatives. These sections, although time consuming to make and often giving disappointing results due to poor preservation and separation of chromosomes, were used for example in meiotic studies in the sheep (Ahmed, 1940; Makino, 1943,a), goat (Makino, 1943,a), bull (Melander and Knudsen, 1953) and boar (Henricson and Bäckström, 1964).

(B) Squash Preparations

A technique for obtaining satisfactory cytological preparations directly from mammalian gonads was first developed in 1952 by Makino and Nishimura. These authors applied to animal material the squash technique used in plant cytology. This technique had several advantages over the histological methods in common use. The procedure was simpler and gave morphologically better chromosomes than those from paraffin sections.

Various modifications of the squash technique have been used by many authors. Meiotic chromosomes prepared by squash methods have been described for example, in the cat (Ohno, Stenius, Weiler, Trujillo, Kaplan and Kinoshita, 1962), bull (Melander and Knudsen, 1953; Knudsen, 1954; Treadwell, Kieffer and Cartwright, 1968; Gustavsson, 1969),

sheep (Berry, 1941; Loir, 1971), man (Ford and Hamerton, 1956; Böök and Kjessler, 1964; Ohno, 1965) and rodents (Welshons, Gibson and Scandlyn, 1962; Brooks and Lengemann, 1967).

Teasing of testis tubules in a hypotonic solution prior to fixation and squashing, gave well spread chromosomes at the diakinesis and metaphase stages (Ford and Hamerton, 1956; Böök and Kjessler, 1964; Ohno, 1965), while prophase stages were easier to study if hypotonic treatment was omitted (Refer section III. 2 (ii)).

The advantages of squashing as described earlier are outweighed by the disadvantages of this method. These include -

- (1) Cell loss and damage when coverslip is removed (Eicher, 1966).
- (2) Repeated observations under the microscope are required for control of squashing by thumb (Rothfels and Siminovitch, 1958).
- (3) Clusters of cells (Ford and Hamerton, 1956).
- (4) When hypotonic treatment is used, few spermatogonial cells are found and those present may be broken (Ford and Hamerton, 1956).
- (5) Not enough well spread figures of a particular stage of meiosis are found in the limited number of slides which can be made in the 40 minute time limit suggested by Welshons et al. (1962).
- (6) Chromosome morphology is often poor, and nuclei are often damaged (Melander and Knudsen, 1953).

In general, squashing techniques are inferior as a chromosome spreading method to the now well accepted air-drying methods.

(C) Air-Dried Preparations

Air-drying of films of cells as described by Rothfels and Siminovitch (1958) produces well spread metaphases with superior chromosome morphology.

The first air-drying method for male mammalian meiotic chromosomes was published in 1963 by Benirschke and Brownhill. Subsequently other air-drying methods were published (Evans, Breckon

and Ford, 1964; Sasaki and Makino, 1965; Meredith, 1969; Williams, Hagen, Runyan and Lafferty, 1971). Modifications of these methods have been widely used for mammals including man and the domestic animals (Eicher, 1966; Hultén, Lindsten, Ming and Fraccaro, 1966; Ferguson-Smith, 1966, pers. comm. to Bruère; Ford and Evans, 1969; Clendenin, 1969; Hoo and Bowles, 1971; Luciani, Devictor-Vuillet and Stahl, 1971; Page, pers. comm., 1971; Polani, 1972).

The method of Evans et al. (1964) has been used successfully in cattle (Popescu, 1971,a) while modifications have been used for example, in the dog (Eliasson, Gustavsson, Hultén and Lindsten, 1967) and bull (Gustavsson, 1969). Bruère (1969) used a simplification supplied by Ferguson-Smith (1966, pers. comm. to Bruère) of the method of Evans et al. (1964), while Nadler et al. (1973) used Meredith's (1969) method for meiotic preparations from rams. Meiosis in the goat was studied by Datta (1970) who used the method of Benirschke and Brownhill (1963).

Table III. 3 summarizes the pertinent points concerning the basic air-drying techniques used for meiotic chromosome preparation.

Evans et al. (1964) attempted to overcome some of the technical hindrances produced by the "wave" nature of the spermatogenic cycle. By converting the contents of the tubules into a suspension, the clumps of spermatogonia and spermatocytes were separated into single cells and thoroughly mixed. The formation of a suspension also allowed advantage to be taken of the more even spreading of cells on the slide afforded by the air-drying procedure of Rothfels and Siminovitch (1958). Evans et al. (1964) did not recommend their method for prophase stages, and suggested that the scarcity of spermatogonial cells was due either to their greater fragility or, more likely, to the fact that they were more firmly bound to the basement membranes of the tubules and therefore less easily released into suspension.

Meredith's (1969) method which employed neither centrifugation nor fragmentation of tubules was found suitable for spermatocyte metaphases in both the rat and Syrian hamster. Meredith (1969) confirmed Benirschke and Brownhill's (1963) comment that slides could be made successfully from tubules which had been stored in fixative (at 0 to 4°C) for several weeks. This could be useful in the collection and subsequent examination of samples collected on expeditions. Like

TABLE III. 3

SUMMARY OF SOME AIR-DRYING TECHNIQUES

AUTHOR AND DATE	BENASCHKE, K. and BROOKHILL, L.E. (1965)	EVANS, S.P.: BRISCAON, G. and FORD, C.E. (1964)	FENICOM-SMITH, M.A. (1966, pers. comm. to Dr. A.N. Braide)	SASAKI, M. and MAKINO, S. (1955)	MEREDITH, R. (1969)	WILLIAMS, D.L.: HOGRE, A.A.: BUNYAN, J.W. and LAFERTY, D.A. (1971)
SPECIES	Monkey.	Nice.	Man	Man	Man, Rodent.	Dog, Rat, Hamster.
SAMPLE	Whole testis.	Whole testis or biopsy.	4-mm diameter pieces of testis	Biopsy from testis	100mg piece of testis	2-mm slices from different areas of testis.
PRE-TREATMENT	Colchicine injected prior to sacrifice. Capsules of testes removed, tubules teased and cut finely.	Suspension made in isotonic 2.2% Na citrate.	Teased in hypotonic Na citrate (as below) transferred to centrifuge tube and tubules ground with glass rod.	Seminiferous tubules minced without saline then hypotonic added.	Tubules washed in hypotonic 1% Na citrate and tunica removed.	0.9% NaCl at 38°C for 20 mins. 1 portion put into fix, other into hypotonic solution.
CENTRI-FUGATION	-	500 r.p.m. (5 mins) Discard supernatant.	-	-	-	-
HYPOTONIC	Barle's Solution.	Na citrate (3 ml).	Na citrate	Na citrate (2ml)	Na citrate (fresh) (20:1 fluid:tissue).	Buffered Na citrate.
CONCENTRATION	20%	1%	1.12%	0.6%	1%	0.8%
TOTAL TIME	30 mins. (at 37°C)	12 mins. (room temp.)	30 mins.	30 mins. (room temp.)	6 mins. (rat, hamster, rabbit, man). 12 mins. (mouse, guinea pig).	90 mins. (room temp.).
CENTRI-FUGATION	-	500 r.p.m. (5 mins)	500 r.p.m. (5 mins.)	-	-	-
	Residue pipetted and washed 3x	Discard supernatant.	Discard supernatant.	-	Tubules removed from hypotonic solution and 2 inch length tubule put into fix.	-
FIXATIVE	Methanol : Acetic Acid 3 : 1	Absolute ethyl alcohol : 3 glacial acetic acid 1 + 1 part chloroform (to 40 parts mixture)	Absolute alcohol : glacial acetic acid 3 : 1 containing 1% chloroform.	Alcohol : acetic acid 3 : 1 6 ml added to cells suspended in hypotonic.	ethyl alcohol : acetic acid 3 : 1	ethyl alcohol : glacial acetic acid 3 : 1
NUMBER OF CHANGES	3	2	2	3+	Can be stored in fix at 4°C.	3
TOTAL TIME IN FIXATIVE	30 mins + Centrifuged 1000 r.p.m. between each change of fix.	1-3 hours.	30 mins.	50 mins. Centrifuged 1000 r.p.m. 5 mins. Supernatant discarded and fix changed.	15 mins.	4 mins. 1st, placed in 2nd, 3rd change (see below).
PREPARATION OF SLIDES	Cell aggregation disrupted by vigorous pipetting. Air-dried.	Air-dried.	Cells in 2 ml fresh fix. Air-dried.	Fixative allowed to burn off slides.	Transfer tubules to 0.5 ml 60% acid acid, cells fall into suspension. Drops onto slide from micropipette. Air-dried.	Minute drops of suspension dropped onto cold wet slide allowed to dry 1 min. then place in 3rd fix in Coplin jar 10 mins. Air-dried.
STAIN	2% Acetic-orcein.	Lactic acetic-orcein.	2% natural orcein in 60% acetic acid (6 mins.).	Dilute Giemsa (20 mins.).	Lacto acetic-orcein.	Giemsa.
STAGES	All stages.	Diplotene to second metaphase.	All stages.	All stages.	All stages.	Diakinesis to second metaphase

Meredith (1969), Williams et al. (1971) considered that cell loss was reduced by not centrifuging at any stage. They commented that consistency in their results was enhanced by having the aqueous solutions used at a constant pH of 7.2 to 7.4.

Eicher (1966) found that although flaming of slides produced good spreading of mitotic metaphases and of first and second metaphase figures, some loss of bivalents at diakinesis and first metaphase occurred. She considered that 1 part acetic acid to 3 parts methyl alcohol, commonly used as a fixative for mitotic chromosomes from leucocyte cultures gave inferior results on testicular material. Better results were obtained with a 1 : 1 mixture of acetic acid and methyl alcohol (Eicher, 1966).

Eicher (1966) commented on the large number of good mitotic metaphases seen when colchicine was injected intra-peritoneally prior to the sacrificing of animals for meiotic study. Benirschke and Brownhill (1963), Clendenin (1969) and Hoo and Bowles (1971) also utilized colchicine pre-treatment of animals.

The use of colchicine in studies of meiotic chromosomes was based on the premise that since it arrested somatic cells at mitotic metaphase, it should also arrest germ cells at first and second meiotic metaphase (Clendenin, 1969). A marked increase in cells at first meiotic metaphase after one and two hour intervals of pre-treatment with colchicine was demonstrated by Clendenin (1969). His results showed that three hour pre-treatment increased second meiotic metaphase cell counts. Because colchicine would cause pain to the ram, the effects of pre-treatment with this drug were not studied. Clendenin (1969) suggested that in vitro exposure to colchicine of intact fragments or suspensions of cells in tissue culture would be an obvious modification of treatment of the live animal with this drug.

Sodium citrate is commonly used as a hypotonic agent in meiotic preparations to cause swelling of cells, disruption of the spindle and dispersion of the chromosomes. Recently however, several workers have reported advantages in using potassium chloride as the hypotonic solution. Clendenin (1969) and Luciani et al. (1971) reported sharper definition of chromosome outline and less disruption of individual chromosomes than occurred with other hypotonic solutions.

Although the fixation process was unchanged, Hungerford (1965) had noted prior to these conclusions that the mitotic chromosomes appeared better fixed after hypotonic treatment with potassium chloride. The reason for this is not understood.

A preliminary report by Sperling and Kaden (1971) suggested the use of seminal fluid as an alternative to testicular material as a source of immature germ cells for studies of human meiosis. Sperling and Kaden (1971) noticed that the percentage of immature cells increased as the sperm count decreased. Using Evans et al.'s (1964) method, they were able to make preparations of primary spermatocyte cells from seminal fluid.

Yet another method for the preparation of spermatogonial cells and spermatocytes of all stages from solid tissue was described in 1972 by Stock, Burnham and Hsu.

(D) Method Used In This Study

The method used in this study was a modification of those of Evans et al. (1964) and Ferguson-Smith (1966, pers. comm. to Dr. Bruère).

Immediately after removal from the body, the testis was taken to the laboratory and weighed. Within five minutes, pieces of tissue 3 to 4 mm in diameter were cut from the testis, the tunica albuginea removed and testis sections quickly washed in a few ml of warm (37°C) sodium tri-citrate solution (2.2 per cent) in a watch glass. The pieces were then transferred to another watch glass containing about 3 ml of 1.2 per cent sodium tri-citrate and teased and chopped, using fine forceps and a scalpel blade, until a cell suspension was obtained. This suspension was transferred to a graduated centrifuge tube. The process was repeated and more hypotonic solution was added until 10 ml of cell suspension were produced. A glass rod was used to crush the larger fragments of tubules. Two centrifuge tubes of material were processed together. They were put into a water bath (37°C) and incubated. When the tubules had been exposed to the hypotonic solution for 20 minutes, the centrifuge tubes were removed from the water bath and spun for 10 minutes at 1000 r.p.m.. The total time of exposure of the cells to hypotonic solution was thus 30 minutes. At this stage the supernatant was carefully removed with

a pipette and discarded until only 1.5 ml remained. The cells were gently resuspended in the hypotonic solution and 0.5 ml of freshly made, cold fixative (absolute ethanol : glacial acetic acid, 3 : 1 + 3 drops chloroform) was slowly added and allowed to run down the sides of the tube until a layer formed on top of the hypotonic cell suspension. Gentle pipetting mixed the fixative with the cell suspension. More fixative was added until the 10 ml mark on the tube was reached. At this stage, any large tubular fragments were removed from the tube. The tubes were then placed in a coplin jar in an ice bath and allowed to stand for 30 minutes in the refrigerator.

After fixation, the cells were again centrifuged at 1000 r.p.m. for 10 minutes and the supernatant fixative discarded. About 1 to 1.5 ml of fixative was retained and the cells resuspended in this. A few drops of fresh fixative were added in the same way as previously described until about 1.5 ml had been added. After 15 to 20 minutes the cells were again resuspended. Figure III. 2 illustrates the meiotic technique diagrammatically.

Four or five drops of the cell suspension were pipetted from a height of about 30 cm onto chilled, wet but well-drained slides.

Air was gently blown onto the slides which were then placed on a warm (37°C) thermostatically controlled hot plate until dry. The number of the meiotic preparation, the year in which it was made, and the slide number were all recorded on the slide, using a diamond-tipped pencil, for example:

M_1 /71/3.

Up to 50 slides were prepared at each session. These slides were stained and mounted as described in section III. 3 (i).

The materials used in making meiotic preparations are listed in Appendix VII.

Wash in 2.2% Na tri-citrate

OR

Chop and tease in 2.2% Na tri-citrate

Centrifuge 5 mins. at 1000 r.p.m.

Discard supernatant

Cell deposit

Chop and tease in few ml 1.2% Na tri-citrate

10 ml cell suspension
Tubules crushed.

Incubate 37°C

Centrifuge 10 mins. at 1000 r.p.m.

Discard supernatant
(leave 1 to 1.5 ml.)Resuspend cells in
1.5 ml hypotonicFixative added
(absolute alcohol:
glacial acetic
acid + trace
chloroform)

10 ml Cell suspension

Stand 30 mins. at 4°C

Centrifuge 10 mins. at 1000
r.p.m.

Discard supernatant

Cell deposit

Few drops fix-
ative added

1 - 1.5 ml cell suspension

Stand 15 to 20 mins. at 4°C

Prepare Slides

Air-Dry

Total of
30 mins.
exposure
to
hypotonic
solution

Figure III. 2: Flow-diagram of main technique used in this study for the preparation of meiotic chromosomes.

(E) Discussion

Some air-drying methods produce excellent results with meiotic stages of specific animals but these methods are not necessarily directly applicable to other animals (Meredith, 1969; author; and others). Modifications were therefore required before a satisfactory number of cells were produced at each preparation.

There are only two reports in sheep of the use of a technique other than histological or squash techniques. Bruère (1969) and Nadler et al. (1973) studied sheep meiotic cells but the results from both studies judging from published photographs, were poor.

Many factors affect the quality and quantity of meiotic cells. These include:-

- (a) The length of time between removal of testicular material from the animal and its subsequent processing.

Best results were obtained when the time interval between castration and initiation of the hypotonic treatment was minimal.

- (b) Effect of time of year.

The ram, a seasonal breeder, undergoes a partial physiological involution of spermatogenic tissue in the non-breeding season. During this time the testes become smaller and lose their tone. Meiotic preparations made at this time were often poor. As spermatogenic activity increases with the onset of the breeding season (Autumn) the testes increase in size and become firm and elastic. Consequently meiotic preparations obtained during this time were usually good.

- (c) Teasing of tubules.

Ford (pers. comm., 1971) does not consider this step to be critical. The aim is to release the cells from the seminiferous epithelium as rapidly as possible with as little damage as possible (Ford, pers. comm., 1971). Too much teasing will produce a suspension containing many small fragments of tubule; inadequate teasing will result in many cells, particularly spermatogonia, remaining within the

discarded tubules (Ford and Evans, 1969).

(d) Hypotonic fluid

Ford and Evans (1969) stated that the optimum concentration, exposure time and temperature at which the hypotonic solution acts, probably differ from one species to another and therefore have to be determined empirically for each new set of conditions.

Ideally, the shortest exposure time consistent with obtaining adequate spreading of chromosomes is required. Excessive exposure to hypotonic fluid reduces the proportion of useful cells in the final preparation. It is believed that the membranes of cells in mitosis or meiosis are more prone to rupture than non-mitotic cells. This occurs because of the lower viscosity of the cytoplasm, which presumptively would mean that the cell membrane had less physical support (Ford, pers. comm., 1971). Ford (pers. comm., 1971) suggested that the lysosomes might be progressively ruptured on exposure to the hypotonic fluid and their contents released into the cytoplasm, and that autolysis could begin while the cell was still living. He further suggested that if this were true, optimal hypotonic exposure would be carried out at low temperatures although for practical purposes, ambient temperatures are used.

The author used fresh sodium citrate solution for hypotonic treatment as suggested by Ford and Evans (1969). In contrast, Page (pers. comm., 1971), on the advice of Chandley (pers. comm., 1970), found that three to four day old sodium citrate gave better results. Aged solutions were tried by the author without any noticeable improvement in results.

Concentrations of hypotonic solution were varied from 0.7% to 1.1%, 1.12%, 1.2%, 1.3% and 1.4%. Exposure times of the cells to hypotonic solution ranged from five to 55 minutes. An exposure time of 30 minutes gave the best spreading and morphology of chromosomes.

Various concentrations of potassium chloride were experimented with as a hypotonic solution, but the author did not observe the improvements in chromosome morphology noted by Hungerford (1965), Clendenin (1969) and Luciani et al. (1971). Consequently the use of sodium tri-citrate solution was continued.

(e) Centrifugation

Ford (pers. comm., 1971) considered it a disadvantage to expose the cells to a great centrifugal force as fixation was difficult if the cells in the pellet were too compacted and too many cells were discarded in the supernatant.

The author collected, centrifuged and processed the supernatant on several occasions as was suggested by Ford and Evans (1969) and Ford (pers. comm., 1971). As very few cells were seen on the slides made from these preparations it was considered that the supernatant did not contain valuable material and it was discarded henceforth.

Times of centrifugation were varied and ten minutes was found to be adequate.

(f) Fixation

This step is considered by Ford (pers. comm., 1971) to be the most critical of all the steps in obtaining satisfactory preparations. He cited a great number of possible empirical variations such as the volume, shape and density of the pellet of cells; the volume of the supernatant fluid retained; the size and shape of the tube used for fixation; the rate at which the fixative is added and its total volume; the time interval between fixative changes; the number of changes of fixatives; the effects of temperature and the addition of adjuvants such as chloroform.

Ford and Evans (1969) noted that speed of penetration of the fixative is probably the most important variable. Maximum speed of penetration is probably achieved when the cells are in suspension at the time when the first fixative is added. The greater the volume of the pellet, if cells are fixed in this way, the slower the rate of penetration of fixative. Ford and Evans (1969) considered that the optimum size for a pellet would be one 2 mm in depth. These authors also noted that the total yield of cells was often less when cells were fixed in suspension than when they were fixed as a pellet. They suggested that many cells were probably disrupted in suspension.

The author found that marked loss of cells occurred if the fixative was changed more than twice, therefore only two changes of fixatives were used. Bruère (pers. comm., 1973) also noted this cell

loss with leucocyte cultures.

(g) Preparations

Ford and Evans (1969) found that much better spreading of chromosomes was achieved with dilute suspensions than with dense suspensions. The author confirmed this result before adopting their method.

(h) Stages visible

Satisfactory preparations of cells at diplotene and good preparations at diakinesis and first and second metaphase were obtained by the author's method. However, at either first or second anaphase very few cells were seen. These results are explained by Ford and Evans' (1969) statement that exposure to hypotonic fluid causes arrest of cell division at metaphase and therefore the progressive disappearance of spermatocytes in first and second anaphase. These authors believed that anaphase figures could be obtained if the period of hypotonic treatment was reduced to a minimum or eliminated altogether.

The observation (Eliasson et al., 1967; Ford and Evans, 1969) that few spermatogonial metaphases are normally present in preparations made by the above methods was confirmed by the author's results. Methods for increasing the number of spermatogonial cells have been suggested by Ford and Evans (1969).

(ii) PROPHASE STAGES

(A) Review of Literature

Prior to the 1960's very little work had been done on the topological mapping of individual mammalian chromosomes according to the chromomere patterns characteristic of each of them at the pachytene stage of meiosis. Schultz and St. Lawrence (1949) using a squash procedure after aceto-orcein staining, were the first to attempt a standard diagram of chromomere pattern in a pachytene bivalent in man. Several years later Yerganian (1957) who described a method involving the use of a Waring blender and centrifugation was

able to analyse chromomere number, size and sequence in many of the bivalents he collected.

Squash techniques without hypotonic pre-treatment (Böök and Kjessler, 1964; Ferguson-Smith, 1964; Gardner and Punnett, 1964; Griffen, 1955; 1960; Ohno, 1965; Bordjadze and Prokofieva-Belgovskaya, 1971) and with hypotonic pre-treatment (Ferguson-Smith, 1964; Ford, Cacheiro, Norby and Heller, 1968; Ford, 1969; Anneron, Berggren, Stahl and Kjessler, 1970) have been used in meiotic prophase studies.

Both types of technique had their disadvantages. Chromosome breakage and overlapping frequently occurred in squash techniques and hypotonic pre-treatment tended to rupture the nucleoli and alter the positions of individual chromosomes within the cell (Ferguson-Smith, 1964). Hypotonic treatment had the advantage however of spreading chromosomes and making them easier to analyse (Ferguson-Smith, 1964; Hungerford, 1971).

Although prophase stages are seen in meiotic preparations made by the air-drying techniques in common use (e.g. Evans et al., 1964; Sasaki and Makino, 1965), their morphology rarely meets the minimal criteria proposed by Hungerford (1971) for chromomeric mapping. Hungerford (1971) considered that preparations should be good enough to allow accurate chromosome counts, the recognition of centromere position and the pattern of discrete chromomeres, and he described a method which met these requirements.

(B) Methods Used In This Study

An attempt was made to study the chromosomes at first meiotic prophase using two different methods. The first was that of Ferguson-Smith (1964) and the second was based on Hungerford's (1971) method.

1. Small pieces of testicular material were teased in fresh 3 : 1 ethyl alcohol : glacial acetic acid fixative and allowed to stand for one hour in a universal container. The tubules were removed from the container and finely chopped in a few ml of fresh fixative. The suspension was dropped onto cold, wet slides, air-dried and stained with aceto-orcein in the same way as other meiotic stages (III. 3(1)).
2. Testicular material was teased in a few ml from 10 ml of warm

(37°C) tissue culture medium containing 200 I.U. of heparin (Appendix VIII). The suspension was transferred from the petri dish to a centrifuge tube. The process was repeated until 10 ml of suspension were obtained after which the tube was centrifuged at 1000 r.p.m. for three minutes to separate out the larger tubular fragments. The supernatant was transferred to a second centrifuge tube and after spinning for ten minutes at 1000 r.p.m. the supernatant was discarded.

200 I.U. heparin were added to 10 ml of 0.125 M Potassium chloride (Appendix IX). The cell pellet was suspended in 1 ml of this solution and incubated for one hour at 37°C. After centrifugation and removal of the supernatant, the cells were fixed in 3 : 1 methanol : glacial acetic acid for 15 minutes. Finally, the suspension was again centrifuged and the supernatant removed, leaving 1 ml of cell suspension. A few drops of fresh fixative were added, the cells resuspended and slides made and stained in the same manner as that described for meiotic stages in sections III. 2 (D) and III. 3 (i).

III. 3 STAINING TECHNIQUES

(i) MEIOTIC CELLS - GENERAL

Slides were stained in refiltered aceto-orcein (Appendix X), for at least two hours. They were then passed through two changes of 95 per cent alcohol (30 seconds in each), dipped in absolute alcohol and allowed to stand for 15 to 20 minutes in two consecutive dishes of xylol before being permanently mounted, in D.P.X. (Appendix V(c)).

Initially all meiotic slides were stained with aceto-orcein. Latterly only a few were stained in this way, the majority being stained with Giemsa after the "C-banding" technique had been carried out.

(ii) CENTROMERE STAINING

(A) Review of Literature

Centromere position is difficult to locate in sheep meiotic chromosomes stained by conventional methods.

In most bivalents examined from human spermatocytes at the late diplotene stage Chen and Falek (1969) observed two round bodies stained dark with Giemsa, which they believed were centromeres. The author of the present study occasionally observed in regions on the bivalent where the centromere was expected, dark staining areas with Giemsa, and light staining areas with aceto-orcein. These findings were neither consistent for all the bivalents in a cell, nor for the other cells on the same slide at the same stage of meiosis. Some bivalents showed three dark staining bodies whereas only two were expected. These results differed from those of Chen and Falek (1969).

During experiments on the chromosomal location of mouse satellite DNA, Pardue and Gall (1970) noticed that the centromeric heterochromatin of mouse mitotic chromosomes which had been treated with sodium hydroxide and stained with Giemsa, stained more densely than the rest of the chromosome. This observation was confirmed by Yunis, Roldan, Yasminah and Lee (1971). Since these initial observations were made, a number of papers have appeared concerning the staining of constitutive heterochromatin at and adjacent to the centromeric region of mitotic chromosomes of many species of animals, (Arrighi and Hsu, 1971; Chen and Ruddle, 1971; Hsu, Cooper, Mace and Brinkley, 1971; Takagi, 1971; Sumner et al., 1971; Sumner, 1972; Polani, 1972; Hansen, 1973,b; Evans et al., 1973; McKenzie and Lubs, 1973; Vosa, 1973).

Centromeric staining ("C-banding") techniques have also been used successfully with meiotic chromosomes (Hsu et al., 1971; Bobrow, Madan and Pearson, 1972; Dev, Miller, Allderdice and Miller, 1972; Falek, Chen and Chan, 1972; Polani, 1972; Sumner, 1972; Chandley and Fletcher, 1973; Gagne, Vagner-Capodano and Devictor-Vuillet, 1973).

While some methods are more tedious than others, all appear to give comparable results. In this study therefore a simple, reliable, method was chosen by which to stain the centromeres of both mitotic and meiotic chromosomes.

(B) Method Used In This Study

The centromeres of sheep meiotic chromosomes were

stained using the method of Chandley and Fletcher (1973) which was based on that described by Sumner (1972).

Slides were prepared as described in section III. 2 (D), and allowed to age for up to eight days. The author found that three to four day old slides gave results similar in quality to those produced by older slides. Consequently slides were processed, five at a time, three and four days after their preparation.

After standing for one hour at room temperature in a coplin jar containing 0.2 M hydrochloric acid (to remove surplus protein from the slide (Sumner, 1972)), the slides were rinsed in deionized water. They were then placed in another coplin jar containing a freshly prepared solution of 5 per cent barium hydroxide ($\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$) at 50°C for ten minutes, a much longer time than the ten seconds used by Chandley and Fletcher (1973) for human meiotic chromosomes. The slides were rinsed at least three times in deionized water, after which they were incubated for one hour at 60°C in 2 x SSC. Both the barium hydroxide and the 2 x SSC solutions were heated to the required temperature in the water bath prior to the time the slides were put into them. Following a rinse with fresh deionized water, the slides were stained for up to one hour with Giemsa. Finally, the slides were rinsed in deionized water, dried, soaked in xylol and mounted in D.P.X.. Details of the materials used for centromere staining are given in Appendix XI.

Slides representative of each of the three different Robertsonian translocation types discussed in this thesis were stained by the centromere-staining technique. The findings were used to elucidate the structure of the trivalent configurations seen at the diakinesis and the metaphase I stages of meiosis (Chapter V).

III. 4 HISTOLOGICAL SECTIONS

Immediately after excision, pieces of testis 5 mm thick were placed in fixative. Two pieces were fixed in Bouin's fluid (Culling, 1963) and two pieces in 10 per cent neutral buffered formalin (Thompson, 1966). After approximately 24 hours and 36 hours respectively, the pieces were removed from the fixative and washed in 70 per cent alcohol. The alcohol was decanted, and the tissue placed

overnight in a Shandon Elliott automatic tissue processor. The next morning, the tissues were embedded in paraffin blocks and cut into sections 4 μ to 5 μ thick.

Sections fixed in Bouin's were stained with H. & E. (Ehrlich's haematoxylin + 1% aqueous Eosin -Y) while sections fixed in formalin were stained with Feulgen (Thompson, 1966).

III. 5 ANALYSIS OF RESULTS

(i) MICROSCOPY

All slides were scanned completely using the 10 x objective (Appendix IV). Each cell which was to be examined more closely had its position on the slide read from a vernier scale and recorded. Cell position could be determined to within an accuracy of 0.1 mm.

When a cell was seen, it was examined under the 20 x objective. If the chromosomes were too contracted or not adequately spread, the cell was rejected. The other cells were examined under oil immersion with the 500 x or 1000 x objectives. A Balzers K-4 green filter was placed over the light source to improve chromosome definition and to reduce glare particularly when large numbers of slides were being scanned. The filter was not used for semen or histology slides.

The chromosomes in each cell at diplotene, diakinesis and metaphase I were counted and the number recorded. The presence, nature and position of the sex chromosomes and any other unusual features of the cell were noted. Both the number of chromosomes and the number of chromosome arms, were counted for each secondary spermatocyte. The type of sex chromosome, and of translocation chromosome when present, was recorded. The analysis of these cells is discussed in greater detail in Chapters IV and V.

For easy reference, all slides were labelled and filed in groups. Each group consisted of slides prepared from one animal.

Cells were counted using a hand operated counter (Appendix XII). If any doubt occurred as to a count, the cell concerned was excluded from the results. A number of the cells from each meiotic preparation were photographed and karyotypes prepared.

(ii) PHOTOGRAPHIC TECHNIQUES

For photography, the wide field (15x) eye-pieces on the microscope were exchanged for one ordinary (10x) eye-piece, and one (10x) photographic eye-piece with a focusing sight and frame. Photographs were taken at both 500x and 1000x with the green filter whenever possible. A Leitz exposure meter interchangeable with one eye-piece was used to make light readings. All photographs were taken at a constant exposure time of one second on Copex-pan rapid film 37 A.S.A. (Agfa-Gevaert). Records were kept of each photograph taken.

Each film was developed and printed by the author with some technical help. Films were developed as described in Appendix XIII and the negatives were labelled and stored in book form.

Each negative was enlarged (Appendix XIV) and printed on Ilfobrom (Ilford, Australia Proprietary Ltd.) Number 3 or 2 paper, depending on whether the negative was light or dark (refer Appendix XV for details of developer and fixer).

Histological preparations were photographed on Pan F film (32 A.S.A. Ilford). (The way in which the film was processed is noted in Appendix XVI).

(iii) KARYOTYPES AND DRAWINGS

Ademco dry mounting tissue was tacked onto the back of each print with a heated fixing iron. The chromosomes on each print were cut out making sure no accidental omissions or duplications occurred. They were arranged on ruled sheets of cardboard in decreasing order of size with centromeres approximately aligned. The sex bivalent or sex chromosomes were placed last. The bivalents, or chromosomes were labelled and the karyotype placed in a press (70°C to 80°C) for 10 to 15 seconds. An entire photograph of each cell was also pressed onto the card which was labelled and filed.

In several karyotypes at the prophase, diakinesis and metaphase stages, an attempt was made to draw the chromosomes. They were traced from photographs, with tracing paper over a light source, and the drawing placed beside the corresponding chromosome on the karyotype. In this way chiasmata positions were more easily defined,

and centromere position, which was accurately seen after the cells were stained by the "C-banding" technique, was marked on the drawings. Trivalent structure was also defined.

- (iv) SEMEN ANALYSIS was described in section III. 1
(i) (D).

EXAMINATION OF HISTOLOGICAL SECTIONS

Histological sections of the testes of all rams were assessed for spermatogenic activity.

CHAPTER IV

MEIOSIS IN THE NORMAL (54,xy) RAM

IV. 1 NOMENCLATURE OF MEIOTIC CHROMOSOMES

The notations used to describe single cells and to summarize the meiotic analysis in the ram follow the recommendations of the Paris Conference (1971) for human cytogenetics.

"The stages of meiosis are indicated by abbreviations:

- PI - prophase of first division
- MI - first diakinesis and metaphase
- AI - first anaphase
- MII - second metaphase
- AII - second anaphase

These are followed by a total count of separate chromosomal elements. The sex chromosomes are then indicated by XY or XX when associated and X,Y when separate. Any additional, missing or abnormal element follows, with that element specified within parentheses and preceded by the Roman numeral I, II, III or IV to respectively indicate if it is a univalent, bivalent, trivalent, or quadrivalent. The absence of a particular element is indicated by a minus sign. The plus sign is used in first metaphase only when the additional chromosome is not included in a multivalent. The chromosomes involved in a rearrangement are listed numerically within parentheses and separated by a semicolon (;)."

For example:

MI,27,XY

A primary spermatocyte at diakinesis or metaphase I with 27 chromosome elements including an XY bivalent.

MI,28,X,Y

A primary spermatocyte at diakinesis or metaphase I with 28 elements including X and Y univalents.

MI,26,XY,III(5q26q)

A primary spermatocyte at diakinesis or metaphase I from a balanced Massey I Robertsonian translocation heterozygote with 26 elements including an XY bivalent and a trivalent which represents the translocation submetacentric paired with two acrocentric autosomes.

MI,27,XY,III,+I+I

A primary spermatocyte at diakinesis or metaphase I with 27 elements including an XY bivalent, a trivalent and two univalents.

MI,26,X,Y,III,-A

A primary spermatocyte at diakinesis or metaphase I with 26 elements including X and Y separated and a trivalent. An autosomal bivalent is missing.

The nomenclature used in descriptions of cells at second metaphase is described in section IV. 3 (c)a.

IV. 2 LITERATURE REVIEW

(i) MEIOTIC STUDIES IN MALE DOMESTIC ANIMALS WITH NORMAL KARYOTYPES

The meiotic chromosomes of domestic animals have been less extensively studied than those of man or rodents. Consequently most of the data on them is qualitative.

(A) Horse

Makino (1943,b) recorded counts of 33 in both primary and secondary spermatocytes and described the morphology of meiotic chromosomes in two breeds of horse. It is now known that the diploid chromosome number for the horse is 64 (Trujillo, Stenius,

Christian and Ohno, 1962) and yet no further meiotic studies have been reported in this species.

(B) Cattle

Cattle meiotic chromosomes have been investigated by several authors including Krallinger (1931), Makino (1944), Melander and Knudsen (1953), Knudsen and Bryne (1960), Treadwell et al. (1968), Gustavsson (1969) and Popescu (1971,a). A modal chromosome number of 60 was counted in spermatogonial metaphases (Krallinger, 1931; Makino, 1944; Melander and Knudsen, 1953; Knudsen and Bryne, 1960; Gustavsson, 1969; Popescu, 1971,a) and Popescu (1971,a) observed a proportion of hyperdiploid cells (7.5 per cent) in his preparations.

Studies revealed that prior to the onset of meiotic prophase in the bull, the chromosomes were contracted (Melander and Knudsen, 1953; Knudsen and Bryne, 1960; Popescu, 1971,a) but they became filiform at leptotene. The "bouquet" arrangements described by Melander and Knudsen (1953) and Knudsen and Bryne (1960) were not seen by Popescu (1971,a) who attributed this to the disturbance of chromosomal relationships by the hypotonic solutions employed during slide preparation. Staining intensity of chromosomes was observed to increase from leptotene to zygotene (Gustavsson, 1969; Popescu, 1971,a). Pachytene nuclei were more numerous than any other stage (Treadwell et al., 1968; Popescu, 1971,a) and Popescu (1971,a) reported only one out of 100 cells examined at pachytene where the number of bivalents deviated from 30. The sex chromosomes were present as a darkly-stained, peripherally-located heteropyknotic body until late pachytene (Treadwell et al., 1968; Gustavsson, 1969; Popescu, 1971,a) when the "sex vesicle" disappeared and the constituent chromosomes straightened out and became isopyknotic with the autosomes at diplotene (Gustavsson, 1969).

Diplotene stages were rare (Treadwell et al., 1968; Popescu, 1971,a) but Gustavsson (1969) counted 76 cells at diakinesis-metaphase I. Melander and Knudsen (1953) and Popescu (1971,a) also analysed a number of cells at these stages. Separated sex chromosomes were found in 33.3 per cent of diakinesis-metaphase I cells examined by Popescu (1971,a) who estimated the average

chiasmata number per cell (from 38 cells) as 53.73 with the average number per bivalent as 1.78. The average coefficient of terminalization was 0.61 per cent and the index of recombination, which Popescu (1971,a) calculated according to Ford's (1969,a) method, was 82.73. There is no other comparable data for the bull.

Makino (1944) gave the first description of the sex bivalent and described an end-to-end association of the X and Y chromosomes. Melander and Knudsen (1953) maintained that the sex chromosomes had homologous and non-homologous segments and that chiasma formation occurred between homologous segments. Popescu (1971,a) who observed an end-to-end association of the X and Y chromosomes considered that their union involved a terminal chiasma.

(C) Goat

Goat meiotic chromosomes were investigated by Makino (1943,a) and more recently by Datta (1970). Both authors counted 30 bivalents at metaphase I including a sex bivalent and Datta (1970) from a study of 200 cells in all stages of meiosis, concluded that the process was similar to that in other animals. Chiasmata frequencies were calculated from 15 nuclei by Datta (1970) who recorded the average number per cell at diplotene, diakinesis and metaphase I as 72, 54.2 and 49 respectively, with an average of 2.4, 1.8 and 1.6 chiasmata per bivalent.

Datta's (1970) interpretation of the sex bivalent differs from that of Makino (1943,a) who found the X to have a tripartite structure and be connected at its end with the Y chromosome. Datta (1970) proposed that chiasma formation occurred between the short arm of the X and one of the arms of the Y chromosome and postulated that the gap between the two chromosomes sometimes seen at metaphase I was proof of the occurrence of chiasma terminalization.

(D) Sheep

The early studies on sheep meiotic chromosomes, now of historical interest only, were concerned with determining the diploid chromosome number (Wodsdalek, 1922; Krallinger, 1931;

Pchakadze, 1936). Ahmed (1940), Berry (1941) and Makino (1943,a) counted 27 bivalents at metaphase I and Makino (1943,a) gave the first descriptions of the chromosomes in spermatogonial cells as well as in primary and secondary spermatocytes. No indication of either the number of meiotic cells analysed, or of the range of chromosome counts obtained, was given by Berry (1941) and Makino (1943,a).

Chiasmata counts from 25 cells were recorded by Ahmed (1940) who must therefore have analysed at least that number of cells. Loir (1971) reported observing "several hundreds of nuclei of spermatocytes I over a series of eight to ten preparations" but did not substantiate his observations with any tables of chromosome counts. There were 27 bivalents in diakinesis-metaphase I cells (Loir, 1971), the same number as Nadler et al. (1973) counted in each of 45 cells from a wild ram.

(E) Pig

Bryden (1933) summarized previous authors' work on pig chromosomes and reported a haploid chromosome number of 19 for the domestic pig. He commented on chromosome morphology in the prophase, metaphase and anaphase stages but did not reveal the number of cells analysed, nor provide any information on chiasmata counts.

The type of association between the sex chromosomes could not be ascertained by Bryden (1933) but Crew and Koller (1939) described pairing and non-pairing segments on the X chromosome and chiasma formation between the homologous sections of the X and Y chromosomes. Crew and Koller (1939) whose information was obtained from a study of histological sections studied chiasmata frequencies in five boars of different age and genetical constitution. They found no relationship between chiasmata frequency and age of boar and calculated the average number of chiasmata per bivalent as 2.16 at diplotene and 2.06 at metaphase I (data from five boars and 36 cells). Crew and Koller (1939) illustrated their observations with good diagrams but their photographs were poor and difficult to interpret.

(F) Dog and Cat

Minouchi (1928), Ahmed (1941), Eliasson et al. (1967), Ford (1969) and Williams et al. (1971) have all contributed information on morphological aspects of dog meiotic chromosomes.

Ahmed (1941) found a similar chiasma frequency for each of the three breeds of dog he studied; at metaphase I the mean number of total chiasmata per nucleus varied between 78.2 and 82.6. He described a sex chromosome pair in which one or more chiasmata were formed in the homologous regions of X and Y and commented on the inconstancy of the morphology of the sex bivalent.

Eliasson et al. (1967) studied two dogs while Williams et al. (1971) examined preparations from 150 dogs; both groups of workers commented on all stages of meiosis. Eliasson et al. (1967) were unable to demonstrate the "sex vesicle" at leptotene, zygotene or pachytene, but it was clearly shown by Williams et al. (1971) at pachytene. The latter authors also observed at this stage, non-homologous associations of bivalents, usually appearing as end-to-end attachments. The diplotene stage was rare (Eliasson et al., 1967; Williams et al., 1971). Separated X and Y chromosomes were observed by Williams et al. (1971), and Eliasson et al. (1967) who studied 92 diakinesis-metaphase I bivalents in detail and found separation in 17.4 per cent of the cells.

The X and Y chromosomes were usually associated terminally (Eliasson et al., 1967; Williams et al., 1971) and Eliasson et al. (1967) thought the union involved the short arm of the X chromosome.

A detailed study of pachytene stages from the dog was made by Ford (1969) who attempted to identify and characterize the 39 bivalents seen at this stage.

Studies on cat meiotic chromosomes are few. Koller (1941) examined histological sections from the testes of several cats and described in detail, the behaviour of the chromosomes during meiosis. He counted an average of 2.7 chiasmata per bivalent at diakinesis and 2.4 at metaphase I. Three types of sex bivalent at meiotic metaphase were identified and described by Koller (1941). One of these was a ring-shaped bivalent having two terminal chiasmata while the remaining two were rod-shaped and each had one terminal chiasma.

Ohno et al. (1962) who studied squash preparations from foetal cat testis, reported normal (by comparison with similar stages from an adult cat) pachytene, diplotene and diakinesis figures. No chromosome counts were given, but these stages were demonstrated photographically.

(ii) MEIOTIC STUDIES IN "NORMAL" MEN

(A) Introduction

Considerably more data has been accumulated on human male meiotic chromosomes than on the meiotic chromosomes of any of the male domestic animals.

Tjio and Levan's (1956) finding of 46 as the correct diploid chromosome number for man, was confirmed in the same year by Ford and Hamerton who demonstrated 46 chromosomes in spermatogonial metaphases, and 23 bivalents including a sex bivalent in primary spermatocytes. In addition an estimation of the genetic length for man was calculated by Ford and Hamerton (1956) from chiasmata counts in cells at the late diplotene to mid-diakinesis stages. Subsequent to these findings, there has been a great increase in knowledge on the morphology and behaviour of meiotic chromosomes in men with a normal karyotype (46,xy) and normal spermatogenesis (Eberle, 1963; 1966; Böök and Kjessler, 1964; Sasaki and Makino, 1965; McIlree, Tulloch and Newsam, 1966,a; Falek and Chiarelli, 1968; Fraccaro, Hultén and Lindsten, 1968; Luciani, 1970; Luciani, Carlon and Stahl, 1970; McDermott, 1971; Ferguson-Smith, 1972; Skakkebaek, Bryant and Philip, 1973).

With increasing interest in meiotic chromosomes has come the publication of many papers concerning meiotic chromosomes in men with various clinical conditions including infertility. This aspect of meiotic study is further considered in Chapters V and VI, while some of the findings from studies of normal human meiosis are summarized below.

(B) The Stages of Meiosis

McDermott (1971) who described the pre-meiotic stages of meiosis reported pairing of mitotic chromosomes during

spermatogonial prophase, a phenomenon not previously observed in man.

Ferguson-Smith (1972) considers that identification of the various early prophase stages of primary spermatocytes is difficult; the information summarized here is from the descriptions given by several authors who have recognized these stages.

a Leptotene

The chromosomes appear as a network of fine threads bearing numerous dark-staining chromomeres (Luciani, 1970; McDermott, 1971). Two heteropyknotic bodies which they thought could represent the sex chromosomes were reported by Sasaki and Makino (1965), Solari and Tres (1967) and McDermott (1971). Luciani (1970) did not see these bodies although he reported the appearance of a "sex vesicle" (Sachs, 1954) at the end of the leptotene stage. However in a later paper (Luciani *et al.*, 1970) the existence of "one or perhaps two" heteropyknotic bodies in the pre-leptotene stage is acknowledged.

McDermott (1971) provided evidence which indicated that some pairing between chromosomes occurred at leptotene.

b Zygotene

Sasaki and Makino (1965) were unable to demonstrate a zygotene nucleus. Photographic representation of cells at this stage has been given by several authors including Luciani (1970), Eberle (1966), McDermott (1971) and Ferguson-Smith (1972). The pairing of the homologous chromosomes and the heteropyknotic "sex vesicle" was demonstrated in some of these photographs (for example, McDermott, 1971).

The distribution of small heterochromatin bodies throughout the nucleus at zygotene was reported by Eberle (1966) and Luciani (1970). Heterochromatic bodies have been previously reported in meiotic prophase chromosomes of several species including man by Woollam and Ford (1964) who observed that these were situated in the vicinity of the region occupied by the centromere in mitotic metaphase chromosomes.

c Pachytene

The pachytene stage has been more intensively studied than any of the other early meiotic prophase stages.

Homologous chromosomes are completely paired. As pachytene progresses the bivalents condense by developing a spiral structure (Luciani, 1970; McDermott, 1971) until by late pachytene they are about half their original length (McDermott, 1971). The "sex vesicle" is heteropyknotic (Sasaki and Makino, 1965; Reitalu, 1970; Ferguson-Smith, 1972) situated peripherally in 79 per cent of cells (Luciani, 1970) and visible until the end of pachytene when it loses its heteropyknotic character and becomes indistinguishable from the autosomes (McDermott, 1971).

Woollam and Ford (1964), Luciani (1970), McDermott (1971, 1973,a) and Ferguson-Smith (1972) reported lateral "hair-like" projections on mid to late pachytene bivalents giving these a "furry" appearance which Woollam and Ford (1964) and McDermott (1971; 1973,a) found was enhanced by treatment with potassium chloride. Luciani (1970) suggested that while technical procedures could be responsible for this effect, he could not discount the possibility that the projections were due to inflexions and loops in the DNA chain. McDermott (1971) could not decide whether the processes were true loops or the result of loop rupture. He observed and described the nature of the apparent interconnection of lateral fibres between adjacent non-homologous chromosomes in intact cells (McDermott, 1973,a). A similar phenomenon was reported by Chen (1973) who noticed interchromosomal fibres connecting the centromeric heterochromatin of pachytene bivalents.

The chromomere pattern characteristic of each of the bivalents at pachytene in man is being determined slowly; contributors towards the mapping of the human complement include Schultz and St. Lawrence (1949), Yerganian (1957), Ferguson-Smith (1964), Eberle (1966), Ford et al. (1968), Ford, Cacheiro, Norby and Heller (1969), Luciani (1970), Bordjadze and Prokofieva-Belgovskaya (1971), Hungerford (1971), Hungerford, La Padie and Balaban (1971,a), Hungerford, La Padie, Balaban, Messatzzia, Haller and Miller (1971,b) and Page (1973).

d Diplotene

At this stage, the autosomal bivalents are usually described as thin threads loosely coiled around one another and connected by chiasmata (Böök and Kjessler, 1964; Sasaki and Makino, 1965; Luciani, 1970). Ferguson-Smith (1972) emphasizes that this is theoretically the best time to examine and count chiasma as they have not yet begun to terminalize. The "sex vesicle" has disappeared (Böök and Kjessler, 1964; Sasaki and Makino, 1965; Luciani, 1970) and been replaced by an X-Y bivalent. Böök and Kjessler (1964), Sasaki and Makino (1965) and Eberle (1966) stated that the sex chromosomes were positively heteropyknotic at this stage. In contrast, Falek and Chiarelli (1968) Reitalu (1970) and Luciani (1970) observed that the sex chromosomes were no longer heteropyknotic.

e Diakinesis and metaphase I

All workers agree that bivalent morphology is most clearly shown at the diakinesis and first metaphase stages.

Bivalents can be classified at this stage on the basis of size (Sasaki and Makino, 1965; McIlree et al., 1966,a) and chiasmata position (Luciani, 1970). More recently Caspersson, Hultén, Lindsten and Zech (1971) have shown that all bivalents can be recognized by their banding patterns after quinacrine mustard staining, as each of the bivalents show different fluorescent patterns similar to those of mitotic chromosomes. Identification of meiotic bivalents is also being attempted on the basis of their banding patterns produced with Giemsa stain (Bath and Gendel, 1973), as well as by the location of their centromeres (Chen and Falek, 1969; Chandley and Fletcher, 1973).

Chiasmata between homologous chromosomes although visible at the diplotene stage, are usually counted at the diakinesis stage. Estimates of chiasmata frequency per cell (Table IV. 1) vary among authors.

TABLE IV. 1

CHIASMATA FREQUENCIES IN MAN

Author	Number of Cells Counted	Average Number Chiasmata/Cell
Ford, C.E. and Hamerton, J.L. (1956)	23	55.9
McIlree, M.E. <u>et al.</u> (1966,a)	96	49.8 - 58.3
Eberle, P. (1966)	4	55.7 (Diplotene)
	60	44.0 (Diakinesis)
Falek, A. and Chiarelli, B. (1968)	8	49-58 -(53.3 Average)
Luciani, J.M. (1970)	88	47.5
Hultén, M. (Paris Conference, 1971)	41	50.61 $\pm$ 3.87 *
Chandley, A.C.; Christie, S.; Fletcher, J.; Frackiewicz, A and Jacobs, P.A. (1972)	93	49
Ferguson-Smith, M.A. (1972)	849	51.11 $\pm$ 2.97 *
McDermott, A. (1973,b)	518	53.7
Skakkebaek, N.E. <u>et al.</u> (1973)	57	51.2

* Standard deviation

The number of chiasmata per bivalent varies. From one to six chiasmata were reported by Böök and Kjessler (1964), Sasaki and Makino (1965) and McDermott (1973,b); one to five by Ford and Hamerton (1956) and Luciani (1970), while Eberle (1966) counted up to eight chiasmata in bivalents at diplotene.

Estimates of genetic length also vary between authors; for example Ford and Hamerton (1956) calculated the genetic length for man as 27.9 morgans, McDermott (1973,b) calculated it as 27 morgans, while Ferguson-Smith's (1972) estimate was 25.6 morgans.

The bivalents continue to contract until the metaphase I stage is reached. There is also a reduction in chiasmata number.

Ferguson-Smith (1972) states that it is uncertain whether this represents terminalization or enumeration difficulties due to the bivalent condensation.

Anomalies including separated X and Y chromosomes (Table IV. 2), separation of autosomal bivalents (Sasaki and Makino, 1965; Skakkebaek *et al.*, 1973) and interlocked bivalents (McDermott, 1966), have been reported in diakinesis-metaphase I cells from apparently normal men. Proposals concerning the origin and importance of these anomalies have been made by the authors who reported them.

TABLE IV. 2

SEPARATED SEX CHROMOSOMES AT DIAKINESIS-METAPHASE I
IN MAN

Author	Total Number of Cells Counted	% X and Y as Univalents
Ford, C.E. and Hamerton, J.L. (1956)	174	14.3
Sasaki, M. and Makino, S. (1965)	162	27
Hultén, M. <i>et al.</i> (1966)	100 100	24 18
Falek, A. and Chiarelli, B. (1968)	11	9.1
Fraccaro, M. <i>et al.</i> (1968)	—	20
Luciani, J.M. (1970)	145	7.5
Luciani, J.M. <i>et al.</i> (1970)	—	6
McDermott, A. (1971)	389	3.2
Ferguson-Smith, M.A. (1972)	849	21.84 $\pm$ 14.16 *
Skakkebaek, N.E. <i>et al.</i> (1973)	492	15

* Standard deviation

f Metaphase II

The chromosomes of metaphase II are discussed in Chapter V.

(C) The Sex Chromosomes

There has been considerable speculation regarding the morphology, behaviour and type of union of human sex chromosomes at meiosis.

The "sex vesicle", a heteropyknotic complex (Sachs, 1954) appears at late leptotene and disappears during diplotene (Böök and Kjessler, 1964; Eberle, 1966; Ford and Woollam, 1969; Luciani, 1970; Luciani et al., 1970). These workers found it difficult to identify the components of this densely staining body, although Sachs (1954) then Reitalu (1970) suggested that it contained two differentially stained bodies which could represent the X and Y chromosomes. Electron microscope studies by Solari and Tres (1967; 1970) confirmed the existence of a sex chromosome pair forming a "sex vesicle" at meiotic prophase and described in detail (Solari and Tres, 1970) the fine structure of this "vesicle."

Although it has been generally agreed upon that the sex chromosomes form an end-to-end bivalent association at diplotene, the type of union was uncertain prior to the introduction of techniques which enabled the determination of centromere position in meiotic chromosomes.

Sasaki and Makino (1965) thought the link involved the distal ends of both arms, while Reitalu (1970) considered that the association was between the medial part of the long arm of the Y chromosome and the distal part of the short arm of the X chromosome and included homologous segments on both chromosomes.

Ford and Hamerton (1956), Ferguson-Smith (1966), Hultén et al. (1966) and Luciani (1970) thought a terminal union existed between the short arms of the X and the long arms of the Y chromosome.

In 1969, Zech found that the Y chromosome in man was easily recognized by an intense fluorescence with certain fluorescent compounds. The same year, Chen and Falek identified the centromere position in human diplotene cells and demonstrated an association between the short arms of both sex chromosomes. This finding was partially confirmed by Pearson and Bobrow (1970) who showed that after staining with quinacrine dihydrochloride, in all cases where the X and Y chromosomes were associated, a highly fluorescent region was situated at one end of the sex bivalent; this they interpreted from mitotic studies (Pearson, Bobrow and Vosa, 1970,a) to mean an association between the short arm of the Y chromosome and the X chromosome. Later, Chen and Falek (1971)

and Chandley and Fletcher (1973) gave unequivocal evidence for the association of the X and Y chromosomes by their short arms.

Light microscope studies have been unable to give conclusive evidence as to the type of union between the sex chromosomes in man. Luciani (1970) and Eberle (1963; 1966) considered the link to be achiasmatic. In contrast, Ford and Hamerton (1956) thought that terminal chiasmata could exist and Ferguson-Smith (1972) is convinced that terminal chiasmata occur in some associations at least. Böök and Kjessler (1964) and Sasaki and Makino (1965) found no real evidence of chiasmata although the latter authors considered they could not exclude the possibility. Hultén et al. (1966) although finding some configurations suggestive of chiasmata formation could not prove this.

Evidence from electron microscope studies (Solari and Tres, 1970) while not conclusive, suggests that the sex chromosomes are joined by a chiasma and that there may be homologous regions in the human sex chromosomes. Some implications of this hypothesis are discussed by Solari and Tres (1970).

IV. 3 MEIOSIS IN THE NORMAL RAM (54,xy)

(i) INTRODUCTION

The author examined meiotic preparations from 14 rams (Ovis aries). An example of a mitotic karyotype from a normal ram is shown in Plate IV. 1. The rams aged from one to three years fulfilled the criteria discussed in Chapter III, section 1 (i). Data on semen analyses and histological examination of the testes from these rams are given in Appendix XVIII, a and XVIII, b. The identification and breed of each ram used in the study is summarized in Table IV. 3. Every ram was identified by a number and usually a letter (for example X435), and the year of its birth was indicated when known (69 = 1969).

Plate IV. 1: Mitotic karyotype from a normal ram (54,xy).
There are six large metacentric chromosomes,
an acrocentric X chromosome and a submetacentric
Y chromosome.
(Aceto-orcein).

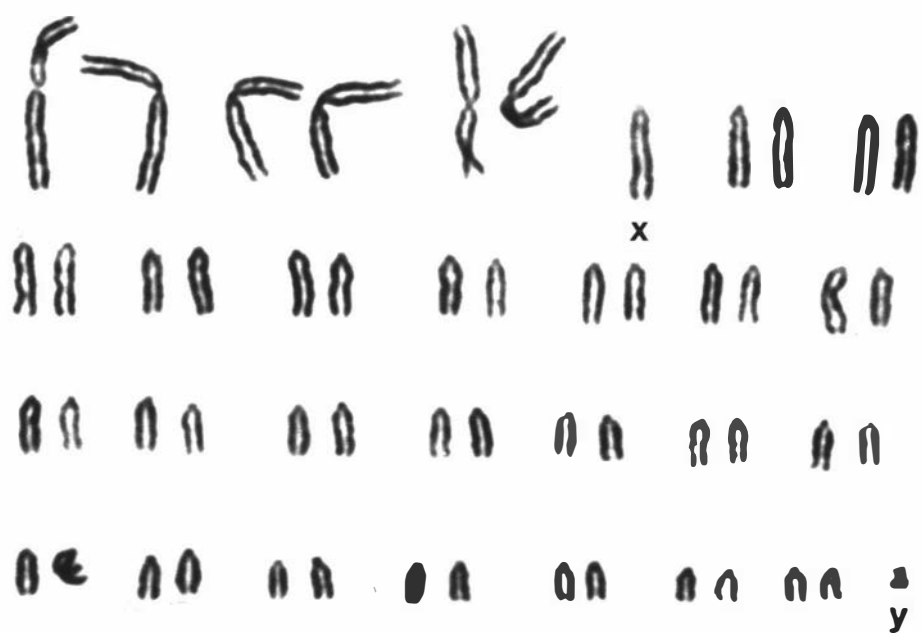


TABLE IV. 3IDENTIFICATION AND BREEDS OF RAMS USED IN STUDY

X435/69	Romney
R285/69	"
X117/69	"
X214/69	"
84/68	Drysdale
B157	Romney
B158	"
R195/69	"
X452/69	"
R294/69	"
R 16/69	"
R244/69	"
PT195/70	"
R225/69	"

The techniques used by the author gave a predominance of analysable diakinesis-metaphase I stages. Quantitative analysis of some of the other meiotic stages was impossible because of the small numbers of cells available for study from many of the rams.

(ii) RESULTS OF ANALYSES OF SPERMATOGONIAL AND PRIMARY SPERMATOCYTE CELLS

(A) Spermatogonial Cells

The numbers of cells and the distribution of chromosome counts in these cells is recorded in Table IV. 4. The modal number was 54; there were no hyperploid cells but several hypoploid cells and chromosome loss in these probably occurred during slide preparation.

Five polyploid cells were seen (21.7 per cent of the total number of spermatogonial cells), but the degree of ploidy could not be determined.

TABLE IV. 4

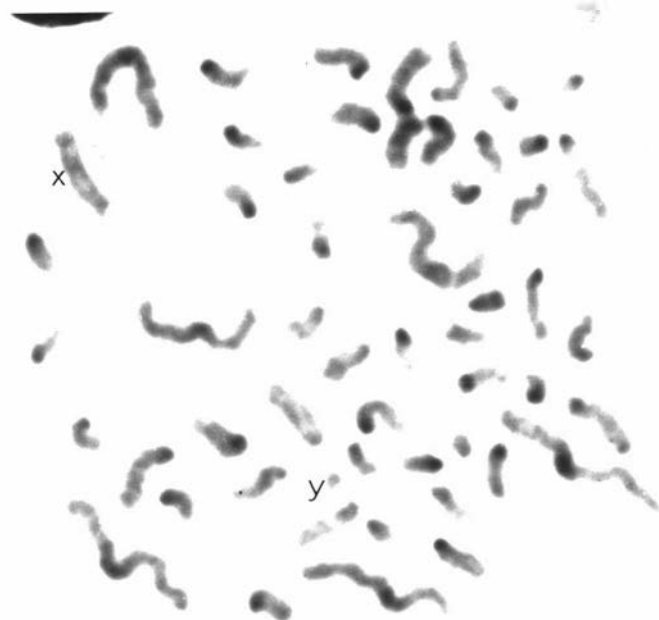
DISTRIBUTION OF CHROMOSOME COUNTS FROM SPERMATOGONIAL
METAPHASES IN NORMAL RAMS ($2n = 54, xy$).

Ram Identification	Number of Cells With Chromosome Number					Number of Polyploid Cells	Total
	52	52	53	54	55		
X435/69				1		2	3
R285/69				1			1
X117/69			1	3			4
X214/69				2		1	3
84/68							0
B157				1			1
B158	2					1	3
R195/69				1			1
X452/69							0
R294/69							0
R 16/69	2			1		1	4
R244/69	1			1			2
PT195/70			1				1
R225/69							0
Total	5	1	1	11	0	5	23

The degree of condensation of the chromosomes varied between metaphases (Plates IV. 2 and IV. 3) but because so few cells were available for analysis it was not possible to decide whether these were in fact different types of spermatogonia (A or B) or merely extremes of the same pre-meiotic division. Similar effects have been observed in man (Ferguson-Smith, 1972).

Plate IV. 2: Spermatogonial metaphase from a normal ram.
Note dark staining areas in region of
centromere. Sex chromosomes are
identified.
(Aceto-orcein. x 1,750).

Plate IV. 3: Spermatogonial metaphase from normal ram.
Chromatid separation is evident in some
chromosomes.
Sex chromosomes identified.
(Aceto-orcein. x 1,750).



2



3

(B) The Primary Spermatocytes

Each of the meiotic stages when fully developed was recognizable, but the classification of many of the intermediate stages was arbitrary.

a Leptotene and zygotene

It was difficult to identify the leptotene and zygotene stages of meiosis in the ram. Two examples of cells thought to be in these stages are shown in Plates IV. 4 and IV. 5. Individual chromosomes cannot be delineated and the cell appears as a mass of fine threads (Plate IV. 4). Parts of some chromosomes can be recognized at the periphery of the leptotene-zygotene cell illustrated in Plate IV. 5. The outline of the chromosomes in Plate IV. 5 is more distinct than that of the chromosomes shown at leptotene in Plate IV. 4.

The sex chromosomes were not identified in early meiotic prophase cells in the ram; neither was a true zygotene stage demonstrated.

b Pachytene

Numerous cells at the pachytene stage were seen both in preparations made by the main technique used (Chapter III section 2(i) (D)) as well as by the special methods for prophase stages (Chapter III section 2(ii) (B)). Pairing of the homologous chromosomes is complete but the dual nature of the bivalents can be seen clearly in many cells (example Plate IV. 12) although it is difficult to distinguish the chromatids in the tetrad as separate entities.

When cells at the pachytene stage were karyotyped, in all cases, 27 bivalents were evident. Three of the bivalents were distinctive in that they were considerably longer than the remainder which could be arranged in decreasing order of size. Plate IV. 12 and Figure IV. 2 are examples of ^acell karyotyped at pachytene.

A distinct chromomeric pattern could be seen on the bivalents in some cells but the author did not attempt to map bivalents on the basis of their chromomere patterns.

The initiation of separation of the chromosomes forming the bivalents was observed in some late pachytene cells (Figure IV. 1).

Dark staining areas (often bipartite) on the autosomal bivalents were sometimes seen in early and mid pachytene cells stained with aceto-orcein (Plates IV. 6, IV. 9). These were in the terminal region on all but the three large autosomal bivalents which had a similar dark stained area at their mid-points.

During the latter stages of zygotene and the early stages of pachytene, the sex chromosomes were in the form of an heteropyknotic "mass" (Plates IV. 7 and IV. 8) often situated near the periphery of the nucleus as is shown in Plate IV. 11 and Figure IV. 3. The shape of this "sex vesicle" varied between cells but was occasionally triangular or oval in form and had a clear outline with dark and light staining regions.

