AN INVESTIGATION OF A CENTRIC FUSION (ROBERTSONIAN) TRANSLOCATION OF SHEEP

by

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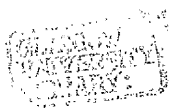

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DECLARATION

I hereby declare that this thesis embodies the results of my own personal work, that it has been composed by myself and that it does not include work offered for any degree at the University of Glasgow or another University.

SUMMARY

The thesis is divided into nine sections. Section I presents a review of the important developments in the field of cytogenetics applicable to the investigation. In this context, the mechanisms involved in leucocyte cultures were discussed, together with theories concerning the mode of formation of differential staining patterns on chromosomes. Also included in Section I is a review of veterinary cytogenetics which emphasized the relative importance of centric fusion translocations in domestic animals.

Section II concerns the examination of the chromosome complement of pre-implantation blastocysts. Blastocysts were collected both from heterozygous male x normal female and normal male x heterozygous female matings. The majority of collections took place post mortem but collection from the live animal by laparotomy was investigated. Information on sex ratio and translocation segregation was obtained from this work.

Section III describes the effect, on lambing performance, of heterozygosity for the Massey I translocation in the male. Information was obtained on lambing percentage and incidence of abortion and stillbirths. In addition, sex ratio and translocation segregation in the live-born lambs were calculated.

Section IV is concerned with male meiosis. Preparations were made from a normal ram and rams heterozygous and homozygous for the Massey I translocation. The degree of non-~~disjunction~~ DISJUNCTION at the second meiotic metaphase was calculated and compared in the different groups of animals, together with the chiasmata frequencies at diakinesis.

Section V describes the identification of the individual chromosomes comprising the Massey I translocation. Identification was by means of G-bands and as a corollary of this a G-band idiogram was produced for sheep chromosomes. The Massey I translocation was further categorised by means of C-banding.

Section VI is the tables, VII an appendix which describes in more detail some of the routine and less important procedures, VIII is the list of references and Section IX the figures.

The work represents the first extensive study of a centric fusion translocation in one of the larger domestic animals. It is argued that whilst the results from the examination of individual aspects of the problem were in themselves inconclusive, collectively, they demonstrated that in the male, heterozygosity for the Massey I translocation did not result in a reduced fertility. Examination of pre-implantation blastocysts failed to reveal zygotes with an unbalanced karyotype and no other chromosomal abnormalities were detected. Examination of lambing performance showed no deviation from the normal sex ratio or the expected translocation segregation ratios. Meiotic studies did show, however, that non-disjunction was occurring at a higher level than in the normal ram and that it was associated with the translocation chromosome. The question remained, therefore, as to whether the unbalanced secondary spermatocytes failed to develop to spermatozoa or whether, on maturation, they were incapable of fertilisation.

The Massey I translocation was identified as a 4/26 translocation by comparison with the G-band idiogram. C-band staining showed that there were two blocks of centromeric heterochromatin, one on either side of

the centromere, indicating little or no loss of DNA. It was stressed however, that this could not be taken, per se, to indicate retention of both centromeres. It was suggested that a better understanding of the structure of the centromere of centric fusion translocations would lead to a better understanding of the likely behaviour of such chromosomes during cell division. This would enable a more accurate predication to be made of the effect on fertility.

SECTION I

INTRODUCTION

INTRODUCTION

1.1. Leucocyte Cultures

One of the most important developments in the field of cytogenetics has been the utilisation of peripheral blood leucocytes as a source of cells for chromosome analysis. The technique depended on stimulating the thymic-dependent, "T" leucocytes to divide in culture and then blocking these dividing cells at metaphase. The breakthrough occurred when it was discovered that a mucoprotein extract of the Red Kidney Bean (Phaseolus vulgaris), Phytohaemaglutinin (PHA) acted as a mitogenic agent.

Whilst working on the problem of human leucaemia, Nowell (1960) noted the development of normal leucocytes in cultures. He found that after the third day of culture the number of mitoses rose dramatically. Subsequent investigations into this phenomenon showed that it was due to the use of PHA to separate white blood cells from whole blood. (Nowell 1960a). PHA as well as having haemaglutinating properties was also a strongly mitogenic agent.

Following this discovery, Nowell and his co-workers developed a method of leucocyte culture which combined the use of PHA with the hypotonic sodium citrate treatment first described by Hsu (1952) and the air drying technique of Rothfels and Siminovitch (1958). This has become the basic technique for blood cultures. (Moorehead, Nowell, Mellman, Batipps and Hungerford 1960).

Since this is such an important technique and one that is used extensively throughout the present work, it is perhaps worthwhile to discuss some of the principles involved in some detail.

Originally the buffy coat of separated whole blood samples was used as the source of leucocytes. This procedure was tedious and although effective for human peripheral blood cultures, it was not always satisfactory for use for blood cultures of domestic animals, particularly those of sheep and cattle where the buffy coat separation was difficult. The problem was overcome by using whole blood. Arakaki and Sparkes (1963) showed that satisfactory chromosome preparations could be obtained from very small inocula of whole blood. The following comments on leucocyte culture techniques are relevant to either separated or whole blood techniques.

1.1.1. Media

A variety of tissue culture media have been used for leucocyte cultures (Table I) and most workers have supplemented the basic tissue culture medium with plasma or serum. This is interesting in the light of recent theories concerning the mode of action of PHA which is discussed in detail later. There has been one report of inhibition of mitotic activity due to the addition of serum to the medium (Yoshikura 1972). Yoshikura found that when fresh medium supplemented with calf serum and colcemid was added to a culture of human kidney cells there was a delay in the mitotic accumulation using concentrations of 25%, 10%, 20% and 40% calf serum. Yoshikura found that the delay was directly dependent on the concentration of the added fresh serum. The same result was obtained, not only with different batches of calf serum but also with mouse serum. This is the only paper known to the present writer concerning serum-inhibition of cultures. It may well be that the phenomenon is more common but because of the transient nature of the inhibition (the maximum effect was seen 1.5 hours after treament) it is not usually detected.

Genest and Auger (1963) made a systematic comparison of two media used

for leucocyte cultures, Eagle's MEM and medium TC 199, and found both produced adequate cultures although TC 199 was at a disadvantage in that variations of pH under culture conditions necessitated daily re-adjustments. Both media tended to produce a plaque formation of leucocyte growth which was attributed to a relatively high calcium concentration in the medium. To overcome this drawback Genest and Auger recommeded the use of Eagle's MEM for suspension culture.

Peter (1971) compared three types of Eagle's medium; Eagle's MEM; Eagle's Modified Medium and Eagle's Basal Medium. They found the Basal Medium to be unsatisfactory for human leucocyte cultures. Harvey (1969) found that swine lymphocyte growth rates were somewhat erratic in TC 199 and the Weymouth's medium gave a more consistently satisfactory result.

It would seem therefore, that different media should be tested for suitability when bloods from various species are to be cultured and that there is no absolute recommendation to be made as to the type of medium to be used.

1.1.2. Action of Phytohaemaglutinin

The Phytohaemaglutinin (PHA) used by Nowell was a partially purified mucroprotein extract prepared from the Red Kidney Bean, Phaseous vulgaris. Nowell had shown that only those cultures containing leucocytes which had been obtained from whole blood separated using PHA showed mitotic activity. Heating the PHA at 100°C for 30 mins. completely abolished its mitogenic activity as well as its ability to agglutinate erythrocytes, whereas heating at 65°C for 30 mins. did not affect either activity. Careful systematic analysis of the cell types found in the leucocyte cultures demonstrated that only one cell type, lymphocytes, was responding to the presence of PHA.

MacKinney, Stohlman, Brecher, 1962) In early culture samples small lymphocytes, monocytes and neutrophilic and eosinophilic granulocytes were easily identifiable but by 24 hours the granulocytes had degenerated. Between 26 and 72 hours of cell culture, the cell type remaining was mainly small lymphocytes with large mononuclear cells which were dissimilar to normal haemic or lymphoid cells. In addition, after 24 hours of culture the percentage of cells synthesising DNA increased rapidly as demonstrated by tritiated thymidine uptake. MacKinney and his co-workers (1962) were able to show that the increase in the number of cells was not due solely to those cells in the stage of DNA synthesis at the beginning of the culture period, and therefore some cells must have been stimulated to divide. The first mitotic figures were seen between 40 and 45 hours and thenceforth their numbers increased rapidly. The authors concluded that the dividing cells in cultures of peripheral blood were derived from a relatively large population of cells and since the granulocytes were shown to degenerate during the first 24 hours it was concluded that the lymphocytes were responsible for the growth. Later work has shown that PHA stimulates a population of lymphocytes dependent on or influenced by the thymus. (Keast and Bartholomaeus, 1972).

Younkin (1972) studied the action of PHA using a specific PHA antiserum. He found that individual lymphocytes began to synthesis DNA at various times after exposure to PHA. At least 6 hours of exposure to PHA was required before some cells were synthesising DNA at 72 hours. The PHA-responding cells required exposure to PHA for a variable period after which their response was independent of the presence of PHA. Eighteen to 24 hours after primary exposure, the lymphocytes could divide in the absence of PHA. Interestingly, Younkin also found that the daughter cells from PHA-stimulated cells could synthesis DNA in the presence of anti-PHA. That is, neither cell-bound PHA nor soluble

PHA was required to keep daughter cells in their cell division cycles.

Younkin concluded that the evidence suggested that PHA exerted its stimulatory effect while on the cell surface. Earlier, Simons, Fowler and Fitzgerald (1968) had suggested that the mitogenic moiety of the PHA molecule was closely related to, or identical with, the antigenic determinant sites and that some of these PHA antigenic sites were similar to those found amongst the antigenic configurations on the surface of lymphocytes.

An alternative theory has been put forward by Beckman (1962) who suggested that the primary site of reaction was located in the serum surrounding the cells. The role of serum in activation of lymphocytes by PHA has been investigated by Forsdyke (1973). He found that an optimum response required an optimum ratio of PHA to nondiffusable serum macromolecules. Lymphocyte activation was impaired in a macromolecule-depleted medium. The magnitude of activation in the depleted medium was only 39 $\pm$ 6% of that in the medium containing the serum macromolecules. The response was dependent on the PHA macromolecule ratio and Forsdyke suggested that a possible role of serum in the activation of lymphocytes was one in which macromolecules both competitively buffered cells against reaction with PHA and facilitated either the reaction of cells with PHA or the immediate response of cells once reaction with PHA had occurred.

There appear to be a number of other factors which affect the PHA-lymphocyte reaction. The dose response curve was found to be bell-shaped (Soren, 1973), but opinions on optimum concentrations have varied between 2.0 μg and 5.6 μg PHA /1x10^6 cells. (Simons et al., 1968; Keast and Bartholomaeus, 1972; and Soren, 1973). In addition, it has been shown that there is a significant reduction in the response of

lymphocytes of pregnant women. (Purtilo, Hallgren and Yunis, 1972). The reduction was most pronounced between 26 and 31 weeks of pregnancy and serum from such women could reduce the response of leucocyte cultures from non-pregnant women. It has also been shown that a porcine mycoplasma, M. arthritidis inhibited the lymphocyte transformation induced by PHA. The mycoplasma, which utilised argenine as an energy source inhibited PHA stimulation although the lymphocytes in these argenine-depleted cultures were not killed and would resume growth once the mycoplasma was removed. Dextrose utilising mycoplasma also inhibited PHA stimulation but to a much lesser degree.

As well as having mitogenic and haemagglutinating properties, PHA stimulates the production of interferon and lymphotoxin. It has been shown that the factors responsible for stimulating nucleic acid synthesis were entirely separate from those which induced interferon and lymphotoxin. (Haber, Rosenau and Goldberg, 1972). Hence it is possible that the multiple activities of PHA could be present in different preparations in various ratios. This is possibly the explanation for the results of Naspitz and Richter (1968) who compared the responses of human peripheral lymphocytes to three types of PHA and pokeweed, another mitogen. The three PHA types had different activities in terms of blastogenic potency.

1.1.3. Action of Colchine

The plant alkaloid, colchicine, has been used for a number of years by workers in the field of plant cytogenetics and their techniques were readily adapted for use with animal tissue. The mode of action of colchicine and its effects on the dividing cell have been extensively studied and are now understood in some detail. The following review of the literature is presented in order to indicate that under certain specific conditions, colcnicine and its analog, deacetymethylcolchicine,

(Colcemid) can be used to accumulate cells in metaphase and that these cells have the same chromosomal complement as untreated cells.

The blocking effect of colchicine was first recognised by Dustin (1934). Other workers had considered that colchicine accumulated metaphases by stimulating cells in prophase to enter metaphase. Levan (1938) introduced the term "c-mitosis" to indicate the mitotic deviation induced by colchicine treatment. He showed that the cell penetration of colchicine was rapid and that chromosomes lost their regular arrangement on the spindle within ten minutes of treatment with strong concentrations. He also confirmed that colchicine had no stimulatory effect on the cell. It was postulated that colchicine produced its effect by disrupting the spindle function. Inoue (1952) showed that the mitotic apparatus of the egg of Chaetopterus pergamentaceous, a marine annelid, was completely disrupted and eventually apparently disappeared, after colchicine treatment. The time taken for complete disorganisation of the micelles in the astral rays and spindle fibres depended on the concentration of colchicine used.

Later workers have shown exactly how colchicine acts on the mitotic spindle. The mitotic spindle fibres extend from the chromosomal centromeres towards the spindle poles. Bajer (1965) showed that at prometaphase the centromere had already divided and two sets of fibre bundles passed from one daughter centromere towards the same pole. The fibres from the other daughter centromere passed to the alternative pole. Using tritiated colchicine, Taylor (1965) demonstrated that there was an initial colchicine-penetration phase which was completed within ten to fifteen minutes. This confirmed Levan's earlier observations. There was then a second, rate-limiting step, in which colchicine was bound to a cellular component. Later workers showed that

the binding sites were the subunit proteins of the microtubules. (Borisy and Taylor, 1967). Binding occurred irrespective of whether a cell was dividing and did not involve chemical modification of the colchicine. The colchicine-binding protein was only approximately 10-20% of the total extractable protein isolated from the mitotic apparatus and was not, therefore, the main component of the mitotic apparatus. (Borisy and Taylor, 1967a) In summary these workers put forward the following theory in relation to the action of colchicine. Colchicine penetrated the cell membrane quite rapidly and became bound to protein subunits in the microtubules of the mitotic spindle. This was reversible but there was a critical level of binding above which the colchicine prevented the assembly of the subunits into microtubules and the cell was unable to form a functional mitotic spindle. The chromosomes thus accumulated at metaphase, being unable to pull apart in a normal anaphase. Electron microscope examination demonstrated that the microtubules of such cells were either absent or reduced in number.

The effect of this action has been shown to vary with the concentration of colchicine and the duration of action, (Inoue, 1952; Levan, 1954; Sasaki, 1961; Taylor, 1965; Herreros, Guerro and Romo, 1966; Cox and Puck, 1969; Rizzoni and Palitti, 1973), and also among species and type of tissue. (Fraser, 1963; Behnhe, 1965; Malawista and Bensch, 1967; Sohrab, 1972.) At concentrations greater than $5x10^{-8}$M mitoses accumulated at a maximum rate and the only effect of increasing the concentration was to reduce the time interval before accumulation began. (Taylor, 1965) At concentrations of below $2.5x10^{-8}$M there was an incomplete mitotic block, even when the cultures were allowed to continue for over 20 hours.

Herreros et al. (1966) deliberately produced polyploidy and endoreduplication in human lymphocyte cell cultures using colcemid at a final concentration of 1 µg/ml. ($1x10^{-6}$M). Two hours after the colcemid treatment, 90% of the culture medium was withdrawn and replaced and the cells were incubated for a further two to three days before harvesting. Rizzoni and Palitti (1973) examined the endoreduplication phenomenon in some detail in a Chinese hamster cell line. At a concentration of 10^{-4}M of colchicine the quantity and quality of endoreduplication was independent of the time of exposure. However, the endoreduplication did not appear until 19-20 hours after exposure. At colchicine concentrations of below $2.4x10^{-8}$M endoreduplicated mitoses were not observed.

Again using Chinese hamster cells, Cox and Puck (1969) investigated the induction of polyploidy by various concentrations of colcemid. They found that at concentrations of greater than 0.03 µg/ml. ($3x10^{-8}$M) with exposure times of 24 hours, most of the cell population was polyploid. However, concentrations of 0.01 µg/ml. ($1x10^{-8}$M) produced no visible effect on the chromosome number of the cell population.

The accumulated evidence suggests, therefore that high concentrations and prolonged exposure to colchicine or colcemid can cause abnormal metaphases in culture. These abnormalities develop when the affected cell is allowed to continue into a new cell cycle. In leucocyte cultures for the examination of metaphase chromosomes, exposure to colchicine or colcemid is not usually longer than from 2-3 hours, after which the cells are immediately harvested and fixed. This is a shorter period than any of the stages in the cycle of cells investigated by Bender and Prescott, (1962) so that abnormal figures do not have time to develop. Therefore, the use of colchicine for examination of

normal chromosome complements is justifiable.

1.1.4. Hypotonic Treatment

The arrested leucocyte cultures are subjected to a hypotonic solution before fixation. This has the double effect of haemolysing red blood cells if whole blood cultures have been used and also of swelling the lymphocytes and enhancing chromosomal separation. The technique was first described by Hsu (1952) who discovered by accident that a hypotonic tryode solution produced good spreading of the chromosomes. Since then a number of different solutions at various concentrations and length of incubation have been described. (Table II)

A number of hypotonic solutions used by various workers were compared by Genest and Auger (1963). Their main conclusion was that solutions containing sodium chloride were ineffective. Bruere (1966) found that exposure to 1% sodium citrate improved the staining of sheep chromosomes as compared with a one in ten solution of Hank's balanced salt solution. Harvey (1969) preferred a 0.3% solution of sodium citrate for 25 mins. for pig leucocyte cultures. Hungerford (1965) recommended 0.075 M potassium chloride as hypotonic treatment for human leucocyte cultures. He considered that the potassium chloride enhanced the stainability of the chromosomes with aceto-orcein. Potassium chloride is probably now the most commondly used hypotonic solution.

1.1.5. Fixation

The final fixation of the leucocyte cultures is an important process in that it affects the morphology of the chromosomes, their ability to stick to the slide and their receptivity to the stain. Rothfels and Siminovitch (1958) used Carnoy's fixative and recommended that this should be added slowly to the pellet of cells and that it was better to delay breaking up the pellet since rapid and immediate dispersal of

the cells caused clumping of the chromosomes. Genest and Auger (1963) agreed that better spreading of the chromosomes was obtained by fixing the intact clump of cells with acetic ethanol. However, other workers, whether using Carnoy's or a 1:3 acetic acid:methyl alcohol fixative have stressed the necessity of rapid fixing by dispersal of the cells. (Arakaki and Sparkes, 1963; Hungerford, 1965) There is also a divergence of opinion as to the value of chilling the fixative before use. Ford and Hamerton (1956) and Hungerford (1965) recommended chilling, whereas Rothfels and Siminovitch (1958) and Genest and Auger (1963) found that chilling had no advantage over treatment at room temperature.

1.2. Chromosome Identification by Means of Differential Staining.

Once techniques were available to produce well spread and well defined metaphase chromosomes it became possible to establish with certainty the normal chromosome number and morphology for a species. This in turn meant that any gross deviations from normal could be detected. However, the problem of identification of individual chromosomes remained.

Individual chromosomes can be characterised by the length of their arms or by their total length and arm ratio. However, there is considerable variation between homologues of the same nucleus, (Patau, 1965) and the errors involved in measurement can be quite high. Bruere and McLaren (1967) recorded a marked dissimilarity in length between homologues of chromosome number 1 in the sheep.

Chromosome markers such as satellites on the short arm of acrocentric chromosomes have been used to identify homologous pairs in man. (Denver Report, 1960) However, satellites were subsequently

identified on all the smaller chromosomes of the D and G groups. Furthermore, homologous chromosomes often differed in the size of their satellites (Patau, 1965) so that subdivision on this basis was not possible in man. Identification of one pair of the small group of E chromosomes in the cat was identifiable on the basis of satellites. (Chu, Thuline and Norby, 1964)

Secondary constrictions have also been used to identify homologous chromosomes in man. (London Report, 1963) Bruere and McLaren (1967) described secondary constrictions in the idiogram of sheep chromosomes but felt that they did not assist chromosome identification. These authors found that the incidence of secondary constrictions increased when hypotonic sodium citrate solution was used rather than hypotonic Hank's balanced salt solution. Saskeela and Moorhead (1962) found that a fixative of 1:1 acetic acid:methyl alcohol enhanced secondary constrictions in man.

A fourth method of chromosome identification is by autoradiography. This relies on the fact that different chromosomes and parts of chromosomes replicate DNA at different periods in the 'S' phase. The incorporation of tritium, a β emitter of low penetrance , into the thymidine in the culture medium allows identification of DNA replication areas by the effect on a photographic plate. If the labelled thymidine is added to the culture at different periods of time for a variable duration, then early and late replicating chromosomes can be identified. The disadvantage of autoraiography is that it is a prolonged and time consuming procedure.

With the development of differential staining techniques, not only was

it possible to identify individual chromosomes and their homologues, but also segments of chromosomes because of their specific staining pattern. These techniques produced bands of darkly staining areas along the chromatid, interspersed with lightly staining bands. The pattern produced was unique for any given chromosome and its homologue.

The various techniques produce five basic types of bands; Q-bands; C-bands; G-bands; R & T-bands and N-bands. The C-band and G-band techniques have been used in the present work in an attempt to characterise the Massey I translocation and this is discussed in detail in section V.

1.2.1. Q-Bands

The first technique for differential staining of chromosomes was described by Caspersson, Faber, Foley, Kudynowski, Modest, Simonsson, Wagh and Zech (1968). These workers compared the staining properties of ~~quinacrine~~ QUINACRINE and the alkylating agent, quinacrine mustard. They argued that since it was known that alkylating agents attacked the N-7 atom of guanine, the quinacrine mustard might interact preferentially and accumulate at the guanine-rich segments of DNA and thus fluoresce more brightly at these areas. Using chromosomes from the English broad bean and the Chinese hamster they found that quinacrine, which is not capable of forming co-valent bonds with DNA, showed uniform staining along the whole chromosome length, whereas quinacrine mustard showed distinctive bands of high intensity fluorescence, separated by regions of lower intensity fluorescence. The quinacrine mustard had shown preferential binding to certain chromosomal areas. These segments were later shown to be in areas of heterochromatin. (Casperson, Zech, Modest, Foley, Wagh, and Simonsson, 1969) In the same paper various fluorochromes were

assessed using both plant and animal chromosomes. The fluorescence showed good correlation with areas of heterochromatin in other plant chromosomes. Similar but weaker and less stable patterns were produced with acroflavine and proflavine staining of human and Chinese hamster chromosomes as well as plant chromosomes. However, ethidium bromide produced patterns in an opposite manner to the pattern obtained with quinacrine mustard. That is, the areas which were brightly fluorescent when stained with quinacrine mustard were dull with ethidium bromide and vice versa.

In the succeeding three years Caspersson and his co-workers produced a number of papers describing the use of this technique. In 1971 they described in detail the fluorescent banding pattern of the human karyotype (Caspersson, Lomakka and Zech, 1971) and this description was adopted as the basic banding pattern for use in distinguishing human chromosomes. (IVth Chromosome Standardisation Conference; Paris Conference, 1972)

Caspersson's work naturally evoked considerable interest. A group of workers in Oxford used quinacrine dihyrochloride to stain human interphase cells and showed that the fluorescence of the Y chromosome could be distinguished in these cells. (Pearson, Bobrow and Vosa, 1970) The marked fluorescence of the Y chromosome was present in only one other species examined, the gorilla. (Pearson, Bobrow, Vosa and Barlow, 1971) The fluorescent identification of the Y chromosome of man has meant that this technique could be used, together with the sex chromatin test, in clinical diagnosis of inter-sexes.

Sumner, Robinson and Evans (1971) showed that the Y fluorescence could be distinguished in Y bearing human spermatozoa and presumptive 24YY

spermatozoa with two fluorescent spots were identified. No Y fluorescence was demonstrable in bull, rabbit or mouse spermatozoa which correlated with the results of Pearson et al. (1971) in other interphase cells. Salamanca, Guzman, Barbosa and Martinez (1972) showed similar fluorescence to that of quinacrine mustard using a new fluorochrome, chlorinetacrine.

Quinacrine mustard has now been used to identify individual metaphase chromosomes in, amongst others, the pig (Gustavsson, Hageltorn, Johansson and Zech, 1972; Hansen, 1972) the ox (Hansen, 1972a; 1973; Schnedl, 1972; Evans, Buckland and Sumner, 1973) the sheep (Evans et al. 1973; Hansen, 1973a), the goat (Evans et al., 1973; Hansen, 1973) and the mouse (Dev, Grewal, Miller, Kouri, Hutton and Miller, 1971; Franke and Nesbitt, 1971; Hutten and Linden, 1971; Schnedl, 1971; Nesbitt and Donahue, 1972; Zech, Evans, Ford and Gropp, 1972).

Although the use of fluorochromes has made possible the identification of individual chromosomes the technique has two major disadvantages. Firstly, a fluorescent microscope is required and secondly, the quinacrine fluorescence fades under illumination so that the time available to scan a slide and examine a given metaphase in detail is very limited. Both these disadvantages were overcome by techniques producing C, G and R - bands.

1.2.2. C - Bands

Whereas Q-bands were named because of the stain used to produce them, C-bands are so called because it is the centromeric regions of the chromosomes that are stained by this technique. They have been defined in the Paris Nomenclature (1972) as the prominently stained centromeric region of each human chromosome, the secondary constriction

regions of chromosomes 1, 9 and 16 and the distal half to two thirds of the long arms of the Y. Similar prominent staining of centromeric regions occurs in chromosomes of a number of other species.

Preferential staining of the centromeric regions with Giemsa was first noted by Pardue and Gall (1970) when they were hybridising RNA into mice chromosomes. Their technique involved the separation of the DNA into single strands (denaturation) followed by reconstitution of the DNA into double strands (renaturation). It had earlier been shown by Britten and Kohne (1968) that separated DNA strands reassociated at different rates. In particular, one fraction of mouse DNA, about 10% of the total DNA content, reassociated extremely rapidly. This was identified as mouse satellite DNA which was located at the centromeric region. Britten and Kohne further showed that this fraction consisted of highly repetitive nucleotide sequences. Pardue and Gall had discovered therefore, a staining method of identifying highly repetitive DNA which reassociated rapidly after denaturation. The incidence of this repetitive DNA was investigated in man by Arrighi and Hsu (1971). They found that almost all the darkly staining regions were located near the centromere as in the mouse and that the amount of heterochromatin varied in different chromosomes. In the Y chromosome, the heterochromatin was located in the long arms and not at the centromere. One significant fact was that, in females, no markedly heteropycnotic X chromosome representing the inactive X was identified. Thus the procedure identified only constitutive heterochromatin and not ~~facultative~~ FACULTATIVE heterochromatin. (The term heterochromatin was first used by Heitz (1928) when describing chromosomes of liverworts and mosses. He called the heteropycnotic regions, heterochromatin and the rest of the chromosome, euchromatin. In later work on heterochromatin in Drosophila melanogaster he postulated that there might be a general

correspondence between cytological heteropycnosis and genetical inertness (Heitz 1933). The term constitutive heterochromatin implies that the chromatin is in a permanently inactive state, whilst facultative heterochromatin is potentially functional.) Saunders, Hsu, Getz, Simes and Arrighi (1972) demonstrated that the stained areas did not depend on the base sequence, but purely on the fact that they were highly repetitive. Saunders et al., showed that human chromosome No.9 had a fraction of a repetitive DNA which differed in its base sequences from any of the other highly repetitive centromeric regions.

The original techniques described by Pardue and Gall (1970) and Arrighi and Hsu (1971) were rather involved, requiring denaturation with hydrochloric acid, RNase and sodium hydroxide, followed by prolonged incubation in saline sodium citrate (SSC) to allow reassociation. This technique was greatly simplified by Sumner, Evans and Buckland (1971). They tested various hydroxides and other denaturing agents and then incubated the slides in 2 x SSC at 60°C for one hour. It was found that barium hydroxide was less destructive to the chromosomes and this became the reagent of choice.

The C-banded karyotype has been examined in the ox by Evans et al., (1973), Hansen (1973c), Popescu (1973) and Schnedl and Czaker (1974), and in the sheep by Evans et al., (1973)

One modification of the C-banding method is the "Giemsa-11" technique. This identifies particularly the centromeric heterochromatin of the human chromosome No.9. Staining is carried out in Giemsa at pH 11.0 (Bobrow, Madan and Pearson, 1972; Gagne and Laberge, 1972) The technique is capable of demonstrating the presence of the No.9 chromosome in interphase cells, including spermatozoa. Recently it

has been used to compare the human and chimpanzee karyotype. (Bobrow, and Madan, 1973) The Giemsa 11 technique produced darkly stained segments adjacent to the centromeres of one large pair of submetacentrics, one very submetacentric pair and three metacentric pairs of chimpanzee chromosomes. In man, chromosome arms 1q; 5q; 7p; 9q; 10q; 17p; and 20q were stained. These results, together with the trypsin banding analysis by Turleau and Grouchy (1972) have led to various hypotheses of how the human karyotype may be related to that of the chimpanzee.

1.2.3. G - Bands

G-banding developed as a natural consequence of the C-banding techniques which occasionally produced a banding pattern along the whole chromosome similar to that produced by quinacrine mustard fluorescence. To distinguish them from Q-bands and as a result of their being produced by Giemsa, they were named G-bands. G-bands have been produced by a multiplicity of methods in a variety of species.

Drets and Shaw (1971) were the first workers to demonstrate G-banding in human chromosomes by a denaturation technique. They treated preparations with 0.7N NaOH for 30 seconds and then incubated the cells in an SSC solution at 65°C for 60 - 72 hours. Schnedl (1971a) used an exposure time to NaOH of between 90-120 seconds. This was followed by incubation in Sorensen buffer at pH 6.8 at 59°C for 24 hours. Both methods utilised Giemsa as the final stain. With one or two exceptions the G-banding pattern of human chromosomes resembled that of the Q-bands. The major contrast in the two techniques was that G-banding produced stained regions at the centromere of most chromosomes and that the secondary constrictions of chromosomes 1 and 16 stained markedly.

It was then shown that the bands could be produced by incubation at 62°C in Sorenson buffer without prior treatment with NaOH. (Chandhuri, Vogel, Voiculescu and Wolf, 1971) Similar results were produced by other workers using different salt solutions. Sumner, et al.,(1971) incubated in SSC at 60°C. Their technique was called the acetic/saline/Giemsa technique (AGS) and was adopted by a number of other workers to examine the human karyotype. (Lomholt and Mohr, 1971; Vass and Sellyei, 1972) A similar result was produced using 0.9% sodium chloride buffered to pH 7.5 at 20-22°C. (Bosman and Schaberg, 1973) These workers found that staining with either Giemsa or Leishman would produce the banding patterns. Meisner, Chuprevich, Johnson, Inhorn and Carter,(1973) used an 0.2M solution of caesium chloride at 65°C. In every instance the banding patterns were broadly similar to those produced by quinacrine fluorescence. Utakoji (1972) used a dilute potassium permanganate solution to oxidize preferentially the pyrimidine residues of heat denatured DNA. After short incubation in 10mM potassium permanganate and staining in Giemsa, clear banding patterns were formed on chromosomes of man, rat, mice and Chinese hamster. Other workers have used phosphate buffered urea solution. (Shiraishin and Yosida, 1971; 1972) Banding patterns have also been produced simply by using a very dilute solution of Giemsa stain. (Sanchez, Escobar and Yunis, 1973) They have even been reported as appearing spontaneously on aged Giemsa stained preparations. (Zuelzer, Ottenbreitt, Inoue and Zuelzer, 1973) These workers examined slides that had been stained with Giemsa without pretreatment, between two and nine years previously and found a definite banding pattern on some of the metaphase spreads.

Despite the various methods available the technique most commonly adopted in routine analysis of human chromosomes is one which depends

on the use of enzyme digestion as a pretreatment. The method has the advantage of producing distinct G-band patterns without the prolonged incubation time required by alternative techniques. The first description of enzyme digestion producing bands in human chromosomes was by Dutrillaux, de Grouchy, Finaz and Lejeune, (1971). These workers used the enzyme pronase. In the same year Seabright published her preliminary report of the use of trypsin to produce G-bands in human chromosomes after Leishman staining. (Seabright, 1971) A more detailed report appeared in the following year together with the identification of the segment involved in a 1-18 translocation, using the technique. (Seabright, 1972) Slight modifications of the original technique were published at intervals (Seabright, 1972a; 1973). The great advantages of trypsin banding techniques compared to others described are that the procedures can be carried out at room temperature on routine air-dried preparations, and can be completed for examination within ten minutes or less. Even the original disadvantage which was that the preparations required to be left for five to seven days before trypsinisation in order to obtain optimum resolution, was overcome by treating the preparations with hydrogen peroxide (Seabright, 1973). Other workers quickly investigated Seabright's technique. Wang and Fedoroff (1972) used trypsin and trypsin-versene in Ca^{2+} and Mg^{2+} free balanced salt solutions. Control preparations treated solely with Ca^{2+} and Mg^{2+} did not form bands when stained with Giemsa. Some Austrian workers used the proteolytic enzyme Pankretin, a combination of protease, amylase and lipase and produced banding patterns essentially the same as those produced by trypsin (Muller and Rosenkranz, 1972).

The trypsin banding technique had, until recently, been used almost exclusively for the examination of human chromosomes. However, it

has now been used to compare the human and chimpanzee karyotype (Turleau and Grouchy, 1972; Bobrow and Madan, 1973). Other workers have examined the karyotype of the sheep (Nadler, Hoffmann and Woolf, 1973) and compared cattle, goat and sheep chromosomes (Evans et al., 1973; Schnedl and Czaker, 1974).

1.2.4. R and T Bands

R-bands were first described by the French workers, Dutrillaux and Lejeune, (1971). The banding patterns produced by these workers were in reverse contrast to both Q and G-bands. The technique involved immersion in phosphate buffer at pH 6.5 at 87°C for 10-12 minutes, followed by Giemsa staining. The procedure produced rather pale bands corresponding to the non-fluorescent areas in the human karyotype described by Caspersson at al., (1971). Further comparison with G-bands (produced by heating or proteolytic digestion) confirmed this observation, (Dutrillaux, Finaz, de Grouchy and Lejeune, 1972). Although reverse banding has been reported by other workers (Buhler, Tsuchimoto and Stadder, 1973) it is not a technique that has been used widely.

A variation of the technique has been described recently by Dutrillaux (1973). With this method the terminal parts of human chromosomes were preferentially stained. These bands were named T-bands and their formation would seem potentially useful in identifying terminal translocations. T-bands could be produced with the same techniques as those employed for R-bands except that the procedure was carried out at pH 5.1. With this method the T-bands were only faint and sometimes difficult to differentiate from R-bands but it had the advantage of producing bands visible under a light microscope. An alternative method was to incubate in phosphate buffer at pH 6.7 at 87°C and stain

with acridine orange. This method produced good differentiation with a terminal green fluorescence whilst the remainder of chromosome fluoresced orange.

1.2.5. N-Bands

This is the latest of the differential staining techniques to be described and identifies the nucleolus organisers in metaphase chromosomes. (Matsui and Sasaki, 1973) The procedure involves extraction of some DNA and RNA with appropriate enzymes and removal of the histone protein in acid. In Giemsa stained preparations the N-bands then appear as purplish spots. The technique has been used in human, rat, kangaroo, Indian muntjac, Chinese hamster and donkey chromosomes. Since the technique extracted nucleic acids and histones the authors concluded that the N-band substances were acidic proteins.

To summarise, there are a number of techniques which will produce differential staining of the chromosomes, but they can be broadly grouped into four methods:-

1) Quinacrine fluorochromes: These produce good, well differentiated patterns but have the disadvantage of requiring a fluorescent microscope for visualisation and fading after brief illumination.
2) Alkali-heat techniques: These have the advantage of producing bands visible under an ordinary light microscope but some of the techniques are time consuming and some of the reagents damaging to the chromosomes. However, simplified C-band techniques have been useful in identifying polymorphisms of the centromeric region.
3) Proteolytic enzymes: These techniques have proved to be most useful in that they produce distinct and characteristic patterns in a relatively short time.

4) Miscellaneous: This group includes such reagents as urea, detergents and other denaturing agents. They tend to produce the least consistent results.

A review of the use of new staining techniques has been presented by Pearson (1972) and a bibliography published by Nilsson (1973).

1.2.6. Mechanism of Band Formation

The mechanism of band formation along the chromosome arms has been the subject of considerable debate for some time. It has been generally agreed that the different staining intensities reflect a variable structure along the chromosome but it has been uncertain whether this variability was of the DNA base sequences or of the associated chromosomal protein.

Caspersson et al., (1969) originally suggested that the fluorescent bands were produced by preferential accumulation of the dye at quinine-rich segments of DNA. It was later shown that highly fluorescent regions were in fact rich in adenine and thymine (A/T) and not guanine and cytosine (G/C) (de la Chapell, Schroder, Selander and Stenstrand, 1973). These workers investigated the temperatures at which various parts of human, mouse and vole chromosomes, denatured in formamide. A/T regions denatured at lower temperatures than G/C regions, and the early denaturing regions were associated with bright quinacrine mustard fluorescence. There were, however, certain contradictory results. Some areas which denatured at a relatively low temperature, indicating A/T areas, did not show bright fluorescence. In particular, centromeric regions of mouse chromosomes, known to be A/T rich (Pardue and Gall, 1970) did not show good fluorescence. Weisblum (1973) suggested that this discrepancy was due to the distribution of G/C

pairs within the A/T fraction. He suggested that it was not so much the absolute A/T : G/C ratio that was important, but rather that the degree of regular G/C dispersion produced increased quenching of fluorescence and hence a dull area on the chromosme.

The formation of C-bands was believed to be due to the differential renaturation rates of various base sequences. Highly repetitive areas renatured extremely quickly and stained darkly. However, McKenzie and Lubs (1973) examined the procedure involved in C-band production and questioned whether denaturation and renaturation was the major mechanism. They found that exposure to HCl alone did not produce C-bands and yet HCl treatment followed by SSC incubation did produce good C-bands. This was without the intervening stage of exposure to NaOH, a procedure considered to be a critical step because of sodium hydroxide's denaturing properties. (Arrighi and Hsu, 1971) McKenzie and Lubs suggested that differential loss of DNA and/or protein was the more likely mechanism of C-banding. However, Feulgen staining indicated that although DNA removal did occur during the procedures, it was unrelated to C-band staining. (Comings, Avelino, Okada and Myandt, 1973) These workers also concluded that although denaturation and preferential renaturation of centromeric DNA was occuring under the conditions of C-banding, it had little to do with the mechanism of differential staining. They concluded that DNA-protein interactions were important in C-band production.

Chromosomal proteins and the denaturation/renaturation process were also thought to be involved in the production of G-bands. (Chaudhuri, et.al., 1971; Lomholt and Mohr, 1971; Schnedl, 1971a; Seabright, 1971; Sumner et al., 1971; Wang and Fedoroff, 1972) Examination of trypsin banded human metaphase chromosomes with the electron microscope

revealed a complex network of chromatin fibres. (Ridler and Ohara, 1972) There was no sharp demarkation between band and interband areas but band regions did have a greater network density. These workers suggested that it was release of protein from the chromatin fibres which reduced the affinity for staining and produced swelling, chromatid fusion and formation of interchromosomal connections. They postulated that the band regions were composed of chromatin containing proteins relatively resistant to proteolytic agents. A similar result of trypsin treatment was seen with the electron microscope by Burkholder (1974). Kato and Moriwaki (1972) studied various factors involved in the production of bands in Chinese hamster chromosomes. Their main finding was that alkalis, strong bases and protein denaturants were the most potent band producers and from this they concluded that protein extraction was of prime importance in chromosomal banding. Dev, Warburton and Miller (1972) suggested that since Ca^{2+} and Mg^{2+} free solutions were required to produce Giemsa banding and trypsin itself bound to Ca ions, the elimination of these cations was important in Giemsa banding. They compared Ca^{2+} and Mg^{2+} free Hank's basic salt solution (BSS) with BSS containing Ca^{2+} and Mg^{2+} and found that although treatment with both solutions would produce bands, BSS + Ca^{2+} and Mg^{2+} only did so after prolonged incubation. They suggested that Giemsa banding of chromosomes would be produced by any pretreatment which removed bivalent cations. The Feulgen banded pattern was compared with that of G and Q bands in the mouse (Rodman and Tahiliani, 1973) These workers found a good correlation between Feulgen banding, the intensity of Giemsa staining and the brightness of fluorescence. It was known that histones determined the extent of cross linkage of DNA and they suggested that the dark Feulgen bands could represent loci of inherently greater DNA density or those at which histones had become aggregated and caused DNA concentration. Since alkaline

treatment is an essential pretreatment for good contrast of bands and alkalis aggregate histone, the latter proposition was considered more likely. They further suggested that the similarities between Feulgen banding and G and Q bands indicated that the fundamental basis for the patterns displayed by all three methods was that of differential densities of DNA or of a component whose concentration closely followed that of DNA, this component being the chromosomal protein. The results of the investigations of Comings et al., (1973) also suggested that it was the non-histone protein interactions that were the important factors in G-banding since absolute DNA content and strandiness of the DNA were shown to be irrelevant.

Daniel and Lam-Po-Tang , (1973) suggested that the R-bands were produced because of the selective denaturation of the A/T bases at the specific temperature ($87^{o}C$) of the prestaining treatment. They suggested that protein in these disrupted areas would be more easily extracted and so the regions stained less intensely.

Recently, workers in Edinburgh have put forward a theory of band formation that seems to explain the results produced by all the different techniques as well as explaining some of the contradictory results found by other workers. Firstly, it was shown that after methanol/acetic acid fixation of standard human chromosome preparations from leucocyte cultures almost all of the histone proteins were extracted from the chromosome. (Sumner, Evans and Buckland, 1973) Sumner and his co-workers concluded that the banding pattern of chromosomes was the result of an interaction of the dye with DNA or non-histone proteins and examined the mechanism of this interaction. (Sumner and Evans, 1973) They showed that nuclei and chromosomes from which DNA had been completely removed did not stain with Giemsa nor

with quinacrine to any significant extent. Furthermore, blocking protein amino groups by acetylation had no effect on banding with either Giemsa or quinacrine. This indicated that the dyes in banded chromosomes were bound to DNA. The staining mechanism of Giemsa was then examined in more detail. Nuclei and chromosomes fixed in methanol/acetic acid and stained with Giemsa developed a magenta colour. This colour was not produced when the chromosomes were stained with the individual dyes from which Giemsa is composed, (methylene blue, azure A, azure B and corin Y). However, a solution of methylene blue and eosin (1:1 by weight) showed similar absorption peaks to the magenta dye when measured on a spectrophotometer.

Combining the results of this and their previous work (Sumner et al., 1973; Sumner and Evans, 1973) the following hypothesis to account for differential binding of quinacrine and Giemsa was put forward.