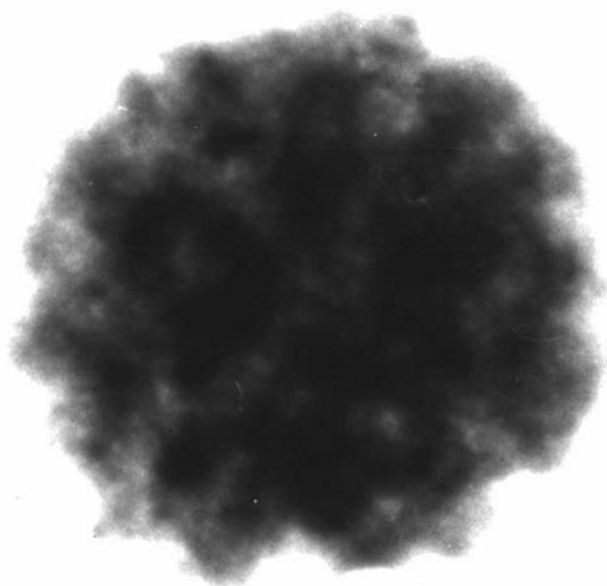
In early to mid pachytene cells the sex chromosomes appeared in a compact figure-of-eight configuration (Plate IV. 9, IV. 12, **A**) which was usually similarly stained to the autosomes. In some cells, a lighter staining tail-like structure composed of two threads could be seen attached to a more condensed structure (Plate IV. 10) and this stage probably preceded the distinctive figure-of-eight stage. In a few cells it could be seen that these configurations opened out to form a circle, with a dark staining heteropyknotic protuberance at the junction of the two free ends in at least one cell which is shown in Plate IV. 11.

c Diplotene

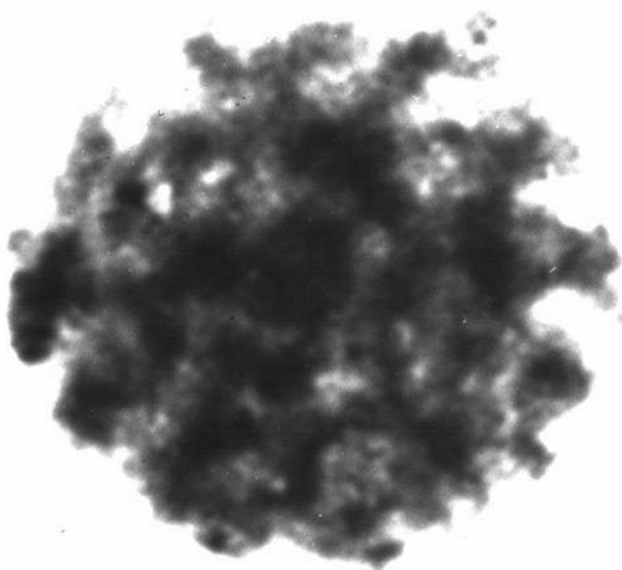
Cells at the diplotene stage were rare and it was not possible to analyse the majority because of the attenuated form of the bivalents which were often overlapping and very lightly stained. An example of an early diplotene cell is shown in Plate IV. 13. This polyploid cell has three deeply staining structures which are probably the sex chromosomes. In the few diploid diplotene cells studied, a sex bivalent isopyknotic with the autosomes was identified. However these cells could have been in the transition stage, between diplotene and diakinesis. The dark staining areas visible at the centromeric region of bivalents in some pachytene cells were also seen on early diplotene autosomal bivalents in areas adjacent to, or at the region corresponding to the centromere.

Plate IV. 4: Leptotene stage.
(Aceto-orcein. x 5,700).

Plate IV. 5: Leptotene-zygotene stage.
(Aceto-orcein. x 5,700).



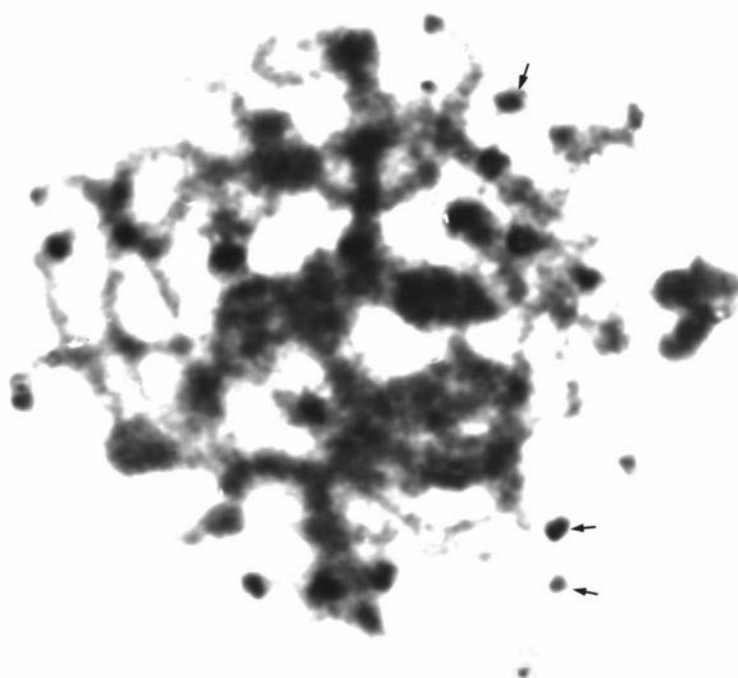
4



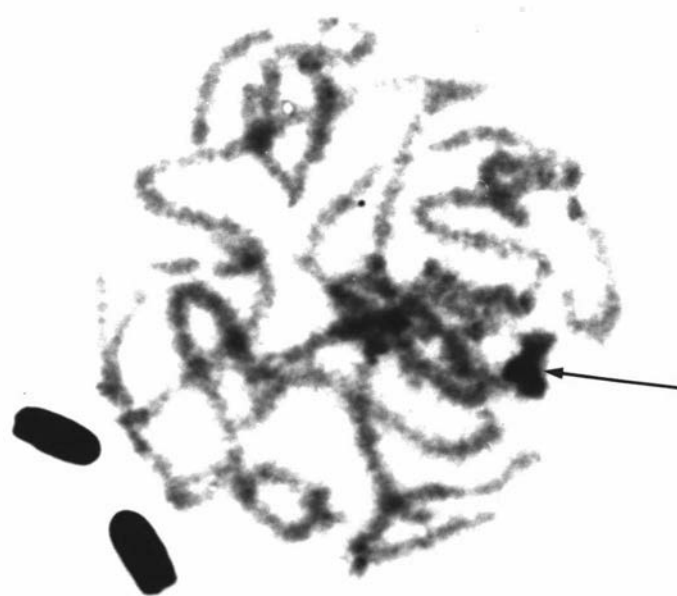
5

Plate IV. 6: Late zygotene - early pachytene stage showing "basal knobs" (some examples are arrowed). (Aceto-orcein. x 3,500).

Plate IV. 7: Early pachytene stage. The "sex vesicle" (arrowed) is situated near the periphery of the cell. (Aceto-orcein. x 2,850).



6



7

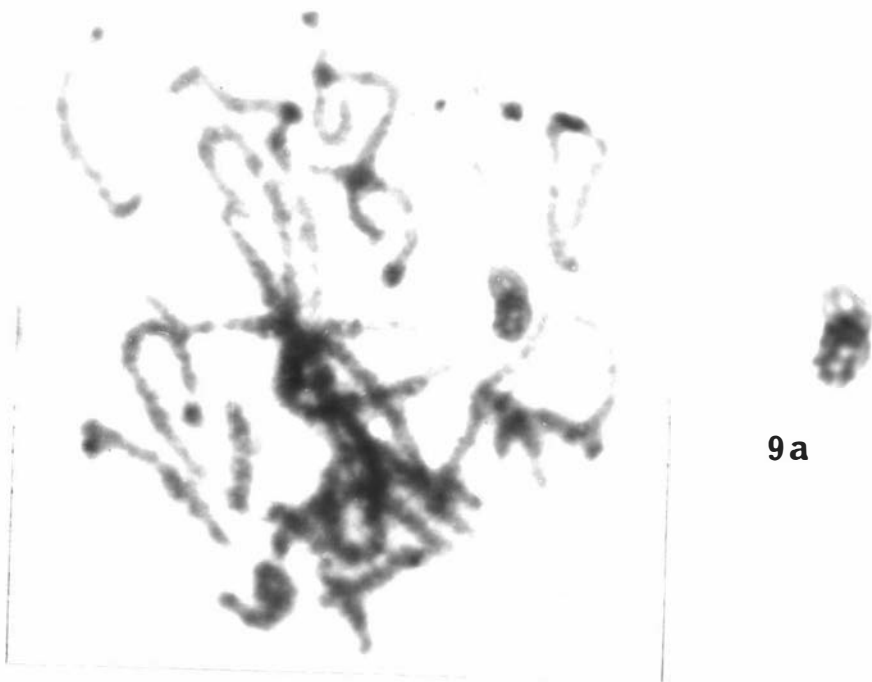
Plate IV. 8: Early pachytene stage. The "sex vesicle"
is arrowed. The chromomeric pattern
can be seen on several of the autosomal
bivalents.
(Aceto-orcein. x 4,000).

Plate IV. 9: Mid-pachytene stage. The sex bivalent
is in a figure-of-eight configuration.
(Aceto-orcein. x 2,000)

9a: Sex bivalent enlarged (x 8,000).



8

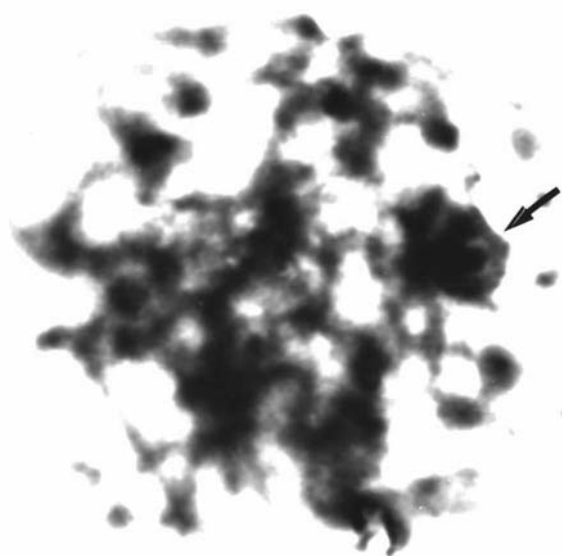


9a

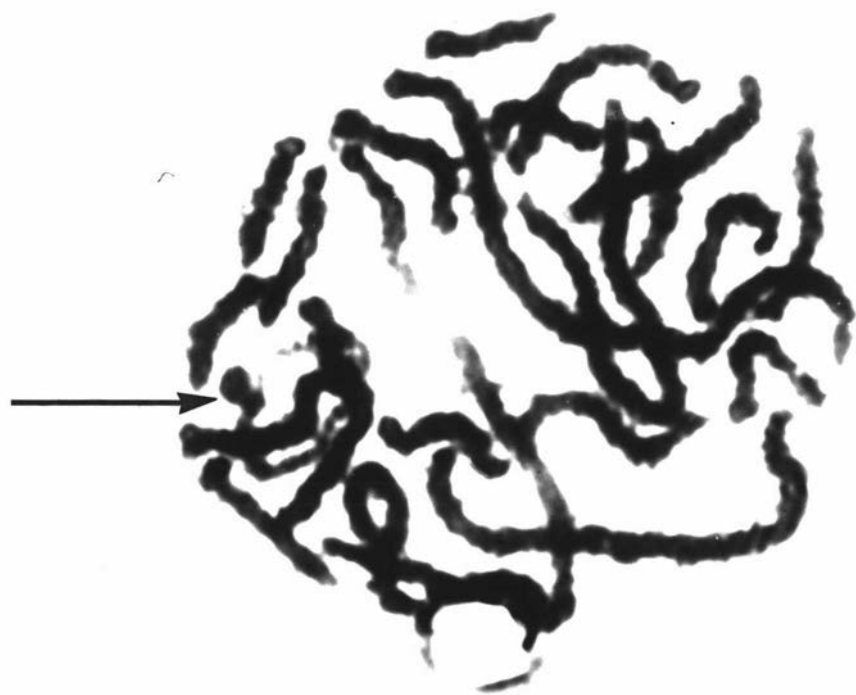
9

Plate IV. 10: Early pachytene stage. A "tail-like" dual thread structure protrudes from the "sex vesicle" (arrowed).
(Aceto-orcein. x 4,000).

Plate IV. 11: Late pachytene stage. Bivalents are contracted. Sex bivalent (arrowed) is in form of a circle.
(Aceto-orcein. Phase contrast. x 4,000).



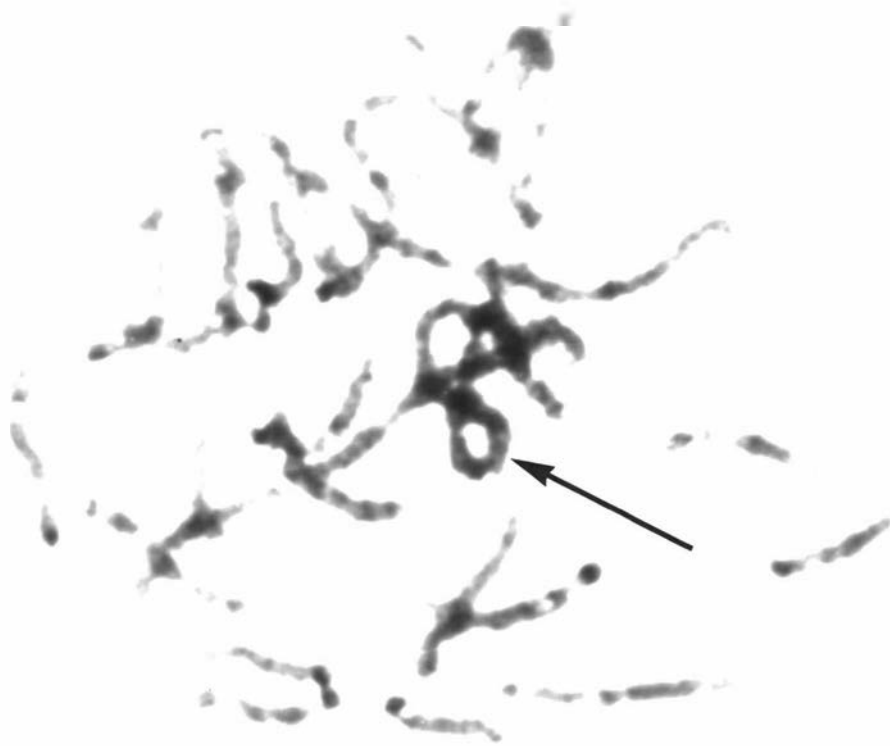
10



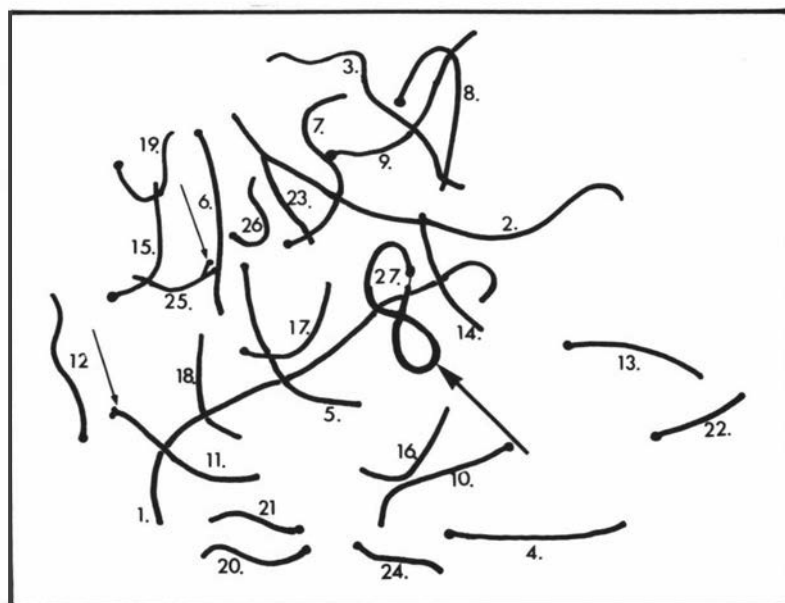
11

Plate IV. 12: Mid-late pachytene stage. Sex bivalent (arrowed) is in figure-of-eight configuration and is isopyknotic with the autosomal bivalents. (Aceto-orcein. x 2,000).

Figure IV. 1: Drawing of pachytene bivalents illustrated in Plate IV. 12. Small arrows point to part of a bivalent in which the chromosomes are separated at one end (No.25) and to bivalent number 11, the dual nature of which can be seen. Large arrow points to sex bivalent.



12



1

Figure IV. 2: Karyotype of pachytene bivalents illustrated
in Plate IV. 12.

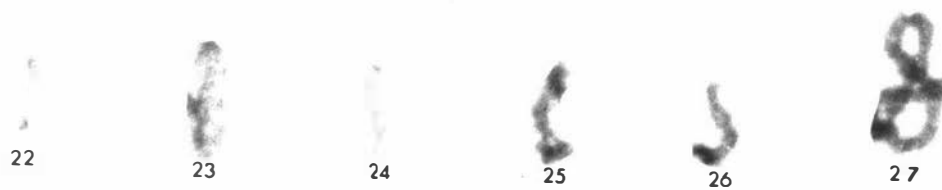
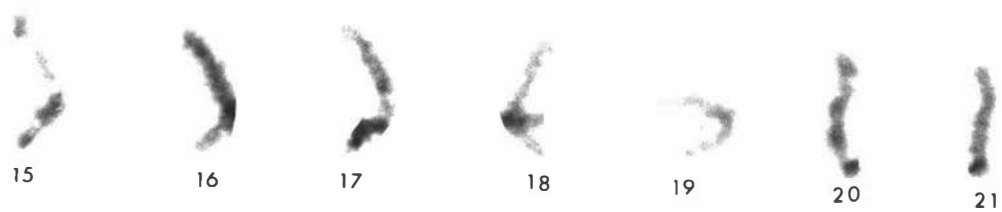
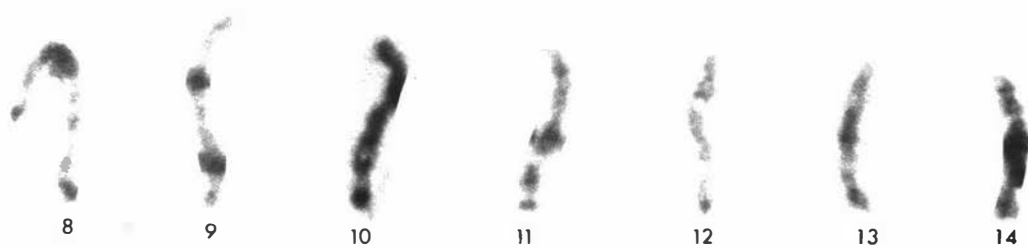
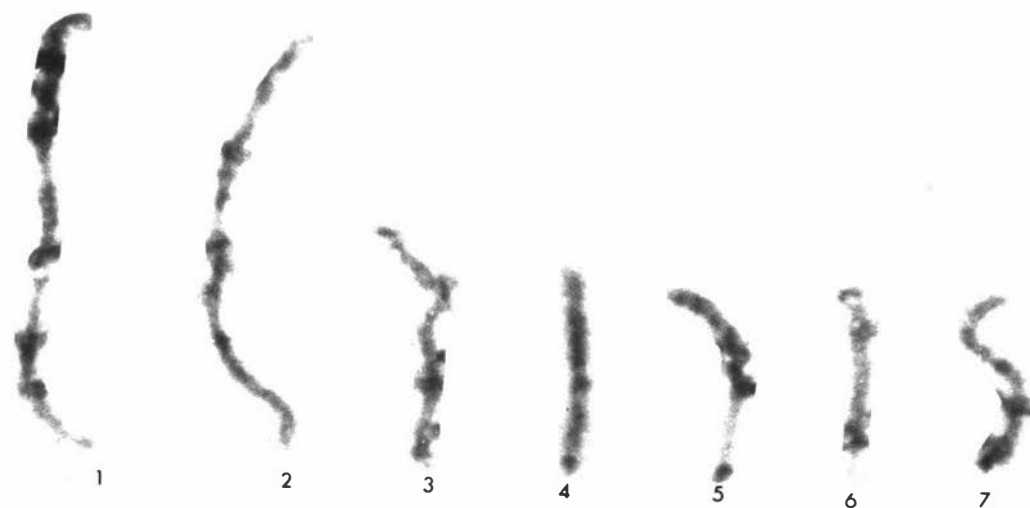
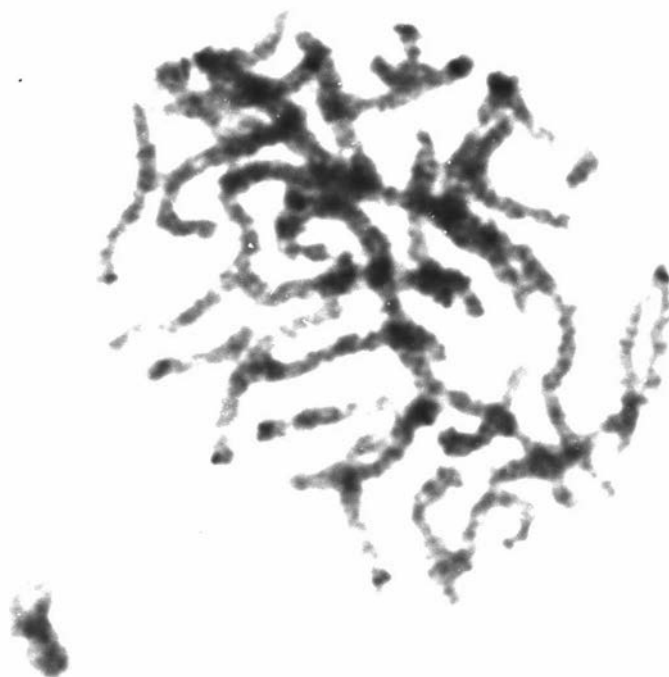
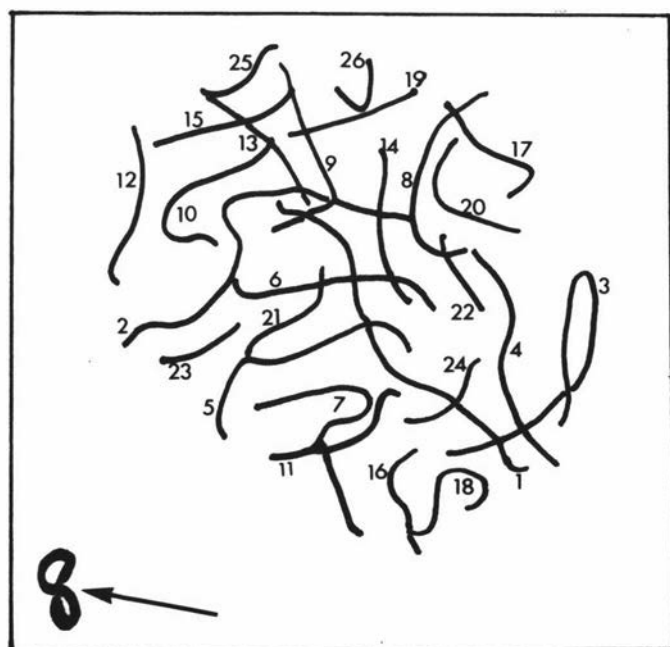


Plate IV. 12A: Mid-late pachytene stage.
(Aceto-orcein. x 2,000).

Figure IV. 3: Drawing of pachytene bivalents illustrated in Plate IV. 12A. The sex bivalent (arrowed) is in a figure-of-eight configuration and is situated at the periphery of the cell. The bivalents are numbered in approximate order of size as determined from an analysis of the karyotype.



12,A



3

Plate IV. 13: Polyploid cell at early diplotene.
Three sex bivalents are identified
(arrows).
(Aceto-orcein. x 1,000).

d Diakinesis-metaphase I

These two stages were combined for the analysis of chromosome counts as it was difficult to determine the transition stage.

The chromosomes in cells at the diakinesis-metaphase I stages were easy to count and identify. They were invariably well separated and rarely overlapping of bivalents occurred.

A total of 309 cells of which 56 were polyploids (18.1 per cent) were recorded. It was possible to accurately count the chromosomes in all but four (polyploid) cells. These counts are summarized in Tables IV. 5 and IV. 6. The modal number of 27 bivalents was recorded in 81.8 per cent of the cells analysed. There were five hypermodal cells out of the 253 analysed (1.98 per cent). In three of these the X and Y chromosomes were separated (Plate IV. 14), in one cell two autosomal univalents were observed (Plate IV. 15), while in the fourth, two extra chromosome elements were present (Plate IV. 16). Loss of one autosomal bivalent was the most common cause of hypoploidy; the remainder of the hypoploid cells were those in which one or more autosomal bivalents and/or occasionally the sex bivalent were missing. Approximately 16.2 per cent of the cells analysed were hypomodal.

The 27 bivalents counted in the majority of cells comprised 26 autosomal bivalents and a sex bivalent. It was difficult to identify individual bivalents in a spread and relate them to their mitotic counterparts when conventional staining techniques (aceto-orcein or giemsa stain) were used. However the six metacentric chromosomes distinctive in mitotic metaphases (Plate IV. 1), were also easy to distinguish in meiotic metaphases. These chromosomes formed three large bivalents, one slightly shorter than the other two which in some cells were of similar length. This length difference was noted by Gimenez-Martin and Lopez-Saez (1966) in mitotic metaphases. In contrast, Bruere and McLaren (1967) and Roca and Rodero (1971) found that the size of the second chromosome pair was midway between that of the first and third pairs. The difference in length between the first two and third bivalents was most often seen in early diakinesis cells. The largest bivalent was more than twice the length of the fourth largest bivalent and four to five times the length of the smallest bivalent in the majority of cells. The three large bivalents appeared as a series of paired loops, the number of

TABLE IV. 5

NORMAL RAMS (2n = 54, XY)

CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS - METAPHASE I STAGES

Ram Identifi- cation	Number of Chromosome bodies/cell							Total	Number of Polyploid Cells	Total including Polyploids	Analysis of Cells Excluding Polyploids Number of Cells with :-					Total Anal- ysed	X-Y Associated with Auto- somal Bivalent	X and Y Separated			
	≤23	24	25	26	27	28	29				27,XY	28,X,Y	26,XY,-A	≤25,XY	Other Aberrant cells			Number	%		
X435/69	Number of Cells	0	0	0	1	5	0	0	6	1	7	5	0	1	0	0	6	0	0	0.0	
R285/69		0	4	2	2	4	0	1	50	13	63	41	0	2	5	29,XY,+II+II? 24,-XY,-2A	1 1	50	5	0	0.0
X117/69		1	0	2	0	9	0	0	12	2	14	9	0	0	3	0	12	0	0	0.0	
X214/69		0	1	1	1	13	1	0	17	7	24	13	0	1	1	28,XY,+I+I 25,-XY,-A	1 1	17	3	0	0.0
84/68		0	0	0	0	8	0	0	8	1	9	8	0	0	0	0	8	3	0	0.0	
B157		0	0	0	0	6	0	0	6	4	10	6	0	0	0	0	6	0	0	0.0	
B158		0	0	1	1	4	0	0	6	1	7	4	0	1	1	0	6	0	0	0.0	
R195/69		0	0	3	6	31	1	0	41	5	46	31	1	6	3	0	41	1	1	2.4	
X452/69		0	0	0	1	3	0	0	4	1	5	3	0	1	0	0	4	1	0	0.0	
R294/69		2	0	1	1	17	0	0	21	2	23	17	0	1	1	25,-XY,-A 20,-XY,-6A	1 1	21	3	0	0.0
R16/69		0	0	0	0	7	0	0	7	5	12	7	0	0	0	0	7	0	0	0.0	
R244/69		0	1	2	0	4	2	0	9	4	13	4	2	0	3	0	9	0	2	22.2	
FT/195/70	1	0	0	3	25	0	0	29	4	33	25	0	3	1	0	29	0	0	0.0		
R225/69	0	0	0	3	34	0	0	37	6	43	34	0	3	0	0	37	3	0	0.0		
TOTAL		4	6	12	19	207	4	1	253	56	309	207	3	19	18	6	253	19	3	1.2	

TABLE IV. 6

NORMAL RAMS (54,xy) POLYPLOID CELLS AT DIAKINESIS-METAPHASE I

Ram Identification	Number of cells showing chromosome complement				Total	Analysis of Polyploid Cells									
						4n				6n			8n		Total
	4n	6n	8n	N.A.		54,2xy	53,xy,-xy	≤53,2xy	≤53,xy,-xy	81,3xy	≤80,3xy	≤80,2xy,-xy	108,4xy	N.A.	
X435/69	1				1	1									1
R285/69	12	1			13	5	1	6			1				13
X117/69	2				2			2							2
X214/69	3	2		2	7	2		1		1		1		2	7
84/68	1				1	1									1
B157	3			1	4	2		1						1	4
B158	1				1	1									1
R195/69	2	3			5	2				3					5
X452/69	1				1			1							1
R294/69	2				2	2									2
R16/69	4	1			5	3			1		1				5
R244/69	1	2	1		4	1					2		1		4
PT195/70	3			1	4	1			2					1	4
R225/69	6				6	5		1							6
Total	42	9	1	4	56	26	1	12	3	4	4	1	1	4	56

N.A. = Not Analysed

which varied with the stage of the meiotic cycle. Their morphology as determined by the number and position of chiasmata present, was very similar in different nuclei.

The tetrad character of many of the bivalents was clear in some cells and chiasmata could be seen involving two of the four chromatids. (Examples are shown in Plates IV. 17, IV. 22 and IV. 26). The sex bivalent was the only other bivalent which could consistently be identified in every cell.

The form of the remaining autosomal bivalents varied according to the stage of meiosis of the cell. Several forms were apparent including a series of paired loops (bivalents with three or more chiasmata), ring-shaped bivalents (two chiasmata, often terminalized), cross-shaped bivalents (one chiasma) and rod-shaped bivalents (one terminalized chiasma). Some of these bivalent forms are illustrated in the series of Plates IV. 17 to IV. 26 which are typical of the diakinesis-metaphase I cells examined in this study.

The shape of the bivalents changed as the metaphase I stage was reached and developed. There was a progressive shortening of the bivalents which became very difficult to analyse. It was still possible to count chromosomal elements at this stage but bivalent contraction precluded accurate chiasmata counts. Examples of cells at the metaphase I stage are shown in Plates IV. 14, IV. 15 and IV. 16.

Chiasmata were counted in 34 diploid cells. These counts were made from photographic karyotypes similar to those illustrated in Figures IV. 4 and IV. 6, and confirmed by visual checking of the cell under the microscope. Only those cells in which an estimation of chiasmata number could be made on every bivalent were included in the results. There were many cells in which it was possible to count the chiasmata from one or several bivalents but as yet it is not possible to designate every bivalent in the meiotic karyotype, and therefore difficult to compare these individual bivalents with their counterparts in other nuclei. The data on frequency and distribution of chiasmata was derived from diakinesis-metaphase I cells with one exception, a diplotene cell. The results are summarized in Table IV. 7. The number of chiasmata per bivalent varied from one to seven with the latter number being observed in one diplotene and several early diakinesis cells. The majority of bivalents had two chiasmata; the next most frequent number was one chiasma, with from three to six

chiasmata being found in varying numbers of bivalents depending on the cell being studied. A chiasmata number of greater than four was restricted to the three largest bivalents. The small autosomal bivalents had either one or two crossover points, with the majority having two. (The counts for individual bivalents are recorded in Appendix XII). The average number of chiasmata per bivalent ranged from 1.9 to 2.8. The total number of chiasmata per cell varied between 42 and 73 with the extremes being counts recorded for a metaphase I and diplotene cell respectively. Where more than one cell was counted per animal, the average chiasmata count per cell ranged between 50.00 and 58.00. Similarly when terminal chiasmata were considered a range of between 15 to 30 was recorded, but when averages were calculated the total terminal chiasmata ranged from 20.7 to 25.2. The terminalization coefficient which is an index of chiasmata movement and an indication of the initial chiasmata frequency in a particular bivalent was calculated according to the formula:

$$\text{Terminalization coefficient} = \frac{\text{number of terminal chiasmata}}{\text{total number of chiasmata}}$$

(per cell). This varied from 0.25 to 0.64 and the average estimates ranged from 0.35 to 0.49.

The mean total number of chiasmata per diploid cell at diakinesis-metaphase I for a normal ram was 55.4. This corresponds to an approximate minimum genetic length of 27.7 morgans. A morgan is defined as "the map-length of a chromatid segment that has experienced, on average, one crossover event per meiosis" (Renwick, 1971). Each chiasma corresponds to 50 "map-units" (White, 1961). A morgan is equal to 100 centimorgans or 100 "map-units" (Renwick, 1971) thus a chiasma is equivalent to 50 centimorgans.

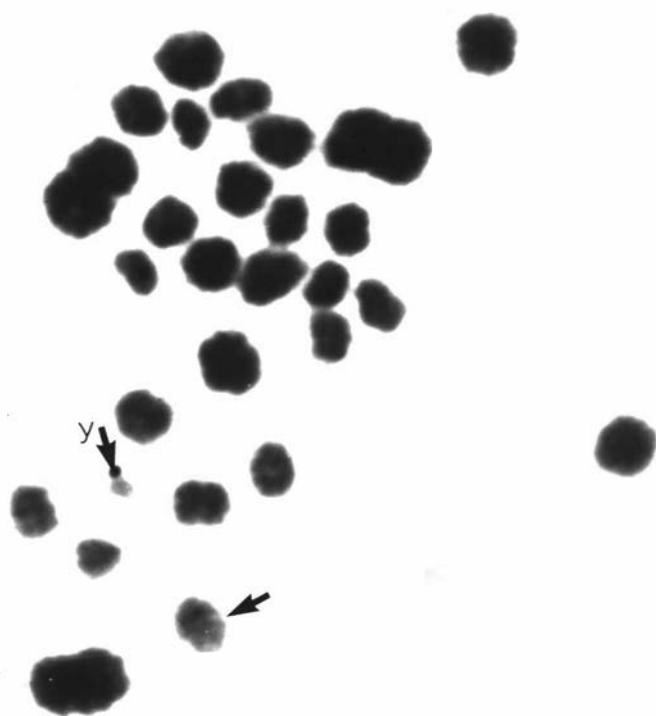
TABLE IV. 7
FREQUENCY AND DISTRIBUTION OF CHIASMATA IN NORMAL RAMS (54,X X)
(Table design after McDermott, 1973,b)

Ram Identification	Total Number Counted		Distribution of Chiasmata Number Average Number of Bivalents With Given Number of Chiasmata								Average Number of Chiasmata/ Bivalent	Total Chiasmata/Cell		Total Terminal Chiasmata		Terminalization Coefficient	
	Cells	Bivalents	1	2	3	4	5	6	7	Average		Range	Average	Range	Average	Range	
X214/69	3	78	6.7	14.3	2.0	0.7	1.0	0.7	0.7	2.2	57.7	53 - 65	21.7	19 - 26	0.38	0.29 - 0.49	
B157	1	26	8.0	13.0	2.0	2.0	1.0	0	0	2.0		53		26		0.49	
X452/69	1	26	4.0	16.0	3.0	0	1.0	2.0	0	2.4		62		23		0.37	
PT195/70	3	78	6.0	14.3	2.7	0.7	1.3	1.0	0	2.2	58.0	55 - 64	20.7	15 - 25	0.35	0.27 - 0.40	
X117/69	1	26	1.0	14.0	7.0	1.0	0	2.0	1.0	2.8		73		24		0.33	
R244/69	1	26	4.0	19.0	0	1.0	1.0	1.0	0	2.2		57		18		0.32	
R225/69	4	104	6.8	14.5	1.7	0.2	1.2	1.5	0	2.2	57.3	53 - 65	24.8	24 - 26	0.43	0.37 - 0.49	
R195/69	10	260	5.8	15.6	1.7	1.3	0.7	0.8	0	2.2	56.3	49 - 65	24.3	16 - 30	0.44	0.25 - 0.61	
84/68	2	52	8.0	15.0	0.5	2.0	0.5	0	0	1.9	50.0	47 - 53	23.0	22 - 24	0.46	0.45 - 0.47	
R285/69	5	130	8.0	13.4	2.0	1.6	0.8	0.2	0	2.0	52.4	47 - 60	25.2	21 - 30	0.49	0.37 - 0.64	
X435/69	3	78	8.7	12.3	3.3	0.3	1.0	0.3	0	2.0	51.7	42 - 64	23.3	22 - 26	0.47	0.34 - 0.62	

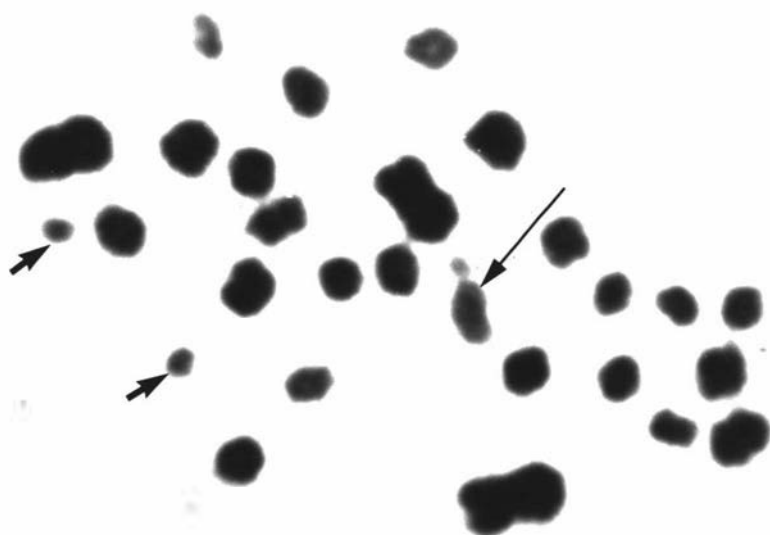
* Diplotene Cell

Plate IV. 14: Metaphase I cell (from normal ram, R244/69)
with 28 chromosomal elements. Arrows
point to separated X and Y chromosomes.
(Aceto-orcein. x 2,000).

Plate IV. 15: Metaphase I cell (from normal ram, X214/69)
with 28 chromosomal elements. Large arrow
points to sex bivalent and small arrows point
to autosomal univalents.
(Aceto-orcein. x 2,000).



14



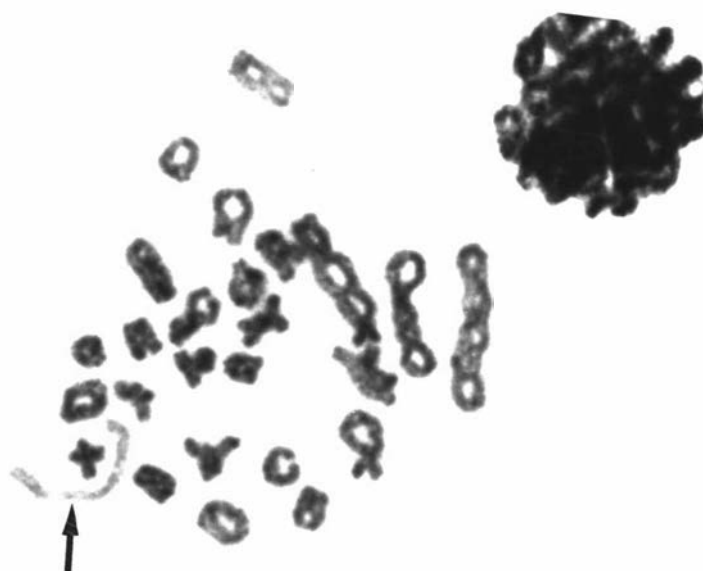
15

Plate IV. 16: Metaphase I cell (from normal ram, R285/69) with 29 chromosomal elements including a sex bivalent (arrowed).
(Aceto-orcein. x 2,800).

Plate IV. 17: Cell in diakinesis (from a normal ram, R195/69) with 27 bivalents including an XY bivalent (arrowed). The tetrad structure of many bivalents can be seen.
(Aceto-orcein. x 2,000).



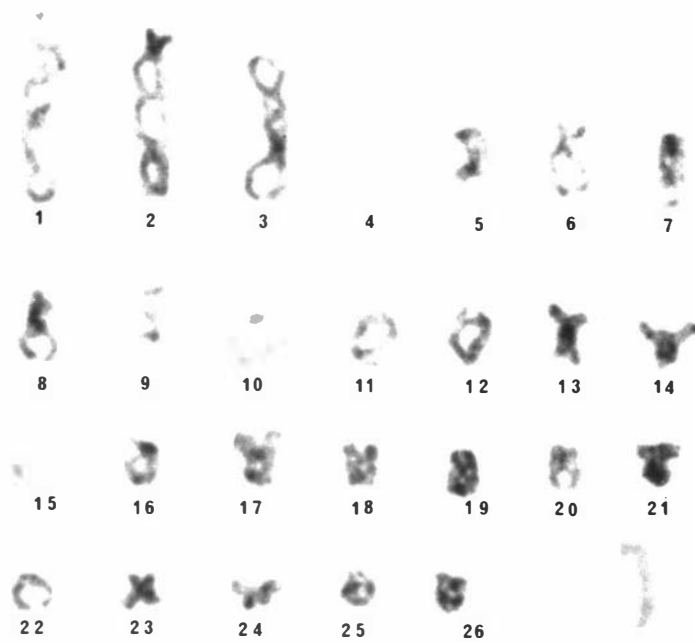
16



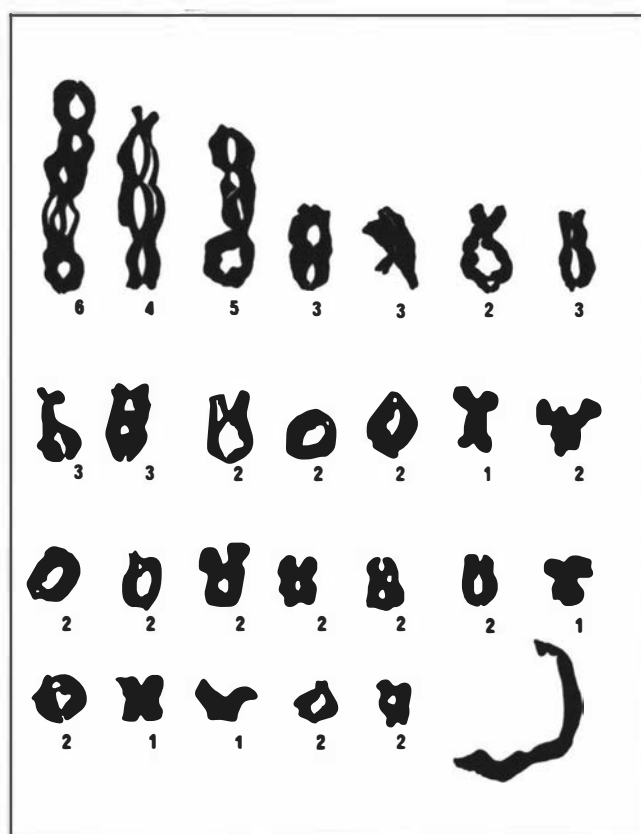
17

Figure IV. 4: Karyotype of cell illustrated in Plate IV. 17. Bivalents are arranged approximately in decreasing order of size and the sex bivalent is placed last. The three largest bivalents, made up of the paired metacentric chromosomes, are distinctive.

Figure IV. 5: Drawings of bivalents from cell illustrated in Plate IV. 17 and arranged in same order as in Figure IV. 4. The number of chiasmata per bivalent is recorded beneath each autosomal bivalent.



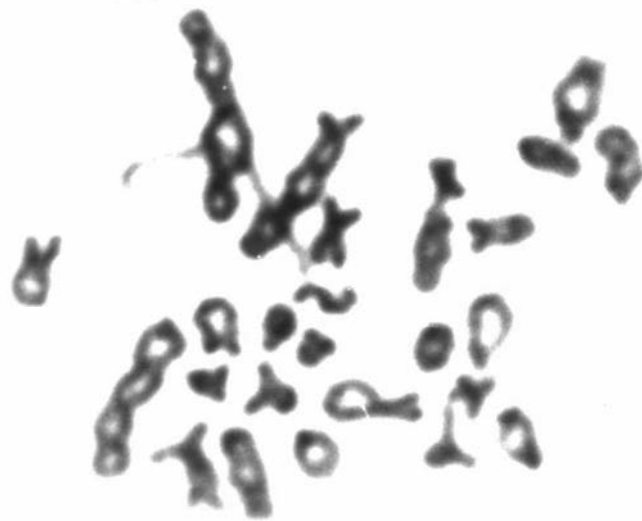
4



5

Plate IV. 18: Cell in diakinesis (from a normal ram, X435/69)
with 27 bivalents including a sex bivalent.
(Aceto-orcein. x 2,850).

Figure IV. 6: Karyotype of cell illustrated in Plate IV. 18.
The approximate numbers of chiasmata per bivalent
are recorded beneath each autosomal bivalent.



18

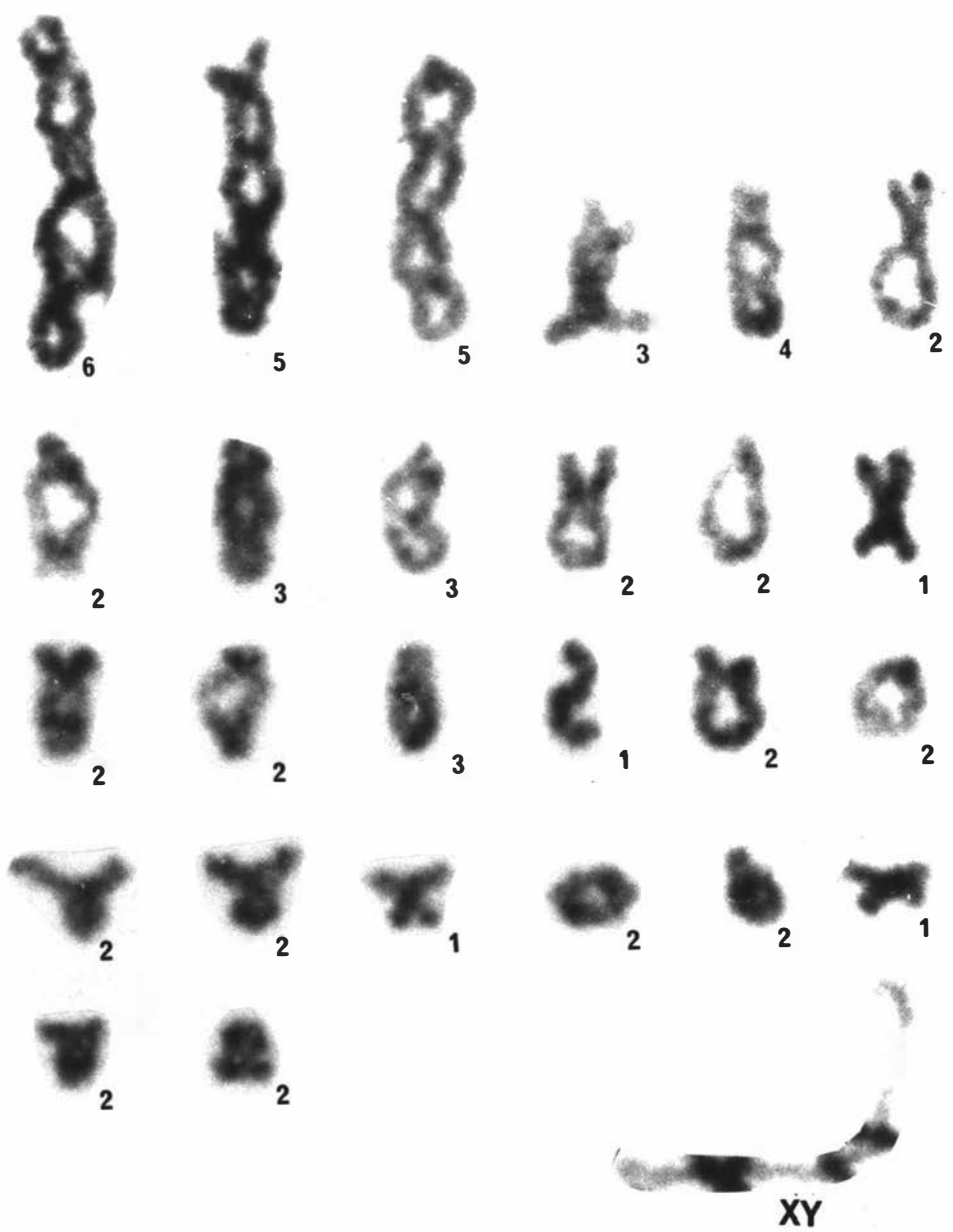
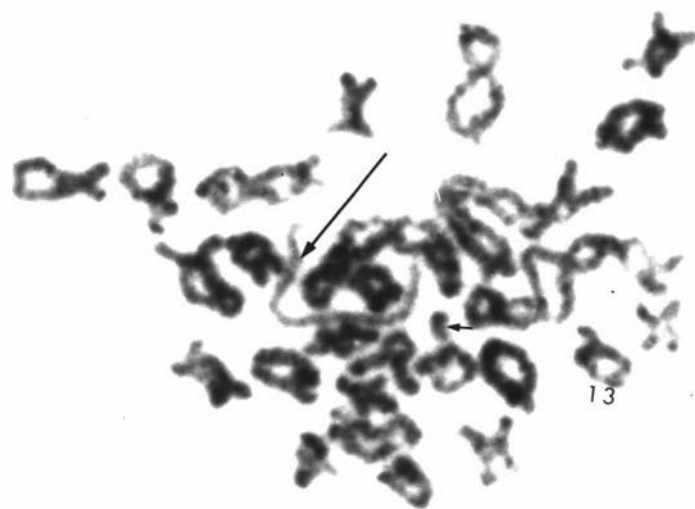
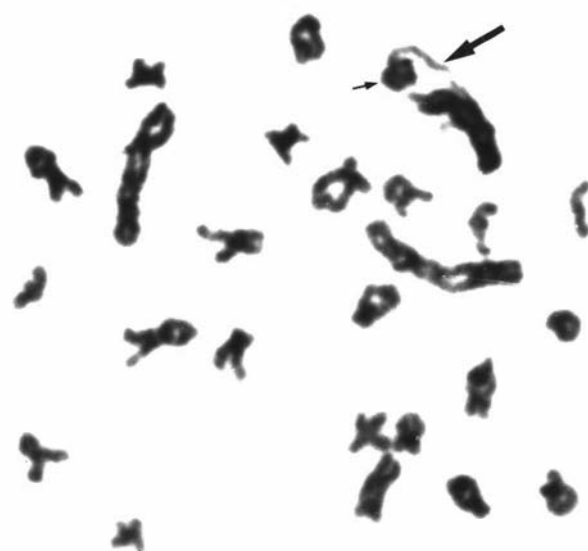


Plate IV. 19: A cell in diakinesis (from a normal ram, R195/69)
with 27 bivalents including a sex bivalent
() , and a fragment () which could
have come from bivalent 13.
(Aceto-orcein. x 2,850).

Plate IV. 20: A cell in diakinesis (from a normal ram, X214/69)
with 27 bivalents including a sex bivalent
() which is associated with a small
autosomal bivalent ().
(Aceto-orcein. x 2,850).



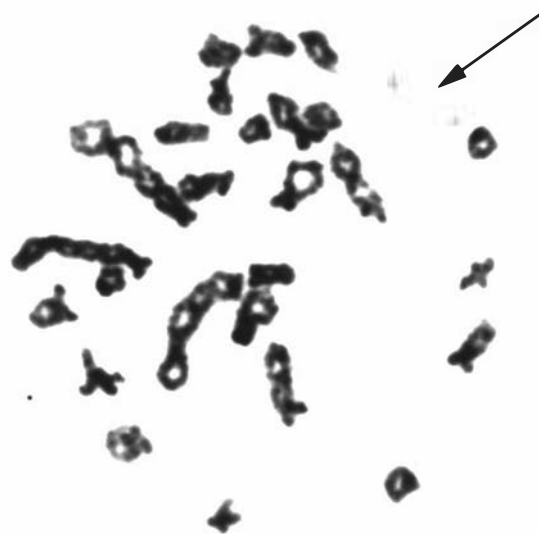
19



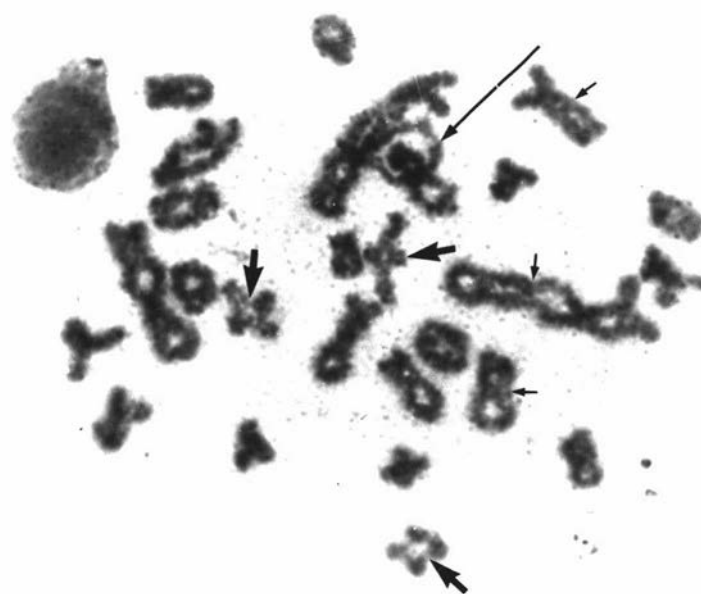
20

Plate IV. 21: A cell in diakinesis (from a normal ram, R225/69)
with 27 bivalents including a sex bivalent
(arrowed).
(Aceto-orcein. x 2,200).

Plate IV. 22: A cell in diakinesis (from a normal ram, R285/69)
with 27 bivalents including a sex bivalent in the
form of a loop ($\longleftrightarrow$). The chromatid structure
of some bivalents can be seen ($\rightarrow$). Note cross-
shaped bivalents ($\rightarrow$).
(Aceto-orcein. x 2,500).



21



22

Figure IV. 7: Drawing of autosomal bivalents from cell illustrated in Plate IV. 22. The approximate number of chiasmata per bivalent are recorded beneath each drawing. Note twisted chromatids in second largest bivalent.

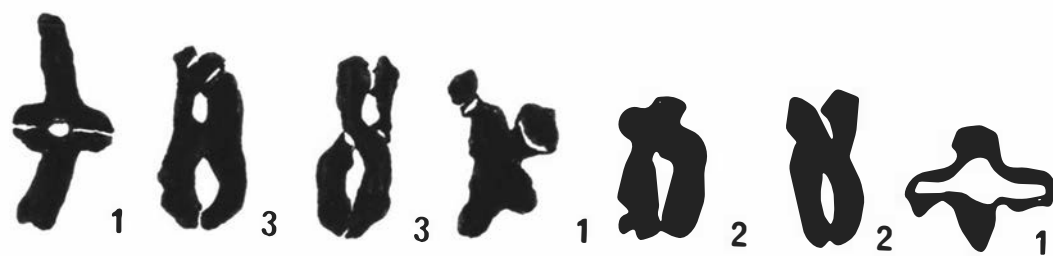
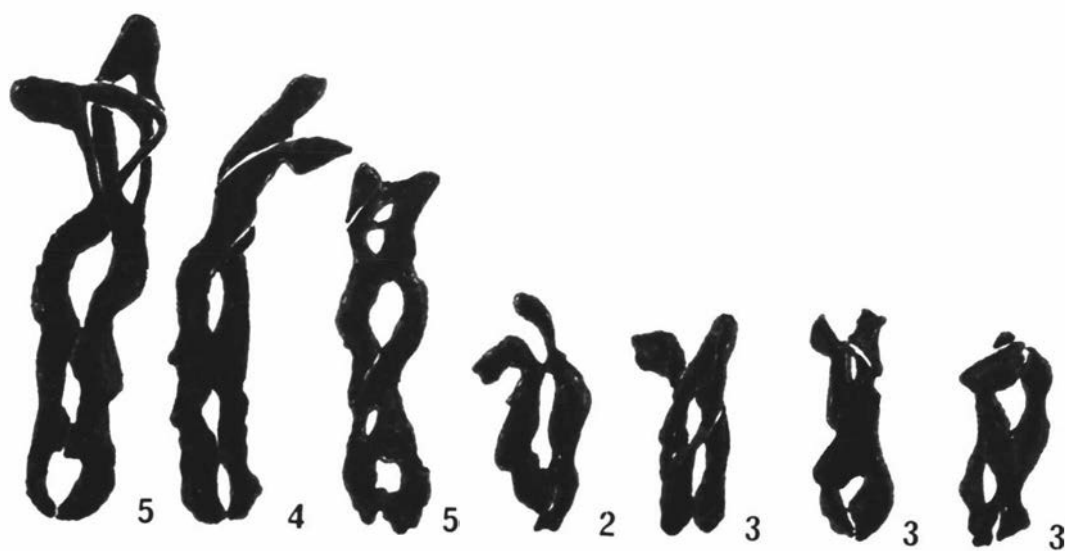
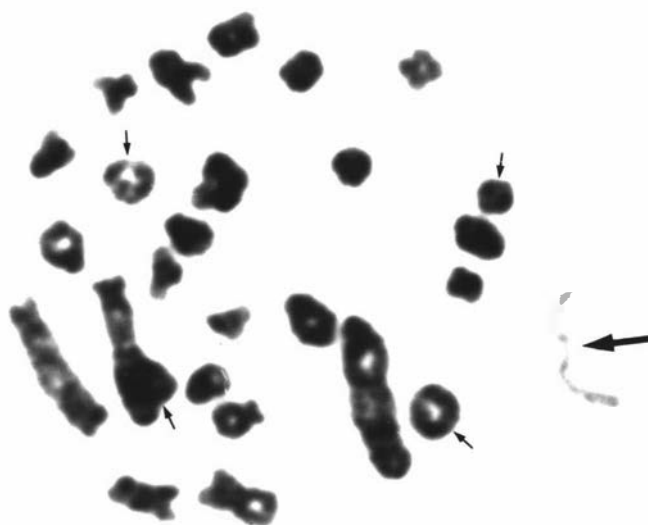
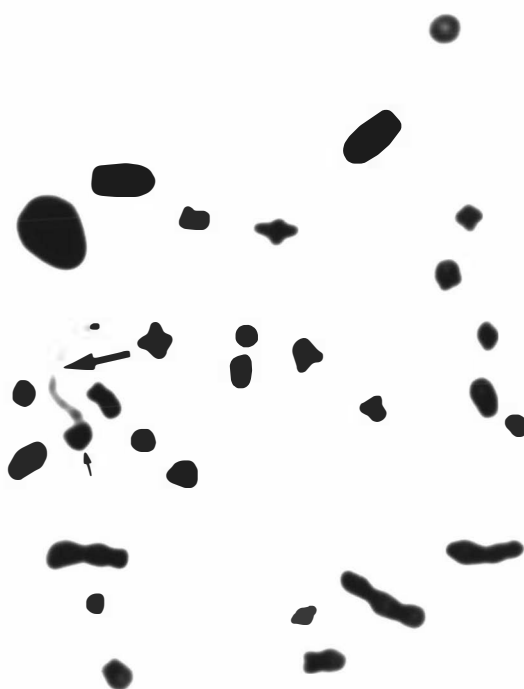


Plate IV. 23: Cell in diakinesis (from normal ram, R225/69)
with 27 bivalents including a sex bivalent
which is peripherally located ().
Note the small ring-shaped bivalents ().
(Aceto-orcein. x 2,200).

Plate IV. 24: Cell in diakinesis (from normal ram, 84/68)
with 27 bivalents including a sex bivalent
() which is associated with a small
autosomal bivalent ().
(Giemsa. x 2,000).



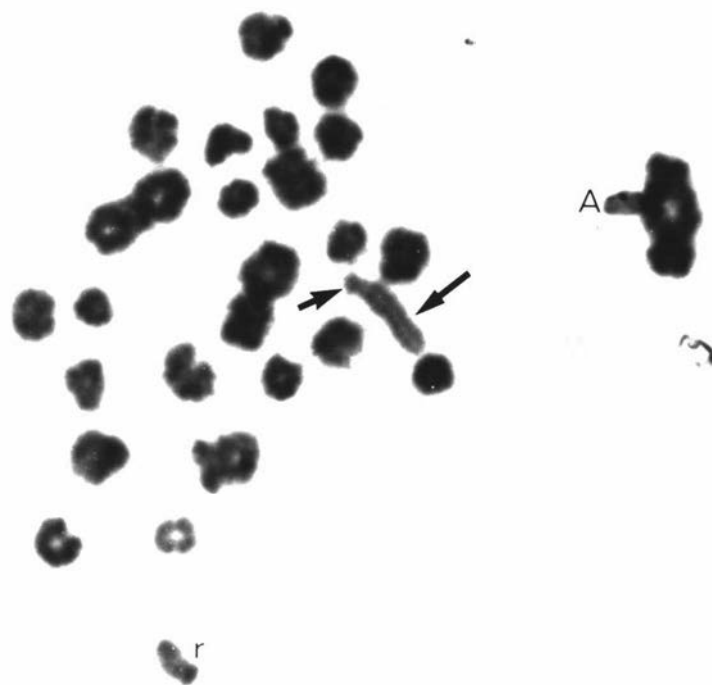
23



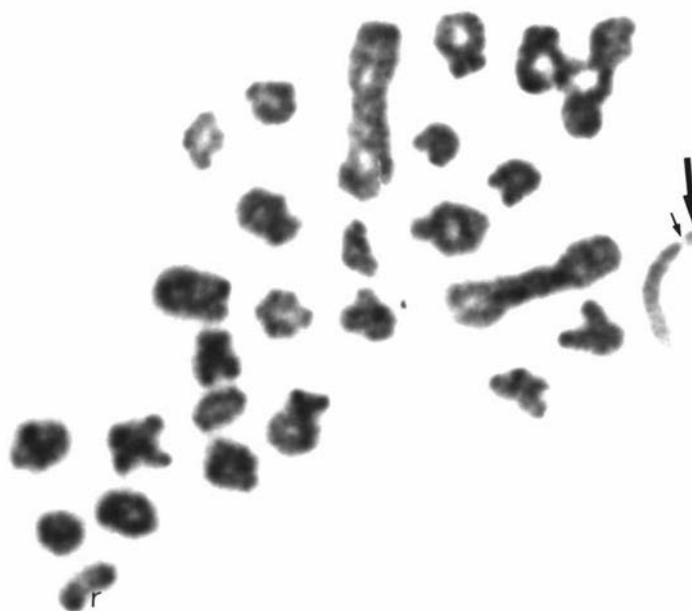
24

Plate IV. 25: Cell in diakinesis-metaphase I (from normal ram R285/69) with 26 bivalents including a sex bivalent. Two thin threads separate the Y chromosome (small arrow) from the X chromosome (large arrow). A small rod-shaped bivalent (r) is situated at the periphery of the spread. (A) is a technical artefact. (Aceto-orcein. x 2,800).

Plate IV. 26: Cell in diakinesis-metaphase I (from normal ram R285/69) with 27 bivalents including a sex bivalent. The Y chromosome (large arrow) is separated from the X chromosome by a pale-staining area (small arrow). Chiasmata can be seen on some autosomal bivalents including the largest. A small rod-shaped bivalent (r) is similar to that in Plate IV. 25. A small bivalent is overlapping part of a large bivalent in the top right hand corner of the photomicrograph. (Aceto-orcein. x 2,800).



25



26

Fifty-two polyploid diakinesis-metaphase I cells were analysed (Table IV. 6). Of this number, 26 were tetraploid, four hexaploid and one was octaploid. The remainder were hypoploid cells divided between the tetraploid (16 cells) and hexaploid (five cells) categories.