The magenta compound formed when Giemsa banding was produced consisted of one molecule of eosin Y and two molecules of methylene blue, since the molecular weight of eosin was approximately twice that of methylene blue. These dye molecules were bound to the DNA since the magenta dye could not be isolated in solution following extraction of stain from the chromosome. There was no correlation between the intensity of staining and amount of DNA so that the differentiation was due to a variable state of DNA. In addition, since the banding patterns produced by Giemsa and quinacrine mustard staining were almost identical, it was probable that the same structural organisation was involved in both processes. Quinacrine mustard intercalated in the DNA molecule since the staining was blocked by ~~concentated~~ CONCENTRATED salt solutions and so it was suggested that quinacrine mustard bound most strongly when the DNA phosphate groups were at the correct distance apart for both of

the amino groups of the quinacrine to combine with them. A differential binding would then occur if the average spacing of DNA phosphate groups differed from one part of the chromosome to another.

The magenta compound from Giemsa was bound to the DNA by hydrogen bonds since staining was lost in the presence of urea. However, the initial staining of chromosomes with methylene blue was ionic, since it was blocked by salt solutions. It was suggested therefore, that Giemsa staining involved two molecules of methylene blue, bound to DNA ionically. If the methylene blue molecules were then the correct distance apart an eosin molecule attached to the methylene blue molecules through the acidic groups of eosin and the magenta complex remained in a position by means of hydrogen bonding and partial intercalation into the DNA. Thus, as with the quinacrine mustard staining, two bonding sites, the correct distance apart were involved. Theoretically the two dye binding sites could be either on the same or separate DNA molecules, or involve longitudinally separated regions on one DNA chain. However, Sumner and Evans, (1973) suggested that the most likely mechanism was that the dye molecules cross-linked adjacent DNA molecules or adjacent sites on a folded molecule. In methanol/acetic acid-fixed chromosomes the DNA chains would be held together by non-histone proteins and any loosening of the structure would result in loss of affinity for the dyes. It was shown that reduction of the protein disulphide bonds prior to routine ASG G-banding procedure resulted in uniform pale staining of the chromosomes. (Sumner, 1974) By contrast, cross-linking of the sulphydryl groups prior to ASG G-banding tended to obscure the banding patterns by producing dark staining throughout the chromosome. It was concluded therefore, that G-banding was a consequence of a varying concentration of protein disulphides and sulphydryls along the chromosomes.

Regions of disulphide bridges held the DNA in a compact state suitable to receive the dye and thus appeared as a dark band. However, alteration of the disuphide bridges or sulphydryl groups had no effect on Q-banding.

The above hypothesis proposed by Sumner and his co-workers was also used to explain C-banding since alkali treatment caused considerable swelling of chromosomes so that DNA chains would be far apart and staining reduced. Furthermore, it explains the correlation between chromosome bands and late replication, (Garner and Evans, 1971) since late replicating regions would be in a compact state suitable for the uptake of the dye. Such late replicating, genetically inactive chromatin appears to be particularly rich in protein disulphide groups (Sadgopol and Bonner, 1970). It was also suggested that the formation of bands in old Giemsa stained preparations was due to the reduction of the dye by the suphydryl groups producing pale areas.

It would seem, therefore, that this hypothesis of differential staining answers a number of outstanding questions regarding the various techniques previously described. Even without a complete understanding of the processes involved, differential staining techniques have been very important in the understanding of various chromosomal abnormalities.

1.3. CHROMOSOME ABERRATIONS

Deviation from the normal chromosome complement can occur in the sex chromosomes or amongst the autosomes and this deviation may be numerical or structural.

1.3.1. Structural Sex Chromosome Aberrations

There are very few reports of structural aberrations of sex

chromosomes in domestic animals. Payne, Ellsworth and DeGroot (1968) reported a phenotypically normal mare, showing irregular oestrous cycles, which had a chromosome complement of 2n= 63 and no identifiable X chromosomes. An extra, small, subterminal chromosome, absent from the normal equine karyotype was present and the authors suggested that this was a modified X chromosome. Enzyme concentrations for the sex linked G-6-PD (Glucose-6-phosphate dehydrogenase) were within the normal limits so that at least part of one of the X chromosomes was present. In addition, the mare had been seen in oestrus and ovarian follicles had been palpated. The mare, however, had failed to conceive when bred to four different stallions over a period of four years. Payne et al., (1968) suggested that the abnormal chromosome had arisen during gametogenesis in the dam and that it resulted from a breakage and rearrangement of the X^m chromosomes. The irregular chromosome would then contain fragments from both X^m chromosomes. It was further suggested that the ococyte containing the abnormal X had been fertilised by a spermatozoon lacking a sex chromosome. The probability of such occurrences coinciding is very low and the authors conceded that they were unable to exclude the possibility of mosaicism with the normal female karyotype of 64 XX, which would explain the normal phenotype and enzyme levels.

McFeely, Hare and Biggers (1967) reported a case of bovine male pseudohermaphrodism in which the diploid number was 60. The karyotype contained one normal X chromosome, an unusually large acrocentric and no distinguishable Y chromosome. It was suggested that the abnormal acrocentric was formed by a pericentric inversion of one of the X chromosomes or by a translocation of the short arm of the X to an autosome.

Martin and Shaver (1972) reported a male rabbit with a minute Y chromosome. The animal was phenotypically normal and was of normal fertility. The minute chromosome in the complement was designated the Y on the basis of late replication, demonstrated by autoradiographic studies, and on the absence of a normal Y. The authors considered it possible that this Y morphology was a normal variant, analogous to the Y polymorphism of man. (Unnerus, Fellman and de la Chapelle, 1967) The polymorphism in man occurs in the long arm of the Y which is composed largely of heterochromatin and genetically inert. (Arrighi and Hsu, 1971) Cases of true structural abnormalities, as opposed to polymorphism, of the Y chromosome of man were reviewed by Ferrier, Ferrier and Bill (1968). Only three of the twentythree cases reported were phenotypically normal males with normal testicular histology. Most of the patients reviewed by Ferrier et al., (1968) lacked either the long or short arm of the Y and the phenotype ranged from normal male with oligospermia through to normal female with gonadal dysgenesis. The latter cases were all mosaics for a 45 XO cell line.

Ferrier et al., (1968) also reported a case of 45 XO / 46 XY pseudohermaphrodite with a dicentric Y chromosome. The putative father of this patient had a normal Y chromosome so that the authors suggested that the dicentric Y resulted from fusion of two Y chromosomes at the short arms. They considered that either the fusion occurred in an XYY cell from which the 46 XY dic. cell line developed, or it was the result of a breakage and reunion of a single Y following chromatid duplication.

An apparent pericentric inversion of the Y chromosome was detected in a phenotypically normal Ayrshire bull. (Harvey, 1974- personal

communication.) The diploid number of 60 chromosomes consisted of 29 pairs of acrocentrics, a submetacentric X and a small unpaired acrocentric chromosome comparable in size to the smallest pair of acrocentrics. The unpaired chromosome was presumed to be the Y chromosome which had undergone inversion. It resembled the acrocentric Y chromosome of Bos indicus cattle, described by Kieffer and Cartwright (1968). The karyotype of the sire of this animal was unknown.

Another form of sex chromosome aberration involves an X - autosome translocation. Two examples have been reported in the mouse. In Cattanach's translocation there was an insertion of an autosomal segment into the X chromosome. (Cattanach, 1961) The translocation X was recognisable as the longest acrocentric of the complement. It was a non-reciprocal translocation and the inserted fragment behaved as an integral part of the X chromosome. The normal and translocation X chromosomes were shown to be late replicating in approximately the same proportion of cells. (Cattanach and ~~Isaacson~~ Isaacson, 1967)

Searle's translocation (Searle, 1962) was a reciprocal translocation between the X and the distal region of chromosome number 16. It was therefore designated T(X;16)16H. (Eicher, Nesbitt and Francke, 1972) This translocation was unusual in that it interfered with the normal process of X inactivation. The translocation appeared to be the active X in most or all cells. (Lyon, Searle, Ford and Ohno, 1964)

Other X autosome translocations were induced in mice by irradiating males. (Lyon and Meredith, 1966) Male offspring of irradiated mice were either sterile or showed greatly reduced fertility. No sterile daughters were produced but some did show reduced litter size. The

sterile sons, and daughters with reduced fertility were found to have an X-autosome translocation.

A presumptive X-autosome translocation was reported in the ox. (Gustavsson, Fraccaro, Tiepolo and Lindsten, 1968) The translocation occurred in a heifer which was also heterozygous for the 1/29 centric fusion translocation described by Gustavsson and Rockborn (1964). The chromosome complement of the heifer consisted of 56 normal acrocentrics, the metacentric 1/29 translocation, one normal submetacentric X and an abnormal, metacentric chromosome which was presumed to be the second X chromosome. Autoradiographic studies consistently showed the abnormal X to be replicated earlier than the normal X. That is, there seemed to be preferential inactivation of the normal X chromosome as in Searle's translocation in the mouse. The heifer was phenotypically normal and gave birth to a stillborn male calf which inherited the normal X chromosome.

1.3.2. Numerical Sex Chromosome Aberrations

Numerical aberrations of sex chromosomes are apparently more frequent than structural abnormalities. The X monosome karyotype, analogous to that of women with Turner's syndrome, is well known in the mouse. (Russel, Russel and Gower, 1959; Welshons and Russel, 1959) Whereas in women, XO individuals have phenotypical abnormalities such as short stature, and webbing of the neck, together with ovarian dysgenesis (Turner, 1938), XO mice are phenotypically normal and fertile. However breeding programmes with XO mice revealed a much lower frequency of XO offspring than would be expected on the basis of normal segregation. (Cattanach, 1962; Morris, 1968) Overall litter size was smaller than the average litter size of normal XX mice. This reduction was presumed to be due to embryonic loss of XO and YO individuals. All the YO individuals and approximately one third of the XO individuals

would be expected to die prenatally. (Russel et al., 1959; Welshons and Russel, 1959) This would result in a litter size only approximately 68% of normal. Cattanach (1962) considered that his results showed a larger litter size than the expected and suggested that preferential selection of the X carrying gamete was taking place. Dissection studies by Morris (1968) in normal and XO mice showed that most of the embryonic loss occurred prior to implantation. The post-implantation loss was greater in XO females. It was suggested that the deficiency in the number of expected XO offspring was due both to a deficiency of nullo gametes at fertilisation and to a loss during embryonic development. The possibility of non-random segregation during oogenesis in XO mice was also investigated by Kaufman, (1972). He collected oocytes from XO and XX females and examined the chromosome complement at second metaphase. One hundred and forty-five oocytes from XO females and 105 from XX females were collected. The number of scorable preparations was not reported but Kaufman stated that there were approximately twice as many oocytes with twenty chromosomes (X bearing gametes) as with 19 chromosomes. This strongly suggested a non-random segregation at the first meiotic division. Kaufman (1972) commented on having observed a YO karyotype at the first cleavage division in a mouse zygote but this appears to be the only report of a YO complement, which is considered to be nonviable. (Welshons and Russel, 1959)

The XO condition has been reported in an XO/XY true hermaphrodite mouse. (Lyon, 1969) The animal had a male phenotype but with a poorly developed scrotum. Upon dissection, the left gonad was found to resemble an abdominal testis and the right an ovary. Histologically, the ovary contained numerous follicles but no corpora lutea. The ovarian stroma was hypertrophied and tended to be

arranged in cords. The testis showed a number of areas of abnormal spermatogenesis and some empty tubules contained hyaline material. The XO/XY karyotype has not been reported in other species apart from man, (Jacobs, 1966).

An XO/XX constitution was reported in the mouse by Cattanach (1967) and Green (1967, quoted by Lyon, 1969). Evans, Ford and Searle (1969) described an XO/XYY mouse, the offspring of a genotypically normal male that had undergone irradiation. The XO/XYY complement was established from bone marrow cultures. Only the XYY cell line was found in the meiotic preparations. The authors suggested that the animal was a true mosaic, having developed from an XY zygote in which there had been non-disjunction of the Y chromosome at the first cell division giving rise to XO and XYY cell lines. The significant finding was the absence of an XO cell line in the testes. It was suggested that, although this could have arisen by chance distribution of cells during organogeny, it was more likely to reflect a true physiological difference in the two cell lines. They postulated that the primordial germ cells of the XO line had failed to establish themselves as spermatogonia, and that the failure was due to the absence of a Y chromosome. This they considered essential to ensure normal spermatogenesis.

The only other report of the XYY constitution in the mouse was by Cattanach and Pollard, (1969). They reported an animal which was phenotypically normal but infertile. After slaughter the testes of the animal were found to be abnormally small. Meiotic preparations produced 16 cells with 20 normal bivalents, including the X-Y bivalent with end to end association, plus a small extra element which resembled the Y chromosome. A further eight cells had 19 bivalents,

a univalent X and two small elements. In two other cells, these two small elements were paired to form a bivalent and the X remained univalent. Chromosome counts on seven spermatogonial mitoses confirmed the diploid number as 41. This animal was not completely aspermic since a few spermatids and spermatozoa were observed in the testes and the authors speculated on the possibility that fertile XYY mice might exist.

In man, estimates of the incidence of XYY males from surveys of newborn populations ranged from 1 in 250 (Sergovich, 1968) to 1 in 1,100, (Court Brown, 1968). The extensive literature on the topic has recently been reviewed by Kessler and Moos (1970), and they cast doubt on the postulated association of the XYY karyotype and agressive and criminal tendencies.

A syndrome in man involving excess X chromosomes with a single Y chromosome is characterised by increased height, hypo-orchidism and azoospermia. The clinical condition was originally described by Klinefelter, Reifenstein and Albright, (1942) and was named the Klinefelter syndrome. Later workers demonstrated that affected individuals had a 47XXY chromosome complement. (Ford, Jones, Mittwoch, Penrose, Ridler and Shapiro, 1959). This particular type of chromosome complement has been described in a variety of domestic animals.

Russel and Chu (1961) reported the first cytogenetically confirmed case of XXY in the mouse. This animal was of normal size and sterile.

Bruere, Marshall and Ward (1969) described two rams with testicular

hypoplasia and an XXY sex chromosome constitution. They described this to be the ovine equivalent of the Klinefelter syndrome in man. Both animals showed bi-lateral testicular hypoplasia but libido was unimpaired, suggesting that there was no androgen deficiency. Histological examination of the testes revealed seminiferous tubules lined by a single layer of well differentiated Sertoli cells, but there was no evidence of hyalinization of the tubular basement membrane that had been reported in man. (de la Balze, Bur Scarpa-Smith and Irazu, 1954) Behavioural studies with these and a further 4 Klinefelter rams failed to show a lower "mental" performance in the Klinefelter animals (Kilgour and Bruere, 1970; Bruere and Kilgour, 1974). Plasma ~~progesterone~~ TESTOSTERONE levels were lower than in normal rams and within the range of normal ewes (Bruere and Kilgour, 1974).

A dog with an XXY sex chromosome constitution has been described by Clough, Pyle, Hare, Kelly and Patterson (1970). The animal was a phenotypic male which had been obtained for use in a study of congenital heart diseases and was known to possess a subaortic, interventricular septal defect. When fully grown the dog was small for the breed (German shorthair pointer) and the testes were only half the normal size. Histological examination revealed testicular hypoplasia and aspermatogenesis. Blood and fibroblast cultures and bone marrow preparations revealed a chromosome complement of 79XXY.

A similar testicular histological picture was found in a pig with a 39XXY karyotype. (Breeuwsma, 1968) The animal was an intersex with a uterus-like organ and two small testes. This was the first report of an XXY sex chromosome constitution in the pig. In the same year Harvey (1968) reported a case of 39XXY/40XXXY in a pig suffering from lymphosarcoma. The animal was a castrated male and therefore no histological information on the status of the testes was available.

An intersex horse with an XXXY sex chromosome constitution has also been reported, (Gluhovschi, Bistriceanu, Suciu and Bratu, 1970) and another intersex horse with a 64XX/65XXY mosaicism was described by Bouters, Vandeplassche and de Moor (1972). The animal was registered in the studbook as a female but showed strong male behaviour in the presence of other mares. Erectile tissue was located at the entrance of a rudimentary vagina from which the processus urethralis emerged. Rectal examination revealed a short penis but no gonads were palpable. Two small, intra-abdominal testes were located and removed at laparotomy. Histological examination revealed hypoplastic, inactive seminiferous tubules. Spermatogonia could not be identified. Blood leucocyte cultures demonstrated a chimeric karyotype in that 95% of the cells examined were of the normal female constitution, 2n=64XX whilst 5% of the cells were 2n=65XXY. Chromosome studies were not made from other tissues but it would have been interesting to know whether the low incidence of 65XXY cells in the blood was reflected in gonodal tissue. Another horse intersex, described by Basrur, Kanagawa and Gilman (1969) had a complex, four cell line mosaicism, one of which was XXY. The animal was phenotypically male but the left testis was apparently undescended and the right, hypoplastic. There was a well developed vulva within which was a short penis. Histological examination of the descended testis revealed an absence of germ cells. Chromosome studies from testicular tissue showed a complex mosaicism consisting of 29.6% 64 XX cells, 17.6% 64XY cells, 2.8% 65 XXY cells and 7.4% 63 XO cells. The remaining 2.5% of cells had undiagnosed sex chromosome complements. Basrur et al., (1969) suggested that this mosaic could have arisen as a result of mitotic abnormality of an XY or XXY zygote. However, more extensive chromosome analysis, together with blood typing and a calculation of the incidence of "drumsticks" in blood neutrophils indicated the horse to be a chimera rather than

a mosaic. (Basrur, Kanagawa and Podliachouk, 1970)

It would seem, therefore, that an excess of X chromosomes produces a similar syndrome in domestic animals and in man. In particular there is complete aspermatogenesis.

In cats, the XXY sex chromosome complement has been recognised as one of the mechanisms for the development of male tortoiseshell cats. The tortoise-shell coat pattern normally only occurs in a female having the genotype OO+ at the X - linked orange locus and the variegated pattern is produced as the result of random inactivation of one of the X chromosomes as hypothesized by Lyon (1961). The first report of an XXY male tortoise-shell cat was by Thuline and Norby (1961). They described two male cats, one of which lacked gonadal tissue and the other showed aspermatogenesis in the descended testes. Both animals had the tortoise-shell coat pattern, and both showed chromatin-positive buccal smears. Blood cultures revealed a karyotype of 2n= 39XXY. A similar case has been reported in a Himalayan cat with tortoise-shell points. (Pyle, Patterson, Hare, Kelly and Digiulio, 1971) The left testis of this animal was in the inguinal region, near the scrotum, and the right was just inside the internal inguinal ring. Histological examination of the left testis showed seminiferous tubules lined by Sertoli cells but an absence of spermatogenesis. Other XXY male tortoise-shell cats have been triploid-diploid chimeras. (Chu, Thuline and Norby, 1964; Thuline and Norby, 1968; Gregson and Ishmael, 1971) Most of these animals were XX/XXY sex chromosome chimeras. Gregson and Ishmael reported a cat with 38XY/57XXY cell mosaic. This animal possessed normal sized testes and histological examination revealed seminiferous tubules lined by cells showing all stages of spermatogenesis including spermatozoa.

Skin cultures revealed only two triploid cells out of 109 counted, the predominant cell line being 38XY. This presumably explains the male phenotype, but it is interesting that such a low incidence of 57XXY cells was sufficient to produce the tortoise-shell coat pattern. Loughman, Frye and Condon, (1970) reported three male tortoise-shell cats with 38XY/39XXY cell lines. One animal had testes slightly smaller than normal, a second had grossly underdeveloped testes and the genital status of the third was unrecorded. Histological preparations were made from only the grossly abnormal testis and the proportion of XY and XXY cells in the bone marrow cultures was unrecorded. None of the three animals appears to have been considered fertile by the authors.

Another method of development of a male, tortoise-shell cat is by simple XX/XY chimerism. Unlike the XX/XXY chimeras, the testes of an XX/XY individual described by Malouf, Benirschke and Hoefnagel (1967) showed some seminiferous tubules in which spermatogenesis was taking place. The proportion of XX and XY cells from various tissues was 57% and 43% respectively.

The most common example of blood cell sex chromosome chimerism is the bovine freemartin. Whilst sex chromosome chimerism is neither a structural nor numerical abnormality, it is included in this section and will be discussed briefly because of its importance in domestic animals.

1.3.3. Freemartins

A freemartin has been defined as a sexually imperfect, sterile female partner of a pair of heterosexual twins. (Swett, Matthews, Graves, 1940) Only about 8% of heifers born with male co-twins are normal. (Marcum, 1974) The freemartin heifer has female external genitalia but the internal genitalia show various degrees of masculisation.

Characteristically, there is a normal vulva but with excess vulval hair and an enlarged clitoris and shortened vagina. The gonads are small, often ovotestes, and seminal vesicles may be present. As early as the beginning of this century, it had been suggested that for freemartins to occur the twins must be of unlike sex and vascular anastomosis must occur, permitting tansplacental passage of hormones from the male to the female foetus. (Tandler and Keller, 1911; Lillie, 1917) It was suggested that these hormones caused masculinisation of the female genital tract. Owen (1945) found that when placental anastomosis occurred, bone marrow precursor cells were exchanged and the co-twins showed erythrocyte chimerism.

The hormonal theory of freemartin development was accepted until Ohno, Trujillo, Stenius, Christian and Teplitz, (1962) demonstrated sex chromosome chimerism in bone marrow cells and Fechheimer, Herschler and Gilmore (1963) demonstrated XX/XY chimerism in leucocyte cultures. Fechheimer et al., (1963) suggested that the freemartin phenotype was produced by the sex chromosome chimerism. Jost, Vigier and Prepin, (1972) examined early foetuses from multiple pregnancies in cattle and found that there was normal development up to 48 days of gestation after which development of the ovaries of presumptive freemartins ceased. Inhibition of the Mullerian duct occurred at the same stage. Ohno et al., (1962) had shown that the male co-twin also possessed blood cell chimerism, and in addition XX cells were detected in the testes. However, no XY cells were found in the freemartin gonads. The absence of XY cells in the freemartin gonad was confirmed by other workers. (Short, Smith, Mann, Evans, Hallett, Fryer and Hamerton, 1969) Nevertheless, the gonads of these freemartins were shown to be secreting testosterone. Short et al., (1969) suggested that the freemartin was probably masculinised by the secretions from its own gonads.

It was originally suggested that the proportion of XY cells in the freemartin was related to the degree of masculinisation (Herschler and Fechheimer, 1967)but a more recent report has failed to confirm this correlation. (Vigier, Prepin and Jost, 1972) Indeed there has been one interesting report of a phenotypic, fertile bull, born co-twin to a phenotypic freemartin, whose karyotype from leucocyte cultures was 60 XX. (Kosaka, Kanagawa and Shikawa, 1969) The total number of cells counted was 1291 so that the possibility of leucocyte chimerism can almost be excluded. The sex ratio of the offspring of this bull did not differ significantly from the expected 1:1 ratio. This suggests that the blood cell precursors had originated solely from the cross-over of the co-twins XX cell line. It is unlikely that the culture technique was giving preferential advantage to the XX cells of an XX/XY population.

In sheep, the incidence of freemartinism is much lower than in cattle. It has been estimated that placental anastomosis occurs in only 5% of ovine twin pregnancies. (Stormont, Weir and Lane, 1953) Dain (1971) found an incidence of two freemartins in the offspring of 870 ewes and from her data she estimated that the sort of placental anastomosis which caused freemartinism occurred in only 1.2% of twin conceptions. Both freemartins occurred in inbred lines and it has been suggested that vascular anastomsis occurs more frequently in inbred flocks. (Alexander and Williams, 1964) Dain (1971) diagnosed freemartinism on the basis of sex chromosome chimerism in leucocyte cultures. Chromosomal evidence of the freemartin condition in sheep was first provided by Gerneke (1965) but erythrocyte chimerism between heterosexual twins had been demonstrated more than ten years earlier. (Stormont et al., 1953) Bruere (1966) and Bruere and MacNab (1968) examined six intersex sheep. Leucocyte cultures revealed a sex

chromosome chimerism but fibroblast cultures showed only XX cell lines. Similar results were obtained by Jonsson and Gustavsson (1969) who examined various tissues from an intersex lamb, originally thought to be a female sib to two male siblings but which was in fact a freemartin. Despite these reports of blood cell chimerism, vascular anastomosis has only once been demonstrated definitely, (Alexander and Williams, 1964) and yet fusion of adjacent chorions in sheep multiple pregnancies appears to be normal. (Mellor, 1969) Mellor found that only minor vessels crossed the fusion line of two chorions. They were usually less than 0.5 mm in diameter, and very few of these formed an anastomosis with the vasculature of the adjoining chorion. No anastomoses of placental circulation from shared cotyledons were found. He concluded that the minor anastomoses that do occur between neighbouring foetuses do not permit mixing of the two circulations in detectable amounts. It is obvious therefore, that for the condition of freemartin to develop in sheep far greater anastomoses must take place and the stimulus for this process remains obscure.

Bruere and MacNab (1968) found no correlation between the percentage of male cells and the degree of masculinisation. Dain and Tucker (1970) reported an increase in the number of aneuploid cells in freemartins. Bruere, (1967) reported an increase in aneuploidy in freemartins over 4 years old but considered that the aneuploidy was due to the age of the animals and not the fact that they were freemartins. An 18 month old freemartin had a model chromosome count near to that of normal animals. Furthermore, Bruere and MacNab (1968) did not find abnormal aneuploidy in the six freemartin sheep they examined.

In the pig there have been three reports of intersex pigs with an XX/XY sex chromosome chimerism. (McFee, Knight and Banner, 1966; Bruere, Fielden and Hutchings, 1968; Vogt, 1968) However, the condition is

not common and most cases of intersexuality in the pig have been genetic females. (Breeuwsma, 1969)

Only one report of XX/XY sex chromosome constitution in the horse is known to the writer. This case was a complex XX/XY/XXY/XO chimera although the XX/XY cell lines consisted of 47.2% of the cells counted and 25% had a doubtful sex chromosome complement. (Basrur et al., 1969)

To summarise, hypermodal numbers of sex chromosomes have been reported from a number of species and are compatible with life. By contrast, absence of an X chromosome appears to render the OY zygote inviable whilst absence of one of the X chromosomes from an XO zygote greatly reduces its chance of survival. Structural abnormalities of the sex chromosomes appear to be rare. Not only are there few reports of such findings in neonatal or adult populations, but chromosome studies of abortuses have not shown a higher incidence than in liveborn individuals. (Carr, 1967) This tends to suggest that structural aberrations of sex chromosomes are indeed rare occurrences. This finding is perhaps not surprising, since a system that allowed for frequent structural abberrations of such important elements would quickly be self-limiting. It is significant too, that there is a greater incidence of supernumery X chromosomes than Y, since according to the Lyon hypothesis (1961) only one X chromosome remains genetically active throughout the cell's life. Without this gene compensation mechanism, it is difficult to imagine how such X chromosome polyploidy could be tolerated.

1.3.4. Numerical Autosomal Aberrations

Compared with numerical aberrations of the sex chromosomes, numerical aberrations of autosomes are not common. Indeed, there is good evidence from cytological study of human abortuses that many such aberrations are incompatible with life. Carr (1965) found 22 of 44 spontaneous human abortuses to be trisomic for an autosomal chromosome. A further 9 specimens were triploidies with a chromosome number of 3n =69. A further study on spontaneous abortuses following conception after cessation of oral contraceptives (Carr, 1970) showed a rise in the incidence of polyploidy. In particular triploid abortuses were 4½ times more common in the post-oral-contraceptive group than in the control group, and tetraploid abortuses were six times more common. In an investigation of abortuses following induced ovulation Boue and Boue (1973) found that 61% of 1457 spontaneous abortuses had abnormal karyotypes and 97% of these were numerical abnormalities.

Comparable data on abortuses from domestic animals are not available. However, karyotypic analysis of pre-implantation blastocysts of a number of laboratory animals has revealed an incidence of autosomal abnormalities far in excess of that which is found in the live born population. Shaver and Carr (1967) examined rabbit blastocysts after delayed fertilisation and found that the commonest chromosome anomaly was polyploidy. Further work by the same authors (Shaver and Carr, 1969) confirmed the high incidence of triploidy following delayed fertilisation. A similar result was reported in the mouse by Vickers (1969) who found that there was a nine-fold increase in triploidy following delayed fertilisation and Fiko and Bomsel-Helmreich (1960) reported that almost all triploid foetuses in their experimental rats were lost before or soon after implantation.

Trisomy has been identified in a macerated foetal cat. (Benirschke, Edwards and Low, 1974) Three phenotypically normal foetuses were also present in the uterus and fibroblast cultures demonstrated a normal karyotype. The authors suggested that death of the macerated foetus was due to the autosomal trismony.

Despite the efficient elimination of numerical autosomal anomalies during gestation, such chromosome complements are occasionally found in the population. The commonest autosomal trisomy is that found in individuals suffering from Down's syndrome. The condition is characterised by mental retardation, skeletal abnormalities with facial disorders, congenital heart conditions and a high incidence of leucaemia, and was shown to be associated with the additional submetacentric chromosome in the complement. (Lejeune, Gautier and Turpin, 1959) Two other autosomal trisomies are recognised in man. One is trisomy 13 (Patau, Smith, Therman, Inhorn, and Wagner, 1960) which is characterised by facial defects, such as cleft palate, kidney and cardiac defects and skeletal abnormalities. The second is trisomy 18. This is also characterised by cardiac defects and facial abnormalities with severe mental retardation. Both conditions usually result in death of the individual in early infancy. However, there has been one report of a 10 year old girl with trisomy D, (Morden and Yunis, 1967) and a 15 year old girl with trisomy 18 (Hook, Lehrke, Rosener and Yunis, 1965) Both were severely mentally retarded. A detailed examination of D trisomy patients has been made in an attempt to relate the morphological abnormalities with the extra chromosomes. (Marin-Padilla, Hoefnagel, and Benirschke, 1964) These authors considered that the duplication of tissue such as the Mullerian duct derivatives and digits, together with excess tissue expressed as supernumerary lobules of the liver, lungs, pancreas and kidney were directly attributable to the extra chromosome. They thought that the

skeletal and facial abnormalities were a secondary effect of some other alteration in early embryonic life.

An autosomal trisomy has been reported in a water vole (Arvicola terrestris L.) caught in southern Sweden (Fredga, 1968). The animal died shortly after capture so that no assessment of fertility was made. The animal was phenotypically normal.

There have been five reported cases of autosomal trisomy in cattle, each associated with brachygnathia (Herzog and Holn, 1968; Mori, Sasaki, Makino, Ishikawa and Kawata, 1969; Holn and Herzog, 1970; Dunn and Johnson, 1972). Three of the animals were males and two were females. Herzog and Holn (1968) suggested the extra chromosome was either 17 or 18 in their case. The extra chromosome in the case reported by Dunn and Johnson (1972) was larger than the largest normal autosome. The case reported by Mori et al., (1969) was that of a grossly abnormal calf with multiple skeletal abnormalities and abdominally retained testes. Herzog (1974) reviewed the literature on autosomal trisomy and brachygnathia in cattle.

A cryptorchid twin calf was reported by Hoffman (1967) to have a 60XY/62XX chimerism of cells of the testis.

By comparison to the ox, autosomal trisomy in the mouse does not appear to be lethal. There were two reports in the same year from independent workers of phenotypically normal mice with an autosomal trisomy. Cattanach (1964) reported a sterile male, which had normal sized testes but histological and cytogenetical examination revealed spermatogenic arrest at the first meiotic division. This animal was

trisomic for one of the smaller acrocentric autosomes. Since the father had been treated with a mutagen it was assumed that non-disjunction had occurred in a meiotic division of the father.

Griffe and Bunker (1964) reported three mice which were trisomic for three different autosomes. Each was phenotypically normal but two were completely sterile and one had reduced fertility. The sires of each of the three animals had been irradiated in the area of the testes some weeks prior to coitus and it was assumed that non-disjunction had occurred due to this irradiation.

The apparent lack of phenotypic effect of the extra chromosome in mice, compared to other species is extremely interesting. However, since the reports were all of artificially induced abnormalities it may well be that the extra chromosome was not completely functional.

Autosomal monosomy is even more rare than trisomy. Cases of partial monosomy are known in man, for example the cri du chat syndrome. (Lejeune, Lafourcade, Berger, Vialatte, Boeswillwald, Seringe and Turpin, 1963) These individuals have a partial deletion of the short arm of chromosome number 5. There has been a report of monosomy G in an infant with clinical features very similar to those described in other cases of partial G monosomy. (Lejeune, Berger, Rethore, Archambault, Jerome, Thieffry, Aicardi, Broyer, Lafourcade, Cruveiller and Turpin, 1964)

There has been one report of a possible monosomy plus trisomy of chromosomes in a bovine Anidian twin monster. (Dunn, Lein and Kenney, 1967) The male twin had a normal male karyotype of 60 XY, whilst the monster had a 61XX/60XX chromosome complement. Definitive

karyotypic identification of the abnormal chromosomes was not possible but since both cell lines had an unpaired acrocentric autosome, the largest of the complement, it was suggested that both cell lines were monosomic for a large acrocentric, the 60XX line trisomic for a small acrocentric and the 61XX line had two extra small acrocentrics. No other report of autosomal monosomy is known to the present author.

An extra chromosome appears to be tolerated better than the loss of a chromosome, but both conditions seem likely to be incompatible with life.

Triploidy was the second most common chromosomal abnormality found in spontaneous human abortuses (Carr, 1965). It has been suggested that death is due to the delay in growth, brought about by prolongation of the time required to complete one or more stages of the mitotic cycle (Mittwoch and Delhanty, 1972). Dunn, McEntee and Hansel (1970) reported a Holstein intersex, anatomically a true hermaphrodite which was a diploid XX/triploid XXY chimera. Cultures from a variety of tissues reflected a higher incidence of triploid, Y-bearing cells of mesodermal origin from the right side of the animal, ipsolateral with the ovotestis. The percentage of triploid cells was different in the different tissues but they were always rare.

1.3.5. Structural Autosomal Aberrations

Structural rearrangement may occur within one chromosome or involve the exchange of material between two or more chromosomes. Types of aberrations in the first category include deletions, peri and paracentric inversions and ring formations. Those involving more than one chromosome are reciprocal translocations, insertions, tandem

and centric (Robertsonian) fusions and centric fission.

1.3.6. Intra-chromosomal Rearrangement

Deletions may be terminal with resultant loss of the acrocentric fragment at the next mitotic division, or interstitial with loss of the fragment and reunion of the two break points. If a terminal deletion occurs in both arms of the chromosome the union of these arms produces a chromosome with a ring formation.

A chromosome deletion has been reported in four unrelated sheep born with Brachygnathia superior. (Luft, 1972) Each animal (two ewes and two rams) was apparently a mosaic for a deletion of one of the acrocentric chromosomes, designated number 13 by the author. The same author reported a fifth ram with a deletion of one of the chromosomes in the group 16-21. (Luft, 1973) This animal was phenotypically normal and a mosaic for the deleted chromosome. In a number of cells the deleted fragment was identified. Differentiation of the individual acrocentric chromosome in the karyotype of the sheep is not possible with normal aceto-orcein staining so that localisation of a defect to a particular chromosome is difficult. In addition, unless the preparations provide a clear indication of the centromeric position, one chromatid may appear longer than the other by the configuration of the metaphase spread on the slide. For this reason an interpretation of apparent deletions, particularly when not present in every cell counted, has to be made with caution. Nevertheless, it is theoretically possible for such a mosaic to occur. If the break and deletion in one chromatid occurred during cell division then one of the daughter cells would carry the deleted chromosome and the other daughter cell would be normal.

Halnan (1972) reported deletions in one or both chromatids of one chromosome in the group 14-26 in the ox. The deletions were seen in 5-10% of cells in six bulls with a history of infertility or subfertility and in one Freemartin and a relationship between chromosomal abnormality and infertility was claimed. No indication was given as to whether the deletion appeared in both cell lines in the Freemartin and the correlation with infertility was doubtful. Furthermore, photographs of the affected chromosomes presented the appearance of marked secondary constrictions rather than deletions.

Multiple chromosome breaks have been reported in a set of bovine quintuplets. (Basrur and Stoltz, 1966) One heifer calf died at birth but three males and one female survived and all were XX/XY chimeras. Distinct chromosome breaks were seen in the donor cells in all four animals, but the male cell line of the heifer showed a greater percentage of affected cells (approximately 20%) than the female cell line in the males, (less than 10%). The authors suggested that the abnormality was possibly due to attempted antibody production of the host against the donor cells.

Chromosome breaks have also been induced after prolonged consumption of phenylbutazone in both man (Stevenson, Bedford, Hill and Hill, 1971) and the horse. (Stevenson, Hastie and Archer, 1972).

Inversions involve two breaks in the same chromosome arm, with inversion and reunion of the intervening fragment. A pericentric inversion includes the centromere whilst paracentric inversions do not. Since pericentric inversions include the centromere their occurrence is more easily detected because of the alteration of chromosome morphology. In man, pericentric inversion of chromosome number 9 is a relatively

common polymorphism. (de la Chapelle, 1974: personal communication) A similar polymorphism, involving pericentric inversion, occurs in the deer mouse (Peromyscus maniculatus) (Chno, Weiler, Poole, Christian, and Stenius, 1966) Two cases of pericentric inversion have been reported in cattle. Short et al., (1969) described a metacentric marker chromosome, presumably due to a pericentric inversion, in the XY cell line of a pair of heterosexual bovine twins. Popescu (1972) described a pericentric inversion of one of the small acrocentric chromosomes, possibly number 14, in a four year old bull. The animal had been referred for examination because of reduced fertility. Hamerton (1971) has suggested that infertility, as a consequence of unbalanced duplication or deficiency of the inversion, is more likely to be demonstrated in the male since the unbalanced crossover chromatid may be included in the polar body in female gametogenesis.

The difference between the Y chromosome of European cattle (Bos taurus) which is a small submetacentric chromosome and Asian cattle (Bos indicus) which is a small acrocentric chromosome (Kieffer and Cartwright, 1968) may be due to a pericentric inversion.

1.3.7. Interchromosomal Rearrangement

Reciprocal translocation involves the exchange of a chromosome segment between two non-homologous chromosomes. The affected individual is a translocation heterozygote but with a balanced chromosome complement. If the exchanged fragments are of approximately even size, such rearrangement would pass undetected in routine chromosome examination. Even unequal reciprocal translocations often require meiotic studies to confirm their existence. At meiosis, reciprocal translocations can be identified by their multivalent associations. Malsegregation of these multivalents can result in chromosomally unbalanced offspring.

In man, it has been estimated that the risk of malsegregation, and the production of unbalanced karyotypes in the offspring is of the order of 10-20% of the progeny of carrier mothers. The risk involved when the father is the carrier is probably only 5-10%. (Lejeune, Dutrillaux and de Grouchy, 1970) The incidence of reciprocal translocations in the general population has been estimated at 0.3% (Court Brown, 1967) and appears to be the commonest structural rearrangement.

There have been only two reports of reciprocal translocation in domestic animals and both of these have been in the pig. (Henricson and Backstrom 1964; Hansen-Melander and Melander, 1970) Henricson and Backstrom described a boar which was phenotypically normal but with a reduced fertility. The number of piglets in litters from sows sired by this boar were approximately half that in litters sired by other boars. (Average litter size of 5.1 as compared to 12.7) The subfertile boar was found to have a structural rearrangement involving one of the chromosomes of pair number 3 or 4 and one of pair number 14. Although there was no evidence that the small chromosome had received some material from chromosome number 14, the authors suggested that a non-reciprocal translocation was unlikely.

Hansen-Melander and Melander (1970) reported a translocation mosaicism in a stillborn, malformed pig. There was lateral asymmetry, skeletal abnormalities, heart and liver abnormalities and although there were male reproductive organs, a penis was missing. Fibroblast cultures from various tissues showed the animal to be a 38XY/38 t (1q+;13?q-)XY mosaic. The translocation bearing cells average 66% of all those counted from various tissues.

Although Henricson and Backstrom (1964) considered that it was

chromosome number 14 that was involved in the case they described, definite identification of individual chromosomes in the pig is not easy and it is tempting to speculate as to the possibility that the same chromosome was involved in their case and that described by Hansen-Melander and Melander (1970). The latter authors suggested that chromosomes 12-13 might have a weak zone with a tendency to break.

Hansen, (1969) has described a tandem fusion in Red Danish milk cattle. He suggested that the translocation was a fusion of an acrocentric chromosome on to the broken ends of another acrocentric chromosome with the loss of a centromere. However, such a configuration could also be produced if there was an unequal reciprocal translocation, before the division of the chromatid, between a large and small acrocentric chromosome. It is likely that the small fragment product of the translocation would be lost at the succeeding mitotic division.

The translocation appeared in both males and females of the breed. Although the males were phenotypically normal their fertility was reduced by approximately 10% whilst that of the females appeared unaffected.

The final class of structural aberrations involves the centromere. In the pig, the difference in chromosome number in the wild pig 2n=36, (McFee, Banner and Rary, 1966; Rary, Henry, Matschke and Murphree, 1968) and domestic pig 2n=38; (McConnell, Fechheimer and Gilmore, 1963; Harvey, 1969) is probably due to centric fission. A case of presumed centric fission was reported in an Indian Langur (Presbytis entellus) (Egozcue, 1971) Centric fission has been reported in a pseudodiploid Chinese hamster cell line, involving the X chromosome. (Kato, Sagai and Yosida, 1973) In this cell line, when deletion of

one of the fission elements occurred, all the cells were found to possess the short arm only. The long arm of the X was heterochromatic and its loss was presumably not incompatible with life. In no case were cells detected without the short arm component. The telocentric chromosomes were extremely stable and remained in culture for three months showing no tendency to form isochromes or metacentric chromosomes through centric fusion.

Centric fusion, or Robertsonian translocation, is much more common than centric fission. Since the present work is concerned with one such centric fusion, the Massey I translocation, this type of chromosomal rearrangement is considered in detail.

1.3.8. Centric fusion (Robertsonian) translocation

Structure

Centric fusion translocations were first recognised as such by Robertson (1916) In the course of his studies on the chromosomes of grasshoppers he noted that chromosome number and morphology throughout the subfamily Acrididae were remarkedly uniform with the exception of the members of the genus, Chorthippus. Grasshoppers belonging to this genus possessed less chromosomes and some of them were "V" of "J" shaped. This was at variance with the rod shaped chromosomes of the rest of the subfamily. Robertson suggested that the "V" and "J" shaped chromosomes were formed by the fusion of two rod chromosomes, each arm of the "V" and "J" chromosome corresponding to a single rod. On the basis of gene linkage studies he suggested that the fusion was between the centromeres of non-homologous chromosomes.

The exact nature of the fusion mechanism is still not entirely understood and the problem is exacerbated by the dearth of information on

the structure of the centromere. The centromere, or kinetochore, has been defined as that region of the chromosome with which the spindle fibres become associated during mitosis and meiosis. (Rieger, Michaelis and Green, 1968) Early workers using the light microscope described the centromere as a quadripartite structure (Tijo and Levan, 1950; Lima-de-Faria, 1956) consisting of four chromomeres, of compact fibres arranged to form a square or parallelogram. One chromomere was located in each arm of the chromatid of a metacentric chromosome and they were joined to each other by less dense fibres.

It had originally been suggested that the centromere could not be truly terminal. (Navashin, 1916) It was thought that the centromeric structure required two chromosome arms and that apparent telocentric chromosomes had minute short arms, invisible with the light microscope. (White, 1957) This concept was not accepted by all workers. (Lima-de-Faria, 1956; Marks, 1957) Marks (1957) suggested that telocentric chromosomes could be formed from the centric fission of a metacentric chromosome. He ascribed their apparent rarity to the fact that telocentric chromosomes would arise in cells with a high possibility of genetic imbalance, due to malsegregation following the fission, so that their chance of survival was low. Later workers, using the electron microscope have been able to confirm that true, telocentric chromosomes do exist and are stable. (John and Hewitt, 1966; Southern, 1969; Comings and Okada, 1970; Kato, Sagai and Yosida, 1973)

Application of electron micrograph examination of the structure of the centromere has shown it to be a much more complex region than originally suspected. Brinkley and Stubblefield (1966) demonstrated that in dividing Chinese hamster fibroblast cells the centromere was a

distinct entity by the prophase stage, before the dissolution of the nuclear membrane even although spindle fibre association did not occur until after the disappearance of the membrane.

The fully developed, metaphase centromere has been described as consisting of two daughter centromeres (Brinkley and Stubblefield, 1966; Jokelainen, 1967; Brinkely and Stubblefield, 1970) separated by a region devoid of interconnecting fibres. (Brinkley and Stubblefield, 1966; Jokelainen, 1967). Sister centromeres were located on opposite sides of the centromeric region and orientated towards the spindle pole. They were connected to this pole by from four to seven spindle filaments. (Jokelainen, 1967) Brinkley and Stubblefield (1966) described each centromere as having a dense central core, 200-300Å wide, enclosed on each side by a less dense zone, 200-600Å thick composed of fine fibrils. Each sister centromere occupied a relatively small area on the surface of the chromatid. Jokelainen (1967) envisaged each sister centromere as being disc-like with a diameter of between 2000 and 2450Å and composed of three layers.

The centromere is located in an obvious constriction, the primary constriction of the chromosome. In this centromeric region some of the chromatid fibres crossed over to the sister chromatid. (Abuelo and Moore, 1969; Comings and Okada, 1970) Some fibres crossed over and continued to run in the same direction whilst others crossed over, reflected back and ran in the opposite direction. This crossing over left a central space between the chromatids which was devoid of fibres. Comings and Okada (1970) described this area as a halo-like, clear area between chromatid associations. They considered the centromere of telocentric metaphase chromosomes to be bipartite (one part for each chromatid) and the centromere of metacentrics to be quadripartite.

The various interpretations of the mechanism of the centric fusion translocation have to be viewed in the light of these complexities of centromeric structure. White (1957) proposed that a centric fusion translocation was a reciprocal exchange between two acrocentric chromosomes. He suggested that the long arm of one acrocentric chromosome joined to the second acrocentric chromosome by its short arm. The centromere and short arm of the first chromosome plus a fragment of short arm from the second chromosome then fused to form a minute metacentric chromosome which was lost at the next cell division. This theory required that some loss of DNA occurred in metacentric formation. Comings and Avelino (1972) investigated centric fusion in the mouse using the electron microscope and found that both centromeres were retained after fusion. They suggested that there was a reciprocal translocation between the chromatin fibres that made up the centromeres. To investigate whether any DNA was lost during centric fusion the same authors compared centromeric heterochromatin in the laboratory mouse (M. musculus) whose karyotype consists entirely of acrocentric chromosomes and that of the tobacco mouse (M. poschianious), which is homozygous for seven centric fusion translocations. They found no significant difference between these two species which suggested that less than 0.2% of the genome could have been lost (limits of experimental error) following fourteen centric fusion translocations. However, the retention of centromeric heterochromatin does not necessarily indicate the retention of both centromeres. Crouse (1960) showed that centromeric heterochromatin was a separate entity from the centromere and could be translocated to various parts of the chromosome without upsetting centromeric function.

Comings and Okada (1970) examined mouse metacentric chromosomes which had arisen in culture due to centric fusion of two telocentric

chromosomes. They found that at the centromere of metacentric chromosomes there were two regions of chromatin fibre assocation with an area between relatively devoid of fibres. In contrast, the telocentric chromosomes had only one area of chromatin fibre association at the centromere and this was half the size of that in the metacentric chromosome. Similar quadripartite centromeres were seen in human, Chinese hamster and sheep metacentric chromosomes. (Comings and Okada, 1970) In the human, not all metacentrics showed two separate areas of association but the authors considered it likely that the difference was a technical artifact.

Centromeric heterochromatin was studied in mouse cell lines in which metacentric chromosomes were believed to have arisen by centric fusion translocation whilst in culture. (Chen and Ruddle, 1971) They found that practically all the centromeric heterochromatin of the acrocentric chromosomes was incorporated into the new metacentrics and found blocks of heterochromatin, one in each chromatid, could be observed. However, some mice metacentric chromosomes were observed with ~~substantially~~ SUBSTANTIALLY less than twice the centromeric heterochromatin found in acrocentric chromosomes. It is possible that in these instances some loss of DNA had occurred. The loss of even small proportions of DNA would be important if the DNA contained essential genes and it has been suggested that positioning of non-essential repetitive satellite DNA at the centromeric regions plays a role in protecting the organism from deleterious effects of such DNA loss. (Mattocia and Comings, 1971)

Evans et al., (1973) described a goat, heterozygous for a centric fusion translocation, with the chromosome complement of 2n= 59xy,T+. Centromeric banding revealed a large mass of heterochromatin at the centromere of the metacentric chromosome. This made an interesting

comparison with the metracentic chromosomes of the sheep which had very little centromeric heterochromatin. By the examination of aminoacid sequences of fibrinopeptides from members of the Order Artiodactyla, Doolittle and Blomback (1964) estimated that the evolutionary divergence of the goat and sheep occurred approximately 5,000,000 years ago. Presumably, excess centromeric heterochromatin has been eliminated from the metacentric chromosomes of sheep during their evolutionary divergence from goats.

In man, centric fusion tranlocations are mainly, if not exclusively, spontaneous rearrangements. (Hecht and Kimberling, 1971) It has been suggested that non-~~homlogous~~ HOMOLOGOUS chromosomes which form centric fusion translocations in man could have homologous segments in areas of secondary constrictions. (Ferguson-Smith, 1967) Pachytene association in man has been noted and it was suggested that it may represent pairing of homologous areas on non-homologous chromosomes. Reciprocal exchange at these points may lead to the formation of dicentric "centric fusion" translocations. (Ferguson-Smith, 1967) Rowley and Pergament (1969) suggested that there was a non-random selection of D-group chromosomes involved in centric fusion translocations in man. However, Cuevas-Sosa (1970) considered that there was a random association of acrocentric chromosomes. The evidence relating to this hypothesis has recently been reviewed by Ferguson-Smith (1971).

Incidence

Centric fusion translocations have been identified in a number of species, but apart from man incidence figures are not available except in relatively small numbers.

The resultant genotype of possible theoretical segregations of a

centric fusion translocation and its acrocentric homologues are shown in Fig.23a. It has been postulated that the production of such monosomic and trisomic zygotes leads to a reduced fertility in heterozygous carriers. This reduced fertility was thought to be due to a reduced viability of unbalanced zygotes leading to early embryonic death.

Man

Three types of centric fusion translocations are known in the human population, t(DqGq), t(DqDq), and t(GqGq), (Hamerton, 1971) with an incidence of 1 per thousand adults for the t(DqDq) translocation (Court Brown, 1967) and 0.05 per thousand adults for the t(DqGq) and t(GqGq) translocations. (Polani, Hamerton, Giannelli and Carter, 1965) In each instance, the balanced heterozygotes were phenotypically normal. The diagnosis of the defect in an affected family was usually due to the production and subsequent diagnosis of an offspring with an unbalanced chromosome complement. This was particularly true for families carrying a (DqGq) translocation when the offspring was trisomic for chromosome number 21 and hence had clinical signs of Down's syndrome. Estimates of the degree of non-disjunction and malsegregation were complicated by the fact that data were biased towards identifying unbalanced offspring. However, Hamerton (1971) examined past case records, corrected the information for this bias and found that t(DqGq) heterozygous parents produced an excess of heterozygous offspring which was entirely accounted for by an excess of heterozygous offspring from heterozygous fathers. In addition, when the father was heterozygous the frequency of unbalanced offspring was only 2.4% compared with 10.8% of unblanced offspring from heterozygous mothers.

Information on t(DqDq) heterozygous families again showed an excess of heterozygous offspring resulting from heterozygous fathers but there was almost a complete absence of progeny with an unbalanced karyotype. The frequency (0.6%) was too low to determine any difference in segregation between the parents. Information on t(GqGq) families was sparse but there did seem to be only a low level of adjacent segregation.

A low incidence of unbalanced offspring in the population may indicate a low incidence of adjacent segregation, or alternatively, a low viability of unbalanced heterozygotes. There was no increased incidence of spontaneous abortion in t(DqDq) families as compared to normal families (Hamerton, 1971; Chandley, Christie, Fletcher, Frackiewicz and Jacobs, 1972). Whilst the frequency of spontaneous abortions in t(DqGq) families was the same as the general population, t(DqGq) mothers married to normal men did have a slightly higher frequency of abortions than normal women married to t(DqGq) fathers. (Hamerton, 1971) Hamerton considered this to be due to the fact that heterozygous mothers tend to produce more unbalanced gametes. It seems likely therefore, that the low incidence of unbalanced offspring reflects a low level of adjacent segregation.

These data show that different translocations involving different chromosomes have different frequencies of adjacent segregation. It would seem therefore, that the incidence of adjacent segregation is a function of the chromosomes involved rather than of the presence of a centric fusion translocation per se. The difference may reflect different centromeric structure in the different translocations. The possible break positions in the centromere of metacentric chromosomes have been discussed by Gimez-Martin, Lopez-Saez and Marcos-

Moreno, (1965) If it is assumed that a centric fusion translocation can only take place following some sort of break in the centromeric region then there are ten possible recombination centromeric structures of the translocation chromosomes. Three of these configurations would have double the centromeric area, four would have one and a half times and three would have the equivalent of one normal centromere. It would seem likely that different configurations would behave differently at meiosis and this may account for the variability of behaviour of centric fusion translocations in both wild and domestic animals, as well as man.

Tobacco House

The tobacco mouse (Mus poschiavinus) was first described as a separate species by Fatio (1869) and has since been shown to have a karyotype consisting of 14 metacentric chromosomes and 12 acrocentric chromosomes in contrast to the 40 acrocentric chromosomes of the laboratory mouse (Mus musculus). (Gropp, Tettenborn and Lehmann, 1970) Meiotic studies (Tettenborn and Gropp, 1970) and differential staining examination (Zech et al., 1972) demonstrated that the tobacco mouse was homozygous for seven centric fusion translocations. Subsequent work revealed that the F_1 tobacco mousex laboratory mouse hybrids had a marked reduction in fertility. Analysis of meiotic metaphas II in the male revealed a high level of meiotic non-disjunction in the F_1 hybrids. (Gropp, et al., 1970; Tettenborn and Gropp, 1970)

DNA measurements showed a greater variation in DNA content in morphologically normal spermatozoa of F_1 hybrids than in the laboratory mouse. (Doring, Gropp and Tettenborn, 1972; Stolls and Gropp, 1974) This range of DNA content was thought to be due to the presence of aneuploid spermatozoa. In the female, F_1 hybrids showed

a marked reduction in litter size due to losses of both monosomic and trisomic foetuses. (Gropp, 1971)

Cattanach and Moseley (1973) and Ford and Evans (1973) isolated each of the seven tobacco mouse metacentrics as homozygous lines upon predominantly Mus musculus genetic backgrounds. Examination of cells from homozygous and heterozygous animals at meiotic metaphase II clearly demonstrated that heterozygosity for the metacentric chromosome was a major factor leading to non-disjunction. In addition, the frequency of non-disjunction associated with each different chromosome was different, although the two groups of workers varied slightly in their estimates of frequency for each chromosome. Furthermore, the degree of zygotic loss associated with each translocation was of the same order as the degree of aneuploid spermatozoa produced in the male. Heterozygotes for each of the seven metacentrics gave a higher frequency of zygotic loss than either the normal or homozygous animals.

The level of non-disjunction in F_1 hybrids of the tobacco mouse and laboratory mouse was much higher than non-disjunction in other mice centric fusion translocations which are discussed below. Cattanach and Moseley (1973) suggested that the raised level of non-disjunction in their stock was probably not a consequence of the centric fusion per se but more likely resulted from some other chromosmal or genetic variation. They suggested that minor differences between the tobacco mouse and house mouse could have arisen during speciation and that the zygotic loss in the F_1 hybrids could be the result of interspecific crossing. If this hypothesis is correct it would be misleading to apply the findings in the tobacco mouse to centric fusion translocations in other species.

Laboratory Mouse

Three centric fusion translocations have been reported in separate strains of Mus domesticus. (Evans, Lyon and Daglish, 1967; Leonard and Deknudt, 1967; White and Tjio, 1967) Both male and female heterozygotes for the T163H translocation (Evans et al., 1967) were fertile but heterozygous males produced smaller litters when mated to normal females than did the normal control males. The heterozygotes had approximately 69% fecundity when compared to normal males. Meiotic studies on two of the male heterozygotes showed that 98% of the gametes formed had a balanced karyotype. It would seem therefore, that the zygotic loss was not due entirely to the production of unbalanced gametes.

Some mice belonging to the AKR strain were found to be homozygous for a centric fusion translocation (Leonard and Deknudt, 1967). No meiotic studies were reported but fertility was claimed to be low as judged by litter size.

The third translocation was discovered accidently in an inbred line of albino mice. (White and Tjio, 1967) Both homozygous and heterozygous animals were identified. Meiotic studies on heterozygous males revealed that 91% of cells at second metaphase were balanced with either 20 acrocentrics or 18 acrocentrics and one submetacentric. The average size of litters from matings between heterozygotes was lower than that of most other matings. However, since both males and females had undergone surgery prior to the test matings the reduction of fecundity may well have been due to factors unrelated to the presence of the translocation.

The reports of centric fusion translocations in mice, therefore,

present a similar picture, irrespective of whether heterozygous crosses are intraspecific or interspecific. All of the male heterozygotes showed some degree of non-disjunction at meiotic second metaphase, but none at such high frequencies as the T_4 of the tobacco mouse. This has important implications for those cases of centric fusion described in domestic animals. If the reduction in fertility is due to the effects of the individual chromosomes it may well be that translocations involving different chromosomes could have neutral or advantageous effects. It is necessary, therefore, to examine each translocation in each species separately in order to assess the effects adequately.

Dog

In the dog, centric fusion translocations have been identified in animals with lymphosarcoma, (Basrur and Gilman, 1966; Froget, Fontaine, Nain and Michaillard, 1972) congenital cardiac defects (Shive, Hare and Patterson, 1965; Patterson, Hare, Shive and Luginbuhl, 1966) bone chondroplasia (Hare, Wilkinson, McFeely and Riser, 1967), and ectopic ureters (Hare and Bovee, 1974). The two dogs with ~~lymphosarcoma~~ LYMPHOSARCOMA, the single case with congenital heart defects and the young poodle with bone chondroplasia were all presumed to be balanced heterozygotes. The diploid number in each case was 2n= 77. Shive et al.,(1965) reported 13 other cases with cardiac defects without chromosomal abnormalities and Basrur and Gilman (1966) reported four cases of lymphosarcoma in dogs without centric fusion translocations. In addition, Ma and Gilmore (1971) reported a 7 month old female setter-retriever cross which was phenotypically normal although heterozygous for a centric fusion translocation. There is no evidence therefore, that in the dog, centric fusion translocations are related to any specific clinical syndrome. No data are available regarding the effects on fertility. Unfortunately, the case reported by Ma and

Gilmore (1971) had been overiectomised before the karyotype had been established.

Goat

Limited work has been carried out to investigate the fertility of goats heterozygous for centric fusion translocations. (Padeh, Wysoki and Soller, 1971; Popescu, 1972a) Padeh and his co-workers found that when heterozygous males were crossed with normal females, the proportion of multiple births was reduced. There was a similar tendency when heterozygous males were crossed with heterozygous and homozygous females although the difference from normal male x female was too small to be statistically significant. The test matings produced a total of 30 offspring. All had balanced karyotypes and there was no significant deviation from the normal sex ratio. All the offspring from matings of heterozygous males with normal females were males, and the ratio, normal to heterozygote was 3 to 9. Popescu, (1972a) found that 14 of 19 male offspring (heterozygous male x normal female) were heterozygotes. The remaining five males had a normal karyotype. Only 6 of the 11 female offspring of a similar cross were heterozygotes. There is some suggestion therefore, both from the report of Padeh et al.,(1971) and Popescu, (1972) that there is an excess of male heterozygotes from a male heterozygous sire. However, with such a small breeding programme conclusions must be guarded. Padeh et al.,(1971) gave no indication of which chromosomes were involved in the translocation but Popescu, (1972a) on the basis of idiogram measurements, estimated that the two autosomes involved in the translocation were numbers 2 and 14. It is interesting to note that these are not the chromosomes suggested by Evans et al., (1973) to be involved in the formation of any of the metacentric chromosomes in the sheep. The case of centric fusion translocation

in the goat reported by Evans et al.,(1973) involved chromosomes number 5 and 15.

Ox

In cattle, considerable work has been carried out on one centric fusion translocation, generally known as the 1/29 translocation. (Gustavsson, 1969) Two other centric fusion translocations have been reported; a 2/4 translocation in the Friesian breed (Pollock, 1972) and an 11-12/15-16 translocation in the Simmental breed. (Bruere and Chapman, 1973) More recent G-band studies on this translocation suggest that it is an 11/21 translocation (Logue, 1974, personal communication). A 1/28 translocation has been described in an achondroplastic calf of the Romagnola breed. (Rugiati and Fedrigo, 1968)

The 1/29 translocation was first reported by Gustavsson and Rockborn (1964) in three leucaemic cattle of the Swedish Red and White breed. A similar translocation has now been reported in cattle from a number of breeds (Table III) but its effects have only been studied in detail in the Swedish Red and White breed. (Gustavsson, 1969; 1970; 1971; 1971a; and 1971b)

In a survey of Swedish Friesian cattle, Swedish polled cattle and Swedish Red and White cattle, animals with the 1/29 translocation were found from the Swedish Red and White breed. (Gustavsson, 1969) In this breed 366 (14%) of the animals studied were heterozygotes and 8 (0.4%) were homozygotes. In a later survey of the distribution of the 1/29 translocation in the bull population used for artificial insemination (A.I.) 12.29% (316) were found to be heterozygotes and 0.42% (4) were homozygotes. (Gustavsson, 1971) There was no

difference in incidence among A.I. associations. It was suggested that non-disjunction between the translocation chromosome and the homologous acrocentric chromosomes at meiosis of heterozygous individuals could result in the production of unbalanced gametes. These gametes, if viable, would give rise to unbalanced zygotes (Fig.23a). Reduced viability of these unbalanced zygotes would then lead to a reduced fertility or fecundity in translocation heterozygotes. The chromosomes of repeat breeder heifers were examined. (Gustavsson, 1971a) One hundred and eighty two animals, (69.2%) showed the normal (2n=60) chromosome complement whilst 81 (30.8%) carried the translocation chromosome. The incidence of the 1/29 translocation in the general female population was approximately 14% (Gustavsson, 1969) so that there was a statistically significant difference between the general population and the group of repeat breeder heifers. The pedigrees of these heifers were not reported, but they were offspring of bulls from four different A.I. centres so that it is unlikely that the results were unduly weighted by the offspring of one bull.

These results would seem to confirm Gustavsson's original findings that daughter groups of heterozygous sires had both significantly lower conception rates to first service and non-return rates at 56 days, as compared with daughter groups of genotypically normal sires. (Gustavsson, 1969). Neither heifers, nor cows born of sires heterozygous for the translocation had higher numbers of stillbirths than normal (Gustavsson, 1969) so that if unbalanced zygotes were being formed by heterozygous animals these zygotes were being lost before full term. This, together with the information regarding repeat breeders suggests that the loss occurs around the time of implantation.

Gustavsson (1969) made a very limited study of male meiosis and found no evidence of non-disjunction at meiotic second metaphase. Further investigation suggested that some spermatocytes with unbalanced karyotypes were being produced, (Gustavsson, 1970) and work in Glasgow indicated a non-disjunction rate of 8.2%. (Logue, 1974, personal communication) Female meiosis has not been investigated. It may be that non-disjunction occurs more frequently in the female than the male. This would account for the higher culling rate in daughters from sires heterozygous for the translocation than those from sires with a normal karyotype. (Gustavsson, 1971b)

Sheep

In contrast to cattle, centric fusion translocations have only been identified in three closely related breeds of sheep, all in New Zealand. These were the New Zealand Romney, (Bruere, 1969; Bruere and Mills, 1971) the Drysdale (Bruere, Chapman and Wyllie, 1972) and the Perendale (Bruere, 1974; personal communication). In addition, Nadler, Lay and Hassenger (1971) have described a polymorphic system in wild sheep of Northern Iran involving the normal metacentric chromosomes of sheep.

Bruere (1969) first reported a centric fusion translocation, the Massey I translocation, in a New Zealand Romney ram with small, abnormally shaped testes. Subsequent investigation of a random sample of a 100 ewes and 52 rams from the same flock revealed a further 7 (4.6%) heterozygotes. (Bruere and Mills, 1971) In addition, one ewe was found with a centric fusion translocation involving different acrocentric chromosomes. This was named the Massey II translocation. A further survey of 309 New Zealand Romney ewes revealed three more animals heterozygous for the Massey II (Bruere, 1973) and in one flock

of progeny test sheep at the Massey University the incidence was 9%. (Bruere, 1974, personal communication) A third translocation, the Massey III translocation was found in flocks of Drysdale sheep, with an incidence of 23.7% heterozygotes and 1.9% homozygotes. (Bruere, et al., 1972)

Karyotype analysis suggested that the Massey I translocation was formed from one of the largest acrocentric chromosomes, possibly number 4 or 5, and one of the smallest acrocentric chromosomes. (Bruere, 1969; Bruere et al., 1972) G-banding analysis showed it to be a 5/26 translocation in their nomenclature. (Bruere, 1974 personal communication) The Massey III translocation appeared to be formed from chromosome number 7 or 8 and one of the smallest acrocentrics, possibly the same one as that involved in the Massey I translocation. (Bruere et al.,1972) More recent G-band analysis has, in fact, shown that the Massey III is a 7/25 translocation. (Bruere, 1974,personal communication) Both the Massey I and III translocations were submetacentric chromosomes. In contrast, the Massey II translocation was a metacentric chromosome (Bruere, 1973), and G-banding analysis showed it to be an 8/11 translocation. (Bruere, 1974, personal communication)

The few cases of Massey II translocations identified were not associated with phenotypic abnormalities. However, in the Massey I and possibly the Massey III translocation, there was an apparent association with testicular abnormalities. The Massey I translocation was first identified in a ram with small, abnormally shaped testes (Bruere, 1969). The abnormal shape was due to a constricting band of the tunica vaginalis, producing an "hour-glass" shape to the testis. The animal was azoospermic and histological examination showed complete

cessation of spermatogenesis in nearly all the tubules at or before the primary spermatocyte stage. In a further group of seven heterozygotes, two rams had palpably normal testes, two had "hour-glass" testes, two were unilateral cryptorchids and one was destroyed prior to examination because of its failure to breed. (Bruere and Mills, 1971) One animal with a normal karyotype was also reported to have abnormal "hour-glass"testes. (Bruere and Mills, 1971)

Despite the high incidence of chromosome polymorphism in the Drysdale breed there has been only one report of an animal with testicular abnormalities. (Bruere et al., 1972) Both testes of this animal were small and atrophic and the epididymis was absent from the right gonad. In addition, a heterozygous ewe was identified with segmental aplasia of the reproductive tract. The horns and body of the uterus were absent.

Nadler et al.,(1971) described a polymorphic system in wild sheep in Northern Iran. A total of 34 animals were examined from seven wild park reserves located along the northern borders of Iran. Whilst the 15 sheep from the three western localities had a modal number of 2n=54 and a karyotype consisting of three pairs of metacentric chromosomes and 23 acrocentric chromosomes, the 7 sheep from the two eastern reserves had a modal number of 58 and the karyotype contained only one pair of metacentric chromosomes. Of the remaining 12 animals from the two central areas, two had a modal number of 2n=57 with three metacentric chromosomes, one had 2n=56 with two pairs of metacentric chromosomes, six had 2n=55 with five metacentric chromosomes and one had 2n=54 and three pairs of metacentric chromosomes. The authors suggested that either the system represented a sequence of differentiation of taxa with hybridization of structurally homologous

populations, or more likely, there was crossing between two taxa of 2n=54 and 2n=58 with the formation of hybrid animals of 2n=55,56 and 57.

SECTION II

CYTOGENETIC EXAMINATION OF

PRE-IMPLANTATION BLASTOCYSTS OF SHEEP.

II CYTOGENETICAL INVESTIGATION OF PRE-IMPLANTATION BLASTOCYSTS OF SHEEP.

2.1. Introduction

2.1.1. Chromosome Anomalies and Prenatal Loss

Studies on human abortions have indicated that a very high percentage have one or more chromosomal abnormalities. Most of these abortions occurred in chromosomally normal parents and were, therefore, caused by abnormal events during gametogenesis or at the time of fertilisation. (Boue and Boue, 1973a) Carr (1965) found 22% of 200 specimens from spontaneous abortions had such defects. Kerr (1966) cited incidences varying between 2-65%. This wide variation was probably due to the differences of maturity of aborted material and the difficulty in ascertaining whether the abortions were genuinely spontaneous or induced. Workers in Denmark, using quinacrine banding to identify individual chromosomes, found that in cases of spontaneous abortion occurring during the first sixteen weeks of pregnancy, 50% had chromosome abnormalities. (Lauritsen, Jonassen, Therkelsen, Lass, Lindsten, and Petersen, 1972) More recent studies in France suggested that the frequency was as high as 65%. (Boué and Boué, 1973a)

Information on women who have had more than one abortion is limited. One report showed that the incidence of second abortions (23%) was higher in women after an abortion with a normal karyotype than after an abortion with chromosomal anomalies. (16.5%) Furthermore, when both abortuses had chromosome anomalies, there was no correlation between the two karyotypes. (Boué, Boué, Lazar and Gueguen, 1973)

Prenatal Loss

Comparable information is lacking in domestic animals although

estimates of prenatal losses have been made. In the pig, most zygotic loss was found to occur before the 25th day of gestation and was between 30-40% of the total fertilised ova. (Hanly, 1961)

Very little information is available on prenatal death in the mare. Day, (1957) reported that 11% of 400 mares diagnosed as pregnant at 40 days failed to complete gestation. However, this is probably a low estimate of total zygotic loss since it does not take into account the preimplantation losses. Platt (1973) reported an overall abortion rate of 12.8% in the thoroughbred mare. One well recognised cause of embryonic loss is twin ovulation. The yearly incidence of twin ovulations was variously reported as 14.5% (Arthur, 1958) and 18.5% (Osborne, 1966). Arthur and Allen (1972) reported a much lower level of twin ovulations in a group of Welsh mountain ponies. The incidence of twin births in the mare has been reported to be only between 0.5% and 1.5% (Roberts, 1971) which represents a considerable zygotic loss. Herman and Routers, (1965) reported that 95% of mares with twin ovulations lost one or both ova during early embryonic development.

In cattle, embryonic loss between the 9th and 26th day of gestation was found to be as high as 30%. (Boyd, ~~Bascich~~ Basich, Young and McCracken, 1969) These workers found that most of the loss occurred prior to the 12th day. Hawk, Wiltbank, Kidder and Casida (1955) found that in repeat-breeder cows most of the embryonic loss occurred between 16-34 days after service. One interesting investigation found a statistical significant difference between prenatal death in inbred cows (28.4%) and outbred dams (19.2%). (Mares, Menge, Tyler and Casida; 1958)

The estimates of prenatal mortality in the sheep have been reviewed by Edey (1969). He concluded that between 20 - 30% of fertilised ova

were lost during gestation with most of the loss occurring in the first month. Quinlivan, Martin, Taylor and Cairney (1966) also reported that the maximum loss occurred within the first 30 days of gestation. A proportion of this loss occurred prior to day 17 without an increase in the inter-oestral period. It has been shown that embryonic material within the uterine horn beyond day 12 delays corpus luteum regression and hence prolongs the oestrous cycle (Thwaites, 1972), so that the loss reported by Quinlivan et al. must have occurred prior to day 12. Sittmann (1972) found that in the sheep embryonic loss was 1.5 times higher in twin ovulations than in single, and that loss of both embryos was more likely when twin ovulations were from a single ovary. Similar results were reported by Doney, Gunn and Smith (1973).

Until recently, cytogenic evidence linking chromosome anomalies with prenatal loss in domestic or laboratory animals was lacking. Bishop (1964) was the first to emphasise the importance of genetic load in prenatal mortality in domestic animals. He suggested that a large proportion of embryonic death could be ascribed to genetic causes and that this death was a natural mechanism for the elimination of certain genetic loads at low biological cost. David, Bishop and Cembrowicz, (1970; 1971) suggested that in cattle, a high proportion of embryonic death was part of the natural mechanism for the removal of genetic abnormalities from the population. In cattle, there was a difference in the conception to first service rates of heifer daughters of bulls heterozygous for the 1/29 translocation and heifer daughters of normal bulls. Gustavsson, (1969) suggested that the reduced fertility of daughters of sires heterozygous for the 1/29 translocation was due to embryonic death in the heterozygous females and that the death was the result of unbalanced karyotype in some of the zygotes. Chromosome

analysis of early zygotes was not, however, undertaken. Such a difference was not obvious in the older cows but this may have been because heifers of reduced fertility had been culled.

2.1.2. Cytogenetic Investigation of Early Embryos in the Mouse

In the mouse, early work indicated that the incidence of heteroploidy in 3½ day old zygotes was about 4.9% but that it varied in different stocks. (Beatty and Fischberg, 1951) Triploid embryos were found to be capable both of implanting and developing to an advanced embryonic stage. The incidence of abnormalities at first cleavage has been reported as 2.6% (Vickers, 1969) and 5.5% with 0.3% tetraploid zygotes and 1.2% triploid zygotes. (Donahue, 1972) Kaufman (1973) found a much higher incidence of 4.1% triploid zygotes in a series of 193 first cleavage metaphases.

Influence of Delayed Fertilisation

Vickers (1969) examined the effects of delayed fertilisation on the incidence of chromosome anomalies in 3-4 day embryos. She found that whereas the total incidence of heteroploidy rose from 2.6% in the controls to 3.9% in those animals with delayed fertilisation, the incidence of triploidy rose dramatically from 0.32% to 2.90%.

Influence of Maternal Age

Gosden (1973) found that immature and aged female mice produced a higher proportion of chromosome anomalies in 3½ day zygotes as compared to young adults, (8.5% and 12.1% respectively.) The proportion of triploid zygotes did not change significantly, (1.4% in immature animals, 4.8% in young adults, 3.4% in aged adults). The increase in abnormalities in aged adults was entirely due to a rise in the incidence of trisomy. This is interesting in view of the similar findings with regard to

maternal age and Down's syndrome in man. (Hamerton, 1971)

Miscellaneous

Yamamoto, Endo, Watanabe and Ingalls (1971) found that there was an increased incidence of trisomic and ~~monosomic~~ MONOSOMIC blastocysts from female mice with an artificially raised blood sugar level. In addition, although the incidence of triploidy was similar to that of the controls (0.5%) the incidence of tetraploidy was approximately four times as great in treated animals. (4.0% compared to 1%)

2.1.3. Cytogenetic Investigations in Early Embryos of the Golden Hamster

Yamamoto and Ingalls (1972) examined delayed fertilisation in 2 and 9 day zygotes. In control animals there were only 0.7% of the zygotes with an abnormal karyotype whereas 15.3% of blastocysts had such defects following a delay in fertilisation.

2.1.4. Cytogenetic Investigations in Early Embryos of the Rabbit

Estimates of the incidence of chromosome anomalies have varied. Martin-Deleon, Shaver and Gasmal (1975) found an incidence of 0.8% whilst Shaver and Carr (1969) reported an incidence of 6.8% in 6 day blastocysts. In the latter investigation the does had been treated with chorionic gonadotrophin. An untreated control group produced blastocysts with 2% abnormalities. The difference was not statistically significant and later observations showed that excess chorionic gonadotrophin did not adversely affect the chromosome complement of blastocysts. (Shaver, 1970) Martin and Shaver (1972a) found 1 of 105 blastocysts had an abnormal karyotype.

Both sperm ageing in utero (Martin and Shaver, 1972a) and ageing in the male reproductive tract by ligation of the corpus epididymis (Martin-

Deleon et al., 1973) increased the incidence of chromosome abnormalities. Aging of the ova also increased the incidence of abnormalities. (Shaver and Carr, 1969) The most common anomaly was triploidy. It appears, however, that triploid zygotes were not normally produced by fertilisation by diploid sperm. (Fechheimer and Beatty, 1974)

2.1.5. Cytogenetic Investigations in Early Embryos of the Pig

There are only four reports of chromosome analysis of pig embryos. McFeely (1967) found that 9 of 88 ten day old blastocysts had detectable chromosome abnormalities. The most common aberrations were triploidy (4) and tetraploidy (3). Smith and Marlow (1971) examined 68 twenty-five day old pig embryos and found all but one had the normal chromosome complement. This suggested that chromosomally abnormal blastocysts did not survive beyond implantation. Bomsel-Helmreich (1961) examined the effects of delayed fertilisation on the chromosome complement of embryos. Mating was delayed by 44-78 hours after the onset of oestrus, and 13 sows were slaughtered at 18 days post service. A further 14 sows were slaughtered 26 days post service. In the first group, 6% of the embryos were triploid whereas no abnormal embryos were found in the second group. This suggested that delayed fertilisation led to an increased incidence of triploidy and that triploid embryos died before the 26th day.

Akesson and Hennicson (1972) examined 113 embryos of various ages from gilts sired by a boar heterozygous for a reciprocal translocation. 101 offspring were either normal or balanced translocation carriers and 12 (10.7%) were unbalanced. No offspring with an unbalanced karyotype were found at full term so that zygotes with such unbalanced karyotypes presumably died later in gestation.

2.1.6. Cytogenetic Investigations in Early Embryos of the Ox

There has been only one report in the ox. McFeely and Rajakoski (1968) found one 16 day old blastocyst with a diploid/tetraploid karyotype and 11 others with a normal karyotype. The authors suggested that chromosome anomalies may be associated with embryonic death in the bovine.

2.1.7. Cytogenetic Investigations in Early Embryos of the Sheep

The present work is the first cytogenetic investigation of pre-implantation blastocysts in the sheep. This work is of particular significance in the understanding of the effects on fertility of centric fusion translocations, particularly since the incidence in cattle and some sheep appears to be quite high. A preliminary report on pre-implantation blastocysts was published by Long, (1974).

Present Investigation

There are a number of factors influencing the number of embryos at any point in the gestation. Ovulation rate is depressed in ewes on a low plane of nutrition, (Gunn, Doney and Russel, 1972) and embryo survival rate is less in underfed ewes. (Gunn et al., 1972) Environmental stress has also been shown to depress mean ovulation rate. (Doney, Gunn and Griffiths, 1973) All these factors were taken into consideration in the present investigation.

The use of a vasectomised tup was abandoned after the first year. The best conception rates occur when insemination takes place 16-24 hours after the onset of oestrus, (Schindler and Amir, 1973) and it was felt therefore that better results would be obtained by running the fertile rams with the flock. This also minimised the danger of producing chromosome anomalies in zygotes due to aging of the ovum. (Witschi and Laguens, 1963; Austin, 1967; Butcher and Fugo, 1967; Shaver and Carr,

1969; Vickers, 1969; Yamamoto and Ingalls, 1972.)

It was decided that blastocyst collection should take place after day 11 post coitum because after this time the blastula begins to elongate and form a ~~trophloblast~~ TROPHOBLAST (Rowson and Moor, 1966; Bindon, 1971) and a higher proportion of dividing cells would be available for collection, (Figure 7a). Although implantation begins at day 15 (Boshier, 1969) detachment is still possible as late as day 18. Since the oestrous cycle of the sheep is 16.5 days (Roberts, 1971) collection must be before this The collection time in the present work was mainly at the 15/16 day and 13/14 day stage.

2.2. MATERIALS AND METHODS

2.2.1. Tupping Management. Heterozygous Male x Normal Female

In the first breeding season 1971-1972, 30 ewes were run with a vasectomised tup as previously described. Twelve days after their first detected oestrus, seventeen ewes were given 2,000 I.U. P.M.S.* (Pregnant Mare Serum Gonadotrophin) subcutaneously and at the next oestrus, detected by the vasectomised tup, they were served by one of the New Zealand Romney rams. Each ewe was then slaughtered between 10 and 13 days post coitum; the day of service being counted as day 0. The remaining thirteen ewes were not given P.M.S. and were served by one of the New Zealand Romney rams at the first detected oestrus. Each ewe was slaughtered between 11 and 15 days post coitum; the day of service being taken as day 0. (Table XIII)

In the second and third seasons, 1972-1973 and 1973-1974, none of the ewes were treated with P.M.S. The Romney rams were run with the flock as previously described, and a total of 98 ewes were slaughtered between day 13 and 18 post coitum, the day of service being day 0.

*Burrows Wellcome.

(Tables XIV and XV)

In addition to the Scottish Blackface ewes described above, the New Zealand Romney rams were allowed to serve eight Finnish Landrace ewes, and three Blackface ewes running at grass. The four rams were run with the ewes at separate times. Each ram wore a sire-sine harness with a different coloured keel so that it was known which ram served which ewes. The ewes were then used in a pilot programme to attempt to collect blastocysts by laparotomy from the anaesthetised live animal. Once again, the day of service was counted as day 0 and the animals were operated on between 11 and 13 days post coitum.

2.2.2. Tupping Management. Normal Male x Heterozygous Female

Eight of the Romney x Blackface ewes, born in the spring of 1972 and known to be heterozygous for the Massey I translocation, were housed in a covered pen and run with an entire, Scottish Blackface ram, known to have a normal karyotype, 2n = 54XY. The ram was keeled and the ewes checked daily to note which had been served. Each ewe was slaughtered between 12 - 13 days post coitum the day of service being day 0 (Table XIX.)

2.2.3. Blastocyst Collection Post Mortem

Each ewe was killed with an intravenous injection of 4g pentobarbitone sodium (20 ml of 200 mg/ml euthetal). The ovaries, uterus and cervix were removed immediately after death. The uterus was rinsed with warm water to remove extraneous blood and dirt and the mesovarium and mesometrium cut so that the uterus could be laid out flat. The cervix was cut, just posterior to the anterior os, which was then ruptured with blunt ended scissors. The uterine horns were flushed with warmed culture medium which was collected from the cervix in a

warmed petri dish.

The flushing medium was 20 ml of Weymouths medium at 37°C, variably supplemented with 20% lamb's serum, penicillin, streptomycin and glutamine, (Table IV). A 19 gauge needle was inserted through the uterine wall at the utero-tubal junction and the medium flushed through each horn using a 20 cc syringe. Great care was required to ensure that the end of the needle was free in the lumen of the uterine horn and not buried in a caruncle. Intramural injection of the medium caused swelling of the horn and occlusion of the lumen so that flushing was impossible. Both uterine horns were flushed in this manner, irrespective of whether corpora lutea were present in both ovaries.

The contents of each horn were flushed into separate petri dishes placed on a black formica-topped hot plate at 37°C. The formica provided a dark background against which the blastocyst could easily be identified. When collection was between 15 - 18 day post coitum, the elongation of the trophoblasts had taken place and each zygote had to be carefully separated and disentangled using a Pasteur pipette. The 12 - 14 day old blastocysts were easily separated but the older ~~one's~~ ONES often broke up on manipulation.

Chromosome Preparation - 1972

After flushing, each blastocyst was transfered to a separate watchglass and cut into small fragments with fine scissors. If sufficient were available, material from each blastocyst was divided and placed into three centrifuge tubes with 7 ml of Weymouth's medium. To this basic medium was added between 0.3 - 0.7 ml colcemid (0.8 μg/ml), 2 ml lamb's serum, 10 iu penicillin; 10 mg streptomycin and 0.024 mM glutamine, (Table IV). The cultures were incubated at 37°C in a water bath for

intervals of either 1 hr., 2 hrs., 3 hrs., 7½ hrs. or 24 hrs. After incubation the material was centrifuged at 800 rpm for 8 mins., the supernatant removed and the cells resuspended in 5 ml of 0.125% KCl at 37°C for 10 mins. Following hypotonic solution treatment, the material was re-centrifuged at 800 rpm for 10 mins., the supernatant removed and the cell button resuspended in 2 ml of cold fixative. (3:1, methanol: acetic acid) Cultures remained in the first fixative at 4°C for 15 mins., in 1 ml of second fixative for 30 mins., and 1 ml of third fixative for 30 mins. Slides were prepared by dropping ½ ml of the cell suspension onto cold, clean slides. These were air dried and stained with 2% aceto-orcein for 3 hrs. before mounting.

The slides were scanned with a Wild microscope using a low power lens and metaphase spreads were counted under oil. A minimum of five good cells were counted before karyotype diagnosis was considered confirmed. Blastocysts diagnosed on less than five cells were denoted by an asterisk, (Table XIV).

Chromosome Preparation - 1973

The procedure was similar to that of the previous year except that the blastocysts were not disaggregated until placed in hypotonic solution. Only one culture was therefore made of each blastocyst.

2.2.4. Blastocyst Collection from the Live Animal

Blastocysts were collected by a modification of the method described by Rowson and Moor (1966). Anaesthesia was induced by an intravenous injection of 10% thiopentone (1 gm/200 lbs. B.W), the animal intubated, and anaesthesia maintained by halothane delivered via a semi-closed inhalation system. The ewe was placed in dorsal recumbency, and the posterior part of the abdomen clipped, shaved, scrubbed clean, washed

with antiseptic and draped in the usual manner. A midline skin incision was made approximately 2 inches long, immediately anterior to the mammary glands. Subcutaneous tissue was reflected by blunt dissection until the linea alba was visible. Care was required to avoid the left subcutaneous abdominal vein which ran very close to midline at this point. The vein was reflected with the subcutaneous tissue and skin. A $1\frac{1}{2}$ in. long incision, was made through the linea alba and into the abdomen (Figure 17). Two fingers were inserted through the opening, the uterus identified by palpation and exteriorised. Both ovaries were examined and the number of corpora lutea noted. Bowel clamps were placed just anterior to the cervix and a 19 gauge needle was inserted into the lumen of one uterine horn at the utero-tubal junction. 10 ml of sterile Weymouth's medium was flushed through into the uterine body. This uterine horn was then clamped with bowel forceps at the horn/body junction. A stab incision was made into the second horn approximately $\frac{1}{2}$ in. posterior to the utero-tubal junction and a catheter inserted. The catheter was fixed by a ligature of 2/0 catgut. A 19 gauge needle was then passed into the lumen of the uterine body and 20 ml of sterile Weymouth's medium was flushed back along the uterine horn, out through the catheter and collected in a universal bottle (Figure 17). The catheter and clamps were then removed, the incision in the uterine horn closed with a single Lembert's suture using 2/0 catgut and the uterus replaced in the abdomen. The linea alba was closed with continuous sutures using thick black nylon and the skin closed with interrupted horizontal sutures using thin blue nylon. Post operative antibotics were given for three days and the skin sutures removed after seven days.