A polyploid cell was defined as "one whose chromosome complement is an exact multiple of the basic haploid number characteristic of the species" (Swanson, Merz and Young, 1967). The following terminology was used by the author when referring to polyploid diakinesis-metaphase I cells:

diploid cells	(2n) have 54 chromosomes or 27 bivalents	
tetraploid cells	(4n) have 108 chromosomes or 54 bivalents	)
hexaploid cells	(6n) have 162 chromosomes or 81 bivalents	) Polyploid
octaploid cells	(8n) have 216 chromosomes or 108 bivalents	)

The sex chromosomes were in the form of a bivalent in all polyploid cells examined. They were present in multiples of the diploid number, that is two sex bivalents in a tetraploid cell and three in a hexaploid cell, with four being observed in an octaploid cell. An example of polyploid diakinesis cells is shown in Plate IV. 27.

The sex chromosomes which were recognizable in every diakinesis-metaphase I cell examined, formed a bivalent in all but three of the 253 chromosome spreads studied. The three atypical cells were in metaphase and in them the sex chromosomes were separated. Examples are shown in Plates IV. 14 and IV. 28t.

There was no difference between the autosomes and the sex chromosomes in their affinity for stain in early diakinesis cells but towards the end of diakinesis and in metaphase I cells, the sex chromosomes were consistently less intensely stained by comparison with the autosomes (Plate IV. 14). In some spreads, the Y chromosome was negatively heteropyknotic when compared with the X chromosome. These findings are illustrated in Plate IV. 28m and IV. 28g.

The morphology of the sex bivalent varied between cells and according to the stage of the meiotic cycle.

In early diakinesis it was thread-like, sometimes straight

but often in a loop or curved at one end (Plate IV 28a-f). It was difficult and in most cases impossible at this stage to identify the X and Y components. In some bivalents a constricted region was observed near one end which was thought to be the region of association between the two chromosomes (Plate IV. 28c, IV. 28g and IV. 28h). As the diakinesis stage progressed the bivalent contracted and appeared more rod-like. It became clear that the chromosomes in the bivalent were in an end-to-end association. In some cells the dual structure of part of the sex bivalent could be seen. This feature illustrated in Plates IV. 25 and IV. 28k-s, was identified as the chromatids of the long arms of the X chromosome. The Y chromosome was thus associated with the short arms of the X chromosome. In mid to late diakinesis cells, a constricted pale staining area was observed between the sex chromosomes. In late diakinesis and metaphase I cells the X and Y appeared as distinct entities joined by two fine thread-like connections (Plate IV. 28i-s).

Preparations stained by the "C-banding" technique (Chapter III section 3(ii) (B)) were scrutinized in an effort to obtain more detailed information on the nature of the X-Y chromosome association. Unfortunately this technique was of no value as the centromeric regions of the sex chromosomes, unlike those of the autosomes, were not stained differentially from the chromosome arms.

An association between the sex bivalent and one of the small autosomal bivalents was seen in 7.5 per cent ($19/253$) of cells at the diakinesis-metaphase I stage. Some examples of these associations are shown in Plate IV. 29. It was not possible to ascertain either the exact nature of the association or the identity of the involved autosome and sex chromosome. It was thought that in some cases the Y chromosome was implicated. The numbers of cells in which some form of relationship was seen varied between rams.

Although the sex bivalent was seen at the periphery of some cell spreads, there was no evidence to indicate that its location in the nucleus was not random.

Plate IV. 27: Polyploid diakinesis cell (from normal ram,
B158) with 54 bivalents. Arrows point to
two sex bivalents.
(Aceto-orcein. x 1,750).

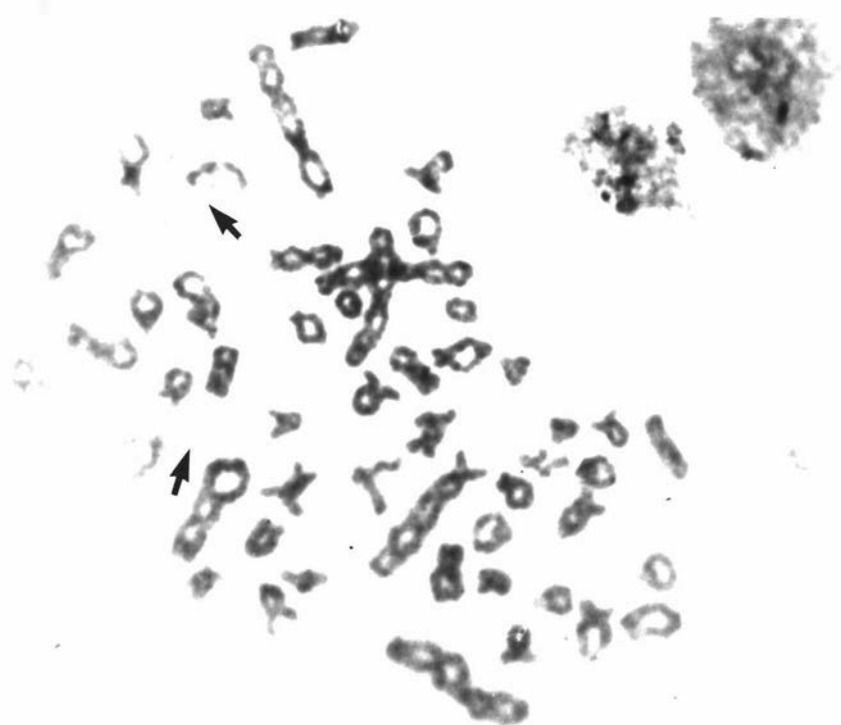


Plate IV. 28 a-t: Demonstrates the variation in morphology of the sex bivalent in representative cells in early diakinesis to late metaphase I. Arrow points to Y chromosome in cells in which it can be identified. Thin chromatin "threads" can be seen joining the X and Y chromosomes in i to s. The sex chromosomes are separated in t. Variation in the intensity of staining is seen on both chromatids of the X chromosome in n. (Aceto-orcein. x 4,750).

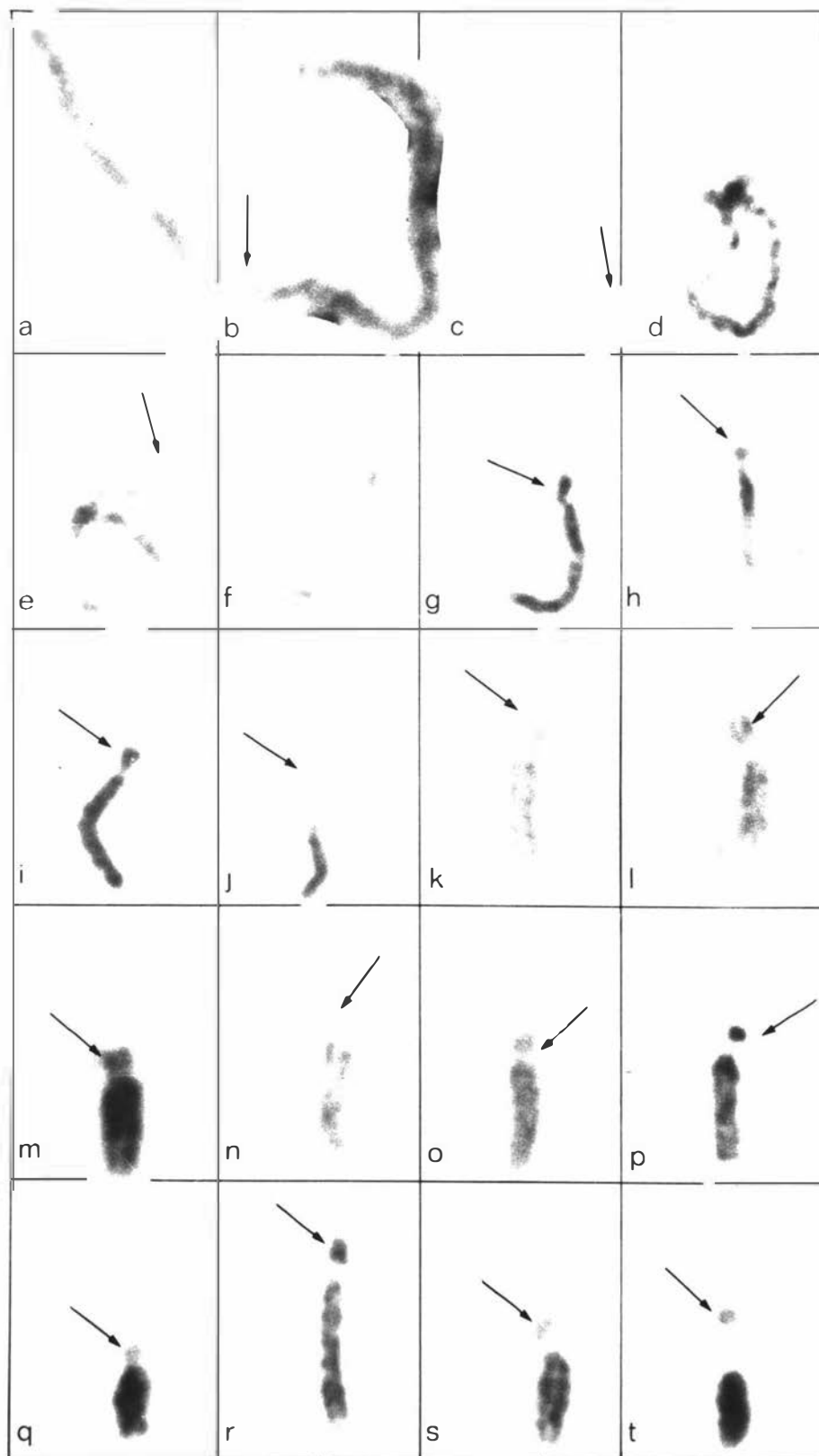
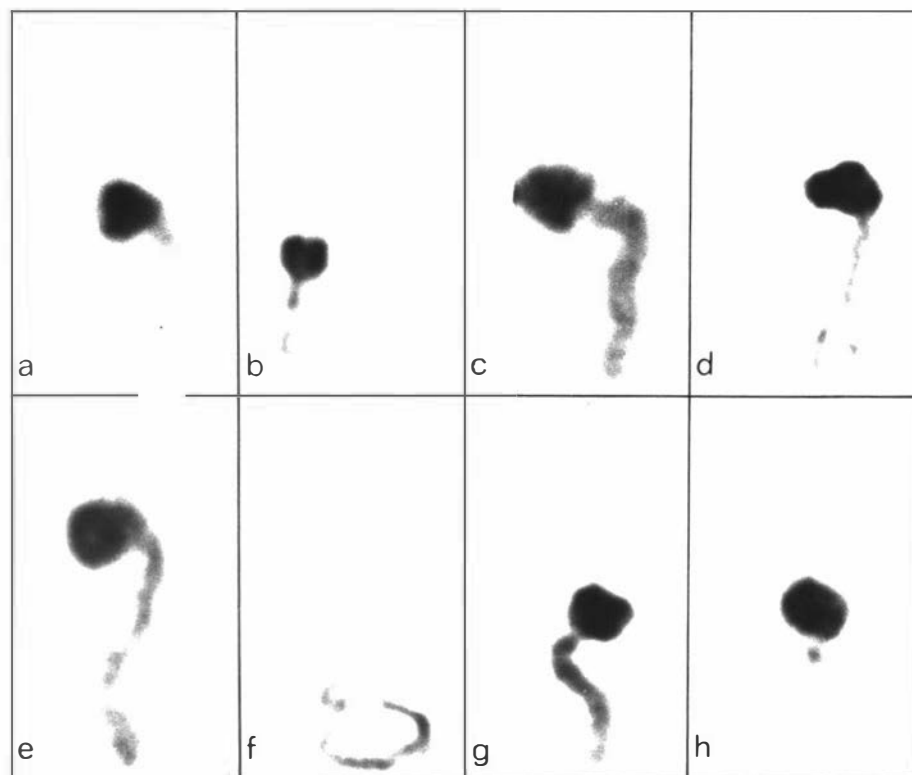
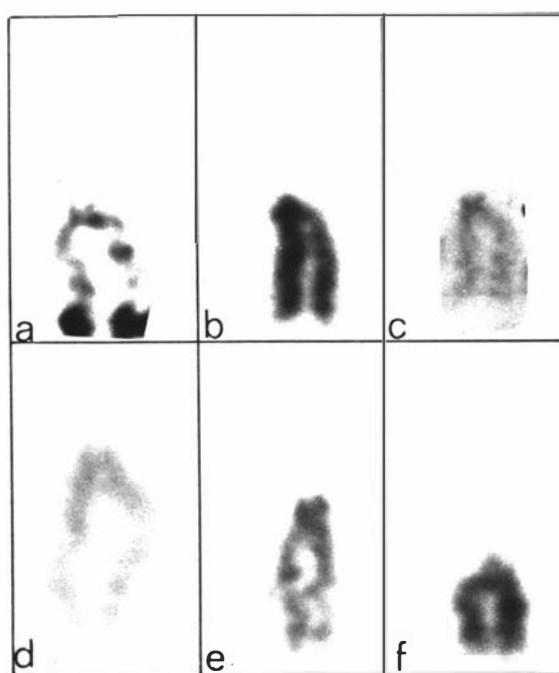


Plate IV. 29 a-h: Series of photomicrographs to demonstrate the association of the sex bivalent with a small autosomal bivalent at diakinesis-metaphase I in normal rams.
(Aceto-orcein. x 4,750).

Plate IV. 30 a-f: Cut-out X chromosomes from metaphase II cells to show chromosome morphology. Short arms are visible on each chromosome.
(Aceto-orcein. x 4,750).



29



30

(C) Analysis Of Secondary Spermatocytes

a Introduction

The method for analysis of chromosomes of second metaphase (metaphase II = MII) figures was based on that used by Tettenborn and Gropp (1970).

At metaphase II, the two sister chromatids of an individual chromosome are often separated from their centromere. A single chromatid from a metacentric or acrocentric chromosome could be easily confused with the two chromatids of an acrocentric chromosome still held together at the centromere. This is illustrated diagrammatically in Figure IV. 8 below.

- 1 (a) and (b) Acrocentric chromosomes with two sister chromatids held together at the centromere



- 2 (a) Metacentric chromosome

- (b) Metacentric chromosome with two sister chromatids separated



Figure IV. 8:

An acrocentric chromosome (1b) could be confused with a single chromatid from a metacentric chromosome (2b).

Tettenborn and Gropp (1970) suggested that counts of total chromosome arms rather than counts of chromosomes themselves were the most reliable method of determining whether a given secondary spermatocyte contained a complete haploid chromosome set.

The author counted both the chromosome number and the number of chromosome arms in her analyses of cells at metaphase II. All acrocentric chromosomes were counted as having one chromosome arm. Metacentric and submetacentric chromosomes were considered to have two chromosome arms. The X chromosome was considered to have one chromosome arm, as was the Y chromosome even though this is known to be a submetacentric chromosome (Chapter II, section 1).

For example:

Normal ram ($2n = 54, xy$). The karyotype is made up of:

$$\begin{array}{rclcl} 46 \text{ acrocentrics} & + & 6 \text{ metacentrics} & + & X + Y = 54 \text{ chromosomes} \\ 46 & & + 12 & & + 1 + 1 = 60 \text{ chromosome arms} \\ & & & & = \text{N.F.} \end{array}$$

The haploid complement then is:

$$\begin{array}{rclcl} 23 \text{ acrocentrics} & + & 3 \text{ metacentrics} & + & \text{sex chromosome} = 27 \text{ chromosomes} \\ 23 & & + 6 & & + 1 = 30 \text{ chromosome} \\ & & & & \text{arms} \\ & & & & = \text{N.F.}/2 \end{array}$$

The author has adopted the practice of writing the haploid number as

$$\frac{\text{number of chromosomes, including sex chromosomes}}{\text{number of chromosome arms}}$$

for example $\frac{27.X}{30}$ or $\frac{27.Y}{30}$ (where $X \equiv x$ and $Y \equiv y$).

Definition of Terms:

Euploid

- A euploid cell is defined as a cell that has a balanced set of chromosomes, in any number (Dorland's Medical Dictionary, 1965).

Aneuploid

- An aneuploid cell is defined as a cell having more or less than the normal diploid number of chromosomes (Dorland's Medical Dictionary, 1965).

Throughout this thesis, the author refers to euploid secondary spermatocytes as those having a fundamental number divided by two (i.e. $\frac{\text{N.F.}}{2}$) of 30 and aneuploid secondary spermatocytes as those with an $\frac{\text{N.F.}}{2}$ of greater than, or less than 30.

Polyploid secondary spermatocytes - A polyploid cell is defined as one whose chromosome complement is an exact multiple of the basic haploid number characteristic of the species (Swanson et al., 1967).

The author used the following terminology when referring to polyploid secondary spermatocyte cells in the sheep:

	monoploid	n =	27 chromosomes	
Polyploid cells	(diploid	2n =	54	"
	(triploid	3n =	81	"
	(tetraploid	4n =	108	"

b Results - chromosome morphology

The metacentric chromosomes and the sex chromosomes, either an X or a Y, were easily distinguished in every cell examined. These three types of chromosome had a similar appearance to that in mitotic cells. The metacentrics were the largest and the two chromatids were usually attached in the region of the centromere although in some cells the two sister chromatids were separated from one another but remained in the vicinity (Plate IV. 34).

The acrocentric chromosomes varied in appearance with the stage of meiosis of the cell being viewed. Generally in cells at metaphase II, the acrocentric chromosomes had a seagull-in-flight-like appearance with both chromatid arms spread widely apart and held together only at the centromere region (Plate IV. 31). As metaphase proceeded and the chromatids became more contracted they often appeared as two "blobs" separated by a lightly staining area (Plate IV. 34) or sometimes completely separated as anaphase occurred.

The chromatids of all chromosomes showed a coiled appearance. The Y chromosome consistently stained more lightly than the autosomes. It was the smallest chromosome visible in the haploid complement and frequently its submetacentric nature could be seen. In other cases it appeared as a "blob".

The X chromosome was distinctive by the fact that both chromatid arms remained more or less in apposition and it was rare to

see them widely divergent as was the norm for other acrocentrics. A coiled appearance was often evident and on many occasions clear short arms were visible (Plate IV. 30). This confirms in meiotic tissue the observations made by Brùère (1966) on the morphology of the X chromosome of the sheep.

Rarely were any cells at second anaphase observed and none at second telophase.

c Results - analysis of metaphase II cells

Eighty seven metaphase II figures (Table IV. 8) were recorded in rams with a karyotype ($2n = 54,xy$). This total included 25 polyploid cells of which 11 could not be analysed and were recorded under the heading N.A. in the tables of results.

The analyses of metaphase II cells from normal rams are recorded in Tables IV. 9 and IV. 10.

When metaphase II counts from all the normal rams were pooled, (62 cells) 84 per cent of the figures examined were euploid. There were no hypermodal cells and 16 per cent of the cells were hypomodal or aneuploid. The figure for the aneuploid cells probably represents mainly chromosome loss during slide preparation.

Twenty five polyploid cells were recorded. One cell with 34 chromosome arms probably represented a diploid secondary spermatocyte with extensive loss of chromosomes. One triploid and one tetraploid cell were recorded, the remainder being diploid. The polyploid cells appeared to be true polyploids and not just two or three cells lying in apposition.

Because of the low total numbers of secondary spermatocytes examined it is difficult to draw conclusions regarding such parameters as the percentage of polyploid cells. No polyploid cells were recorded in four rams but in one of these no metaphase II cells were recorded and in the other three very small numbers. Likewise in rams with a relatively high percentage of polyploid cells only a few cells were analysed.

The pooled results of the rams were analysed by the Chi-square test and it was found that there was no departure from the 1 : 1 ratio of male : female metaphase II cells (χ^2_1 non-significant).

Plates IV. 30 to IV. 38 are representative of metaphase II cells from normal rams and demonstrate some of the features mentioned in this section.

TABLE IV. 8

COUNTS OF MII CELLS AND NUMBER OF MII CELLS KARYOTYPED
FROM NORMAL RAMS (54,xy)

Ram Identification	MII Cells	Polyploid MII Cells	Total Including Polyploids	Number of Karyotypes
X435/69	1	2	3	1
R285/69	13	5	18	9
X117/69	2	1	3	0
X214/69	1	4	5	3
84/68	3	0	3	1
B157	1	0	1	0
B158	0	0	0	0
R195/69	5	1	6	5
X452/69	2	1	3	1
R294/69	1	1	2	0
R16/69	1	0	1	0
R244/69	0	2	2	1
PT195/70	13	6	19	5
R225/69	19	2	21	2
Total	62	25	87	28

TABLE IV. 9

DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN NORMAL RAMS ($2n = 54.xy$)

Ram Identification	27,x 30	27,y 30	Total	22,x 25	24,x 26	24,x 27	24,y 27	25,y 27	25,y 28	26,x 29	26,y 29	27,x 29	28,y 30	Total	Total MII/Ram
X435/69		1	1											0	1
R285/69	5	6	11							1		1		2	13
X117/69		1	1					1						1	2
X214/69		1	1											0	1
E4/68	1	1	2			1								1	3
B157	1		1											0	1
B158			0											0	0
R195/69	2	2	4		1									1	5
X452/69	1		1	1										1	2
R294/69		1	1											0	1
R16/69			0				1							1	1
R244/69			0											0	0
PT195/70	2	9	11						1		1			2	13
R225/69	8	9	17				1						1	2	19
Total	20	31	51	1	1	1	2	1	1	1	1	1	1	11	62

TABLE IV. 10

DISTRIBUTION OF CHROMOSOME COUNTS IN POLYPLOID MII CELLS IN NORMAL RAMS ($2n = 54,xy$)

Ram Ident- ification		<u>28.x</u> 34	<u>52.x.y</u> 58	<u>53.x.y</u> 58	<u>2n</u> <u>53.x.y</u> 59	<u>54.x.y</u> 60	<u>54.x.x</u> 60	<u>3n</u> <u>81.x.x.y</u> 90	<u>4n</u> <u>105.vvvv</u> 117	<u>N.A.</u>	<u>Total/Ram</u>
X435/69			1			1					2
R285/69				1	1	1				2	5
X117/69										1	1
X214/69		1					1	1		1	4
84/68	Number of Cells										0
B157											0
B158											0
R195/69						1					1
X452/69						1					1
R294/69										1	1
R16/69											0
R244/69									1	1	2
PT195/70						2				4	6
R225/69						1				1	2
Total		1	1	1	1	7	1	1	1	11	25

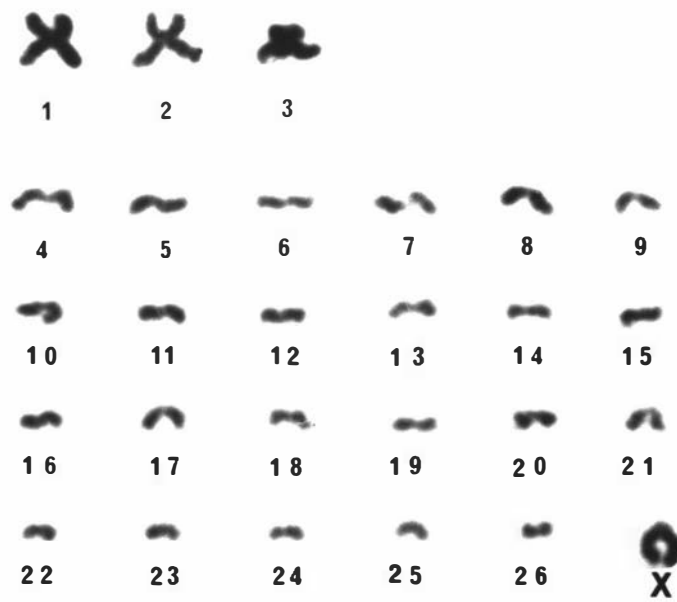
N.A. = Not Analysed

Plate IV. 31: Metaphase II cell (from a normal ram R225/69)
with 30 chromosome arms and 27 chromosomes
including three metacentric chromosomes and
an X chromosome (arrowed).
(Aceto-orcein x 4,000)

Figure IV. 9: Karyotype of cell illustrated in Plate IV. 31.



31



9

Plate 1V. 32:

MII cell (from normal ram, R285/69) $\frac{27.x}{30}$
Arrow points to X chromosome.
(Aceto-orcein. x 4,000).

Plate 1V. 33: MII cell (from normal ram, R195/69)
with 30 chromosome arms and 27 chromosomes
including an X chromosome. There is a
pale staining area in the centromeric
region of some of the acrocentric
chromosomes.
(Aceto-orcein. x 4,000).



32



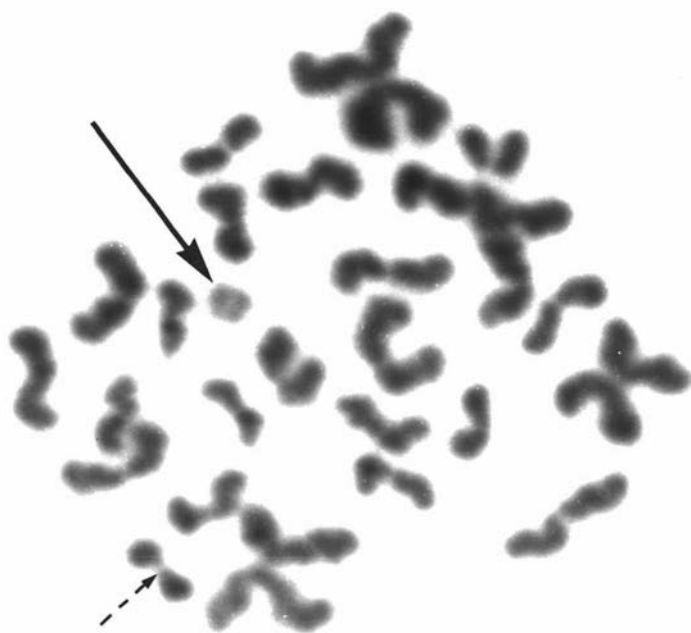
33

Plate IV. 34: MII cell from a normal ram R195/69

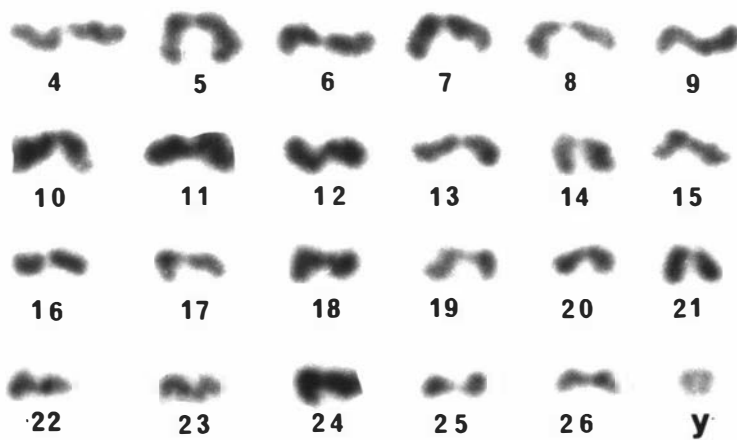
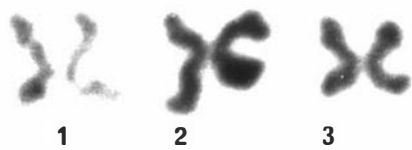
There are 27 chromosomes including three metacentrics and a Y chromosome (large arrow) which is more lightly stained than the autosomes. One of the smallest acrocentric chromosomes (small arrow) appears as two "blobs" separated by a lightly stained centromeric region.
(Aceto-orcein. x 4,750)

Figure IV. 10: Karyotype of cell illustrated in Plate IV. 34.

There are 30 chromosome arms. The chromatid arms of the three metacentric chromosomes are slightly separated and could be confused with one of the large acrocentric chromosomes.



34



10

Plate IV. 35: Metaphase II cell (from normal ram PT195/70) with 30 chromosome arms and 27 chromosomes including a Y chromosome (large arrow) and three metacentric chromosomes. Two of the acrocentric autosomes are overlapping (small arrow).
(Aceto-orcein. x 4,000).

Plate IV. 36: Aneuploid metaphase II cell from a normal ram, PT195/70. There are 28 chromosome arms and 25 chromosomes including three metacentric chromosomes and a Y chromosome (arrowed). The coiled nature of some chromatids is evident.
(Aceto-orcein. x 4,000).



35



36

Plate IV. 37: Diploid metaphase II cell from a normal ram PT195/70.
There are 60 chromosome arms and 54 chromosomes
including six metacentric chromosomes, an X
chromosome and a Y chromosome.
(Aceto-orcein. x 2,050).

Plate IV. 38: Polyploid (triploid) metaphase II cell from a normal
ram X214/69. There are 90 chromosome arms, and
81 chromosomes including nine metacentric chromosomes,
two X chromosomes and a Y chromosome.
(Aceto-orcein. x 1,500).



37



38

Figure IV. 11: Karyotype of the diploid secondary spermatocyte
illustrated in Plate IV. 37.



11

(D) Cell Distributions At Meiosis In Normal Rams

Cell distributions in each of the categories, spermatogonial metaphase, diakinesis and metaphase I and metaphase II are shown in Table IV. 11.

TABLE IV. 11

DISTRIBUTION OF CELLS IN SPERMATOGONIAL METAPHASE (SM), DIAKINESIS AND METAPHASE I (MI) AND METAPHASE II (MII) IN NORMAL RAMS (54,xy)

(After Skakkebaek *et al.*, 1973)

* Polyploid cells included in counts are recorded in brackets after counts.

Ram Ident- ification	SM	MI	MI I	Total	MII/MI Ratio
X435/69	3 (2)	7 (1)	3 (2)	13 (5)	0.43
R285/69	1 (0)	63 (15)	18 (5)	82 (18)	0.29
X117/69	4 (0)	14 (2)	3 (1)	21 (3)	0.21
X214/69	3 (1)	24 (7)	5 (4)	32 (12)	0.21
84/68	0 (0)	9 (1)	3 (0)	12 (1)	0.33
B157	1 (0)	10 (4)	1 (0)	12 (4)	0.10
B158	3 (1)	7 (1)	0 (0)	10 (2)	0.00
R195/69	1 (0)	46 (5)	6 (1)	53 (6)	0.13
X452/69	0 (0)	5 (1)	3 (1)	8 (2)	0.60
R294/69	0 (0)	23 (2)	2 (1)	25 (3)	0.09
R16/69	4 (1)	12 (5)	1 (0)	17 (6)	0.08
R244/69	2 (0)	13 (4)	2 (2)	17 (6)	0.15
PT195/70	1 (0)	33 (4)	19 (6)	53 (10)	0.58
R225/69	0 (0)	43 (6)	21 (2)	64 (8)	0.49
Total	23 (5)	309 (56)	87 (25)	419 (86)	0.28

The proportion of cells in each category varied between rams. Relatively few spermatogonial cells were seen.

The average ratio of metaphase II to metaphase I cells was 0.28 with a range of zero (no cells observed at second metaphase) to 0.60.

IV. 4 DISCUSSION

(i) INTRODUCTION

Meiosis is an integral part of the spermatogenic cycle in the ram. An A-type spermatogonial stem cell produces two daughter cells one of which divides four times to produce 16 primary spermatocytes, the cells which undergo meiotic division. The other daughter cell becomes a dormant A-type cell during a fraction (0.68) of the seminiferous cycle and afterwards ensures the renewal of the spermatogonial population (Ortavant, 1959). The primary spermatocytes at metaphase I in a normal ram each have 27 bivalents or tetrads (108 chromatids). At the first meiotic division 27 dyads (54 chromatids) pass to each pole. The two daughter secondary spermatocytes thus formed are equationally separated in anaphase II with 27 monads (27 chromatids) going to each of the four haploid nuclei formed at the end of the second meiotic division.

(ii) CELL DISTRIBUTIONS AT MEIOSIS IN NORMAL RAMS

Few spermatogonial cells were observed in meiotic preparations from the normal rams studied. This was similar to the results of studies on men (Ford and Hamerton, 1956; McIlree *et al.*, 1966,a; Skakkebaek *et al.*, 1973), dogs (Eliasson *et al.*, 1967) and rodents (Ford and Evans, 1969; Hultén, Karlman, Lindsten and Tiepolo, 1970,a).

The observed ratio of metaphase II to metaphase I cells in normal rams (Table IV. 11) was less than the expected theoretical ratio of 2.0. In the absence of any other data on rams, it is interesting to note that in one study on normal men (Skakkebaek *et al.*, 1973), although a theoretical ratio of 2.0 was expected, in fact a mean ratio of 0.9 with a range of 0.6 to 1.4 was found. The data from the normal rams probably underestimates the true proportion of cells at first and second metaphase as only those cells which were analysed were included in the counts and more metaphase II than metaphase I cells were rejected as being unsuitable for analysis. By comparison Skakkebaek *et al.*'s (1973) data was based on all the first and second metaphase stages seen; only a proportion of these were actually analysed.

Information on metaphase II to metaphase I ratios could be

useful in detecting disturbances of spermatogenesis in rams. This use was suggested and utilized by Skakkeback et al. (1973) in studies in normal and infertile men. These authors pointed out that the spermatocyte arrest sometimes observed in histological sections is not always easy to localize to a particular stage of the spermatogenic cycle, because it is often difficult to distinguish between the first and second meiotic divisions in histological sections, whereas these can be identified in meiotic preparations.

(iii) EARLY MEIOTIC PROPHASE STAGES

The compact heavily-stained regions observed on some bivalents at the zygotene or pachytene stages in the ram, are similar to the "basal knobs" reported in other mammals including man, mouse (Woollam and Ford, 1964; Jagiello and Polani, 1969) and wallaby (Fredga, 1964; Woollam and Ford, 1964). Their location on ram prophase bivalents is consistent with Woollam and Ford's (1964) proposal that these "knobs" correspond to the heterochromatic regions adjoining the centromere in mitotic metaphase chromosomes.

Several authors have commented on the occurrence of "lateral outgrowths" on bivalents at the late zygotene and pachytene stages (Fredga, 1964; Woollam and Ford, 1964; Luciani, 1970; Pogossianz, 1970; Loir, 1971; McDermott, 1971; 1973,a; Ferguson-Smith, 1972). In some cells at early meiotic prophase in the ram, the bivalents had a "furry" outline which could be due to outgrowths similar to those observed by Loir (1971). The latter author used a special preparative technique which may have enhanced this feature on the bivalents he studied.

No evidence of a nucleolus was seen in any of the meiotic preparations examined in this study. Gustavsson (1969) and Popescu (1971,a) reported a similar finding in cattle and suggested that the hypotonic solutions used in slide preparation, caused nucleoli to rupture. Likewise it was suggested by Bruère (1966) that the same technical factors disrupted the delicate satellite associations which probably occur in sheep mitotic chromosomes.

(iv) DIPLOTENE, DIAKINESIS AND METAPHASE I STAGES

Diplojene nuclei were rare and difficult to analyse in the ram, a similar finding to that of Loir (1971) and several authors working with species other than sheep (Treadwell et al., 1968; Popescu, 1971,a; Ferguson-Smith, 1972).

Ahmed (1940) who claims to have counted chiasmata from ten late diplojene cells, found a variation in total number of from 60 to 73 per nucleus. The author counted the chiasmata in one late diplojene cell and found 73 chiasmata, a total consistent with Ahmed's (1940) estimations. The largest bivalent had seven chiasmata as was the case in one of the cells examined by Ahmed (1940) who observed that bivalents with two chiasmata occurred more frequently than those with fewer or greater numbers, similar findings to those in this study. Loir (1971) although not stating total cell counts, reported finding five chiasmata on the two largest bivalents at diplojene and three or four on the third largest bivalent.

The proposal that the scarcity of diplojene nuclei could be due to the rapidity with which this stage occurs in the ram was made by Loir (1971); his observations showed that this stage and the diakinesis stage would probably take about the same length of time as metaphase I, less than two hours. This time limit is in marked contrast to that suggested by Ortavant (1959) who quoted 2.4 to 3.5 days as the time for the diplojene stage, but is much shorter than the duration of the pachytene stage in the ram, which has been quoted as nine days (Loir, 1971) and four to six days (Ortavant, 1959).

In addition to counting chiasmata at diplojene, Ahmed (1940) examined five diakinesis nuclei and ten metaphase I nuclei and reported chiasmata ranges of 58 to 64 and 56 to 62 per nucleus, which is a range of between 56 and 64 for the two stages considered together. The corresponding values obtained in this study were 42 to 65 with an average range of 50 to 58, with metaphase I chiasmata estimations being lower than those counted by Ahmed (1940).

The approximate genetic length of the sheep estimated from Ahmed's (1940) data (diakinesis-metaphase I stages) was 29.4 morgans. This is greater than the author's estimation of 27.7 morgans, which is a minimum value as a proportion of the chiasmata had terminalized by the diakinesis-metaphase I stage and the numbers of terminal chiasmata

are greater at metaphase than diakinesis.

No other data with which these results can be compared is available from normal sheep, but it has been shown that estimates of human genetic length vary between researchers.

It is interesting to note that although Ahmed (1940) based his studies on histological sections which were 16 μ in thickness, his results were very similar to those obtained by the author using modern cytological methods.

(v) METAPHASE II

The secondary spermatocytes of normal rams are discussed in Chapter V of this thesis and will not be considered here.

(vi) THE SEX CHROMOSOMES

Compared with those of man and some rodents, the sex chromosomes of domestic animals have not been studied extensively.

The results obtained in this thesis are in accord with the descriptions of the sex chromosomes in early meiotic prophase given by Ahmed (1940) and Loir (1971). The progressive change in appearance of the sex chromosomes from a figure-of-eight configuration at zygotene, to a circle at late pachytene and a rod at diplotene noted in this study were also reported in sheep by Loir (1971) and in cattle by Popescu (1971,a).

This study confirmed the bivalent association of the sex chromosomes at diakinesis as reported by Ahmed (1940), Berry (1941), Makino (1943,a) and Loir (1971). From this study the description of the sex bivalent however, was different from that of Ahmed (1940). Ahmed (1940) identified two different configurations at diakinesis and metaphase I. One type resembled an autosomal bivalent with two or three interstitial chiasmata while the other, an asymmetrical bivalent had terminally or sub-terminally associated chromosomes. By comparison, in this study, no sex bivalent resembling an autosomal bivalent was recorded and the existence of homologous pairing segments in the X and Y chromosomes could not be established. It was not possible to determine the relationship between the chromosomes in early prophase and it was difficult to demonstrate the point at which the X chromosome terminated and the Y began in many bivalents at the diakinesis stage. Consequently the possibility of homologous pairing

segments in sheep sex chromosomes cannot be excluded.

The occurrence of homologous regions in sex chromosomes has been considered in man (Solari and Tres, 1970) and cattle (Melander and Knudsen, 1953), and demonstrated in the Armenian hamster (Lavappa and Yerganian, 1970) and in the Chinese and European hamsters by Fredga and Santesson (1964) who suggested that the end-to-end association between the X and Y chromosomes in these species was the result of chiasma terminalization.

The author has established that the short arms of the X chromosome are involved in a terminal association with the Y chromosome from a study of cells at the late diakinesis and metaphase I stages. Other workers on sheep chromosomes did not comment on this aspect of bivalent morphology. The controversy as to which arms of the sex chromosomes of man united was solved using fluorescent and centromere staining techniques (Chapter IV. 2 (ii) (C)). There are no reports in the literature on Giemsa banding, fluorochrome staining or centromere staining of sheep meiotic chromosomes. The author used the "C-banding" technique to study centromere position in both mitotic chromosomes (Bruère, Zartman and Chapman, 1974) (Appendix XX) and meiotic chromosomes. By this method the sex chromosomes were either very lightly stained in mitotic cells (Bruère et al., 1974), or uniformly stained in meiotic cells. Evans et al. (1973) and Hansen (1973,a) observed similar non-differential staining of the centromeric regions compared with the chromosome arms in sheep mitotic chromosomes.

Evans et al. (1973) claimed faint staining of the centromeric region of sheep mitotic chromosomes following a Giemsa banding technique. Using the same technique Zartman and Bruère (1974) showed similar faint staining of the centromeric regions of all chromosomes except the metacentrics.

It was shown by Pearson, Bobrow, Vosa and Barlow (1971) that the intense fluorescence seen on the human Y chromosome did not occur on those of the domestic animals they examined. In contrast, Evans et al. (1973) reported fluorescence of sex chromosome centromeres in sheep, goats and cattle and Hansen (1973,a) described an intense fluorescence of the long arms and faint staining on the short arms of the sheep Y chromosome.

It is impossible to decide from the light microscope examination of cells in this study whether or not the union between

the sex chromosomes involves a chiasma. Further work utilizing the Giemsa staining method, fluorescent studies and electron microscope examination should be done in order to describe sheep sex bivalents.

Dissociation of the X and Y chromosome was found in 1.2 per cent of the 253 diakinesis-metaphase I cells analysed from normal rams. The occurrence of sex chromosomes as univalents has been reported in other species including man (Ford and Hamerton, 1956; Sasaki and Makino, 1965; Hultén et al., 1966; Falek and Chiarelli, 1968; Fraccaro et al., 1968; Luciani, 1970; Luciani et al., 1970; McDermott, 1971; Ferguson-Smith, 1972; Skakkebaek et al., 1973), dog (Eliasson et al., 1967), cattle (Popescu, 1971,a), mice (Lin, Tsuchida and Morris, 1971; Furnell, 1973), hamster (Emmons and Husted, 1963) and hare (Gustavsson, 1971,a).

The percentages of separated sex chromosomes were higher in species other than the sheep; for example in man a range of 3.2 to 27 per cent was found (refer Table IV. 2) while in the dog 17.4 per cent, cattle, 33.3 per cent, mice 6.2 to 14.4 per cent, and hamster 6 per cent of the sex chromosomes were present as univalents.

The cause of the non-association of the sex chromosomes has been considered by several authors. Ford and Hamerton (1956) thought that while most cases could be due to precocious non-disjunction, rupture of the terminal connection between the chromosomes during slide preparation, could account for some cases. Hultén et al. (1966), Fraccaro et al. (1968), Skakkebaek et al. (1973) and the author, all found that separation of the sex chromosomes tended to occur towards the latter stages of metaphase I which suggested that precocious disjunction of the chromosomes could have occurred (Hultén et al., 1966; Fraccaro et al., 1968; Skakkebaek et al., 1973). Hultén et al. (1966) considered premature terminalization of chiasmata was a possibility in man. Another alternative cause of non-association could be a failure of the chromosomes to pair at zygotene (Hamerton, 1961). Ford and Evans (1964) noticed univalent sex chromosomes in mice in both air-dried and squash preparations and attributed this dissociation largely to the effect on the cells of manipulations during slide preparation. However as suggested by Skakkebaek et al. (1973) the fact that different investigators, using differing techniques and species, have all observed this phenomenon tends to suggest that it is not artefactual.

Kihlberg, Gustavsson and Sundt (1969) reported an association between the "sex vesicle" and an autosomal bivalent in some pachytene cells from the muskrat. They considered that these associations were "probably fortuitous and caused by stickiness", an explanation which could also apply to the previously unreported sex bivalent - small autosomal bivalent associations at diakinesis-metaphase I in the sheep.

(vii) OTHER ANOMALIES OBSERVED AT MEIOSIS IN NORMAL RAMS

A separated autosomal bivalent was seen in only one cell in one ram and could have been due to premature chiasma terminalization as it involved the chromosomes of one of the smaller bivalents in a first metaphase cell. Alternatively, this separation could be interpreted as a technical artefact.

Sasaki and Makino (1965) reported finding a precocious terminalization in 60 per cent of the human metaphase I cells they examined. These authors observed that the smallest autosomal bivalents were involved and proposed that these products of precocious terminalization could be confused with supernumerary chromosomes. Further reports on non-pairing of autosomal bivalents in men were made by Luciani (1970), Luciani *et al.* (1970) and Skakkebaek *et al.* (1973) who commented on the role these abnormalities could have in the production of aneuploid spermatozoa and subsequently, aneuploid zygotes.

The proportion of hypomodal and hypermodal cells was approximately the same in diakinesis and first and second metaphase cells in the normal rams examined. Values of 16.2 per cent for hypomodal diakinesis-metaphase I cells and 16 per cent for hypomodal metaphase II cells were recorded. No hypermodal metaphase II cells were recorded but approximately two per cent of diakinesis-metaphase I cells were hypermodal.

(viii) POLYPLOIDY

Poikiloploidy is the term given to "the state of having varying numbers of chromosomes in different cells" (Dorland's Medical Dictionary, 1965). The term includes diploid, aneuploid and polyploid cells, all of which may be found in the same animal.

The occurrence of apparently polyploid cells in squash and air-dried preparations has been reported in many mammals (Table IV. 12).

TABLE IV. 12

INCIDENCE OF POLYPLOID CELLS IN MEIOTIC PREPARATIONS
FROM SOME MAMMALS

Author and Date	Species	Spermato- gonial Cells	Primary Spermato- cytes *	Secondary Spermato- cytes *	Type of Preparation Comments
Fechheimer, N.S. (1961)	Mouse	$\frac{31}{431}$ 7.2% triploid tetraploid hexaploid octaploid	-	-	Squash
Darlington, C.D. and Haque, A. (1962)	Man	-	$\frac{17}{46}$ 37%	-	Squash Polyploidy associated with chromo- some breakage.
Sasaki, M. (1964)	Man	$\frac{48}{594}$ 8.1% mainly tetraploid	$\frac{27}{910}$ 3.0% mainly tetraploid	$\frac{54}{734}$ 7.4% mainly diploid	Air-dried
Sasaki, M. and Makino, S. (1965)	Man	$\frac{66}{923}$ 7.1% mainly tetraploid	$\frac{30}{1190}$ 2.5% mainly tetraploid	$\frac{57}{818}$ 6.9% mainly diploid	Air-dried Some polyploid pachytene cells.
McIlree et al. (1966,a)	Man	$\frac{32}{520}$ 6.2%	$\frac{12}{746}$ 1.6%	$\frac{19}{210}$ 9.1%	Air-dried
Eliasson, K. et al. (1967)	Dog	-	$\frac{5}{98}$ 5.1% Tetraploid	$\frac{8}{138}$ 5.8% 6-Diploid 2-Triploid	Air-dried
Pogosianz, H.E. and Brujako, E.T. (1969)	Steppe Lemming	-	$\frac{146}{1429}$ 10.2% All tetraploid	-	Air-dried
	Striped hairy footed hamster	Some polyploid cells	Tetraploid and hexaploid cells	Some polyploid cells	Numbers of cells not given.
Hultén, M. et al. (1970,a)	Chinese hamster	$\frac{20}{225}$ 8.9% 14 tetraploid 4 hexaploid 2 octoploid	$\frac{3}{174}$ 1.72%	$\frac{5}{392}$ 1.3%	Air-dried Squash
Luciani, J.M. (1970)	Man	$\frac{11}{110}$ 10%	$\frac{5}{150}$ 3.3% Tetraploid	$\frac{7}{63}$ 11.1% Diploid	Air-dried
Lin, C-C. et al. (1971)	Mouse	Some polyploid cells	$\frac{18}{586}$ 3.1%	Some polyploid cells	Air-dried 2.7% of pachytene cells were polyploid.
Pogosianz, H.E. and Brujako, E.T. (1971)	Djungar- ian hamster	-	29 tetraploid 4 hexaploid	18 diploid 1 triploid 5 tetraploid	Air-dried
Popescu, C.P. (1971,a)	Cattle	7.5% mainly tetraploid	-	-	Air-dried
Skakkebaek, N.E. et al. (1973)	Man	$\frac{8}{166}$ 4.8%	$\frac{11}{856}$ 1.3%	$\frac{27}{752}$ 3.6%	Air-dried

* Number of polyploid cells
Number of cells counted

In general, the percentage variations between individuals appear to be random, although Fechheimer (1961) found one genetic strain of mice with a significantly higher proportion of polyploid spermatogonial cells than was found in three other strains examined at the same time.

The percentage of apparent polyploid cells in spermatogonial and secondary spermatocyte cells from the normal rams used in this study was higher than the values reported in other species, but relatively few cells at these stages were counted from the rams. In ram primary spermatocyte cells the percentage of polyploid cells was greater than that reported by other authors.

Most spermatogonial and primary spermatocyte polyploid cells were tetraploid and diploid cells were common among the secondary spermatocytes. Cells of higher ploidy were recorded rarely. These findings are similar to those of Sasaki (1964), Sasaki and Makino (1965), Hultén et al. (1970,a) and Pogosianz and Brujako (1971).

The cause of the apparent polyploidy is uncertain. In some cases it may be an artefact resulting from the overlapping of adjoining cells (Fechheimer, 1961; Sasaki, 1964; Sasaki and Makino, 1965; Hultén et al., 1970,a; Ford and Evans, 1971; Lin et al., 1971) although the similar appearance and behaviour of the chromosomes in many of the cells suggests other factors may be involved (Hultén et al., 1970,a; Ford and Evans, 1971).

Disturbances of cytokinesis in spermatogonial cells (Lin et al., 1971; Pogosianz and Brujako, 1971), endomitosis and fusion of spermatogonial cells could be involved (Fechheimer, 1961; Pogosianz and Brujako, 1971). Alternatively cells may fuse at pachytene (McIlree et al., 1966,a; Pogosianz and Brujako, 1971) and produce polyploid primary spermatocytes.

Polyploid secondary spermatocytes may be a consequence of the normal division of tetraploid primary spermatocytes, while cells of higher ploidy could be explained on the basis of cell fusion as suggested by Pogosianz and Brujako (1971).

Fechheimer (1961) suggested that the majority of polyploid spermatogonia may never complete meiosis. However diploid spermatozoa have been demonstrated in mice, rabbits, bulls (reviewed by Beatty, 1970) and men (Sumner, 1971) which means that some polyploid cells must develop into spermatozoa and these could be one

of the causes of triploid zygotes (Saseki and Makino, 1965; Sumner, 1971; Jonasson, Therkelsen, Lauritsen and Lindsten, 1972; Niebuhr, 1974). Although this mechanism of triploid formation is probably rare (Austin, 1969) it could contribute towards embryonic loss, as most triploids are aborted (McFeely, 1967; Carr, 1971; Niebuhr, 1974).

CHAPTER V

ROBERTSONIAN TRANSLOCATIONS IN SHEEP

V. 1 REVIEW OF LITERATURE(i) ROBERTSONIAN TRANSLOCATION

The term "translocation" (Bridges, 1923) implies the shifting of a segment or fragment of one chromosome into another part of a different chromosome. It is believed that most simple translocations originate through chromosome breakage followed by the reunion of the non-related broken ends. This description does not however exactly fit all translocation types. Robertsonian translocations, first described in insects by Robertson (1916), involve the association of two acrocentric or telocentric chromosomes to form a single metacentric or submetacentric chromosome.

The mechanism of formation of Robertsonian translocations is uncertain. White (1954) described their formation in the following way. Two acrocentric chromosomes break at or near the centromere and the non-homologous segments reunite to form a metacentric or submetacentric chromosome plus a centric fragment which is commonly lost during subsequent cell divisions. The minute centric fragment is composed of the centromere from one and short arm material from both acrocentric chromosomes. This mechanism assumes that the loss of the centric fragment has little or no effect on the genome of the individual (White, 1954). There is one chromosome less than normal in the resultant karyotype but the number of chromosome arms, N.F.. (Matthey, 1945) remains the same.

White (1954) also proposed the process in reverse with two new acrocentric chromosomes being produced by a process of "dissociation" in which a translocation produces two acrocentric chromosomes from a large metacentric chromosome plus a hypothetical "donor" chromosome which provides the necessary extra centromere and two telomeres.

Other alternatives to White's hypotheses have been suggested in plant and insect species as well as animals. Fission within the centromere of a metacentric or submetacentric chromosome may take place to form telocentric chromosomes and these may produce metacentric chromosomes by simple fusion (John and Hewitt, 1968; McFee and Banner, 1969). Hsu and Mead (1969) suggested that none

of the centromeres are lost in the Robertsonian process but one is not visible under the light microscope.

Recently evidence was presented which seemed to indicate that Robertsonian translocations in man might be due to breaks in the short arms of both acrocentrics with formation of dicentric translocation chromosomes (Niebuhr, 1972). Niebuhr (1972) also suggested that one of the centromeres was "more or less frequently suppressed and no longer visible in light microscopy". The mechanics of the suppression are not known. That this hypothesis is possibly correct is shown by the fact that although dicentric chromosomes have been reported rarely in Robertsonian translocations (Cohen, Takagi and Harrod, 1968; Niebuhr, 1972; Ferguson-Smith, 1972) their existence had been suggested previously (Barnicot, Ellis and Penrose, 1963). Further evidence that centric fusions may involve both centromeres has been given from electron microscope studies by Comings and Okada (1970) who demonstrated that the centromeres of metacentric and submetacentric chromosomes were quadripartite in structure while those of telocentric chromosomes were bipartite in man, mouse, chinese hamster, sheep and goat chromosomes. Unfortunately although studied, no mention was made by these authors of the exact nature of the centromere in the acrocentric chromosomes of the sheep.

Preliminary studies on the centromeric staining of the mitotic chromosomes of the three Robertsonian translocations found in domestic sheep suggest that two of these (Massey I and Massey III) are dicentric. This is discussed in a paper, to which the author was a contributor, in Appendix XX of this thesis.

Centromere staining methods have shown the possible dicentric nature of a translocation chromosome in mice (White, Tjio, Van de Water and Crandall, 1972,a).

Comings and Avelino (1972) concluded that the process of Robertsonian translocation is a reciprocal translocation resulting from a crossover within the chromatin fibres that go to make up the centromere region.

Most workers have assumed that there is negligible loss of genetic material in the formation of a Robertsonian translocation. This is emphasized by the work of Comings and Okado (1970), Chen and Ruddle (1971) and Comings and Avelino (1972).

Whatever their method of formation may be, it is known that Robertsonian translocations occur frequently in the phylogeny of many groups of animals and are now considered to be a regular feature of karyotype alteration in the eukaryotes.

(ii) ROBERTSONIAN TRANSLOCATIONS IN CATTLE, PIGS, GOATS
SHEEP AND DOGS

Sheep, cattle, goats and pigs belong to the order Artiodactyla. The chromosomes of these animals have shown widespread Robertsonian variation, possibly due to the large number of acrocentric or telocentric chromosomes found in each species. Within this Order is the super family Bovoidea in which the chromosomes of over 50 species have now been investigated (Wurster and Benirschke, 1968). Although the diploid number varies from 30 to 60 among those investigated, the N.F. varies (with only three exceptions) from 58 to 62. Wurster and Benirschke (1968) claim that this indicates an almost exclusive use of the Robertsonian fusion mechanism of karyotype evolution in this group of species which represent over 30 genera.

The complexity of chromosome variation and its relationship to evolutionary patterns has been summarized further by Taylor, Hungerford and Snyder (1969), Nadler et al. (1971) and Wurster (1972). These reports emphasize that consideration must be given to the evolutionary purpose of Robertsonian translocations as well as to their possible deleterious effects.

(A) Cattle

The presence of a Robertsonian translocation in cattle was first reported by Gustavsson and Rockbern in 1964. These authors described a translocation between the largest autosome and probably the smallest autosome of the karyotype resulting in a diploid chromosome number of 59 instead of the usual 60. In later studies Gustavsson (1966; 1969) showed that some animals were homozygous for the translocation and had a chromosome number of 58. Translocations the same as, or similar to the 1/29 translocation have since been recognized in a variety of breeds from many different

countries and are listed in Table V. 1.

Recently, two different Robertsonian translocations of domestic cattle were reported. The first in British Friesian cattle was a translocation between chromosomes two and four to form a metacentric chromosome (Pollock, 1972). The second type of Robertsonian translocation found in Simmental cattle imported into New Zealand from Scotland was also a metacentric chromosome made up of chromosomes 11 or 12 and 15 or 16 (Bruère and Chapman, 1973). A similar translocation to the latter has been reported by Harvey (Pers. comm. to Dr. Bruère, 1973) in the same line of Simmental cattle.

ROBERTSONIAN TRANSLOCATION STUDIES ON CATTLE

Author and Date	Breed	Country	Translocation	Number of Cattle Studied (Number with Translocation)
Gustavsson, I. and Rockborn, G. (1964)	?	Sweden	¹ /29	- (3)
Gustavsson, I. (1966)	Swedish Friesian (SLB) Swedish Red and White (SRB)	Sweden	¹ /29	1,134 (126)
Herschler, M.S. and Fechheimer, W.S. (1966)	Holstein	U.S.A.	¹ /29	4 (3)
Rugiati, S. and Fedrigo, M. (1957) (1968)	Romagnola "	Spain "	¹ /28 ? "	1 (1) 4 (2)
Rieck, G.W.; Hühn, H. and Herzog, A. (1968)	German Red Pied	Germany	¹ /29	5 (4)
Amrud, J. (1969)	Norwegian Red	Norway	¹ /29	430 (18)
Gustavsson, I. (1969)	Swedish Red and White	Sweden	¹ /29	1,916 (374)
Gustavsson, I. (1971,b)	Swedish Red and White Swedish Ayrshire	Sweden	¹ /29	944 (120)
Popescu, C. P. (1971,b)	Montbéliard	France	¹ /29	2 (2)
Fisher, H. (1971)	Siamese	Thailand	¹ /29 ?	4 (1)
Darré, R.; Quéinnec, G. and Berland, H.M. (1972)	Limousin, Blonde d'Aquitaine	France	¹ /29	175 (31)
Froget, J.; Coulon, J.; Nain, M.-C. and Dalbiez, J.-M. (1972)	Charolais	France	¹ /29	1 (1)
Pollock, D. (1972)	Friesian	Scotland	² /4	? (1)
Harvey, M.J.A. (1971 and 1972)	Charolais Simmental Limousin Offspring of Charolais bull* Red Poll Blonde d'Aquitaine	Scotland { origin Continent Scotland Britain France	¹ /29 " " " " "	143 (1)* 42 (2) 5 (1) 44 (23) ? (1) ? (2)
Bruère, A.N. and Chapman, H.M. (1973)	Simmental Blonde d'Aquitaine	New Zealand (origin Scotland) New Zealand (origin France)	¹¹ or ¹² / ₁₅ or 16 ¹ /29	13 (1) 5 (1)

(B) Pigs

Chromosome polymorphism has been reported in European wild pigs (McFee, Banner and Rary, 1966). Of the 36 pigs these authors studied, none was found with 38 chromosomes, the characteristic number for domestic pigs. Seventy three per cent of the animals were homozygous (with 36 chromosomes) and 27 per cent were heterozygous (with 37 chromosomes) for a Robertsonian translocation. In a further study Rary, Henry, Matschke and Murphree (1968) examined 103 wild pigs and found diploid chromosome numbers of 36 (31.5 per cent), 37 (53.7 per cent) and 38 (14.8 per cent). Intermating experiments between pigs carrying the different chromosome numbers produced the expected ratios of progeny with the three chromosome types (McFee and Banner, 1969).

(C) Goats

Soller, Wysoki and Padeh (1966) reported a familial centric fusion of two acrocentric autosomes in a family of Saanen goats. A similar translocation was also observed in an apparently unrelated XX/XY pseudohermaphrodite (Padeh, Wysoki, Ayalon and Soller, 1965). Padeh, Wysoki and Soller (1971) in further studies of the translocation observed that both translocation heterozygote and homozygote animals born in a series of matings were phenotypically normal.

Fourteen of 19 male and six of 11 female offspring of a male heterozygous carrier of a Robertsonian translocation carried the submetacentric translocation chromosome (Popescu, 1972). No homozygous carriers were observed by Popescu (1972).

Hulot (1969) and Evans et al. (1973) also reported Robertsonian translocations in a goat and Evans et al. (1973) identified the individual chromosomes involved in the translocation.

(D) Sheep

Although there have been many studies on sheep chromosomes involving large numbers of animals from many different countries it is only recently that chromosome polymorphism has been extensively described in domestic sheep (Ovis aries) (Bruère, 1969;

Bruère and Mills, 1971; Bruère et al., 1972; Bruère, 1973; Bruère, 1974; Bruère and Chapman, 1974). Work on Robertsonian translocations in domestic sheep commenced following the discovery by Bruère (1959) of a translocation between a large and small autosome in leucocyte cultures from a sterile ram which had abnormally shaped testicles. Four phenotypically normal relatives of the ram also carried the translocation. Results from cytogenetic examination of 180 Romney sheep from which the translocation-carrying family was originally isolated, identified 25 animals (17 ewes and 8 rams) heterozygous for the translocation. Of the eight translocation-carrying rams examined, five had testicular abnormalities. Bruère and Mills (1971) named this submetacentric translocation chromosome the Massey I translocation.

A phenotypically normal Romney ewe was found with a Robertsonian translocation involving two of the larger acrocentric chromosomes during the same survey. When 309 ewes were screened for the presence of a normal karyotype, three more ewes with a similar translocation chromosome were found (Bruère, 1973). Bruère (1973) reported that these ewes were phenotypically normal and that the translocation was familial but of unknown origin. The metacentric translocation chromosome was named the Massey II translocation (Bruère and Mills, 1971).

A third type of Robertsonian translocation was reported in the Drysdale breed of sheep by Bruère et al. in 1972. Both structurally homozygous ($2n = 52$) and structurally heterozygous ($2n = 53$) Massey III translocation animals were found as well as sheep with the normal diploid chromosome number for domestic sheep ($2n = 54$). This translocation involved a fusion between one of the larger and one of the smaller acrocentric autosomes to form a submetacentric chromosome (Bruère et al., 1972). The overall incidence of chromosome polymorphism in this study of 327 sheep which included 224 ewes and 103 rams, was 26.3 per cent (Bruère et al., 1972). With the exception of two sheep with genital abnormalities, all were phenotypically normal. Bruère et al. (1972) concluded from a study of pedigree data that the translocation chromosome had been present in the Drysdale breed for a number of years and that its breed derivation would be difficult to determine.

The suggestion was made by Bruère et al. (1972) that

chromosomal polymorphism in domestic sheep is confined to certain breeds and strains within these breeds in cases where close mating has been practiced.

The incidences of Robertsonian translocations in domestic sheep are summarized in Table V. 2.

The arm components of the three translocations have now been identified by a Giemsa-banding technique (Appendix XX). If the three pairs of large metacentric chromosomes are numbered one to three and the first acrocentric is number four, the composition of the translocation chromosomes is as follows:

Massey	I :- t_1	(5 ; 26)
Massey	II :- t_2	(8 ; 11)
Massey	III :- t_3	(7 ; 25).

The diploid chromosome number varies between 52 and 58 in members of the genus Ovis (Schmitt and Ulbrich, 1968; Wurster and Benirschke, 1968; Nadler, 1971; Nadler et al., 1973). In all cases the N.F. was 60, or 58 if only the autosomal arms were counted (Nadler et al., 1971).

In addition to numerical variations between different members of the same genus of wild sheep, Nadler et al. (1971; 1973) reported wild sheep in different localities with different chromosome numbers. In the case of two localized sheep populations in Central Iran, Nadler et al. (1971) suggested that this variation in chromosome number represented a form of "intergradation between two chromosomally homogenous but distinctive taxa, rather than chromosome polymorphism, an event which occurs within a population and not at the zone of contact between two forms". Table V. 3 summarizes the variation in chromosome number occurring in wild sheep.