Chromosome Preparations

Amorphous cellular material was collected from the Blackface ewes operated on day 16 and 17 post coitum. It was processed in a similar manner to that described for blastocysts, collected post mortem, at day 14 - 18 post coitum. Two morulae, collected at laparotomy from one Finnish Landrace ewe were cultured in 10 ml Weymouth's medium to which was added 0.4 µg of colcemid. The medium was maintained at 37°C in a waterbath for 2 hrs. and then centrifuged at 800 rpm for 8 mins. The supernatant was discarded and the cellular material resuspended in 5 mls of 0.125% KCl. It was at this stage that the morulae were identified as they floated to the top of the hypotonic solution. The morulae were left in the hypotonic solution at 37°C for 10 mins. and then placed in fixative (3:1; methanol:acetic acid) for 15 mins. Each morula was then dropped on to a clean, chilled slide and bombarded with fixative to try and spread the cells. The fixative was evaporated as quickly as possible and more fixative dropped. This procedure was repeated several times and then the preparations stained in 2% aceto-orcein for three hours.

2.2.5. Examination of the Ovaries

In the first year, the ovaries from the slaughtered ewes were examined after the removal of the blastocysts from the uterus. A note was made of the number of corpora lutea present. In the second and third years, in addition to counting the number of corpora lutea, each ovary was fixed in Bouin's fixative or formol saline and histological preparations made of the corpora lutea.

2.2.6. Histological Preparations

The material was prepared in the usual way using a histokine. Sections were cut 5 µ thick and stained in haematoxylin and eosin.

2.3. RESULTS

2.3.1. Blastocyst Recovery, Post Mortem. Heterozygous Male x Normal Female

The results of blastocyst collection for the three seasons are shown in Tables XIII - XV. In the first season, although a total of 46 blastocysts was collected, a recovery rate of 33.1%, the quality of chromosome preparation was poor. The main defects were low mitotic index and poor spreading of cells at metaphase which made chromosome counting difficult. The results from the first year's collection are therefore not included in later calculations.

In the season 1972-1973 the recovery rate was 87.3% as judged by the number of corpora lutea, and the percentage of collected blastocysts successfully analysed was 78.18%. In the season 1973-1974 the recovery rate was 72.3% and percentage successfully analysed was 68.1% (Tables XIV and XV).

2.3.2. Blastocyst Collection by Laparotomy

The approach and exposure of the uterus was satisfactory but application of clamps to the uterine horn and body did lead to considerable congestion if the procedure was prolonged. In particular the ovary, became severely congested.

The recovery rate of blastocysts was disappointing. Although material was obtained from all three animals operated on day 16 and 17 *post coitum*, it was impossible to determine whether this was pieces of trophloblast or cellular debris from the ewe. Collection at between day 11 and 13 *post coitum*, when the blastocyst would be beginning to elongate, (Rowson and Moor, 1966; Boshier, 1969; Bindon, 1971) was also difficult. One problem was that the medium did not always flush easily through the uterus. This may have been due to bad

positioning of the catheter which could have become occluded against the uterine wall. The two zygotes collected were morulae/blastulae and only identified once the hypotonic solution was added to the culture. Chromosome preparations were poor from both the specimens. In one blastula, the metaphase chromosomes were insufficiently spread to enable counting whilst in the second the cells had burst, dispersing metaphase chromosomes throughout the field of observation, again making counting impossible.

2.3.3. Sex Ratio. Heterozygous Male x Normal Female

In the season 1972-1973, there were 26 blastocysts with a male karyotype and 17 with a female karyotype. In 1973-1974 there were 21 males and 15 females with an overall sex ratio of male:female, 1:0.72. This was not a statistically significant difference from a theoretical 1:1 ratio ($x^2 = 2.85$: $P > 0.05$).

2.3.4. Translocation Segregation. Heterozygous Male x Normal Female

Only normal or balanced translocation heterozygotes were identified (Figures 8-11). One blastocyst from ewe SE80/2, apparently had a complex karyotype of 52XY/53XY/54XY in the proportion of 20.45%, 15.91% 34.09%. A translocation chromosome was not observed in any of the cells counted. A comparison of the spread of chromosome number in cells from the blastocyst collected from ewe SE80/2 and blastocysts collected from two other ewes is shown in Figure 15.

Significantly, not only were no unbalanced translocation carriers identified, but neither were any other abnormalities, with the exception of the blastocyst from ewe SE80/2.

There was a total of 20 balanced male heterozygotes and 14 balanced

female heterozygotes with 25 normal male and 18 normal female blastocysts. There was no significant difference in segregation of the translocation between male and female zygotes, (x^2 = 0.002; P > 0.9) neither was there a significant deviation from the expected 1:1 ratio of normal and balanced heterozygotes (x^2 = 1.052; P > 0.3) (Table XVIII) The karyotype of blastocysts sired by each ram are shown in Table XVII.

2.3.5. Blastocyst Recovery. Normal Male x Heterozygous Female

The blastocyst data for ewes heterozygous for the Massey I translocation are shown in Table XIX. There was a recovery rate of only 33.3% based on the number of corpora lutea and a successful analysis was made of 3 of the 4 blastocysts collected.

2.3.6. Examination of Ovaries

a) Effect of P.M.S. Treatment

One striking point about the effect of 2,000 iu of P.M.S. on the ovary was the tremendous variation in response. (Table XIII) Some animals only had one corpus luteum whilst one had a total of 23. The second point of note was that those ovaries with no corpora lutea were small and pale with no, or only tiny follicular formation. The general impression was that 2,000 iu was too high a dose and upset the balance of follicle stimulation and ovulation.

b) Distribution of Corpora Lutea

Excluding the results from the first season, there was a total of 78 corpora lutes identified on the right ovary and 50 on the left. This was a statistically significant difference (x^2 = 6.13; P > 0.01). When the results of the first season were considered separately, 82 corpora lutea were found on the right ovary of animals stimulated with P.M.S. and only 53 on the left ovary. The corresponding figures for

untreated ewes were 9 corpora lutea on the right ovary and 5 on the left. In ewes treated with P.M.S. the difference in the number of corpora lutea on the right and left ovaries was statistically significant. (X^2 = 6.23; P 0.01) whereas the difference in ovaries from untreated ewes was not. (X^2 = 1.143: P >0.2). However, the group of untreated ewes was small and only just within the limits of application of the chi-squared test, for which the numbers in the groups to be compared must exceed five. (Moroney, 1973)

c) Histological Examination of Corpora Lutea

The corpora lutea were examined histologically to try to determine whether they were corpora lutea of pregnancy or regressing corpora lutea of an oestrous cycle. This was attempted by subjectively assessing the number of type IV lutein cells (Thwaites and Edey, 1970) The 11-13 day corpus luteum of pregnancy (i.e. C.L. from animals from which a blastocyst had been collected) could not be distinguished from a corpus luteum of the oestrous cycle at this stage by this method. However 16 - 18 day corpora lutea of pregnancy could be distinguished from regressing corpora lutea (Figure 18). The ewes found to be empty when slaughtered all had corpora lutea with a histological picture typical of a late cycle, regressing corpus luterum, i.e. mainly type IV luteum cells. The corpus luteum from SE19/2, from which a degenerating blastocyst was obtained, presented a histological picture similar to that of a pregnant ewe.

2.4. Discussion

2.4.1. Sex Ratio of Pre-implantation Blastocysts

The sex ratio of pre-implantation blastocysts from heterozygous males x normal females showed an excess of Y bearing blastocyst (47) over female blastocyst, (32). This gave a sex ratio for 13 - 15 day sheep

blastocyst of male:female of 1:0.72. These results were not, however, statistically significant - (X^2 = 2.85; P > 0.05). The results could have been biased in that the matings were between male translocation heterozygotes and normal females. This would influence the sex ratio if there were to be a differential fertilisation of ova by X or Y translocation-bearing spermatozoa. This does not appear to have occurred as there was no statistical significance between the number of male (22) and female (14) translocation heterozygous blastocysts, (X^2 = 0.123; P > 0.70). Therefore the use of males, heterozygous for a centric fusion translocation is unlikely to have influenced the sex ratio.

Examination of the sex ratio in early embryos in other species has generally shown a ratio close to 1:1. In the rabbit the sex ratio has been examined in 5 - 6 day old blastocysts. Shaver (1970) examined 75 blastocysts, 42 of which had an XY complement and 31 an XX with 2 undetermined. This gave a ratio of male:female of 1:0.738, which was not statistically significantly different from a 1:1 ratio, (X^2 = 1.66; P > 0.30). Fechheimer and Beatty (1974) examined 440 blastocysts. Two hundred and eleven were XY and 223 XX with 6 undiagnosed. This was not significantly different from a 1:1 ratio.

In the mouse, Vickers (1967) sexed 98 three day old blastocysts and found an exact 1:1 ratio. Kaufman (1973) examined mice zygotes at metaphase of the first cleavage. Of 168 cells, 123 were sexed and 62 were male and 61 female. This is the only report known to the writer of examination of the sex ratio so close to conception.

In the pig, Smith and Marlowe (1971) examined 68 twenty-five day old embryos with the normal diploid number of 38 chromosomes. The

distribution of sex was reported as 57% XY and 43% XX. This was a ratio of male:female of 1:0.816.

The only report of sex ratio widely differing from 1:1 was in the golden hamster. (Sundell, 1962) Five hundred and two blastocysts were collected 3½ days after mating. Ninety-eight were suitable for sexing and 63 were classified as male and 35 female giving a sex ratio of male:female of 1:0.5. This was a significant difference from 1:1. The large percentage of undiagnosed material makes interpretation difficult. If this ratio was true in the undiagnosed material it suggests that either there was a differential production of X and Y spermatozoa in the testes or a preferential fertilisation of the ova by Y bearing gametes. Alternatively, there could have been a higher percentage loss of female zygotes in the pre-implantation stage. At birth, the sex ratio of 167 offspring was 86 males and 81 females. For this to occur following a sex ratio of 1:0.5 in favour of males at 3½ days, there must have been a higher percentage loss of male zygotes in the post-implantation period. Such a differential loss of male and female zygotes in the pre- and post-implantation periods would still be consistent with a primary and secondary sex ratio of 1:1. Since the report by Sundell (1962) in the golden hamster was at variance with findings in other species it would be interesting to investigate the situation further. The high level of undiagnosed material in the report by Sundell suggests that the squash technique used to produce cells for chromosome examination was inadequate for consistent identification of the chromosomes. It is possible that more consistent results would be obtained using the recent technique of Tarkowski, (1966) which produced good spreading of chromosomes and reduced the amount of cytoplasmic background, making identification

of morphology and number easier.

2.4.2. Translocation Segregation and Chromosome Anomalies

The most important finding was the absence of blastocysts with an unbalanced karyotype. Of 102 blastocysts recovered, 75 (73.14%) were diagnosed of which 40 had the normal ~~diploid~~ DIPLOID number of 54 chromosomes and 35 were balanced translocation heterozygotes. Indeed, with the possible exception of one blastocyst (SE80/2 B_1) which is discussed below, there was a complete absence of chromosome abnormalities. There are a number of explanations for these findings.

Firstly, it may be that the technique used for the preparation of chromosomes from pre-implantation blastocysts favoured those with a normal karyotype and any blastocyst with an abnormal karyotype remained undiagnosed. A very short culture time in colcemid of between $1\frac{1}{2}$ and 3 hours was selected so as to avoid the danger of producing chromosome anomalies during divisions in culture. However, if cells from blastocysts with an abnormal karyotype were dividing less frequently than those with a normal karyotype less cells would accumulate at metaphase. The chances of being able to diagnose these blastocysts would therefore be reduced.

In the examination of human aborted material Curé, Boué and Boué (1973) found that embryonic cells with chromosome abnormalities had a longer generation time and shorter life-span than normal cells and trisomy "C" material was particularly difficult to culture. Mittwoch and Delhanty (1972) compared the relative percentage of diploid and triploid fibroblast cells from a 46XX/69XXX woman after various intervals in culture. They found that the proportion of triploid cells reduced with time and concluded that this was due to delayed

growth caused by the extra set of chromosomes which prolonged the time necessary to complete the mitotic cycle. Therefore, slower division rates may have been one factor causing chromosomally abnormal blastocysts, if any were present, to remain undiagnosed. Trisomic embryos have been identified in the tobacco mouse F_1 hybrids but in these experiments colcemid was injected into the dam a number of hours prior to blastocyst collection so that there would have been time for mitotic cells to accumulate. With the present data, the failure to detect unbalanced blastocysts cannot, on its own, be taken as definite evidence that such blastocysts did not exist. However this in conjunction with the lambing results (Section III), strongly suggests that their existence was unlikely. Unbalanced, live born lambs were not found and there was no evidence of abnormal embryonic loss.

It is unlikely, however, that all the undiagnosed material was chromosomally abnormal. Of the 102 blastocysts collected 27 (26.56%) were undiagnosed. Four of these were categorised as cellular debris and a fifth was an entire blastocyst but considered to be degenerating. It had a mucoid nature and the cells were cloudy rather than having the more normal translucent appearance. The possibility that these five samples were the remains of chromosomally abnormal zygotes cannot be excluded. However, the remaining 22 blastocysts were morphologically normal and the dispersed cells on the slide preparations did not appear to be degenerating. The failure to obtain suitable chromosome spreads could have been due to some small unrecorded variation in technique which produced inferior preparations.

Secondly, in the 75 blastocysts diagnosed it is just possible that such abnormal karyotypes as mosaics or chimeras were missed. Mosaics are formed by divisional errors producing two or more cell

lines from a single zygote whereas chimeras are formed by cell lines from two independent zygotes or by double fertilisation. (Ford, 1969) When one cell line greatly exceeds the frequency of the others these may be missed if only a small sample of cells is examined. At the beginning of the present programme 10 cells were counted from each blastocyst. For practical reasons this was reduced to five cells. The standard procedure was to count the first five cells that appeared, under low power scanning, to be diploid. If the quality of chromosome morphology was poor, more than five cells were counted and diagnoses based on less than five because of the paucity of cells have been so recorded. (Table XIV and XV) It is conceded that this technique might not have identified mosaics or chimeras with a second cell line of low frequency. However, Bruere and Mills (1971) identified a 54XX/53XXT+ ewe by routinely counting 5-10 cells at metaphase. More extensive counts showed there were 65.6% 53XXT+ and 34.4% 54XX cells in the leucocyte cultures. A similar technique was adopted by Ford and Evans (1973) in the examination of pre- and post-implantation mouse embryos. These authors also recognised the possibility of mis-diagnosing mosaics, but considered the chances of doing so were low. Ideally the maximum number possible should be counted but practical considerations usually limit this to between 5 and 10 cells. In individual cases more cells were counted when definite diagnosis was difficult.

An example of just such a case was blastocyst number SE30/2 B_1. The first five cells counted were either diploid, 54XY, or hypodiploid. A total of 44 cells were counted, giving a distribution of chromosome number as shown in Figure 1b. A translocation chromosome was not observed in any of the cells. The distribution of cell number was compared with that in blastocyst SE4/2 B_2, diagnosed as 54XY, and

SE41/2 B_2 diagnosed as 53XYT+. The scatter of cell number in all three blastocysts was predominantly hypodiploid but in SE80/2 B_1 the hypodiploid cells comprised a much higher proportion of the total cells counted. (54.5% compared to 25.0% and 10%) In such a situation it is difficult to determine what was the correct karyotype. Hypo-modal cells can be produced artificially during the treatment with hypotonic solution and the application of the spreading technique. Hyper-modal cells were less likely to be produced by such procedures. If blastocyst SE80/2 B_1 was more fragile than the other blastocysts, treatment with hypotonic solution might have caused more disruption of cells than in other blastocysts. In the absence of a marker chromosome to identify a mosaic or chimera, it is impossible to make a definite diagnosis from the above results. However, since no translocation chromosome was found in any of the presumptive cell lines it was not an unbalanced translocation carrier.

An alternative reason for the absence of blastocysts with an abnormal karyotype could be that they were all eliminated early in the pre-implantation stage. The time of collection was chosen to obtain blastocysts after elongation of the trophloblast which occurs around day 12. (Bindon, 1971) This provides a large number of cells for examination. However, it may have been later than the stage at which abnormal zygotes were lost. In the second year of the programme 8 (12.7%) corpora lutea were not represented by a zygote and in the third year 18 (27.7%)were unrepresented. These figures represent fertilisation failure plus early zygotic death, and the two conditions could not be differentiated. Thwaite (1972) examined the time course of embryonic resorption in the ewe. He found that traces of embryos killed by an intra-uterine injection of colchicine on day 13 post coitum could still be detected in the uterus by day 17 in four of six

animals examined. It is reasonable to assume, therefore, that in the present work most of the embryonic death occurring after day 13 would have been recognised. However, if zygotic death occurred before day 10, when the zygote was only a spherical blastula, approximately 340 µm in diameter, (Bindon, 1971) it is unlikely to have been recognisable by day 15 or 16 when collection took place. Even when collection was on day 13 and 14 as in the third year it is doubtful whether a disintegrating blastula would have been identified. The degree of early zygotic loss is, therefore, unknown and it is possible that some of the early losses were chromosomally abnormal.

Heterozygosity for a centric fusion translocation in the male has been shown to be associated with early zygotic death in the female mouse. Monosomic zygotes, the products of gametes formed by non-disjunction at meiotic metaphase I of translocation heterozygous males, were lost in the pre-implantation stage whilst trisomic individuals survived for some time post-implantation. (Cattanach and Mosely, 1973; Ford and Evans, 1973 and Gropp, 1973) These mice were heterozygous for one of the translocation chromosomes of the tobacco mouse (M.poschiavinus) and there was a marked difference between the degree of zygotic death and the different chromosomes involved in the translocation. However, with all the translocations the incidence of foetal death was higher when the female was the heterozygous partner. This suggests that either more unbalanced gametes were being formed by the female or a smaller proportion of unbalanced spermatozoa were fertilising normal ova. In the human, females heterozygous for the DqGq centric fusion translocation produced a higher proportion of offspring with an unbalanced karyotype than male heterozygotes. (10.8% compared to 2.4%) (Hamerton, 1971) This again suggests that either the female produces more unbalanced gametes or unbalanced male gametes fertilise fewer

ova than normal or balanced translocation carriers.

In the present work only eight heterozygous females were available for blastocyst collection. Blastocyst recovery from normal male x heterozygous female matings was markedly below that of heterozygous male x normal female. (33.3% compared to 87.3% and 78.18%) This did suggest that a higher incidence of either fertilisation failure or zygotic loss occurred when the female was heterozygous for the Massey I translocation. However, in the absence of identification of zygotes with an unbalanced karyotype the cause of such differences remainedspeculation.

More extensive matings of normal males with females heterozygous for the Massey I translocation, than was possible in the present programme did not verify the finding. Translocation females were equally as fertile as normal females with no evidence of increased embryonic loss. (Bruere, 1974; personal communication)

The final possibility for the absence of blastocysts with unbalanced karyotypes is that unbalanced spermatozoa were incapable of fertilisation. Such unbalanced spermatozoa are known to be capable of fertilisation in the mouse (Cattanach and Mosely, 1973; Ford and Evans, 1973; Gropp, 1973) and in man (Hamerton, 1971) and there is no evidence to suggest that they would not be capable of fertilising in the sheep.

To summarise, although no blastocysts with an unbalanced karyotype were identified, this in itself is not sufficient evidence that they were not being produced. However, lambing results (Section III) failed to reveal any unbalanced liveborn lambs and there was no evidence of an increased incidence of stillbirths or abortions.

Futhermore, there were no prolonged or irregular oestrous cycles indicating early embryonic death so that the existence of unbalanced blastocysts seems unlikely.

It is interesting to note that the level of undiagnosed material (26.8%) was within the range of the estimated prenatal loss in sheep, (20-30%) (Edey, 1969). Bishop (1964) suggested that a high proportion of prenatal loss in any species was due to genetic factors and in the human 65% of spontaneous abortions have been found to have chromosomal aberrations. (Boué and Boué, 1973a). Even if all the undiagnosed material in the present work were to be chromosomally abnormal and destined not to survive, the prenatal loss would not be greater than in animals presumed to have a normal karyotype. Therefore, males heterozygous for the Massey I translocation did not cause a rise in prenatal loss in ewes to which they were mated.

2.4.3. Ovarian Response to High Levels of Pregnant Mare Serum Gonadotrophin

In the present work, ewes were superovulated with Pregnant Mare Serum Gonadotrophin, (PMSG) with the view to obtaining the maximum number of blastocysts from a limited number of ewes. A dose level of 1,000 iu PMSG was recommended in the Scottish Blackface ewe to produce the highest mean litter size. (Newton, Denehy and Betts, 1972) However, it was thought by the present writer that the limitation on the maintenance of multiple conceptions in the ewe would be uterine space and that this factor would not apply to pre-implantation blastocysts. It was therefore decided to use the higher dose level of 2,000 iu PMSG. However, the premise proved to be false. There was marked variation in ovarian response and blastocyst recovery rate was poor. The average number of corpora lutea from 14 animals was 3.3 per ovary but

values ranged from 1 to 15. (Table XIII) Whilst ovulation rate was higher than in the untreated animals the percentage blastocyst recovery was lower. A total of 106 corpora lutea were represented by only 38 blastocysts in the PMSG treated group, and 16 of these came from one animal. Comparable figures in the untreated group were 14 corpora lutea represented by 7 blastocysts.

These data show that following the subcutaneous injection of 2,000 iu PMSG there was a marked individual response and a higher degree of either failure of fertilisation or early embryonic death.

The variation in response to subcutaneous injections of PMSG has been noted in a number of breeds of sheep (Averill, 1958; Cummings and McDonald, 1967; Newton et al., 1972) and occurred even at levels of between 500 - 1,500 iu. Tervit and McDonald (1968) reported some failure of fertilsation in New Zealand Romney ewes injected with 1,500 iu PMSG. The low fertilisation rates were particularly noticeable in ewes with the greater number of corpora lutea, a tendency found in the present work. It was suggested that this could be due to the fact that with a high rate there would be a spread of ovulation over a period of time. This may lead to an impairment of fertilisation and development of the later ova by the earlier ones, (Newton et al., 1972), since Moore and Shelton (1964) had shown that the development of fertilised eggs was impaired when the reproductive tract was out of phase.

Newton et al.,(1972) also suggested that the variation in response between breeds might have been due to different brands of PMSG used by different workers. This however, does not explain the variation of response within each trial. Such variation was probably due to the

differing hormonal status of the individual ewes. At present PMSG is administered a certain number of days after the onset of oestrus, usually 13 days. This may not be a sufficiently precise timing for constant results to be obtained. Perhaps a more consistent result would be obtained if PMSG were to be injected at a given time after the last ovulation. This would, of course, severely restrict its application in commercial flocks.

2.4.4. Comparative Ovulation Rates of the Right and Left Ovary

In 97 Scottish Blackface sheep, not treated with PMS, there was a statistically significant difference in the number of corpora lutea in each ovary. A total of 78 (61.0%) corpora lutea were located on the right ovary and 50 (39.0%) on the left ($X^2 = 6.13$; $P > 0.01$). In 80 ewes, ovulation occurred in one ovary only. In these animals 60 (65.2%) corpora lutea were on the right ovary and 32 (34.8%) were on the left. Single ovulations occurred in 70 ewes, of which 62.8% were on the right ovary and 37.2% were on the left. The remaining 27 ewes produced a total of 58 corpora lutea of which 34 (58.6%) were on the right and 24 (41.4%) were on the left. That is, if there was to be a single ovulation, it was 1.7 times more likely to occur in the right ovary than the left. For multiple ovulations there were 1.4 times more corpora lutea on the right than the left. It is evident, therefore, that in both single and multiple ovulations there was a dominance of the right ovary. Similar findings were reported by Casida, Woody and Pope (1966) in Hampshire and Columbia sheep. These authors also found that in single ovulations only 21.7% of corpora lutea on the right ovary were not represented by embryos in the uterus whilst 26.4% of corpora lutea from the left ovary were not represented. Such a difference was not found in the present work. In single ovulations, 7 corpora lutea (15.9%) from the right ovary and 4 (12.5%) corpora

lutea from the left ovary were not represented by blastocysts. However cellular debris was found in three cases where there was a corpus luteum in the right ovary and once when the corpus luteum was in the left ovary. If this debris is counted as a zygote then only 9.1% of corpora lutea from the right ovary were not represented by a blastocyst whilst 11.5% from the left ovary were not represented. There is some suggestion, therefore, that there is some sort of biological bias in favour of the right ovary.

2.4.5. Histological Differentiation of the Corpus Luteum of an Oestrous Cycle and that of Pregnancy.

In the present work, histological preparations of corpora lutea were examined and correlated with the presence or absence of a blastocyst in the uterus. The aim was to distinguish between a degenerating corpus luteum and one of pregnancy and to apply this when considering whether cellular debris collected from the uterus was a degenerating zygote. If the zygote had died after day 12 of the oestrous cycle the normal degenerative process of the corpus luteum would have been delayed. (Moor and Rowson, 1966)

Histological differentiation of the corpus luteum of pregnancy and of oestrus has been studied in detail by a number of authors. (Deane, May, Moor, Rowson and Short, 1966; Thwaites and Edey, 1970; Rinder, 1971). All agreed that after day 15 of the oestrous cycle there were progressive signs of luteal degeneration. Thwaites and Edey (1970) based their identification of this cyclic change on the relative proportion of five different types of lutein cells. This was the approach used in the present work. In the 15-day corpus luteum of the oestrous cycle the predominating cells were type III lutein cells. These were large but irregular in outline and the nucleus was still

and darkly staining. By day 17 most of the cells were type IV and V. These were small, shrunken cells with very little cytoplasm and dark, hyperchromatic nuclei. In contrast, the 16 - 17 day corpus luteum of pregnancy possessed mainly types I, II and III lutein cells with very few type IV and V cells. Characteristically, in pregnancy, the cells were large with pale cytoplasm and round, centrally located nuclei. Figure 18 shows the histology of a 16 day corpus luteum of oestrus and pregnancy.

The only animals in which cellular debris was collected, were all slaughtered on day 13 post coitum and the degenerating blastocyst came from a ewe slaughtered 14 days post coitum. It proved impossible to distinguish the 13 - 14 day corpus luteum of the oestrous cycle, of pregnancy and of those ewes containing cellular debris or a degenerating blastocyst. It was not possible, therefore, to confirm that early zygotic death had occurred in these cases.

SECTION III

THE EFFECT OF THE MASSEY I TRANSLOCATION ON LAMBING PERFORMANCE

III THE EFFECT OF THE MASSEY I TRANSLOCATION ON LAMBING PERFORMANCE

3.1. Introduction.

Very little information is available on the effect of centric fusion translocations on the breeding capabilities of either wild or domestic sheep. The present investigation presents information on mating, pre-implantation blastocysts and secondary spermatocytes so that the Massey I translocation is the most extensively studied of all the centric fusion translocations in domestic animals.

Breeding programmes were run for three successive years between October 1971 and June 1974, using four New Zealand Romney rams (769, 6169, 7369 and 7969 - Figures I, II, III, IV and V) known to be heterozygous for the Massey I translocation and Scottish Blackface ewes. The rams had travelled by ship, leaving New Zealand on 26th August 1971 and arriving in London on 6th October 1971. They spent the requisite two weeks in quarantine and then travelled by road to Glasgow. There was some concern that their breeding performance might be impaired by the disturbance of the long journey and the change of hemispheres. They had left New Zealand in late winter when spermatogenesis is poor in the ram (Bruere, 1974; personal communication) and arrived in Glasgow eight weeks later, at the beginning of the breeding season in the northern hemisphere. However, whilst on board ship the rams had been crated on deck and were exposed to the change of daylight hours. There is evidence that although the male reproductive capacity is not so strictly seasonal as the female, it does reach a peak to coincide with the female breeding season under normal circumstances (Papalko and Clegg, 1965; Lees, 1965). Despite their long journey, three of the four tups were keen to work when presented to the ewes in November 1971 and all four rams worked successfully in

the two succeeding years.

3.2. Materials and Methods

3.2.1. Tupping Management

1) Heterozygous Male x Normal Female - 1971-1972

One hundred and twenty six adult Scottish Blackface ewes were run at grass with two vasectomised tups wearing "sire-sine" harnesses. (Radford, Watson and Wood, 1960) The ewes were gathered daily at 9:00 a.m. to check which animals had been marked by the vasectomised tups, indicating that they were in oestrus. The marked ewes were removed from the general flock and introduced singly to one of the New Zealand Romney rams. The ewe number, ram number and ram tupping behaviour were noted. If the ewe did not stand for the ram she was held until he obtained intromission. After tupping the ewe was returned to the main flock. The colour of the keel carried by the vasectomised rams was changed at 16 day intervals to detect those ewes returned to service.

2) Heterozygous Male x Normal Female - 1972- 1973

Ninety-three Scottish Blackface ewes were run with the four New Zealand Romney rams. Rams 6169 and 769 were running at grass, 7363 was housed in deep litter and 7969 was in a concrete yard. The rams wore "sire-sine"harnesses and the keel was changed for one of a different colour at 15 day intervals over a period of three oestrous cycle lengths. Each flock was gathered daily at 9:00 a.m. and the numbers of the ewes newly marked were noted.

Forty-three of the ewes were allowed to proceed to lambing and the remainder were used for blastocyst collection. (See Section II)

3) Heterozygous Male x Normal Female - 1973-1974

Sixty-nine Scottish Blackface ewes were bought from the livestock market and divided at random into two flocks. One New Zealand Romney ram, wearing a "sire-sine" harness, was run with each flock. The flocks were gathered daily at 9:00 a.m. and checked as described for previous years. The first 57 ewes to be mated were used for blastocyst collection and of the remaining 12 ewes only six were mated by the rams. These six ewes were allowed to proceed to lambing. The remaining unmarked ewes were considered barren and discarded from the experiment.

In addition one crossbred ewe, with a normal karyotype of 2n=54XX was tupped by a New Zealand Romney ram and allowed to proceed to lambing.

4) Heterozygous Male x Heterozygous Female

At the end of the first breeding season there were 13 ewe lambs and seven ram lambs heterozygous for the Massey I translocation. These were allowed to run together loose, in a covered yard, throughout the summer, winter and spring of 1972-1973.

In the autumn of 1973, nine of that year's ewe lambs, heterozygous for the Massey I translocation and three heterozygous hogs born in 1972 were run with one male heterozygote (F_1 74) throughout the winter. Tupping dates were not known in either year and only in the second year was the identity of the sire recorded.

3.2.2. Lambing Management

General lambing management was according to routine husbandry. In addition lambing date and phenotype of each lamb was recorded. Each lamb was ear tagged for identification as soon after birth as possible.

In particular, care was taken that mismothering was avoided. In the first year a rough estimate of birth weights was obtained by weighing the lambs as soon as possible after birth.

3.2.3. Leucocyte Cultures

Blood samples for chromosome analysis were taken from the four New Zealand Romney rams, all the lambs surviving for more than three weeks (173), from 39 Scottish Blackface ewes, 1 Scottish Blackface ram, 4 Finnish Landrace ewes, 1 Border Leicester ewe and 1 Border Leicester tup. Only a random sample of 39 Blackface ewes was screened because of limitations of time. A whole blood culture technique, a modification of that described by Basrur and Gilman (1964) and Bruere (1966) was used.

3.2.4. Testes Examination

In order to assess the incidence of "hour-glass" testes, and any other gross morphological abnormality the testes of the ram lambs were palpated periodically and a subjective assessment made of size and morphology.

3.2.5. Bone Marrow Cultures

In an effort to analyse the karyotype of all the offspring of translocation heterozygotes, bone marrow cultures were attempted from the two foetuses of the dead ewe and the two aborted foetuses.

3.3. RESULTS

3.3.1. Tupping Behaviour of the Rams

In the first breeding season, Ram 769 would not work in the presence of an observer. However, in the second and third breeding seasons, when he was allowed to run at pasture with a flock, his performance

was satisfactory. The other three rams (6169, 7369 and 7969) had similar tupping behaviour to normal rams. At no time during the observations, either in the pens or with the ewes at grass, was the neck-tupping behaviour observed as described by Bruere and Mills (1971). These workers recorded one ram, heterozygous for the Massey I translocation, as persistently attempting to mount the ewe from the side and towards the anterior part of the neck.

3.3.2. Fertility of the Rams

There was no evidence that ewes mated to rams heterozygous for the Massey I translocation had prolonged or irregular oestrous cycles, indicating early embryonic death. In the season 1972-1973, of the 43 ewes left to proceed to lambing, 42 held at the first service and the remaining ewe held to the second service. The inter-oestral period in this animal was normal for the species, being 15 days.

The lambs produced from each ram over the three years period are summarised in Table VI.

3.3.3. Leucocyte Cultures

Chromosome analysis was made on blood samples from 39 Scottish Blackface ewes, 1 Scottish Blackface ram, 4 Finnish Landrace ewes, 1 Border Leicester ewe and 1 Border Leicester tup. All had the normal diploid number for sheep of 2n=54. (Berry,1938; 1941; Ahmed, 1940; Makino, 1943; Melander, 1959; Borland, 1964; McFee, Turner and Morphis 1963; Bruere, 1966) The normal chromosome complement in sheep is discussed in detail in Section V of the present work.

In the first year's breeding programme a Suffolk tup which had been vasectomised and was being used to identify ewes in oestrus proved to

be fertile. A total of 35 Suffolk x Scottish Blackface lambs survived until old enough for karyotype analysis to be made. All had a diploid number of 2n= 54 (Table XXVI).

3.3.4. Lambing Results Heterozygous Male x Normal Female

In the first year's breeding programme, of the 126 ewes only 50 produced lambs sired by a New Zealand Romney ram. Since all the Suffolk-cross lambs were found to have a normal karyotype of 2n=54 they are excluded from the ensuing discussions. All mention of lambs henceforth, refers to those lambs sired by one of the Romney rams, heterozygous for the Massey I translocation, unless specifically stated otherwise.

a) Lambing Percentage

A total of 100 ewes was tupped of which 94 ewes produced a total of 123 lambs (Table VII). This gave an average lambing percentage of 130.9% calculated from the number of ewes lambing or 123% for ewes tupped, with 117% lambs weaned per ewes tupped.

b) Abortion and Stillbirths

Of the 100 ewes tupped, 93 produced live lambs. Of the other 7 two ewes lost their lambs at parturition, 1 ewe, which was carrying twins, died during pregnancy, 1 ewe produced stillborn twins at full term and 3 ewes were found to be empty at term. These three ewes had been assumed to have held to service because they failed to return to oestrus. No evidence of aborted material was found.

c) Birth weights

In the first lambing season each lamb was weighed as soon after birth as possible (Table V). There was no statistical difference between

the mean birth weights of the males (4.54 $\pm$ 1.14 kg) and the females (4.20 $\pm$ 1.13 kg) (t = -1.1688). In addition, there was no statistical difference between the average birth weight of lambs with normal karyotypes (4.45 $\pm$ 1.26 kg) and those heterozygous for the Massey I translocation (4.25 $\pm$ 0.97 kg) (t = -0.6315).

d) Sex Ratio

Over the three breeding seasons a total of 123 lambs was born, of which 59 were males and 64 were females (Table VIII) with an overall sex ratio of male:female of 1:1.08. This was not statistically different from a 1:1 ratio (x^2 = 0.20, P > 0.5).

e) Translocation Segregation

Sixtyfour lambs were born after the first breeding season and of these five died before successful chromosome analysis could be made. Three were males and two females. All the lambs in the second season were successfully analysed but one female lamb died in the third year before chromosome analysis was carried out.

A total of 117 lambs was analysed. Forty-nine were balanced heterozygotes for the Massey I translocation with a diploid number of 2n=53T+. Sixty-eight animals had a normal chromosome complement with 2n=54. No lambs were born with an unbalanced karyotype. (Table IX). The difference between the number of chromosomally normal offspring and balanced heterozygotes was not statistically significant (x^2 = 3.08; P > 0.05). The difference between the number of male (22) and female heterozygotes (27) was not significant (x^2 = 0.172; P > 0.50).

f) Testes Examination

A total of 59 ram lambs was born during the three breeding seasons of which 56 survived beyond the first few days. One ram, $F_1$110 a heterozygous carrier for the Massey I translocation was found to be a unilateral cryptorchid. The left testis was descended into the scrotum and was of normal size and firmness. The cauda epididymis was easily palpable and of a normal firm consistency. No right testis was palpable either within the scrotum or at the external inguinal ring. No testicular abnormalities were found in any of the remaining 55 ram lambs.

g) Bone Marrow Preparations

Bone marrow cultures were set up from the twin lambs removed from the ewe which died, but no metaphase chromosomes were found.

3.3.5. Lambing Results. Heterozygous Male x Heterozygous Female.

a) Sex Ratio

A total of 14 lambs was produced from the crossing of heterozygous males and heterozygous females, of which 7 were male and 7 female.

b) Translocation Segregation

Of the 14 lambs, two were late aborted foetues and 1 died at parturition. All the surviving lambs were blood tested for chromosome analysis. (Table X) There was an overall deficiency of lambs carrying the translocation but the figures were too small to be significant. (7 normal lambs, 3 heterozygotes, 1 homozygote and 3 undiagnosed.)

c) Abortions and Stillbirths

Two of the ewes aborted single foetuses, one male and one female,

shortly before full term. (Date of tupping was unknown.) However, these were probably post trauma abortions since the animals had been transported and dipped a few days earlier. A third lamb died at birth due to malpresentation and cervical constriction of the ewe.

d) Bone Marrow Preparations

No metaphase spreads were observed from the female foetus aborted by the heterozygous ewe. Two metaphase spreads were observed from the male aborted foetus in which two translocation chromosomes were visible. In one cell the chromosomes were not sufficiently spread to count but the other was diagnosed a 52XY T++ (Figure 16)

e) Testes Examination

No abnormalities were detected in the testes of any of the 7 ram lambs after manual examination.

3.4. DISCUSSION

3.4.1. Massey I Translocation and Male Sterility

It had been originally postulated that there may have been some association between heterozygosity for the Massey I translocation and testicular abnormality, in particular the "hour-glass" testis. (Bruere, 1969) The relationship could not have been a simple one since the abnormality was not found in every heterozygous animal and one ram with a normal karyotype was found with the abnormal testes. (Bruere and Mills. 1971)

The results in the present work did not support the view that heterozygosity for the Massey I translocation was related in any way

to testicular abnormality. A total of 59 ram lambs were born during the three breeding seasons as a result of heterozygous male x normal female matings, of which three did not survive beyond the first few days. None of the 34 rams with a normal karyotype (2n= 54XY) nor the 22 rams heterozygous for the Massey I translocation (2n= 53XYT+) had an "hour-glass" testis. All but one of the ram lambs had normal genitalia. The exception was ram F_1 110, a translocation heterozygote (Figure 6) which was also a unilateral cryptorchid. This animal is discussed below.

Bruere and Mills (1971) suggested that the association of testicular abnormality with heterozygosity for the Massey I translocation was a chance phenomenon or a gene effect with the fortuitous association of the "sterility gene" with the translocation chromosome. They postulated that gene interchange at meiosis between the translocation chromosome and the acrocentric homologues could confer the "sterility gene" to the acrocentric chromosome. Hence a cytogenetically normal animal could develop an "hour-glass" testis. An extension of this hypothesis is proposed by the present author.

The condition may be due to a "sterility gene" which is a simple recessive of low frequency in the general population of New Zealand Romney sheep. It is further suggested that the gene is located on one of the chromosomes involved in the Massey I translocation. Translocation heterozygotes would, therefore, be autometically heterozygous for the sterility gene. If these animals were crossed with a cytogenetically normal animal carrying the sterility gene on one of the acrocentric chromosomes the translocation heterozygous offspring would be the only ones to be homozygous for the sterility gene. However, if the cytogenetically normal partner did not carry the gene,

none of the offspring would be homozygous for the gene and so the translocation heterozygotes would be morphologically normal. It would still be possible for cytogenetically normal males to become homozygous for the gene (if they inherited the gene on acrocentric chromosomes from both parents) but the chances of them doing so would be less than the chances of a translocation heterozygote being homozygous for the gene. In contrast, translocation homozygotes would be homozygous for the sterility gene and so have abnormal testes. Bruere et al.,(1972) reported finding males homozygous for the Massey I translocation but did not comment on testicular morphology. In the present work the single homozygous animal, $F_2$200 showed some degree of spermatogenic arrest in both testes. (See Section IV) Homozygous animals have been located in New Zealand with similar testicular abnormalities. (Bruere, 1974, personal communication) This is interesting in the light of the current theories of centric fusion translocation formation. (Ferguson-Smith, 1967; Rowley and Fergament, 1969; Hsch and Kimberling, 1971) If centric fusion translocations are formed due to association of homologous segments of non-homologous chromosomes then the degree of genetic homogeneity in translocation homozygotes may lead to the expression of deleterious recessive genes. One such expression could be the abnormal testicular development in animals homozygous for the Massey I translocation.

If it is further postulated that the sterility gene is absent from or of low frequency in the Scottish Blackface breed this would explain the absence of testicular abnormalities in the heterozygous animals in the present programme. This hypothesis still allows for the fortuitous association of the gene with the translocation chromosome and does not indicate an impairment of fertility due to the centric fusion translocation *per se*.

3.4.2. Massey I Translocation and Cryptorchidism

Ram $F_1$110 was a unilateral cryptorchid. The left testis had descended into the scrotum and the right was located at laparotomy at the internal inguinal ring. $F_1$110 was also heterozygous for the Massey I translocation having received the translocation chromosome from the sire, 7369. Two of the heterozygous animals examined by Bruere and Mills (1971) were also unilateral cryptorchids and the question arises whether there is an association between heterozygosity for the Massey I translocation and cryptorchidism.

Unilateral and bi-lateral cryptorchidism is known to be an inherited condition (Warwick, 1931) and has been shown to be associated with polledness in Merino rams. (Dolling and Brooker, 1964) The method of inheritance is still not fully understood. However, a recent investigation (Claxton and Yeates, 1972) suggested that the condition was caused by an autosomal recessive gene rather than an autosomal dominant with incomplete penetrance.

In order to demonstrate an association between cryptorchidism and heterozygosity for the Massey I translocation it would be necessary to show a higher incidence in the latter group. This has not been shown by other workers and the fact that the only unilateral cryptorchid animal in the present programme was also heterozygous for the Massey I translocation was not in itself sufficient evidence to suggest a relationship between the two.

3.4.3. Massey I Translocation and Tupping Behaviour

There was no evidence in any of the heterozygous rams run with the ewes of the "neck-tupping" behaviour described by Bruere and Mills, (1971). Since such behaviour has been described in only one animal

heterozygous for the Massey I translocation it seems most probable that this was an individual trait and unassociated with heterozygosity for the translocation. Such behaviour is not uncommon in sheep in New Zealand. (Bruere, 1974; personal communication)

3.4.4. Massey I Translocation and Birth Weight

It is known from studies in man that offspring with chromosomal abnormalities tend to have a decreased birth weight. Chen, Chan and Falek (1971) found that both males and females with an extra X chromosome and XO females had lower birth weights than the normal population. More interestingly infants trisomic for chromosome 21, 13 or 18 also had lower birth weights. (Naeye, 1967; Chen, Chan and Falek, 1972) Estimates were made of the birth weights of lambs born in the first lambing season with a view to determining whether trisomic lambs, if produced, would have a lower than average birth weight. However, the recorded weights were not birth weights since some lambs were not weighed until after they had their first suckle. This produced such a wide variation that comparison of normal and balanced heterozygous offspring became meaningless. True birth weights could only have been obtained if the ewes had been observed for 24 hours daily and this was not practicable. No useful conclusions can be drawn, therefore, from the data obtained in this section regarding comparative birth weights of cytogenetically normal and heterozygous lambs.

3.4.5. Massey I Translocation and Fecundity

In the tobacco mouse x laboratory mouse F_1 hybrids, males heterozygous for the seven centric fusion translocations have been shown to have a reduced fecundity. (Gropp, Tettenborn and Lehmann, 1970) Even mice heterozygous for a single tobacco mouse translocation chromosome

showed a higher frequency of zygotic loss than controls. (Cattenach and Moseley, 1973; Ford and Evans, 1973) Evidence from other centric fusion translocations in the mouse presented a similar picture of reduced litter size in females mated to heterozygous males. (Evans et. al., 1967; White and Tjio, 1967) The present work was carried out in an attempt to establish whether a similar phenomenon occurred in sheep heterozygous for the Massey I translocation.

The overall lambing percentage from heterozygous males x normal females was 130.9% calculated from the number of ewes lambing or 123.0% of ewes tupped. This was comparable to the lambing performance of Scottish Blackface ewes kept under similar conditions at the Glasgow Veterinary School in previous years. (Hignett, 1974; personal communication) Whilst the number of animals is conceded to be low it is still possible to conclude that the Massey I translocation was not causing a marked deviation from the expected lambing performance. Furthermore, evidence from tupping data, stillbirths and abortions showed that there was not unusual embryonic loss during gestation. One ewe produced stillborn twins, three ewes of the 100 ewes tupped were found to be empty at term and only one of the 43 ewes failed to hold to the first service and she held at the second service after a normal inter-oestral period. This was well within the estimates of normal prenatal loss in sheep, (Edey, 1969), and so it seems that the reduction in fecundity observed in mice heterozygous for a centric fusion translocation did not occur in sheep heterozygous for the Massey I translocation in sheep.

The number of lambs resulting from heterozygous male x heterozygous female matings was too small in the present programme to derive statistical information on such matings.

Good evidence of the effects of heterozygosity for a centric fusion translocation in other species is limited to man and cattle. In man it has been shown that the frequency of abortion in women heterozygous for a DqGq translocation was slightly higher than the abortion rate in normal women.(Hamerton, 1971) Thus in man, female heterozygotes showed a slightly reduced fertility. In cattle, Gustavsson (1969) found a reduced fertility in daughter groups of bulls heterozygous for the 1/29 translocation compared to daughter groups of normal sires. In his survey the daughter groups of 3 out of 10 heterozygous bulls showed a significantly lower conception rate at first service than normal sires. The weighted means of conception rate at first service, 56 and 273 day non-return rates of daughters of translocation heterozygous sires were also unfavourable compared to daughters of normal sires. Gustavsson suggested that the difference was due to an increased level of embryonic death in heterozygous females because of the production of zygotes with an unbalanced karyotype. This hypothesis was supported by a later survey which showed that there was a higher incidence of heterozygous females in a group of repeat breeder heifers than in the population as a whole. (Gustavsson, 1971a) It seems, therefore, that in cattle, as in man, there is some reduction in the fertility of the heterozygous female. A very limited breeding programme in goats heterozygous for a centric fusion translocation suggested that in both normal male x heterozygous female and heterozygous male x normal female matings the proportion of multiple births was lower than normal.(Padeth et al., 1971) Because of the small numbers of animals involved in the present investigation there was no information available on the fertility of females heterozygous for the Massey I translocation. However, more extensive breeding programmes in New Zealand have failed to show any reduction in fertility in female heterozygotes. (Bruere, 1974,

personal communication) In this respect the Massey I translocation would appear to behave differently from the DqGq translocation in man and the 1/29 translocation in cattle.

As discussed earlier, fertility of males heterozygous for the Massey I translocation was found to be unimpaired. This was also the finding in men heterozygous for the DqGq translocation (Hamerton, 1971). However, perhaps figures in man have to be regarded with caution since it would be difficult to ascertain the result of every mating of the male. No evidence of a reduced fertility in bulls heterozygous for the 1/29 translocation has been published but Gustavsson (1973) mentioned unpublished data purporting to demonstrate this point. If this were to be so, then the Massey I translocation in sheep differs from the 1/29 translocation in cattle, the only other large domestic animal in which centric fusion translocations have been studied extensively.

3.4.6. Segregation of Massey I Translocation in the Full Term Offspring

The most important finding was the absence, in offspring of heterozygous male x normal female, of animals with an unbalanced karyotype. All the lambs karyotyped (117) had a normal or balanced translocation karyotype. However six of the 123 lambs died before chromosome analysis was carried out and it could be argued that some may have had an unbalanced karyotype which predisposed to early post natal death. In man, the incidence of chromosome abnormalities in perinatal deaths was ten times that of the neonatal population. (Sutherland, Bauld and Bain, 1974) However, the few reported cases of trisomy in the veterinary literature have all been associated with marked phenotype abnormalities. Five cases have been reported in cattle (Herzog and Kohn, 1968; Mori et al., 1969; Kohn and Herzog, 1970; Lamm and Johnson, 1972) and all have been associated with brachygnathia. In the cat, a runt foetus

was identified as being trisomic for the D2 chromosomes. (Benirschke, et al., 1974) It is unlikely therefore, that one of the unexamined lambs had an unbalanced karyotype.

Man is the only species in which unbalanced centric fusion translocation carriers have been found in the live-born population. Population studies in cattle (Gustavsson, 1969), domestic sheep (Bruere and Hills, 1971), wild sheep (Nadler et al., 1971), shrews (Hamerton and Ford, 1953; Ford and Hamerton, 1970) and wild mice (Gropp, Winking, Zech and Muller, 1972) failed to reveal unbalanced live-born individuals. There are a number of possible explanations for the apparent difference between man and the other species.

Firstly, it may be, by chance, that in man trisomy for the chromosomes involved in the translocations is less lethal than trisomy in other species. The difference would depend on the genetic load carried by the translocation chromosomes.

Alternatively, it may be an artificial difference, reflecting a difference in the population sample. The type of survey carried out in mice, shrews and wild sheep was unlikely to detect very young animals. Unbalanced translocation animals may have existed but died soon after birth. Their existence in the population would then have remained undetected. Gustavsson's survey in cattle (1969) also only included adults so that calves with an unbalanced karyotype could have existed but been eliminated from the population. In contrast, because the unbalanced karyotype in man was associated with an abnormal phenotype such individuals were identified at an early age. Human unbalanced translocation carriers are less well adapted for life and under conditions of natural selection such individuals might not

be expected to survive.

Thirdly, the difference may reflect a real difference in the incidence of unbalanced individuals. Indeed, in man, the incidence of unbalanced carriers of DqDq translocations was much less than unbalanced carriers of DqGq translocations. (Hamerton, 1971) This was not reflected by a higher incidence of abortion or stillbirths in DqDq carriers (Hamerton, 1971; Chanley, Christie, Fletcher, Frackiewicz and Jacobs, 1972), so that the difference was presumably due to a difference in the frequency of adjacent segregation of the two translocations. If such differences occur with different translocations in the same species, it is reasonable to assume that translocations in different species will have different frequencies of adjacent segregation. The higher incidence in man, of live-born unbalanced translocation carriers, compared to other species is probably due both to differences in frequency of adjacent segregation of translocation chromosomes and to differences in the viability of the unbalanced individuals.

In the present work, a total of 117 lambs from heterozygous male x normal female matings was examined. Sixty-eight had a normal karyotype and 49 were balanced translocation heterozygotes. (Table X) The difference was not statistically significant. ($x^2 = 3.00$, $P > 0.05$) When the figures were combined with those of a comparable breeding programme in New Zealand (Bruere, 1974, personal communication) there was an almost perfect 1:1:1:1 segregation ratio of the Massey I translocation with sex. (Table XI) The segregation data, together with the findings in pre-implantation blastocysts discussed in Section II argues strongly against the production of zygotes with an unbalanced karyotype. If they were being formed they would either

have been detected in the offspring or the pre-implantation blastocysts or their absence, due to early embryonic death would have been reflected by a distortion of the segregation ratios.

In cattle, the segregation ratio of offspring from males heterozygous for the 1/29 translocation was also 1:1, normal to balanced translocation carriers which again suggests that no embryonic loss was occurring.

An apparent excess of heterozygous offspring has been reported in goats heterozygous for a centric fusion translocation (Padeh et al., 1971; Popescue, 1972a), but the number of animals involved was too few for definite conclusions to be drawn.

In man there was an excess of heterozygous offspring from heterozygous fathers. (Hammerton, 1971) Since trisomic offspring exist in man, this suggests that the monosomic zygotes were dying in utero. Such a situation was indeed found in mice. Ford and Evans (1973) and Cattanach and Moseley (1973) found that the monosomic embryos died prior to implantation whilst some of the trisomic embryos survived into the late post-implantation period. However, it would not account for an excess of balanced heterozygous offspring. This would suggest some competitive advantage over karyotypically normal offspring. To date, man is the only species in which such an excess of heterozygous offspring have been shown.

3.4.7. Massey I Translocation and Sex Ratio

The overall sex ratio of 1:1.08 (male:female) was not statistically different from a 1:1 ratio (x^2 =0.20, P > 0.5) Six lambs died before cytogenetic analysis was carried out. Three were phenotypically

male and three phenotypically female so their loss did not alter the sex ratio. Nor was there a significant difference between the number of male heterozygotes (22) and female heterozygotes (27). (x^2= 0.172; P>0.50) (Table IX).

Such an unbiased distribution of translocation chromosome with sex has not been found in all species. Popescu (1972a) reported an excess of male heterozygotes in the offspring of a goat with a centric fusion translocation. However, in that work not all the offspring were examined and of those that were the numbers were small so that it is possible that there was an artificial bias.

In the mouse, males heterozygous for the T163H translocation produced a statistically significant excess of heterozygous daughters. (Evans et al., 1967). There was no association of the translocation trivalent and the sex bivalent at meiosis and no preferential segregation with sex at second metaphase. This suggests that either there was selective loss of Y Tr bearing spermatozoa, or of male translocation zygotes. Such a selective loss might occur if the close association of the acrocentric chromosomes involved in the translocation blocked the action of a gene which triggered a second gene on the Y chromosome. The blocking effect would not be significant in XX zygotes. However, it is unlikely that a functional gene would be affected since the centromeric area of mice chromosomes is occupied by heterochromatin (Chen and Mueller, 1971) so that the euchromatic parts of the translocation chromosome would be well separated. In the light of the results from the breeding programme, the blastocyst analysis (Section II) and meiotic studies (Section IV) of the present work, alteration of sex ratio is unlikely to be associated with heterozygosity for an autosomal centric fusion or dislocation.

There were too few lambs produced as a result of heterozygous male x heterozygous female matings for the results to be statistically significant. One lamb died before chromosome analysis could be made and two were aborted. Seven of the remaining lambs had a normal karyotype, three were balanced translocation heterozygotes and one was homozygous for the Massey I translocation. From bone marrow cultures one of the aborted lambs was believed to be a homozygote. (Fig. 16) The translocation segregation ratio was therefore 3.5:1.5:1 (normal: heterozygous:homozygous). However, when these results are added to those from New Zealand (Bruere, 1974, personal communication) the ratios become 1:2:1 (Table XII) so that again there is no evidence of a segregation distortion.

SECTION IV

STUDIES OF MALE MEIOSIS

IV STUDIES OF MALE MEIOSIS

4.1. Introduction

Spermatogenesis in the ram has been extensively studied both quantitatively and qualitatively. It has been shown that theoretically one type A spermatogonium can produce 16 primary spermatocytes, 32 secondary spermatocytes and 64 spermatozoa. However, this theoretical maximum is hardly ever achieved. (Ortavant, 1958) In rats it has been estimated that there was an approximate 22% loss of cells between late spermatogonial and advanced spermatid stages. (Roosen-Runge, 1973) In rabbits, there was a 24% cell loss during meiotic divisions, (Swierstra and Foote, 1963) whilst in man there was a 35% cell loss between prediakinetic cell stages and spermatids. (Barr, Moore and Paulsen, 1971) Roosen-Runge (1973) suggested that this loss served to remove gametes which were in some way unsuitable for the propagation of the species.

Fechheimer (1961) examined spermatogonia in mice and found that the incidence of polyploidy decreased as the stage of development neared maturity. A total of 431 spermatogonia were counted of which 31 (7.2%) were polyploid. Of 400 primary spermatocytes, none were polyploid but 23 (5.7%) were heteroploid. Lin, Tsuchida and Morris (1971) examined meiotic chromosomes of male mice. These authors found that 9% of cells at pachytene were degenerating whilst only 5% of cells at metaphase I were degenerating. The extent to which chromosomal abnormalities were responsible for these losses was not known.

Popescu (1971a) examined meiotic chromosomes from six genotypically normal bulls and although he did not quantitate the findings of cells at second metaphase he did indicate that some cells were noted with

31 chromosomes suggesting that non-disjunction was occurring in the normal male. Gustavsson (1969) in a count of only 38 metaphase II cells of normal bulls found one with 31 chromosomes and three with 29. Of 151 cells from males heterozygous for the 1/29 translocation two had 31 chromosomes and 52 had 29 chromosomes. His original study (Gustavsson, 1969) found that males heterozygous for the 1/29 translocation showed no impairment of fertility but more recently (Gustavsson, 1973) it was suggested that there was a slight reduction.

The problem of non-disjunction associated with centric fusion translocations has been studied most extensively in the mouse and in particular in the tobacco mouse (Mus poschiavinus) which is homozygous for seven centric fusion translocations. The cross of the tobacco mouse with the laboratory mouse (Mus musculus) produces F_1 hybrids which are heterozygous for seven different centric fusion translocations. Studies of male meiosis in these F_1 hybrids (Tettenborn and Gropp, 1970) showed that less than 50% of the secondary spermatocytes had the regular haploid number of chromosome arms. Feulgen-DNA measurements on morphologically normal spermatozoa from F_1 males showed a much broader variation of values than similar measurements in either the tobacco mouse or laboratory mouse. (Doring, Gropp and Tettenborn, 1972) It seemed likely, therefore, that the majority of aneuploid secondary spermatocytes had developed into mature and morphologically normal spermatozoa. Similar findings were reported by Stolla and Gropp, (1974). In addition, these authors found that morphologically abnormal spermatozoa had a higher mean DNA content and a larger standard deviation of DNA content than morphologically normal spermatozoa.

There is evidence, therefore, both from studies on meiotic disjunction

and from analysis of early embryos (Gropp, 1971), that the male F_1 hybrids have a reduced fertility, directly attributable to the presence of the translocation in the heterozygous state. The effect of each translocation on non-disjunction rates has been investigated separately. (Cattanach and Moseley, 1973; Ford and Evans, 1973) These workers isolated all seven tobacco mouse translocation metacentrics and established them in homozygous lines upon a predominantly Mus musculus genetic background. It was found that there were significant variations in the degree of non-disjunction at second metaphase in male mice, heterozygous for centric fusion translocations involving different chromosomes. This difference was also reflected in the number of foetuses which died before full term in heterozygous male x normal female matings. The degree of zygotic loss was dependent upon which translocation was present in the karyotype of the sire. Cattanach and Mosely (1973) found no evidence of gametic selection against aneuploid spermatozoa and Ford and Evans (1973) showed a good correlation between the percentage of non-disjunction at second metaphase and the percentage pre- and post-implantation losses.

Two other centric fusion translocations have been investigated in two different strains of mice. The level of non-disjunction of both these translocations was far below that of the tobacco mouse translocations. Evans et al., (1977) examined meiotic disjunction in four males heterozygous for the T16H translocation. A total of 600 cells were counted. 309 (51.5%) had the normal haploid number of n=20, 283 (47.3%) cells were balanced translocation carriers, n=19 Rt; 3 cells were n=20 Rt; 3 cells were n=19; and 2 cells were n=18 Rt. This suggested a very low level of non-disjunction. A second translocation, involving different chromosomes, was investigated by White and Ilbo (1977). A total of 314 cells at second metaphase

were counted from 20 males, heterozygous for the translocation. 119 cells (55.5%) had the normal haploid number of n=20; 76 cells (35.5%) were balanced translocation carriers with n=19 T4; one cell was n= 20T4; one cell was n=21; 8 cells were n= 19; four cells were n=18; two were n=17 and three were n= 18 T4. This compares with the rate of non-disjunction of the tobacco mouse translocation chromosome in males heterozygous for T_4 which averaged 29.5%. The lowest incidence of non-disjunction was associated with T_6 and was 6.0%. (Cattanach and Moseley, 1973)

In man, three different centric fusion translocations are known but significant information on non-disjunction is limited to DqGq translocations. The frequency of unbalanced offspring was only 2.4% when the father was heterozygous for the DqGq translocation, compared to 10.8% when the mother was the heterozygote.

Quantitative studies of male meiosis in the sheep have not yet been reported in the literature. A limited description of meiotic configurations in the ram has been presented by Hoar, (1971) but no counts of cells at metaphase II were carried out to estimate the level of non-disjunction in animals with a normal karyotype.

4.2. Materials and Methods

Material for histological and meiotic study was collected from six rams following orchidectomy. Four animals were heterozygous for the Hosey I translocation ($F_1$40; $F_1$101; $F_1$110; and $F_1$113) one was a translocation homozygote, ($F_1$200; Figure 7) and one ($F_1$199) was a ram with the normal karyotype of 2n= 54XY. All the rams except the homozygote were fit & active, Blackface ewe & Hill Cheviot cross.

The testes were removed under local anaesthetic. Five millitres of 2% xylocaine were injected into the epidural space at the lumbar-sacral junction and a further 2 ml. of xylocaine were injected subcutaneously under the line of incision in the scrotum.

4.2.1. Meiotic Preparations

Meiotic preparations were made using a method developed by Logue (1973, personal communication) for use in the bull. This was an adaptation of the method described by Evans, Breckon and Ford (1964).

1) Immediately after removal of the testis two long, thin slivers of material were cut and one placed in 20 ml. of 1% (W/V) sodium citrate and the other in 20 ml of 0.5% (W/V) potassium chloride.

2) The material in 1% sodium citrate was allowed to stand in a water-bath at 37°C for ten minutes before processing whilst the material in 0.5% potassium chloride was used at once. The subsequent method of processing was the same for both sets of material.

3) The piece of testis was placed in a petri-dish with sufficient hypotonic solution to cover the bottom of the dish and minced with fine scissors for ten minutes.

4) More hypotonic solution was added at intervals and the fine cell suspension transferred to a centrifuge tube with a pipette.

5) After ten minutes of mincing all the cell suspension was removed to centrifuge tubes. The large pieces of testis were discarded and the cell suspension incubated at 37°C in a waterbath for ten minutes.

6) Following incubation, the cell suspension was centrifuged at 500 rpm for ten minutes. This precipitated the large cellular elements but allowed most of the spermatozoa to remain in suspension.

7) The supernatant was discarded and the cell button resuspended in six times the volume of freshly made fixative (3:1; methanol:acetic acid) at 4°C.

8) The cell suspension was then placed in the refrigerator at 4°C for 15 minutes.

9) After 15 minutes the fixative was changed and the cell suspension was returned to the refrigerator for a further 30 minutes.

10) After the second change of fixative, fresh fixative was added at irregular intervals.

11) Slides were usually prepared approximately 24 hours after the beginning of the process.

12) Assessment of completion of fixation was made from a small sample of suspension which was dropped on a cold slide and dried rapidly in air. This slide was stained in 1:10 Giemsa in water for 5 minutes, dried in air and scanned under the microscope. If the chromosome morphology was clear with a sharp outline, fixation was considered to be adequate.

13) The slides were made by dropping approximately ½ ml of cell suspension onto a clean, cold slide and dried rapidly in air.

14) Slides were stained in 1:10 Giemsa in water for five minutes or

2% aceto-orcein for 3 hrs.

15) The slides were scanned under the x10 lens and cells counted under oil immersion. In the 2nd metaphase figures each chromosome, rather than each chromosome arm, was counted so that the number of translocation bearing cells was known.

4.2.2. Histological Preparations

Samples were taken from the testis, and head, body and tail of epididymis of each animal, fixed in Bouin's solution and histological preparations made in the usual way. Sections were cut at 5 μ and stained in haemotoxylin and eosin.

Ram $F_1$110 was a unilateral cryptorchid, the right testis being undescended. An exploratory laparotomy was carried out and the right testis located at the internal inguinal ring. This testis was removed, sections cut and fixed in Bouin's solution and histological sections made.

4.3. RESULTS

4.3.1. Gross Testicular Morphology

Gross testicular morphology was normal in all animals except $F_2$200. In both testes of this animal there were adhesions of the tunica vaginalis onto the cauda epididymis (Figure 43). The adhesions extended over the whole of one half of the surface of the cauda and when reflected the line of attachment ran dorso-ventrally.

There was no evidence of fibrous constriction of the tunica vaginalis as described by Bruere (1969) producing an "hour-glass" shaped testis in either the heterozygous animals or the homozygote.

4.3.2. Meiotic Studies of a Normal Male $F_1$99

Preparations in both sodium citrate and potassium chloride produced good pachytene, diplotene and diakinesis figures of first prophase, and first and second metaphase figures. In addition, a few spermatogonial cells at metaphase were also identified. Figures at leptotene and zygotene could not be identified with confidence using this method.

Pachytene figures were most common and in each instance the sex vesicle was easily identified (Figure 24). It was not possible to identify individual bivalents at this stage.

Thirty-three cells in late diplotene or early diakinesis were selected for photography and karyotyping. Each cell contained 26 autosomal bivalents plus the X/Y bivalent which was always markedly less darkly stained than the other bivalents. In addition, the X/Y bivalent could be identified by means of its elongated configuration, indicating an end-to-end association. (Figure 30) It was not possible to determine by this method which ends of the X and Y chromosomes were associating. No X and Y univalents were observed.

The autosomal chiasmata counts are shown for each cell (Table XX). The mean total chiasmata for thirty-three cells was 63.51 x 4.43 (Table XXV). Each of the three large bivalents of the metacentric chromosomes were easily identified and usually had between 4 and 6 chiasmata. The acrocsomal bivalents could not be individually identified. The most common number of chiasmata for these bivalents was two, occasionally there were three and rarely only one.

One hundred cells in second metaphase were counted (Table XXVI) and thirty-five were photographed and karyotyped. Forty-one of the

figures contained a Y chromosome, 50 contained an X and one spread had both an X and Y chromosome. (Figure 36) The X chromosome was easily recognised as the largest acrocentric chromosome and was slightly less condensed and lighter staining than the other acrocentrics. The chromatid arms of the X chromosome also lay in a more parallel manner than the autosomes. The Y chromosome was the smallest chromosome and its submetacentric form was usually clearly visible. In addition, it was also slightly less condensed and lighter stained than the autosomes. The segregation of the X chromosome (58 cells) and the Y chromosome (41 cells) was not significantly different from a ratio of 1:1 ($\chi^2 = 2.92$; $P > 0.05$).

Eighty-six cells had the modal haploid chromosome number of n = 27 (86%). Ten cells had twenty-six chromosomes, two had twenty-five chromosomes, one had twenty-eight chromosomes with both an X and Y chromosome (Figure 36) and one cell had twenty-eight chromosomes with only one X chromosome (Figure 35).

Some metaphase spreads were observed having the diploid number of 2n = 54 XY. These spreads were of two types. The first had the characteristic appearance of metaphase XY chromosomes with widely parted chromatids and elongated centromeric regions, whilst the second had the typical appearance of metaphase spreads from routine leucocyte cultures (Figure 22).

4.3.3. Meiotic Studies of Mules, Determined for the Meiosis I Preparations

Preparations from some F_1 hinnies, the first animal studies, were of poor quality, being inadequate. Hence no results were obtained from material from this animal.

Preparations from the three other animals, ($F_1$49; $F_1$101; $F_1$113) were suitable for study. The most common elements in material from all the rams were cells in pachytene of prophase I. Cells in late diplotene and early diakinesis each had a total of twenty-six figures. These consisted of the elongated X/Y bivalent, three large bivalents formed from the metacentric chromosomes, twenty-one bivalents similar to the small bivalents of the acrocentric chromosomes seen in the normal ram, $F_2$199 and in addition, a large irregular-shaped element not found in cells from $F_2$199. This element had a characteristic morphology and easily identifiable as consisting of three chromosomes. (Figures 31 and 32). This was considered to be a trivalent formed by the translocation chromosome and the two acrocentric chromosomes, homologous with those forming the translocation. A total of thirty-seven cells in late diplotene or diakinesis were photographed and karyotyped. The chiasmata counts for each cell from each animal are shown in Tables XXI - XXIII and the mean chiasmata counts are shown in Table XXV. These were of the same order as chiasmata counts for the normal ram, $F_2$199. At no time was there observed any association between the X/Y bivalent and the translocation trivalent.

A total of one hundred and sixty-three cells at second metaphase were counted (Table XXVII) and ninety-eight of these were photographed and karyotyped. When results from all three animals were pooled there were 79 Y-bearing cells and 84 X-bearing cells. (Table XXVII) This was not significantly different from the expected 1:1 ratio (X^2 = 0.15; $P > 0.50$) so that the presence of the translocation trivalent at diakinesis had not affected the segregation of the X/Y bivalent. No cells at second metaphase were seen with both an X and a Y chromosome.

The distribution of chromosome number for each cell counted is shown

in Table XXVII. Eighty cells had the haploid number of n = 27 (Figure 33) and 56 had the haploid number of n = 26 which included the translocation chromosome (Figure 34). Only five cells had the haploid number of twenty-seven chromosomes including the translocation chromosome. Four of the unbalanced second metaphase cells contained an X-chromosome and on a Y. (Figures 37 - 41)

Of the balanced translocation bearing cells, 31 had an X chromosome and 25 a Y. This was not a statistically significant difference from a 1:1 ratio (x^2 = 0.64; $P > 0.3$) so that there was no preferential segregation of the translocation chromosome with sex.

A total of 66 cells carried the translocation chromsome whilst 97 cells did not. This was a statistically significant difference (x^2 = 5.896; $P > 0.01$).

4.3.4. Meiotic Studies on a Male, Homozygous for the Massey I Translocation $F_2$200.

The predominant cell type was again pachytene of prophase I. Sixteen cells in late diplotene or diakinesis were photographed and karyotyped. Each contained 26 bivalents consisting of the X/Y bivalent, the three large bivalents corresponding to the three metacentric chromosomes, twenty-one small bivalents and a medium sized bivalent not seen in either the normal male or the translocation heterozygotes (Figure 29). This was considered to be the bivalent formed by the Massey I translocation chromosomes.

Chiasmata counts are shown in Table XXIV and the mean total chiasmata count in Table XXV. This was of the same order as the chiasmata count in the normal ram and the three translocation heterozygotes.

There were very few cells at second metaphase in these preparations. This may have been partially a technical artifact, but in addition, histological examination showed poor spermatogenesis in a number of tubules (see below). Seventeen cells were counted at second metaphase (Table XXVII). Eleven contained an X chromosome and six a Y. (Figure 29). This was not significantly different from a 1:1 ratio ($x^2 = 1.47$; $P > 0.20$).

4.3.5. Histological Examination of Testis and Epididymis

1) Normal Male. $F_2$199

This animal was a year old. All stages of spermatogenesis were identifiable in the testis and spermatozoa were within the tubules of the cauda epididymis.

2) Males heterozygous for the Massey I Translocation

Rams $F_1$49; $F_1$101 and $F_1$113 were approximately two years old. No gross abnormalities were detected in the histology of their testes and each showed tubules at various stages of spermatogenesis. Ram $F_1$110 was a unilateral cryptorchid. The descended left testis showed all stages of spermatogenesis (Figure 19) and tubules of the cauda epididymis were packed with spermatozoa. Histological examination of the cryptorchid testis revealed complete absence of spermatogenesis. The seminiferous tubules were lined by a single layer of irregular shaped cells with large nuclei (Figure 20). No spermatozoa were present in the cauda epididymis.

3) Male, homozygous for the Massey I Translocation, $F_2$200.

This animal was a year old. Histology of the left testis showed a number of tubules with spermatogenic arrest. (Figure 21) Spermatogonia, types A and B were identifiable as well as primary

spermatocytes but there were very few developing spermatids and even fewer spermatozoa. Spermatozoa were absent from the tubules of the cauda epididymis.

Fixation and cutting of the right testis was poor but spermatogenesis could be seen to be more active and stages 1, 6 and 8 (Cole and Cupps, 1959) of the seminiferous epithelium cycle were identified. Spermatozoa were also present in the tubules of the cauda epididymis.

4.4. Discussion

The technique developed by Logue (1974, personal communication) for use in the bull proved satisfactory for the examination of male meiosis in the ram.

4.4.1. Spermatogonia

Two morphologically different types of diploid metaphase spreads were observed. One type (Figure 22) closely resembled the metaphase spreads seen in routine leucocyte cultures. The chromatids were joined at the centromere and were aligned in parallel with each other. In contrast, the second type (Figure 23) more closely resembled the haploid spreads seen at meiotic metaphase II. There was marked chromatid repulsion, often to the extent of separation at the centromere. It has been suggested that these represent types A and B spermatogonia respectively. (McDermott, 1971) However, it is possible that the supposed type A spermatogonia were in fact dividing fibroblast cells. In addition, it is possible, although unlikely, that the type B spermatogonia were two haploid cells intermixed. Although in the present programme the total number of diploid cells were not recorded, all those counted had an XY sex chromosome complement. If they were random associations of haploid cells it might be expected that an XX

complement would have been identified. McDermott's hypothesis is interesting in that if type B spermatogonia do indeed have a different chromosome morphology at a mitotic metaphase from that of type A spermatogonia it would suggest that the mechanism instigating a meiotic division had already begun. The extensive chromatid repulsion, characteristic of chromosomes at meiotic metaphase II has not been observed by the present author in any mitotic metaphase chromosomes other than the presumptive type B spermatogonia.

4.4.2. Pachytene

In all the rams examined there was an excess of cells at pachytene compared to those at diplotene and diakinesis. This was in accordance with the findings by Loir (1971) that the stages of diplotene and diakinesis were very brief in the ram. Late Pachytene stages were not observed and identification of individual bivalents was not possible. Such mapping of pachytene bivalents has been possible in man. (Hungerford, 1971)

4.4.3. Diplotene and Diakinesis

Chiasmata counts in cells from ram $F_2$199 with a normal karyotype (2n = 54xy) were in close agreement with those reported by Loir (1971). The three metacentric chromosomes had between 4 and 6 chiasmata and the mean total chiasmata count for 33 cells was 54.31 ± 6.40. The total chiasmata counts for the three rams heterozygous for the Massey I translocation were 56.36 ± 5.27 ($F_1$49), 51.78 ± 4.67 ($F_1$101) and 59.42 ± 6.16 ($F_1$113). The total chiasmata count for ram $F_2$200, homozygous for the Massey I translocation was 49.73 ± 6.27. There was therefore no evidence from these data of reduced chiasmata frequency in the heterozygous animals.

The X/Y bivalent was always elongated with a single, end to end

association. In the rams heterozygous for the Massey I translocation there was never an association between the sex bivalent and the translocation trivalent. This confirmed the original suggestion by Bruere (1969) and Bruere and Chapman (1974) that the Massey I translocation involved only autosomes.

The translocation trivalent had a characteristic shape and was easily identifiable. (Figures 31 and 32) Bruere (1969) described this as being ~~similar~~ SIMILAR to the frying-pan formation in the mouse. (White and Tjio, 1967) In the present work the formation of the trivalent was frequently more asymmetric than that described in the mouse and more closely resembled the configuration in bulls heterozygous for the 1/29 translocation. (Gustavsson, 1969) The Massey I translocation trivalent had between 3 and 6 chiasmata, indicating a high degree of crossover.

There was no trivalent figure at diakinesis in ram $F_2$200 which had two translocation chromosomes. The two translocation chromosomes formed a bivalent in the normal way providing further evidence that $F_2$200 was indeed homozygous for the Massey I translocation and not heterozygous for two different translocations.

4.4.4. Second Metaphase

The degree of non-disjunction at metaphase I was assessed by examination of the chromosome number at metaphase II. However, interpretation of these results must be guarded. In the normal ram $F_2$199 (2n = 54 XY) 86 (86%) cells had the diploid number of n = 27, one cell contained both an X and a Y chromosome and 26 autosomes and one cell contained 27 autosomes plus an X chromosome. Of the remaining 12 cells, 10 had a total of 26 chromosomes and 2 had 25. The total number of aneuploid cells was therefore 14 (14%). However, some of the hypomodal cells

could be technical artifacts due to loss of chromosomes during the spreading process. A cell with one extra chromosome was less likely to have been a technical artifact. Therefore, for the purpose of comparison of non-disjunction in normal and translocation heterozygous males it is proposed that the degree of non-disjunction be calculated as follows:-

$$\text{Percentage Non-disjunction} = \frac{\text{No. of hyperdiploid cells} \times 2}{\text{Total No. of cells counted}} \times 100$$

By this definition it is assumed that the number of hypohaploid and hyperhaploid cells were equal. Hypohaploid cells in excess of the number of hyperhaploid cells are assumed to have been technical artifacts.

The total level of non-disjunction in ram $F_2$199 was therefore, 4%. One of the cells involved non-disjunction of the sex chromosomes so that the level of non-disjunction of the autosomes was 2%. This compares with the level of non-disjunction of 6.13% calculated for the three rams, heterozygous for the Massey I translocation. (Table XXVI) Very few cells were available for counting at second metaphase in ram $F_2$200 so that it is difficult to draw definite conclusions about the effect of the Massey I translocation in the homozygous state. However, it would appear that rams, heterozygous for the Massey I translocation had a higher incidence of non-disjunction at meiotic metaphase I than the ram with the normal karyotype.

All the hypomodal cells also contained a translocation chromosome. Identification of individual chromosomes was not possible, so that the level of non-disjunction of the translocation chromosome and its

homologous acrocentric chromosomes is not known. However, an indirect assessment can be made if it is assumed that the level of non-disjunction of the other acrocentric chromosomes was unaffected by the translocation and remained at 2%. The difference in the level of non-disjunction of 4.13% between the normal and heterozygous animals was presumably due to non-disjunction of the translocation chromosome.

When the results of other workers are compared using the above definition, the centric fusion translocation in the mouse described by White and Tjio (1967) had a level of non-disjunction of 1.87% and the T163H (Evans et al., 1967) a level of 1%. These were low levels compared with non-disjunction of each of the tobacco mouse translocation chromosomes of T_1 = 18.5%; T_2 = 14.5%; T_3 = 13.5%; T_4 = 29.5%; T_5 = 8.0%; T_6 = 6.0%; T_7 = 12.0%. (Cattanach and Moseley, 1973). It is obvious, therefore, that there is considerable variation in the rates of non-disjunction of different centric fusion translocations. It has been suggested that the high level of non-disjunction associated with the tobacco mouse translocations is due not only to the presence of the centric fusion translocation but also to the fact that it is an interspecific cross. (Cattanach and Moseley, 1973). These authors suggested that the major part of the non-disjunction was due to minor differences in genetic or chromosome complement between the metacentrics and their homologous acrocentrics which reflected the fact that the chromosomes were derived from different species. It is perhaps invalid, therefore, to compare the Massey I translocation with the tobacco mouse translocations. When compared to the T163H translocation (Evans et al., 1967) and that reported by White and Tjio (1967) in the mouse, the Massey I translocation has a high level of non-disjunction. In heterozygotes for the Massey I translocation the rise in the level of non-disjunction appears to be attributable to

the centric fusion translocation. In view of the doubts expressed regarding the relationship between centric fusion translocations and raised levels of non-disjunction, this is an important finding. More extensive work on meiosis in males heterozygous for the Massey I translocation, carried out in New Zealand independent of the present work found a similar level of non-disjunction. (Chapman, 1974, personal communication) The level is comparable to that found in bulls heterozygous for the 1/29 translocation of 8.2 % (Hogue, 1974 personal communication)

4.4.5. Testicular Morphology

The abnormal spermatogenesis and adhesions of the tunica vaginalis found in both testes of $F_2$200 are extremely interesting. They may have been due, as suggested in Section I, to homozygosity for a sterility gene located on one of the chromosomes involved in the centric fusion translocation, or to gene homozygosity as suggested by Ferguson-Smith (1967). Similar abnormalities have been found in rams homozygous for the Massey I translocation in New Zealand (Bruere, 1974, personal communication) so that the association seems not to be a chance phenomenon.

Homozygosity for a centric fusion translocation in cattle (Gustavsson, 1969) and mice (Evans et al.; White and Tjio, 1967) did not reduce fertility. There is no evidence that homozygosity *per se* in these species involves a reduction in fertility. Mice homozygous for the T_5 translocation did have a reduced fertility. Indeed, the spermatozoa counts of mice homozygous for each tobacco mouse translocation was lower than normal controls. (Cattanach and Mosely, 1973) However, this was considered to be due to the fact that the tobacco mouse metacentrics were not entirely equivalent to their

homologous house mouse acrocentric chromosomes.

To summarise, it has been shown that males heterozygous for the Massey I translocation have a higher level of non-disjunction at meiotic metaphase I than a normal ram or one homozygous for the Massey I translocation. It is suggested that, unlike the tobacco mouse metacentrics, the non-disjunction can be attributed to the presence of the centric fusion translocation per se and that it is the structural heterozygosity and not genetic differences that are important. This is an important difference from the two other mice centric fusion translocations in which no raised level of non-disjunction was found. (Evans et al., 1967; White and Tjio, 1967) The level of non-disjunction in the Massey I heterozygotes was similar to the level in bulls heterozygous for the 1/29 translocation. (Logue, 1974, personal communication)

The effect of homozygosity for the Massey I translocation requires further investigation, but it would appear that there are some detrimental effects on fertility in some animals.

4.4.6. Fate of Unbalanced Secondary Spermatocytes

It has been shown in this section (IV) that secondary spermatocytes were produced with an unbalanced karyotype in males, heterozygous for the Massey I translocation. However, no pre-implantation blastocysts were found with a similar unbalance karyotype. (Section II) The problem remains, therefore, as to whether the unbalanced secondary spermatocytes mature and develop into spermatozoa, capable of fertilisation. The data are open to three interpretations:

1) Unbalanced secondary spermatocytes develop into fully functional

spermatozoa capable of fertilising ova.

2) Unbalanced secondary spermatocytes develop into mature spermatozoa but these are incapable of fertilising ova.

3) Unbalanced secondary spermatocytes do not develop into mature spermatozoa.

If it is assumed that all the unbalanced secondary spermatocytes matured and that the spermatozoa with an unbalanced karyotype were equally as capable of fertilising ova as normal spermatozoa then one would expect 6.13% of the zygotes to have an unbalanced karyotype. Since there was 12.7% and 27.7% of the corpora lutea in the second and third year respectively not accounted for by blastocysts and 21.82% and 31.9% of collected blastocysts were undiagnosed, the failure to detect blastocysts with an unbalanced karyotype cannot be taken as evidence for their nonexistence. Similarly it would not be possible to demonstrate that the unbalanced secondary spermatocytes matured but were incapable of fertilisation.

There is evidence from the literature that in some species aneuploid spermatocytes develop into mature spermatozoa. In Drosophila melanogaster spermatids will mature in the total absence of a chromosome complement (Lindsley and Grell, 1968) and in the rabbit (Beatty and Fechheimer, 1972) and in cattle (Salisbury and Baker, 1966) diploid spermatozoa have been identified in the ejaculate. However, there is no evidence regarding the fertility of the diploid spermatozoa in cattle and in the rabbit Fechheimer and Beatty (1974) considered it unlikely that the diploid spermatozoa were responsible for the production of triploid zygotes found in their experiment. In

contrast, the F_1 hybrids of the tobacco mouse and laboratory mouse, have been shown by Feulgen staining (Doring, Gropp and Tettenborn, 1972) and UV light (Stolla and Gropp, 1974) to have aneuploid spermatozoa. These aneuploid spermatozoa are known to fertilise ova since unbalanced zygotes have been found. (Gropp, 1971; 1973; Cattanach and Moseley, 1973; Ford and Evans, 1973) Similarly in man, individuals, trisomic for one of the chromosomes involved in a centric fusion translocation do occur. (Hamerton, 1971) It is possible, therefore, that in sheep heterozygous for the Massey I translocation some of the unbalanced secondary spermatocytes may develop into fully functional spermatozoa.

If the unbalanced secondary spermatocytes did not mature to spermatozoa but degenerated, this would have resulted in an increase in the degenerating cells beyond the secondary spermatocyte stage. Quantitative counts of the various cells of the testis in histological section were not made in the present work so that a 6.13% increase in the loss of cells between these stages would not have been detected on routine screening. Degeneration of aberrant gametes is part of the normal process of spermatogenesis, (Roosen-Runge, 1973) and serves to select and remove gametes unsuitable in some way for the propagation of the species. Some or all of the unbalanced secondary spermatocytes in translocation heterozygotes may not, therefore, mature to spermatozoa.

It is not possible from the present data, to determine the fate of the unbalanced secondary spermatocytes. However, information from the literature suggests that there is a high probability that most will degenerate but that those which do not will mature to spermatozoa equally capable of fertilisation as normal spermatozoa.

Another interesting finding was the statistically significant excess of n = 27 compared to n = 26T+ secondary spermatocytes in the heterozygous males. This predominance of the normal karyotype was not reflected in the karyotype of blastocysts or live-born lambs sired by these rams. For each set of data to be compatible there must either have been a lower percentage of normal secondary spermatocytes maturing to spermatozoa or the 26T+ spermatozoa were at a competitive advantage in fertilising ova. Either circumstance would be unusual and a third possibility is that in some meiotic second metaphase spreads the translocation chromosome was mis-interpreted as two acrocentric chromosomes.

To avoid such mis-interpretations some workers count the number of chromosome arms and not the number of chromosomes. This provides information on non-disjunction but not on the proportion of normal and balanced translocation secondary spermatocytes. It was for this reason that an attempt was made to count whole chromosomes in the present work. However, in the light of the findings of the karyotypes of blastocysts and live-born lambs, where identification of metacentric chromosomes is easier, it is likely that the excess of n = 27 secondary spermatocytes was a counting artefact.

SECTION V

IDENTIFICATION OF THE MASSEY I TRANSLOCATION

V Identification of the Massey 1 Translocation

5.1. Introduction

Early workers, using squash preparations from the testes or ovaries found considerable difficulty in determining accurately the diploid number of chromosomes in the sheep. The first report of chromosome analysis in sheep was by Wodsedalek (1922). He examined spermatogonia and concluded that they contained thirty-three chromosomes. He postulated that there was a single sex chromosome in the male and that two types of secondary spermatocytes were formed. One had 16 chromosomes and the other had 16 chromosomes plus the sex chromosome. He also examined oogonia and thought that they had 34 chromosomes. this was explained on the basis that the female had two sex chromosomes whilst the male had only one. Other early workers reported the diploid number as between 50 and 60 (Krallinger, 1931) or 60 (Novikov, 1935; Bruce, 1935 - quoted by Melander, 1959). The difficulty encountered by these workers was due to the poor separation of the chromosomes obtained by the squash technique and the problems which then arose in distinguishing acrocentric and metacentric chromosomes.