TABLE V. 2

INCIDENCE OF ROBERTSONIAN TRANSLOCATIONS IN DOMESTIC SHEEP

Author	Breed	Country	2n	Number Studied (Number With Translocation)	%	Translocation Type
Bruère, A.N. (1969)	Romney	N.Z.	53	6 (4)		Massey I
Bruère, A.N. and Mills, R.A. (1971)	Romney	N.Z.	53	180 (25)	13.88	Massey I
	"	"	"	2 (2)		Massey II
Bruère, A.N.; Chapman, H.M.; and Wyllie, D.R. (1972)	Drysdale	N.Z.	53	{ 321	(76) 23.67	Massey III
	"	"	52		(6) 1.86	" "
Bruère, A.N. (1972, unpublished)	Polled Drysdale	"	53	11	27.2	" "
Bruère, A.N. (1973)	Romney	N.Z.	53	309 (3)		Massey II
Bruère, A.N. (1973, Unpublished)	Romney	N.Z.	53	331	4-5	Massey II

TABLE V. 3

CHROMOSOME POLYMORPHISM IN WILD SHEEP

Author and Date	Species	(Autosomes)				Sex Chromosomes
		NF	2n	Number of Metacentrics	Number of Acrocentrics	
Schmitt, J. and Ulbrich, F. (1968)	<u>Ovis ammon nigrimontana</u> (Servetsov, 1873) (Kara-Tau sheep)	60	56	4	50	X acrocentric Y biarmed
	<u>Ovis ammon musimon</u> (Pallas, 1811) (Mouflon)	60	54	6	46	"
	<u>Ovis ammon Laristancia</u> (Nasonov, 1909)	60	54	6	46	"
	<u>Ovis ammon cycloceros</u> (Hutton, 1842) (Afghanistan sheep)	60	58	2	54	"
Wurster, D.H. and Benirschke, K. (1968)	<u>Ovis canadensis</u> (Bighorn sheep)	60	54	6	46	"
	Nadler, G.F.; Hoffmann, R.S. and Woolf, A. (1973)					
Nadler, C.F. (1971)	<u>Ovis dalli dalli</u> (Nelson)	60	54	6	46	"
Nadler, C.F.; Lay, D.M.; and Hassinger, J.D. (1971)	<u>Ovis ammon</u> populations in Iran	60	58	2	54	"
	Eastern populations	60	54	6	46	"
	Central populations	60	55	5	48	"
		60	56	4	50	"
		60	57	3	52	"
		60	58	2	54	"
	Western populations	60	54	6	46	"
Nadler, C.F.; Hoffmann, R.S. and Woolf, A. (1973)	<u>Ovis musimon</u> (Schreber) (European mouflon)	60	54	6	46	"
	<u>Ovis orientalis</u> (Gmelin) (Asiatic mouflon)	60	54	6	46	"
	<u>Ovis vignei</u> (Blyth) (Urial sheep)	60	58	2	54	"

(E) Dogs

Centric fusion of chromosomes has been observed on at least three different occasions in the dog. Two cases of apparent centric fusion between two autosomes have been reported in dogs with congenital abnormalities (Shive, Hare and Patterson, 1965; Hare, Wilkinson, McFeely and Riser, 1967) and a third case in a phenotypically normal dog (Ma and Gilmore, 1971).

(iii) ROBERTSONIAN TRANSLOCATIONS IN MAN

The human karyotype has been studied more extensively than that of any other mammal. Much of this study has been prompted by the variety of clinical conditions frequently found to be associated with an abnormal karyotype. Robertsonian translocations are the most common type of translocation reported in man and are present in about 0.5 per cent of the population (Court Brown, 1967). The human karyotype contains only two groups (group D and group G) of acrocentric chromosomes (10 out of a total of 46 chromosomes). In contrast to the acrocentric chromosomes of the domestic animals, human acrocentric chromosomes have visible satellites, believed to consist of euchromatin, on their short arms (Ferguson-Smith and Handmaker, 1961; 1963). The satellited chromosomes of man associate short arm to short arm in mitotic metaphase preparations (Ferguson-Smith and Handmaker, 1961; 1963; Cohen and Shaw, 1967; Galperin, 1968; Cooke, 1972) and also during meiosis (Ferguson-Smith, 1964). From this association it has been suggested that these chromosomes are non-disjunction prone (Ohno, Trujillo, Kaplan and Kinosita, 1961; Galperin, 1968) and Ohno et al. (1961) postulated that increased satellite association might also be related to the formation of translocations between the acrocentric chromosomes. Breakages could possibly occur more easily in the attenuated secondary constriction areas and the chance of a translocation occurring would be greater if the broken ends of different members were in proximity in the nucleolar region.

Three types of Robertsonian translocation have been reported in man; between members of the D group of chromosomes

(13, 14 and 15) D/D or
t (DqDq)

between members of the G group of chromosomes

(21 and 22) G/G or
t (21q22q) and

between members of both groups D/G or t(DqGq).

The D/D translocation is the most common, occurring in about one in 1,000 adults in the general population (Court Brown, 1967). It is a metacentric translocation, familial and carriers of it produce offspring which are either normal, balanced carriers, or trisomic for a D group chromosome. Apart from the trisomic state (Taylor, 1968) there appears to be little evidence of phenotypic abnormality in balanced D/D carriers. Individuals who are trisomic for a D chromosome either may be aborted (Lucas, 1969; Dhadiyal, Machin and Tait, 1970; Kajii, Ohama, Niikawa, Ferrier and Avirachan, 1973) or born alive but with severe congenital malformations (Giannelli, 1965; Court Brown, 1967; Taylor, 1968). Kjessler (1964) described a subfertile man with a D/D translocation, but the association if any with sterility, was not clear.

Regular mongolism in man is associated with the presence of an additional acrocentric chromosome of the G group and is referred to as trisomy G or trisomy 21. In this case the mongol has 47 chromosomes instead of the normal 46. However approximately two to five per cent of all mongoloid individuals have 46 chromosomes but carry the extra chromosome in the form of a translocation. The commonest translocation is between a member of the D group and chromosome 21 (D/G) and less frequently between two group G chromosomes.

Between 25 and 50 per cent of translocation mongols have inherited the translocation chromosome from a parent (Giannelli, Hamerton and Carter, 1965; Court Brown, 1967) while in the remainder the translocation has arisen de novo. The parent in cases where the child has inherited the translocation chromosome, is a

phenotypically normal balanced translocation carrier with 45 chromosomes and a normal number of chromosome arms. The translocation mongol with 46 chromosomes is trisomic for the G group chromosome which has presumably entered his karyotype from a non-disjunctional event during gametogenesis.

There are many reports of population studies on the three human Robertsonian translocation types (Hamerton, 1968; Dutrillaux and Lejeune, 1969; Stene, 1970; Jacobs, Aitken, Frackiewicz, Law, Newton and Smith, 1970) and from these studies several points emerge which are relevant to studies in domestic animals.

Firstly, balanced translocation carriers, that is translocation heterozygotes with normal N.F. (Matthey, 1945) but one less chromosome than normal are apparently unaffected phenotypically except under special circumstances such as the case described by Kjessler (1964).

Secondly, all three human Robertsonian translocation types are familial as are those reported in most other species. However in man it has been shown that a high proportion of Robertsonian translocations continually arise on a de novo basis (Court Brown, 1967).

A third important observation from human studies is the tendency for non-random involvement of specific chromosomes in both D and G groups in these translocations (Rowley and Pergament, 1969; Kohno and Makino, 1969; De Capoa and Rocchi, 1970; Hecht and Kimberling, 1971).

(iv) NON-RANDOM INVOLVEMENT OF CHROMOSOMES IN ROBERTSONIAN TRANSLOCATIONS

The reasons for the non-random involvement of acrocentric chromosomes in Robertsonian translocations of man and their method of formation have been discussed recently by Ferguson-Smith (1972) and Chandley and Fletcher (1973). By comparison, there is little evidence for the non-random association of chromosomes in such translocations in the tobacco mouse (Gropp, Winking, Zech and Müller, 1972), but the dispersion of the 1/29 translocation among many breeds of cattle in several countries

suggests that in this species, non-random involvement could take place (Bruère and Chapman, 1973; Bruère, 1974). It seems reasonable therefore to accept the possibility that certain chromosomes in the domestic sheep may have a greater tendency to form translocations than do others.

(v) PHENOTYPIC EFFECTS OF ROBERTSONIAN TRANSLOCATIONS
IN DOMESTIC ANIMALS

Most reports indicate that domestic animals carrying a Robertsonian translocation are phenotypically normal. There are few confirmed reports of phenotypic effects due to Robertsonian translocations in domestic animals, but several reports of phenotypic changes and a Robertsonian translocation occurring in the same animal are summarized in Table V. 4. In most cases the relationship between the chromosomal and phenotypic anomaly was not clear and it may have been coincidental that these were found together.

In Swedish cattle, although no phenotypic abnormalities were observed it was claimed that daughters of bulls heterozygous for the 1/29 translocation showed an increased return to service (Gustavsson, 1969; Gustavsson, 1971,c). This claim was based on statistical evidence only and the extrapolation was made that this relative reproductive inefficiency was probably due to an abnormal karyotype in some of the daughters. Gustavsson (1969) further claimed from the deviating non-return rates within 273 days of pregnancy and the difference observed in heifers between the non-return rates within 56 and 273 days, that the reduced fertility was due to an increased rate of embryonic death. Close examination of Gustavsson's data by Bruère (1974) indicated that of 11 bulls whose daughters were investigated, the progeny of one showed a better than expected conception rate, while the data from seven was not significant and that from the translocation homozygote barely significant. Gustavsson (1969) neither produced cytological evidence of reduced fertility in these bulls nor of embryonic death in the translocation heifers. He did not take into account sire relationships nor any of the inherited factors which could have affected the results.

TABLE V. 4

ANIMALS WITH PHENOTYPIC ABNORMALITIES AND A ROBERTSONIAN
TRANSLOCATION

Author and Date	Species	Number	Phenotypic Abnormality	Karyotype
Gustavsson, I. and Rockborn, G. (1964)	Cattle	3	Lymphatic Leukaemia	59 chromosomes including 1/29 translocation
Rugiati, S. and Fedrigo, M. (1957)	Cattle	1	Achondroplastic dwarfism	58,xy including 2(1/28) translocation
(1968)	"	2	Chondrodystrophic lesions	59,xx including (1/28) translocation
Herzog, A. and Höhn, H. (1971)	Cattle	1	C.N.S. abnormalities	59 chromosomes including translocation
Froget, J.; Coulon, J.; Nain, M.-C. and Dalbiez, J.-M. (1972)	Cattle	1	Crooked forelegs	59,xx including 1/29 translocation
Shive, R.J.; Hare, W.C.D. and Patterson, D.F. (1965)	Dog	1	Congenital cardiac defect Cleft upper lip	77,xy including submetacentric translocation chromosome
Hare, W.C.D.; Wilkinson, J.S.; McFeely, R.A. and Riser, W.H. (1967)	Dog	1	Bone chondroplasia	77,xx including subtelocentric translocation chromosome
Padeh, B.; Wyscki, M., Ayalon, N. and Soller, M. (1965)	Goat	1	Pseudohermaphrodite	xx/xy 59 chromosomes including translocation in xx cells
Bruère, A.N. (1969)	Sheep	1	Small abnormally shaped testicles associated with complete sperma- toocyte arrest.	53,xy including translocation chromosome
Bruère, A.N. and Mills, R.A. (1971)	Sheep	5	2 Rams - monorchids 3 Rams - "hour-glass" testes with spermatocyte arrest	53,xy including translocation chromosome
Bruère, A.N.; Chapman, H.M.; and Wyllie, D.R. (1972)	Sheep	2	1 Ram - atrophic testes and absence of epididymis on right testis. 1 Ewe - segmental aplasia of genitalia.	53,xy including translocation chromosome 53,xx including translocation chromosome

V. 2 MEIOSIS IN ROBERTSONIAN TRANSLOCATION CARRIER RAMS

(i) INTRODUCTION

The incidence of Robertsonian translocations in cattle and sheep is higher than that in man (refer sections V. 1 (ii), (iii)). Multiple Robertsonian translocation heterozygosity is possible in cattle since three different translocations have been demonstrated (Gustavsson and Rockborn, 1964; Pollock, 1972; Bruère and Chapman, 1973). Similarly in sheep, three different Robertsonian translocations are known (Bruère, 1969; Bruère and Mills, 1971; Bruère et al., 1972) and mating experiments carried out by Dr. A.N. Bruère at Massey University have produced sheep which are heterozygous for two different Robertsonian translocation chromosomes with all combinations of the three types being involved (Bruère, 1974; Bruère and Chapman, 1974).

The high incidence of Robertsonian translocations in domestic animals, apparent from the number of new cases continually being reported, and their possible deleterious effects on fertility as has been shown to occur in some species of mice, make an understanding of the behaviour of these translocations important. In addition, data from the sheep could be a useful model for human studies as there has been little published on meiotic chromosomes in Robertsonian translocation carriers in either man or domestic animals.

The Robertsonian translocations of sheep discussed in this thesis were originally referred to as the Massey I, Massey II and Massey III translocations. A scheme of nomenclature based on the recommendations of the Paris Conference (1971) has been drawn up and is summarized in Table V. 5. For example, the term "Massey I translocation heterozygous ram" is synonymous with the nomenclature "53,xy,t₁" and the short designations as listed, are used in the text.

TABLE V. 5

NOMENCLATURE FOR ROBERTSONIAN TRANSLOCATIONS IN DOMESTIC SHEEP
(BASED ON PARIS CONFERENCE, 1971)

	MASSEY I	MASSEY II	MASSEY III
Breed of Origin	Romney	Romney	Drysdale
Chromosomes Involved in Translocation	5;26	8;11	7;25
Type of Translocation Chromosome	Submetacentric	Metacentric	Submetacentric
Heterozygote Designation - detailed	53,xy,-5,-26,+ t(5q26q)	53,xy,-8,-11,+ t(8q11q)	53,xy,-7,-25,+ t(7q25q)
short	53,xy,t(5q26q) or 53,xy,t ₁ 53,xx,t ₁	53,xy,t(8q11q) 53,xy,t ₂ 53,xx,t ₂	53,xy,t(7q25q) 53,xy,t ₃ 53,xx,t ₃
Homozygote Designation	52,xy,t(5q26q), t(5q26q) 52,xy,t ₁ t ₁	52,xy,t ₂ t ₂	52,xy,t ₃ t ₃

Double Translocation Heterozygotes

Massey I, II	52,xy,t(5q26q),t(8q11q) or 52,xy,t ₁ t ₂
Massey I, III	52,xy,t(5q26q),t(7q25q) or 52,xy,t ₁ t ₃
Massey II, III	52,xy,t(8q11q),t(7q25q) or 52,xy,t ₂ t ₃

Representative rams from each of the three Robertsonian translocation heterozygote types as well as a Massey I translocation homozygote, a Massey III translocation homozygote and a Massey I and III double translocation heterozygote were included in this study. The identification, numbers and karyotypes of the rams studied are summarized in Table V. 6.

TABLE V. 6

ROBERTSONIAN TRANSLOCATION-CARRYING RAMS
FROM WHICH MEIOTIC CHROMOSOMES STUDIED

Massey I 53,xy,t ₁ 52,xy,t ₁ ,t ₁		Massey II 53,xy,t ₂	Massey III 53,xy,t ₃ 52,xy,t ₃ ,t ₃		Massey I and III 52,xy,t ₁ ,t ₃
B46/69	B55/70	B25/72	Link 1966	A134	B9/72
B68/69		B87/72	HML 99/58		
B12/69		B68/72	B116/71		
B53/69		B66/72	B13/72		
236/67(B15)			B76/72		
B26/70					
A182					
B8/72					
B71/69					
9	1	4	5	1	1

The criteria by which individual rams were chosen for inclusion in the study have been described in Chapter III, section 1 (i). The results of the semen and histological analysis from each ram are summarized in Appendices XVIIIa and XVIIIb.

(ii) RESULTS OF ANALYSES OF SPERMATOGONIAL
AND PRIMARY SPERMATOCYTE CELLS

(A) Spermatogonial Metaphases

Chromosome counts from the few spermatogonial cells analysed from the three Robertsonian translocation types studied are given in Table V. 7.

TABLE V. 7

DISTRIBUTION OF CHROMOSOME COUNTS IN SPERMATOGONIAL CELLS
IN RAMS HETEROZYGOUS FOR A ROBERTSONIAN TRANSLOCATION

Type of Heterozygote	Ram Identi- fication	Number of Cells With Chromosome Number					Number of Polyploid Cells	Total
		<52	52	53	54	>54		
53,xy,t ₁	B46/69						0	0
	B68/69	1		1			0	2
	B12/69	1					0	1
	B53/69	1		1			0	2
	236/67			2			1	3
	B26/70						0	0
	A182	2		5			0	7
	B8/72						0	0
	B71/69			1			0	1
Total		5	0	10	0	0	1	16
53,xy,t ₂	B25/72		1				0	1
	B87/72			4			0	4
	B68/72	1					0	1
	B66/72			1			0	1
	Total	1	1	5	0	0	0	7
53,xy,t ₃	Link 1966						0	0
	HML 99/58			6			1	7
	B116/71	1	1	1			4	7
	B13/72	3		4			0	7
	B76/72			3			0	3
	Total	4	1	14	0	0	5	24

Four spermatogonial metaphases were recorded from the Massey I homozygote, B55/70. The modal chromosome number was 53 in each of the heterozygote types and 52 in the homozygote; a similar finding to that in somatic metaphases. No hypermodal metaphases were seen, but six polyploid cells were recorded.

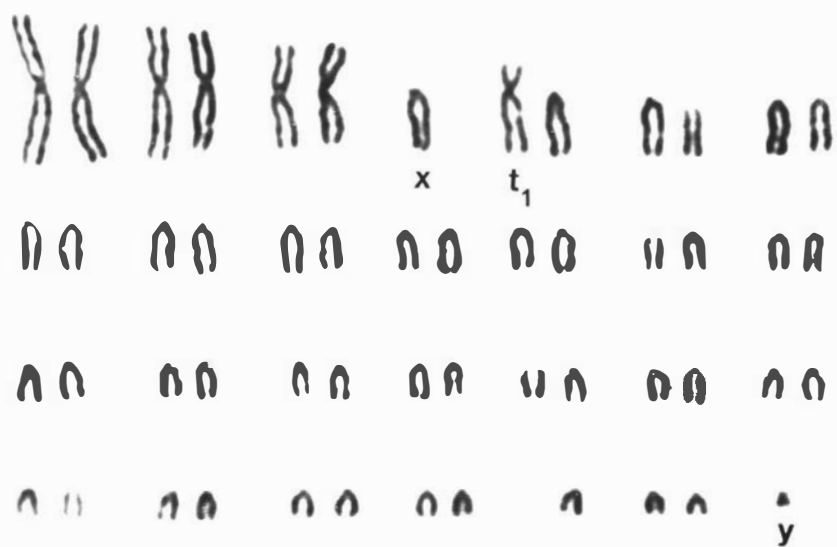
The mitotic metaphase spreads from each of the Massey I, II and III Robertsonian translocation heterozygotes were made up of: 6 metacentric chromosomes, 1 translocation metacentric ($53,xy,t_2$) or submetacentric ($53,xy,t_1$ and $53,xy,t_2$) chromosome, 44 acrocentric chromosomes, 2 sex chromosomes. Karyotypes representative of each of the three types of Robertsonian translocation heterozygote are shown in Plates V. 1, V. 3 and V. 5.

The 52 chromosomes seen in mitotic metaphase spreads from rams homozygous for each of the Robertsonian translocations included: 6 metacentric chromosomes, 2 translocation metacentric or submetacentric chromosomes, 42 acrocentric chromosomes, 2 sex chromosomes.

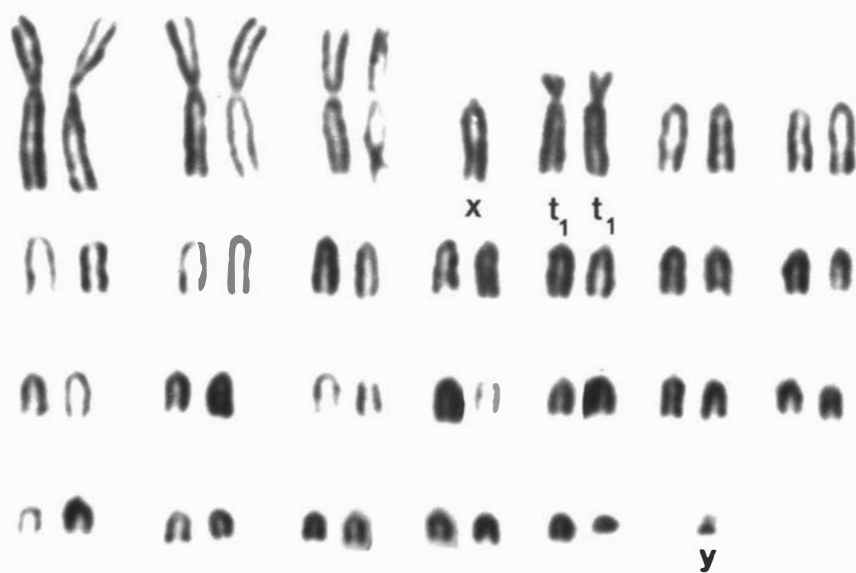
Examples of mitotic karyotypes from rams homozygous for the three types of translocation are given in Plates V. 2, V. 4 and V. 6. Chromosome morphology in the spermatogonial metaphases was similar to that seen in somatic mitotic metaphases. Plates V. 7 and V. 8 show examples of mitotic cells from germinal tissue.

Plate V. 1: Karyotype of a male Massey I Robertsonian translocation heterozygote. The translocation submetacentric chromosome (t_1) and the sex chromosomes are identified. (Giemsa).

Plate V. 2: Karyotype of a male Massey I Robertsonian translocation homozygote. (Giemsa).



1



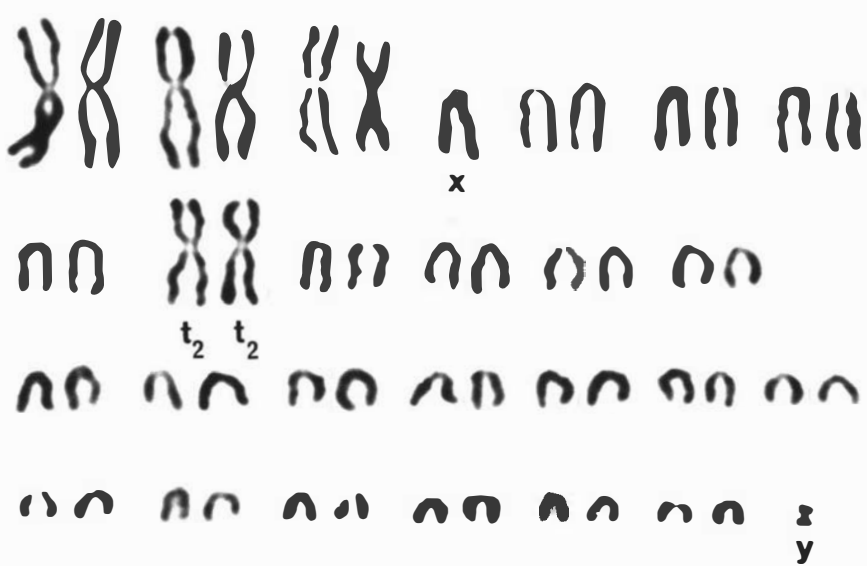
2

Plate V. 3: Karyotype of a male Massey II Robertsonian translocation heterozygote. The metacentric translocation chromosome (t_2) and the sex chromosomes are identified. (Giemsa).

Plate V. 4: Karyotype of a male Massey II Robertsonian translocation homozygote. (Giemsa).



3



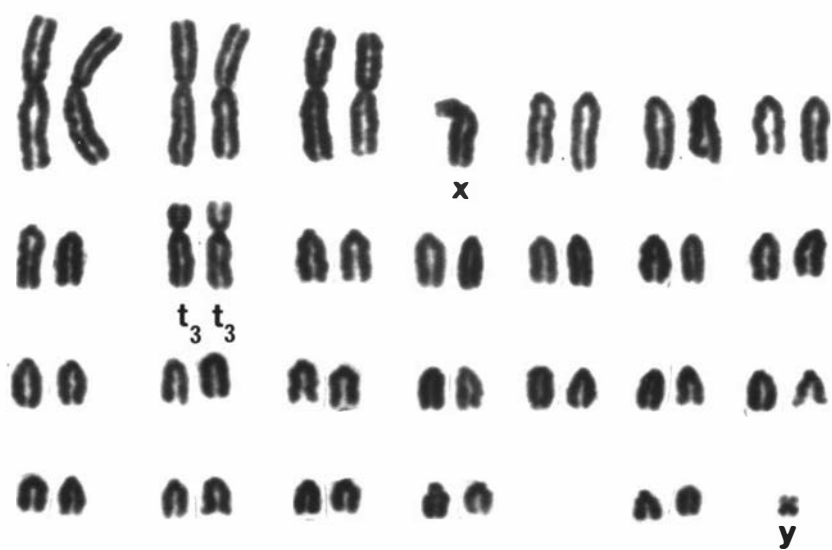
4

Plate V. 5: Karyotype of a male Massey III Robertsonian translocation heterozygote. The submetacentric translocation chromosome (t_3) and the sex chromosomes are identified. (Giemsa).

Plate V. 6: Karyotype of a male Massey III Robertsonian translocation homozygote. (Giemsa).



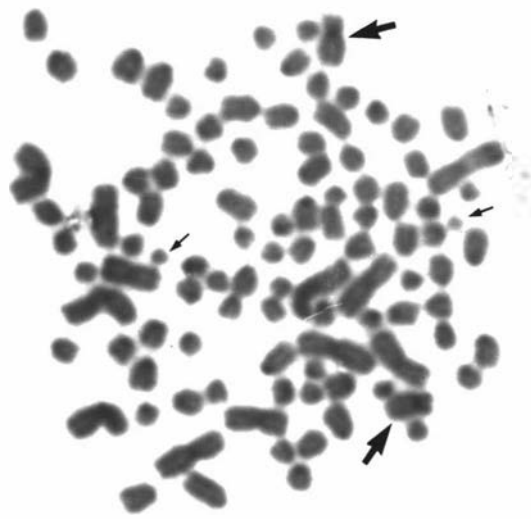
5



6

Plate V. 7: Tetraploid spermatogonial metaphase from 236/67
(53,xy,t₁). Large arrows point to the
translocation chromosomes, and small arrows
point to the Y chromosomes.
(Aceto-orcein. x 1,750).

Plate V. 8: Diploid spermatogonial metaphase from HML99/58
(53,xy,t₃).
(Aceto-orcein. x 3,000).



7



8

(B) Diakinesis And Metaphase I Stages

A total of 2,402 cells at the diakinesis-metaphase I stages were counted in meiotic preparations from the Robertsonian translocation-carrying heterozygous and homozygous rams included in this study. This total included 232 polyploid cells. Karyotypes were made of 67 diakinesis-metaphase I cells.

a 53,xy,t₁

Chromosome counts from diakinesis-metaphase I cells are given in Table V. 8. Of the 534 cells counted, 89 (16.7 per cent) were polyploid cells. Analyses of non-polyploid cells are also shown in Table V. 8. The majority of cells, 82.7 per cent, had 26 chromosomal bodies. Four cells, had 27 chromosomal bodies. No cells with more than 27 chromosomal bodies were recorded among the non-polyploid cells studied. There were 73 hypomodal cells.

Close examination of the 368 cells having chromosome counts of 26, showed that this number included 24 autosomal bivalents, a sex bivalent and a trivalent. The 24 autosomal bivalents comprised three large bivalents and 21 small bivalents. The three large bivalents were formed by the pairing of the metacentric chromosomes and the smaller bivalents were formed by the pairing of the acrocentric chromosomes seen in mitotic metaphases. The trivalent is composed of the translocation submetacentric chromosome paired with its two acrocentric homologues. These morphological features are shown in Plates V. 9, V. 10 and V. 11.

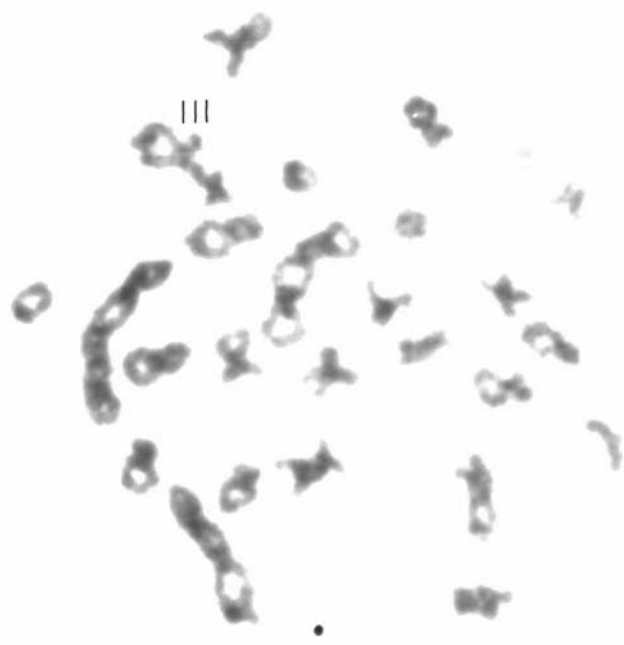
Four of the 445 cells analysed had chromosome body counts of 27. The latter number comprised two cells having 24 autosomal bivalents, a trivalent and separated X and Y chromosomes, and two cells with 23 autosomal bivalents, a sex bivalent, trivalent and two other small structures interpreted as univalents. An example of the latter type of cell is shown in Plate V. 12. The remainder of the cell counts included those with one or more autosomal bivalents missing, as well as cells with hypomodal counts in which the sex bivalent or trivalent was missing. Twenty one of the sex bivalents were associated with one of the small autosomal bivalents. The percentages of cells from the three rams in which this association was recorded varied from 3.5 to 7.4 per cent.

TABLE V.8 ROBERTSONIAN TRANSLOCATION HETEROZYGOTES : 53,xy,t₁
CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS – METAPHASE I STAGES

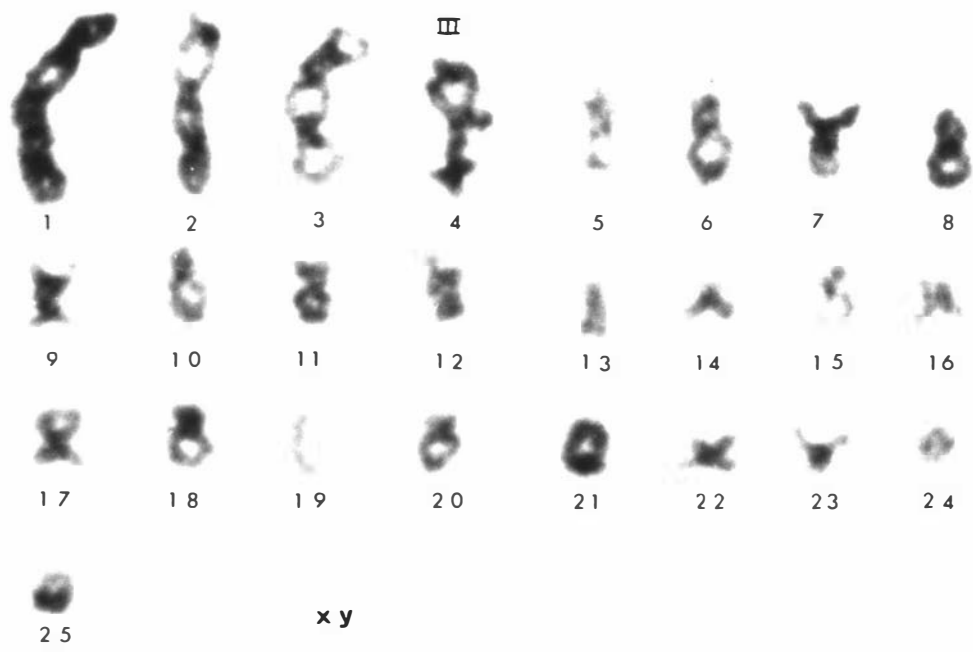
Ram Identi- fication		NUMBER OF CHROMOSOME BODIES/CELL						Total	Number of Polyploid Cells	Total Including Polyploid Cells	ANALYSIS OF CELLS EXCLUDING POLYPOIDS										Total Number Cells Analysed	Number of cells with xy associa- ted with Auto- somal Bivalent	x and y Separated						
											NUMBER OF CELLS WITH:												number	%					
		<24	24	25	26	27	>27				26,xy,III	27,x,y,III	-A 25,xy,III	-2A 24,xy,III	OTHER ABERRATIONS														
B46/69	NUMBER OF CELLS	0	0	0	2	0	0	2	0	2	2	0	0	0	0										2	0	0		
B68/69		2	1	3	51	0	0	57	7	64	51	0	3	0	24, -xy, III, -A 1 21, xy, III, -5A 1 17, xy, III, -9A 1										57	2 (3.5%)	0		
B12/69		1	1	4	26	1	0	33	4	37	26	0	4	0	27, xy, III, +I +I 1 24, xy, -III, -A 1 19, xy, -III, -6A 1										33	0	0		
B53/69		7	6	21	195	2	0	231	48	279	195	1	21	5	27, xy, III, +I +I 1 22, xy, -III, -3A 1 24, -xy, III, -A 1 22, xy, III, -4A 1 23, xy, III, -3A 2 19, -xy, III, -6A 1 23, -xy, -III, -A 1 18, xy, -III, -7A 1										231	17 (7.4%)	1	0.4	
236/67 (B15)		2	0	2	7	0	0	11	2	13	7	0	2	0	23, xy, III, -3A 1 20, xy, III, -6A 1										11	0	0		
B26/70		1	0	0	9	0	0	10	0	10	9	0	0	0	23, xy, -III, -2A 1										10	0	0		
A182		4	3	8	29	1	0	45	24	69	29	1	7	2	25, -xy, III, 1 22, xy, III, -4A 1 24, xy, -III, -A 1 22, -xy, III, -3A 1 23, xy, III, -3A 1 22, xy, -III, -3A 1										45	2 (4.4%)	1	2.2	
B8/72		1	2	2	47	0	0	52	3	55	47	0	2	2	21, -xy, III, -4A 1										52	0	0		
B71/69		1	0	1	2	0	0	4	1	5	2	0	1	0	12, -xy, III, -13A 1										4	0	0		
TOTAL		19	13	41	368	4	0	445	89	534	368	2	40	9	19										7	445	21 (4.7%)	2	0.5(%)

Plate V. 9: Cell in diakinesis from B53/69 (53,xy,t₁). There are three large autosomal bivalents, a trivalent (III), 21 small acrocentric bivalents and a sex bivalent.
(Aceto-orcein. x 2,500).

Figure V. 1: Karyotype of cell illustrated in Plate V. 9.



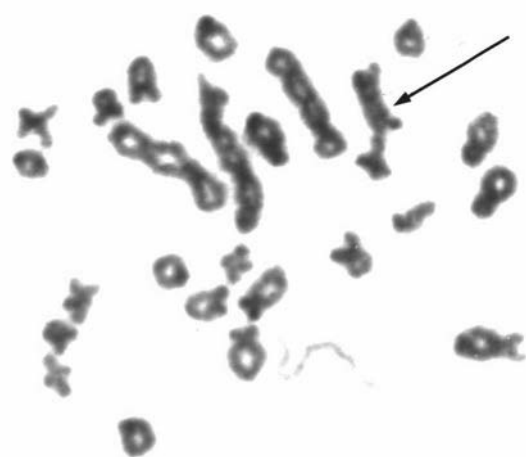
9



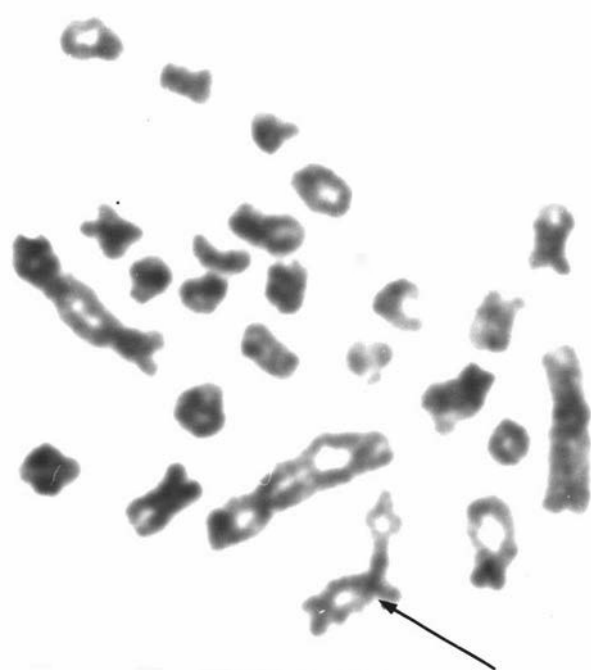
1

Plate V. 10: Cell in diakinesis from A182 (53,xy,t₁) with
25 bivalents and a trivalent (arrowed).
(Aceto-orcein. x 1,750).

Plate V. 11: Cell in diakinesis from A182 (53,xy,t₁) with
25 bivalents and a trivalent (arrowed).
(Aceto-orcein. x 3,500).



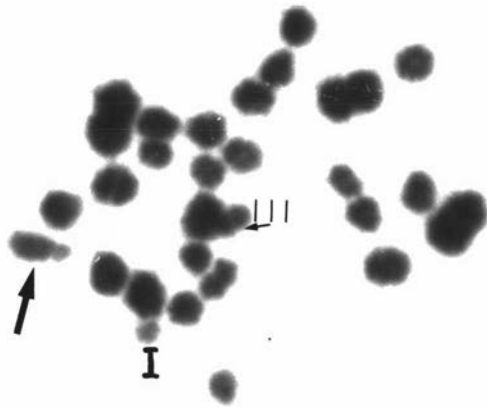
10



11

Plate V. 12: Metaphase cell from B53/69 (53,xy,t₁) with 27 chromosomal elements including a trivalent (III), sex bivalent (arrowed) and an extra element which is probably a univalent (I). (Aceto-orcein. x 3,000).

Plate V. 13: Tetraploid cell in diakinesis from A1S2 (53,xy,t₁). There are two trivalents (III) and two sex bivalents (arrowed). One of the sex bivalents is associated with a small autosomal bivalent (a). (Aceto-orcein. x 3,000).



12



13

Results from chromosome counts of polyploid cells are given in Table V. 9. Eighty nine cells were recorded. Of those cells which were analysed, 57 were tetraploid (Plate V. 13), nine were hexaploid, three were octaploid while one cell had 124 elements including five sex bivalents and five trivalents (Plate V. 14). Apart from the trivalents, no multivalents were observed in any of the apparently polyploid cells examined.

Plate V. 14: Polyploid cell in diakinesis-metaphase I from B53/69 (53,xy,t₁) with a chromosomal count of 124 including five sex bivalents and five trivalents (arrowed).
(Aceto-orcein. x 2,000).



TABLE V. 9
MASSEY I ROBERTSONIAN TRANSLOCATION HETEROZYGOTES (53,xy,t₁):
CHROMOSOME COUNTS FROM POLYPLOID CELLS AT DIAKINESIS-METAPHASE I STAGES

Ram Identi- fication	Number of Cells Showing Chromosome Complement			N.A.	Total	Analysis of Polyploid Cells						N.A.	Total
						4n		6n		8n			
	4n	6n	8n			52,2xy,2III	51,2xy,2III	78,3xy,3III	Other Cells	104,4xy,4III	Other Cells		
	4n	6n	8n										
B46/69	0	0	0	0	0	0	0	0	0	0	0	0	0
B63/69	3	0	0	4	7	2	1	0	0	0	0	4	7
B12/69	4	0	0	0	4	3	1	0	0	0	0	0	4
B53/69	31	7	3	7	48	21	10	6	71,3xy,3III	1	101,4xy,4III? 124,5xy,5III?	7	48
235/67	1	0	0	1	2	1	0	0	0	0	0	1	2
B26/70	0	0	0	0	0	0	0	0	0	0	0	0	0
A182	14	2	0	8	24	11	3	1	73,3xy,3III	0	0	8	24
B8/72	3	0	0	0	3	3	0	0	0	0	0	0	3
B71/69	1	0	0	0	1	1	0	0	0	0	0	0	1
Total	57	9	3	20	89	42	15	7	2	1	2	20	89

N.A. = Not Analysed

b 53,xy,t₂

Chromosome counts in diakinesis-metaphase I cells from Massey II Robertsonian translocation heterozygotes, are summarized in Table V. 10.

Of the 1,022 cells counted, 48 (4.7 per cent) were polyploid. Analysis of the 974 non-polyploid cells revealed that 26, the modal chromosomal element count, was present in 830 of the 974 cells. Fifteen cells had hypermodal counts of 27 while hypomodal counts were recorded in 129 cells. Hypermodal and hypomodal cell counts were found in each of the meiotic preparations from the four rams studied. Twenty six chromosomal bodies including 24 autosomal bivalents, a sex bivalent and a trivalent were found in 827 cells (84.9 per cent). A similar total number of chromosomal bodies was found in three cells, one in each of the preparations from rams B25/72, B68/72 and B66/72. In two of these cells, the sex chromosomes were separated and an autosomal bivalent was missing, while in the third, the sex bivalent was fragmented into two pieces and an autosomal bivalent was absent.

Twenty seven chromosomal elements including 24 autosomal bivalents, a trivalent and separated X and Y chromosomes were observed in nine cells (0.9 per cent). Six other cells, five from ram B68/72 and one from ram B66/72 also showed total counts of 27. Five of these cells were composed of 23 autosomal bivalents, a trivalent (the identity of this was difficult to ascertain in three cells), a sex bivalent and two extra elements thought to be autosomal univalents.

Other aberrant chromosome counts were due to missing elements.

An association between the sex bivalent and an autosomal bivalent was seen in some cells from each of the rams used in the study. The percentage of cells containing this association varied from 3 to 5.7 per cent. A total of 43 of the 974 cells studied showed this phenomenon of sex bivalent-autosomal bivalent association.

Separation of the sex chromosomes was observed in 13 of the 974 cells (1.3 per cent) with a range of from 0.7 to 1.8 per cent in preparations from the different rams. The chromosomal composition of individual cells showing this separation is summarized in Table V. 10.

Polyploid cells were recorded from each ram. Thirty six of the total number of 48 counted, were analysed. The majority of these (68.8 per cent) were tetraploid. Two cells had counts of 77 and 78

TABLE V.10 ROBERTSONIAN TRANSLOCATION HETEROZYGOTES : 53,xy,t₂
CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS – METAPHASE I STAGES

Ram Identi- fication		NUMBER OF CHROMOSOME BODIES/CELL						Total	Number of Polyploid Cells	Total Including Polyploid Cells	ANALYSIS OF CELLS EXCLUDING POLYPOLOIDS										Total Number Cells Analysed	Number of cells with xy associated with Auto- somal Bivalent	x and y Separated	
		NUMBER OF CELLS WITH:									OTHER ABERRATIONS	number	%											
		<24	24	25	26	27	>27							26,xy,III	27,x,y,III	-A 25,xy,III	-2A 24,xy,III							
B25/72	NUMBER OF CELLS	5	2	10	93	1	0	111	9	120	92	1	10	1	26, x,y, III, -A 24, -xy, III, -A 22, xy, III, -4A 20, xy, III, -6A 19, -xy, III, -6A	1 1 1 3 1	111	5 (4.5%)	2	1.8				
B87/72		11	6	42	331	5	0	395	21	416	331	5	38	6	25, xy, -III, 25, x,y, III, -2A 23, xy, -III, -2A 23, xy, III, -3A 23, -xy, III, -2A 22, xy, III, -4A 19, xy, III, -7A 17, xy, III, -9A	2 2 1 4 1 1 3 1	395	12 (3.0%)	7	1.8				
B68/72		6	3	11	151	6	0	177	10	187	150	1	11	3	27, xy, III, +I +I 27, xy, III?, +I +I 26, x,y, III?, -A 23, xy, III, -3A 20, xy, III, -6A 19, xy, III, -7A	2 3 1 3 2 1	177	10 (5.7%)	2	1.1				
B66/72		2	11	20	255	3	0	291	8	299	254	2	20	9	27, xy, III?, 26, xy (fragmented into 2 pieces), III, -A 24, -xy, III, -A 24, xy, -III, -A 23, xy, III, -3A 20, xy, III, -6A	1 1 1 1 1 1	291	16 (5.5%)	2	0.7				
TOTAL		24	22	83	830	15	0	974	48	1022	827	9	79	19		40	974	43 (4.4%)	13	1.3				

chromosomal elements and in one cell, 102 chromosomal elements were seen. These findings are summarized in Table V. 11.

Some of the morphological features of cells in the diakinesis-metaphase I stages from Massey II Robertsonian translocation heterozygotes are shown in Plates V. 15 to V. 20.

TABLE V. 11

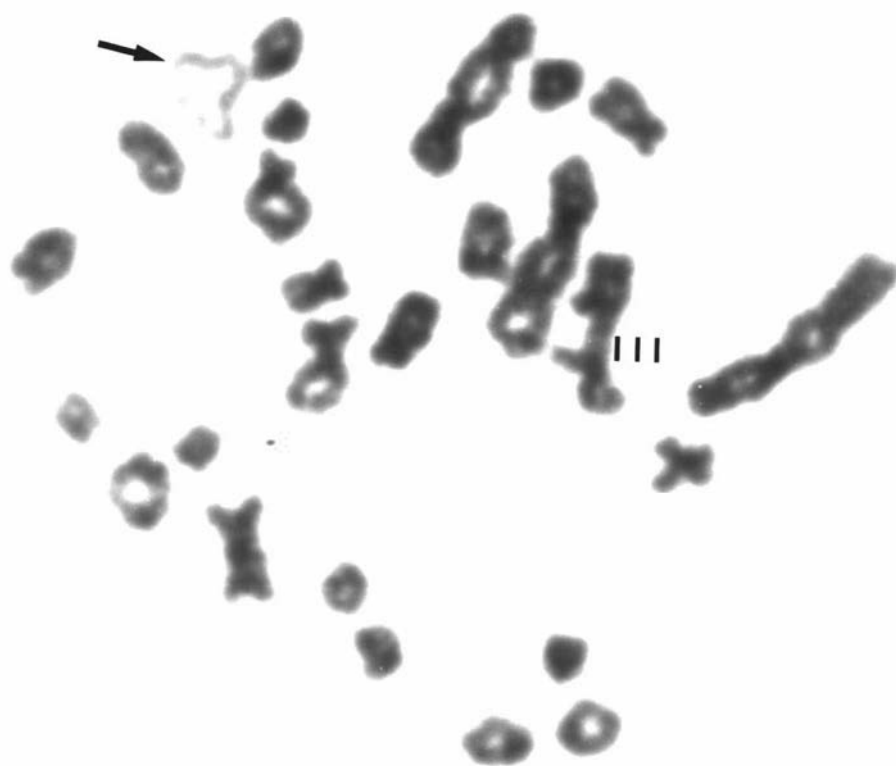
MASSEY II ROBERTSONIAN TRANSLOCATION HETEROZYGOTES ($53,xy,t_2$):
CHROMOSOME COUNTS FROM POLYPLOID CELLS AT DIAKINESIS-METAPHASE I STAGES

Ram Identification	Number of Cells Showing Chromosome Complement			N.A.	Total	Analysis of Polyploid Cells					Other Cells	N.A.	Total
						4n		6n		8n			
	4n	6n	8n			52,2xy,2III	51,2xy,2III	78,3xy,3III	Other Cells	104,4xy,4III			
B25/72	3	1	1	4	9	3	0	0	77,3xy,3III	0	102,4xy,4III	4	9
B87/72	15	1	0	5	21	11	4	1	0	0	0	5	21
B68/72	8	0	0	2	10	5	3	0	0	0	0	2	10
B66/72	7	0	0	1	8	6	1	0	0	0	0	1	8
Total	33	2	1	12	48	25	8	1	1	0	1	12	48

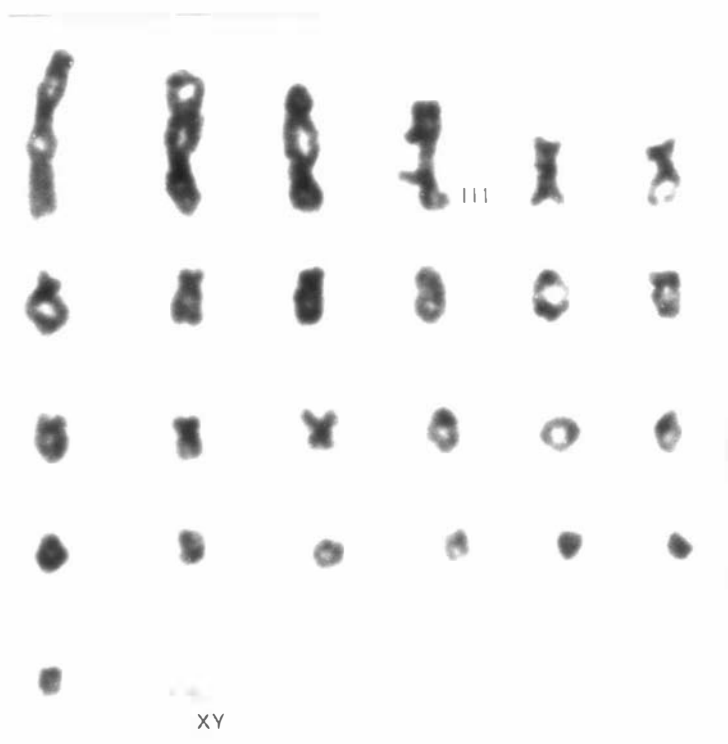
N.A. = Not Analysed

Plate V. 15: Cell in diakinesis from B66/72 (53,xy,t₂). There are three large autosomal bivalents, a trivalent (III), 21 small autosomal bivalents and a sex bivalent (arrowed). (Aceto-orcein. x 3,750).

Figure V. 2: Karyotype of cell illustrated in Plate V. 15.



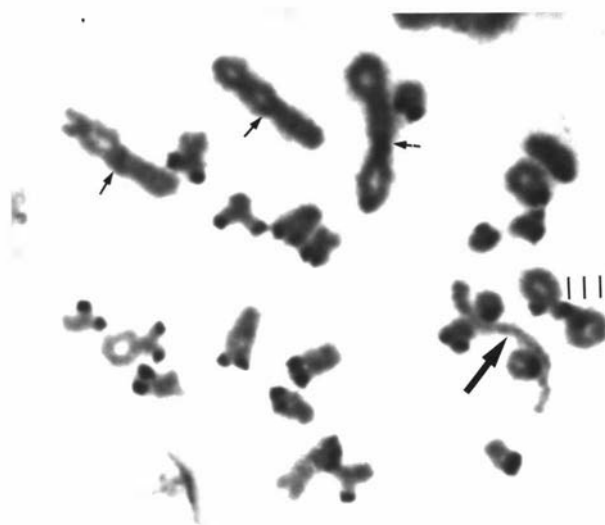
15



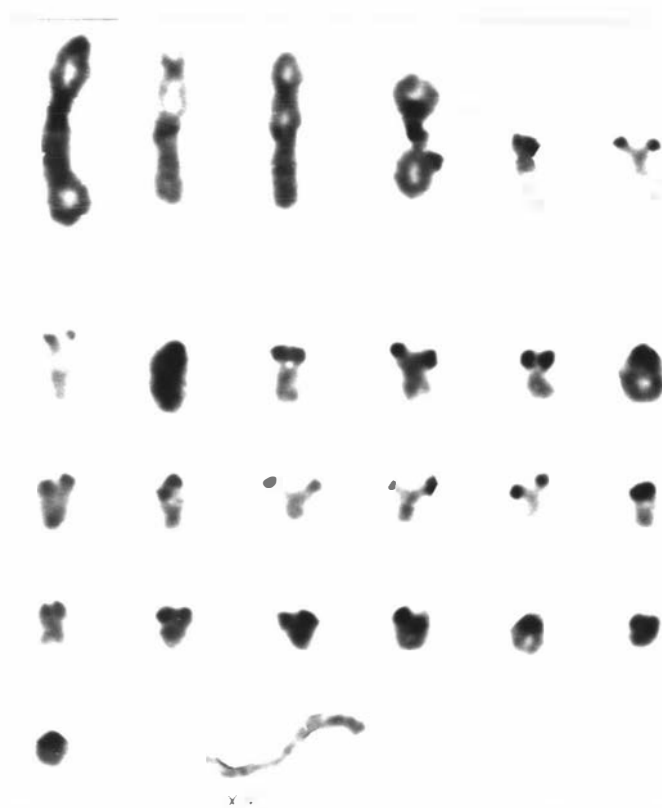
2

Plate V. 16: "C-banded cell" in diakinesis from B66/72
(53,xy,t₂). The three large autosomal bivalents have a narrow faintly stained area in the centromeric region (small arrows) compared with that on the small bivalents and trivalent. In contrast, the centromeric regions on the trivalent (III) and 21 smaller autosomal bivalents are stained more markedly. The sex bivalent (large arrow) is not differentially stained.
(C-banded. x 2,000).

Figure V. 3: Karyotype of cell illustrated in Plate V. 16.



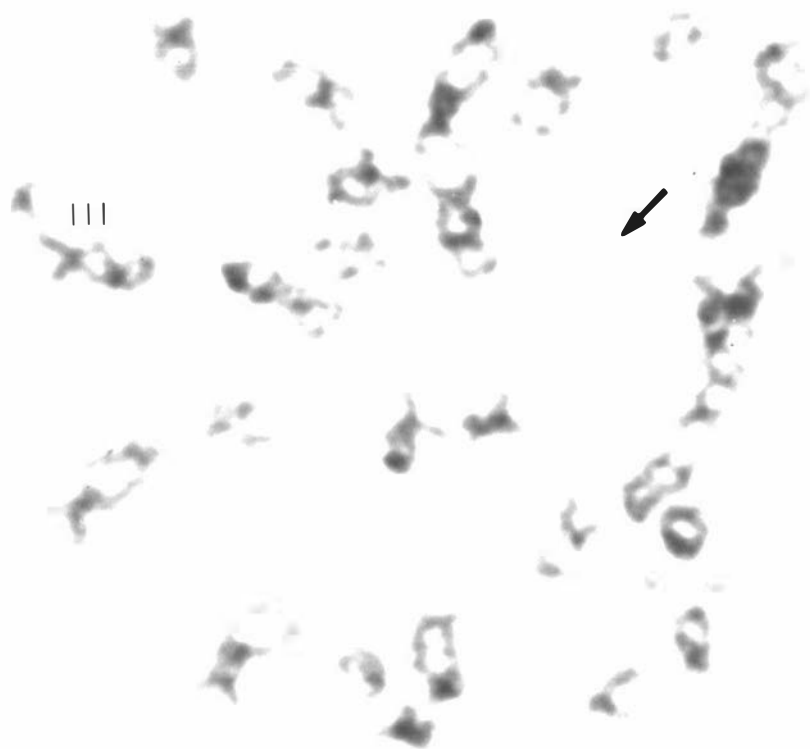
16



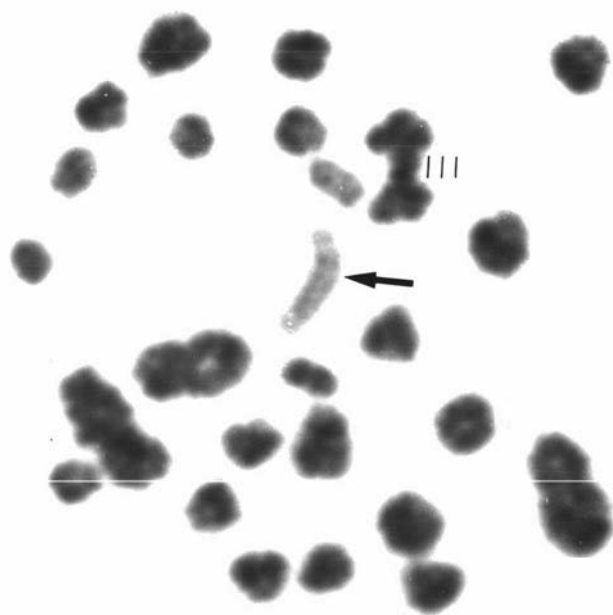
3

Plate V. 17: Cell in diakinesis from B68/72 (53,xy,t₂).
There are 24 autosomal bivalents, a trivalent (III) and a sex bivalent (arrowed).
(Aceto-orcein. x 3,750).

Plate V. 18: Cell from B87/72 (53,xy,t₂) in first metaphase
with 26 chromosomal elements including a
trivalent (III) and sex bivalent (arrowed).
(Aceto-orcein. x 4,000).



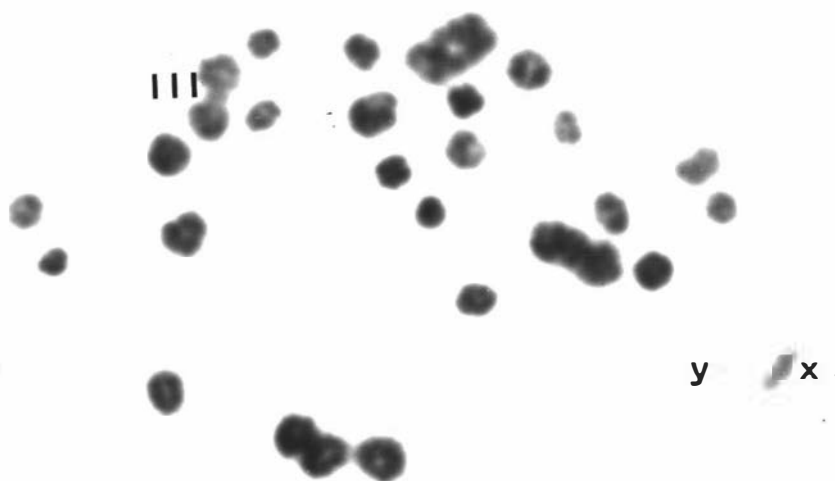
17



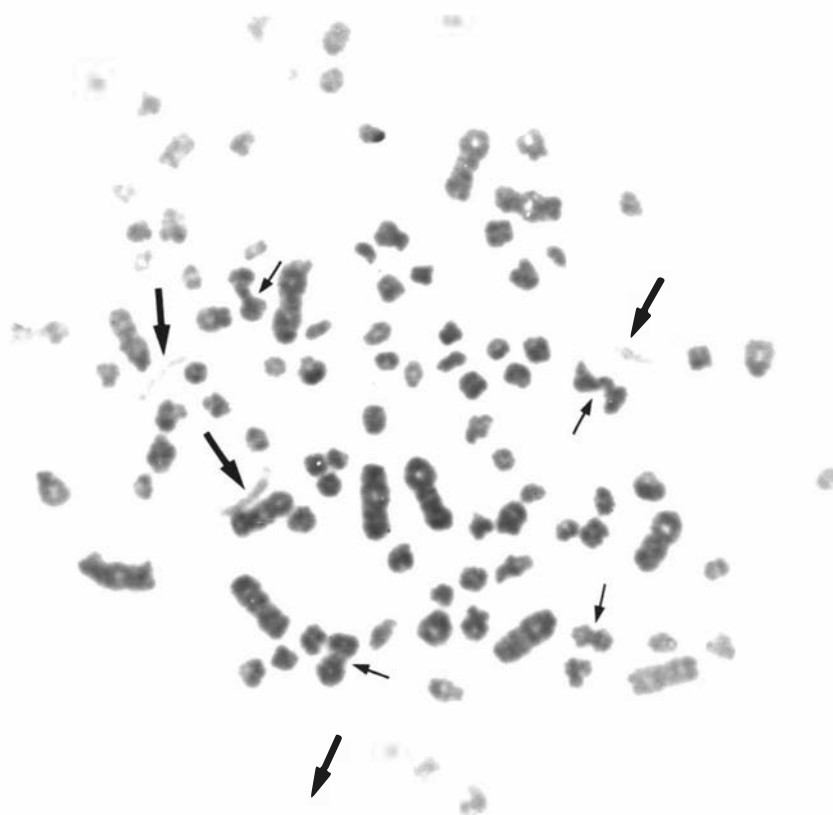
18

Plate V. 19: Metaphase I cell from B25/72 (53,xy,t₂).
There are 27 chromosomal elements including
a trivalent (III) and separated X and Y
chromosomes.
(Aceto-orcein. x 3,000).

Plate V. 20: Polyploid cell from B25/72 (53,xy,t₂).
There are 102 chromosomal elements
including four trivalents (small arrows) and
four sex bivalents (large arrows).
(Aceto-orcein. x 2,000).



19



20

c 53,xy,t₃

Table V. 12 contains the results of chromosome counts from the Massey III Robertsonian translocation heterozygous rams included in this study. Sixty four (11.9 per cent) of the 537 cells counted were polyploid. A modal number of 26 chromosomal bodies per cell was recorded in 389 of the 473 non-polyploid cells. Six cells were hypermodal and 78 cells were hypomodal. Of the 473 cells analysed, 389 had 24 autosomal bivalents, a sex bivalent and a trivalent (Plates V. 21 and V. 22); five had 24 autosomal bivalents, a trivalent and separated X and Y chromosomes (Plates V. 23 and V. 24); one had 27 chromosomal elements including 23 autosomal bivalents, a sex bivalent, a trivalent and two extra autosomal elements thought to be univalents (Plate V. 25), while the remaining cells had one or more chromosomal elements missing.

In 16 of the 473 cells analysed the sex bivalent was associated with an autosomal bivalent. In individual meiotic preparations, this type of association was seen in between 1.3 to 5.9 per cent of cells. The X and Y chromosomes were separated in five cells (1.1 per cent), with a range of from 0.4 to 2.9 per cent being found in preparations from different rams.

The results from counts of polyploid cells are given in Table V. 13. A total of 64 cells were observed and of the 53 analysed, 48 (90.6 per cent) were tetraploid, four (7.5 per cent) were hexaploid and one (1.9 per cent) was octaploid.

TABLE V.12 ROBERTSONIAN TRANSLOCATION HETEROZYGOTES : 53,xy,t₃
CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS – METAPHASE I STAGES

Ram Identi- fication		NUMBER OF CHROMOSOME BODIES/CELL						Total	Number of Polyploid Cells	Total Including Polyploid Cells	ANALYSIS OF CELLS EXCLUDING POLYPOIDS										Total Number Cells Analysed	Number of cells with xy associated with Auto- somal Bivalent	x and y Separated				
											NUMBER OF CELLS WITH:					OTHER ABERRATIONS							number	%			
		<24	24	25	26	27	>27				26,xy,III	27,x,y,III	-A 25,xy,III	-2A 24,xy,III													
Link 1966	NUMBER OF CELLS	1	0	0	5	0	0	6	1	7	5	0	0	0	18, xy, -III, -7A 1					6	0	0					
HML 99/58		2	2	3	26	1	0	34	4	38	26	1	3	2	20, xy, III, -6A 1 22, xy, III, -4A 1					34	2 (5.9%)	1	2.9				
B116/71		3	1	2	59	2	0	67	11	78	59	1	2	0	27, xy, III, +I +I? 1 24, xy, -III, -A 1 23, xy, III, -3A 1 22, xy, III, -4A 1 18, xy, III, -8A 1					67	4 (6.0%)	1	1.5				
B13/72		10	10	22	193	1	0	236	38	274	193	1	21	6	25, -xy, III, 1 21, xy, -III, -4A 1 24, xy, -III, -A 2 21, -xy, III, -4A 1 24, -xy, III, -A 2 20, xy, III, -6A 1 23, xy, III, -3A 4 19, -xy, III?, -6A 1 23, xy, -III, -2A 1 22, xy, III, -4A 1					236	3 (1.3%)	1	0.4				
B76/72		7	8	7	106	2	0	130	10	140	106	2	7	8	23, xy, III, -3A 2 18, xy, -III?, -7A 1 22, xy, III, -4A 1 21, xy, -III, -4A 1 20, xy, -III?, -6A 1 19, xy, III?, -7A 1					130	7 (5.4%)	2	1.5				
TOTAL		23	21	34	389	6	0	473	64	537	389	5	33	16	25					473	16 (3.4%)	5	1.1				

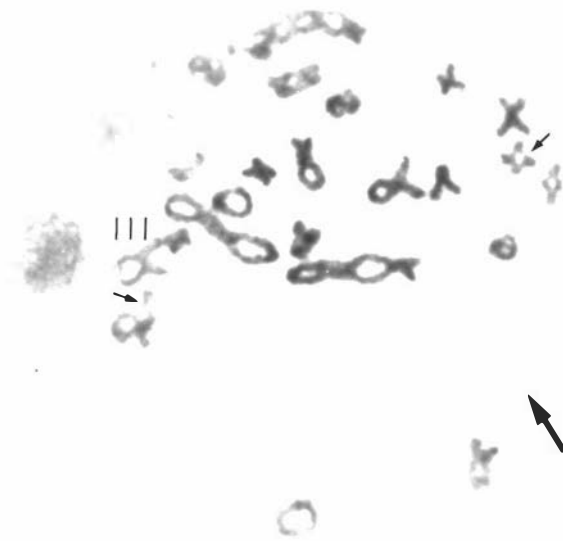
TABLE V. 13
MASSEY III ROBERTSONIAN TRANSLOCATION HETEROZYGOTES (53.xy.t₃) CHROMOSOME
COUNTS FROM POLYPLOID CELLS AT DIAKINESIS-METAPHASE I STAGES

Ram Identification	Number of Cells Showing Chromosome Complement			N.A.	Total	<u>Analysis of Polyploid Cells</u>				8n	N.A.	Total
						4n		6n				
	4n	6n	8n			52,2xy,2III	≤ 51,2xy,2III	78,3xy,3III	Other Cells			
Link 1966	1	0	0	0	1	1	0	0	0	0	0	1
HML99/58	4	0	0	0	4	4	0	0	0	0	0	4
B116/71	7	0	0	4	11	5	2	0	0	0	4	11
E13/72	26	4	1	7	38	15	11	4	0	1	7	38
B76/72	10	0	0	0	10	7	3	0	0	0	0	10
Total	48	4	1	11	64	32	16	4	0	1	11	64

N.A. = Not Analysed

Plate V. 21: Cell in diakinesis from B13/72 ($53,xy,t_3$).
There are 26 chromosomal elements including
a trivalent (III) and a sex bivalent (arrowed).
Individual chromatids are clear in some
bivalents (small arrows).
(Aceto-orcein. x 2,000).