Despite these limitations some workers did describe correctly the diploid number in sheep as 2n=54, notably Shivago (1930); Berry, (1938, 1941); Ahmed (1940); Makino (1943) and Melander (1959).

Shivago (1930) examined cells from the amnion of sheep foetuses. Berry (1938) was examining sheep and goats and their hybrids. He collected the amnion from 30 day sheep embryos and found that the sheep foetuses had a chromosome number of 2n=54 whilst the goat x sheep hybrids had 2n=57. Early embryos from goats were found to have a chromosome number of 2n= 60.

Later work on cells from teased testes of adult rams (Berry, 1941) confirmed the diploid number to be 2n=54. Berry (1941) described the three pairs of metacentric chromosomes. The largest had the short arm 2/3 the length of the long arm; the second metacentric had arms only slightly unequal and the third chromosome was somewhat smaller with arms nearly equal. This description was shown to be remarkably accurate by Bruere and McLaren (1967) who published the first idiogram of sheep chromosomes some 26 years after Berry's description. Berry (1941) was the first worker to describe accurately the diploid number in sheep using testicular material. Other workers, who had correctly reported the diploid number to be 2n=54 had used the amnion as a source of cells.

All the above authors agreed that the Y was a small chromosome but Ahmed (1940) thought it had a subterminal centromere whilst Melander (1959) thought it had a median centromere. The X chromosome was not definitely identified.

It was not until 1964 that satisfactory metaphase spreads were obtained of sheep chromosomes. (Borland, 1964) Borland examined bone marrow cells obtained by sternal puncture from sheep which had received an intraperitoneal injection of colcemid 90-100 minutes prior to collection. His findings confirmed the diploid number as 2n=54 with three pairs of metacentric chromosomes and twenty-four pairs of acrocentric chromosomes. The Y chromosome was described as small and dot-like whilst the X was thought to be one of the smallest acrocentric chromosomes.

The first description of chromosome analysis of sheep using lymphocyte cultures was by McFee, et al., (1965). Their preparations indicated

that the X chromosome was the largest of the acrocentric chromosomes and the Y was a small submetacentric chromosome. The autosomal chromosomes consisted of three pairs of metacentric chromosomes and twenty-three pairs of acrocentric chromosomes. This chromosome complement has been found in a number of different breeds of sheep of Western Europe (Bruere and Mills, 1971). However, it has been shown that wild sheep in the eastern limits of northern Iran have a diploid number of 2n= 58. Those in the western regions have the usual number of 2n= 54, comparable to Western European sheep. (Nadler et al., 1971)

Bruere (1967) noticed an increased incidence of aneuploidy in leucocytes, with increasing age in the sheep. Five freemartins, one aged 4 years, two aged 5 years and two over 7 years were found to have an increase of both hypo and hypermodal cells as compared to normal sheep of less than 1 year old. A yound freemartin only 18 months old showed a modal chromosome count near that of normal young sheep so that it was concluded that age was the influencing factor and not the state of blood cell chimerism. It would be interesting to compare young and old "normal" animals to see whether this apparent age aneuploidy in sheep can be confirmed. Age aneuploidy has been reported in man. (Hamerton, Taylor, Angell, and McGuire; 1965)

There has been one very interesting report suggesting that there was a sex dimorphism in the lengths of some chromosomes of the sheep. (Dain, 1972) The long arm of chromosome numbers one and two were calculated to be longer in the male than in the female. Dain suggested that this might indicate the presence of male determining genes on these arms, which would be non-functional in the female. That part of the chromosome would be more contracted in the female compared to the male.

No information was given on the age of animals examined. It would have been interesting to know whether this was also an influencing factor. More work is required in order to be certain that this is a real sex dimorphism with no other contributary factors.

There have been only a few reports of chromosome polymorphism in the sheep and these have been discussed in detail in section I. (1.3.8.) Five unrelated sheep were identified with a deletion of one of the autosomes. (Luft, 1972; 1973) Four of the animals, two ewes and two rams, were born with brachygnathia superior.

Three different centric fusion translocations have been identified, the Massey I (Bruere, 1969), Massey II (Bruere and Mills, 1971) and Massey III (Bruere et al., 1972).

In recent years identification of individual sheep chromosomes has been attempted by means of Q-bands (Hansen, 1973a; Schnedl and Czaker, 1974), G-bands (Evans et al., 1973; Nadler, Hoffmann and Woolf, 1973; Schnedl and Czaker, 1974) and C-bands (Evans et al., 1973; Schnedl and Czaker, 1974). The present work concerning the identification of the Massey I translocation was begun before these reports appeared.

5.2. Material and Method

Routine leucocyte cultures were prepared as previously described. The air dried preparations were stored in dust-free boxes for between two days and two months before being used for G-band or C-band staining.

For C-band preparations, ~~chromosmes~~ CHROMOSOMES were paired by a subjective assessment of length and degree of centromeric staining. Karyotypes were then prepared by arranging the chromosome pairs in order of decreasing size. For G-band preparations, chromosomes with the same

banding pattern were paired and the karyotype prepared by a subjective assessment of chromosome size.

5.2.1. G-band Preparations

G-band staining was carried out using a modification of the method described by Seabright (1971). Slides were treated in a 1% trypsin solution in Sorensen's buffer at pH 6.8 at 37°C for between 5 and 90 seconds. The length of time depended on the degree of digestion and this was assessed by examination using a phase contrast microscope. The slides were then washed in running tap water and stained in 1:10, Giemsa: Sorensen's buffer at pH6.8 for between $1\frac{1}{2}$ and $2\frac{1}{2}$ minutes. After staining the slides were rinsed in running water, blotted to remove excess water and allowed to dry in air. Permanent mounts were made with DPX and a coverslip. One hundred and sixty-three cells were photographed using a Leitz microscope with an automatic camera. Eighteen karyotypes were made.

5.2.2. C-band Preparations

The method used was a modification of that described by Sumner (1972). Slides were immersed in 0.1N hydrochloric acid at room temperature for one hour. They were then rinsed in warm tap water and treated with 2.5% (w/v) barium hydroxide at 50°C for ten minutes. It was important to rinse well to avoid the formation of barium chloride on the slide. Rinsing in warm water brought the slide to the temperature of the barium hydroxide solution and this avoided the precipitation of barium hydroxide crystals onto the cold slide. After hydroxide treatment the slides were thoroughly rinsed in warm running tap water and stained in 1:10, Giemsa: Sorensen's buffer at pH 6.8, for eight minutes.

Fifty-seven cells were photographed using a Leitz microscope with

automatic camera at a magnification of x 140 with x 10 eyepiece. Twenty-two karyotypes were made, seven from a normal ram, eight from rams heterozygous for the Massey 1 translocation and seven from a ram homozygous for the Massey 1 translocation.

5.3. RESULTS

5.3.1. G-banding

Preparations made and stored for less than three days were very susceptible to the effects of the 1% trypsin solution and were unsuitable for use for G-banding. Even when exposure times were very short structural disruption occurred. The chromosomes appeared extremely swollen and "ghost-like". Only their outlines remained visible and no, or very few banding patterns were visible. The three pairs of metacentric chromosomes appeared least affected in that their banding patterns were more often distinguishable in these preparations. Preparations stored longer than six weeksrequired progressively longer exposure times to the trypsin solution and often only the centromeric regions were affected. The chromosomes failed to swell and the stain attained a bead-like appearance along the chromatids. No regular pattern was formed.

The best results were obtained from preparations stored for between one and four weeks. These were treated with 1% trypsin for between 15 and 25 seconds and stained for $2\frac{1}{2}$ minutes. There was considerable variation in the effect of this treatment on different preparations made from the same animal at the same time and even on different cells on the same slide. One factor consistently noted was that chromosomes on the periphery of a spread were least effected by the trypsin and showed less definite band patterns.

Of the one hundred and sixty-three cells that were photographed, not all had clear band patterns in each chromosome but several homologous chromosomes could be identified from each cell. Certain chromosomes had very distinctive patterns and could be identified very easily.

These were chromosomes number 1, 2, 3, 6 and X. Eighteen full karyotypes were made, four from normal animals, eleven from rams heterozygous for the Massey 1 translocation and three from the homozygous ram. From the analysis of all the cells examined a schematic representation of the G-band pattern of sheep chromosomes was formed (Fig. 45). Centromeric vacuolation was a feature of all the chromosomes except the X and Y chromosomes.

Chromosome No.1

The long arms showed two, broad, darkly staining bands. One was just below the centromere and the second two-thirds of the way down. In well differentiated cells each of these bands could be seen to be formed from two bands close together.

The short arm contained one broad, darkly staining band, two thirds of the length from the centromere, which was again formed by two thin bands lying closely together.

Chromosome No.2

The long arm contained three equally intense bands. One was just below the centromere, one half way down the arm and one near the free end of the chromatid. Each band was formed from two thinner bands close together. The short arm had two bands, one close to the centromere and one more distally placed. Both bands were of equal intensity.

Chromosome No.3

The most noticeable feature of this chromosome was the very darkly staining band on the long arm, proximal to the centromere. This band was quite characteristic and made the chromosome easily identifiable. Two other, less densely stained bands were present on the long arm, one in the centre and one near the distal end. The short arm had three

moderately dense bands, one close to the centromere composed of two thin bands, a central band that seemed to be a single entity and a distal band formed from two lines close together.

Chromosome No.4

This chromosome had a darkly stained band at the centromere, two bands centrally and two bands more distally placed.

Chromosome No.5

This chromosome had a band at the centromere not so darkly staining as that in chromosome number 4. The double band was closer to the centromere than those in number four and distally only one band was commonly seen. Cells at early metaphase sometimes showed a pale band very close to the distal end of the chromatid.

Chromosome No.6

Chromosome number six was characterised by a dark band located one quarter of the way down the arms. This made the chromosome easily recognisable. Two further bands, less darkly stained, were located distally.

Chromosome No.7

This chromosome was characterised by a dark band at the centromere. In addition, there were two sets of less dense double bands, one set centrally located and one set more distally placed.

Chromosome No.8

Chromosome number eight was distinguished from number seven in that the band at the centromere was not so wide as that of 7 and the two sets of bands were more centrally placed.

Chromosome No.9

This chromosome also had a dark band at the centromere but it was accompanied by a second thin band. In addition there were two dark bands approximately two-thirds of the way down the arm.

Chromosome No.10

This chromosome also had a dark band at the centromere accompanied by a thin band but the two additional distal bands were much closer to the free end of the chromosome arm than those in chromosome number 9.

Chromosome No.11

Chromosome number 11 had only a single band at the centromere and two bands approximately two-thirds of the way down the chromosome arm.

Chromosome No.12

The characteristic feature of this chromosome was the darkly staining band at the centromere. In heavily trypsinised preparations no further bands were visible but in some preparations two faint bands were visible close to the free end of the chromosome.

Chromosome No.13

Chromosome number 13 was very similar to number 10 except for the smaller size and slightly thinner band at the centromere.

Chromosome No.14

This chromosome was characterised by a double band at the centre of the chromosome arm.

Chromosome No.15

This chromosome had a pair of bands at the centromere, a second pair in the centre of the arm and a third pair at the distal end.

Chromosome No.16

This chromosome was similar to number 14. In addition to the central two bands there were two thin bands close together at the distal end of the chromosome.

Chromosome No.17

Chromosome number 17 was characterised by two distinct bands close to the centromere and two bands at the distal end.

Chromosome No.18

This chromosome was very similar to number 17 except that the two sets of bands were placed slightly more centrally.

Chromosome No.19

Chromosome number 19 had the same banding pattern as number 14, i.e., two dark bands close together in the centre of the chromosome arm, but was distinguished from number 14 by its smaller size.

Chromosome No.20

This chromosome had three bands, one next to the centromere, one centrally placed and one near the distal end.

Chromosome No.21

This chromosome had one band at the centromere and one near the distal end.

Chromosome No.22

Chromosome number 22 was sometimes difficult to distinguish from number 21 in that differentiation depended on the extent of vacuolation at the centromere. Both chromosomes had dark bands below the centromere and at the distal end.

Chromosome No.23

This chromosome had two dark bands immediately below the centromere.

Chromosome No.24

This chromosome was difficult to distinguish from chromosome number 23 in that differentiation depended on the degree of vacuolation at the centromere. The double band was slightly more centrally placed in chromosome number 24.

Chromosome No.25

This chromosome had a dark band at the centromere and one near the distal end of the chromosome. In addition, there was a faint band proximal to the band at the distal end of the chromosome.

Chromosome No.26

This chromosome had two dark bands placed centrally.

X Chromosome

The X chromosome was quite characteristic and had two bands close to the centromere, a large, dark band placed centrally, and two dark bands towards the distal end of the chromosome with a narrow faint band proximal to the latter bands. There was no centromeric vacuolation.

Y Chromosome

The Y chromosome was such a small body that a distinct banding pattern was difficult to distinguish. However, there appeared to be a band on either side of the centromere. There was no centromeric vacuolation.

By comparison with this idiogram the long arm of the Massey 1 translocation corresponded with the banding pattern of the chromosome most frequently designated number four. More tentatively the short

arm of the Massey 1 translocation was identified as chromosome number 26.

5.3.2. C-banding

The hydrochloric acid and barium hydroxide treatment proved satisfactory for producing C-bands on sheep chromosomes. All the autosomes had clear centromeric staining but there was no evidence of centromeric heterochromatin in the X and Y chromosomes,(Fig. 46). The three metacentric chromosomes could be distinguished by the degree of centromeric staining. Chromosome number one had only faint centromeric staining whilst number two had distinctive blocks of stain. Chromosome number three was intermediate between these two. It was not possible to differentiate all the autosomes on the basis of their centromeric staining but there were different degrees of staining in the various acrocentrics, (Fig.46). Analysis of the staining pattern of the Massey 1 translocation indicated two centres of staining, one either side of the centromere, (Fig.47). There was no evidence that chromosome number 1, 2 and 3 had two centres of stain.

It was not possible to identify the acrocentric chromosomes involved in the Massey 1 translocation on the basis of C-banding alone.

5.4. Discussion

5.4.1. Normal Karyotype of the Sheep

The present work has contributed further evidence that the diploid number of domestic sheep (Ovis aries) is 2n =54. A total of 81 sheep (Table 26) unrelated to the four New Zealand Romney rams heterozygous for the Massey 1 translocation, were examined and all had the normal karyotype of the sheep with 2n =54. There were three pairs of metacentric chromosomes, 23 pairs of acrocentric autosomes and the X

and Y chromosomes. The Y chromosome was the smallest of the karyotype and in good preparations could be seen to be metacentric. The X chromosome was the largest acrocentric chromosome of the karyotype. In cells at late prophase/early metaphase, distinct short arms were visible and facilitated the identification of the X chromosome. In cells at late metaphase the chromosomes were more contracted and the short arms could not always be distinguished. Identification of the X chromosome in these cells relied on comparison of size alone.

When figures from the present work are added to those of Bruere and Mills (1971) and Bruere et al., (1972) the total number of sheep studied is seen to be 1,282 from twentyone different breeds. The number sampled from each breed was small except for the New Zealand Romney and Drysdale breeds.

Centric fusion translocations have been reported only in the three closely related breeds, New Zealand Romney, Perendale and Drysdale. The Massey I was found in the New Zealand Romney and Perendale (Bruere, 1969; Bruere and Mills, 1971; Bruere, 1974, personal communication) the Massey II in the New Zealand Romney (Bruere and Mills, 1971) and the Massey III in the Drysdale flock. (Bruere et al., 1972) These were presumed to have arisen spontaneously and spread through the flock by genetic drift. It may well be that similar polymorphisms have arisen in other breeds of sheep and remain as yet undetected. In particular, there is no reported survey of the Romney Marsh sheep in Britain. It would be interesting to know whether the centric fusion translocations were already present in the British foundation stock or arose during the line breeding to develop the New Zealand breeds. If the centric fusion translocations were to be present only in closely inbred flocks this may provide an indication of the mode of formation and possible function of

such polymorphisms. More extensive surveys of British flocks are necessary to determine the incidence of such polymorphisms in Britain.

5.4.2 G-Banding

Certain of the chromosomes of the sheep karyotype were more consistently and characteristically stained than others. Chromosome number 1, 2, 3, 6, 7, and X could be distinguished easily in most spreads whilst the remaining chromosomes required more careful comparisons. Chromosome number one was characterised by the two bands on the long arm and one on the short arm. The other metacentric chromosomes both had three bands on the long arm and two or three on the short. Even in early metaphase cells, when each band was visible as two bands close together, identification was easy. Chromosome number 3 was recognisable by the prominent dark band just below the centromere on the long arm. This dark band was visible even when the band formation on other parts of the chromatids or on other chromosomes was indistinct. Chromosome number 2 was recognisable more by the absence of the distinguishing features of the other metacentric chromosomes than by a characteristic pattern of its own. It had two sets of bands on the short arm, as opposed to the one on chromosome number 1, and the band below the centromere on chromosome number 2 was less prominent than that on chromosome number 3.

Chromosome number 6 was recognisable by the dark band one quarter of the way down the arm. It was distinguishable from the X chromosome, which it superficially resembled by the vacuolation at the centromere and the position of the band. The X chromosome was larger than chromosome number 6, the band was more centrally placed along the length of the chromosome, there was no vacuolation at the centromere and the short arms of the X were clearly visible in most preparations.

Chromosome number 7 was recognisable by the dark band at the centromere and distinguished from number 12 by the two sets of more faintly staining bands. Chromosome number 12 had only one set of paler bands located distally. Other chromosomes could be identified by comparison with the whole of the karyotype. For example, the patterns of chromosomes 14 and 19 were almost identical but these chromosomes could be differentiated because of their size. Similarly chromosome numbers 17 and 21.

The greatest difficulty was encountered when trying to identify the small acrocentric chromosomes 22, 23, 24, 25 and 26. Most of the material at the centromere had been digested away leaving marked centromeric ~~vaculolation~~ VACUOLATION. This left very little of the chromosome on which banding patterns could be produced. Most of these chromosomes had two darkly staining bands, one at the centromere and one distally located. The distinction had to be made on the spacing of these two bands. Better identification of the small acrocentric chromosomes was obtained when the cells were treated in late prophase/early metaphase. At this time the chromosomes were more elongated and so a greater length was available for comparison. However, even using these cells, complete identification could not be made since the distinction between the band and interband areas was not so precise and the position of the bands was less easily defined. The Y chromosome could always be distinguished from these small acrocentrics by the absence of centromeric vaculolation.

Hence, whilst the long arm of the Massey I translocation was confidently described as corresponding to the chromosome designated number 4, the short arm was only tentatively designated number 26.

There are a number of recent reports of G-band patterns in sheep chromosomes. (Evans et al., 1973; Nadler et al., 1973; Schnedl and Czaker, 1974) Whilst each was in broad agreement with one another and the present work, there were a few discrepancies. For example, the present results showed that the X chromosome had a distinctive pattern and was characterised by a prominent dark band approximately half way down the long arm. Evans et al., (1973) and Schnedl and Czaker (1974) reported similar findings whereas Nadler et al., (1973) considered that the X did not show distinctive bands. This difference may have been due to the fact that the last group of workers were examining sheep other than Ovis aries. The karyotype of a male Ovis canadensis mexicana (Desert bighorn) clearly showed that the X had a banding pattern as described in the present work, but that of O.musimon x O. canadensis did not. In the latter karyotype, none of the chromosomes showed very distinct banding patterns so that the absence of a characteristic pattern on the X may have been due to inferior preparations.

All three groups of workers agreed that chromosome numbers 1, 2 and 3 were easily distinguished and findings in the present work agree almost exactly with the description provided by Evans et al.,(1973). Classification of the acrocentric chromosomes was not so closely paralleled in the different groups of workers. The disparity was not so much in the band pattern but rather in the designation of the chromosome number. Since allocation of chromosome number depends on the basic karyotype of each group of workers the discrepancies are not surprising. The gradual reduction in size of the acrocentric chromosomes in sheep makes it difficult to number the chromosomes consistently. In the present work the long arm of the Massey I translocation was identified as chromosome number 4 by comparison with

the basic karyotype. However, workers in New Zealand identified the long arm as number 5. (Bruere, 1974, personal communication.) In each instance the banding pattern was the same and identification was based on different basic karyotypes. It is obviously necessary to have a standard nomenclature so that confusion in the identification of new translocations does not develop. In man, such a standard has been based on the Q-band pattern and a similar standard could easily be adopted for the sheep based on the work by Hansen. (1973a) Meanwhile, if G-banding is to be used to identify chromosomal abnormalities it will be necessary to define the pattern of the normal karyotype with which it is compared.

The system of nomenclature adopted by Evans et al., (1973), whereby the sheep karyotype is numbered according to the basic karyotype of the goat is considered by the present writer to be unsatisfactory. It implies too great a degree of genetic homology based on similar or even identical band patterns than is necessarily true and leads to confusion.

5.4.3. C-banding

Using a modification of the technique by Sumner (1972) for the demonstration of centromeric heterochromatin it was shown that all the autosomes of the sheep had blocks of heterochromatin at the centromere. The X and Y chromosomes showed no such centromeric staining. The metacentric chromosomes 1, 2 and 3 had smaller stained areas than the acrocentric chromosomes and considerably less than that in the Massey I translocation. In some preparations the block in the Massey I translocation could be seen to be formed by two stained areas, one either side of the centromere. Similar double blocks of centromeric heterochromatin have been described in centric fusion translocations in the goat (Evans et al., 1973), mouse (Chen and Ruddle, 1971) and man,

(Niebuhr, 1972) In contrast, the 1/29 translocation in cattle had only one block of centromeric heterochromatin. (Popescu, 1973; Logue, 1974, personal communication.) These findings have always been interpreted as indicating that the translocations with two blocks of centromeric heterochromatin were dicentric and those with one, monocentric. Relative measurements showing a statistical difference between the length of "monocentric" and "dicentric" chromosomes have been offered as evidence of retention of the second centromere. (Niebuhr, 1972) DNA measurements have indicated that there is little or no loss of DNA after the development of the seven pairs of centric fusion translocations in the tobacco mouse, (Mus poschiavinus) (Comings and Avelino, 1972).

The significance of the differentiation was that dicentric chromosomes in animals were thought to be unstable and therefore more likely to undergo non-disjunction at meiotic metaphse I. (Niebuhr, 1972) However, the evidence pointing to the presence of two centromeres has been all circumstantial. Crouse (1960) and Chen and Ruddle (1971) showed that centromeric heterochromatin was a separate entity from the centromere and could be translocated to various parts of the chromosome without upsetting centromeric function. Gimenez - Martin et al., (1965) discussed the possible break points in the centromere leading to centric fusion translocation. None of these points involved the loss of paracentric material on the long arm so that two blocks of centromeric heterochromatin could be present irrespective of the structure of the translocation chromosome.

Some convincing pictures have been published, notably by Cohen and Marrods (1963) and Subrt, Blehova and Vaborsky, (1971) showing presumptive dicentric, centric fusion translocations in man. These

had elongated centromeric regions highly indicative of two centromeres. Angell, Giannelli and Polani (1970) described three cases of apparent dicentric Y chromosomes in man. Again the published photographs clearly showed an attenuated centromeric region with a central area clear of fibres.

Therefore, evidence is accumulating that dicentric mammalian chromosomes do exist and are stable. Niebuhr, (1972) suggested that the stability may be due to suppression of one of the centromeres by the close proximity of the second. Similar centromeric suppression has been reported in plant dicentric chromosomes which were stable. (Sears and Camara, 1952)

In the Massey I translocation, two blocks of centromeric heterochromatin were visible but the centromeric region was not noticeably larger than that of the metacentric chromosomes 1, 2 and 3. By definition, the centromere is the point of attachment of the chromosome on the mitotic and meiotic spindle. (Rieger et al., 1968) Bajer (1965) showed that the mitotic spindle fibres originated from the centromere itself and extended towards the spindle poles. Thus, if there were two centromeres both functional, one would expect a double set of spindle fibres. Perhaps a more accurate assessment of the number of functional centromeres in a centric fusion translocation could be made by examining the spindle formation.

Alternatively, electron microscope scanning would demonstrate the arrangement of the chromatid fibres in the centromeric region. However, the interpretation of the arrangement is open to debate. Barnicot, Ellis and Penrose (1962) described a centric fusion translocation in a child which they considered to be dicentric.

Electron micrographs of the translocation chromosome were published in support of their conclusion. However, their pictures were very similar to that shown by Comings and Okada (1970) as being the normal quadripartite centromere of metacentric chromosomes.

In conclusion, whilst there is often circumstantial evidence, as with the Massey I translocation, that some centric fusion translocations have two centromeres, it has not been proven conclusively. Furthermore, in cases where a second centromere seems highly likely it is not known whether both are functional. A better understanding of the structure of centric fusion translocations would perhaps lead to a better understanding of the likely behaviour of such chromosomes during cell division.

SECTION VI

TABLES

TABLE I Culture Media Used by Previous Workers for Leucocyte Cultures

Medium	Species	Reference
1. T.C. 199	Human	Osgood and Brooke (1955)
		Rangerford et al., (1959)
		Moorehead et al., (1960)
	Pig	Harvey (1969)
	Dog	Hare et al., (1966)
	Sheep	McFee, Banner & Murphree (1965)
	Cattle	Gustavsson (1969)
2. Eagle's Medium	Human	Genest & Auger (1963)
		Peter (1971)
		Purtilo et al., (1972)
	Dog	Hare et al., (1966)
3. Weymouth's Medium	Human	Fergusson-Smith (1964)
	Pig	Harvey (1969)
	Cattle	Harvey (1971)
4. N.C.T.C. 109	Cat	Hare et al., (1966)
5. Connaught's M597	Cattle	Basrur & Gilman (1964)
6. McCoy's 5A Mod.	Human	Preg (1972)
		McKenzie & Lube (1973)

TABLE II

Hypotonic Solutions Used by Previous Workers in Leucocyte Cultures

Author	Hypotonic Solution
Hsu (1952)	TYRODE ~~Tyrode~~ solution
Moorehead *et al.*, (1960)	Distilled water
Basrur & Gilman (1964)	Distilled water
Ferguson-Smith (1964)	1.12% Sodium citrate
Hungerford (1965)	0.75 M Potassium chloride
Hare *et al.*, (1966)	1:5; Foetal calf serum:distilled water
Bruere (1966)	1.0% Sodium citrate
Harvey (1969)	0.3% Sodium citrate

TABLE III

Incidence of the 1/29 Translocation in Various Breeds of Cattle

Breed	Author	No.of Normal	No.of Hetero-zygotes	No.of Homo-zygotes	Total
1. Swedish Red & White	Gustavsson & Rockborn (1964)	0	3	0	3
	Gustavsson (1966)	1542	366	8	1916
	Total	1542	369	8	1919
2. Charolais	Harvey (1972)	142	1	0	143
	Power & Masterson (1973)	4	0	0	4
	Bruere & Chapman (1973)	21	0	0	21
	Feckheimer (1973)	15	0	0	15
	Total	182	1	0	183
3. Simmental	Holn (1971)	4	2	0	6
	Harvey (1972)	40	1	1	42
	Power & Masterson (1973)	14	0	0	14
	Bruere & Chapman (1973)	13	0	0	13
	Total	71	3	1	75
4. Limousin	Harvey (1972)	4	1	0	5
	+(Larre, Queinnec & Berland (1972))	144	31	0	175
	Bruere & Chapman (1973)	5	0	0	5
	Total	153	32	0	185
5. Blonde d'Aquitaine	+(Larre et al.,(1972))	144	31	0	175
	Harvey (1972)	?	2	0	?
	Bruere & Chapman (1973)	6	1	0	5
	Total	150	34	0	180
6. German Red Pied	Adeck, Holn & Herzog (1968)	4	1	0	5

Breed	Author	No.of Normal	No.of Hetero-zygotes	No.of Homo-zygotes	Total
7. Montbeliaed	Popescu (1971)	0	2	0	2
8. Friesian	Herschler & Fechheimer (1966)	3	1	0	4
	Harvey (1972)	25	0	0	25
	Fechheimer (1973)	537	0	0	537
	Power & Masterson (1973)	1	0	0	1
	Total	566	1	0	567
9. Red Poll	Harvey (1972)	?	1	0	?
	Fechheimer (1973)	1	0	0	1
	Total	1	1	0	1
10. Norwegian Red	Amrud (1969)	412	18	0	430

+(The number of Limousin and Blonde d'Aquitaine not separated.)

TABLE IV

Variations of Culture Medium for Chromosome Analysis of Pre-Implantation Blastocysts - 1972-1973

Culture Medium	% Lamb Serum	10 iu Pen/ 10 mg. Strep.	0.024 iu M Glutaine	µg Colcemid
10 ccs	0	+	+	0.24
10 ccs	0	+	+	0.40
10 ccs	0	+	+	0.56
10 ccs	20	+	+	0.40
10 ccs	20	-	-	0.40
10 ccs	0	-	-	0.40

TABLE V

Birth Weight of Lambs Born from Heterozygous Male x Normal Female

Mean weight of all lambs - = 4.32 ± 1.13 kg

Mean weight of male lambs - = 4.54 ± 1.14 kg

Mean weight of female lambs - = 4.20 ± 1.13 kg

Mean weight of lambs with normal karyotype = 4.45 ± 1.26 kg

Mean weight of lambs heterozygous for the Massey I translocation = 4.25 ± 0.97 kg

TABLE VI

Heterozygous Male x Normal Female - Type of Offspring of Individual Rams

Rams		Offspring			
		No.of Single Males	No.of Single Females	No.of Sets of Twins	Total No. of Lambs
6169	T+	6	5	4	19
	Normal	5	9	8	30
7369	T+	6	5	1½	14
	Normal	4	10	3½	21
7969	T+	4	2	2½	11
	Normal	4	2	4½	15
769	T+	3	0	0	3
	Normal	2	2	0	4

TABLE VII

Lambing Percentage. Heterozygous Male x Normal Female

Year	No.Ewes Tupped	No.Ewes Lambed	No.Lambs	Lambing % age Per Ewe Tupped	Lambing % age per Ewe Lambed
1971-72	-	50	64	-	128.0
1972-73	43	37	52	120.9	140.5
1973-74	7	7	7	100.0	100.0

TABLE VIII

Heterozygous Male x Normal Female. Phenotypic Sex Ratio of Lambs

Year	Males	Females	Total	Sex Ratio Male: Female
1971-72	31	33	64	1:1.06
1972-73	25	27	52	1:1.08
1973-74	3	4	7	1:1.33
Total	59	64	123	1:1.08

TABLE IX

Heterozygous Male x Normal Female. Translocation Segregation in the Offspring.

Year	Karyotype of Offspring			
	2n = 54		2n = 53 T+	
	XY	XX	XY	XX
1971-72	18	16	10	15
1972-73	13	17	12	10
1973-74	3	1	0	2
Total	34	34	22	27

TABLE X

Heterozygous Male x Heterozygous Female. Translocation Segregation in the Offspring

Year	Karyotype of Offspring							
	2n = 54		2n = 53T+		2n = 52T++		Undiagnosed	
	XY	XX	XY	XX	XY	XX	Male	Female
1972-73	2	1	0	0	1	0	1	1
1973-74	2	2	1	2	0	0	0	1
Total	4	3	1	2	1	0	1	2

TABLE XI

Segregation of the Massey I Translocation in Offspring of Heterozygous Male x Normal Female Matings. Combined Data, Scotland & New Zealand

Source of Data	Karyotype of Offspring				Total
	54 XY	53 XY T+	54 XX	53 XX T+	
Long, 1974	34	22	34	27	117
Bruere, 1974	67	72	63	69	271
	101	94	97	96	388

TABLE XII

Segregation of the Massey I Translocation in Offspring of Heterozygous Male x Heterozygous Female. Combined Data; Scotland & New Zealand.

Source of Data	Karyotype of Offspring		
	2n = 52	2n = 53	2n = 54
Long, 1974	1	3	7
Bruere, 1974	20	37	14
Total	21	40	21
Ratio	1	2	1

TABLE XIII

Blastocyst Collection Data - 1971-1972

Heterozygous Male x Normal Female

Ewe No.	No. of i.u. P.M.S.	Slaughtered Days Post Coitum	No. Corpora Lutea R. Ovary	L. Ovary	No. of Blastocysts
1R	0	12	1	1	0
6	2,000	10	0	1	0
9	0	12	0	1	1
15	2,000	12	2	4	0
22	0	12	Not Recorded		0
27	2,000	13	3	2	5
28R	0	12	1	0	1
29R	0	12	1	0	0
31	0	11	1	0	0
40R	2,000	12	10	4	4
45	0	12	1	0	0

Ewe No.	No. of i.u. F.M.S.	Slaughtered Days Post Coitum	No. Corpora Lutea		No. of Blastocysts
			R. Ovary	L. Ovary	
51	0	12	2	0	0
54	2,000	12	6	5	2
54R	2,000	12	15	8	16
56	2,000	12	2	0	0
57	2,000	13	2	1	0
65R	2,000	12	7	7	0
73	2,000	13	1	0	1
77	0	12	1	0	1
83	2,000	12	1	0	1
96	0	15	1	1	2
98	2,000	10	10	7	0
104	0	15	0	0	0
108	2,000	13	4	9	4 or 5
123	0	15	0	1	2
130	2,000	12	2	0	0
134	2,000	10	10	3	0
135	0	12	0	1	0
147	2,000	12	2	0	2
149	2,000	12	5	2	2
Total			91	58	46

TABLE XIV

Blastocyst Collection Data. 1972-1973

Heterozygous Male x Normal Female

Ewe No.	Slaughtered. Days Post Coitum	No. of Corpora Lutea		No. of Blastocysts	Karyotype
		R.	L.		
1/2	14	0	1	1	54 XX
2/2	16	2	0	0	-
3/2	17	1	0	1	53 XX T+
4/2	15	0	1	1	54 XY
5/2	15	1	1	0	-
6/2	16	2	0	2	53 XY T+
					54 XX
7/2	17	1	0	1	53 XY T+
9/2	16	1	0	1	N.D.
10/2	16	1	0	1	54 XY
12/2	17	0	2	1	54 XY*
13/2	16	0	1	1	53 XY T+
16/2	16	1	0	1	53 XX T+
18/2	17	0	1	1	N.D.
19/2	14	1	0	1(Degenerating)	N.D.
21/2	15	2	0	2	N.D.
					N.D.
22/2	16	1	0	1	54 XY
23/2	14	1	1	2	54 XY
					54 XY
25/2	14	1	0	1	53 XX T+
26/2	15	1	1	2	54 XY
					N.D.

Ewe No.	Slaughtered. Days post Coitum	No. of Corpora Lutea R.	No. of Corpora Lutea L.	No. of Blastocysts	Karyotype
28/2	14	0	1	1	54 XX
30/2	14	1	0	1	N.D.
31/2	16	1	1	2	54 XX
					54 XX
33/2	17	1	0	1	54 XY
34/2	17	1	0	1	N.D.
35/2	16	1	0	1	54 XY
36/2	16	1	0	1	N.D.
38/2	16	0	1	0	-
39/2	17	2	0	2	N.D.
					N.D.
40/2	15	1	0	1	53 XY T+
41/2	16	1	1	2	53 XX T+
					53 XY T+
42/2	17	2	0	0	-
44/2	16	0	1	1	53 XX T+
45/2	16	1	0	1	53 XX T+
51/2	13-15	1	0	2	53 XY T+
					N.D.
56/2	16	1	1	2	54 XY
					53 XY T+
61/2	17	1	0	1	54 XY
66/2	15-17	1	0	1	53 XY T+
69/2	16	2	0	2	53 XY T+
					53 XY T+
70/2	17	1	0	1	54 XX
72/2	15	1	0	1	53 XY T+
73/2	15-17	1	1	2	53 XX T+ 53 XX T+

Ewe No.	Slaughtered. Days Post Coitum	No. of Corpora Lutea R.	L.	No. of Blastocysts	Karyotype
79/2	15	1	0	1	54 XX
80/2	16-18	1	1	1	53 XY/54XY
85/2	17	1	1	2	53 XX T+
					53 XX T+
91/2	17	0	1	1	54 XX
93/2	18	0	1	1	54 XY
95/2	17	1	0	1	54 XY
47	TOTAL	43	20	55	

Key: N.D. = Not Diagnosed

* = Less than 5 cells counted

TABLE XV

Blastocyst Collection Data. 1973-1974

Heterozygous Male x Normal Female

Ewe No.	Slaughtered. Days Post Coitum	No. of Corpora Lutea R.	L.	No. of Blastocysts	Karyotype
1/3	14-15	1	2	3	53 XY T+
					N.D.
					N.D.
12/3	13	0	1	1	? Y
15/3	13	1	0	0	-
16/3	13	1	1	2	54 XY
					N.D.
17/3	13	0	1	1	54 XX

Ewe No.	Slaughtered Days Post Coitum	No. of Corpora Lutea R.	L.	No. of Blastocysts	Karyotype
18/3	13	1	0	0	-
21/3	13	1	0	0	-
22/3	13	2	0	1	53 XX ♀+
23/3	13	1	0	0	-
24/3	13	1	0	1	53 XY T+
25/3	13	0	1	C.D.	N.D.
26/3	13	0	1	1	54 XY
27/3	13	0	1	0	-
28/3	13	1	0	C.D.	N.D.
29/3	13	0	1	1	54 XX
30/3	13	1	0	C.D.	N.D.
31/3	13	1	0	1	N.D.
32/3	13	1	0	1	54 XY*
34/3	13	1	0	1	? Y
35/3	14	0	1	1	53 XY T+*
36/3	13	0	2	2	54 XY*
					53 XY ♀+*
37/3	14	1	0	1	54 XY*
38/3	13	0	1	0	-
39/3	13	0	1	1	54 XX*
40/3	13	1	1	0	-
41/3	13	0	2	1	N.D.
43/3	13	0	0	1	53 XY T+
44/3	13	0	1	1	53 XY T+*
46/3	13	1	1	0	-
47/3	14	0	1	1	54 XY
48/3	13	1	0	1	54 XY
49/3	13	1	0	C.D.	N.D.

Ewe No.	Slaughtered. Days Post Coitum	No. of Corpora Lutea		No. of Blastocysts	Karyotype
		R.	L.		
50/3	14	0	1	1	N.D.
51/3	13	1	0	1	54 XX
52/3	13	1	0	1	53 XY T+
54/3	13	1	0	1	? Y T+
55/3	13	1	0	1	N.D.
56/3	13	2	0	2	N.D.
					N.D.
59/3	13	1	0	1	N.D.
60/3	13	1	1	2	53 XX T+*
					53 XY T+*
61/3	13	0	1	1	54 XX
62/3	13	1	0	1	53 XX T+*
63/3	13	0	1	1	53 XY T+
64/3	13	0	1	1	54 XY*
66/3	13	0	1	1	N.D.
67/3	13	1	0	1	54 XX
68/3	13	2	1	2	54 XX
					54 XY
69/3	13	1	0	1	53 XX T+
71/3	13	0	1	1	54 XX*
72/3	13	1	2	1	? Y T+
73/3	13	2	0	2	54 XX
					53 XX T+*
	TOTAL	35	30	47	

Key: C.D. = Cellular Debris

N.D. = Not Diagnosed

* = Less than 5 cells counted.

TABLE XVI

Blastocyst Recovery Data. Heterozygous Male x Normal Female

Season	No. Corpora Lutea	No. Blastocysts	% Recovery	No. Blastocysts Diagnosed	% Diagnosed
1972-73	63	55	87.3	43	78.18
1973-74	65	47	72.3	32	68.1

Key: % Recovery = $\frac{\text{No. Blastocysts} \times 100}{\text{No. of Corpora Lutea}}$

% Diagnosed = $\frac{\text{No. Blastocysts Diagnosed} \times 100}{\text{No. of Blastocysts Recovered}}$

TABLE XVII

Translocation Segregation in Blastocysts from Individual Sires

Sire	2n = 54		2n = 53T+		Undiagnosed	Total
	XY	XX	XY	XX		
6169	6	1	4	4	6	21
7369	3	1	1	1	4	10
7969	7	11	12	8	12	50
769	6	5	3	2	5	21
TOTAL	22	18	20	15	27	102

TABLE XVIII

Blastocyst Data. Sex Ratio and Translocation Segregation

Season	Male		Female		Total
	T+	T-	T+	T-	
1972-1973	11	15	9	8	43
1973-1974	11	10	5	10	36
TOTAL	22	25	14	18	79

N.B. Includes 2 blastocysts where the Y plus translocation chromosome were identified and 2 where the Y chromosome was identified.

TABLE XIX

Blastocyst Collection Data. 1973-1974

Normal Male x Heterozygous Female

Ewe No.	Slaughtered. Days Post Coitum	No.of Corpora Lutea		No.of Blastocysts	Karyotype
		R	L		
$F_1$34	12	1	1	2	53 XY T+ 54 XY
$F_1$54	12	1	0	0	-
$F_1$55	13	0	2	1	N.D.
$F_1$81	13	1	1	1	53 XX T+
$F_1$104	13	0	1	0	-
$F_1$119	13	0	1	C.D.	N.D.
$F_1$129	13	0	2	C.D.	N.D.
$F_1$148	12	1	0	0	-
Total		4	8	4	

Key: C.D. = Cellular Debris N.D.=Not Diagnosed

TABLE XX

Ram F_2 199 Diakinesis Chiasmata Counts

Chromosome No.			Acrocentrics	Total
1	2	3		
7	5	5	47	64
8	7	5	40	60
5	6	6	46	63
6	5	5	50	66
5	5	5	39	54
5	4	4	41	54
6	4	4	37	51
6	4	4	32	45
5	5	5	35	50
4	5	4	32	45
6	5	4	41	56
7	5	7	40	59
7	5	5	45	62
4	6	5	39	54
5	4	4	35	48
5	4	4	34	47
6	4	5	35	50
6	5	5	50	66
3	4	4	36	47
5	5	4	36	50
6	6	4	44	60
7	8	6	35	56
6	6	5	40	57
4	5	4	41	54
4	4	4	34	46

Ram: F_2 199 cont.