Figure V. 4: Karyotype of cell illustrated in Plate V. 21.

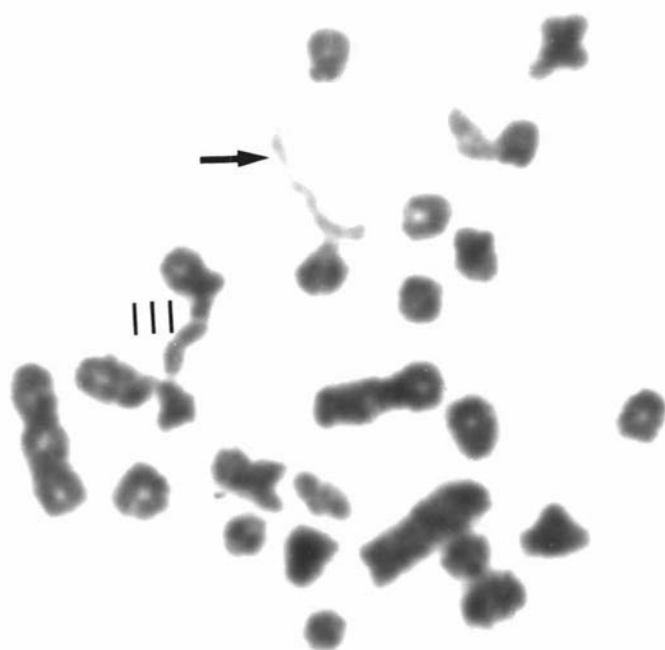


21

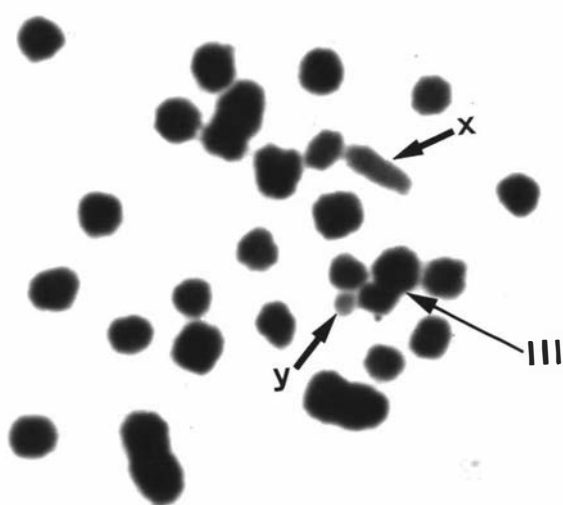


Plate V. 22: Cell in diakinesis-metaphase I from B116/71
(53,xy,t₃). There are 26 chromosomal elements
including a sex bivalent (arrow) and a trivalent
(III).
(Aceto-orcein. x 3,000).

Plate V. 23: Cell at metaphase I from B116/71 (53,xy,t₃).
There are 24 autosomal bivalents, a trivalent
(III) and separated X and Y chromosomes.
(Aceto-orcein. x 3,000).



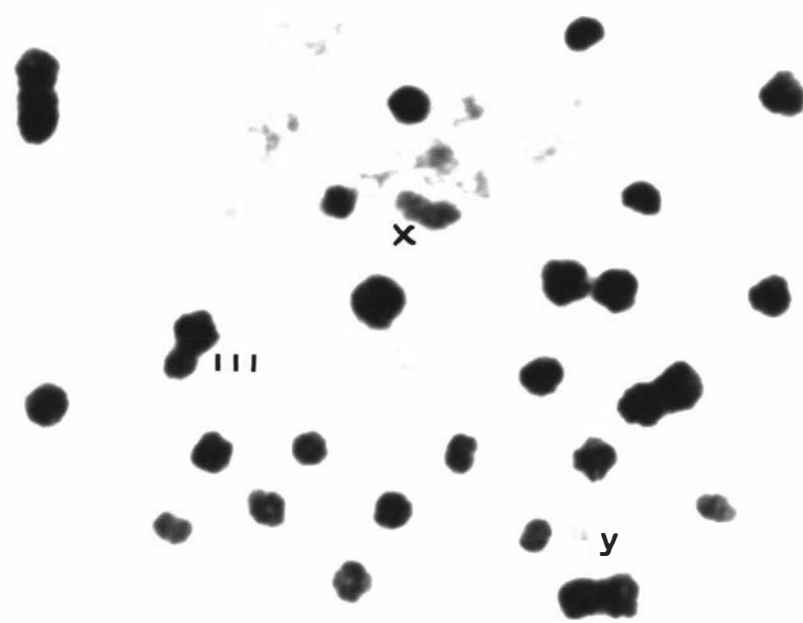
22



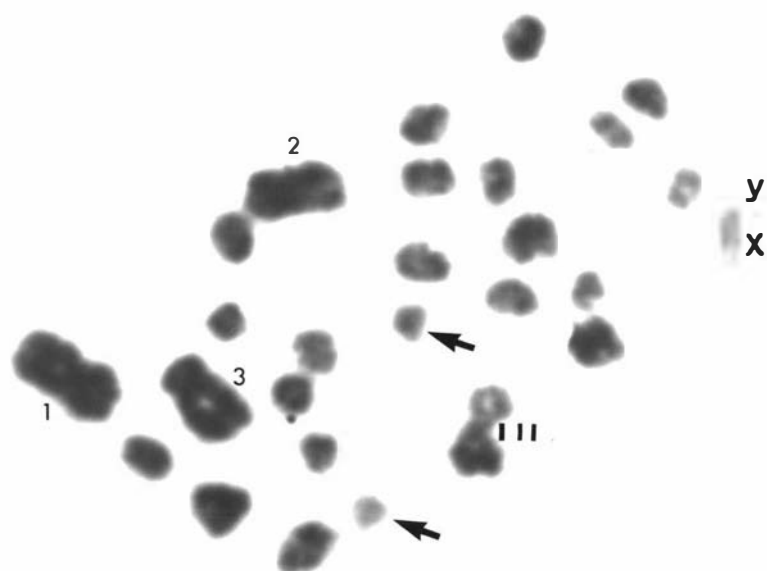
23

Plate V. 24: Cell at metaphase I from HML99/58
(53,xy,t₃). There are 24 autosomal bivalents,
a trivalent (III) and separated X and Y
chromosomes.
(Aceto-orcein. x 2,500).

Plate V. 25: Cell at metaphase I from B116/71 (53,xy,t₃).
There are 27 chromosomal elements including
three large bivalents (1,2,3), a trivalent (III)
and an XY bivalent. Two extra elements which
are possibly univalents are present (small
arrows).
(Aceto-orcein. x 3,000).



24



25

1 The trivalent

The morphological appearance of the trivalent seen in cells at the diplotene, diakinesis-metaphase I stages in each of the Robertsonian translocation heterozygote types varied from cell to cell. This variation was dependent on the type of translocation chromosome involved and on the number and location of the chiasmata in the trivalent.

Some examples of the main types of trivalent observed in cells from Massey I Robertsonian translocation heterozygotes are shown in Plate V. 26. The structure of some of these trivalents has been shown in Figure V. 5. The majority of the trivalents had three chiasmata including one interstitial and one terminal chiasma between the long arms of the translocation submetacentric chromosome and its homologue, and one interstitial or terminal chiasma between the short arms. Trivalents having greater or fewer chiasmata were rare.

Plate V. 27 shows a range of cut-out trivalents from primary spermatocyte cells in the Massey II Robertsonian translocation heterozygotes. Suggested interpretations of a few of the configurations are shown in Figure V. 6. Four chiasmata were commonly seen in these trivalents. One interstitial chiasma and one terminal chiasma were found on each side of the centromere of the metacentric translocation chromosome. Occasionally trivalents with two chiasmata (one interstitial chiasma on each side) and three chiasmata (one interstitial chiasma and two terminal chiasmata) were seen.

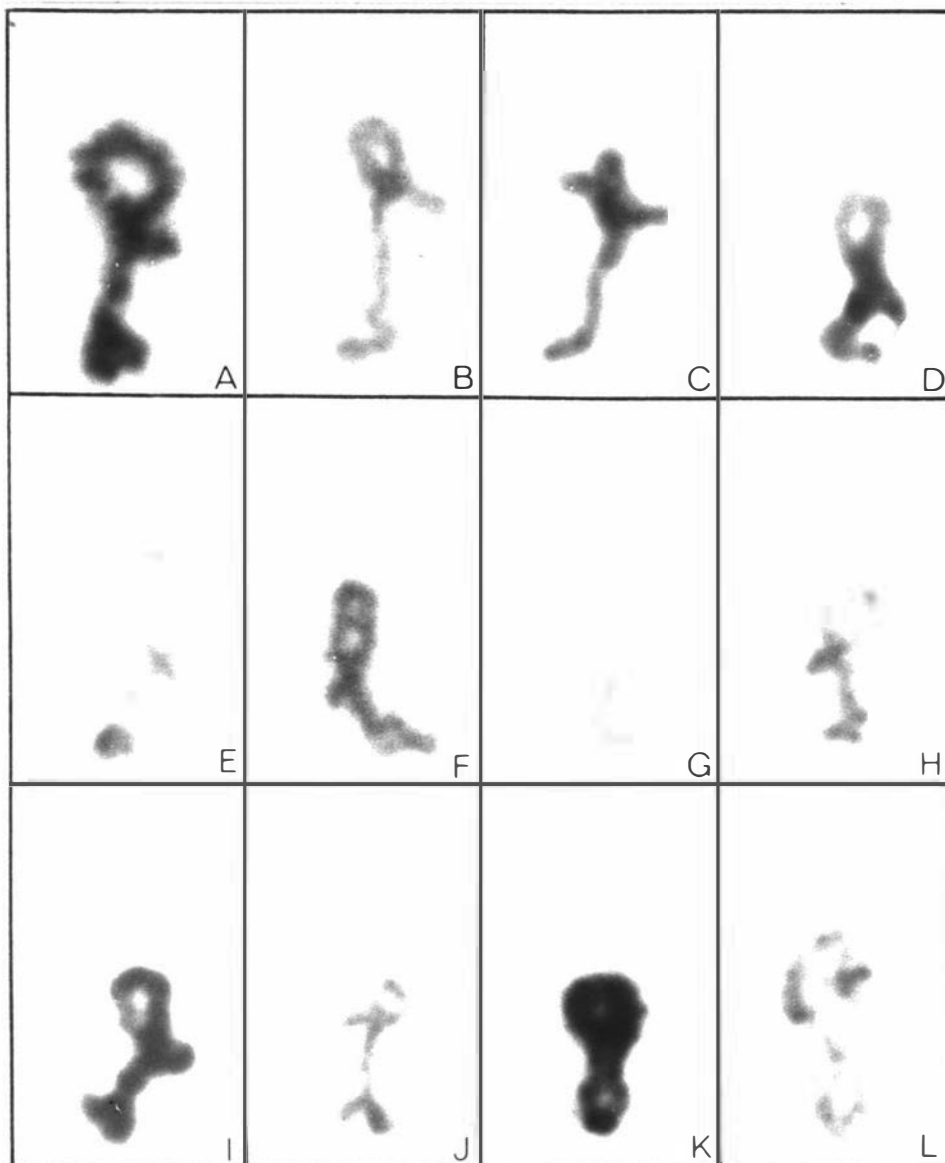
Trivalents from primary spermatocytes in the Massey III Robertsonian translocation heterozygotes like their counterparts in the Massey I Robertsonian translocation heterozygotes, commonly had three chiasmata, one interstitial and one terminal chiasma involving the long arms, and one interstitial or terminal chiasma involving the short arms of the submetacentric translocation chromosome and its acrocentric homologue. Trivalents with two chiasmata were seen but none with a greater number than three were observed. Examples of trivalent configurations and suggested interpretations of some of these are shown in Plate V. 28 and Figure V. 7. No trivalent was found with more than one chiasma on its short arms from either the Massey I or Massey III Robertsonian translocation heterozygote types.

Although trivalents with ^{more} ~~greater~~ than three chiasmata (53,xy,t₃) and four chiasmata (53,xy,t₄) and ^{five chiasmata} (53,xy,t₅) were not identified in this study, their existence cannot be excluded as it was not

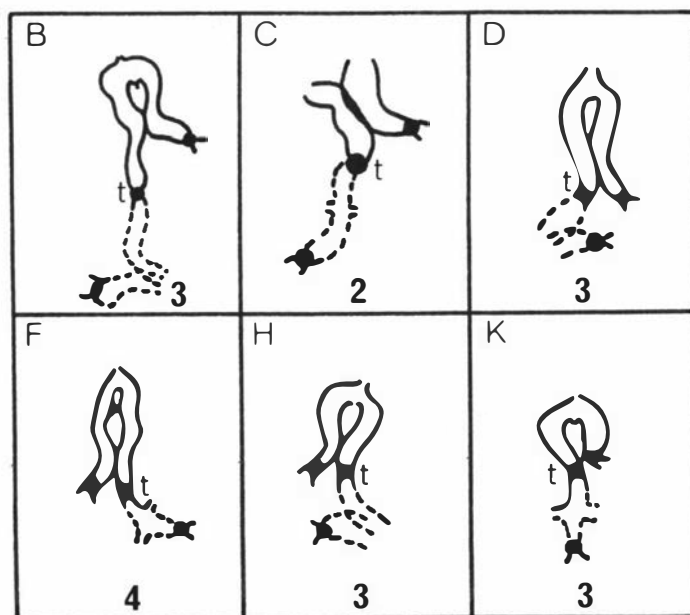
possible to show the exact configuration of many of the trivalents seen. The "C-banding" technique enabled the centromere position to be determined in a few examples of the trivalent types described. The centromeres were present as darkly stained regions (Plates V. 16, V. 26 and V. 27).

Plate V. 26A-L: Cut-out trivalents from diakinesis-metaphase I cells to demonstrate the range in morphology (53,xy,t₁).
(D, C-banded; A-C, E-L, aceto-orcein. x 5,000).

Figure V. 5: Suggested interpretations of several of the trivalents illustrated in Plate V. 26.
Numbers of chiasmata are given below each drawing.



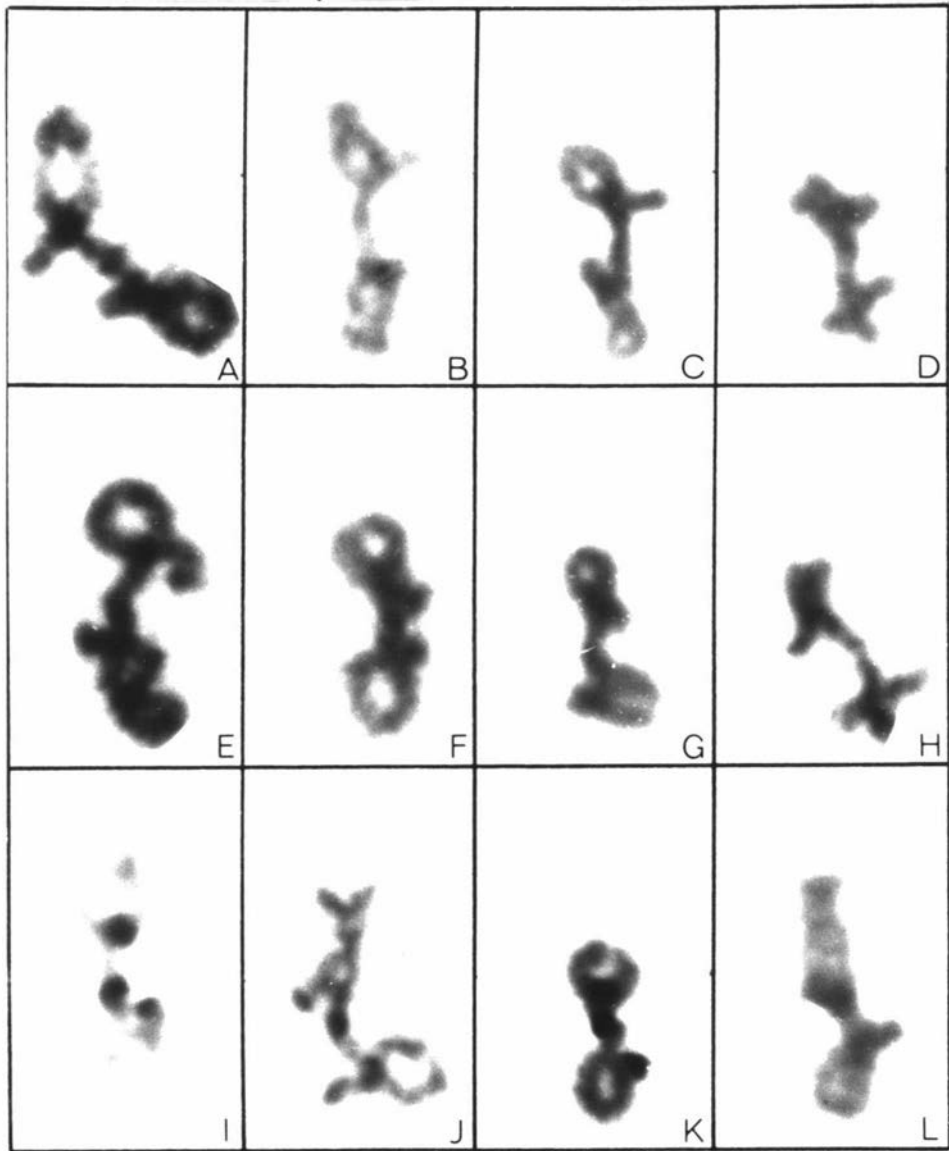
26



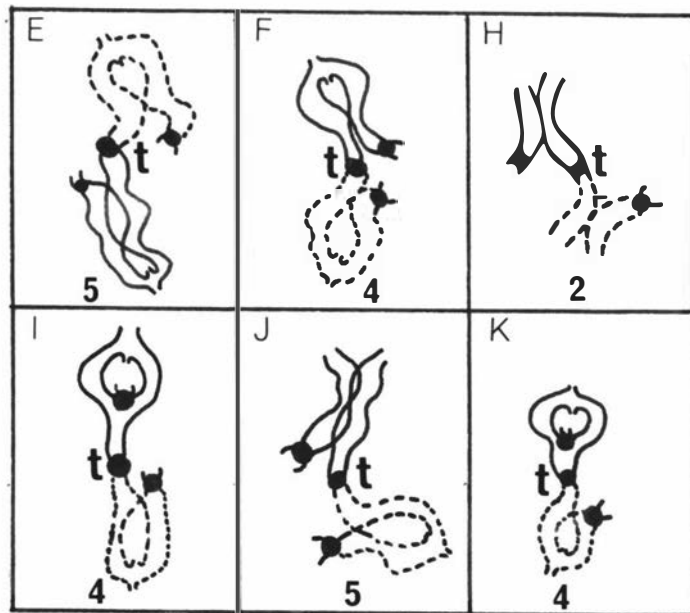
5

Plate V. 27A-L: Cut-out trivalents from diakinesis-metaphase I cells to demonstrate the range in morphology (53,xy,t₂).
(A-H, aceto-orcein; I-K, C-banded. $\times$ 5,000).

Figure V. 6: Suggested interpretations of representative trivalents from Plate V. 27. Numbers of chiasmata are given below each drawing.



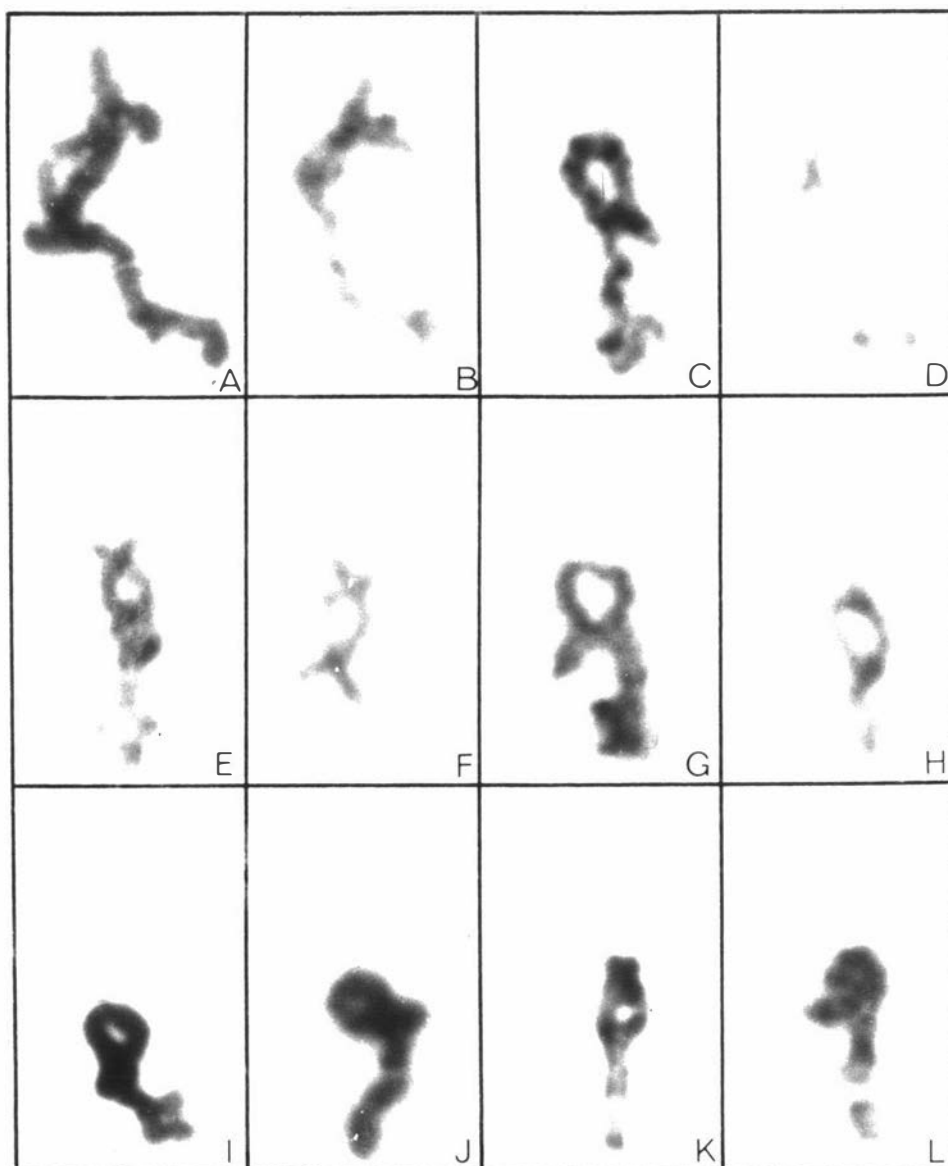
27



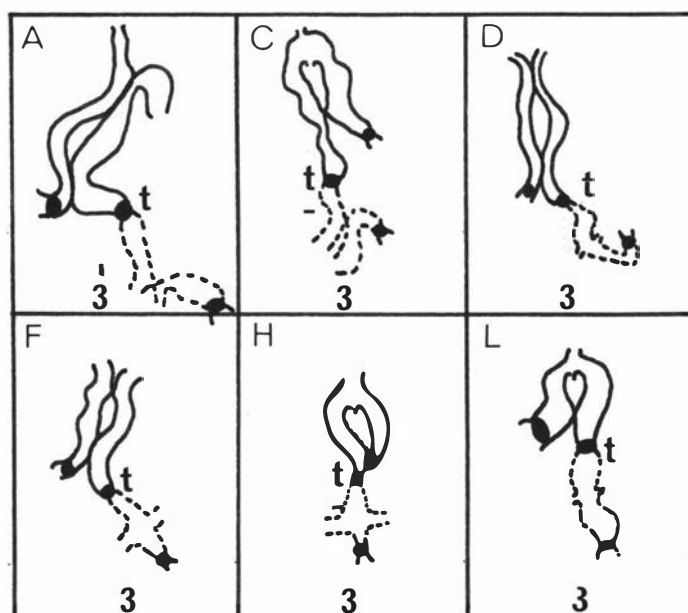
6

Plate V. 28A-L: Cut-out trivalents from diakinesis-metaphase I cells to demonstrate the range in morphology ($53, xy, t_3$). (Aceto-orcein. x 5,000).

Figure V. 7: Suggested interpretations of several of the trivalents illustrated in Plate V. 28. Numbers of chiasmata are given below each drawing.



28



e $52,xy,t_1,t_1$ and $52,xy,t_3,t_3$

Tables V. 14 and V. 15 show the results of chromosome counts from the two homozygous rams included in this study. A total of 213 cells were analysed from the Massey I Robertsonian translocation homozygote and 96 from the Massey III Robertsonian translocation homozygote. These totals included 19 (8.9 per cent) polyploid cells in the Massey I and 12 (12.5 per cent) polyploid cells in the Massey III Robertsonian translocation homozygous ram. In both homozygotes a modal number of 26 chromosomal bodies per cell was observed. Five cells from ram B55/70 were hypernodal.

Close examination of diakinesis-metaphase I cells showed that although a similar number of chromosomal figures to that counted in Robertsonian translocation heterozygous rams is observed in these cells, there are some differences in the number and types of element observed. Four large bivalents, 21 small bivalents and a sex bivalent are usually seen. Three of the large bivalents are the paired metacentric chromosomes as seen in heterozygotes, while the fourth is composed of the paired translocation chromosomes and is really a quadrivalent made up of four acrocentric chromosomes. This cell composition was recorded in 150 cells from B55/70 ($52,xy,t_1,t_1$) and 76 cells from A134 ($52,xy,t_3,t_3$). Examples of diakinesis cells from these rams are shown in Plates V. 29 and V. 30 and Figures V. 8 and V. 9.

The sex chromosomes were separated in three of the five cells in which counts of 27 chromosomal elements were recorded. An example of this is shown in Plate V. 31. It was thought that two univalents were present in two cells with counts of 27 chromosomal elements as a sex bivalent was observed in both cells.

Hypodiploid chromosome counts were due to chromosome loss.

The apparent polyploid cells analysed were all shown to be tetraploid.

TABLE V. 14

ROBERTSONIAN TRANSLOCATION HOMOZYGOTES

CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

Ram Identification	Number of Chromosome Bodies/Cell					Total	Number of Polyploid Cells	Total Including Polyploid Cells	23,x,y,II	26,xy,II	25,xy,II-A	424,±xy,±II,-xA	Total Number Cells Analysed	Number Cells With xy Associated With Autosomal Bivalent	Number of Cells With x and y Separated
	23	24	25	26	27										
Drysdale Massey III Homozygote A134	1	0	7	76	0	84	12	96	0	76	7	1	84	3	0
Romney Massey I Homozygote B55/70	19	6	14	150	5	194	19	213	3	150	14	25 +(2 27,xy,II, +I+I?)	194	7	3
Total	20	6	21	226	5	278	31	309	3	226	21	28	278	10	3

TABLE V. 15

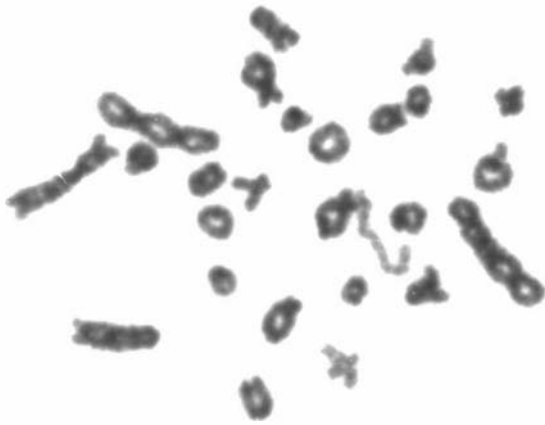
ROBERTSONIAN TRANSLOCATION HOMOZYGOTESCHROMOSOME COUNTS FROM POLYPLOID PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

	Number of Cells		Total Number Polyploid Cells	Analysis of Polyploid Cells	
	4n	N.A.		52,2xy,2II	← 52,2xy,2II
Drysdale Massey III Homozygote (A134)	7	5	12	6	1
Romney Massey I Homozygote (B55/70)	16	3	19	11	5
Total	23	8	31	17	6

N.A. = Not Analysed

Plate V. 29: Cell in diakinesis from B55/70 (52,xy,t₁,t₁).
There are 26 chromosomal elements including
four large autosomal bivalents, 21 small
autosomal bivalents and a sex bivalent.
(Aceto-orcein. x 2,000).

Figure V. 8: Karyotype of cell illustrated in Plate V. 29.
The fourth largest bivalent is formed by the
paired translocation chromosomes.



29

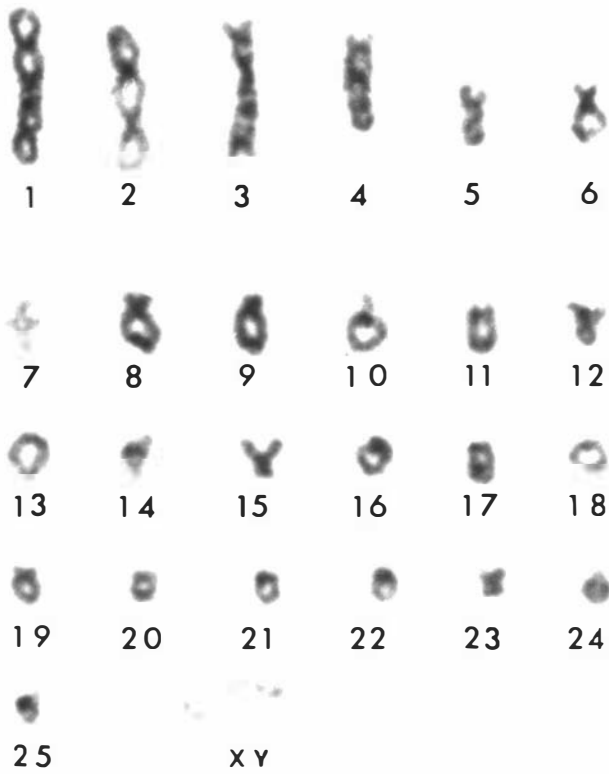
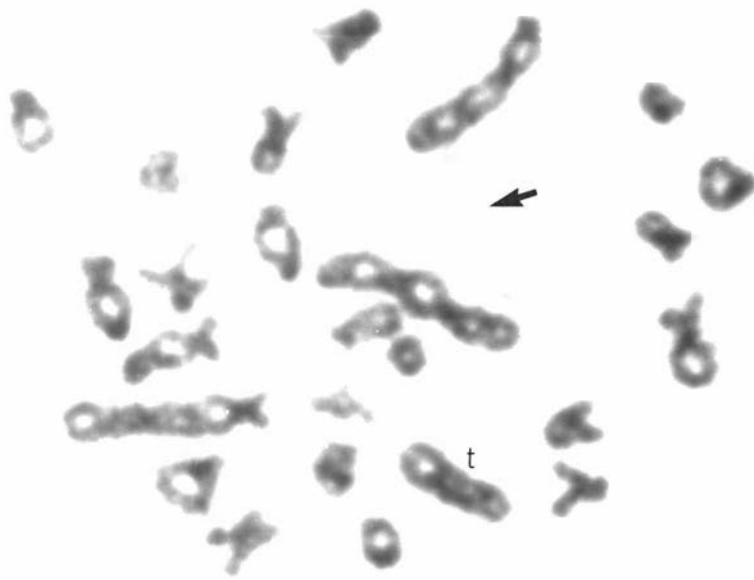


Plate V. 30: Cell in diakinesis from A134 (52,xy,t₃,t₃).
There are 26 bivalents including three large
autosomal bivalents, a translocation bivalent
(t), 21 small autosomal bivalents and a sex
bivalent (arrowed).
(Aceto-orcein. x 3,000).

Figure V. 9: Karyotype of cell illustrated in Plate V. 30.
The translocation bivalent is the fourth
largest autosomal bivalent.



30

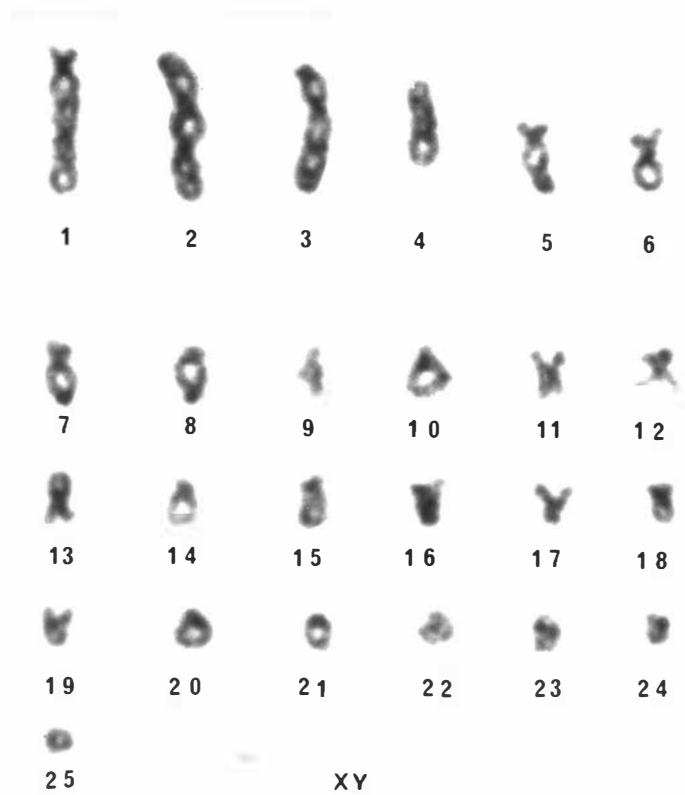
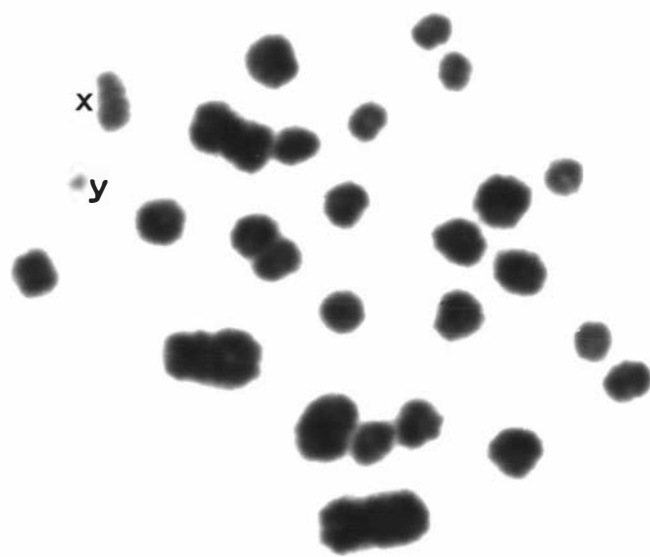


Plate V. 31: Cell in metaphase I from B55/70 (52,xy,t₁,t₁).
There are 27 chromosomal elements including
separated X and Y chromosomes.
(Aceto-orcein. x 3,000).



(C) Discussion

The percentage values for hypomodal, modal and hypermodal cells from the translocation types included in this study are given in Table V. 16.

TABLE V. 16
PERCENTAGES OF HYPOMODAL, MODAL AND HYPERMODAL CELLS AT
DIAKINESIS-METAPHASE I IN ROBERTSONIAN TRANSLOCATION-
CARRYING RAMS

	% Hypomodal	% Modal	% Hypermodal
53,xy,t ₁	16.4	82.7	0.9
53,xy,t ₂	13.2	85.2	1.5
53,xy,t ₃	16.5	82.2	1.3
52,xy,t ₁ ,t ₁	20.1	77.3	2.6
52,xy,t ₃ ,t ₃	9.5	90.5	0.0
[54,xy	16.2	81.8	1.9]

The values recorded in the translocation-carrying rams are similar to and show the same trends as those for normal rams. There are few hypermodal cells but a greater proportion of hypomodal cells. Most hypermodal counts have been shown due to either separation of the sex chromosomes or separation of the chromosomes in autosomal bivalents. No cases of separation of the chromosomes involved in the trivalent were recorded in this study.

Associations between sex bivalents and small autosomal bivalents were observed in 4.7 per cent, 4.4 per cent and 3.4 per cent respectively of the cells in the Massey I, II and III Robertsonian translocation heterozygotes studied. A similar value of 3.6 per cent was recorded for each of the Robertsonian translocation homozygous rams studied. The levels found in the Robertsonian translocation-carrying rams were comparable with those recorded for the normal rams examined.

The sex chromosomes were separated in from 0.5 per cent to 1.5 per cent of the diakinesis-metaphase I cells studied in Robertsonian translocation-carrying rams. These levels were within the range reported in the normal rams.

Various numbers of cells with separated sex chromosomes have been reported in human D/G translocation carriers. Mikkelsen (1966) and Hultén and Lindsten (1970) reported low numbers of cells with this anomaly in D/G translocation carriers compared with the numbers seen in normal men. Hamerton, Cowie, Giannelli, Briggs and Polani (1961) reported a higher level but even this was still within the range reported for normal men. Hultén and Lindsten (1970) have suggested therefore that the translocation chromosome does not exert an "interchromosomal effect". Gustavsson (1959) reported separation of the sex chromosomes in 28 per cent of the cells from one heterozygous carrier of a $1/29$ translocation but did not comment on this phenomenon. This value is less than the value recorded in normal cattle by Popescu (1971,a). The causes of non-association of the sex chromosomes have been discussed in Chapter IV, section 4 (vi). Random segregation of sex chromosome univalents at the first meiotic division could be one of the causes of sex chromosome aneuploidy. Failure of the association between the X and Y chromosomes in the majority of cells at the diakinesis and metaphase I stages has been reported in conjunction with sterility in mice (Beechey, 1973) and man (McIlree et al., 1966,b) and it has been suggested that the association between the sex chromosomes in some species may be necessary for normal spermiogenesis (Beechey, 1973).

Trivalent formation at the diakinesis-metaphase I stage is characteristic for heterozygous carriers of Robertsonian translocations involving non-homologous chromosomes. This was first reported in a ram heterozygous for the Massey I Robertsonian translocation by Bruère (1969) and has been reported in many other species (Kjessler, 1964; Evans, Lyon and Daglish, 1967; White and Tjio, 1967; Gustavsson, 1969; Hultén and Lindsten, 1970; Gropp, Tettenborn and Lehmann, 1970,a; Tettenborn and Gropp, 1970; Chandley et al., 1972). Bivalent formation is characteristic of homozygous carriers of a Robertsonian translocation involving non-homologous chromosomes. This confirms the findings of White and Tjio (1967) and Gustavsson (1969).

Several of the trivalent configurations observed in cells from

the Massey I and Massey III Robertsonian translocation heterozygotes were similar to those described in the human D/G trivalent. A similarity also existed between the configurations reported in human D/D Robertsonian translocations and those seen in the primary spermatocytes from Massey II Robertsonian translocation heterozygotes, although there was a greater variation in trivalent types in the human cells.

There was no evidence of disparity in the size of the blocks of centromeric heterochromatin in the separate homologues when compared with that on the translocation chromosome in the trivalent. Evidence from mitotic metaphases has shown that the Massey I and III Robertsonian translocation chromosomes have a larger block of centromeric heterochromatin than is found on either of the acrocentric homologues, whereas the Massey II Robertsonian translocation chromosome does not show this feature (Bruère et al., 1974; Appendix XX). Chandley and Fletcher (1973) have commented on the size difference between the blocks of centromeric heterochromatin on the fused D chromosomes of a trivalent compared with those on their separate homologues. Further work needs to be done with the "C-banding" technique in translocation-carrying rams in order to see whether or not these features are also characteristic of the Massey I and III trivalents.

(iii) ANALYSIS OF SECONDARY SPERMATOCYTES

Metaphase figures in secondary spermatocytes from Robertsonian translocation-carrying rams were analysed in a manner similar to that already described in Chapter III section 5 (i) and Chapter IV section 3 (ii) (C).

(A) Statistical Analysis

Where relevant, secondary spermatocyte count results were analysed using the standard method for the chi-squared (χ^2) test with the correction for continuity where required (Snedecor and Cochran, 1967). Counts from neighbouring classes were combined if necessary to ensure that the smallest expected number was at least one (Cochran, 1954).

In the comparison of heterozygotes, homozygotes and normal groups, orthogonal comparisons after the manner employed with the analysis of variance were used (Grant, 1968).

The null hypothesis was rejected when probabilities of less than 0.05 ($p < .05$) were obtained.

(B) Theoretical Considerations

a Rams heterozygous for a Robertsonian translocation

At the diplotene, diakinesis and metaphase I stages of meiosis in rams heterozygous for the Massey I, II and III Robertsonian translocations, 26 chromosomal figures are usually seen. This number included:-

3 large bivalents	(12 chromosome arms),
1 trivalent	(4 " "),
21 acrocentric (small) bivalents	(42 " "), and
1 sex bivalent	(2 " ").

The chromosome complement of the secondary spermatocytes, and in particular the second metaphase stages analysed by the author, is affected by the orientation of the chromosomes forming the trivalent at

first metaphase and their subsequent segregation at anaphase I.

Trivalents having three centromeres can orientate in various ways depending in part, on how the three chromosomes are associated. If more than one trivalent is present, the trivalents orientate independently (Lewis and John, 1963). Assuming that regular disjunction of the bivalents occurs at both first and second meiotic divisions, the chromosomal complement of the gamete will depend on the pattern of disjunction followed by the trivalent.

Theoretically there are four possible types of segregation and eight possible segregation products if the chance that fission of the translocation chromosome ever occurs is ignored. The range of chromosome type and number as well as the number of chromosome arms produced by each type of segregation are summarized in Table V. 17.

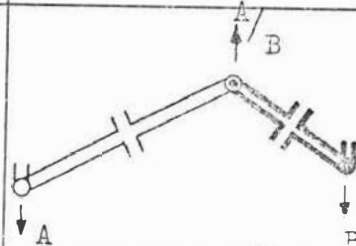
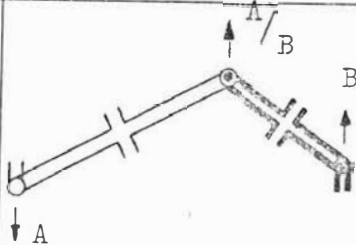
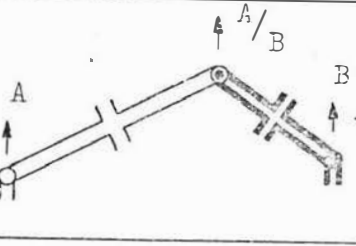
Alternate segregation of the chromosomes of the trivalent leads to the formation of chromosomally balanced metaphase II cells, and provided no disturbances of the meiotic process occur subsequently, to balanced gametes: one a balanced translocation carrier $\frac{26,t,x}{30}$ or $\frac{26,t,y}{30}$, the other a normal $\frac{27,x}{30}$ or $\frac{27,y}{30}$ gamete.

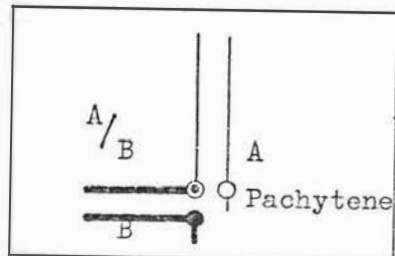
Partial non-disjunction in which the translocation chromosome together with an acrocentric homologue pass to the same pole produces gametes carrying either a duplication ($n+1$) or deficiency ($n-1$) of chromosome material. Theoretically four different types of gamete are possible depending on which of the acrocentric homologues segregates with the translocation chromosome. These segregation types are sometimes referred to in the literature as adjacent non-homologous (non-homologous centromeres pass to the same pole) and adjacent homologous (homologous centromeres pass to the same pole) (Hamerton, 1966).

Total non-disjunction occurs when the translocation chromosome and its two acrocentric homologues pass to the same pole producing gametes with duplication ($n+2$) and deficiency ($n-2$) of chromosomes.

TABLE V. 17

ROBERTSONIAN TRANSLOCATION HETEROZYGOTE : TYPES OF SEGREGATION AT MI AND PRODUCTS FROM TRIVALENT

Type of Segregation	Segregation	Products	Meta.	Acro.	t	X or Y	Total *
Alternate		A/B + $A + B$	$3/6$	$21/21$	$1/2$	$1/1$	$26/30$
		$A/B + B$ + A	$3/6$	$23/23$	0	$1/1$	$53/60$ $27/30$
Partial non-disjunction		$A/B + B$ + A	$3/6$	$22/22$	$1/2$	$1/1$	$27/31$ $53/60$
		$A/B + A$ + B	$3/6$	$22/22$	0	$1/1$	$26/29$
		$A/B + A$ + B	"	"	"	"	$53/60$
Total non-disjunction		$A + A/B + B$	$3/6$	$23/23$	$1/2$	$1/1$	$28/32$ $53/60$
		$A/B + B$	$3/6$	$21/21$	0	$1/1$	$25/28$



Key * $\frac{\text{total number chromosomes}}{\text{total number chromosome arms}}$

Meta = metacentric chromosomes

Acro = acrocentric "

t = translocation chromosome

. b Rams homozygous for a Robertsonian translocation

In rams homozygous for a Robertsonian translocation, at metaphase I 26 chromosomal figures made up of 52 chromosomes having 60 chromosome arms are observed in the majority of cells. This number comprises:

3 large bivalents	(12 chromosome arms),
1 translocation bivalent	(4 chromosome arms),
21 small bivalents	(42 chromosome arms), and
1 sex bivalent	(2 chromosome arms).

The theoretical segregation possibilities at the first meiotic division for the Robertsonian translocation homozygote are summarized in Table V. 18.

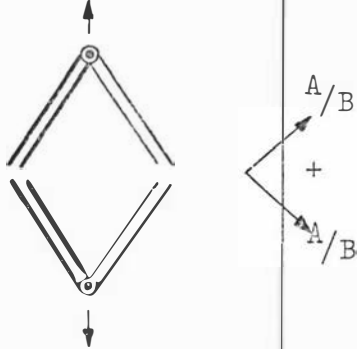
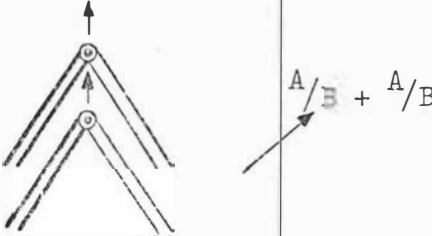
These possibilities assume that malsegregation of chromosomes other than those involved in the translocation chromosomes does not occur.

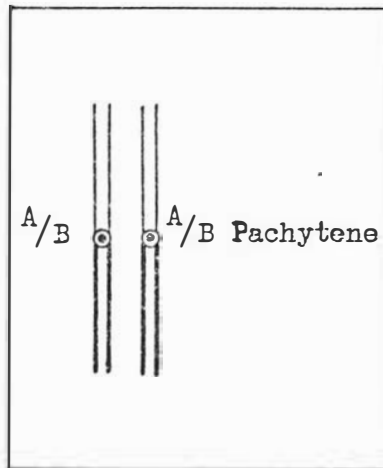
(C) Results

A total of 1,146 secondary spermatocytes from the three different translocation types were recorded. Of these, 1055 including 116 polyploid cells were analysed. A further 91 polyploid cells were recorded but it was not possible to make accurate counts from them. They were recorded in the tables under the heading N.A. (not analysed). Total metaphase II counts from each of the rams from which testicular material was utilized as well as the number of cells karyotyped from representatives of the translocation types, are summarized in Table V. 19.

TABLE V. 18

ROBERTSONIAN TRANSLOCATION HOMOZYGOTE : TYPES OF SEGREGATION AT MI OF TRANSLOCATION BIVALENT

Type of Segregation	Segregation	Products	Meta.	Acro.	t	X or Y	Total*
Normal		$A/B + A/B$	$3/6$	$21/21$	$1/2$	$1/1$	$26/30$
			$3/6$	$21/21$	$1/2$	$1/1$	$26/30$
Total non-disjunction		$A/B + A/B$	$3/6$	$21/21$	$2/4$	$1/1$	$27/32$
			$3/6$	$21/21$	-	$1/1$	$25/28$



* $\frac{\text{total number of chromosomes}}{\text{total number of chromosome arms}}$

Meta = metacentric chromosomes

Acro = acrocentric chromosomes

t = translocation chromosome

TABLE V. 19

COUNTS OF MII CELLS AND NUMBER OF MII CELLS KARYOTYPED
FROM ROBERTSONIAN TRANSLOCATION-CARRYING RAMS

	Ram Ident- ification	MII Cells	Polyploid MII Cells	Total Including Polyploids	Number of Karyotypes
	B46/69	0	0	0	0
	B68/69	7	2	9	2
	B12/69	6	1	7	2
	B53/69	53	12	70	8
	236/67(B15)	9	2	11	8
$2n = 53, xy, t_1$	B26/70	0	0	0	0
	A182	59	22	81	33
	B8/72	21	2	23	2
	B71/69	0	0	0	0
	Total	160	41	201	55
$2n = 53, xy, t_2$	B25/72	61	13	74	3
	B87/72	151	15	166	4
	B68/72	65	18	83	3
	B66/72	54	7	61	10
	Total	331	53	384	20
$2n = 53, xy, t_3$	Link 1966	4	0	4	0
	HML99/58	10	10	20	11
	B116/71	7	10	17	10
	B13/72	206	64	270	29
	B76/72	99	13	112	0
$52, xy, t_1, t_2$	Total	326	97	423	50
	B55/70	99	11	110	12
$52, xy, t_3, t_3$	A134	23	5	28	1
	Total	939	207	1146	138

a Massey I Robertsonian translocation
heterozygotes

Counts of chromosomes and chromosome arms were made in a total of 160 cells at the metaphase II stage in rams ($2n = 53, xy, t_1$). In addition 41 polyploid metaphase II cells were recorded and 17 of these analysed. Counts were arranged where possible under the headings suggested from the theoretical segregation possibilities for the translocation chromosome. Cells with counts which did not fit into the categories alternate segregation, partial non-disjunction or (total) non-disjunction, were listed under the general heading "other cell counts". A detailed analysis of $53, xy, t_1$ cell counts is recorded in Tables V. 20 and V.21.

A modal number of 30 chromosome arms was found for each ram. Hypermodal cells were recorded for four of the six rams and hypomodal cells in all rams.

Diploid cells were recorded (Table V. 21) but no cells with higher ploidy were observed. The percentage of polyploid cells varied from 8.7 to 27.2 per cent (Table V. 22).

Only four of the 13 polyploid metaphase II cells analysed had a euploid chromosome arm complement (60).

TABLE V.20

DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN ROMNEY RAMS ($2n = 53,xy,t_1$)

Ram Identification		ALTERNATE SEGREGATION					PARTIAL NON-DISJUNCTION					NON-DISJUNCTION				OTHER CELL COUNTS																Total M II Cells/ Ram
		<u>27,x</u>	<u>27,y</u>	<u>26,t,x</u>	<u>26,t,y</u>	Total	<u>26,x</u>	<u>26,y</u>	<u>27,t,x</u>	<u>27,t,y</u>	Total	<u>25,x</u>	<u>25,y</u>	<u>28,t,x or y</u>	Total	<u>21,t,y</u>	<u>22,y</u>	<u>22,y</u>	<u>23</u>	<u>24</u>	<u>24,t</u>	<u>25,t</u>	<u>25,t,y</u>	<u>25,t,x</u>	<u>26,t,y</u>	<u>26,y</u>	<u>28,y</u>	<u>28,y</u>	Total			
		30	30	30	30		29	29	31	31		28	28	32		24	25	26	26	26	28	29	29	29	29	30	30	31				
B68/69	NUMBER OF CELLS	3	0	1	1	5	1	1	0	0	2				0														0	7		
B12/69		1	1	0	0	2	1	0	0	2	3				0			1											1	6		
B53/69		5	14	8	8	35	5	6	1	0	12	2			2	1			1	1		1	2	1			1	1	9	58		
236/67 (B15)		1	2	3	1	7	0	1	1	0	2				0														0	9		
A182		11	11	5	12	39	4	4	3	1	12	1	1		2		1				1		1		1	1	1		6	59		
B8/72		5	4	1	6	16	1	2	0	0	3	1			1									1					1	21		
TOTAL		26	32	18	28	104	12	14	5	3	34	4	1	0	5	1	1	1	1	1	1	1	3	2	1	1	2	1	17	160		

TABLE V. 21

DISTRIBUTION OF CHROMOSOME COUNTS IN POLYPLOID MII CELLS IN ROMNEY RAMS ($2n = 53.xy.t$)

Ram Identification	<u>38</u> , tty 42	<u>46</u> , txy 51	<u>46</u> , txy 53	<u>51</u> , txy 55	<u>49</u> , txy 56	<u>50</u> , txy 56	<u>52</u> , xy 57	<u>51</u> , txy 58	<u>52</u> , xy 58	<u>52</u> , txy 59	<u>53</u> , xy 59	<u>52</u> , ttyy 60	<u>53</u> , txy 60	<u>54</u> , xy 60	N.A.	Total
B68/69	Number of Cells								1						1	2
B12/69										1					0	1
B53/69		1										1			10	12
236/67 (B15)											1		1		0	2
A182				1	1	1	1	1			2			1	13	22
B8/72			1											1	0	2
Total		1	1	1	1	1	1	1	1	1	3	1	1	2	24	41

N.A. = Not Analysed

TABLE V. 22PERCENTAGE OF POLYPLOID MII CELLS

Ram Ident- ification	MI I cells	Polyploid	Total	% Polyploid MI I cells
B68/69	7	2	9	22.2
B12/69	6	1	7	14.3
B53/69	58	12	70	17.1
236/67	9	2	11	18.2
A182	59	22	81	27.2
B8/72	21	2	23	8.7

Table V. 23 shows the distribution of euploid and aneuploid metaphase II figures according to the number of chromosome arms per cell.

In the six rams studied, the frequency of euploid cells exceeded that of aneuploid cells and ranged between 33.3 per cent in B12/69 and 77.8 per cent in B15. However in both these animals only a few cells were analysed. If rams from which fewer than 20 metaphase II cells were analysed are excluded from the results, there is still a variation between individuals with regard to the percentage frequencies of the various chromosome arm counts. The modal number of 30 is found in between 62.1 and 76.2 per cent of cells and an asymmetrical variation from the euploid value is observed with an excess of hypomodal cells.

The relative percentage arm counts in the three rams B53/69, A182 and B8/72 from which over 20 cells were analysed are represented in Figure V. 10.

Two estimates of aneuploid spermatocyte frequency were made. Both were based on the method used by Cattanach and Moseley (1973). The first estimate gave the total proportion of cells with either 29 or 31 chromosome arms and the second was derived by doubling the frequency of cells with a chromosome arm count of 31. Cattanach and Moseley (1973) consider that the former frequency may be an overestimate of the frequency of aneuploid spermatozoa while the latter may be an underestimate. The frequencies calculated for the three rams from which at least 20 metaphase II cells were analysed were as follows:

	29+31 (%)	31x2 (%)
B53/69	29.31	6.90
A182	23.73	13.56
B8/72	19.05	0.00

TABLE V. 23

DISTRIBUTION OF EUPLOID (N.F./2 = 30) AND ANEUPLOID (N.F./2 < or > 30) MII FIGURES IN ROMNEY
RAMS (2n = 53,xy,t₁)

Ram Ident- ification	(N.F./2)													Total
	< 28		28		29		30		31		> 31			
	N	%	N	%	N	%	N	%	N	%	N	%		
B68/69	Number of Cells	0	0	0	0	2	28.57	5	71.43	0	0	0	0	7
B12/69		1	16.67	0	0	1	16.67	2	33.33	2	33.33	0	0	6
B53/69		3	5.17	2	3.45	15	25.86	36	62.07	2	3.45	0	0	58
236/67 (B15)		0	0	0	0	1	11.11	7	77.78	1	11.11	0	0	9
A182		1	1.69	3	5.08	10	16.95	41	69.49	4	6.78	0	0	59
B8/72		0	0	1	4.76	4	19.05	16	76.19	0	0	0	0	21
Total		5	3.13	6	3.75	33	20.63	107	66.88	9	5.63	0	0	160

N = number of cells

% = percentage of cells

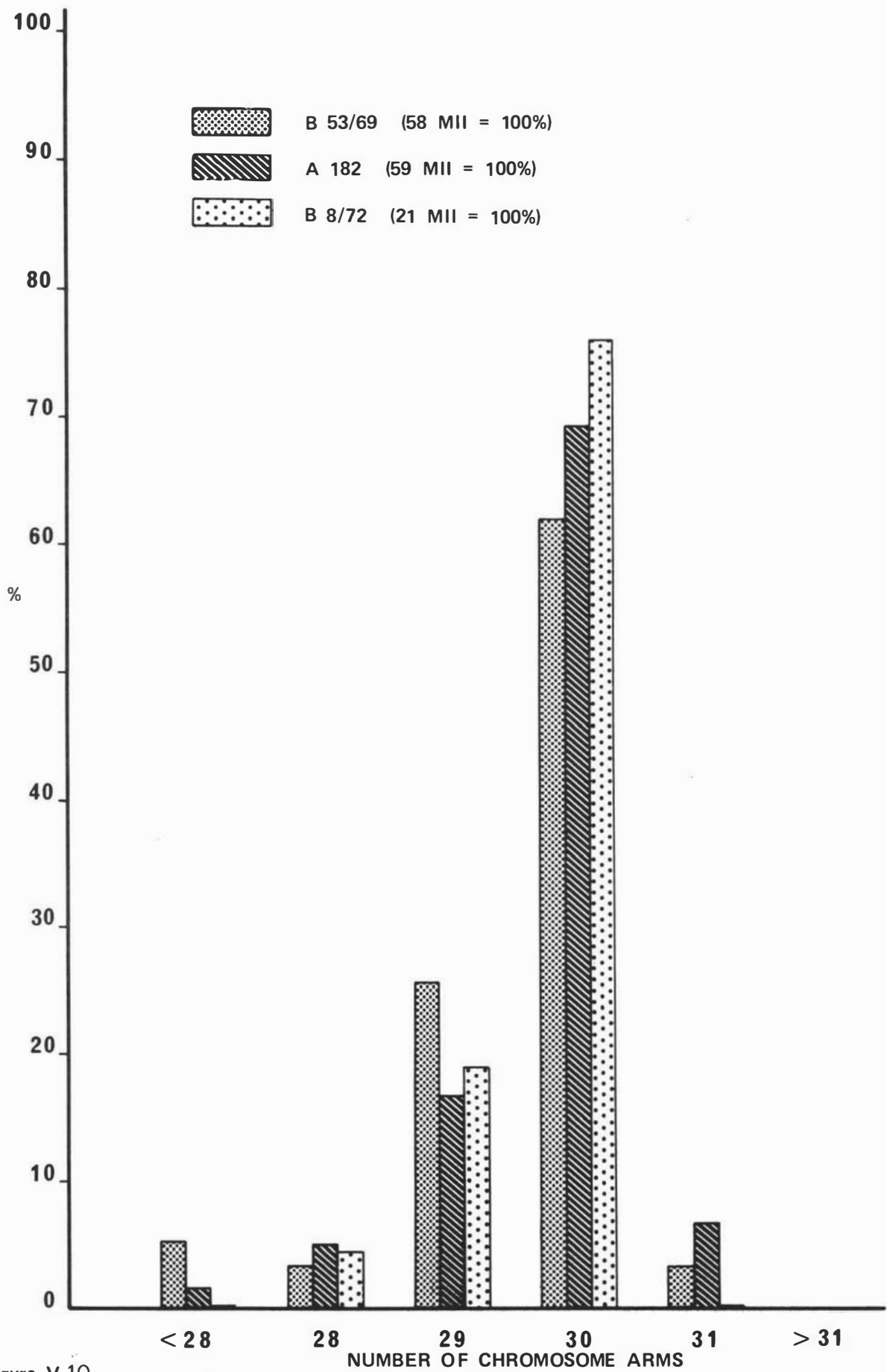


Figure V.10

Frequency histogram showing the distribution of euploid ($N.F./2 = 30$) and aneuploid ($N.F./2 < \text{or} > 30$) MII figures in three Romney ($53,xy,t_1$) rams.

The frequency of aneuploidy varied between rams and in each case the estimation of aneuploid frequency based on the hypermodal cell counts was considerably smaller than the estimate based on the $29+31$ chromosome arm counts.

A chi-squared analysis showed that there were no significant differences between rams ($53,xy,t_1$) with regard to the distribution of euploid and aneuploid cells when these were considered as groups.

Approximately equal numbers of balanced translocation-carrying and normal cells were produced (Table V. 20) (χ^2_1 not significant), and of these translocation-carrying cells, approximately 50 per cent carried a Y chromosome and 50 per cent an X chromosome (χ^2_1 not significant) (Table V. 24).

There was no statistically significant departure from the 1 : 1 : 1 : 1 ratio expected for each of the four possible cell classes produced by alternate segregation.

When cells from all classes (euploid plus aneuploid) were combined, a departure from the expected 1 : 1 : 1 : 1 ratio of translocation-carrying male : translocation-carrying female : male (Y) : female (X) cells was observed ($\chi^2_3 = 10.52$ $p < .05$) with fewer translocation-carrying female (X) cells and more non-translocation-carrying male (Y) cells than expected (Table V. 25).

However, there was no significant departure from the expected 1 : 1 ratio of X and Y carrying cells when data was pooled (Table V. 26).

TABLE V. 24

ASSOCIATION OF TRANSLOCATION CHROMOSOME WITH SEX CHROMOSOMES
AT MII IN ROMNEY RAMS ($2n = 53, xy, t_1$)

Ram Identification		(N.F./2 = 30) Euploid				(N.F./2 < or > 30) Aneuploid			
		+t,y	+t,x	-t,y	-t,x	+t,y	+t,x	-t,y	-t,x
B68/69	Number of Cells	1	1	0	3	0	0	1	1
B12/69		0	0	1	1	2	0	1	1
B53/69		8	8	15	5	3	2	7	7
236/67 (B15)		1	3	2	1	0	1	1	0
A182		12	5	13	11	3	3	6	5
B8/72		6	1	4	5	0	1	2	2
Total		28	18	35	26	8	7	18	16

TABLE V. 25

ASSOCIATION OF TRANSLOCATION CHROMOSOME WITH SEX CHROMOSOMES
AT MII IN ROMNEY RAMS ($2n = 53, xy, t_1$) EUPLOID AND ANEUPLOID
CELLS GROUPED

Ram Identification		+t,y	+t,x	-t,y	-t,x
B68/69	Number of Cells	1	1	1	4
B12/69		2	0	2	2
B53/69		11	10	22	12
236/67 (B15)		1	4	3	1
A182		15	8	19	16
B8/72		6	2	6	7
Total		36	25	53	42

TABLE V. 26

DISTRIBUTION OF SEX CHROMOSOMES IN
MI I CELLS

	(N.F./2)						
	< 28	28	29	30	31	> 31	Total
X	0	4	14	44	5	0	67
Y	3	1	18	63	4	0	89

The results showed that there was no preferential segregation of the translocation chromosome with either of the sex chromosomes in the euploid gamete classes but that when results from euploid and aneuploid cells were pooled, non-translocation X and Y bearing cells predominated.