1	2	3	Acrocentric	Total
5	5	4	40	54
7	5	5	38	55
5	4	4	31	44
4	5	4	41	54
6	6	5	36	53
7	6	5	43	61
7	5	7	40	59
5	5	5	33	48
Mean: 5.57 ± 1.17 ± SD	5.06 ± 0.93	4.72 ± 0.83	38.97 ± 4.99	54.31 ± 6.41

TABLE XXI

Ram: F_1 49 Diakinesis Chiasmata Counts

Chromosome No.					
1	2	3	Trivalent	Acrocentric	Total
7	6	5	4	43	65
5	4	5	3	39	56
6	6	5	3	34	54
5	5	5	3	36	54
6	5	4	3	41	59
5	5	4	3	35	52
7	5	6	4	37	59
7	4	5	3	34	46
5	4	5	3	41	58
6	6	4	3	35	54
6	7	6	3	41	63
Mean ± SD 5.90 ± 0.83	5.13 ± 0.98	4.90 ± 0.79	3.18 ± 0.41	37.82 ± 3.28	56.36 ± 5.28

TABLE XXII

Ram:$F_1$101. Diakinesis Chiasmata Counts

Chromosome No.					
1	2	3	Trivalent	Acrocentrics	Total
7	6	6	3	40	62
5	5	3	3	37	53
6	5	6	4	36	57
5	4	4	3	33	49
3	4	4	2	34	47
4	4	4	3	32	47
5	4	4	3	38	54
6	4	4	3	40	57
4	4	3	3	40	54
6	4	4	3	34	51
6	5	6	3	28	48
7	5	5	3	32	52
5	5	5	3	32	50
5	6	5	3	22	41
6	8	5	3	32	54
5	6	5	3	29	48
6	5	4	3	37	55
6	7	4	2	32	51
6	4	6	3	35	54
Mean ± SD.					
$5.42^{\pm}1.01$	$5.00^{\pm}1.15$	$4.57^{\pm}0.96$	$2.95^{\pm}0.41$	$33.84^{\pm}4.55$	$51.79^{\pm}4.67$

TABLE XXIII

Ram: $F_1$113 Diakinesis Chiasmata Count

Chromosome No.					
1	2	3	Trivalent	Acrocentrics	Total
7	7	6	4	44	68
5	5	5	4	31	50
6	7	6	3	42	64
7	6	6	2	40	61
7	6	6	3	31	53
6	6	6	4	38	60
7	6	6	4	37	60
Mean ± SD					
$6.42^{\pm}0.78$	$6.14^{\pm}0.69$	$5.85^{\pm}0.37$	$3.43^{\pm}0.79$	$37.57^{\pm}5.06$	$59.43^{\pm}6.16$

TABLE XXIV

Ram: $F_2$200 Diakinesis Chiasmata Count

Chromosome No.					
1	2	3	Trans-location	Acrocentrics	Total
5	7	4	4	26	41
4	4	4	3	31	48
5	4	4	4	36	53
4	5	4	4	33	50
5	4	4	4	25	39
4	3	4	3	30	51
6	5	5	5	31	48
5	4	4	4	30	47

Ram: $F_2$200 cont.

Chromosome No.					
1	2	3	Trans-location	Acrocentrics	Total
5	4	4	4	42	59
6	4	4	3	26	43
6	4	5	4	33	52
5	5	5	4	30	49
6	6	5	5	40	62
5	4	5	3	33	50
5	4	4	4	35	52
5	4	4	4	41	61
Mean ± SD.					
$5.06^{\pm}0.68$	$4.43^{\pm}0.96$	$4.31^{\pm}0.47$	$3.87^{\pm}0.61$	$32.00^{\pm}4.69$	$49.73^{\pm}6.27$

TABLE XXV (Corrected)

Mean Chiasmata Counts

Ram	Chromosome No.			Trivalent	Translocation Bivalent	Acrocentric	Total
	1	2	3				
$F_2$199	5.57±1.17	5.06±0.93	4.72±0.83	-	-	38.97±4.99	54.31±6.40
$F_1$49	5.90±0.83	5.18±0.98	4.90±0.70	3.18±0.40	-	37.81±3.28	56.36±5.27
$F_1$101	5.42±1.01	5.00±1.15	4.57±0.96	2.94±0.40	-	33.84±4.54	51.78±4.67
$F_1$113	6.42±0.78	6.14±0.69	5.85±0.37	3.42±0.78	-	37.57±5.06	59.42±6.16
$F_2$200	5.06±0.68	4.43±0.96	4.31±0.47	-	3.87±0.61	32.00±4.69	49.73±6.27

TABLE XXVI

Breeds of Sheep Examined and Found to Have a Chromosome Number of 2 n = 54

Breed	No. Males	No. Females	Total
Scottish Blackface	1	39	40
Finnish Landrace	0	4	4
Border Leicester	1	1	2
Suffolk x Blackface	14	21	35
	16	65	81

TABLE XXVII

Distribution of Chromosome Number in Cells at Meiotic 2nd Metaphase

Ram	Diploid Karyotype	Chromosome Number															Total
		X				Y				XT+			YT+			XY	
		25	26	27	28	25	26	27	28	25	26	27	25	26	27	28	
$F_2$199	54XY	0	6	51	1	2	4	35	0	0	0	0	0	0	0	1	100
$F_1$49	53XYT+	2	4	22	0	0	3	31	0	2	19	1	1	15	0	0	100
$F_1$101	53XYT+	1	1	14	0	0	3	11	0	1	11	3	1	9	1	0	56
$F_1$113	53XYT+	0	0	2	0	0	3	0	0	0	1	0	0	1	0	0	7
TOTAL		3	5	38	0	0	9	42	0	3	31	4	2	25	1	0	163
$F_2$200	52XYT++	0	0	0	0	0	0	0	0	0	11	0	1	5	0	0	17

TABLE XXVIII

Sex Ratio at Meiotic 2nd Metaphase

Ram	Diploid Karyotype	Y Bearing Cells	X Bearing Cells	Total
$F_2$199	54XY	42	57	99
$F_1$49	53XYT+	50	50	100
$F_1$101	53XYT+	25	31	56
$F_1$113	53XYT+	4	3	7
TOTAL		79	84	163
$F_2$200	52XYT+	6	11	17

SECTION VII

APPENDIX

APPENDIX

6.1. Details of Leucocyte Culture Technique

1) The basic medium consisted of 100 ml Weymouth's medium to which was added 10,000 i.u. penicillin; 10,000 µg streptomycin; 1.5 ml Glutamine (200 mM), 20 ml Lamb's serum and 5 ml of reconstituted phytoheamaglutinin.

2) Either 1 or 2 ml of whole blood was added to 10 ml of the above composite medium and incubated at 37°C for 48 hours.

3) After 48 hours incubation, 0.2 ml of colcemid (80 µg/ml) or 0.1 ml of colchicine (25 µg/ml) was added and the cultures incubated for a further 2-3 hours.

4) The cells were harvested by centrifuging at 1,000 rpm for 10 mins. decanting the supernatant and resuspending the button of cells in 10 ml of 0.125% KCl. The cells remained in the hypotonic solution at 37°C for 8 mins.

5) Following treatment with hypotonic solution the cells were centrifuged at 800 rpm for 10 mins., the supernatant removed and the cell button resuspended in 3 ml of chilled fixative. (3:1, methanol: acetic acid.)

6) There were two changes of fixative, one after 15 mins. and the second 30 mins. after the first.

7) After the second fixative the cells were resuspended in 1 ml of fixative and preparations made by dropping 0.5 ml of cell suspension

onto a clean, chilled slide. The slide preparations were left to dry in air.

8) Air dried preparations were stained in either 2% aceto-orcein for three hours or 1:10 Giemsa in buffer for 5 minutes.

6.2. Details of Bone Marrow Cultures

1) The bone marrow was flushed from the femur with 10 ml of Weymouth's medium.

2) 0.5 ml of colcemid (8 µg/ml) was added to 10 ml of culture which was then incubated at 37°C for 2.5 hours.

3) The cells were harvested by centrifuging at 1,000 rpm for 8 mins., decanting the supernatant and resuspending the cell button in 10 ml of 0.125% KCl. The cells were incubated in the hypotonic solution at 37°C for 8 mins.

4) After treatment with hypotonic solution the cells were centrifuged at 800 rpm for 8 mins., the supernatant discarded and the cells resuspended in 5 ml of chilled fixative (3:1, methanol:acetic acid) for 15 mins. at 4°C.

5) After two changes of fixative at 15 min. intervals the cells were left in 3 ml of fixative at 4°C overnight.

6) Slide preparations were made as described for leucocyte cultures and stained in 2% aceto-orcein for 3 hours.

6.3 Details of Histological Preparations

1) The material was dehydrated automatically in a histokine ready for sectioning.

2) Samples were embedded in wax and 5 μ sections cut on a microtome. The cut sections were floated on warm water at approximately 45°C to remove the creases.

3) The section was picked up by placing the slide on top of the water.

4) The slides were left on the hot plate to dry for 30 mins. and then placed in the oven at 56°C overnight to facilitate the sticking of the section to the slide.

5) The wax was removed by dissolution in xylol for 5 mins. and then the slide was washed in absolute alcohol, followed by methylated spirits and finally rinsed in water.

6) Sections were stained with haemotoxylin and eosin.

6.4. Photography

All photomicrographs were taken using a Leitz automatic camera mounted on a Leitz autolux microscope. Black and white photographs were taken on Microneg Pan, film type B (Ilford) and developed in the laboratory using Bromophen developer and Hypam fixative. Prints were made using a Rapidoprint machine.

Colour photographs were taken on Kodacolour X film and developed and printed commercially.

Photographs of the entire rams were taken on an Edixa Prismat camera using Kodacolour X film and developed and printed commercially.

6.5. Glassware

All glassware used for leucocyte and blastocyst cultures underwent careful cleaning after use.

1. Soaked in concentrated chloros solution for 24 hours.
2. Soaked in pyroneg solution overnight.
3. Washed in warm, fresh pyroneg solution.
4. Rinsed approximately 12x in running hot water.
5. Rinsed approximately 12x in running cold water.
6. Left to soak in cold deionised water overnight.
7. Rinsed in running deionised water.
8. Dried in the hot air oven.

6.6. Slides

Absolutely clean slides were essential for good spreading of the chromosomes. New slides were first rinsed in running cold water to remove the dust and soaked in a concentrated pyroneg solution for at least 24 hours. This process removed the grease from the slides. The degreased slides were then placed in slide racks and rinsed in running cold water for at least an hour before being used. Immediately prior to use the slides were placed in the refrigerator or freezer to ensure final chilling.

SECTION VIII

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SECTION IX

FIGURES

Fig. 1

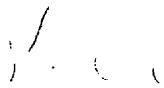
The four New Zealand Romney rams heterozygous for the Massey I translocation.

a) 6169

b) 769

c) 7969 d) 7369

Fig. 2



Karyotype of a metaphase spread from a leucocyte culture of ram 769.

769

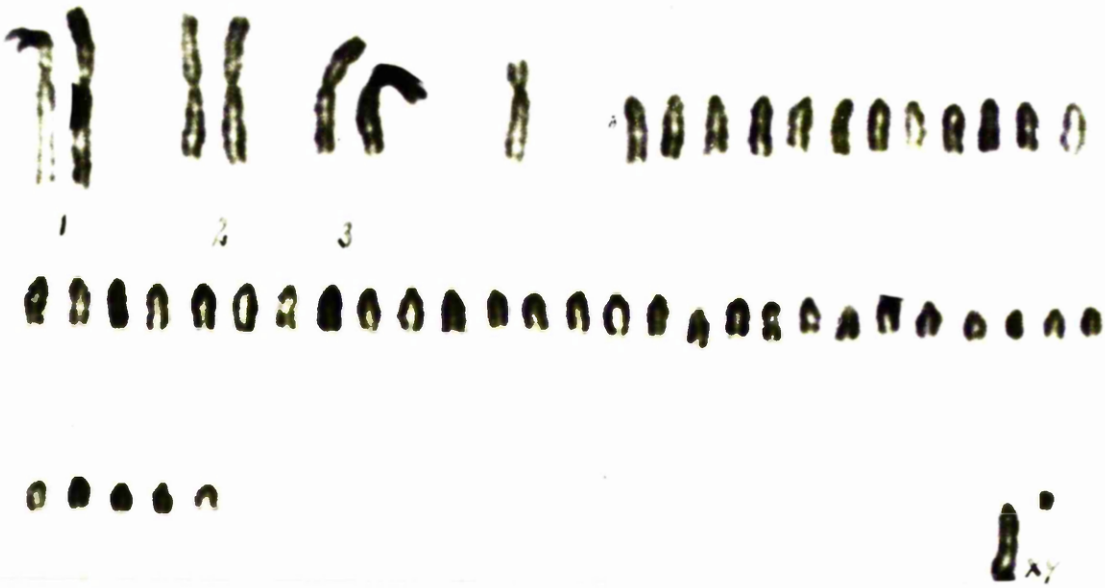
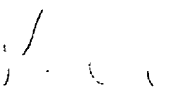


Fig. 3



Karyotype of a metaphase spread from a leucocyte culture of ram 6169.

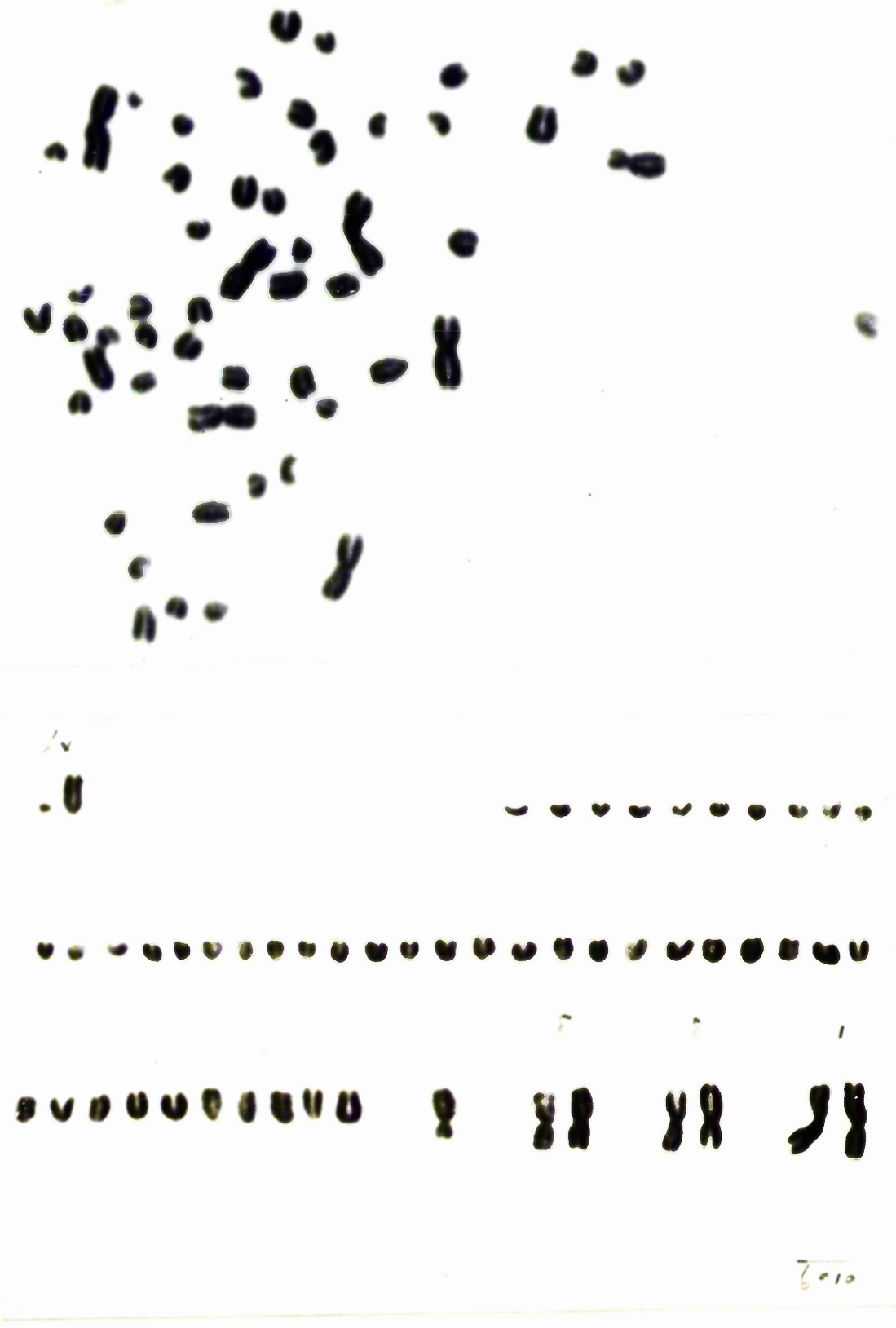
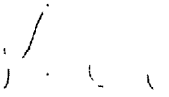


Fig. 4



Karyotype of a metaphase spread from a leucocyte culture of ram 7369.

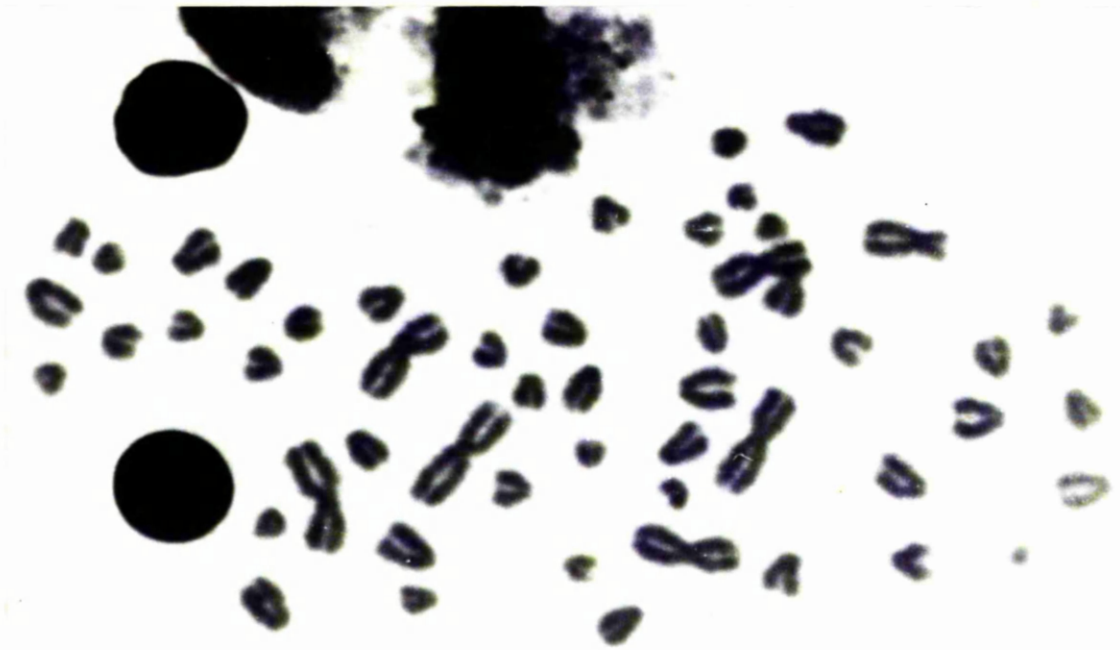


Fig. 5

Karyotype of a metaphase spread from a leucocyte culture of ram 7969.

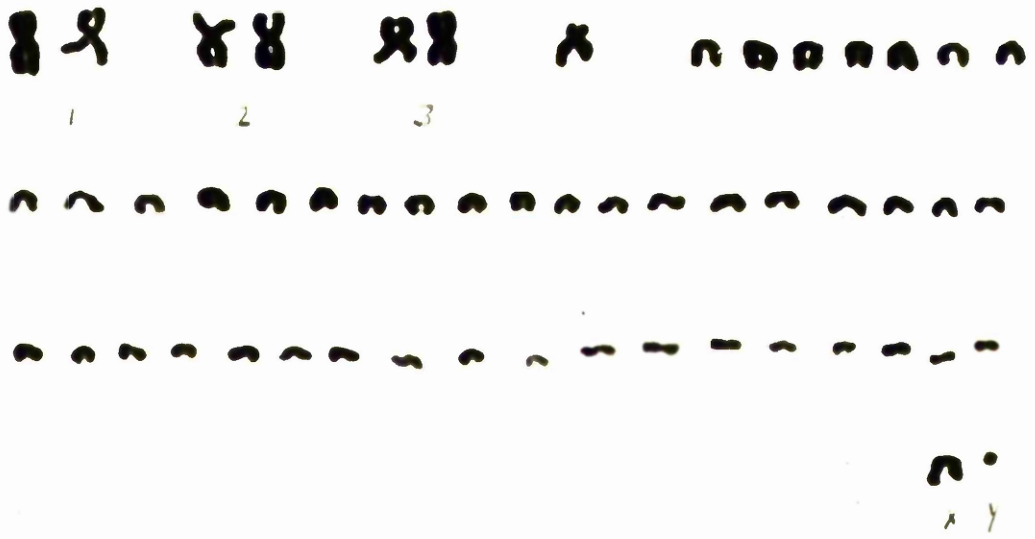
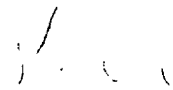

1
2
3
X
Y

Fig. 6

Karyotype of a metaphase spread from a leucocyte culture of ram $F_1$110.

This animal was heterozygous for the Massey I translocation and also a unilateral cryptorchid.

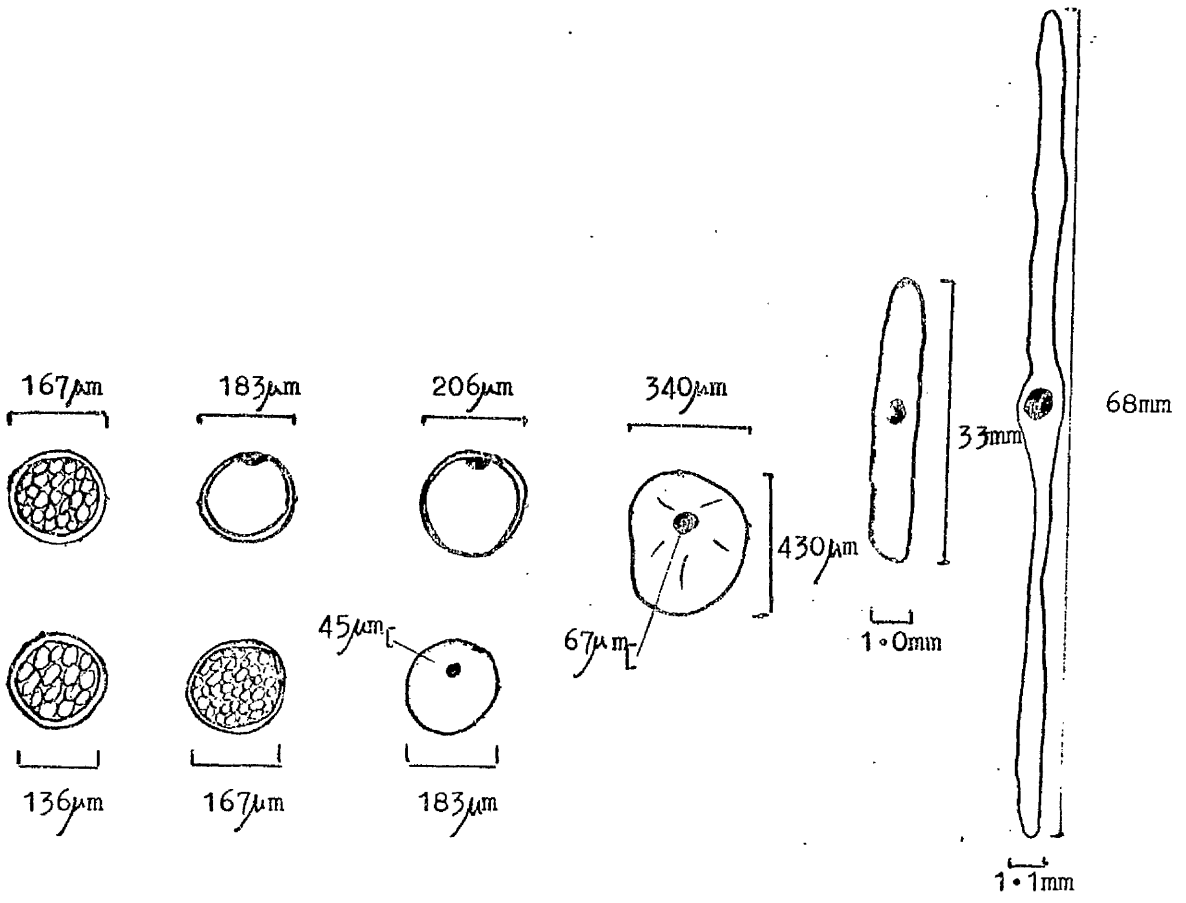
Fig. 7



Karyotype of a metaphase spread from a leucocyte culture of ram $F_2$200, homozygous for the Massey I translocation.

1
2
3

Fig. 7a.



Diagrammatic representation of the growth and development of the sheep embryo before implantation. The day of oestrus was Day 0. (After Bindon, 1971.)

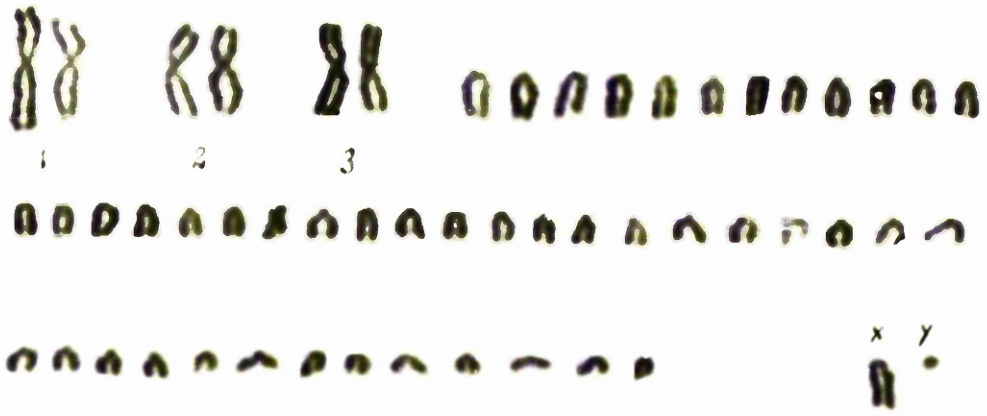
Fig. 8

Heterozygous male x normal female.

a) Karyotype from a 15 day old blastocyst. 2n = 54 xy

b) Karyotype from a 17 day old blastocyst. 2n = 54 xx

SE 26 -/2 B1/2/1 BLASTOCYST 104·0/60+



SE 70 -/2 BLASTOCYST B1/2/1 93·0/23·Y

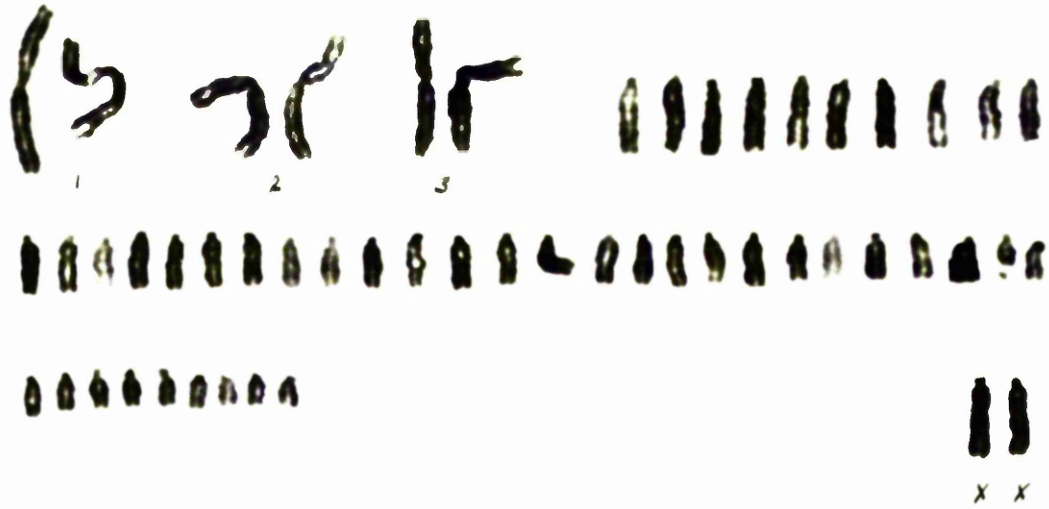


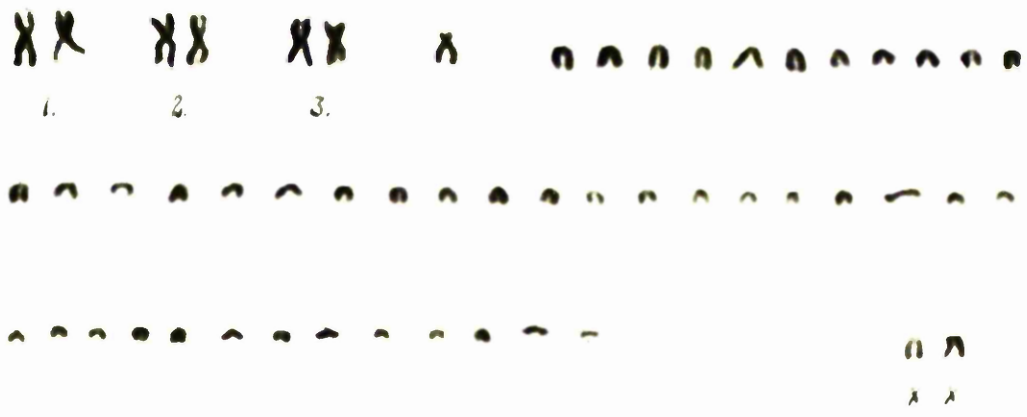
Fig. 9

Heterozygous male x normal female.

a) Karyotype of a 16 day old blastocyst, heterozygous for the Massey I translocation. 2n = 53xx T+

b) Karyotype from a 16 day old blastocyst, heterozygous for the Massey I translocation. 2n = 53xx T+

SE 41 -/2 BLASTOCYST B 1/1/1 106·0/58·3



SE 45 -/2 BLASTOCYST B 2/2/1 114·0/65·3

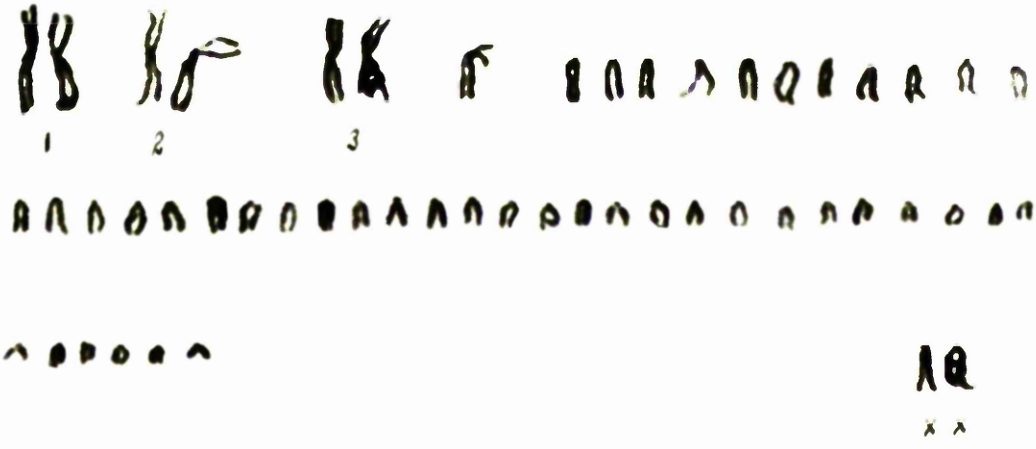


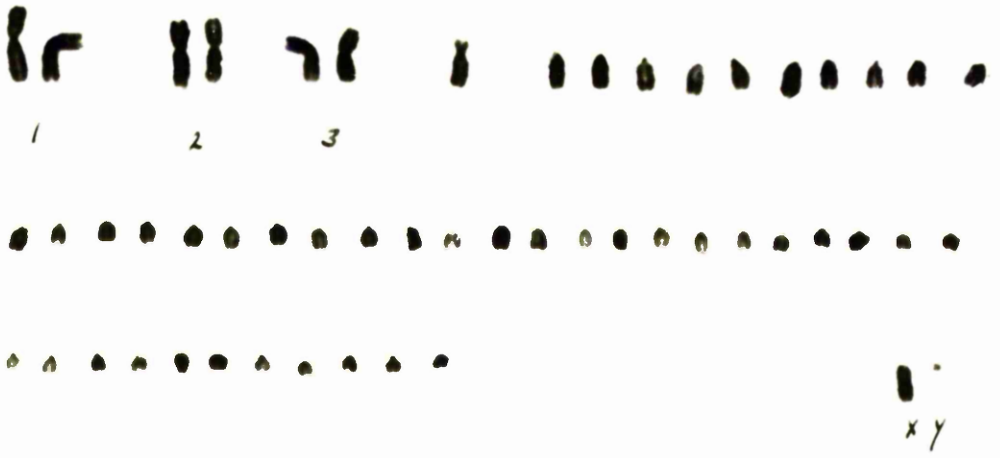
Fig. 10

Heterozygous male x normal female.

a) Karyotype from a 16 day blastocyst, heterozygous for the Massey I translocation. 2n = 53xy T+

b) Karyotype from a 15 day blastocyst, heterozygous for the Massey I translocation. 2n = 53xy T+

SE 13-/2 BLASTOCYST B1/2/2 92·3/29·0



SE 66-/2 BLASTOCYST 31/1/2 [illegible]

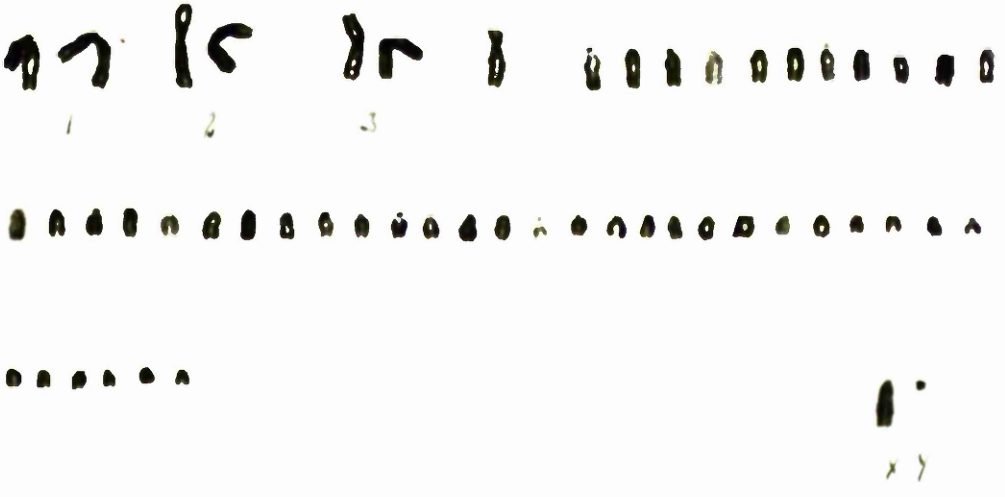


Fig. 11

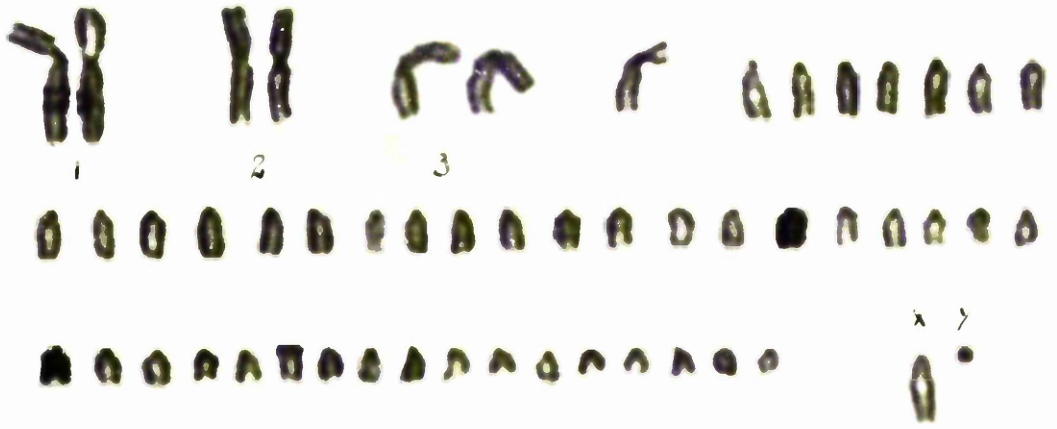
Heterozygous male x normal female.

Karyotype from two 16 day blastocysts recovered from the same ewe.

a) Karyotype of blastocyst, heterozygous for the Massey I translocation. 2n = 53xy T+

b) Karyotype of blastocyst with a normal chromosome complement. 2n = 54xx

SE 6-/2 B1/1/1 BLASTOCYST 45.3/3 F.Y.



SE 6-/2 BLASTOCYST B2/1/1 110.6/SEP.

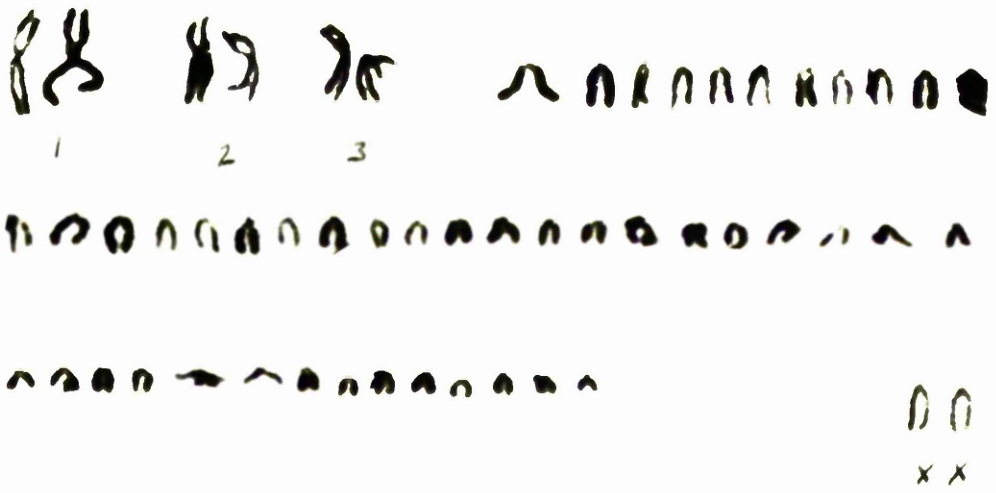
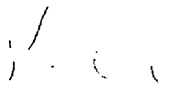


Fig. 12



Normal male x heterozygous female.

Karyotype from a 13 day blastocyst. 2n = 53xx T+

F1 81 BLASTOCYST. B1/1 99·3/38·P.

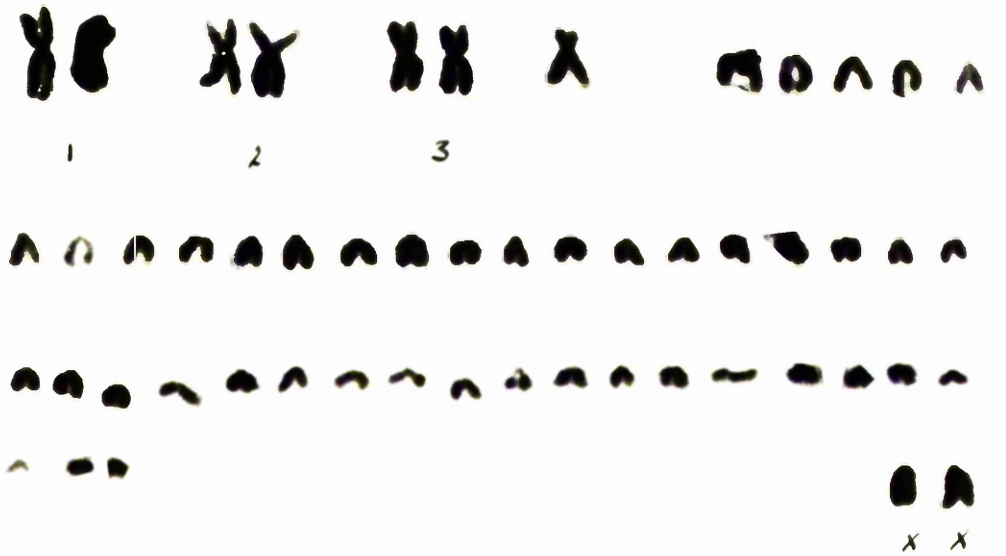
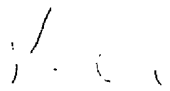


Fig. 13



Normal male x heterozygous female.

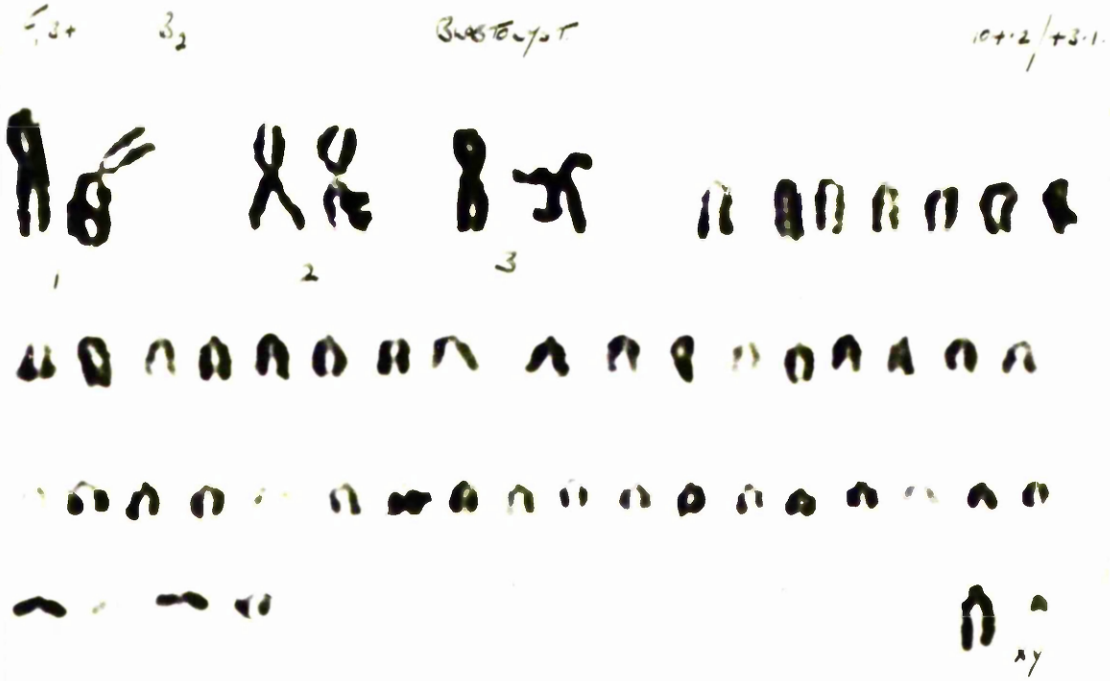
Karyotype from a 12 day blastocyst, one of two, collected from ewe $F_1 34$. 2n = 53xy T+

F 34 BLASTOCYST. B1 100·9/53 y.

Fig. 14

Normal male x heterozygous female.