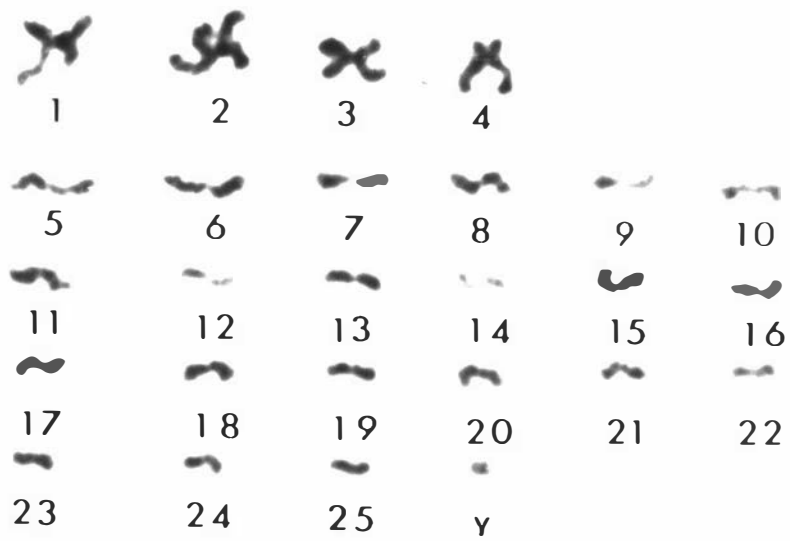
A selection of metaphase II cells including balanced translocation-carrying cells, normal cells and some aneuploid cells are shown in Plates V. 32 to V. 39 and Figures V. 13 to V. 16.

Plate V. 32: Metaphase II cell from A182 (53,xy,t₁).
There are 26 chromosomes including a
translocation submetacentric (t) and a
Y chromosome (arrowed).
(Aceto-orcein. x 4,200).

Figure V. 13: Karyotype of cell illustrated in Plate
V. 32. There are 30 chromosome arms.
The translocation chromosome is the fourth
largest chromosome.



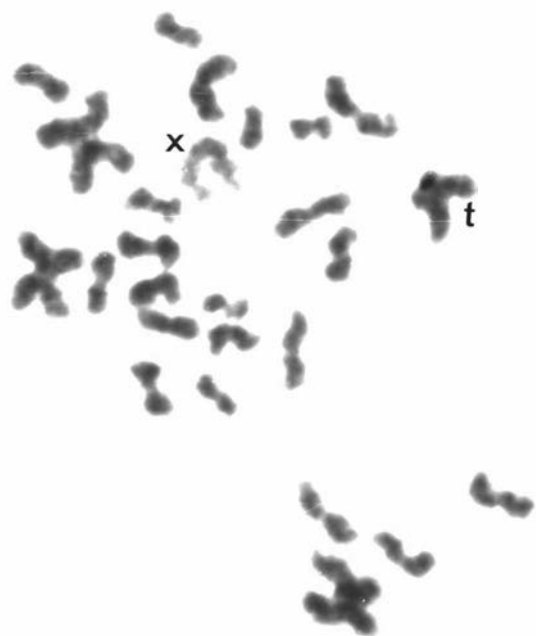
32



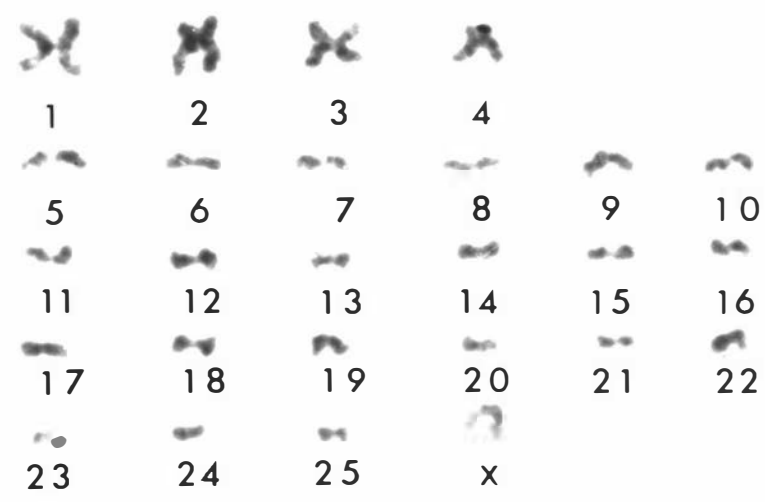
13

Plate V. 33: Metaphase II cell from 236/67 (53,xy,t₁). This is a balanced translocation-carrying cell with 26 chromosomes and 30 chromosome arms. The X chromosome and the translocation chromosome are identified.
(Aceto-orcein, x 4,200).

Figure V. 14: Karyotype of cell illustrated in Plate V. 33.



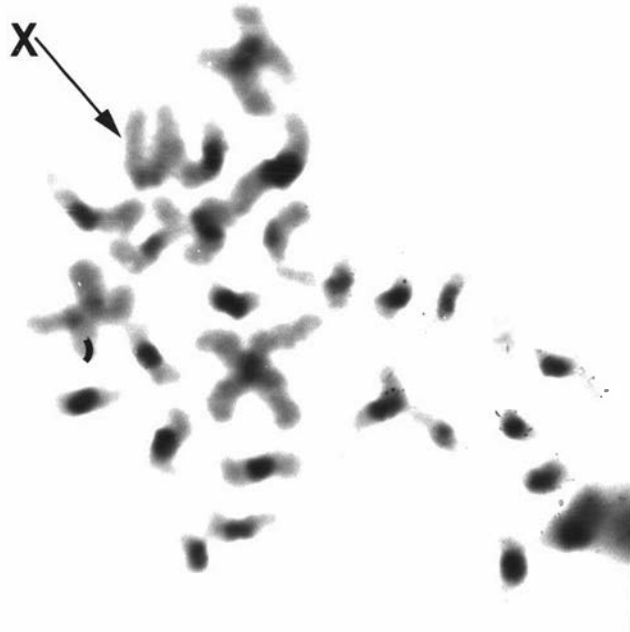
33



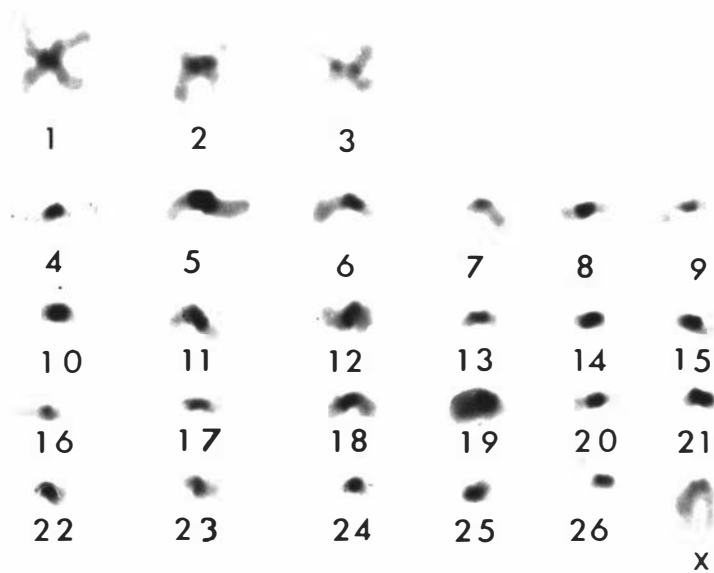
14

Plate V. 34: Metaphase II cell from B8/72 (53,xy,t₁).
There are 27 chromosomes including an X
chromosome, and 30 chromosome arms.
(C-banded. x 4,000).

Figure V. 15: Karyotype of cell illustrated in Plate V. 34.



34



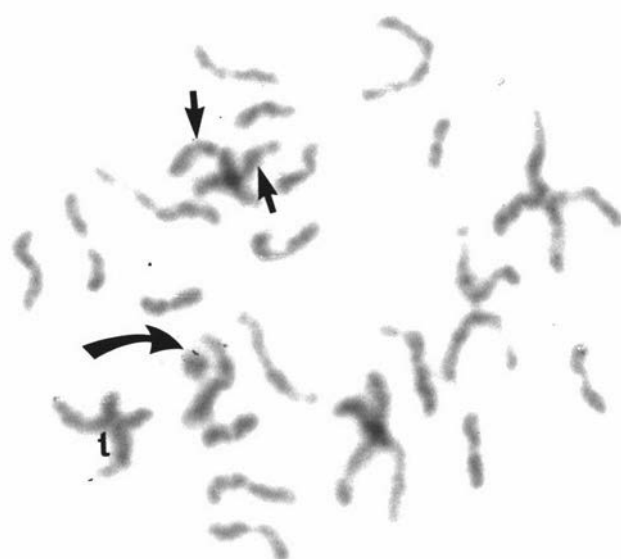
15

Plate V. 35: Metaphase II cell from A182 (53,xy,t₁).
There are 30 chromosome arms and 26
chromosomes including a translocation
submetacentric chromosome (t) and a Y
chromosome (arrowed). Part of one arm of
a metacentric chromosome is obscured.
(Aceto-orcein. x 4,500).

Plate V. 36: Aneuploid metaphase II cell from A182 (53,xy,t₁).
There are 29 chromosome arms and 25 chromosomes
including three metacentric chromosomes, a
submetacentric translocation chromosome (t) and
a Y chromosome (arrowed).
Two acrocentric chromosomes are overlapped (small
arrows).
(Aceto-orcein. x 6,000).



35



36

Plate V. 37: Aneuploid metaphase II cell from A182 (53,xy,t₁).
There are 29 chromosome arms and 26 chromosomes including a Y chromosome (arrowed).
(Aceto-orcein. x 2,500).

Plate V. 38: Aneuploid metaphase II cell from B8/72 (53,xy,t₁).
There are 28 chromosome arms and 25 chromosomes including an X chromosome (arrowed). The chromosome arms of two of the small acrocentric chromosomes are in apposition (small arrows).
(C-banded. x 4,000).



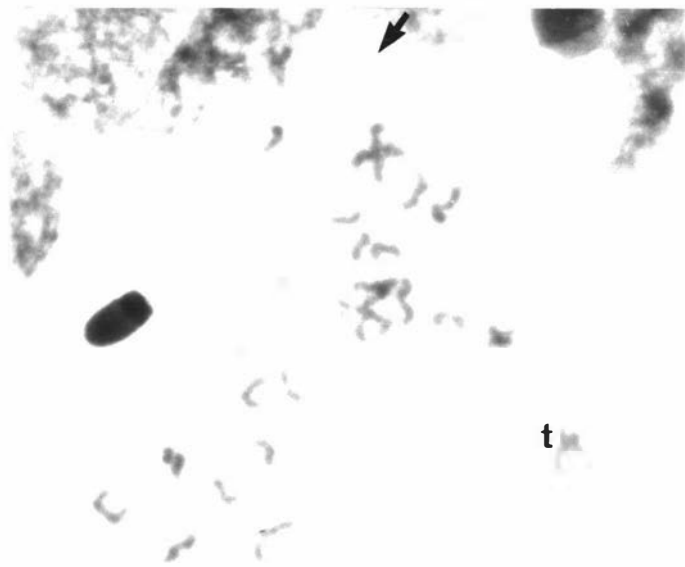
37



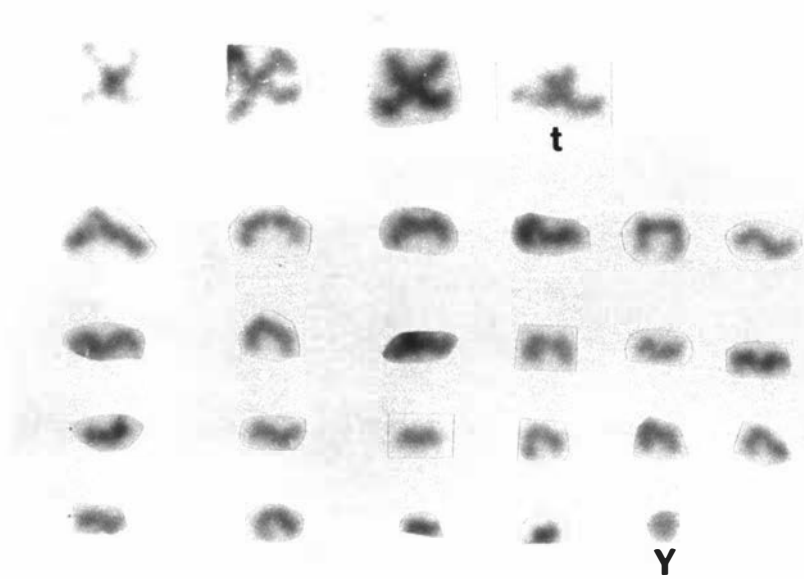
38

Plate V. 39: Metaphase II cell from B12/69 (53,xy,t₁). There are 31 chromosome arms and 27 chromosomes including three metacentric chromosomes, a submetacentric translocation chromosome (t) and a Y chromosome (arrowed).
(Aceto-orcein. x 1,750).

Figure V. 16: Karyotype of cell illustrated in Plate V. 39. The translocation chromosome and Y chromosome are identified.



39



16

b Massey II Robertsonian translocation heterozygotes

A total of 384 metaphase II figures were recorded in these rams (53,xy,t₂). This total included 53 polyploid cells. The majority of these cells (359 metaphase II figures including 28 polyploids) were analysed in the manner previously described for the 53,xy,t₁ rams. Tables V. 27 and V. 28 show a detailed analysis of the cells studied. As was observed in 53,xy,t₁ animals, a modal number of 30 chromosome arms was observed for all rams studied. No hypermodal cells were observed in B66/72 but some were recorded in the other three rams the proportion varying from 4.6 per cent to 6.6 per cent of the total metaphase II cells analysed. Hypomodal cells were recorded from all rams and these showed a wider distribution than that of the hypermodal cells. Three rams B25/72, B87/72 and B68/72 had a higher proportion of cell counts showing 28 chromosome arms than was observed in B66/72 or in any of the 53,xy,t₁ rams.

When the distribution of euploid and aneuploid cells was compared over the four rams a significant ($\chi^2_3 = 8.94$ $p < .05$) difference between rams was shown. Most of this difference could be attributed to one ram B66/72 from which more euploid cells than expected were counted.

No metaphase II cells with chromosome counts exceeding the diploid number were observed, (Table V. 28) a similar finding to that in the 53,xy,t₁ rams. Two diploid cells (both from the same ram) carrying a YY sex chromosome complement were seen (Plate V. 40). The percentage of polyploid cells varied from 9.0 to 21.7 (Table V. 29). Euploid chromosome arm counts were seen in 11 of the 28 polyploid cells analysed.

TABLE V. 28

DISTRIBUTION OF CHROMOSOME COUNTS IN POLYPLOID MII CELLS IN ROMNEY RAMS ($2n = 53, xy, t_2$)

Ram Identification	$\frac{47}{52}, x, y$	$\frac{43}{53}, x, y$	$\frac{52}{57}, x, y$	$\frac{52}{58}, x, y$	$\frac{53}{58}, x, y$	$\frac{53}{59}, x, y$	$\frac{54}{59}, x, y$	$\frac{54}{60}, x, y$	$\frac{54}{60}, y, y$	$\frac{43}{50}, t, x, y$	$\frac{48}{55}, t, x, y$	$\frac{50}{57}, t, x, y$	$\frac{51}{58}, t, x, y$	$\frac{52}{59}, t, y, y$	$\frac{52}{59}, t, x, y$	$\frac{53}{59}, t, x, y$	$\frac{53}{60}, t, x, y$	N.A.	Total
B25/69								2		1					1			9	13
B37/72	1			1		1										1	1	10	15
B62/72		1		1	1	2	1	4	1		1	1		1			1	3	18
B66/72			1					2					1					3	7
Total	1	1	1	2	1	3	1	8	1	1	1	1	1	1	1	1	2	25	53

N.A. = Not Analysed

Plate V. 40: Diploid metaphase II cell with two Y
chromosomes (arrowed) from B68/72
(53,xy,t₂).
(Aceto-orcein. x 3,000).

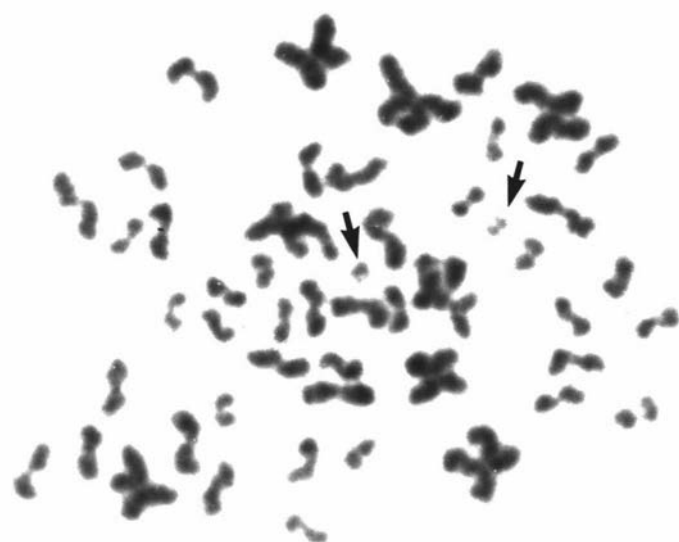


TABLE V. 29

PERCENTAGE OF POLYPLOID MII CELLS

Ram Identification	MI I cells	Polyploid MII cells	Total MII cells	% Polyploid MII cells
B25/72	61	13	74	17.6
B87/72	151	15	166	9.0
B68/72	65	18	83	21.7
B66/72	54	7	61	11.5

The distribution of euploid and aneuploid figures is shown in Table V. 30. In three of the four rams studied, the metaphase II euploid frequency exceeded 50 per cent while in one ram the frequency was less than this figure (47.7 per cent). Thus there are between animal variations of individual cell counts from the various classes of chromosome arm number. This has been commented on in the Massey I Robertsonian translocation heterozygous rams.

The modal chromosome arm number of 30 was found in between 47.7 per cent and 70.4 per cent of the metaphase II cells studied. An excess of hypomodal cells was recorded. The percentage distribution of metaphase II cell counts according to their number of chromosome arms is further illustrated in Figure V. 11.

Aneuploid frequencies based on Cattanaach and Moseley's (1973) method are given in Table V. 31. Again there is between ram variation and one animal, B66/72, has a value of zero when the aneuploidy estimate is based solely on a doubling of the value for the number of cells with 31 chromosome arms.

TABLE V. 30

DISTRIBUTION OF EUPLOID ($N.F./2 = 30$) AND ANEUPLOID ($N.F./2 < \text{or} > 30$) MIL FIGURES IN
ROMNEY RAMS ($2n = 53, xy, t_2$)

Ram Identification		N. F./2												Total
		< 28		28		29		30		31		> 31		
		N	%	N	%	N	%	N	%	N	%	N	%	
B25/72	Number of cells	2	3.28	8	13.11	11	18.03	36	59.02	4	6.56	0	0	61
B87/72		7	4.64	24	15.89	40	26.49	72	47.68	8	5.30	0	0	151
B68/72		1	1.54	8	12.31	19	29.23	34	52.31	3	4.62	0	0	65
B66/72		0	0	2	3.70	14	25.93	38	70.37	0	0	0	0	54
Total		10	3.02	42	12.69	84	25.38	180	54.38	15	4.53	0	0	331

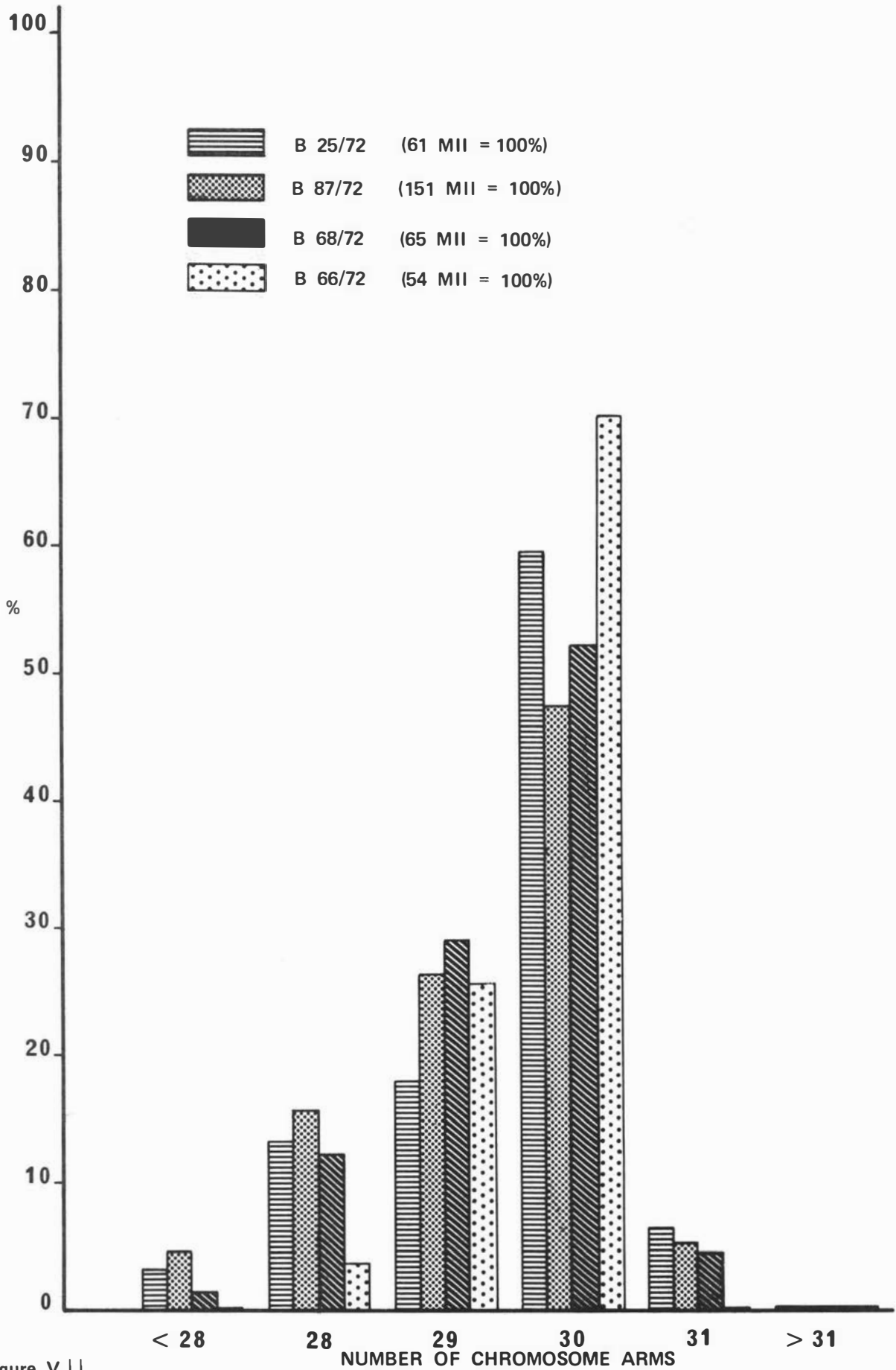


Figure V.11

Frequency histogram showing the distribution of euploid ($N.F./2 = 30$) and aneuploid ($N.F./2 < \text{or} > 30$) MII figures in four ($53,xy,t_2$) Romney rams.

TABLE V. 31

ANEUPLOID FREQUENCIES

	29+31 (%)	31x2 (%)
B25/72	24.59	13.11
B87/72	31.79	10.60
B68/72	33.85	9.23
B66/72	25.93	0.0

Fewer balanced translocation-carrying metaphase II cells than expected for a 1 : 1 ratio with normal cells were recorded ($\chi^2_1 = 6.19$ $p < .05$) but approximately equal numbers of Y-bearing and X-bearing balanced translocation-carrying cells were recorded. There was no statistically significant departure from the expected sex ratios when the aneuploid cells were considered as a group nor when both euploid and aneuploid cells were grouped (Tables V. 32 and V. 33).

When data from euploid cells was analysed there was a departure ($\chi^2_3 = 9.05$ $p < .05$) from the expected 1 : 1 : 1 : 1 ratio of $\frac{26,t,x}{30}$, $\frac{27,y}{30}$, $\frac{26,t,y}{30}$, $\frac{26,x}{30}$ cells produced as a result of alternate segregation of the trivalent. Fewer $\frac{26,t,x}{30}$ cells were recorded than expected.

When euploid and aneuploid data were pooled, there was a significant departure ($\chi^2_3 = 70.39$ $p < .001$) from the expected equal numbers of translocation-carrying X and Y cells and non-translocation-carrying X and Y cells with an excess of non-translocation-carrying cells being found, most of them in the aneuploid group.

Some examples of metaphase II cells from the Massey II Robertsonian translocation carrier rams are shown in Plates V. 40 to V. 49 and in Figures V. 17 to V. 20.

TABLE V. 32

ASSOCIATION OF TRANSLOCATION CHROMOSOME WITH SEX
CHROMOSOMES AT MII IN ROMNEY RAMS ($2n = 53, xy, t_2$)

Ram Identification		N.F./2 = 30				N.F./2 < or > 30			
		+t,y	+t,x	-t,y	-t,x	+t,y	+t,x	-t,y	-t,x
B25/72	Number of cells	6	3	13	14	2	4	10	12
B87/72		19	16	18	19	8	4	39	27
B68/72		10	3	11	10	1	1	13	13
B66/72		7	7	15	9	0	0	10	7
Total		42	29	57	52	11	9	72	59

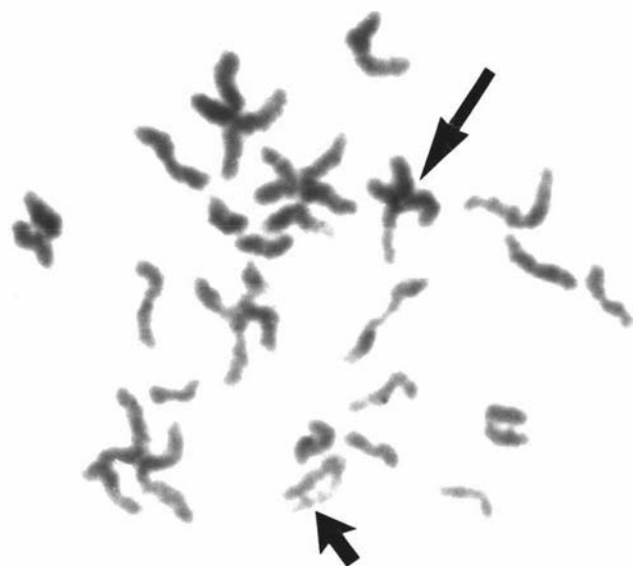
TABLE V. 33

ASSOCIATION OF TRANSLOCATION CHROMOSOME WITH SEX
CHROMOSOMES AT MII IN ROMNEY RAMS ($2n = 53, xy, t_2$)
EUPLOID AND ANEUPLOID CELLS GROUPED

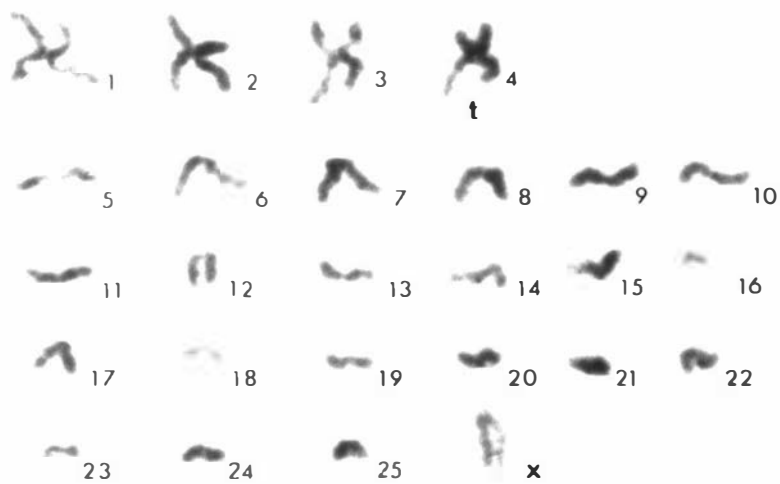
Ram Identification		+t,y	+t,x	-t,y	-t,x
B25/72	Number of cells	8	7	23	26
B87/72		27	20	57	46
B68/72		11	4	24	23
B66/72		7	7	25	16
Total		53	38	129	111

Plate V. 41: Metaphase II cell from B66/72 (53,xy,t₂). There are 30 chromosome arms and 26 chromosomes including a translocation metacentric chromosome (large arrow) and an X chromosome (small arrow). (Aceto-orcein x 4,200).

Figure V. 17: Karyotype of cell illustrated in Plate V. 41.



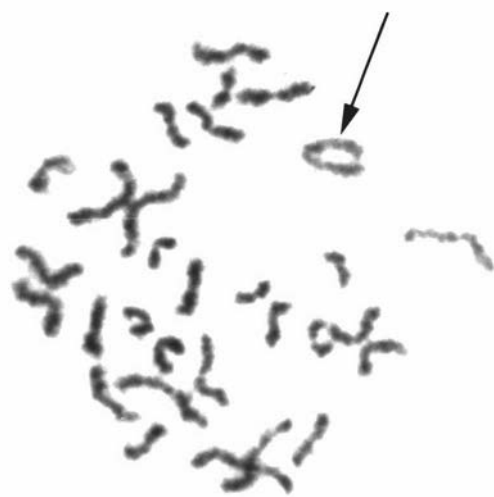
41



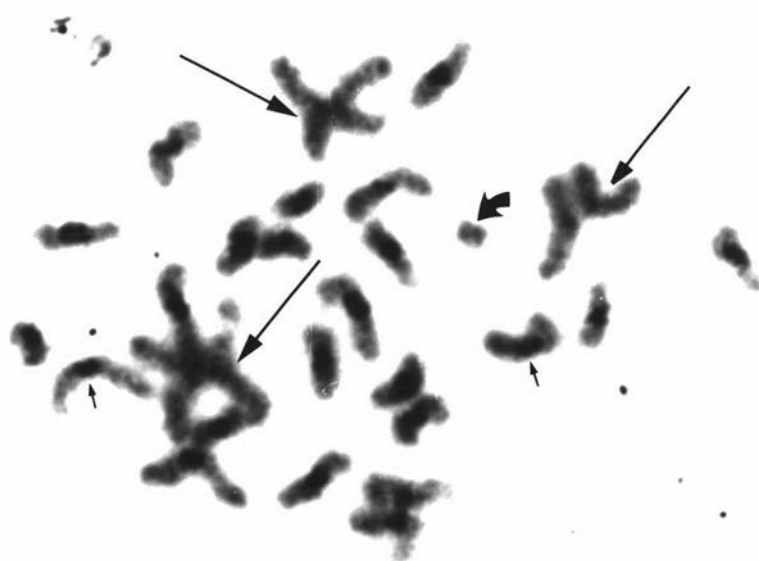
17

Plate V. 42: Metaphase II cell from B66/72 (53,xy,t₂).
There are 30 chromosome arms. The X chromosome is arrowed. Separation of the chromatids of the metacentric chromosomes makes it difficult to determine whether or not a translocation metacentric chromosome is present.
(Aceto-orcein. x 2,500).

Plate V. 43: Metaphase II cell from B66/72 (53,xy,t₂).
There are 27 chromosomes including three metacentric chromosomes and a Y chromosome. The centromeric heterochromatin stains faintly on the three metacentrics (large arrows). Dual blocks of heterochromatin can be seen on some acrocentric chromosomes (small arrows). There is no differential staining on the Y chromosome (curved arrow).
(C-banded. x 4,200).



42



43

Plate V. 44: Metaphase II cell from B66/72 (53,xy,t₂).

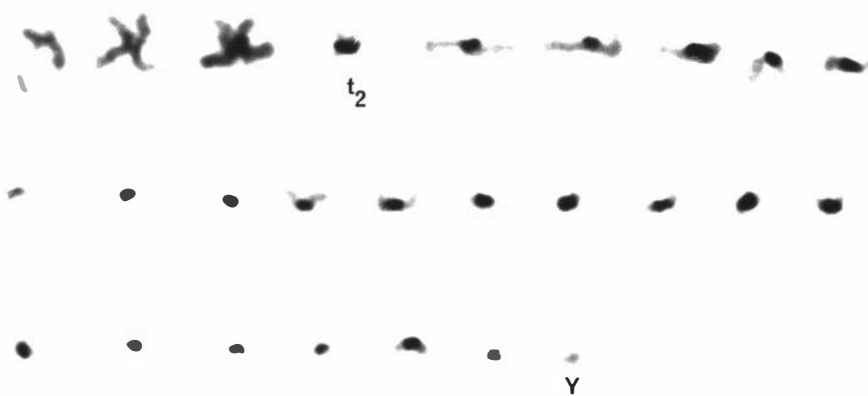
There are 30 chromosome arms and 26 chromosomes including a metacentric translocation chromosome (t₂) and a Y chromosome (arrowed).
(C-banded. x 4,000).

Figure V. 18: Karyotype of cell illustrated in Plate V. 44.

There is a large block of centromeric heterochromatin on the translocation metacentric chromosome. The Y chromosome is uniformly stained.



44



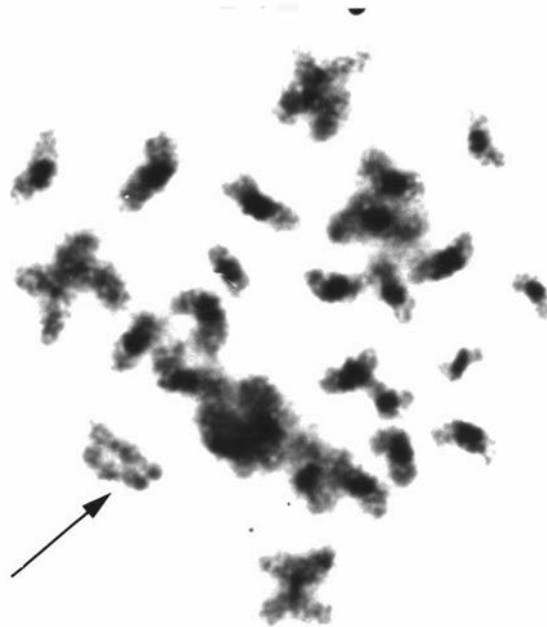
18

Plate V. 45: Metaphase II cell from B66/72 (53,xy,t₂).

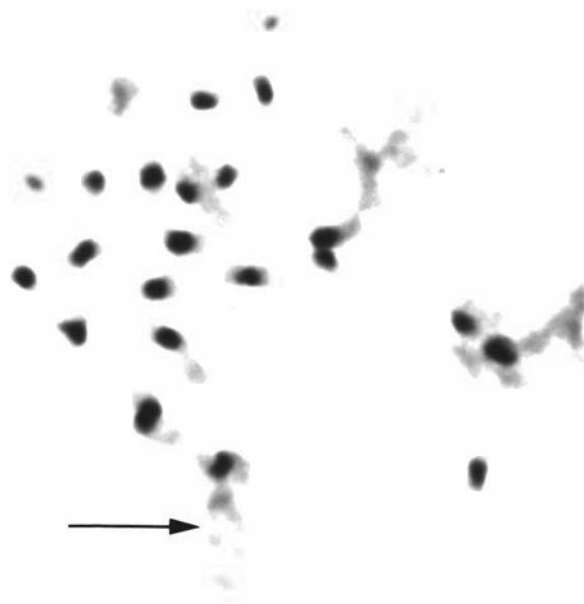
There are 30 chromosome arms and 27 chromosomes including an X chromosome (arrowed) which has a pale staining area in the centromeric region by comparison with that on the acrocentric chromosomes. (C-banded. x 4,000).

Plate V. 46: Metaphase II cell from B66/72 (53,xy,t₂).

There are 29 chromosome arms and 26 chromosomes including an X chromosome (arrowed). The three large metacentric chromosomes and the X chromosome are uniformly stained. (C-banded. x 4,000).



45



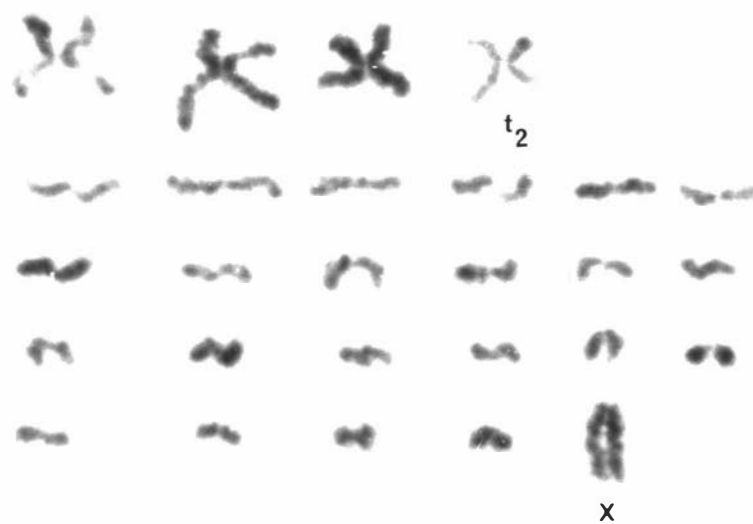
46

Plate V. 47: Metaphase II cell from B55/72 (53,xy,t₂).
There are 31 chromosome arms and 27
chromosomes including a translocation
metacentric chromosome (t₂) and an X
chromosome (arrowed).
(Aceto-orcein. x 4,300).

Figure V. 19: Karyotype of cell illustrated in Plate V. 47.



47



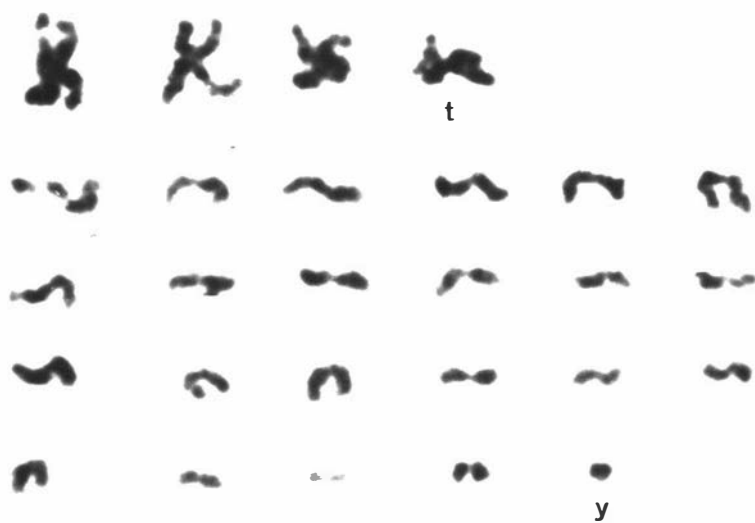
19

Plate V. 48: Metaphase II cell from B87/72 (53,xy,t₂).
There are 31 chromosome arms and 27 chromosomes
including a translocation metacentric
chromosome (t₂) and a Y chromosome (arrowed).
(Aceto-orcein. x 3,500).

Figure V. 20: Karyotype of cell illustrated in Plate V. 48.



48



20

Plate V. 49: Diploid metaphase II cell from B66/72
(53,xy,t₂). There are 60 chromosome
arms. The sex chromosomes are indicated,
X chromosome (large arrow) and Y chromosome
(small arrow).
(C-banded. x 1,900).



c Massey III Robertsonian translocation
heterozygotes

A total of 423 secondary spermatocyte figures were recorded and analyses made of 394 of these. The remaining 29 cells were polyploids and accurate counts were not possible. The results of analyses of metaphase II figures are summarized in Tables V. 34 and V. 35.

A modal number of 30 chromosome arms was again seen in the five rams studied. No hypermodal cells were recorded in the limited material from three of the rams but significant levels were seen in two other rams. Hypomodal cells were seen in all rams. Two cells from one ram (B13/72) were recorded as having a count of $\frac{28,t,x}{32}$.

This was the only record of such counts and it is possible that these counts if not due to technical artefact, could be the complementary cells to the cells with counts of $\frac{25,y}{28}$. Four cells with counts of

$\frac{25,x}{28}$ were recorded in material from B76/72, but no complementary cells were observed.

Of the 68 polyploid metaphase II cells analysed (Table V. 35), 66 could be considered to be diploid, one possibly triploid and one possibly tetraploid. If counts from rams where fewer than 20 cells were evaluated are excluded, the percentage of polyploid cells ranges between 11.6 and 23.7 (Table V. 36). Twenty one of the 68 polyploid cells which were analysed had a euploid chromosome arm complement; six were hypermodal and the remainder hypomodal.

TABLE V. 36
PERCENTAGE POLYPLOID MII CELLS ($53,xy,t_2$)

Ram Identi- fication	MIi cells	Polyploid MIi cells	Total	% Polyploid MIi cells
HML99/58	10	10	20	50.0
Link 1966	4	0	4	0.0
B116/71	7	10	17	58.8
B13/72	206	64	270	23.7
B76/72	99	13	112	11.6

TABLE V.34
DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN DRYSDALE RAMS ($2n = 53,xy,t_3$)

Ram Identification		ALTERNATE SEGREGATION					PARTIAL NON-DISJUNCTION					NON-DISJUNCTION					OTHER CELL COUNTS																				Total M II Cells/ Ram	
		27,x	27,y	26,t,x	26,t,y	Total	26,x	26,y	27,t,x	27,t,y	Total	25,x	25,y	28,t,x	28,t,y	Total	19	19,x	19,x	21,x	21,x	21,y	22,y	23,x	23,y	24,y	25,x	25,y	24,x	28,x	19,t,x	23,t,y	23,t,x	24,t,x	25,t,x	25,t,y		Total
		30	30	30	30		29	29	31	31		28	28	32	32		21	21	22	23	24	24	25	26	26	27	27	27	27	31	22	27	27	27	29	29		
HML 99/58	NUMBER OF CELLS	1	2	3	2	8	1	0	0	0	1					0													1								1	10
Link 1966		1	1	1	0	3	0	1	0	0	1					0																				0	4	
B116/71		3	0	3	0	6	1	0	0	0	1					0																				0	7	
B13/72		33	34	31	17	115	27	13	8	8	56	1	7	2		10	1	1	1	1	1	1	1	2		2	2	1		1	1	1	1	3	1	3	25	206
B76/72		18	11	5	10	44	20	15	6	5	46	4				4								1		1		1			1			1		5	99	
TOTAL		56	48	43	29	176	49	29	14	13	105	5	7	2	0	14	1	1	1	1	1	1	1	2	1	2	3	1	2	1	1	2	1	3	2	3	31	326

TABLE V.35
DISTRIBUTION OF CHROMOSOME COUNTS IN POLYPLOID MII CELLS IN DRYSDALE RAMS ($2n = 53,xy,t_3$)

Ram Identification	30,x 33	33,y 39	34,y 39	44,x 49	51,x,y 57	52,x,y 58	52,y,y 58	53,x,y 59	54,x,y 60	55,x,y 61	29,t,y 33	30,t,x 34	35,t,x 38	35,t,x 40	(2n) 38,t,y 43	43,t,x 50	49,t,x,y 54	48,t,y 55	48,t,x,y 55	48,t,x,y 56	49,t,x,x 56	51,t,x,x 58	51,t,x,y 58	52,t,x,y 59	53,t,x,y 60	52,t,t,x,y 60	52,t,t,y,y 60	54,t,x,y 61	54,t,t,x,y 62	58,t,x,y 62	55,t,x,y 62	(3n) 82,t,t,x,y 100	(4n) 107,t,x,x,y 120	N.A.	Total
HML 99/58																							1	1		2				1	1	1		3	10
Link 1966																																		0	0
B116/71			1			1		1															1		3									3	10
B13/72	1	1		1	1	2		2	4	1	1	1	1		1	1	1	1		1		1	1	6	10	2		2	1				1	19	64
B76/72						1								1				1		1		1	1		2	1								4	13
TOTAL	1	1	1	1	1	3	1	2	5	1	1	1	1	1	1	1	1	1	1	1	1	1	4	8	13	6	1	2	1	1	1	1	1	29	97

N.A. = Not Analysed

The distribution of euploid and aneuploid metaphase II cells in 53,xy,t₃ rams is summarized in Table V. 37. In only one ram (B76/72) was the frequency of euploid cells less than 50 per cent. Again, if animals from which fewer than 20 metaphase II cells were evaluated are excluded, the percentage frequency of euploid cells is 44.4 per cent (B76/72) and 53.8 per cent for B13/72. The frequency distribution of chromosome arm counts for these two rams is further illustrated in Figure V. 12. The frequency of cells with a chromosome arm count of 29 is at a high level (36.4 per cent) in B76/72 and high frequencies of cells with chromosome arm counts of 31 were recorded in B13/72 (8.3 per cent) and B76/72 (11.1 per cent).

When metaphase II counts from rams from which more than 20 cells were analysed are considered according to the method of Cattanach and Moseley (1973) the frequency of aneuploid cells is high (Table V. 38).

TABLE V. 38
ANEUPLOID FREQUENCIES (53,xy,t₃)

	29+31 (%)	31x2 (%)
B13/72	29.61	16.50
B76/72	47.47	22.20

The 31x2 values are considerably higher than those calculated for either the Massey I or Massey II Robertsonian translocation heterozygote types.

An overall chi-squared test of Table V. 37 showed a significant difference in the distribution of euploid and aneuploid cells (pooled counts) ($\chi^2_4 = 10.21$ $p < .05$) but one ram B76/72 had a greater number of aneuploid than euploid cells (44 euploid and 55 aneuploid).

The pooled results of the five rams showed that a significantly greater ($\chi^2_1 = 5.46$ $p < .05$) number of normal cells were analysed than balanced translocation-carrying cells.

TABLE V. 37

DISTRIBUTION OF EUPLOID ($N.F./2 = 30$) AND ANEUPLOID ($N.F./2 < \text{or} > 30$) MII FIGURES
IN DRYSDALE RAMS ($2n = 53, xy, t_3$)

Ram Identification		(N.F./2)												Total
		< 28		28		29		30		31		> 31		
		N	%	N	%	N	%	N	%	N	%	N	%	
HML99/58	Number of cells	1	10.0	0	0	1	10.0	8	80.0	0	0	0	0	10
Link 1966		0	0	0	0	1	25.0	3	75.0	0	0	0	0	4
B116/71		0	0	0	0	1	14.29	6	85.71	0	0	0	0	7
B13/72		20	9.71	8	3.88	44	21.36	115	55.83	17	8.25	2	0.97	206
E76/72		4	4.04	4	4.04	36	36.36	44	44.44	11	11.11	0	0	99
Total		25	7.67	12	3.68	83	25.46	176	53.99	28	8.59	2	0.61	326

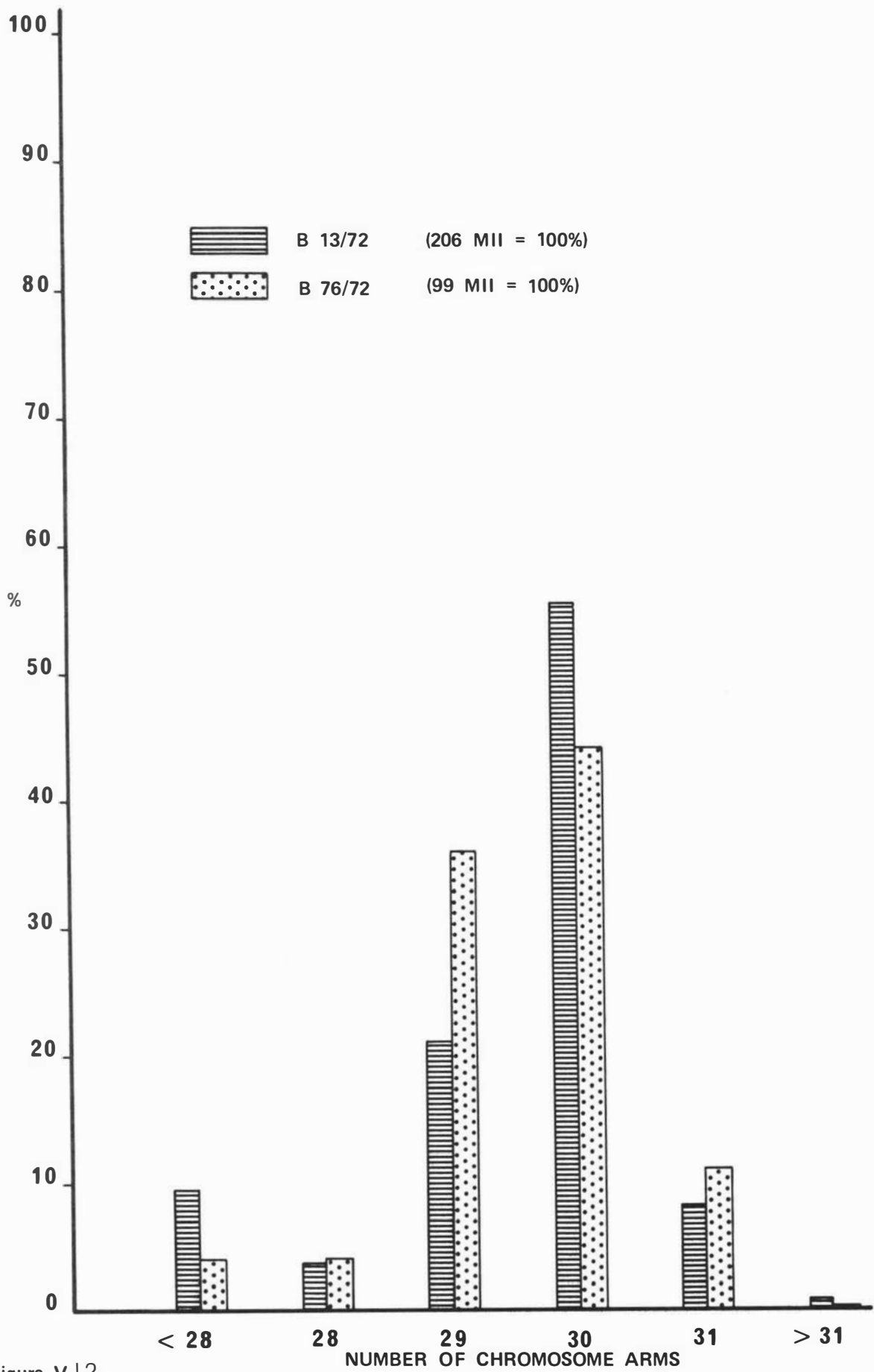


Figure V.12

Frequency histogram showing the distribution of euploid ($N.F./2 = 30$) and aneuploid ($N.F./2 < \text{or} > 30$) MII figures in two Drysdale ($53,xy,t_3$) rams.

Approximately equal numbers of Y-bearing and X-bearing balanced translocation-carrying metaphase II cells as well as aneuploid translocation-carrying cells were recorded. Overall, the ratio of Y-carrying to X-carrying cells differed ($\chi^2_1 = 7.37$ $p < .01$) with an excess of X-carrying cells being observed.

There was a departure from the expected equal proportions of the various cell categories resulting from alternate segregation ($\chi^2_3 = 8.77$ $p < .05$) with fewer translocation-carrying Y cells and more normal X cells than expected (Table V. 39). This trend was observed when the pooled results of both euploid and aneuploid classes over all rams were analysed ($\chi^2_3 = 38.68$ $p < .001$) (Table V. 40).

Examples of euploid, aneuploid and apparent polyploid metaphase II cells from rams heterozygous for the Massey III Robertsonian translocation are shown in Plates V. 50 to V. 56 and Figure V. 21.

TABLE V. 39

ASSOCIATION OF TRANSLOCATION CHROMOSOME WITH SEX CHROMOSOMES
AT MII IN DRYSDALE RAMS ($2n = 53, xy, t_3$)

Ram Identification		(N.F./2 = 30) Euploid				(N.F./2 < or > 30) Aneuploid			
		+t,y	+t,x	-t,y	-t,x	+t,y	+t,x	-t,y	-t,x
HML99/58	Number of cells	2	3	2	1	0	0	0	2
Link 1966		0	1	1	1	0	0	1	0
B116/71		0	3	0	3	0	0	0	1
B13/72		17	31	34	33	12	16	25	37
B76/72		10	5	11	18	6	7	16	26
Total		29	43	48	56	18	23	42	66

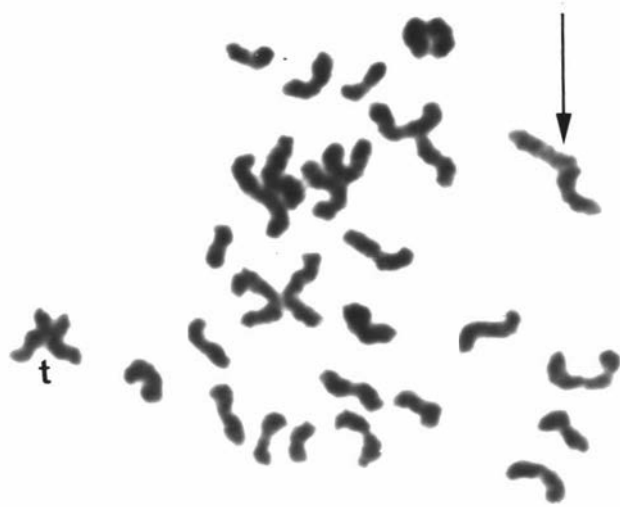
TABLE V. 40

ASSOCIATION OF TRANSLOCATION CHROMOSOME WITH SEX CHROMOSOMES
AT MII IN DRYSDALE RAMS ($2n = 53, xy, t_3$)
EUPLOID AND ANEUPLOID CELLS GROUPED

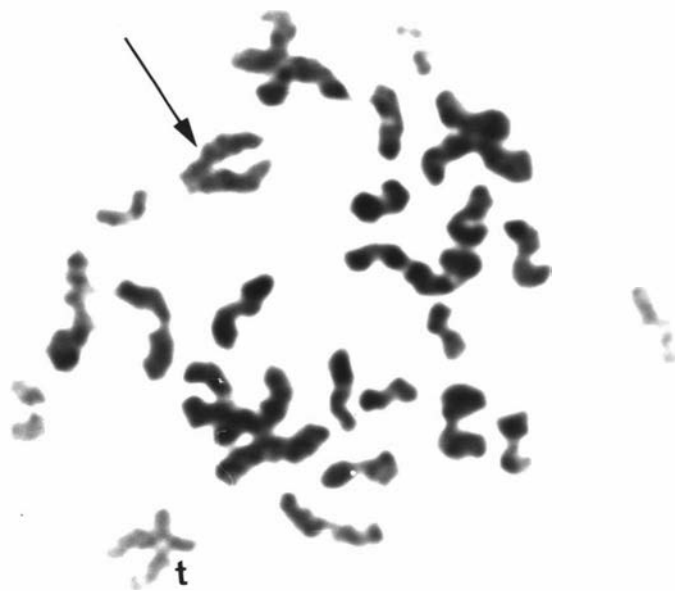
Ram Identification		+t,y	+t,x	-t,y	-t,x
HML99/58	Number of cells	2	3	2	3
Link 1966		0	1	2	1
B116/71		0	3	0	4
B13/72		29	47	59	70
B76/72		16	12	27	44
Total		47	66	90	122

Plate V. 50: Balanced translocation-carrying metaphase II cell
from B116/71 (53,xy,t₃) There are 30 chromosome
arms and 26 chromosomes. The translocation
submetacentric chromosome and the X chromosome are
identified.
(Aceto-orcein. x 3,800).

Plate V. 51: Balanced translocation-carrying metaphase II cell
from B13/72 (53,xy,t₃). The translocation
submetacentric chromosome and the X chromosome are
identified. There are 30 chromosome arms and 26
chromosomes.
(Aceto-orcein. x 4,500).



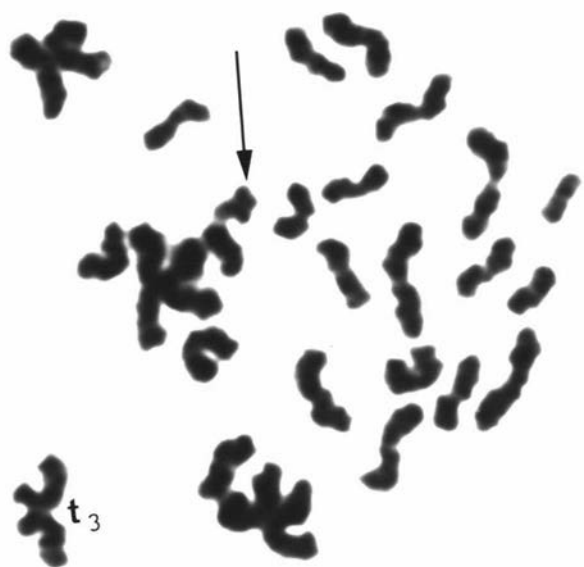
50



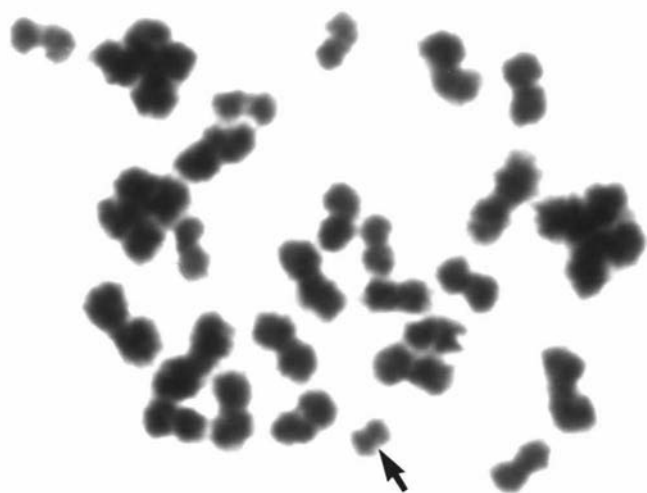
51

Plate V. 52: Balanced translocation-carrying metaphase II cell from B13/72 (53,xy,t₃). There are 30 chromosome arms and 26 chromosomes including three metacentric chromosomes, a submetacentric translocation chromosome (t₃) and a Y chromosome (arrow).
(Aceto-orcein. x 5,000).

Plate V. 53: Metaphase II cell from B13/72 (53,xy,t₃). There are 30 chromosome arms and 27 chromosomes including three metacentric chromosomes and a Y chromosome (small arrow).
(Aceto-orcein. x 4,800).



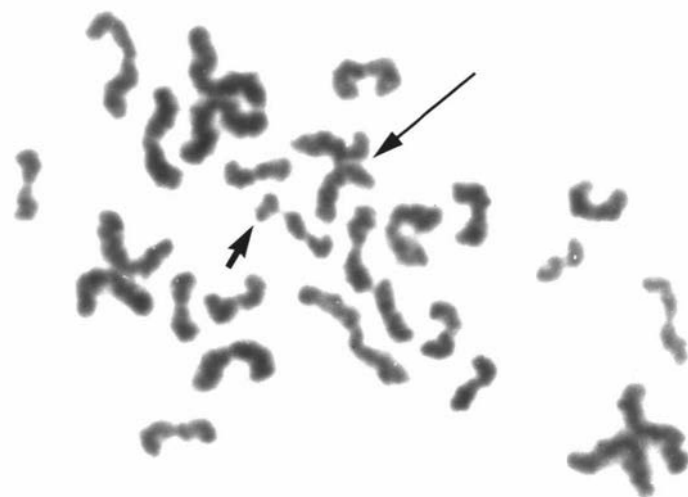
52



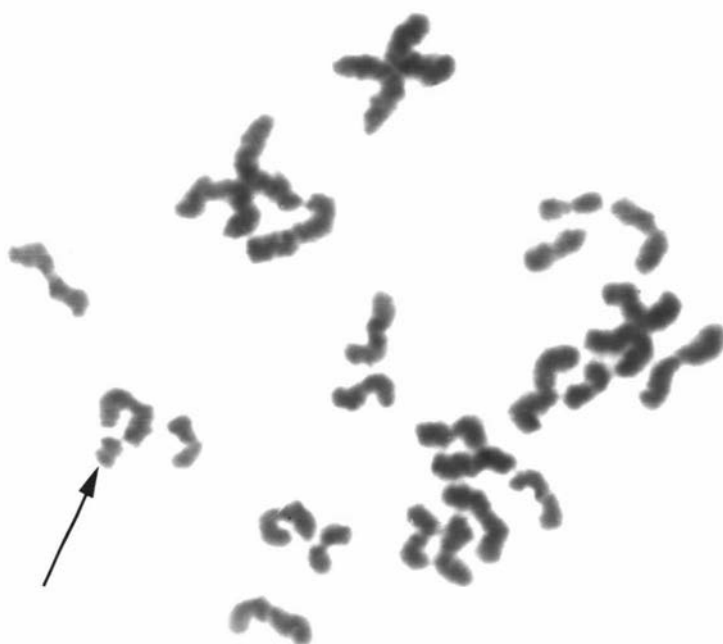
53

Plate V. 54: Metaphase II cell from B13/72 (53,xy,t₃).
There are 29 chromosome arms and 25
chromosomes including three metacentric
chromosomes, a translocation submetacentric
chromosome (large arrow) and a Y chromosome
(small arrow).
(Aceto-orcein. x 4,200).

Plate V. 55: Aneuploid metaphase II cell from B13/72
(53,xy,t₃). There are 28 chromosome arms
and 25 chromosomes including three metacentric
chromosomes and a Y chromosome (arrowed).
(Aceto-orcein. x 4,000).



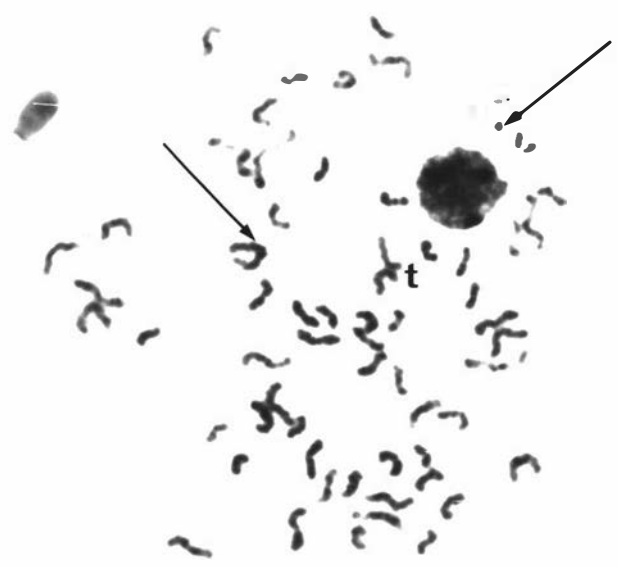
54



55

Plate V. 56: Diploid metaphase II cell from B13/72
(53,xy,t₃). The translocation chromosome
(t) and the sex chromosomes are identified
(arrows).
(Aceto-orcein. x 2,450).

Figure V. 21: Karyotype of cell illustrated in Plate V. 56.
There are 60 chromosome arms and 53 chromosomes
including six metacentric chromosomes, a
translocation submetacentric chromosome (t₃),
an X and a Y chromosome.



56



21

d Rams homozygous for a Robertsonian translocation

A total of 110 metaphase II cells including 11 polyploids were counted in a Romney homozygous ram ($2n = 52, xy, t_1, t_1$). A smaller number of cells, 28 were counted in a Drysdale homozygous ram ($2n = 52, xy, t_3, t_3$). These results are listed in Table V. 41.

A submetacentric translocation chromosome was easily distinguished in 92 of the 110 cells studied from B55/70. Similarly in 21 of the 23 cells from A134, the translocation chromosome was easy to recognize. In both translocation types, the translocation chromosome was the fourth largest of the karyotype.

No hypermodal cells were recorded in either the $52, xy, t_1, t_1$ or the $52, xy, t_3, t_3$. If all hypomodal cells were included 14.1 per cent were recorded in $52, xy, t_1, t_1$ and only 8.7 per cent in $52, xy, t_3, t_3$. If cells with chromosome arm counts of 28 and less were excluded, the percentage of hypomodal cells in B55/70 is 6.1, nearer to the value calculated for A134. In one cell each from B55/70 and A134 a sex chromosome was missing. The X chromosome was present in 52 cells and the Y in 46 cells from B55/70 while nine X-carrying and 13 Y-carrying cells were seen in metaphase II figures from A134. In both cases this does not depart from the expected 1 : 1 ratio.

Polyploid metaphase II cells were recorded in both translocation types but only three out of the total 16 were analysed. Two of these cells carried two translocation chromosomes and an X and Y chromosome, while one cell carried only one translocation chromosome (Table V. 41).

Ten per cent of the cells at metaphase II from B55/70 and 17.9 per cent of the cells from A134 were apparently polyploid.

Plates V. 57 and V. 58 are typical of the metaphase plates seen in meiotic preparations from ram B55/70.

e Double translocation heterozygous ram

Ram B9/72 ($52, xy, t_1, t_3$) was the subject of a paper included in the Appendices (Bruère and Chapman, 1974; Appendix XXI). Insufficient numbers of metaphase II cells were analysed to enable a conclusion to be reached regarding the segregation behaviour of the trivalents at metaphase I.

TABLE V. 41

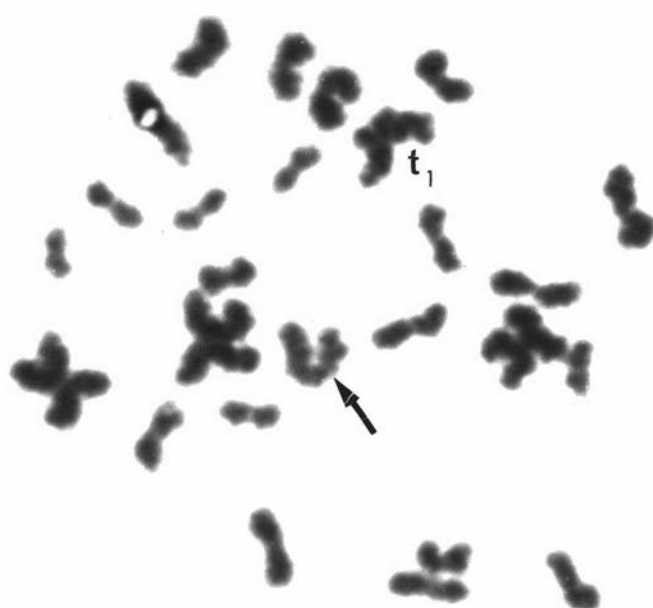
DISTRIBUTION OF CHROMOSOME COUNTS AT MII IN RAYS HOMOZYGOUS FOR A ROBERTSONIAN TRANSLOCATION

Ray Identification and Karyotype		n																	Total/Ram	Polyploid cells				Total/Ram	Total MII Cells/Ram
		<u>26,t,x</u> 30	<u>26,t,y</u> 30	<u>20,t</u> 23	<u>21,y</u> 23	<u>21,t,x</u> 24	<u>21,t,x</u> 25	<u>23,x</u> 25	<u>23,t,x</u> 27	<u>24,t,x</u> 28	<u>24,y</u> 28	<u>25,t,y</u> 29	<u>25</u> 29	<u>25,t,x</u> 29	<u>26,x</u> 29	<u>27,y</u> 29	<u>27,y</u> 30	<u>27,x</u> 30		<u>28,t,x</u> 30	t,t,x,y 50 58	t,t,x,y 52 60	<u>53,t,x,y</u> 60		
B55/70 52,xy,t ₁ ,t ₁	Number of cells	41	39	1	1	1	1	1	1	1	1	2	1	2	1	2	2	1	99	1	1	1	8	11	110
A154 52,xy,t ₃ ,t ₃		8	12										1	1			1			23				5	5

N.A. = Not Analysed

Plate V. 57: Metaphase II cell from B55/70 (52,xy,t₁,t₁).
There are 30 chromosome arms and 26
chromosomes including a translocation
submetacentric (t₁) and X chromosome
(arrowed).
(Aceto-orcein. x 4,200).

Plate V. 58: Metaphase II cell from B55/70 (52,xy,t₁,t₁).
There are 30 chromosome arms and 26 chromosomes
including a translocation submetacentric
chromosome (large arrow) and a Y chromosome
(small arrow).
(Aceto-orcein. x 1,900).



57



58

(D) Discussion Of Results From Secondary Spermatocyte Analyses

a Introduction

The commonly used method of segregation analysis of different chromosomes by the examination of the karyotypes of progeny can be unreliable. Malsegregation of chromosomes with subsequent preferential fertilization of gametes or zygotic selection could occur obscuring some of the unbalanced karyotypes produced. Examination of metaphase II figures is a more accurate method of determining the segregation pattern that occurs at anaphase I. There is little published information on the analysis of metaphase II figures in mammals other than mice. Several authors have discussed the reasons for this; these include technical difficulties involved in obtaining sufficient high quality cells, and difficulties in interpreting the chromosome counts from these cells (Fredga and Santesson, 1964; Sasaki, 1965; Sasaki and Makino, 1965; Fraccaro et al., 1968; Datta, 1970; Hultén, 1970; Ohno, 1970; Pogosianz, 1970; Padeh et al., 1971; Ferguson-Smith, 1972).

Some examples of the numbers of metaphase II cells recorded by mammalian workers are given in Table V. 42. In most cases no information was given as to the actual chromosome counts in each cell and cells were merely recorded as being haploid or polyploid. For example Skakkebaek et al. (1973) recorded a large number of metaphase II cells but gave no tables of accurate counts from these, and Sasaki and Makino (1965) gave accurate counts of only 18 cells from a total of 761. The high numbers of metaphase II cells recorded in some mice studies may have been due in part to the use of colchicine (Tettenborn and Gropp, 1970; Döring, Gropp and Tettenborn, 1972; Cattanaach and Moseley, 1973). Reasons for the non-use of this drug in sheep have already been explained.

The majority of authors who observed secondary spermatocytes in meiotic preparations commented on chromosome morphology (Makino, 1943,a; Böök and Kjessler, 1964; Sasaki and Makino, 1965; Lavappa and Yerganian, 1970; Luciani, 1970; Pogosianz, 1970; McDermott, 1971; Popescu, 1971,a). In general it is considered that metaphase II chromosomes resemble mitotic metaphase chromosomes, at least in man (Luciani, 1970; Luciani et al., 1970; Ferguson-Smith, 1972).

TABLE V. 42

SOME EXAMPLES OF NUMBERS OF MII CELLS STUDIED IN OTHER SPECIES

Author	Species (Number)			Number Cells Counted*
Sasaki, M. and Makino, S. (1965)	Man	(16)	Normal fertility	761(6.9)
Sasaki, M. (1965)	Man	(1)	Down's syndrome	21(4.8)
McIlree, M.E. <u>et al.</u> (1966,a)	Man	(16)	Normal fertility	210(9)
(1966,b)	Man	(50)	Infertile	32(12.5)
Eliasson, K. <u>et al.</u> (1967)	Dog	(2)		157(5.1)
Gustavsson, I. (1969)	Bull	(2)	2n = 60	38(7.9)
	"	(2)	2n = 59	151(7.3)
	"	(1)	2n = 58	62(3.2)
Luciani, J.M. (1970)	Man	(4)	Normal fertility	56(12.5)
		(4)	Infertile	39(5.1)
Gustavsson, I. (1971,a)	Variable hare	(1)	2n = 48	16(18.8)
	Common hare	(1)	2n = 48	19(5.3)
Skakkeback, N.E. <u>et al.</u> (1973)	Man	(18)	Normal fertility	757(3.6)
	"	(74)	Infertile	1909(10.6)

* Percentage polyploid cells in brackets.

No differences in the intensity of staining between the sex chromosomes and autosomes were observed in metaphase II cells in man (Böök and Kjessler, 1964; Luciani, 1970) the Djungarian hamster (Pogosianz, 1970) or the Syrian hamster (Fredga and Santesson, 1964). However, positive heteropyknosis of the sex chromosomes was noticed in the Armenian hamster (Lavappa and Yerganian, 1970), hare (Gustavsson, 1971,a) and in man by Ferguson-Smith (1972). The latter observation differs from that of Böök and Kjessler (1964) and Luciani (1970).

From the only report of metaphase II analysis in the sheep, Makino (1943,a) found a haploid number of 27 chromosomes with two types of secondary spermatocyte, one with an X and the other a Y chromosome. He observed "three very prominent atelomitic chromosomes with a double V-shaped configuration" but did not give any idea of the numbers of cells counted nor the range of chromosome counts in these.

b Chromosome morphology

The morphology of the chromosomes in secondary spermatocytes in both normal and translocation-carrying rams has been described (Chapter IV. 3 (ii) (C) b). The three large metacentric chromosomes described by the author were synonymous with the "atelomitic" chromosomes referred to by Makino (1943,a).

The translocation and sex chromosomes were easily identified both in cells stained with aceto-orcein and by the centromere-staining technique. There are two other reports of the use of the latter technique in sheep (Hsu and Arrighi, 1971; Evans et al., 1973) but these studies did not refer to testicular or ovarian material.

Staining features similar to those observed in mitotic chromosomes were recorded for metaphase II cells stained by the "C-banding" technique. Blocks of heterochromatin were seen on all autosomes. Occasionally a pale staining area was seen in the centromeric region of the sex chromosomes but usually there was no differential staining, a similar situation to that in mitotic chromosomes. Hsu and Arrighi (1971) and Evans et al. (1973) also reported a lack of differential staining between the centromeric region and the chromosome arms in sheep mitotic sex chromosomes with this staining method, although the

centromeric region of cattle sex chromosomes was stained. Non-differential staining of the sex chromosomes may be species-specific, as Hsu *et al.* (1971) and Polani (1972) reported staining of the centromeric heterochromatin on the X chromosome in metaphase II cells in mice, and Polani (1972) also thought he occasionally observed it on the Y chromosome in the same species.

The blocks of centromeric heterochromatin visible on the autosomes varied in size and staining intensity among chromosomes. The three large metacentrics had narrow, relatively lightly staining bands, similar to mitotic metacentric chromosomes. In some metacentric chromosomes a block of heterochromatin was visible on each chromatid both in metaphase II and mitotic metaphases, while in others where the chromatid arms were in apposition there appeared to be a single block. The centromeric heterochromatin in the acrocentric autosomes was neither of uniform staining intensity nor were the blocks always similar in size.

The large block of heterochromatin seen in the centromeric region of the three types of translocation chromosomes was a similar finding to that of Polani (1972) in mouse metaphase II cells.

c Non-disjunction and aneuploidy

Table V. 43 shows the distribution of euploid and aneuploid metaphase II cells (polyploids excluded) for the three different types of Robertsonian translocation heterozygotes, the two types of Robertsonian translocation homozygotes and the normal rams. The results tabulated for the heterozygotes and normal, are the pooled results for all the animals in that category.

The percentage distribution of euploid cells is lowest for the heterozygous animals: 54.0, 54.4 and 66.9 for the $53,xy,t_3$, $53,xy,t_2$ and $53,xy,t_1$ respectively. The $54,xy$, $52,xy,t_1,t_1$ and $52,xy,t_3,t_3$ all had euploid percentage distributions of greater than 80.0.

TABLE V. 43

DISTRIBUTION OF EUPLOID ($N.F./2 = 30$) AND ANEUPLOID ($N.F./2 < \text{or} > 30$) MII FIGURES
IN NORMAL RAMS AND RAMS HETEROZYGOUS FOR A ROBERTSONIAN TRANSLOCATION (POOLED DATA)

Andromeda

Karyotype		N.F./2												Total
		< 28		28		29		30		31		> 31		
		N	%	N	%	N	%	N	%	N	%	N	%	
53,xy,t ₁ (6 rams)	Number of cells	5	3.13	6	3.75	33	20.63	107	66.88	9	5.63	0	0	160
53,xy,t ₂ (4 rams)		10	3.02	42	12.69	84	25.38	180	54.38	15	4.53	0	0	331
53,xy,t ₃ (5 rams)		25	7.67	12	3.68	83	25.46	176	53.99	28	8.59	2	0.61	326
52,xy,t ₁ ,t ₁ (1 ram)		6	6.06	2	2.02	6	6.06	85	85.86	0	0	0	0	99
52,xy,t ₃ ,t ₃ (1 ram)		0	0	0	0	2	8.70	21	91.30	0	0	0	0	23
54,xy (10 rams)		6	9.68	1	1.61	3	4.84	52	83.87	0	0	0	0	62
														1001

There were no hypermodal cells in homozygous or normal rams but from 4.5 to 9.2 per cent of these cells for the heterozygous animals (pooled data). The percentage of cells with counts of 29 chromosome arms was higher in the heterozygotes than in the homozygotes and normals:

20.6% in 53,xy,t ₁	6.1% in 52,xy,t ₁ ,t ₁
25.4% in 53,xy,t ₂	8.7% in 52,xy,t ₃ ,t ₃
25.5% in 53,xy,t ₃	4.8% in 54,xy

This trend is illustrated in Figures V. 22 and V. 23. Percentage values of from 6.9 to 15.7 for the distribution of cells with fewer than 29 chromosome arms, were recorded for the three types of heterozygotes. Values within this range were also recorded for the normal rams (11.3 per cent) and Massey I Robertsonian translocation homozygote (8.1 per cent). No cells with fewer than 29 chromosome arms were observed in preparations from the Massey III Robertsonian translocation homozygote.

Estimates of the percentage frequency of aneuploid metaphase II cells were calculated in two different ways based on the methods used by Cattanaach and Moseley (1973). In addition, an estimate of the percentage non-disjunction was calculated in a similar way to that by Ford and Evans (pers. comm., 1973). The results of these calculations are summarized in Table V. 44.

TABLE V. 44
ESTIMATIONS OF ANEUPLOID MII FREQUENCY AND PERCENTAGE NON-DISJUNCTION IN RAMS

Karyotype	Aneuploid MII Frequency (Cattanaach and Moseley, 1973)		% non-disjunction (Ford and Evans, pers. comm., 1973). $\frac{\text{£ 29,31 classes}}{\text{£ 29,30,31 classes}} \times 100$	Total MII Cells Scored
	29+31 (%)	31x2 (%)		
53,xy,t ₁ (6 rams)	26.25	11.25	28.19	160
53,xy,t ₂ (4 rams)	29.91	9.06	35.48	331
53,xy,t ₃ (5 rams)	34.05	17.18	38.68	326
52,xy,t ₁ ,t ₁ (1 ram)	6.06	0.00	6.59	99
52,xy,t ₃ ,t ₃ (1 ram)	8.70	0.00	8.70	23
54,xy (10 rams)	4.84	0.00	5.45	62

Counts of less than 28 chromosome arms per cell probably represent chromosome loss and cell breakage during slide preparation,

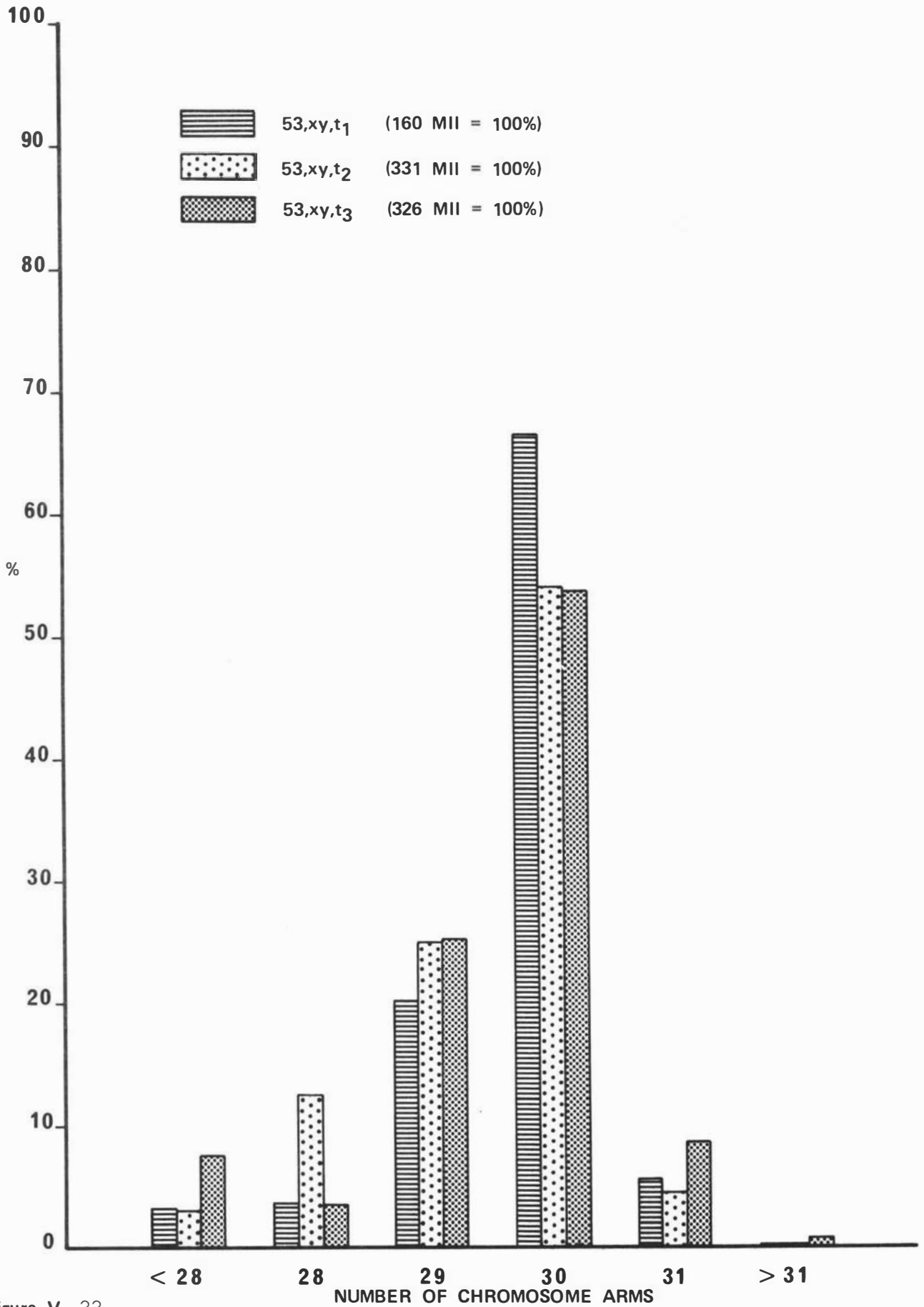


Figure V. 22

Frequency histogram showing the distribution of euploid (N.F./2 = 30) and aneuploid (N.F./2 < or > 30) MII figures in rams heterozygous for t₁, t₂ and t₃ translocation chromosomes. (Results for each translocation - type are pooled results from individual rams).

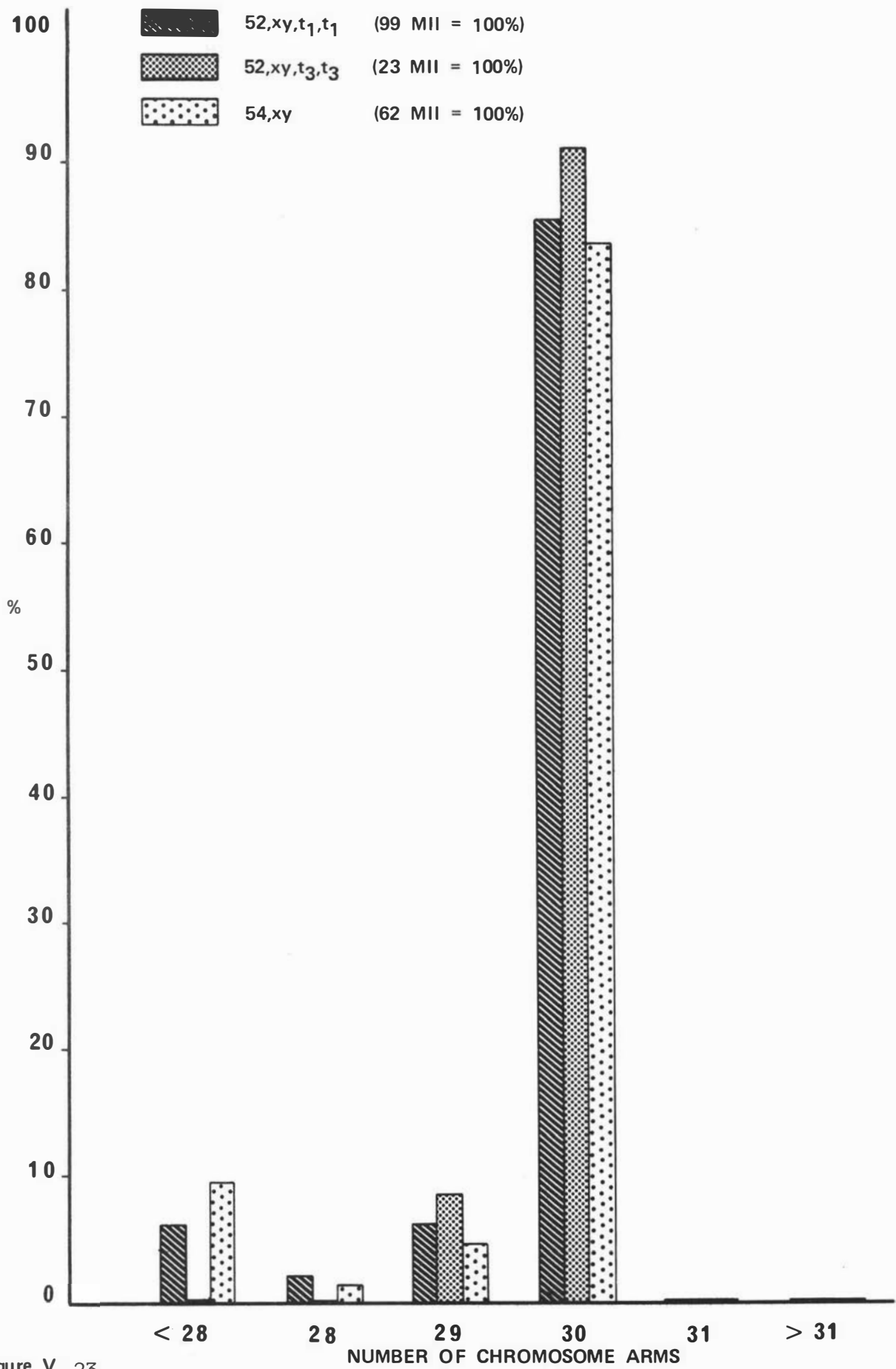


Figure V. 23
Frequency histogram showing the distribution of euploid (N.F./2 = 30) and aneuploid (N.F./2 < or > 30) MII figures in two homozygous rams and the pooled results from ten normal rams.

and these counts were not included in the above calculations. A proportion of the counts of 29 and 31 chromosome arms may also represent cell loss, or gain due to chromosome breakage, during technical manipulations as well as true aneuploid cells.

The observations of Cattanach and Moseley (1973) working with tobacco mouse crosses suggested that the first estimate of aneuploid metaphase II frequency (29+31 classes as a percentage) is an overestimate while the second (31x2 classes as a percentage) is an underestimate of the true occurrence. The results obtained in this thesis show that this is also probably true for the ram. There are significantly greater numbers of cells in the 29 chromosome arm class than in its complementary 31 chromosome arm class in all three heterozygotes (Table V. 45) which tends to suggest that some chromosome loss, possibly due to lagging at first anaphase, must have occurred in addition to non-disjunction. Ford and Evans (pers. comm., 1973) reported finding approximately equal numbers of cells in the two analogous classes in mice heterozygous for each of four different Robertsonian translocations, and deduced that anomalous chromosome counts could therefore be wholly attributed to non-disjunction. Tettenborn and Gropp's (1970) data for two Mus musculus x Mus poschiavinus F₁ hybrids showed no significant differences between the numbers of cells in the 19 and 21 chromosome arm classes. Similar results were seen with the pooled results of Döring et al. (1972) and for the T₄ translocation heterozygote of Cattanach and Moseley (1973). When data from mice singly heterozygous for the T₁ Bnr, T₂ Bnr, T₃ Bnr, T₅ Bnr, T₆ Bnr and T₇ Bnr translocations (Cattanach and Moseley, 1973) were examined, it was found that significant differences existed between the numbers of cells in the two complementary classes. The differences varied between different heterozygotes but the results suggested that as in the ram, some chromosome loss probably also occurred in meiotic preparations from mice singly heterozygous for a Robertsonian translocation. Table V. 45 summarizes these results.

TABLE V. 45

NUMBER OF CELLS WITH HYPER- AND HYPOMODAL CHROMOSOME ARM COUNTS

Author	Number of Chromosome Arms *		χ^2_1
	19	21	
Ford and Evans (pers. comm., 1973) ($T_1+T_2+T_3+T_7$ - totals)	160	182	1.29 $p > .2$
Tettenborn and Gropp (1970) (F_1 hybrids - totals)	62	48	1.54 $p > .2$
	29	22	0.71 $p > .3$
Döring <u>et al.</u> (1972) (F_1 hybrids - totals)	150	124	2.28 $p > .1$
Cattanach and Moseley (1973)	T_1 63	37	6.25 $p < .05$
	T_2 66	29	13.64 $p < .001$
	T_3 58	27	10.59 $p < .01$
	T_4 81	59	3.15 $p > .05$
	T_5 56	16	21.13 $p < .001$
	T_6 49	12	21.23 $p < .001$
	T_7 48	24	6.35 $p < .05$
Author (totals)	Number of Chromosome Arms **		
	<u>29</u>	<u>31</u>	
	t_1 33	9	12.60 $p < .001$
	t_2 84	15	46.71 $p < .001$
	t_3 83	28	26.27 $p < .001$

* Mice

** Sheep

The calculated values of the three estimates show that marked differences exist between animals which are heterozygous for a Robertsonian translocation, and animals which are either homozygous for a similar Robertsonian translocation or which are normal rams. Differences also exist between groups of rams heterozygous for a different Robertsonian translocation.

An overall chi-squared test of the three translocation heterozygotes (pooled data for each), the two translocation homozygotes and the normal, showed that ($\chi^2_{20} = 105.59$ $p \ll .001$). Statistically significant differences exist between these groups with regard to the distribution of chromosome arm counts. The data was analysed as described in Chapter V. 2 (iii) (A). The results are summarized in Table V. 46.

TABLE V. 46
STATISTICAL COMPARISONS OF MII CELL COUNTS IN RAMS

Comparison	Chi-squared value	d.f.	Probability
Homo t_1t_1 + Homo t_3t_3 Vs Hetero $t_1+t_2+t_3$ +normal	40.02	4	< .001
Homo t_1t_1 Vs Homo t_3t_3 (within homozygotes)	0.12	1	> .70
Homo t_1t_1 + Homo t_3t_3 Vs Normal	1.67	3	> .50
Hetero $t_1+t_2+t_3$ Vs Normal	26.25	4	< .001
Hetero t_2 Vs Hetero t_1 + Hetero t_3 (within heterozygotes)	29.98	4	< .001
Hetero t_1 Vs Hetero t_3 (within heterozygotes)	9.41	4	> .05

Additional comparisons are recorded in Appendix XXV.

There is a statistically significant difference between the homozygotes and the heterozygotes plus normal rams, but no difference between the two types of homozygote nor between the homozygotes and normal rams in the distribution of euploid and aneuploid cell frequencies. Similarly, there is a significant difference between the pooled results from the three heterozygote types and the normal rams. No difference exists between $53,xy,t_1$ and $53,xy,t_3$ but significant differences between $53,xy,t_2$ and both the other types of heterozygotes. Most of this difference could be attributed to the greater number of cells with arm counts of 28 in the $53,xy,t_2$ rams.

A comparison of the data in Tables V. 44 and V. 46 indicates that heterozygosity for a Robertsonian translocation appears to be a major factor leading to an increased frequency of aneuploid secondary spermatocytes and also the major factor associated with non-disjunction. This trend was shown in tobacco mouse hybrids by Cattanach and Moseley (1973) and Ford and Evans (pers. comm., 1973).

The tobacco mouse and its various crosses are a useful model with which to compare data from sheep Robertsonian translocation carriers. Seven different metacentric chromosomes derived from the tobacco mouse were studied by Cattanach and Moseley (1973) and four of these were also examined by Ford and Evans (pers. comm., 1973). These metacentric chromosomes originated in the following way: the domestic mouse (Mus musculus, Linn.) has 40 acrocentric chromosomes while the tobacco mouse (Mus poschiavinus, Fatio) has a karyotype of 26 chromosomes of which seven pairs are metacentric. The N.F. in both species is the same (40). It has been shown in both mitotic studies (Zech, Evans, Ford and Gropp, 1972) and meiotic studies (Gropp et al., 1970,a; Tettenborn and Gropp, 1970) that the tobacco mouse metacentric chromosomes were derived from the domestic mouse acrocentric chromosomes by a process of Robertsonian translocation.

The F_1 hybrid has 33 chromosomes including seven metacentric chromosomes (Gropp et al., 1970,a) designated T_1 Bnr to T_7 Bnr (Gropp, Tettenborn and Léonard, 1970,b). Each metacentric has been isolated as a homozygous line upon a predominantly Mus musculus genetic background (Cattanach, Williams and Bailey, 1972; Cattanach and Moseley, 1973).

In addition several different Robertsonian translocation chromosomes have been identified in mice. These include T163H-

(Evans et al., 1967), T1 Ald- (Léonard and Deknudt, 1967), T1 Wh- (White and Tjio, 1967), T8 Bnr (Gropp, Olert and Maurizio, 1971), T1 IEM (Baranov and Dyban, 1971), T9 Bnr, T "Bonde", T "Chiav" 1-3 and T "Rov" 1-6 (Gropp et al., 1972). Arm homologies exist between some of these (Gropp et al., 1970,b; 1971; 1972). This is in contrast to the sheep where it has been shown that the chromosomes involved in the t_1 , t_2 and t_3 translocation chromosomes do not have any arm homologies (Bruère, 1974; Bruère and Chapman, 1974).

Studies on meiotic disjunction of translocation chromosomes, and zygotic analyses from various matings of mice carrying Robertsonian translocations, have shown that non-disjunction at anaphase I of translocation chromosomes does occur (Tettenborn and Gropp, 1970; Döring et al., 1972; Cattanach and Moseley, 1973; Ford and Evans, pers. comm., 1973). Döring et al. (1972) examined metaphase II figures from NMRI (Laboratory mice), Mus poschiavinus and the F_1 hybrids between these, and noted the average frequency of euploid metaphase II plates as 95.2 and 93.1 per cent respectively for the parent strains and 46.2 per cent in the F_1 males. In all three groups there was an excess of hypomodal counts. When the data of Döring et al. (1972), which included earlier counts made by Tettenborn and Gropp (1970), was analysed using the methods of Cattanach and Moseley (1973) and Ford and Evans (pers. comm., 1973), similar trends were observed to those seen in the ram. Table V. 47 shows that percentage estimations of aneuploid metaphase II frequency and non-disjunction were low for NMRI and Mus poschiavinus strains but high for the F_1 hybrid.

TABLE V. 47
ESTIMATIONS OF ANEUPLOID MII FREQUENCY AND PERCENTAGE
NON-DISJUNCTION IN MICE

(Based on data of Döring et al., 1973)

	Average Aneuploid Frequency 19+21 (%)	21x2 (%)	Average % Non-dis- junction	Number Cells Scored
NMRI	3.13	0.99	3.18	607
<u>Mus poschiavinus</u>	6.19	1.13	6.24	533
F_1 hybrid	36.29	32.85	43.98	755

Döring et al. (1972) found that there was no significant difference between individual specimens within each of the three groups nor between the NMRI and Mus poschiavicus males, but significant differences were shown to exist when F_1 hybrids were compared with NMRI and Mus poschiavicus.

When mice which were heterozygous and homozygous for each of the seven metacentric translocations T_1 Bnr - T_7 Bnr, were studied (Cattanach and Moseley, 1973) it became clear that the frequency of non-disjunction associated with the different metacentric chromosomes varied. This was also noted by Ford and Evans (pers. comm., 1973). The average values for aneuploid frequencies calculated by Cattanach and Moseley (1973) are listed in Table V. 48.

TABLE V. 48

ESTIMATIONS OF ANEUPLOID MII FREQUENCIES IN MICE

(Cattanach and Moseley, 1973)

Genotype of ♂	Average 19+21 (%)	Average 21x2 (%)	Number of Cells Scored	Average % Non-dis- junction *
T 1/+	25.0	18.5	400	26.04
T 2/+	23.8	14.5	400	24.55
T 3/+	21.3	13.5	400	21.85
T 4/+	35.0	29.5	400	38.78
T 5/+	18.0	8.0	400	18.80
T 6/+	13.3	6.0	400	15.84
T 7/+	18.0	12.0	400	18.70
Metacentric Homozygotes	7.28	2.86	700	7.42
+ /+ Normal control	8.50	0.00	400	7.51

* Calculated according to method of Ford and Evans (pers. comm., 1973).

Σ 19, 21 classes x 100

Σ 19, 20, 21 classes

Comparison of these figures with those for mice homozygous and heterozygous for all seven (T_1 Bnr- T_7 Bnr) Robertsonian translocation chromosomes (Table V. 47), shows that the percentage aneuploid frequency and non-disjunction is considerably higher in (NMRI x Mus poschiavinus) F_1 hybrids than in mice heterozygous for a single translocation chromosome. The metaphase II aneuploid frequencies also varied with the different translocation chromosomes. $T_4/+$ had the highest frequency of aneuploid cells (also confirmed by Gropp et al., 1972) and $T_6/+$ the lowest (Cattanach and Moseley, 1973). When the results in Table V. 48 are compared with those calculated for rams (Table V. 44), similar trends are seen. The figure calculated for 53,xy, t_3 of 35.1 per cent based on the 29 and 31 class was similar to that for the T_4 Bnr heterozygote but the complementary figure based on the 31x2 classes was lower for the ram (17.2 per cent), than for the mouse (29.5 per cent). Similarly with the 53,xy, t_1 and 53,xy, t_2 , figures for the 29+31 classes were recorded which were higher than those for any of the other six types of mouse heterozygote. However those for the 31x2 classes were lower than calculated for the mouse.

Individual rams varied in the estimations of aneuploid metaphase II frequency. For example B76/72 (53,xy, t_3) from which 99 cells were evaluated, had values of 47.5 and 22.2 per cent, while B13/72 (53,xy, t_3) from which 206 cells were evaluated had values of 29.6 and 16.5 per cent and B87/72 (53,xy, t_2) from which 151 cells were evaluated had values of 31.8 and 10.6 per cent. Differences between different mice heterozygous for any one metacentric chromosome were observed by Evans et al. (1967), White and Tjio (1967), Cattanach and Moseley (1973) and Ford and Evans (pers. comm., 1973).

Hyperploid cells were not seen in either of the two homozygous rams studied but low levels were found by Döring et al. (1972) and Cattanach and Moseley (1973). The percentages of aneuploid cells based on the sum of the 29 and 31 classes (rams) and 19 and 21 classes (mice) were similar for the homozygous rams and mice (Tables V. 44 and V. 48). Significantly lower levels of aneuploid metaphase II cells were recorded in both homozygotes and normal rams when compared to heterozygous rams. Similar trends were reported in mice (Döring et al., 1972; Cattanach and Moseley, 1973).

It was interesting to note that in the 53,xy, t_2 there were fewer balanced translocation-carrying female metaphase II cells (26,t,x) than expected in three of the four rams studied, and the

difference was statistically significant (pooled data, page 149). Bruère (pers. comm., 1974) found a significantly lower number of translocation-carrying ewe lambs ($53,xx,t_2$) born in 1972 from matings of $53,xy,t_2$ x $54,xx$, and a higher number of ram lambs $53,xy,t_1$ born in 1971 from $53,xy,t_1$ x $54,xx$ matings than expected. Bruère (pers. comm., 1974) is continuing his study of these matings with larger numbers of sheep in an attempt to explain these discrepancies.

The author also noticed that non-translocation-bearing X and Y cells predominated at metaphase II in all three translocation types when all metaphase II cells were considered, and greater than expected numbers of X-carrying cells were found in both euploid and aneuploid cell classes in the t_3 group (page 157). This is in contrast to the data of Tettenborn and Gropp (1970) which indicated that there was no preferential sex chromosome distribution in any of the aneuploid cell types. Several other reports on anomalies of this kind have been made. White and Tjio (1967) noted an excess of balanced non-translocation carrying metaphase II cells in T1 Wh- heterozygous mice but could neither determine the reason for this, nor its relationship to the karyotypes of progeny from matings involving these mice. Evans et al. (1967) observed an excess of T163H daughters and a deficiency of sons in matings of heterozygous males to normal females. No sex linkage nor any associations of the translocation chromosome with sex chromosomes at metaphase II were recorded. Although the numbers of animals involved were small, Hamerton (1968) suggested that perhaps the sex imbalance in the progeny could be explained as a result of the T163H- X-bearing sperm having a selective advantage over the Y-bearing sperm.

d Cause of aneuploidy in Robertsonian translocation heterozygotes

Several theories have been suggested in explanation of the non-disjunction observed at metaphase II in structural heterozygotes. Tettenborn and Gropp (1970) proposed that either genetic heterozygosity per se or the structural heterozygosity resulting in trivalent formation was responsible for the induction of meiotic ~~non-disjunction~~ ^{non-disjunction}. Cattanaach and Moseley (1973) however, consider that "while non-disjunction can be associated with heterozygosity for Robertsonian translocations, it is not a necessary consequence". The differences in levels of non-disjunction for mice heterozygous for different metacentric translocation chromosomes and the observation that non-disjunction may also be occurring in mice which are homozygous for a metacentric translocation chromosome, as well as data on zygote lethality and linkage groups, led Cattanaach and Moseley (1973) to propose that genetic differences associated with the species (Mus musculus x Mus poschiavinus) cross may be an important factor in malsegregations.

e Fate of aneuploid cells and effects of heterozygosity

Evidence from different species suggests that aneuploid secondary spermatocytes have several possible fates:

- (1) They may degenerate during spermatogenesis (Roosen-Runge, 1973) or
- (2) develop into mature spermatozoa capable of fertilizing an ovum (Döring et al., 1972; Ford, 1972). These spermatozoa even although chromosomally unbalanced may be morphologically similar to genetically balanced spermatozoa (Henricson and Bäckström, 1964; Hultén and Lindsten, 1970) and may participate in fertilization in numbers related to the total numbers produced rather than according to whether their genome is normal or unbalanced (Ford, 1972).
- (3) The product of ovum fertilization by an aneuploid sperm, an aneuploid zygote, may die before implantation (Evans et al., 1967; Gropp et al., 1970,a; Cattanaach and Moseley, 1973; Ford and Evans, pers. comm., 1973) and this pre-implantation loss could be confused with

losses occurring because of reduced rate of ovum fertilization (Ford and Evans, pers. comm., 1973).

- (4) Post-implantation death, recognizable as abortions, may occur; many of the abortuses could be monosomic or trisomic embryos or foetuses (Waxman, Arakaki and Smith, 1967; Gropp et al., 1970,a; Tettenborn and Gropp, 1970; Cattanaach and Moseley, 1973; Ford and Evans, pers. comm., 1973).
- (5) A proportion of the aneuploid zygotes may survive until term and produce individuals which are monosomic (Say, Tuncbilek, Yamak, Balci, 1970) or trisomic for a particular chromosome (Griffen and Bunker, 1964; Schröder, Lydecken, de la Chapelle, 1971; White, Tjio, Van de Water and Crandall, 1972,b).

The effects of heterozygosity for a Robertsonian translocation in the male are manifested in various ways in different species. Fertility depression has been recorded in mice (Evans et al., 1967; White and Tjio, 1967; Gropp et al., 1970,a; Tettenborn and Gropp, 1970; Cattanaach and Moseley, 1973; Ford and Evans, pers. comm., 1973). In man, it has been proposed that the D/D translocations may be heterogeneous in their clinical effects with some carriers having normal fertility while others are sterile (Palmer, Morris, Thompson and Nance, 1973). Only about four per cent of the progeny of male D/G translocation carriers are chromosomally unbalanced whereas 50 per cent might be expected (Ferguson-Smith, 1972), and Hamerton (1966; 1968) has attempted to explain this on the basis of selection against aneuploid spermatozoa at fertilization. Similarly the virtual absence of adjacent segregants in the offspring of D/D translocation carriers has been suggested due to preferential alternate segregation of the chromosomes involved in the trivalent, and this is supposedly related to the similar arm lengths of the chromosome pair involved in the translocation as opposed to the unequal lengths of the chromosomes forming the D/G translocation where adjacent segregation is more common (Hamerton, 1966; 1968).

From limited metaphase II data and the identification of balanced karyotypes only, Gustavsson (1969) suggested that preferential alternate segregation of the chromosomes in the trivalent occurred in 1/29 translocation heterozygous cattle. This type of segregation is also thought to predominate in mice heterozygous for the T1 Wh- (White

and Tjio, 1967) and T163 H (Evans et al., 1967) translocations where aneuploid levels of only nine and two per cent respectively were found in metaphase II cells, and Ford and Evans (pers. comm., 1973) suggested that the fertility depression recorded by Evans et al. (1967) could perhaps have been due to the reduced fertilization capacity of the sperm.

It appears that the situation in the sheep may differ from that so far described in other mammals.

The possible chromosomal composition of zygotes produced in translocation heterozygote rams x normal ewe matings are listed in Table V. 49. In fact, no cases of lambs with unbalanced karyotypes associated with Robertsonian translocations have been recorded. Neither are there any reports of monosomic or trisomic cattle (Gustavsson, 1969) or goats (Soller et al., 1966; Popescu, 1972) associated with Robertsonian translocations.

It is known from mating experiments that the fertility of the three types of Robertsonian translocation-carrying rams when mated with normal ewes is at least as good as that recorded for other Romney mating flocks at Massey University and equivalent to National Romney fertility data (Bruère, pers. comm., 1974). Since the majority of the ewes conceived to their first service this suggests that only chromosomally balanced (euploid) spermatozoa were involved in fertilization. In addition the three translocations in the homozygous state behaved as normal autosomal bivalents with regular segregation at anaphase I predominating, and all lambs produced from homozygous x normal matings were translocation heterozygotes (Bruère, pers. comm., 1974), confirming the regular alternate segregation pattern at anaphase I. There is some evidence for malsegregation of chromosomes in the form of hyperploid cells in the metacentric mouse homozygotes (Cattanach and Moseley, 1973) but this was not seen in the homozygous rams.

Bruère's data (pers. comm., 1974) showed that there was a significant decrease in fertility, seen as a lower lambing percentage, when translocation heterozygous rams were mated with translocation heterozygous ewes and this was especially noticeable for the Massey I translocation. In view of the fact that heterozygous and homozygous Massey I, II and III Robertsonian translocation-carrying rams mated with normal ewes (Bruère, pers. comm., 1974) and a double heterozygote ($52,xy,t_1,t_3$) ram when mated with normal ewes, (Bruère and Chapman, 1974)

were of normal fertility, the fertility depression could not be attributed to the heterozygous rams.

It seems most likely that the absence of unbalanced karyotypes and the normal fertility of translocation heterozygous rams is explicable on the basis of a degeneration of aneuploid spermatocytes (Ortavant, 1958 cited by Roosen-Runge, 1973) which is species-specific and occurs at some stage prior to spermatocyte maturation (Roosen-Runge, 1973). Bishop (1964) and Roosen-Runge (1973) suggested that the loss of male germinal cells was one way of ensuring that unsuitable gamete-types were not disseminated.

Although a significant level of aneuploidy occurs in secondary spermatocytes of all three translocation heterozygous rams, the conception rates and progeny are not affected. By comparison the fertility of tobacco mouse crosses (heterozygotes) is significantly reduced because aneuploid sperm are produced and fertilize, resulting in zygotic loss. The reason for the different fate of aneuploid spermatocytes in the ram and tobacco mouse crosses is probably due to the fact that the chromosomes of the latter are derived from two genetically different species (Cattanach and Moseley, 1973).

TABLE V. 49

POSSIBLE ZYGOTES : ROBERTSONIAN TRANSLOCATION HETEROZYGOUS
RAMS MATED WITH NORMAL FWEES

		Gamete	Ovum	
			$\frac{27,x}{30}$	$+$ $\frac{27,x}{30}$
S P E R M A T O Z O A	Alternate segregation	$\frac{27,x}{30}$	$\frac{54,xx}{60}$	Normal ♀
		$\frac{27,y}{30}$	$\frac{54,xy}{60}$	Normal ♂
		$\frac{26,t,x}{30}$	$\frac{53,t,x,x}{60}$	Balanced ♀ 't' carrier
		$\frac{26,t,y}{30}$	$\frac{53,t,x,y}{60}$	Balanced ♂ 't' carrier
	Partial non-disjunction (adjacent segregation)	$\frac{26,x}{29}$	$\frac{53,x,x}{59}$	♀ monosomic for an autosome
		$\frac{26,y}{29}$	$\frac{53,x,y}{59}$	♂ monosomic for an autosome
		$\frac{27,t,x}{31}$	$\frac{54,t,xx}{61}$	♀ 't' carrier trisomic for an autosome
		$\frac{27,t,y}{31}$	$\frac{54,t,x,y}{61}$	♂ 't' carrier trisomic for an autosome
	Total non-disjunction	$\frac{25,x}{28}$	$\frac{52,xx}{58}$	♀ double monosomy for autosome
		$\frac{25,y}{28}$	$\frac{52,x,y}{58}$	♂ " "
		$\frac{28,t,x}{32}$	$\frac{55,t,x,x}{62}$	♀ 't' carrier with double trisomy for autosome
		$\frac{28,t,y}{32}$	$\frac{55,t,x,y}{62}$	♂ " "

f Polyploid secondary spermatocytes

The possible modes of formation of polyploid secondary spermatocytes have already been discussed in Chapter IV, section 4 (viii). The abnormal sex chromosome complements seen in several metaphase II cells as well as the aneuploid polyploid cells could be explained on the basis of cell fusions at telophase I thus:

$\frac{26,x}{29}(\text{or } y)$ fused with $\frac{27,x}{30}(\text{or } y)$ to give $\frac{53,xy}{59}$ or $\frac{53,xx}{59}$,
 $\frac{26,x}{29}(\text{or } y)$ fused with $\frac{26,t,x}{30}(\text{or } y)$ to give $\frac{52,t,x,x}{59}$ $\frac{52,t,x,y}{59}$,
 and $\frac{26,t,y}{30}$ fused with $\frac{26,t,y}{30}$ to give $\frac{52,t,t,y,y}{60}$.

The percentages of polyploid cells estimated from the totals for each of the translocation types were 20.4 per cent ($53,xy,t_1$), 13.8 per cent ($53,xy,t_2$), 22.9 per cent ($53,xy,t_3$), 10 per cent ($52,xy,t_1,t_1$) and 17.9 per cent ($52,xy,t_3,t_3$) (Table V. 19 page 132). A variation in the proportions of polyploid cells between rams was observed. These findings were similar to those for the normal rams.

g Univalent sex chromosomes

Separated sex chromosomes were recorded in preparations from both normal and Robertsonian translocation-carrying rams at diakinesis and metaphase I, but no evidence was observed of the sex chromosome aneuploidy expected in secondary spermatocytes if the two univalents disjoined at random at first anaphase. Similar findings have been reported in mice (Ford and Evans, 1964; Ohno, Kaplan and Kinoshita, 1959; Lin *et al.*, 1971). The exceptional sex chromosome complements seen in some polyploid cells in the ram were probably the products of cell fusion at telophase I. Beechey (1973) has suggested that "cells with X-Y dissociation do not usually proceed beyond metaphase I" and the results reported in this thesis confirm his observations.

(E) Cell Distributions At Meiosis In
Robertsonian Translocation-carrying
Rams

Cell distributions in each of the categories, spermatogonial metaphase, diakinesis-metaphase I and metaphase II are given in Appendix XXII. The proportion of cells in each category varied between rams. On average the MII : MI ratio was greater than that found in the normal rams, but in only ram A182 did it approach the expected value of 2.0, and the rams with values of zero were producing semen of acceptable quality. The preponderance of cells in the diakinesis and metaphase I stages over those at metaphase II are not attributable to spermatogenic disturbances but rather to technical variables.

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APPENDICES

Appendix

I Restraint of ram:

Begg's Foot Trimming Cradle (G.N. Begg Engineering Co. Ltd., Southland, New Zealand).

II Electro-ejaculation:

Kuakura Mk.IV Ram Probe, Plastic Products, Hamilton, New Zealand.

III Examination of semen for motility:

- (a) Warm Stage Reichert, Austria.
- (b) Wild MII Microscope (Wild Heerbrugg Ltd., Switzerland).

IV Examination of semen, histological and chromosome preparations:

Wild M20 Microscope fitted with photographic attachment and camera (Wild Heerbrugg Ltd., Switzerland).

V Materials for blood leucocyte culture:

- (a) For each culture: sterilized 1 x 10cc syringe + 20g x 2" needle
10 ml Venoject tube with sodium heparin (Jintan Terumo Co., Ltd., Tokyo, Japan) + 20g x 1" needle
universal jar + lid.

Adapter for venoject tube.

Methylated spirits + swabs.

Media TC 199 (Glaxo Laboratories (N.Z.) Ltd., Palmerston North, New Zealand).

20cc syringe + 18g x 1" needle.

Phytohaemagglutinin (Wellcome Reagents Ltd., Wellcome Research Laboratories, Beckenham, Kent, England).

5 ml syringe + 18g x 1" needle.

Centrifuge (Bench centrifuge Watson Victor Ltd., Australia and New Zealand).

Incubator set at 37°C.

Appendix V
cont.

(b) To harvest cultures:

For each culture - 1 graduated centrifuge tube.
 1 pasteur pipette + rubber bulbs.

Colchicine (Aqua-Colchin, 1 ml contains 0.5 mg of
Colchicine) Park, Davis and Company, Sydney,
Australia.

Fixative - 15 ml methanol + 5 ml glacial acetic acid
(for each culture).

0.075 M KCl (Hungerford, 1965).

To make basic solution hypotonic KCl: Add 7.46 g
KCl to 100 ml glass distilled H₂O.

To make working solution KCl: Take 7.5 ml of
basic solution and make up to 100 ml with
glass distilled water.

Water bath at 37°C (Grant water bath, Grant's Instruments
(Cambridge) Ltd., Barrington, Cambridge, England).

Beaker for supernatant.

Cleaned slides (5/culture in beaker iced water).

Bunsen Burner.

Hot plate.

Centrifuge (as above).

Refrigerator.

(c) To mount slides:

Materials: Cover glasses (Matsunami 22 x 64 No.2, Watson
Victor Ltd., Wellington, New Zealand).

D.P.X. (De PeX mounting medium, G.T. Gurr
Ltd., London, England).

Glass rod, Xylol, absolute alcohol and swabs.

- (1) Cover glass should be soaked in alcohol and dried
with swab.
- (2) Place drop of D.P.X. on cover slip.
- (3) Remove slide to be mounted from Xylol and invert
over cover slip.

Appendix

- (4) Turn right side up and gently express any air bubbles.
- (5) Leave to dry overnight.

VI Materials used in anaesthesia of ram and preparation of scrotal area:

Nembutal Sodium (Pentobarbitone Sodium) 60mg/ml.

Abbot Laboratories (N.Z.) Ltd., Naenae, New Zealand.

Acetyl promazine (2mg/ml).

Boots Pure Drug Co. Ltd., Nottingham, England.

Xylocaine containing HCl 20 mg/ml and adrenaline 1:80,000.

Astra Pharmaceuticals (Australia) Pty. Ltd.

Hibitane (Chlorhexidine) 1% solution : 60 ml distilled

H₂O + 15 ml 5% concentrate

solution Hibitane

(Imperial Chemical Industries Ltd.,
Cheshire, England.)

Hibitane tincture: 7 fluid oz. 5% concentrate solution

10.5 " " distilled H₂O

52.5 " " methylated spirits

VII Materials for meiotic preparations:

Tri-sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$)

2.2% and 1.2% solutions made in 100 ml
flask with glass distilled water.

Watch glasses - (x2).

Forceps.

Scalpel blades (x2).

Graduated centrifuge tubes (x4).

Glass rod.

Water bath at 37°C (see Appendix V).

Centrifuge (" ").

Alarm clock.

Pasteur pipettes, one for fixative, one for hypotonic solution.

Coplin jars (x3).

Appendix

Ice bath.

Slides (3" x 1", .8 to 1mm thickness). Up to 10 for each centrifuge tube of material processed.

Fixative, 5 ml glacial acetic acid + 15 ml absolute alcohol in universal jar (for each pair centrifuge tubes of material).

Chloroform.

VIII Tissue culture medium for meiotic prophase stages:

10 ml TC 199 (Glaxo Laboratories, N.Z. Ltd., Palmerston North, New Zealand) to which Heparin (Pularin B.P. Evans Medical Co. Ltd.). 200 I.U. added.

IX 0.125 M KCl : 0.93 g KCl in 100 ml glass distilled water.

X Aceto-orcein Stain:

Materials	Synthetic Orcein (G.T. Gurr Ltd., London, England)	8 g
	Glacial acetic acid	240 ml
	Glass distilled water	160 ml

Method (1) Heat acetic acid to 100°C.
 (2) Add orcein powder and stir well.
 Keep at 90°C+ for 1 hr.
 (3) Cool and add glass distilled water.
 (4) Filter before use.

XI Materials used for centromere staining:

(1) 5% Barium hydroxide ($\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$) 5 g in 100 ml glass distilled water.

(2) 2xSSC (0.3 M Sodium chloride containing 0.03 M tri-sodium citrate)

NaCl	17.53 g
$\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$	8.82 g
Deionized H_2O	1 litre

Store in refrigerator and do not use hot 2xSSC more than two days old.

Appendix

(3) 0.2 M hydrochloric acid

19.64 ml HCl in 1 litre of deionized H_2O .

(4) Giemsa stain

Dissolve 0.5 g Giemsa powder (Allied Chemical Corporation, New York) in 33cc glycerine at $55^{\circ}C$ to $60^{\circ}C$ for 1.5 to 2 hrs. To this add 33cc of methyl alcohol. Dissolve 1 ml of this stock solution in 50 ml buffer at pH 6.8 (Buffer tablets pH 6.8, Geo. W. Wilton and Co. Ltd., Lower Hutt, New Zealand: 1 tablet to 100 ml distilled water).

XII Counting cells:

Hand operated Tally counter (English Numbering Machines Ltd., England).

XIII Developing Film:

- (1) Place film in tank in neat Dektol print developer (Kodak, New Zealand Ltd.) at $20^{\circ}C$ for 3 minutes with continuous agitation for first minute).
- (2) Wash film under running tap water for 1 minute.
- (3) Fix in Universal fixer 2 minutes.
- (4) Wash in running tap water 5 minutes.
- (5) Wipe and hang up to dry.

XIV Enlarger:

Focomat IIc, Leitz, Germany.

XV Developing Prints:

- (1) Develop prints in solution of 1 part Dektol diluted with 3 parts water at $20^{\circ}C$ for 2 minutes.
- (2) Transfer prints to stop bath (water + 1 teaspoon acetic acid).
- (3) Transfer prints to hypo bath ($2\frac{1}{2}$ lb hypo crystals + 8oz metabisulphate + 1 teaspoon alum).

Appendix

- (4) Transfer prints to water bath and wash for 20 minutes.
- (5) Glaze.

XVI Histology photographs - developing film:

- (1) Develop film in Microdol-X at 20°C for 9 minutes.
- (2) "Stop bath" 2 minutes.
- (3) Fix, wash 20 minutes and glaze.

XVII Washing of Glassware:

All glassware was soaked for 24 hrs in Decon 75 concentrate (20 ml/litre), rinsed in tapwater, washed in Fyroneg, rinsed, drained and dried.

Universal jars and lids used in blood leucocyte cultures were rinsed in 1 N HCl then given 3 rinses with glass distilled H₂O before being dried and autoclaved.

Appendix XVIII(a) SEMEN ANALYSIS

Ram Identification	Testes		SEMEN					MORPHOLOGICAL EXAMINATION							Date
	Size	Tone	Volume	Colour	Motility %	Rate	% Live/dead	Normal %	Tail Abnorm- alities %	Free Heads %	Mid-Piece Abnorm- alities %	Cytoplas- mic Droplets %	Head Abnorm- alities %	Degen- erate Sperm %	
<u>54,xy</u>			cc					%	%	%		%	%	%	
x435/69	L	Fi & El	1.5	Creamy	80	4	86/14	96	1	2.5	0.5	-	-	-	24/4/72 200 spermat zoa cou -tec
R285/69	L	Fi & El	1.0	Milky	70	3	78/22	86	2	11	0.5	0.5	-	-	17/4/72 200 spermat zoa cou -tec
x117/69	M	Fi & El	2.5	Creamy	80	4	80/20	79	6	7	-	8	-	-	12/4/72
x214/69	L	Fi & El	1.0	Creamy	80	4	70/30	90	1	9	-	-	-	-	29/11/7
84/68	L	Fi & El	Not ejaculated												
B157	M	Fi & El	Not ejaculated												
B158	M	Fi & El	Not ejaculated												
R195/69	M	Fi & El	Not ejaculated												
x452/69	S	Fi & D	Not ejaculated												
R294/69	M	Fi & El	0.5	Creamy	90	4	99/1	86	1	-	-	13	-	-	16/10/7
R 16/69	L	Fi & El	0.7	Creamy	90	5	95/5	94	6	-	-	-	-	-	16/10/7
R244/69	L	Fi & El	0.5	Milky	75+	3	97/3	95	4	-	-	1	-	-	29/11/7
"	"	"	1.0	"	75	3	85/15	64	8	15	3	-	1	9	15/3/72
PT195/70	L	Fi & El	1.5	Creamy	90	4+	79/21	95	4	1	-	-	-	-	23/5/73
R225/69	M	S & Fl	2.0	Creamy	90	5	90/10	74	11.5	3	1.5	0.5	0.5	9	15/3/72 200 spe -natozoa counted
"	M	"	1.0	Milky	70	3	44/56	89	8	3	-	-	-	-	21/6/72
"	M	Fi & El	1.5	Creamy	90	4	60/40	99	-	1	-	-	-	-	2/7/73

Key: Fi = firm. El = elastic. D = dull. S = soft. Fl = flabby. L = large. M = medium. S = small.

XVIII (a) cont.

53.xv.t₁

B46/69	M	Fi & El	0.4	Creamy	80	4	98/2	95	4	1	-	-	-	-	5/7/72
B68/69	L	Fi & El	1.0	Milky	80	4	88/12	93	4	2	-	1	-	-	15/2/72
B12/69	M	Fi & El	0.7	Creamy	90	4	95/5	96	2	-	-	1	-	1	3/2/72
B53/69	M	Fi & El	1.0	Creamy	80	4	95/5	99	-	-	-	1	-	-	21/2/72
"	"	"	1.7	Milky	70	4	77/23	96	2	2	-	-	-	-	12/4/73
236/67 (B15) 26/70	L	Fi & El	1.5	Creamy	75	3	79/21	98	1	1	-	-	-	-	7/6/72
A182	M	Fi & El	Not ejaculated												
"	L	Fi & El	0.8	Milky	70	3	93/7	99	-	1	-	-	-	-	26/4/72
"	M	"	0.8	Creamy	90+	5	81/19	98	-	2	-	-	-	-	21/6/72
B8/72	M	Fi & El	0.7	Creamy	60	3	42/58	94	5	1	-	-	-	-	5/4/73
B71/69	L	Fi & El	1.0	Creamy	90	4	72/28	100	-	-	-	-	-	-	21/6/72
"	"	"	1.5	"	70	3	71/29	73	2	24	1	-	-	-	5/7/72
"	"	"	1.5	Milky	50+	3	70/30	97	1	1	-	-	1	-	25/5/73
B8/72	M	Fi & El	0.5	Milky	70	3+	73/27	71	3	1	1	24	-	-	27/11/73

XVIII (a) cont.

53.xv.t₂

B25/72	M	Fi & El	0.5	Milky	70	3	90/10	91	2	5	-	-	2	-	3/4/73 13/4/73 electro- ejaculated but no sample ob- tained.
B87/72	M	Fi & El	0.7	Creamy	80	4	85/15	98	1.0	1.0	-	-	-	-	10/4/73
B68/72	M	Fi & El	1.0	Milky	70+	3+	62/32	92	3	1	3	1	-	-	31/5/73
B66/72	M	Fi & El	1.5	Creamy	90+	5	80/20	96	-	4	-	-	-	-	6/6/73
"	L	"	0.5	Creamy	85+	4+	73/27	85	9	4	-	2	-	-	4/10/73

53.xv.t₃

Link 1966	L	Fi & El	0.7	Creamy	90	5	92/8	92	7	-	-	1	-	-	28/2/72
HML99/58	L	S & Fl	0.7	Creamy	70	3	84/16	83	10	6	1	-	-	-	28/2/72
B116/71	M	Fi & El	0.8	Creamy	85	4	87/13	82	4.0	1.0	2.0	-	-	11.0	15/3/72
B13/72	S	Fi & El	Not	ejaculated											
B76/72	M	Fi & El	0.5	Creamy	90	5	85/15	96	-	3	-	-	1	-	14/3/73
"	L	Fi & El	0.5	Creamy	70	3+	74/26	79	13	1	2	2	3	-	15/10/73

52.xv.t₃.t₃

A134	L	Fi & El	0.7	Milky	40	2	31/69	80	6	13	-	-	-	1	17/4/73
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52.xv.t₁.t₁

B55/70	Ejaculated but semen sample unobtainable.														
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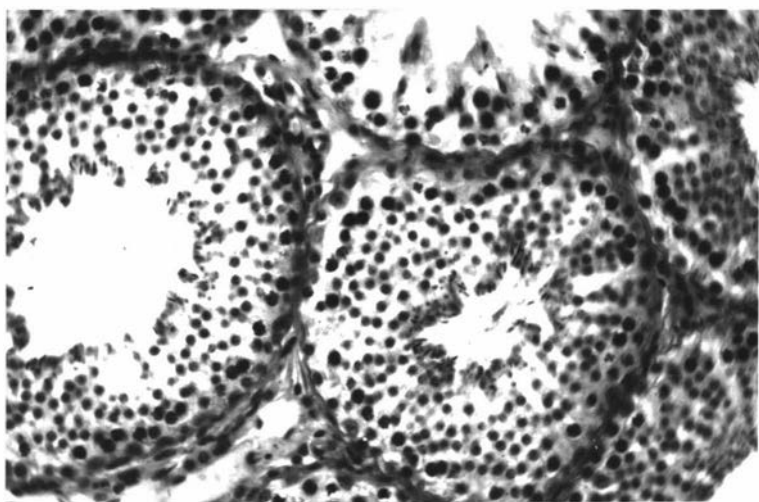
52.xv.t₁.t₃

B9/72	M	Fi & El	0.6	Creamy	90	4+	82/18	94	1	4	-	-	1	-	21/3/73
"	"	"	0.7	Thin-creamy	75	3	80/20	98	1	1	-	-	-	-	3/4/73

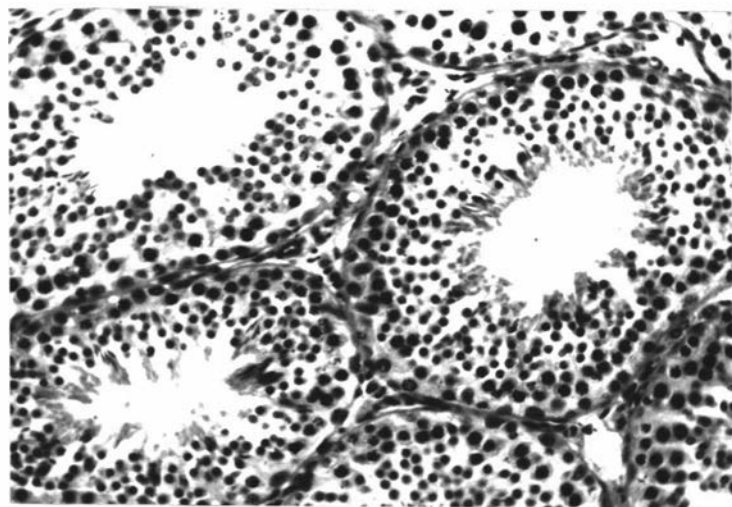
Appendix XVIII(b)

Examination of Histological Sections From Normal
and Robertsonian-translocation Carrying Rams

Histological sections of the testes of all rams, both normal and translocation-carrying, were examined microscopically. No differences were detected between these samples. In all rams a normal spermatogenic process was observed. Photomicrographs of the testis sections are shown in Plates 01 and 02.



01



02

Appendix XIX CHIASMATA COUNTS IN NORMAL RAMS (54,xy)

Bivalent Number	Number of		Chiasmata/Bivalent		X117/69	R244/69	R225/69
	X214/69	B157	X452/69	PT195/70			
1	7 6 7	4	6	6 5 5	7	6	6 5 6 6
2	6 3 5	4	5	6 5 5	6	5	6 5 4 6
3	5 5 4	5	6	6 4 4	6	4	5 5 5 6
4	2 1 4	2	3	2 3 3	4	2	3 3 2 3
5	2 3 2	3	2	2 2 3	3	2	3 2 1 3
6	1 2 2	3	1	3 2 2	3	2	2 2 2 2
7	2 2 2	2	2	1 2 3	2	2	2 2 1 2
8	2 2 3	2	2	2 2 2	3	2	2 2 2 2
9	2 2 2	2	3	2 2 2	3	2	2 1 2 2
10	1 2 2	1	2	2 2 2	2	1	2 2 2 2
11	1 2 3	1	3	2 2 2	2	2	2 2 2 3
12	2 1 2	2	2	3 2 2	3	2	2 2 2 2
13	2 2 1	1	1	1 2 2	3	2	1 1 1 2
14	2 2 2	2	2	1 1 2	2	2	1 1 1 2
15	1 2 2	1	2	2 2 1	2	2	1 2 2 1
16	2 2 3	2	2	2 1 1	2	2	2 2 1 2
17	1 2 2	2	2	3 2 1	2	2	1 2 1 1
18	2 1 3	1	1	1 2 2	2	2	2 1 2 2
19	2 2 1	2	2	2 1 2	1	2	2 2 2 3
20	1 1 2	2	2	3 2 2	3	1	2 1 2 2
21	1 2 2	2	1	2 2 1	2	2	2 1 1 1
22	2 2 2	2	2	2 1 2	2	2	1 1 2 2
23	1 1 1	1	2	2 1 1	2	2	1 2 1 2
24	1 1 2	1	2	2 2 1	2	2	1 1 2 2
25	2 1 2	1	2	2 1 1	2	1	1 2 2 2
26	1 1 2	2	2	2 2 1	2	1	2 2 2 2

XIX cont.

Bivalent Number	R195/69										84/68	R285/69					X435/69			
1	6	5	6	4	6	6	5	6	4	4	5	4	4	4	5	6	2	3	6	5
2	6	4	5	7	4	6	3	4	5	4	4	4	4	4	5	5	4	3	5	3
3	5	4	4	4	5	6	4	4	5	4	4	3	4	4	5	4	3	3	5	3
4	3	2	2	3	3	3	1	3	2	2	2	2	2	3	2	2	2	2	3	2
5	2	2	2	2	3	3	2	2	1	2	2	1	2	2	3	2	2	2	4	2
6	2	2	2	2	2	3	2	2	2	3	2	2	2	2	3	2	2	2	2	3
7	2	2	2	2	3	2	1	2	2	2	2	2	2	2	3	2	2	1	2	2
8	2	2	3	2	3	3	2	1	2	2	2	2	2	2	1	2	2	1	3	2
9	2	2	2	2	3	2	1	2	2	2	2	2	2	3	3	2	1	2	3	1
10	2	1	1	2	2	2	2	2	2	2	1	1	2	2	3	2	2	2	2	2
11	2	1	2	1	2	2	2	1	2	2	1	2	2	1	1	1	2	1	2	1
12	2	2	1	1	2	2	2	2	2	1	2	2	2	2	2	2	2	2	1	2
13	2	2	2	2	2	2	2	1	2	2	2	1	1	1	2	2	2	1	2	2
14	2	2	2	2	2	2	2	2	2	2	1	1	2	2	1	1	2	1	2	1
15	1	2	2	2	2	2	2	2	2	1	2	1	1	2	2	1	1	1	3	1
16	2	2	2	1	2	1	2	2	1	1	2	2	1	2	3	1	2	2	1	2
17	3	2	1	2	2	1	2	2	2	2	2	2	2	2	2	1	2	1	2	2
18	2	1	2	2	2	1	2	2	2	2	2	2	1	1	1	2	1	2	2	1
19	2	1	2	1	2	2	1	2	2	2	1	2	1	1	2	2	2	1	2	2
20	2	1	1	1	2	2	1	2	1	2	2	2	1	2	3	1	2	2	2	2
21	2	1	1	2	1	3	1	2	2	1	2	1	2	1	1	2	1	1	1	2
22	2	1	2	2	2	2	2	2	2	2	1	1	2	1	2	1	1	1	2	1
23	1	1	2	2	1	2	2	2	2	2	2	1	2	1	2	2	1	1	2	2
24	2	1	1	1	1	2	2	1	1	2	1	1	1	2	1	2	2	2	1	1
25	1	2	1	1	2	2	1	1	2	1	2	2	2	2	1	1	1	1	2	1
26	2	1	1	2	2	1	1	2	2	1	2	1	1	2	1	1	1	1	2	1

Appendix XX

Abstract of the following paper which is In Press

"The significance of the G-bands and C-bands of three different Robertsonian translocations of domestic sheep (*Ovis aries*)

A.N. BRUERE, D.L. ZARTMAN, HELEN M. CHAPMAN

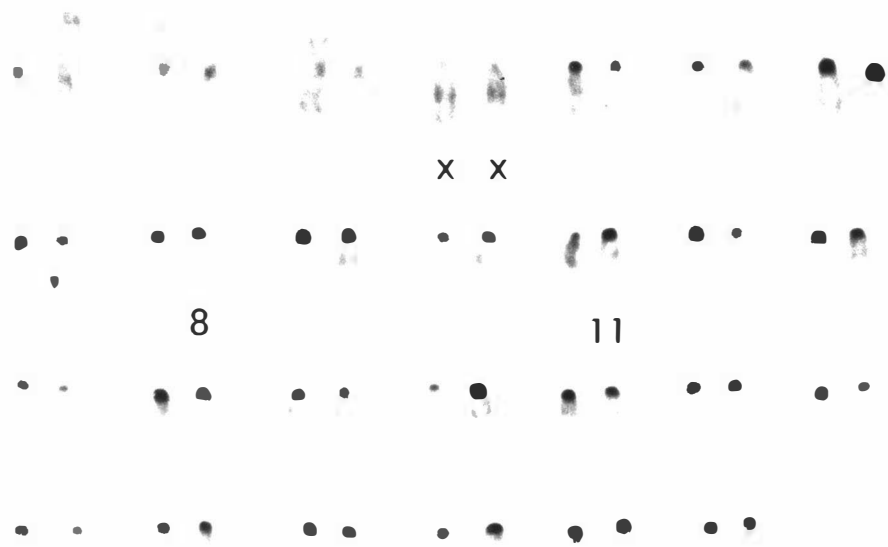
Abstract

G-banding of three different Robertsonian translocations of domestic sheep (MI, MII and MIII) has shown that the arm components of each are non-homologous. The C-bands of the MI and MIII translocation chromosomes showed heavy blocks of centric heterochromatin which were distributed evenly on either side of the centromeric constriction and suggested a dicentric structure. The C-band pattern of the MII translocation was regularly exocentric, suggesting that its method of formation may be that of a reciprocal translocation. The need for caution on the use of the terms Robertsonian translocation is discussed.

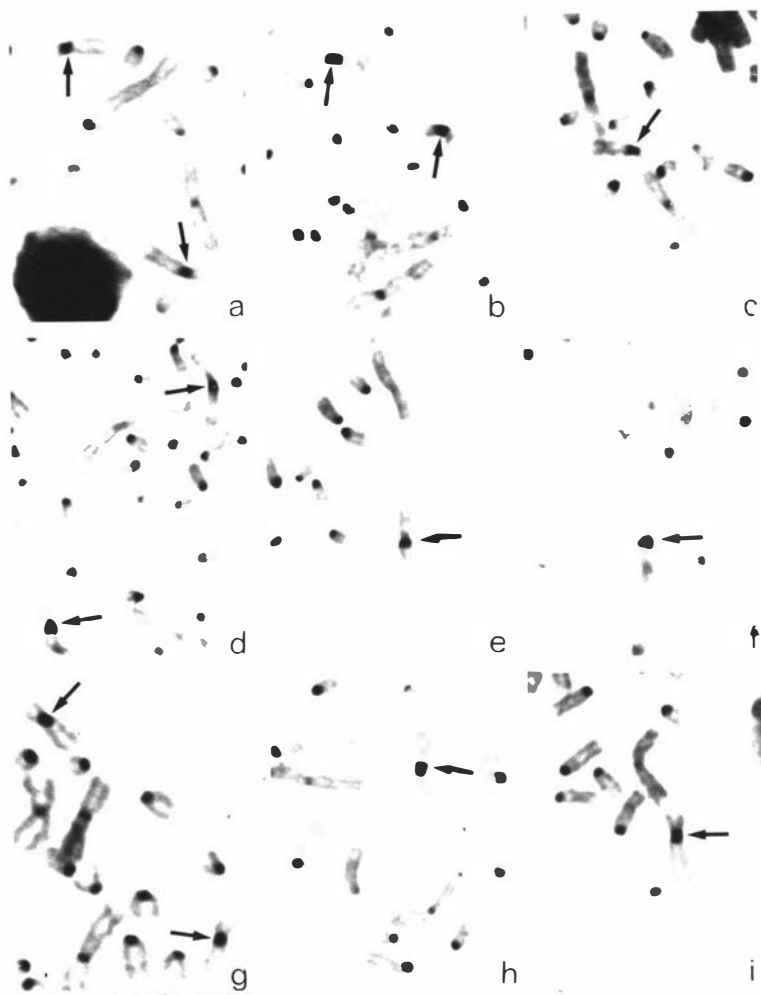
The amounts of centromeric heterochromatin in each of the translocation chromosomes is apparently greater than in the regular metacentrics, which suggests that they are of a more recent origin."

Fig. 2. The C-band karyotype of the domestic sheep (female)
 $2n = 54$. Note the absence of centromeric staining
in both X chromosomes. X 1500.

Figs. 3a-c. The C-bands of the 3 Massey translocations (arrowed)
a-c MI, a and b from homozygotes. d-f MII, d from
a homozygote. ~~g-i~~ MIII, g from a homozygote.
Note the large block of centric heterochromatin in
both MI and MIII and the exocentric heterochromatin
in MII, also the narrow centric region of the regular
metacentrics in all photomicrographs. X 2500.



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Double translocation heterozygosity and normal fertility in domestic sheep¹

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Abstract

Intermating of sheep heterozygous for what was believed previously to be two different submetacentric translocations (Massey I and Massey II) and subsequent meiotic studies on one of the ram progeny have confirmed that these translocations are completely nonhomologous. A ram heterozygous for the two translocations, when mated to ewes of normal karyotype, produced an above-normal number of progeny. During mating there was no undue return to service. The fertility of double translocation heterozygous ewes was also normal. An hypothesis is suggested to explain the odd anomalies of fertility that have been described in sheep and humans carrying Robertsonian translocations.

The high incidence of Robertsonian translocations in some populations of domestic sheep (BRUËRE and MILLS, 1971; BRUËRE et al., 1972; BRUËRE, unpublished) and domestic cattle (GUSTAVSSON, 1969; HARVEY, 1972, 1973, personal communication; DARRÉ et al., 1972; BRUËRE and CHAPMAN, 1973) has raised important issues for breeders of these species. Although in most instances these translocations are considered to be a normal evolutionary process associated with species phylogeny (WHITE, 1969), there are a number of reports from both man (WILSON, 1971; CHANDLEY et al., 1972; FRACCARO et al., 1973; PALMER et al., 1973) and domestic animals (GUSTAVSSON, 1969, 1971; BRUËRE, 1969; BRUËRE and MILLS, 1971) of their possible association with infertility. Unfortunately, the association between these translocations and infertility is rather inconclusive and the cause of the infertility somewhat speculative.

¹ Supported by a grant from the Wellcome Trust of Great Britain.

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The tobacco mouse (*Mus poschiavinus*) is the only comparable example of translocation heterozygosity in higher mammals in which the mechanism that affects fertility is understood (Gropp et al., 1970). This species has 7 Robertsonian translocations and these multiple translocations in the heterozygous state cause the production of a high level of aneuploid gametes (Tettenborn and Gropp, 1970; Döring et al., 1972; Cattanach and Mosley, 1973). These mature and are capable of fertilization, but the chromosomally unbalanced zygotes usually die in early embryogenesis (Tettenborn and Gropp, 1970; Cattanach and Mosley, 1973; Ford and Evans, 1973, personal communication).

A few cases of double translocation heterozygosity have been reported in man. Orye and Deliri (1967) described a D/D and D/G translocation in a boy with Down's syndrome whose brother, a balanced carrier of both translocations, was normal.

The following report from domestic sheep (*Ovis aries*) describes what are believed to be the first cases of multiple Robertsonian translocation heterozygosity in domestic animals. It also gives details and discusses the fertility of both rams and ewes carrying two translocations.

Materials and methods

The sheep

The sheep heterozygous for the two translocations (Massey I and Massey III) were bred by mating in 1972 a Drysdale ram that was homozygous for the Massey III translocation ($2n = 52$) to Romney ewes heterozygous for the Massey I translocation and by mating Massey I heterozygous rams to Massey III heterozygous ewes. The results of these matings will be described in another publication dealing with the intermating of heterozygotes. One of the ram lambs (B9/72), born in July, 1972, was a double translocation heterozygote (fig. 1). In March, 1973, following puberty, one testis was removed from this ram for meiotic chromosome studies. Two weeks later, when the castration wound had healed and his semen production considered satisfactory, ram lamb B9/72 was mated to 10 four-year-old Romney ewes of normal karyotype ($2n = 54$). These ewes had all produced lambs previously.

During the mating, ram lamb B9/72 was harnessed with a brisket raddle, so that as each ewe came into estrus and was served by him, her service date could be recorded. The color of the raddle was changed every 15 days, which is just short of the average estrus cycle of the sheep. By this means, sheep which returned to estrus could be recognized.

Three ewe lambs seven months of age and also heterozygous for the same two translocations as the ram lamb were mated to the Massey II translocation homozygous ram lamb (B62/72).

Cytogenetic methods

Leukocyte cultures were prepared by a whole blood technique and cultured for 48 h. Chromosome metaphases were prepared as required.

The meiotic chromosome studies were carried out on testicular tissue derived from one testis of ram lamb B9/72 by a technique developed for the sheep by one of us (H.M.C.) and modified from the method of EVANS *et al.* (1964). The meiotic preparations were stained with aceto-orcein before mounting and microscopic viewing. Histological sections of testis were prepared and stained with hematoxylin and eosin. The semen samples were collected by electro-ejaculation and were assessed for contrast, motility, percentage of live/dead spermatozoa, and morphology.

Results

A number of early and late diakinesis metaphases were viewed and counted from the meiotic chromosome preparations. Of 46 such metaphases, 96% showed 25 chromosome associations, including 23 bivalents and two characteristic trivalents (fig. 2*a, b*). Secondary spermatocyte metaphases were also seen, but an insufficient number of these was clearly discernable to give a segregation pattern. Several metaphases were seen in which both translocation chromosomes had segregated together (fig. 2*c*).

No abnormalities of spermatogenesis were seen in the histological sections of testis from ram B9/72. Semen from this ram, examined both before unilateral castration and mating, was of a good quality for the factors scored.

All 10 ewes mated to ram lamb B9/72 had come into estrus and been served by 21 April 1973. Only 1 of the 10 ewes returned to service on 16 May 1973. The first of the 9 ewes which did not return to service lambed on 13 August 1973; the last of these lambed on 14 September 1973. The one ewe which returned to service lambed 10 October 1973. All ewes gave birth to normal live lambs; four lambed twins and six lambed singles. This gave an overall lambing percentage of 140, which is higher than the national average for New Zealand Romney sheep and above average for New Zealand stud Romney flocks (QUINNIVAN and MARTIN, 1971*a, b*). The mean total lamb production of stud Romney ewes which lambed is quoted by the former authors as 153.5%, with a range of 121.9% to 138.4%.

The chromosome segregation patterns from this mating, as recorded from the lamb progeny, are shown in table I.

Of the three ewe lambs which were mated to ram lamb B62/72, B1/72 produced a single lamb, B18/72 produced twin lambs, and one B116/72

failed to lamb. Although these numbers are few, they represent a very satisfactory performance from ewe lambs which are not normally mated under New Zealand conditions. The majority of sheep in New Zealand are first mated as "two tooths," at 18 months of age. The progeny of the ewe lambs were all ewes, one was a Massey II heterozygote (BRUIER and MILLS, 1971; BRUIER, 1973) and two were Massey II and Massey I double translocation heterozygotes (fig. 3).

Discussion

Although the number of animals used in these mating experiments was small, it is interesting to note that an excess of double translocation heterozygous males was produced. No females of the same category were bred (table I). Conversely, three females but no males of normal karyotype were produced. Further experimentation is needed before significance can be attached to this observation.

Nevertheless, the presence of 5 out of 14 animals carrying both translocations suggests that gametes of this type are at no disadvantage. Furthermore, the theoretical possibilities of aneuploid gametes are numerous, if partial and complete nondisjunction are considered in gametogenesis. The present segregation data suggests no evidence of this possible high level of aneuploidy in either gametes or zygotes. This is also clearly shown by the nonreturn to service of the majority of ewes mated and the comparatively high lambing percentage recorded. This reproductive performance is even better than might have been expected, since the translocation animals were all lambs and the ram had undergone a unilateral castration only 14 days before mating. These results are in direct contrast to those associated with multiple heterozygosity in the tobacco mouse, viz., gamete and zygote aneuploidy and reduced fertility.

Although odd anomalies of fertility have been described in sheep in association with Robertsonian translocations (BRUIER, 1969; BRUIER and MILLS, 1971), these are clearly not a result of chromosome aneuploidy. Similarly, in man the possible associations of Robertsonian translocations with infertility are also not explicable by these means (WILSON, 1971; CHANDLEY et al., 1972; FRACCARO et al., 1973; PALMER et al., 1973). The cause of these anomalies of fertility reported in animals and man is possibly genetic (WILSON, 1971) and, therefore, may be explained by a consideration of the factors by which these special translocations are formed.

There is evidence from the mouse for the random involvement of acrocentric chromosomes in Robertsonian translocations (GROFF et al., 1972). However, in man the majority of Robertsonian translocations are clearly formed by the nonrandom association of acrocentric chromosomes (ROWLEY and PERGAMENT, 1969; HECHT and KIMBERLING, 1971). In domestic animals, although there is a dearth of information on the involvement of specific chromosomes, there is evidence from the geographical distribution of the 1/29 translocation of cattle that nonrandom involvement of acrocentric chromosomes could occur, as in man (BRUIRE, 1974). The nonrandom involvement of specific chromosomes in some Robertsonian translocations could have a genetic basis. FERGUSON-SMITH (1967), ROWLEY and PERGAMENT (1969), and HECHT and KIMBERLING (1971) have all mentioned that nonhomologous chromosomes which form Robertsonian translocations could have homologous segments and that this is a likely explanation for their formation. FERGUSON-SMITH (1971, 1973) has reviewed the evidence for this contention.

Therefore, the explanation of the odd anomalies of fertility which on occasion are associated with Robertsonian translocations may be a direct result of the cause of their formation. Fusion between nonhomologous chromosomes which have gene homologies could, in some instances, impose an increased homozygosity on an individual. This could mean that under certain circumstances the chance of duplicating undesirable genetic material is increased, thus leading to a deleterious effect on the fertility of the individual so affected.

The recent work on *Mus poschiavinus* by CATTANACH and MOSELEY (1973) has shown that nondisjunction is not an obligatory consequence of structural heterozygosity. These authors conclude that the variable high level of aneuploidy and reduced fertility in different heterozygotes of *M. poschiavinus* probably result from minor genetic differences between the metacentrics of *M. poschiavinus* and *M. musculus*. This reflects the fact that the chromosomes are derived from different species. Although nondisjunction of secondary spermatocytes does occur in rams heterozygous for all three Massey translocations, at a level comparable to individual tobacco mouse heterozygotes (CHAPMAN, in preparation; CHAPMAN and BRUIRE, in preparation), the subsequent fertility of these rams is not affected (present data and BRUIRE, in preparation). Furthermore, the breeds of sheep so far examined are not distinct species, as compared to *M. poschiavinus* and *M. musculus*. The Drysdale breed (Massey III) was derived from the Romney breed (Massey I), and only relatively few gene-

rations separate them. On this basis, it is unlikely that a reduction in fertility, such as occurs in the tobacco mouse, would be expected in sheep heterozygotes, other than sporadic anomalies such as the "hour glass" testes described previously (BRUÈRE, 1969; BRUÈRE and MILLS, 1971). Such anomalies may well be explained on the basis of the proposed hypothesis associated with the formation of Robertsonian translocations.

We believe that the results of this example from the sheep emphasizes the need to reconsider the conclusions reached by GUSTAVSSON (1969, 1970) that animals (cattle) heterozygous for Robertsonian translocations necessarily produce aneuploid gametes which lead to reduced fertility because of embryonic loss. DARRÉ et al. (1972) have also shown that bulls carrying the 1/29 translocation were among the best bulls at an insemination center for service in regard to growth index and conformation.

Mating experiments with sheep are to be extended to investigate these matters further and to attempt the identification of linkage groups in sheep, as has been done in the mouse by the use of translocation-bearing animals (CATTANACH et al., 1972).

Acknowledgements

The authors wish to acknowledge the technical assistance of ROSALIE J. MORRIS and BETSY F. BRULIN and the care and shepherding of the sheep by W. M. DUGGILLON and P. H. WINTERHEAD. Mrs. JOY B. PLANCE typed the manuscript.

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Manuscript received 21 November 1973; accepted for publication 12 February 1974

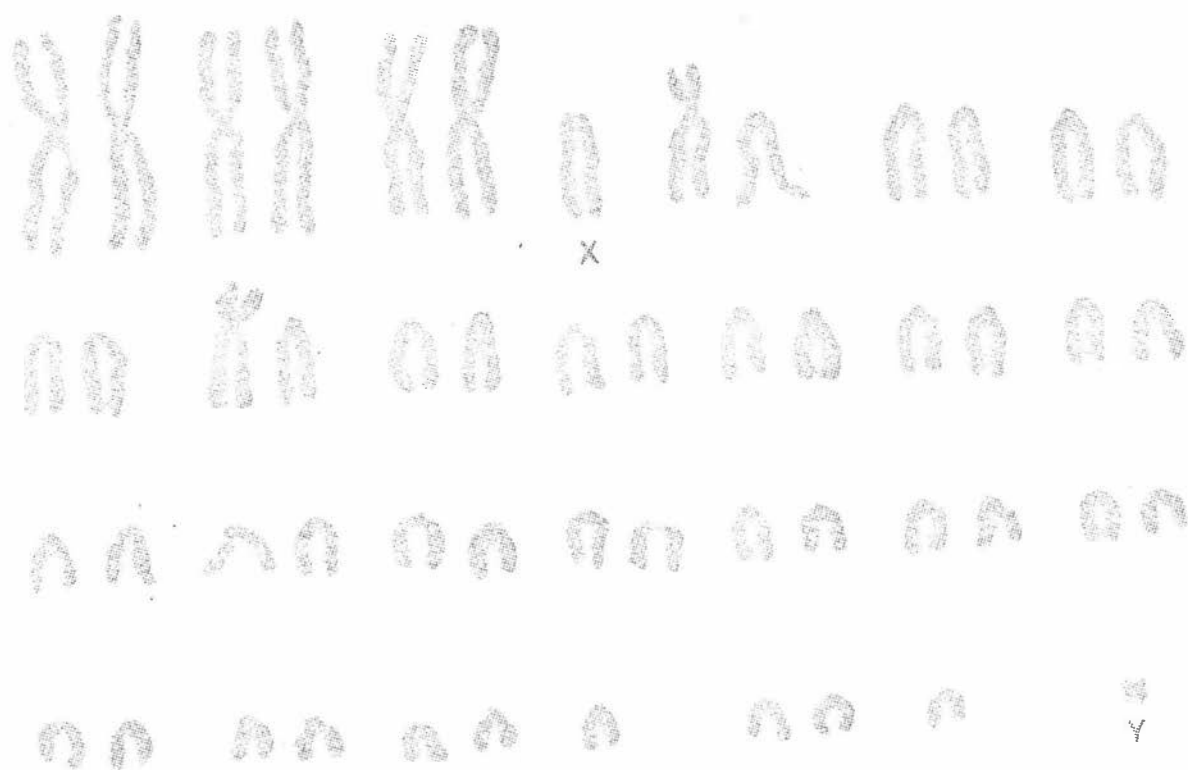
Table 1. Numbers of progeny of each category born as a result of mating B9/72^{a} to ewes of normal karyotype¹.

Karyotype	54XY	54XX	53XYt ₁ +	53XXt ₁ +	52XYt ₁ + t ₃ +	52XXt ₁ + t ₃ +
Expected ratio of progeny	1/8	1/8	2/8	2/8	1/8	1/8
Observed number of progeny	0	3	3	3	5	0

¹ The symbol

t₁ refers to the Massey I and t₃ to the Massey III translocations.

These were not distinguished in the single translocation carriers, i.e., 53XYt₁ + and 53XXt₁ +.



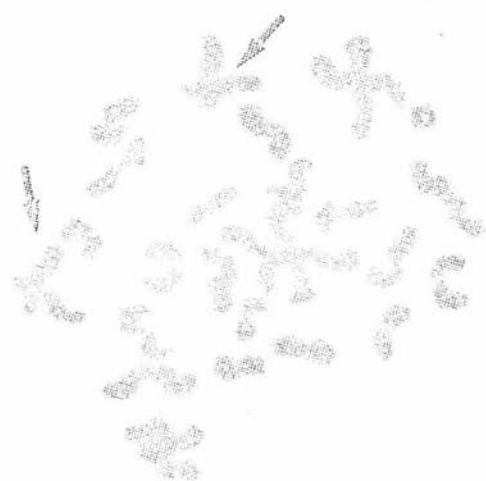
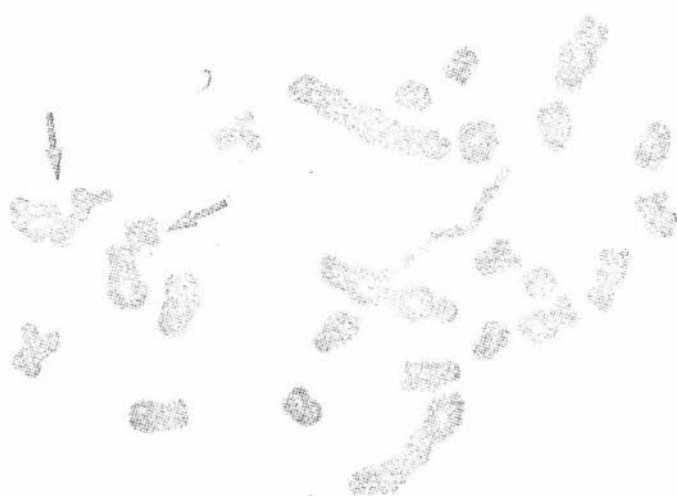
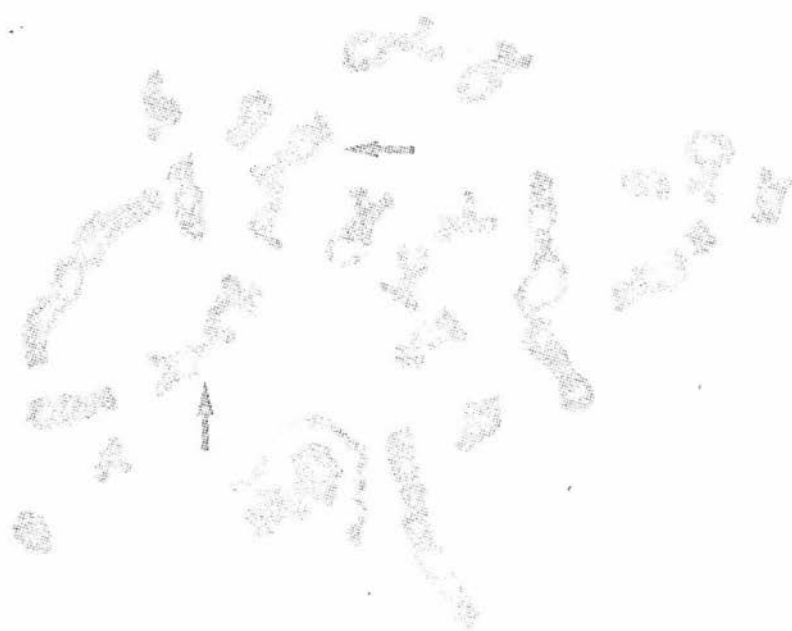
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Appendix XXII

DISTRIBUTION OF CELLS IN SPERMATOGONIAL METAPHASE (SM),
DIAKINESIS AND METAPHASE I (MI) AND METAPHASE II (MII)
IN ROBERTSONIAN TRANSLOCATION-CARRYING RAMS.

(After Skakkeback et al., 1973).

* Polyploid cells included in counts are recorded in brackets.

Ram Identi- fication	SM	MI	MII	Total	MII/MI Ratio
B46/69	0(0)	2(0)	0(0)	2(0)	0.0
B68/69	2(0)	64(7)	9(2)	75(9)	0.14
B12/69	1(0)	37(4)	7(1)	45(5)	0.19
53,xy,t ₁ B53/69	2(0)	279(48)	70(12)	351(60)	0.25
235/67	3(1)	13(2)	11(2)	27(5)	0.85
B26/70	0(0)	10(0)	0(0)	10(0)	0.0
A182	7(0)	69(24)	81(22)	157(46)	1.17
B8/72	0(0)	55(3)	23(2)	78(5)	0.42
B71/69	1(0)	5(1)	0(0)	6(1)	0.00
Total	16(1)	534(89)	201(41)	751(131)	0.38
53,xy,t ₂ B25/72	1(0)	120(9)	74(13)	195(22)	0.62
B87/72	4(0)	416(21)	166(15)	586(36)	0.40
B68/72	1(0)	187(10)	83(18)	271(28)	0.44
B66/72	1(0)	299(8)	61(7)	361(15)	0.20
Total	7(0)	1022(48)	384(53)	1413(101)	0.38
Link 1966	0(0)	7(1)	4(0)	11(1)	0.57
HML99/58	7(1)	38(4)	20(10)	65(15)	0.53
B116/71	7(4)	78(11)	17(10)	102(25)	0.22
B13/72	7(0)	274(38)	270(64)	551(102)	0.99
53,xy,t ₃ B76/72	3(0)	140(10)	112(13)	255(23)	0.80
Total	24(5)	537(64)	423(97)	984(166)	0.79
B55/70	4(0)	213(19)	110(11)	327(30)	0.52
52,xy,t ₁ .t ₁					
A134	0(0)	96(12)	28(5)	124(17)	0.29
52,xy,t ₃ .t ₃					

Cytogenetics 11: 233-246 (1972)

Chromosome polymorphism and its possible implications in the select Drysdale breed of sheep¹

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Abstract

Within the elite Drysdale breed of sheep, an incidence of 26.3% of chromosome polymorphism, among a total of 327 animals studied cytogenetically, is described. Sheep were found which were both heterozygous, $2n = 53$, and homozygous, $2n = 52$, for a Robertsonian-type translocation. Pedigree examination of one flock suggests that the translocation chromosome had been present in some sheep of the Drysdale breed for several generations and probably since its formation. The long arms of the translocation chromosome are believed to incorporate a different acrocentric chromosome from that seen in the previously described Massey I translocation. Tentatively, the translocation has been named the Massey III translocation, and the implications of this high level of chromosome polymorphism are discussed in respect to the likely effects of impaired fertility and alternatively hybrid vigor. Two sheep which were translocation heterozygotes were found with varying degrees of segmental aplasia of their genitalia.

A familial Robertsonian translocation (Massey I) between a large and a small acrocentric autosome is now recognized in the New Zealand Romney sheep (BRUÈRE, 1969; BRUÈRE and MILLS, 1971). Of 180 Romney sheep studied previously, the incidence of the Massey I translocation was 4.6% (BRUÈRE and MILLS, 1971). A second autosomal translocation in sheep, also reported in the same study, has now been proved familial and also seen in yet another sheep (BRUÈRE, unpublished data).

In the present paper a much higher incidence of a morphologically similar autosomal translocation, though one we believe to be different from the Massey I translocation, is described in the several elite flocks of the Drysdale sheep. In this survey both structurally homozygous ($2n = 52$) and

¹ Supported by a grant from the Wellcome Trust of Great Britain.

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structurally heterozygous ($2n = 53$) translocation animals were found, as well as some sheep with the normal chromosome number of the domestic sheep (*Ovis aries*), namely, $2n = 54$. These findings of a possibly third type of Robertsonian translocation among domestic sheep raise both academic points on speciation and important questions on the likely effects on fertility both within this breed and in crossbreeding.

Materials and methods

The Drysdale sheep

The Drysdale sheep, which was evolved in New Zealand, produces a particularly coarse, noncrimped wool which is used extensively in the carpet manufacturing industry. The breed had its origin in research by Dr F. W. DRY on the presence of halo-hairs in the wool of the New Zealand Romney (RAE, 1969). DRY (1955a-c) was able to show that the abundance of halo-hairs was due to a dominant *N*-gene, and ultimately he was able to standardize the breed, so that the present purebred Drysdale carries a pair of *N*-genes. Besides dominance for halo-hairs, the presence of the *N*-gene also produces horns, which are present in both *NN* sheep and heterozygous *Nn* sheep (DRY, 1955b; FRASER, 1955). Horn growth is sex-limited, however, with large horns seen in Drysdale rams and usually small and fragile horns in ewes. Although derived mainly from two sources of Romney sheep, the Neilson Romney and Massey University Romneys, the Drysdale in its formation has been crossed with the Lincoln and Cheviot breeds (DRY, 1956).

In New Zealand the number of Drysdale sheep is strictly controlled by the requirements of the carpet industry. Four stud flocks are responsible for the production of all rams used in the multiplying flocks. These flocks originally derived their rams and ewes from the Massey University flock. Among the four flocks studied, only in the Massey flock were reliable pedigree records kept. In the other three pedigree flocks, the rams used and ewes mated in any one year were recorded only on a non-individual basis. In all four flocks only true homozygous, or *NN*, rams have been mated to *NN*ewes. The progeny of these matings are sold to commercial breeders for both pure breeding and crossbreeding with the New Zealand Romney. For purposes of discussion, the four pedigree flocks concerned are named by their respective districts, Massey, Utuwai, Pahiatua, and Pohangina.

The propositus ram

The present study resulted from a single structurally heterozygous Drysdale ram from the Utuwai flock (fig. 2). This ram was presented for examination because he had small testicles similar to those described by BRUÈRE (1969) and BRUÈRE and MILLS (1971). The right testis of this ram had no epididymis. Both testes were atrophic and smaller than those of a normal ram (fig. 1). Histologically, they showed severe degeneration and hyalinization of the seminiferous tubules.

Pedigree follow-up

A total of 100 sheep, 71 ewes and 29 rams, from the Utuwai flock, 49 sheep, 24 ewes and 25 ram hoggets, from the Pahiatua flock, and 26 rams from the Pohangina flock were



Fig. 1. Left and right testes of the propositus ram showing atrophy of both and absence of an epididymis of the right testis.

examined cytogenetically. Because of the very reliable records of the Massey University flock, it was possible to examine the entire *NN* Drysdale mating-ewe flock and a number of *NN* rams. In all, a total of 327 animals, 224 ewes and 103 rams, were studied (table I).

Cytogenetic methods

Leukocyte cultures were prepared by a standard whole-blood technique and cultured for 48 h. Usually, from 5 to 10 metaphases were counted from each culture, but in certain cases many more were examined. For comparative karyotype work, up to 20 photographic karyotypes were prepared from both the homozygous, $2n = 52$, and heterozygous, $2n = 53$, rams and ewes. A number of karyotypes were prepared also of the Massey I translocation. Karyotyping was done without prior knowledge of which animal the operator was karyotyping. Before their final mounting, all karyotypes were examined by two persons. It was appreciated that this was not a finite method for chromosome identification, but it proved as accurate as present means of identification of individual sheep chromosomes would allow. Autoradiography did not help in the present identification because of similarities of chromosome replication of the larger acrocentric chromosomes. It did show, however, that the short arms of both translocations are early replicators, as described previously for the Massey I translocation (BRUÈRE and MILLS, 1971).

Mitotic metaphases of both Romney and Drysdale homozygotes were processed for Giemsa banding by the techniques of SUMNER et al. (1971) and SEABRIGHT (1971). Meiotic chromosome studies were carried out on testicular material from both Romney and Drysdale heterozygotes using a modification of the technique of EVANS et al. (1964).

Results

The overall incidence (26.3%) of chromosome polymorphism from this survey is summarized in table I. These data do not include the 1971 progeny

Table 1. Incidence of translocation heterozygotes and homozygotes from three Drysdale flocks (overall incidence = 26.3 %).

Chromosome number	Rams			Ewes			Incidence (male and female)	
	54	53	52	54	53	52	2n = 53	2n = 52
Massey							27	3
22 M	16	4	2	100	23	1	146	146
124 F							(18.5%)	(2.1 %)
Utuwai							32	3
29 M	21	7	1	44	25	2	100	100
71 F							(32%)	(3.0%)
Pahiatua							13	0
25 M	17	8	0	19	5	0	49	49
24 F							(26.5%)	(0.0%)
Pohangina							4	
26 M	22	4	0	—	—	—	26	—
							(15.4%)	
Total							76	6
(321)	76	23	3	163	53	3	321	321
							(23.7%)	(1.9%)

of the Massey translocation-bearing ewes, because addition of these to the data would have created a bias. From the sample of 321 sheep, including 102 rams and 219 ewes, a total of 82 translocation animals was recorded, including 76 heterozygotes with 53 chromosomes and 6 homozygotes with 52 chromosomes. In addition to the 82 translocation-bearing animals listed in table 1, a further four such animals were found among six lambs from the 1971 progeny of the Massey translocation-bearing heterozygous ewes, the latter being mated in this instance with chromosomally normal rams. In all, 86 translocation animals were recorded among the 327 animals studied.

Photographic karyotypes of the translocation from the Drysdale sheep, including karyotypes of both homozygous, $2n = 52$, and heterozygous, $2n = 53$, rams and ewes were compared with karyotypes from similar animals with the Massey I translocation. In all cases, the translocation chromosomes karyotyped in different positions. The Massey I translocation chromosome or chromosomes in the homozygous animals had larger long arms than the Drysdale translocation chromosome and karyotyped so that their long arms

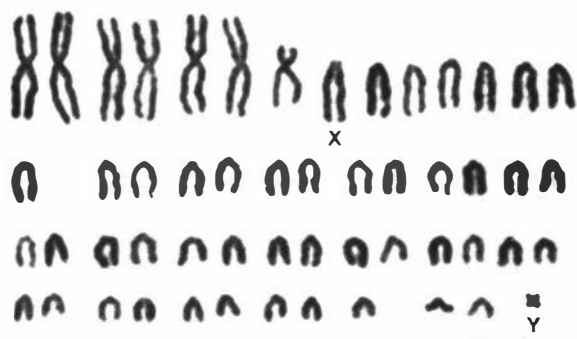


Fig. 2. Karyotype of the propositus ram which was heterozygous ($2n = 53$) for the Massey III translocation. Aceto-orcein; reproduced at $1000\times$.



Fig. 3. *a*. Karyotype of the Massey I translocation from a homozygous ram ($2n = 52$). *b*. Karyotype of the Massey I translocation from a homozygous ewe ($2n = 52$). In both instances the first pair of acrocentric chromosomes probably corresponds to the long arms of the translocation chromosomes. Aceto-orcein; reproduced at $1000\times$.



Fig. 4. *a*. Karyotype of the Drysdale (Massey III) translocation from a homozygous ram ($2n = 52$). *b*. Karyotype of the Massey III translocation from a homozygous ewe ($2n = 52$). In both instances either the fourth or fifth pair of acrocentric chromosomes probably corresponds to the long arms of the translocation chromosomes. The short arms may be identical to those of the Massey I translocation. Aceto-orcein; reproduced at $1000\times$.

corresponded to chromosomes 1 or 2 of the acrocentric group (fig. 3). The Drysdale translocation, though morphologically similar, karyotyped at position 4 or 5 of the acrocentric group (fig. 4). The short arms could not be differentiated and thus, from present observation, are similar to the Massey I translocation. Similarly, the many metaphases from both breeds of sheep, stained for Giemsa banding, showed indistinguishable patterns on the larger acrocentric chromosomes likely to be involved in the present translocations. In both breeds the short-arm banding was similar and inconclusive.

Meiotic studies of 165 primary spermatocytes in the Romney heterozygotes and 64 primary spermatocytes in the Drysdale heterozygotes showed 144 trivalents in the Romney and 46 trivalents in the Drysdale which could be distinguished clearly. The majority of these metaphases were in the later stages of diakinesis (fig. 5). In both breeds of sheep two different

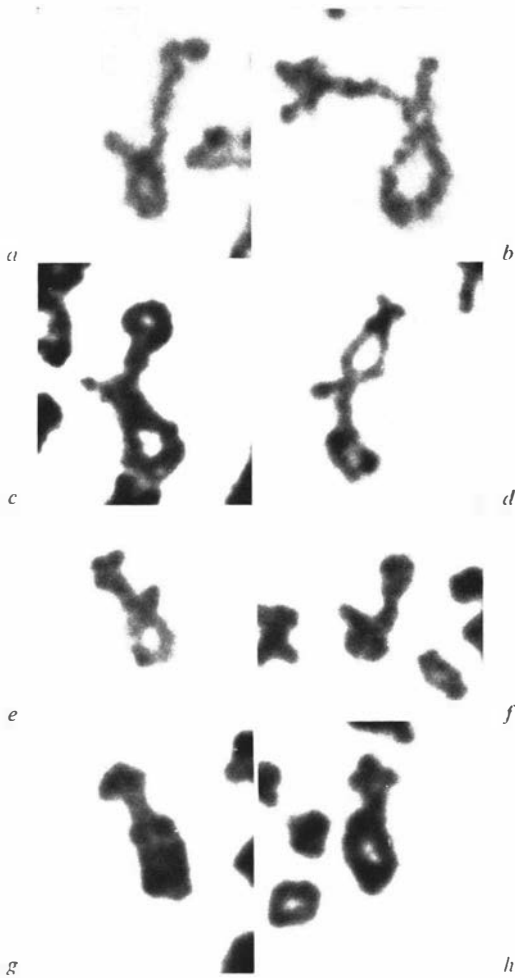


Fig. 5. a. Late diplotene trivalent (Romney). *b.* Late diplotene trivalent (Drysdale). *c, e, g.* Diakinesis metaphase trivalents (Romney). *d, f, h.* Diakinesis metaphase trivalents (Drysdale). Aceto-orcein; reproduced at 2000 $\times$.

trivalent configurations were predominant. None of these trivalents showed characteristics indicating that the chromosome components were different. Although the trivalents seen in both breeds were morphologically similar, it is considered not unlikely that the chromosomes involved were different, because Robertsonian translocations of the submetacentric type in both

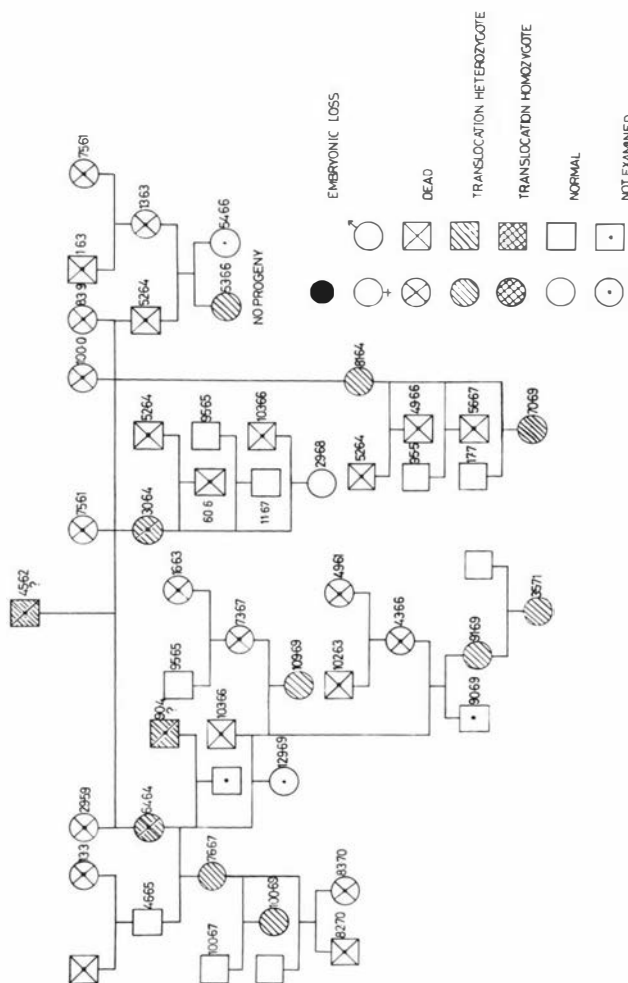


Fig. 6. Recorded pedigree from the Massey Drysdale flock showing the relationship of the various translocation animals.

cattle (GUSTAVSSON, 1969) and man (HULTÉN and LINDSTEN, 1970) have shown similar trivalent morphology to those in the Romney and Drysdale breeds of sheep. Therefore, from present evidence it is concluded that the translocation demonstrable in the Drysdale sheep is possibly different from the Massey I translocation because of a different long-arm component, albeit the short-arm component is similar and perhaps even identical. For identification, it is tentatively named the Massey III translocation.

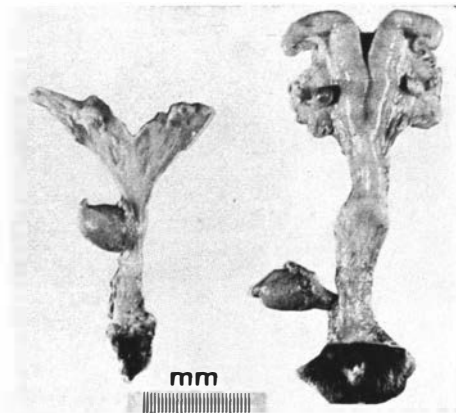


Fig. 7. Genitalia of ewe 97/66, left, compared with the genitalia of a normal ewe, right.

From the pedigree (fig. 6) ewe 97/66, a translocation heterozygote, had never left a lamb. This ewe apparently came into estrus and is reported to have taken the ram. A laparotomy revealed that two normal ovaries were present. However the horns and body of the uterus were absent (fig. 7). The genitalia of the ewe showed segmental aplasia similar to that recorded in white heifer disease of cattle (SPRIGGS, 1946).

The extensive pedigree follow-up (fig. 6) showed that at least three rams, two certainly (90/64 and 83/68) and one probably (45/62), were translocation heterozygotes and had been responsible for the dissemination of the translocation chromosome in their respective generations. From the present pedigree there is insufficient data to draw any conclusions concerning meiotic drive of this translocation chromosome. Where the karyotype of the parent was known, a total of 12 translocation heterozygotes and 13 normal animals resulted from matings of heterozygous ewes to normal rams. Three homozygous sheep, two rams (12/70 and 20/70) and one ewe (92/68), resulted from heterozygous $\times$ heterozygous matings.

Discussion

The 26.3% level of chromosome polymorphism described is higher than any previously reported in domestic animals. (RARY et al. [1968] reported an incidence of 53.7% of chromosome polymorphism among European wild pigs.) From the available pedigree data it is evident that the translocation

chromosome has been present in the Drysdale breed for a number of years and can be traced positively back to 1962. Since the breed had its first origins in 1931 (RAE, 1969), it is not unlikely that the translocation chromosome was either present in or arose in one of the earlier generations. Its breed derivation will never be known, for both Romney and Cheviot breeds have been used in the development of Drysdale sheep.

Genetic drift in such a small group of animals as the Drysdale sheep could be quite rapid. This is emphasized by the presence of several homozygous rams and ewes and no evidence of selection against the translocation for phenotypic reasons, as in the case of the Massey I translocation (BRUÈRE and MILLS, 1971). From the pedigree follow-up, two rams (83/68 and 90/64), both translocation heterozygotes, had been sold and used extensively in commercial flocks. Therefore, it becomes evident that this translocation, like the Massey I translocation, is distributed widely among the Drysdale breed and its crosses. Accumulated data from both within New Zealand and from overseas would suggest that chromosome polymorphism in domestic sheep is confined possibly to certain breeds and strains within breeds where close mating has been practiced (table II). From these data it can be seen that a total of 19 different breeds of domestic sheep (*Ovis aries*), totaling 1201 animals from seven different countries, have been examined. In only two instances has chromosome polymorphism been established, in the Drysdale breed and in one flock within the New Zealand Romney (BRUÈRE and MILLS, 1971).

The possible morphological difference between the present chromosome translocation (Massey III) and the previously described Massey I translocation is likely to have reproductive implications, particularly if the short arms of both translocation chromosomes are homologous. The recent, excellent model of mating widely divergent karyotypes in the tobacco mouse (*Mus poschiavinus*) described by TETTENBORN and GROPP (1970) and GROPP et al. (1970) shows that multiple chromosome polymorphism, even though the N. F. (MATTHEY, 1945) is constant, may not lead necessarily to normal segregation and fertility. In the previous example, F_1 mice resulting from mating *M. poschiavinus* ($2n = 26$) with *M. musculus* ($2n = 40$), when themselves mated, showed a 50% fertility reduction. Meiotic nondisjunction and embryonic aneuploidy were considered the major cause of the reduced fertility. The present chromosome translocations, if each were present in the heterozygous state in either one or the other of both mated breeds of sheep, could lead to chain quadrivalent formation involving the two translocation chromosomes and their long-arm homologs. Present evidence

Table II. Known chromosome studies on domestic sheep (*Ovis aries*) with $2n = 54$ and the incidence of Robertsonian translocations.

Reference	Breed	No. of sheep studied	Chromosome number
BRUÈRE and MILLS (1971) (recorded from six authors)	various	246	all animals $2n = 54$
DAIN (1970) and DAIN (1971, personal communication)	Clun Forest, Clun Forest $\times$ Hampshire, Finnish Landrace, Merino, Welsh Mountain, Soay	322	all animals $2n = 54$
BRUÈRE (unpublished)	Tasmanian Merino	4	all animals
	N.Z. Romney	120	$2n = 54$
BRUÈRE and MILLS (1971) (Massey I translocation)	N.Z. Romney	180	4.6% of animals $2n = 53$
BRUÈRE and MILLS (1971) and BRUÈRE (unpublished) (Massey II translocation)	N.Z. Romney	2	$2n = 53$
Present study (Massey III translocation)	Drysdale	327	26.3% of animals $2n = 53$ and $2n = 52$
Total		1201	

would suggest that such a quadrivalent may become disorientated during meiosis and lead to spermatocyte arrest (LEWIS and JOHN, 1963). It must be emphasized also that although reduced fertility could result from meiotic misdivision of a structural heterozygote, genetic heterozygosity too could have an effect on the incidence of misdivision during gametogenesis or cause an early embryonic death (TETTENBORN and GROPP, 1970). This point is emphasized also by the reduced fertility recorded in male mice heterozygous for a metacentric chromosome (EVANS et al., 1967) and further by the increased return to service of daughters of bulls heterozygous for the $1/29$ translocation of Swedish cattle (GUSTAVSSON, 1969). To test these possibilities, mating experiments are now in progress.

In view of the absence of either regularly detrimental phenotypic changes of impaired fertility in association with the Massey III translocation, it

would be presumptuous to cull translocation-bearing animals from the present flocks in spite of the above possibilities, particularly since there could be an implicit advantage from the translocation. This last point has been suggested in some species, such as the white-throated sparrow (*Zonotrichia albicollis* [GMELIN]), where an extremely divergent karyotype may act as a form of selective breeding, ensuring heterozygosity and possibly preserving hybrid vigor (THORNEYCROFT, 1966). Furthermore, under conditions of natural selection in the plains woodrat (*Neotoma micropus* [BAIRD]), it is suggested by BAKER et al. (1970) that natural selection may actually favor a polymorphic chromosome system for the above reasons. The wide diversity of karyotypes in the common rat (*Rattus rattus*) (YOSIDA et al., 1971) may suggest also the same point, since this species of rodent is developing with tremendous potency.

The large pedigree is published here in part to emphasize the fact that, as in man, a deeper understanding of the effects of Robertsonian translocations in domestic animals is likely to come from cumulative data. Similarly, the recent work of NADLER et al. (1971) in wild sheep populations show further the divergent karyotypes present within the genus *Ovis* and emphasize that, as with this study, more information is needed on the actual chromosomes involved in the various translocations described.

Acknowledgements

The authors wish to acknowledge the cooperation of B. EBBET, R. L. GARDNER, J. LINKLATER, and M. UDY for access to their pedigree flocks, Prof. A. L. RAE for pedigree details and other information on the Massey flock, BETSY F. BRUÈRE and ROBYN A. MILLS for some technical assistance, and MAUREEN MACDONALD for typing the manuscript.

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Reprinted from THE VETERINARY RECORD, June 9th, 1973. Pp. 615-618.

Autosomal Translocations in Two Exotic Breeds of Cattle in New Zealand

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Vet. Rec. (1973). **92**. 615-618

SUMMARY.—From a survey involving 48 cattle of several exotic breeds imported into New Zealand, two translocation bearing bulls have been found. One bull was of the Simmental breed and carried a chromosomal translocation distinct from those reported previously in cattle. The other chromosomal translocation was detected in a bull of the Blond d'Aquitaine breed from France and was similar to the 1/29 translocation already reported from several countries. The implications of these findings are discussed with a view to international co-operation on identification, distribution and effect of such translocations on domestic cattle and sheep.

Introduction

SINCE 1970 beef cattle from several exotic breeds have been imported into New Zealand from Britain, France and Australia. These animals have been and are to be

used in beef cattle improvement programmes under both private and government sponsorship. Breeds of cattle imported include: Blond d'Aquitaine, Charolais, Limousin, Maine Anjou, Pie Rouge (French Simmental), Simmental, South Devon, Santa Gertrudis and Murray Grey. The extension of the Charolais breed has taken place also by the importation of semen and ova from the United Kingdom and the number of Charolais cross cattle within New Zealand is now considerable. Main extensions of the other exotic breeds of cattle have not yet taken place.

In view of the report by Harvey (1971) on the presence of a 1/29 chromosome translocation in Charolais cattle in Scotland, it was decided to under-

take a pilot survey of some recently imported breeds in New Zealand. Through the courtesy of the New Zealand Ministry of Agriculture and Fisheries and certain private individuals, it has been possible to carry out cytogenetic studies on 48 animals. Already several studies, both extensive and individual, have shown the presence of two different types of Robertsonian translocations in domestic cattle (Table I). The exact reproductive and genetic implications of such translocations are still somewhat obscure, although Gustavsson (1969 and 1971) has reported an increased return to service of daughters of translocation carrying bulls of the Swedish Red and White breed. Studies in other domestic species including pigs (McFee, Banner and Rary, 1966; McFee and Banner, 1969) and sheep (Bruère, 1969; Bruère and Mills, 1971; Bruère, Chapman and Wyllie, 1972, and Bruère, 1972) have shown no conclusive evidence of deleterious effects of Robertsonian translocation. The two translocations described here in two additional breeds of cattle, add further information to much needed data on these apparently frequent chromosome abnormalities of domestic animals.

TABLE I
TRANSLOCATION STUDIES ON DOMESTIC CATTLE

Author	Breed	Country	Translocation	No. of cattle of breed with translocation studied
Amrud (1969)	Norwegian Red Breed	Norway	1/29	430
Gustavsson (1969)	Swedish Red & White	Sweden	1/29	1916
Harvey (1971)	Charolais	Scotland	1/29	23
Herschler & Fechheimer (1966)	Friesian	U.S.A.	1/29	4
Pollock (1972)	Friesian	England	2/4	—
Rieck <i>et al.</i> (1968)	German Red	Germany	1/29	4
Present Study	Pied Cattle	New Zealand	11-12/15-16	13
	Simmental (origin Scotland)			
	Blond d'Aquitaine (origin France)		1/29	5

Materials and Methods

Blood samples for lymphocyte cultures were collected from cattle at various centres, all of which were over 200 miles from our laboratory. The samples were transported by air in ice and in each case the maximum time from collection to culture preparation was 10 hours. This latter point is of interest, as satisfactory results were obtained from 48 of 51 samples collected. Lymphocyte cultures were prepared by a standard whole blood technique (Basur & Gilman, 1964), incubated at 37° C and harvested at 48 hours in the usual way. Mitotic chromosomes from suitable metaphases were counted in each case and six photographic karyotypes were prepared from the animals with the translocations.

Results

The diploid chromosome number for cattle is $2n=60$. The 58 autosomes are all acrocentric and the two sex chromosomes are distinctively submetacentric. The X chromosome is larger than the Y chromosome. The results of the cytogenetic survey of the five different breeds of cattle are summarised in Table II. Two animals from different breeds, namely the Simmental and the Blond d'Aquitaine, were found to be translocation heterozygotes with $2n=59$ chromosomes.

TABLE II
NUMBERS AND BREEDS OF CATTLE EXAMINED

Breed	Number of cattle examined (Number of translocations*)		
	Bulls	Cows	Total
Charolais	11 (0)	10 (0)	21 (0)
Simmental	12 (1*)	1 (0)	13 (1*)
Limousin	5 (0)	0	5 (0)
Blond d'Aquitaine	5 (1*)	0	5 (1*)
Maine Anjou	4 (0)	0	4
Total	37 (2*)	11	48 (2*)

In each a centric fusion of two autosomes was found forming a translocation chromosome. From the Simmental bull a total 75 metaphases from two cultures were counted and in each the translocation chromosome was seen. The translocation in the Simmental bull was metacentric (Fig. 1) and consistently karyotyped in the position corresponding to chromosomes 11 or 12 for the long arm component and chromosomes 15 or 16 for the short arm component. Although metacentric in nature, this translocation chromosome is different from that reported by Pollock (1972). In the latter case, the long arm chromosome was probably number 2 and the short arm chromosome number 4.

In the Blond d'Aquitaine bull, the translocation included the largest of the acrocentric autosomes fused with probably the smallest acrocentric auto-



Fig. 1. — Karyotype from the heterozygote 59, XY+ Simmental bull.

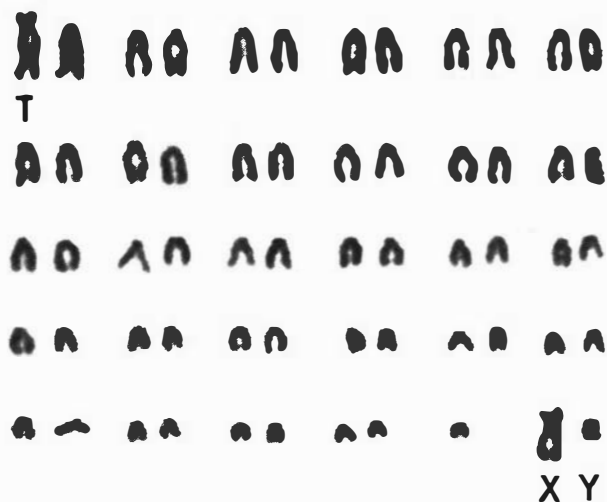


FIG. 2.—Karyotype from the heterozygote 59, XYt+ Blond d'Aquitaine bull.

some and was seen in a total of 89 counted metaphases from two cultures. Studies of six karyotypes showed these two chromosomes to be involved consistently in the translocation. (Fig. 2.) It is concluded, therefore, that this latter translocation is the same as the 1/29 translocation first reported by Gustavsson and Rockborn (1964) and now reported in several breeds of cattle with a wide geographical distribution. (Table I.)

Neither bull showed any phenotypic abnormality and as both animals were yearlings their fertility was not assessed.

Discussion

The presence of a third distinct type of Robertsonian translocation in another breed of European cattle is of considerable interest. In the present study it was not possible to trace the sire and dam of the Simmental bull with the chromosome translocation. However, it would be possible for such studies to be pursued on these cattle, which are in Scotland. At present, no progeny of this bull are available in New Zealand, so that the familial nature of this translocation is unconfirmed.

The chance finding of the previously reported 1/29 translocation in another different and widely disseminated breed of cattle is particularly interesting. Although this translocation has been transmitted through many generations of Swedish cattle (S.R.B.) (Gustavsson, 1969), its presence in six other diverse breeds of cattle renews the speculation about its origin. Gustavsson (1969) has suggested that there is little evidence for its *de novo* formation in domestic cattle. He has suggested further that in the Swedish Red and White cattle this translocation may have had its origin from the Ayrshire breed in Scotland, sometime prior to 1930. However, in domestic sheep the finding of translocation/normal mosaicism (Bruère & Mills, 1971) and the several examples of such mosaicism in man (Turpin & Lejeune, 1964; Zellweger & Abbo, 1965) suggest that *de novo* formation of such

translocations is possible during either gametogenesis or embryogenesis.

The many cases of the 1/29 translocation now reported in several breeds of cattle in different countries, also strengthen the argument for the occasional *de novo* formation of centric fusion translocations in cattle, sheep and dogs which have a high number of acrocentric chromosomes. Further, its presence in yet another breed of cattle supports the suggestion of Bruère (1972) that, as in man, specific chromosomes are probably involved in such translocations on an almost non-random basis (Hecht & Kimberling, 1971).

The implications of Robertsonian translocations in domestic animals are not clearly understood and the findings of Gustavsson (1969) and Gustavsson (1971) need to be assessed in other breeds of cattle. Further, the points raised by Bruère *et al.*, (1972) from studies on sheep suggest that such translocations could have distinct advantages as well as disadvantages in some breeds. Therefore the exclusion of translocation bearing cattle by some importing countries could be unwarranted; also if such translocations do arise on a *de novo* basis the problem of control by chromosome screening of cattle would be difficult. Therefore it is suggested that the breeding performances of translocation bearing cattle and sheep need closer study and the cytogenetic screening of bulls used for artificial breeding is in this respect very important. From present increasing evidence there is clearly a distinct need for international co-operation on reporting, identification and accumulation of data on Robertsonian translocations of domestic cattle and sheep.

Acknowledgments.—The authors wish to thank the Wellcome Trust for financial assistance, also Miss Rosalie Morris for expert technical assistance and Mrs. S. West for typing the manuscript.

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Résumé

Dans une étude comportant 48 boeufs de plusieurs races exotiques, importés en Nouvelle Zélande, on a trouvé deux boeufs qui démontraient une translocation. Un boeuf était de la race Simmentale et portait une translocation chromosomale différente de celles trouvées dans les comptes rendus préalables. L'autre translocation chromosomale fut aperçue dans un taureau de la race Blond d'Aquitaine venue de la France, et ressemblait à la translocation 1/29 déjà exposée dans plusieurs pays.

On va discuter ci-dessous la portée de ce qu'on a découvert dans l'intention d'encourager la coopération internationale au sujet de l'identification, la distribution et l'effet que telle translocation ont chez les boeufs et les moutons domestiques.

Zusammenfassung

Von einem Überblick, 48 Rinder von mehreren exotischen Rassen enthaltend, die in Neuseeland eingeführt wurden, sind zwei Rinder mit Translokation gefunden worden. Ein Stier war von der simmentalischen Rasse und trug eine Chromosomen-Translokation, unterschieden von denen die früher in Vieh berichtet worden sind. Die andere Chromosomen-Translokation wurde in einem Stier von der blonden d'Aquitaine Rasse aus Frankreich und glich der Translokation 1/29 von vielen Ländern schon berichtet. Die Folgerungen dieser Entdeckungen werden erörtert in der Absicht gegen internationale Mitwirkung von solchen Translokationen in inländischen Rinder und Schafe zustande zu bringen.

Appendix

XXV Analysis of MII cell count distributions (Chi-square test)

Comparison			χ^2	d.f.	p
Hetero t_1	Vs	Hetero t_2	12.99	4	< 0.05
Hetero t_1	Vs	Hetero t_2 + hetero t_3	9.93	4	< 0.05
Hetero t_2	Vs	Hetero t_3	28.12	4	< 0.001
Hetero t_3	Vs	Hetero t_1 + hetero t_2	24.85	4	< 0.001
Hetero t_1 +		Normal +			
Hetero t_2 +	Vs	Homo t_1 +			
Hetero t_3		Homo t_3	64.89	4	< 0.001

Appendix XXVI

Personal Communications

The personal communications cited in the text, involved the following persons:

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