Karyotype from a 12 day blastocyst, one of two, collected from ewe $F_1 34$. $2n = 54xy$



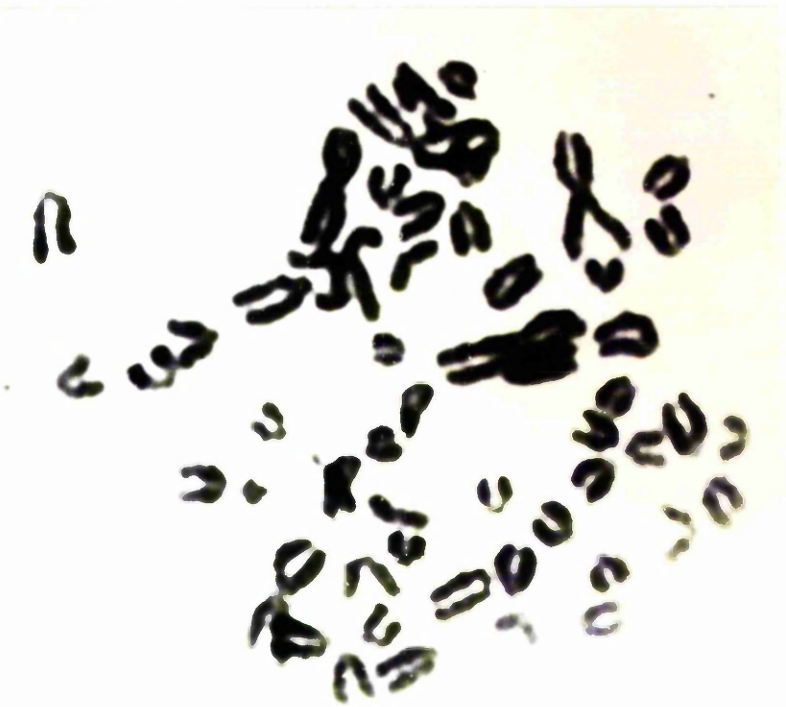


Fig. 15

Distribution of Chromosome Number in Metaphase Cells from Blastocysts.

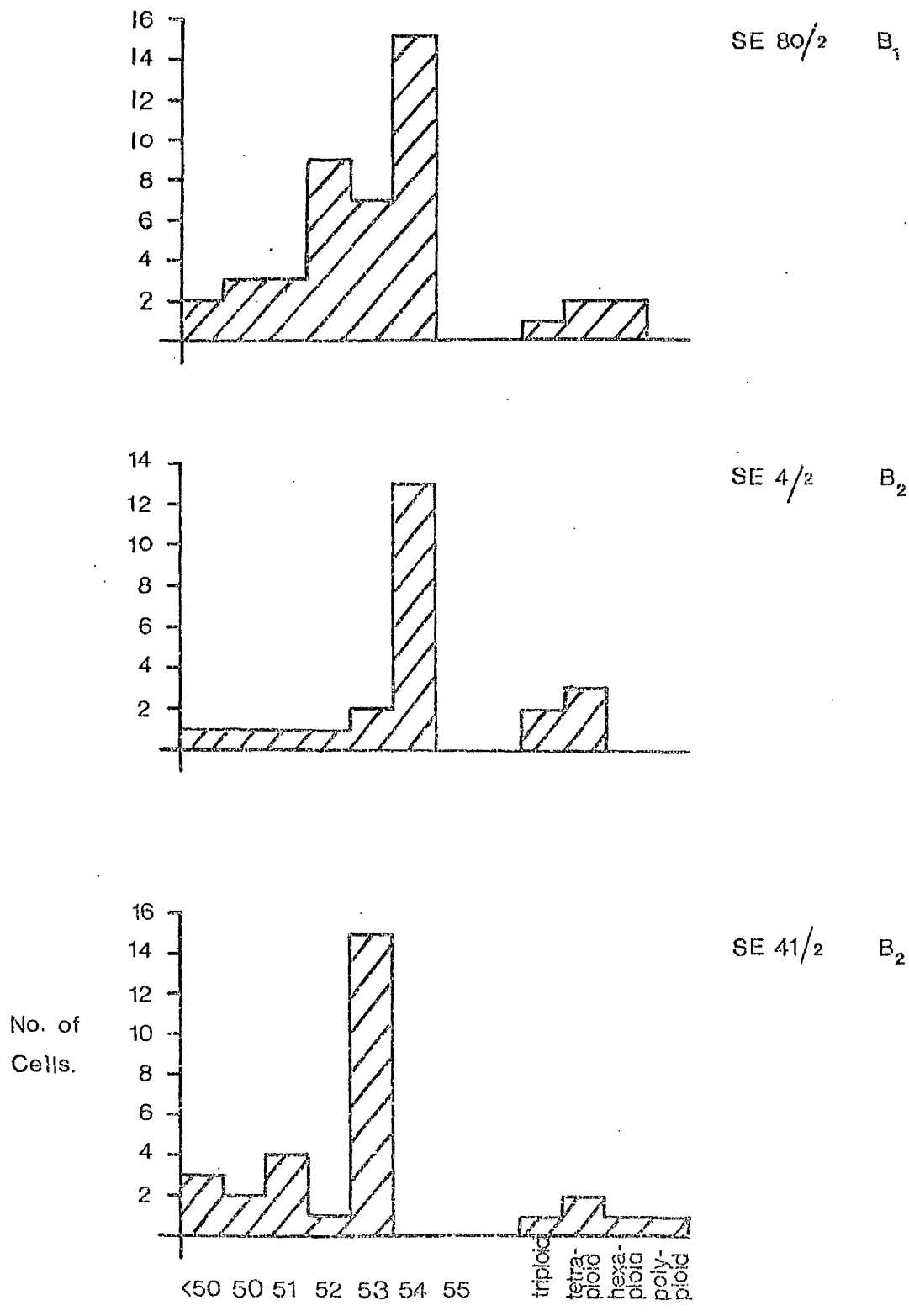


Fig. 16

Metaphase spread from a short term bone marrow culture of an aborted lamb. 2n = 52xx T++

The arrows indicate the Massey I translocation chromosomes and the black triangles indicate the metacentric chromosomes of the normal sheep karyotype.

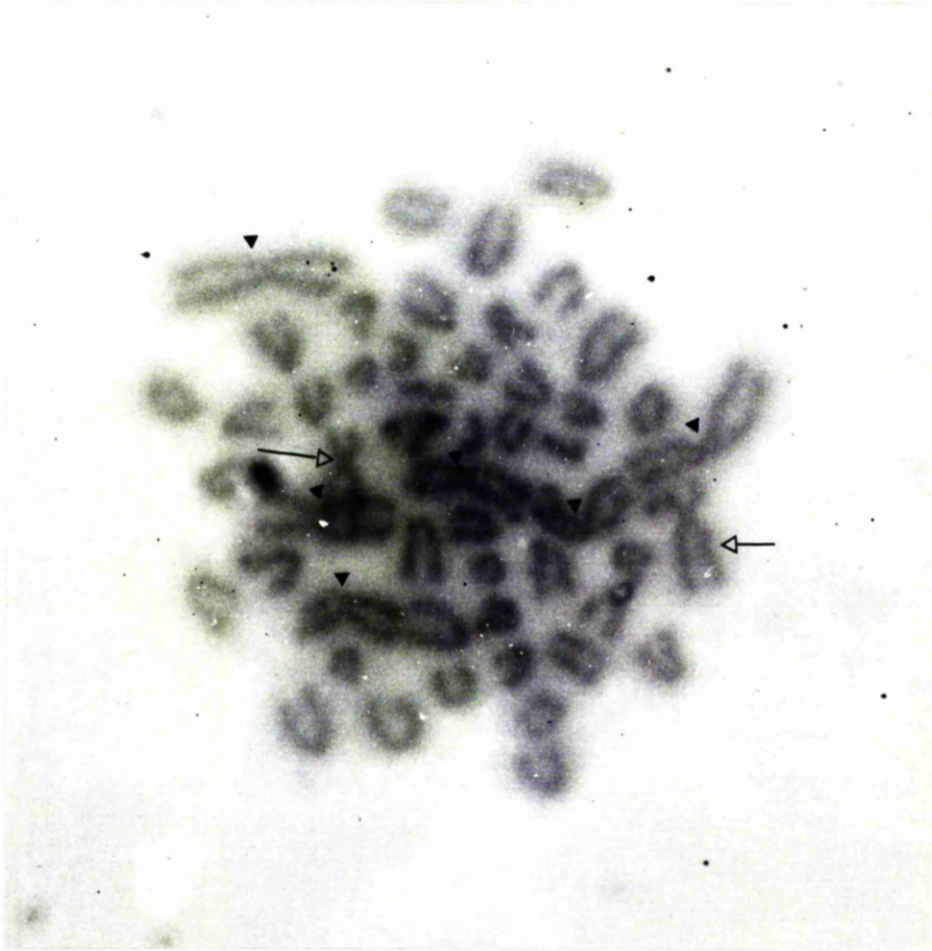
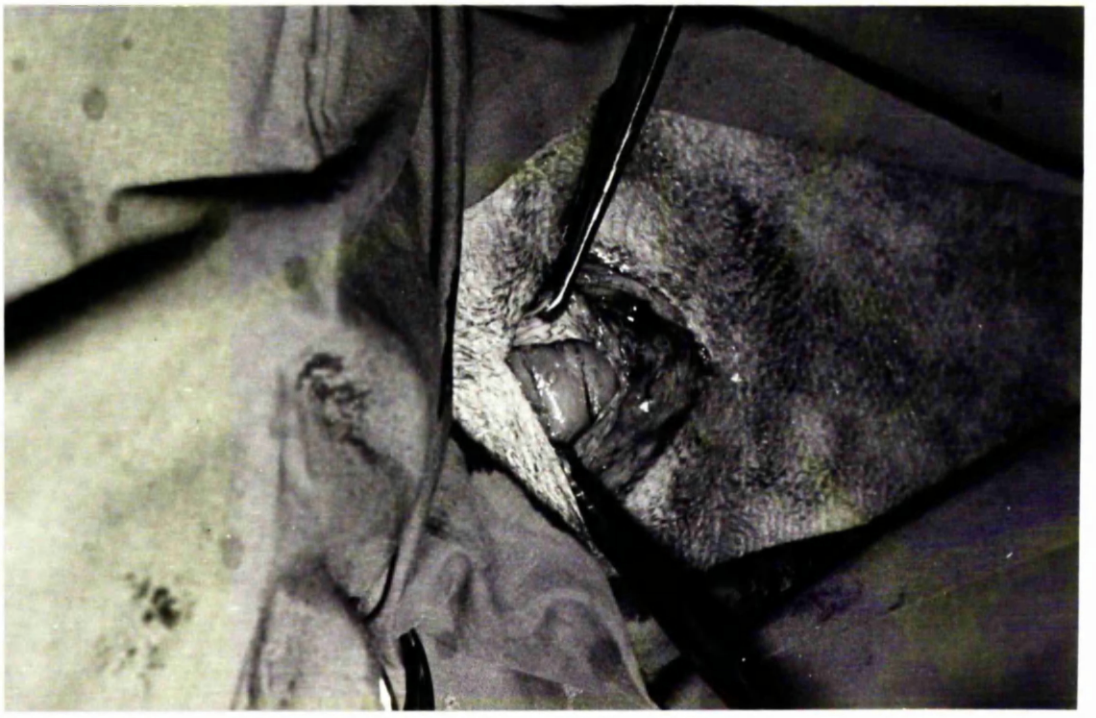


Fig. 17

Surgical approach for the collection of blastocysts by laparotomy.

a) Mid-line incision through the linea alba just anterior to the mammary gland and extending approximately two inches anteriorly.

b) The exteriorised uterus with a glass catheter inserted near the utero-tubal junction. Fluid was flushed through the horn and collected in the universal bottle via the catheter.



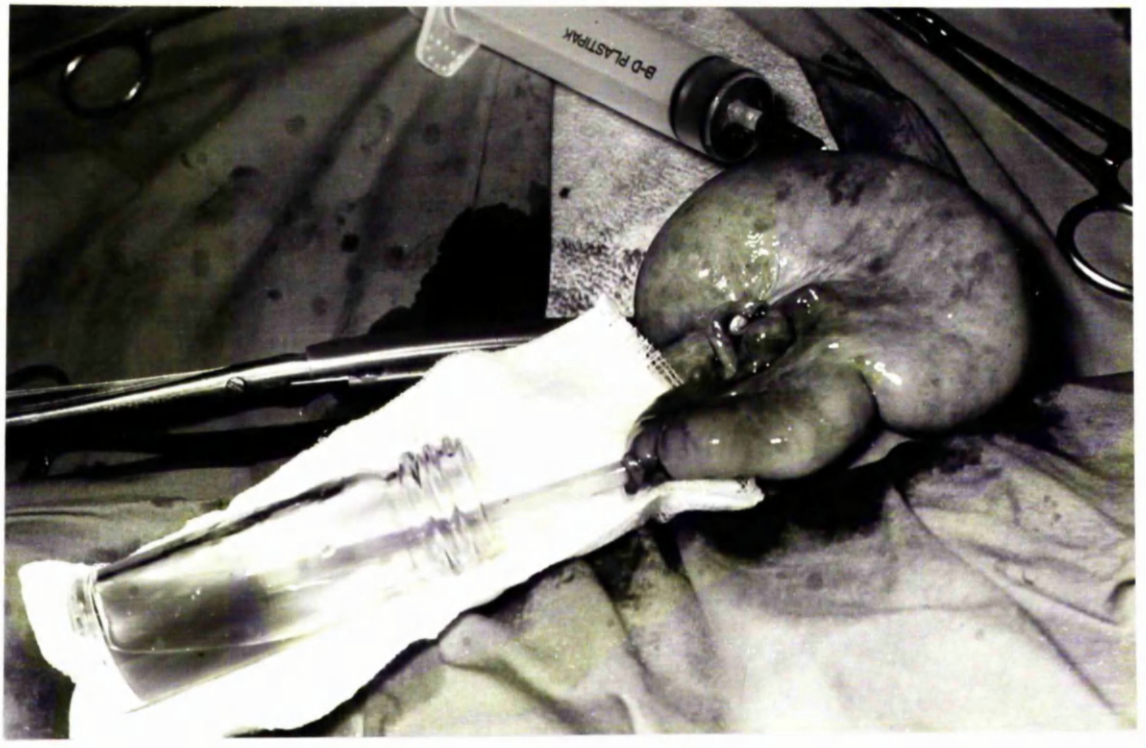

B-D PLASTIPAK

Fig. 18

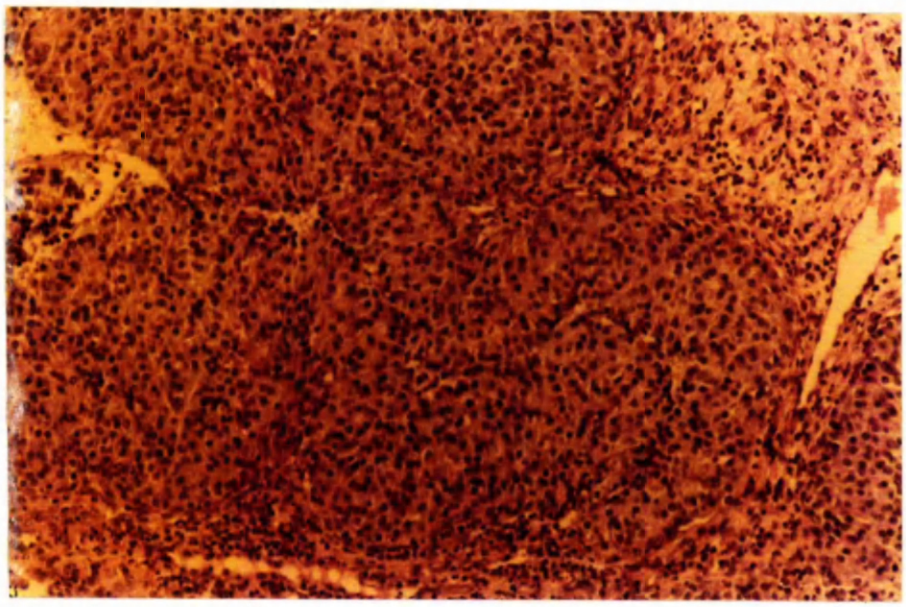
Histological sections of corpora lutea of the sheep.

Magnification: x 32. approx.

Stain: Haematoxylin and Eosin.

a) Section from a corpus luteum on Day 16 of oestrous cycle.

b) Section from a corpus luteum 16 days post coitum.



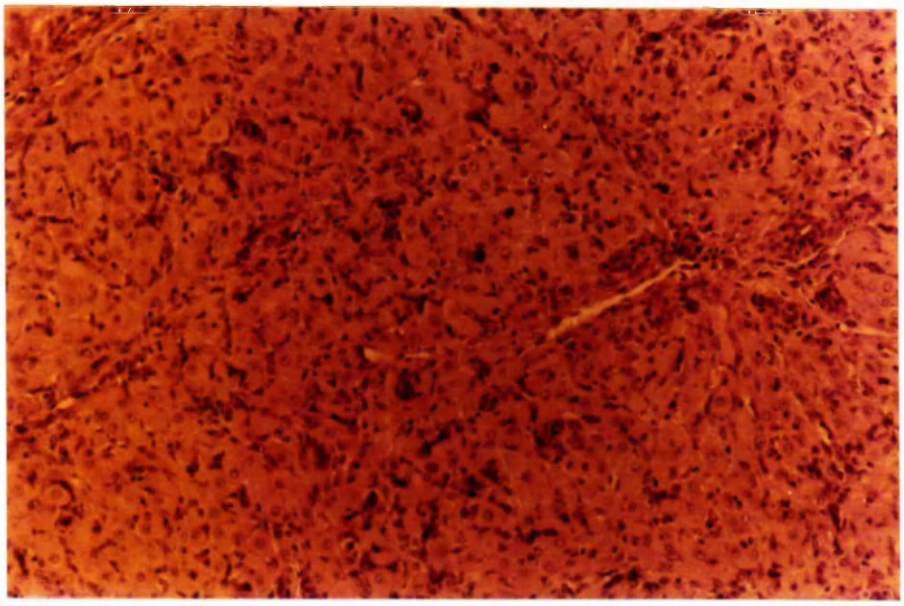


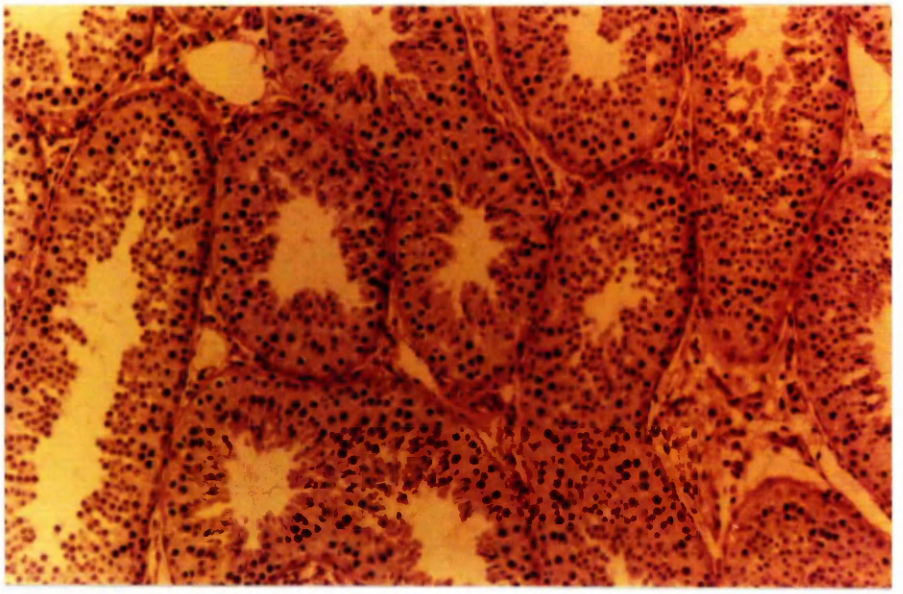
Fig. 19

Histological sections of the descended left testis of Ram $F_1$110.

Stain: Haematoxylin and Eosin.

a) Section through seminiferous tubultes.
Magnification approx. x 32.

b) Section of a seminiferous tubule at stage 8 of the seminiferous cycle.
Magnification approx. x 173.



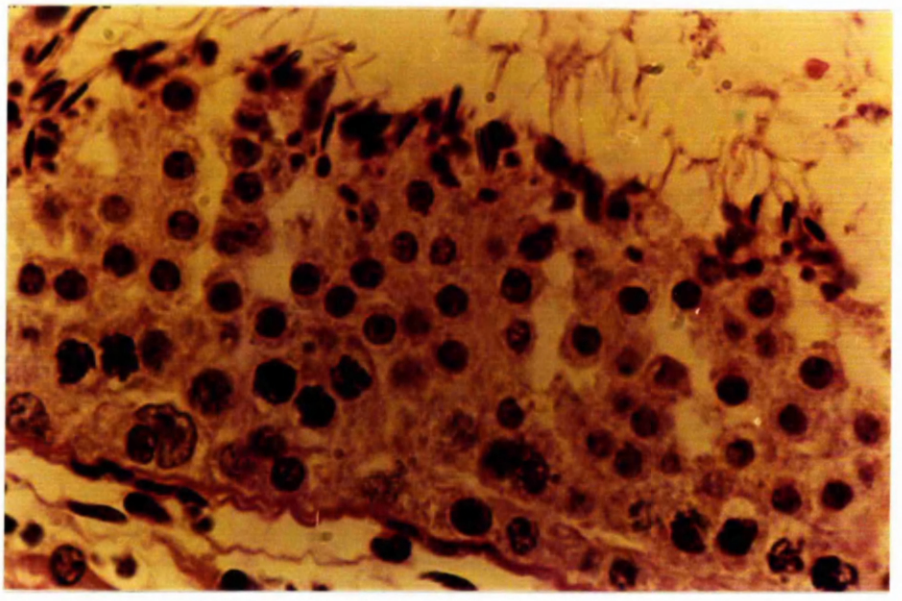


Fig. 20

a) Histological section of seminiferous tubules of the abdominal testis of Ram $F_1$110.

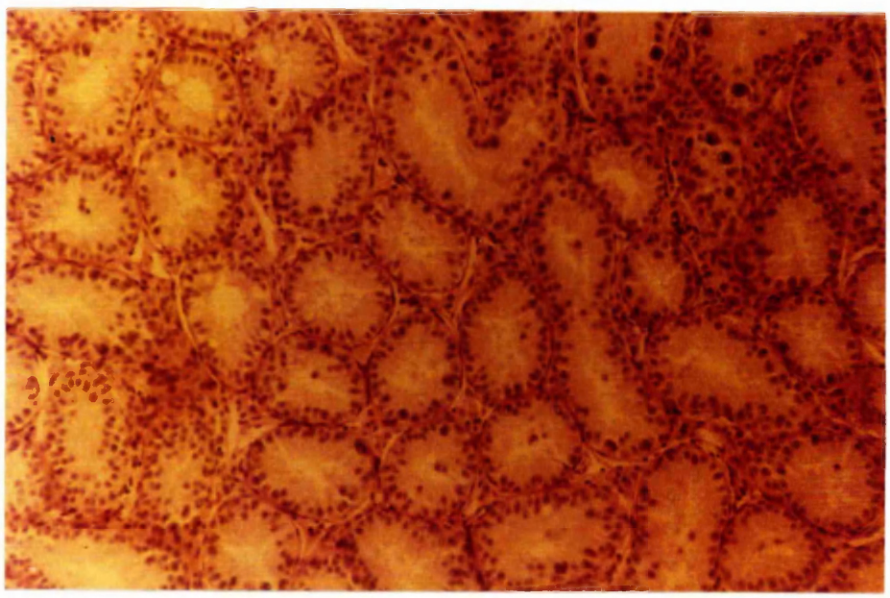
Magnification: x 32 approx.

Stain: Haematoxylin and Eosin.

b) Histological section of the cauda epididymis of the abdominal gonad of Ram $F_1$110.

Magnification: x 32 approx.

Stain: Haematoxylin and Eosin.



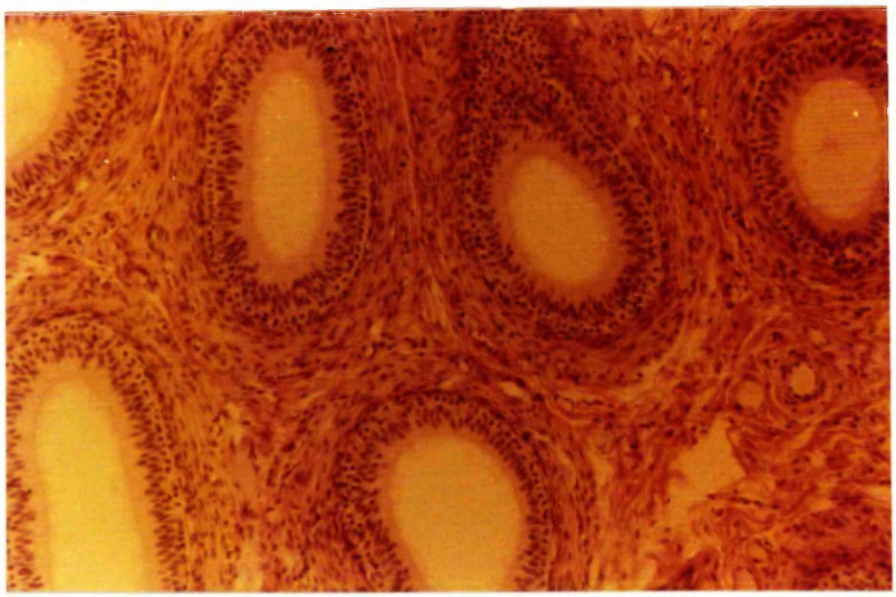


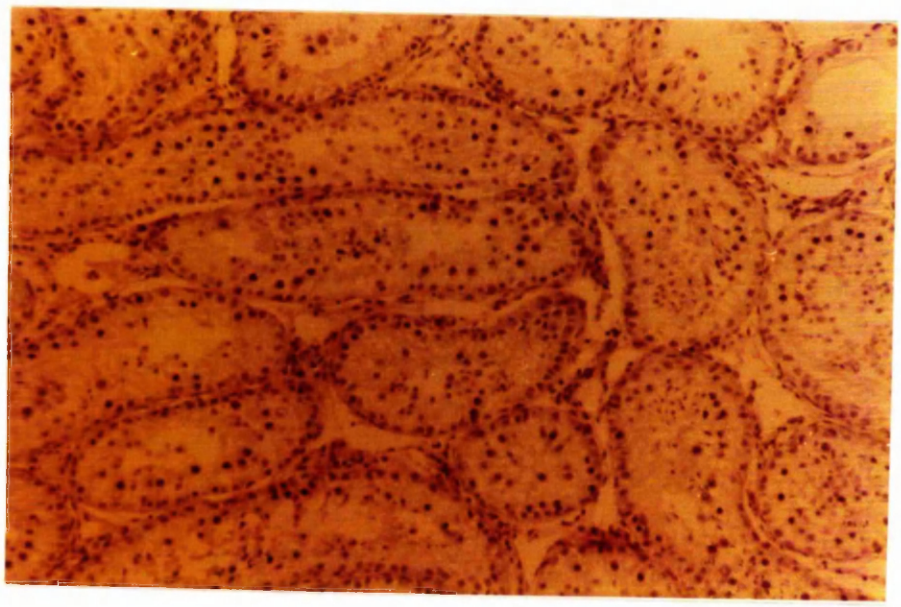
Fig. 21

Histological sections of the left testis of Ram $F_2$200, homozygous for the Massey I translocation.

Stain: Haematoxylin and Eosin.

a) Section through seminiferous tubules.
Magnification approx. x 32.

b) Section through seminiferous tubule.
Magnification approx. x 173.



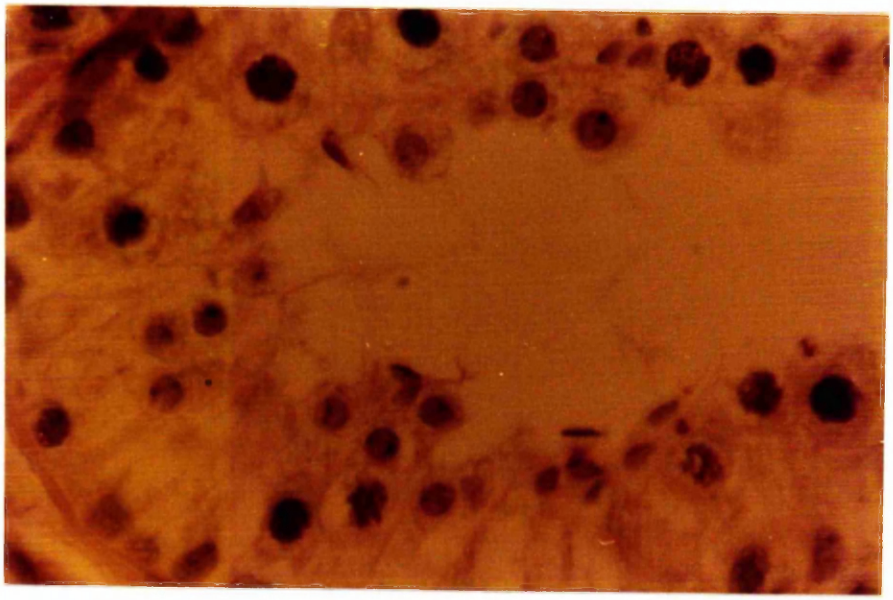


Fig. 22

Metaphase spread, containing the diploid number of $2n = 54xy$, from meiotic preparations of the left testis of Ram $F_2$199.

Note: Absence of chromatid repulsion and the general appearance of the spread similar to those of leucocyte cultures.

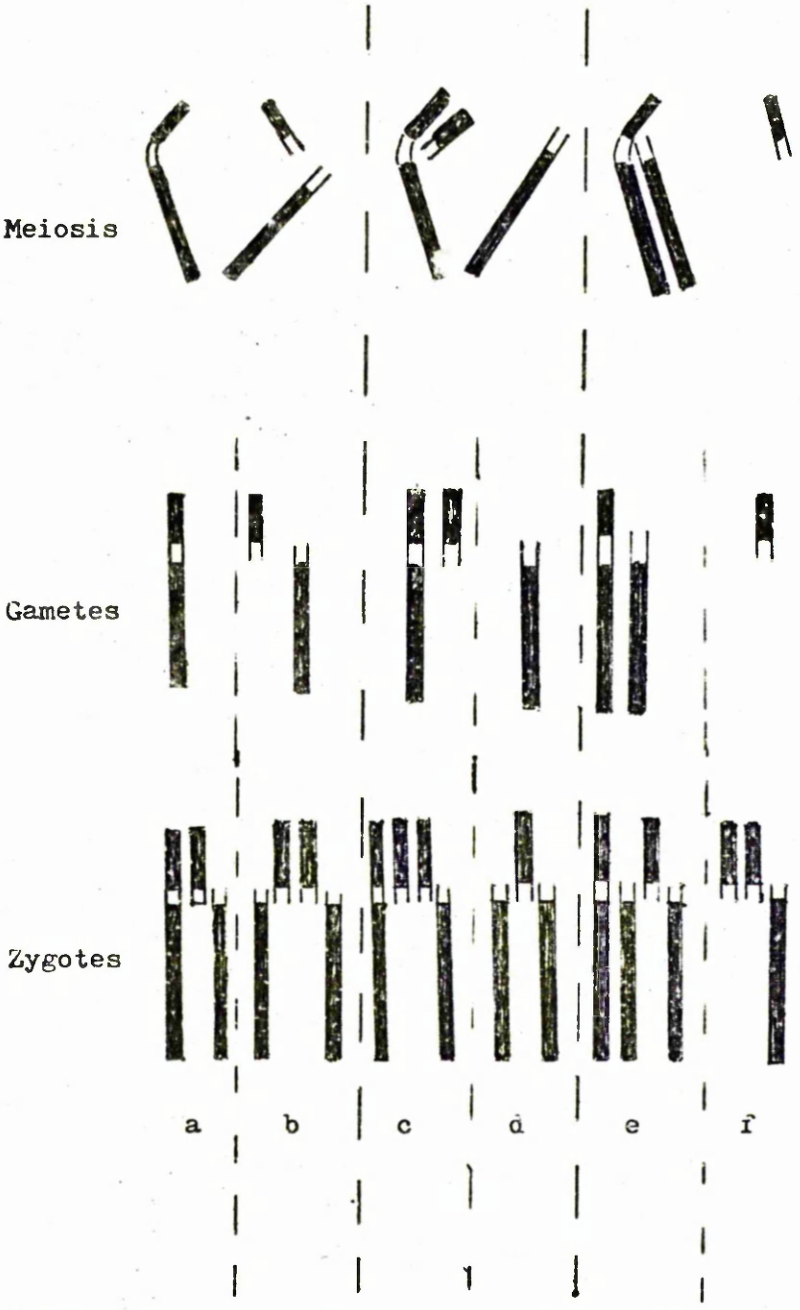
The origin of these chromosomes is presumed to have been a fibroblast cell or possibly a type A spermatogonium.

Fig. 23

Karyotype and spread of type B spermatogonium from Ram $F_2$49.

Note: The Massey I translocation (arrowed) and the marked chromatid repulsion.

Fig. 23a.



Types of segregation of chromosomes in the trivalent and the zygotic products after fertilisation with a normal gamete. (After Gustavsson, 1969.)

a. Balanced carrier, b. Normal, c. Trisomy, d. Monosomy, e. Trisomy, f. Monosomy.

Fig. 24

Pachytene figures in meiotic preparations from the left testis of Ram $F_1$49.

Note: The pycnotic sex vesicle (arrowed).

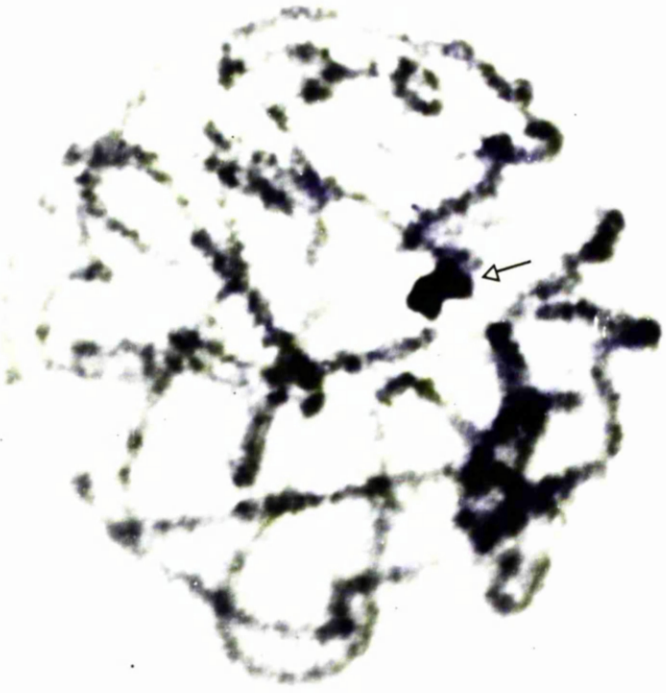


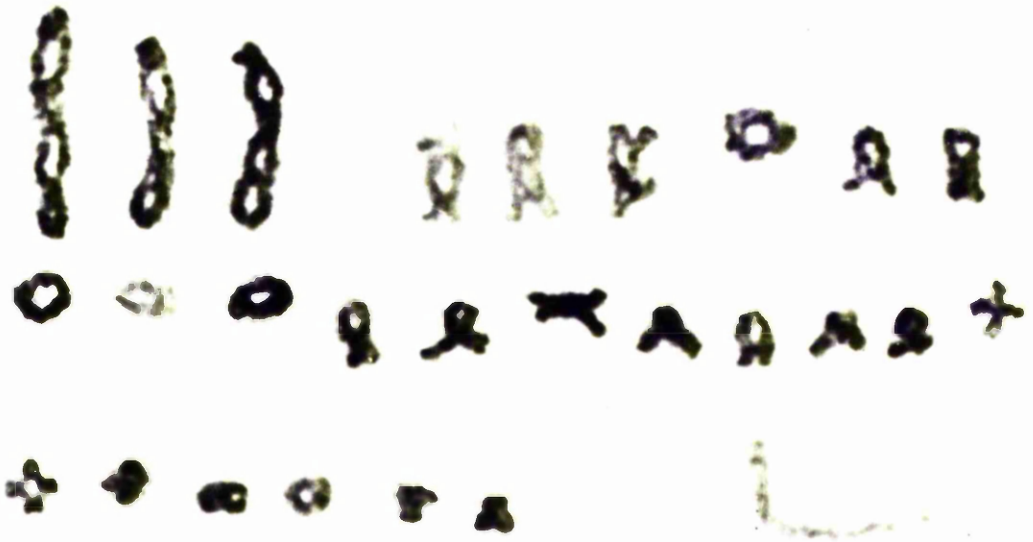
Fig. 25

Karyotype and spread of a cell at diakinesis from meiotic preparations of the left testis of Ram $F_2$199.

2n = 54xy

Note: Elongated and pale staining x/y bivalent. The individual chromatids are visible in some of the autosomal bivalents.

F1 199 DIAKINESIS 45/1 ♂♀.



X/Y BIVALENT.

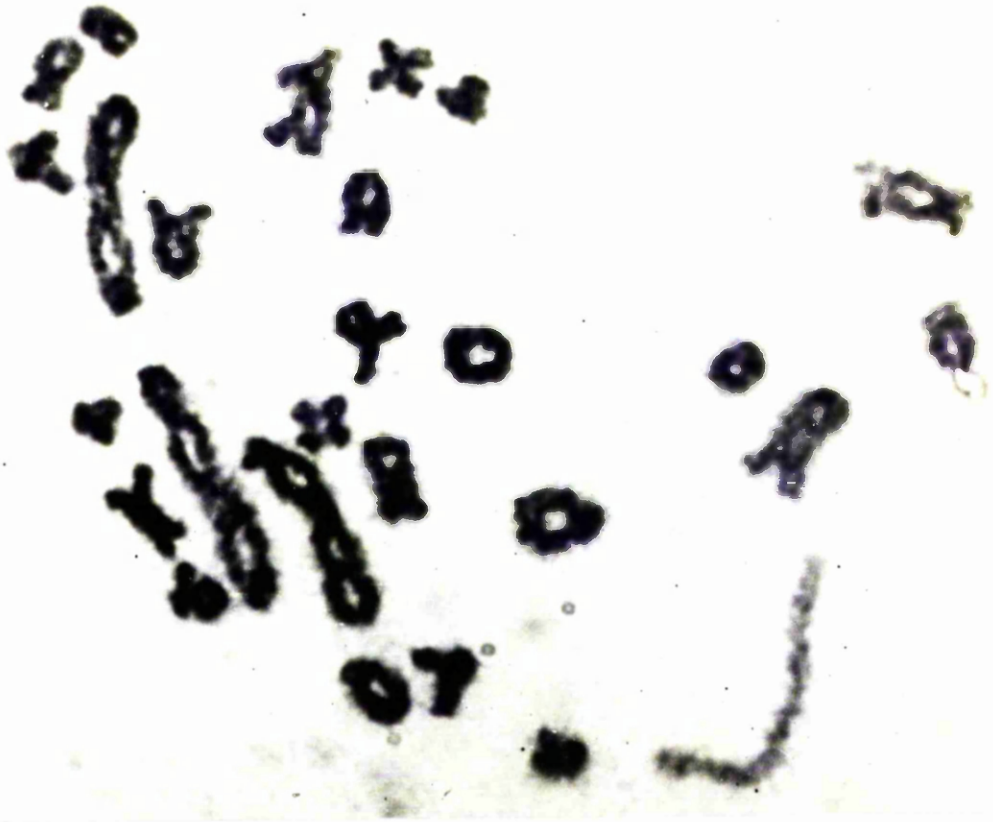


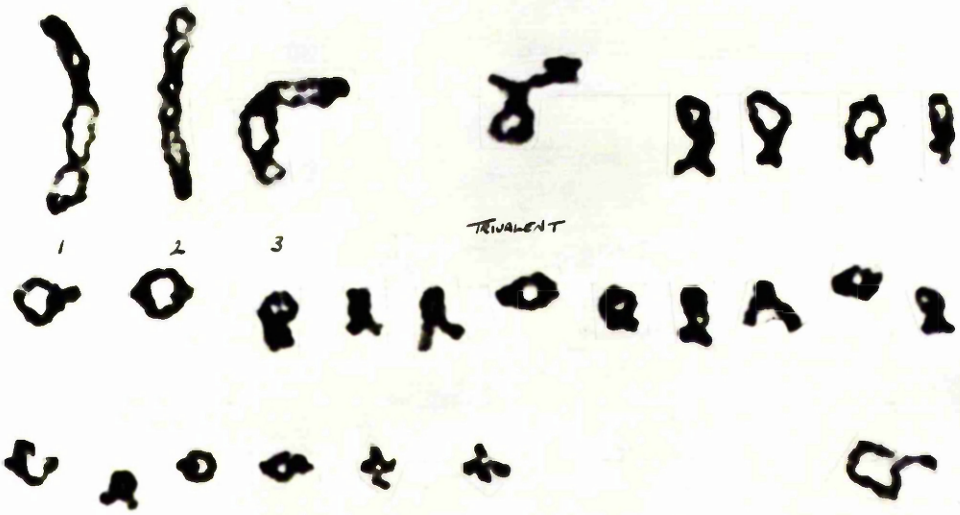
Fig. 26

Karyotype and spread of a cell at diakinesis from meiotic preparations of the left testis of Ram $F_1$49.

2n = 53xyT+

Note: Twenty-five bivalents and one trivalent.

F.49 DIAKINESIS MB/R 91-3/32-Y



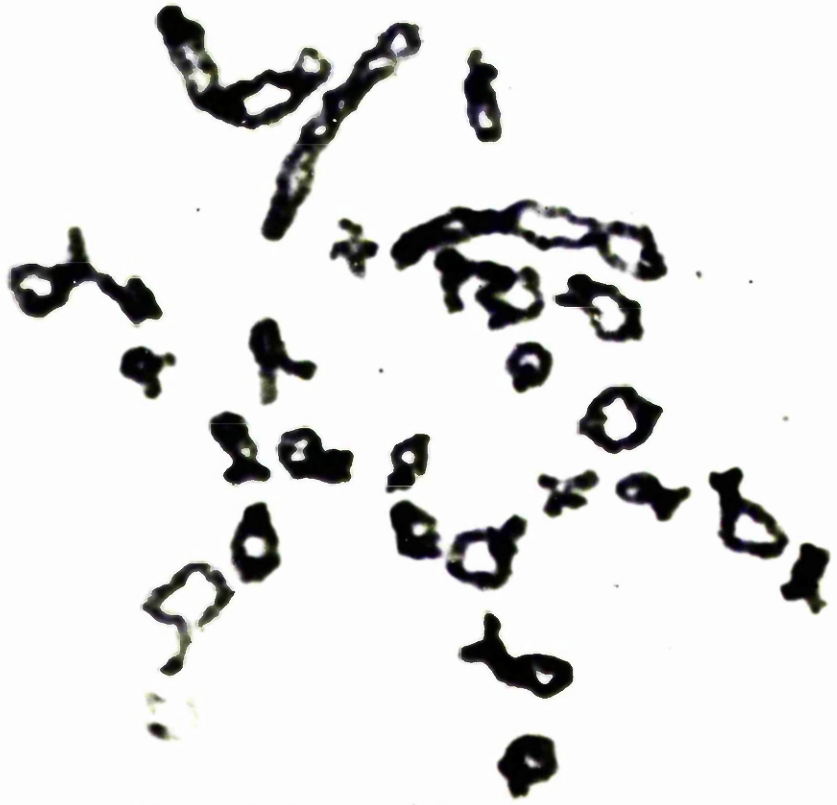


Fig. 27

Karyotype and spread of a cell at diakinesis from meiotic preparations of the left testis of Ram F_1 101.

2n = 53xyT+

Note: Twenty-five bivalents and one trivalent.
The individual chromatids are visible in a number of the bivalents and in the trivalent.

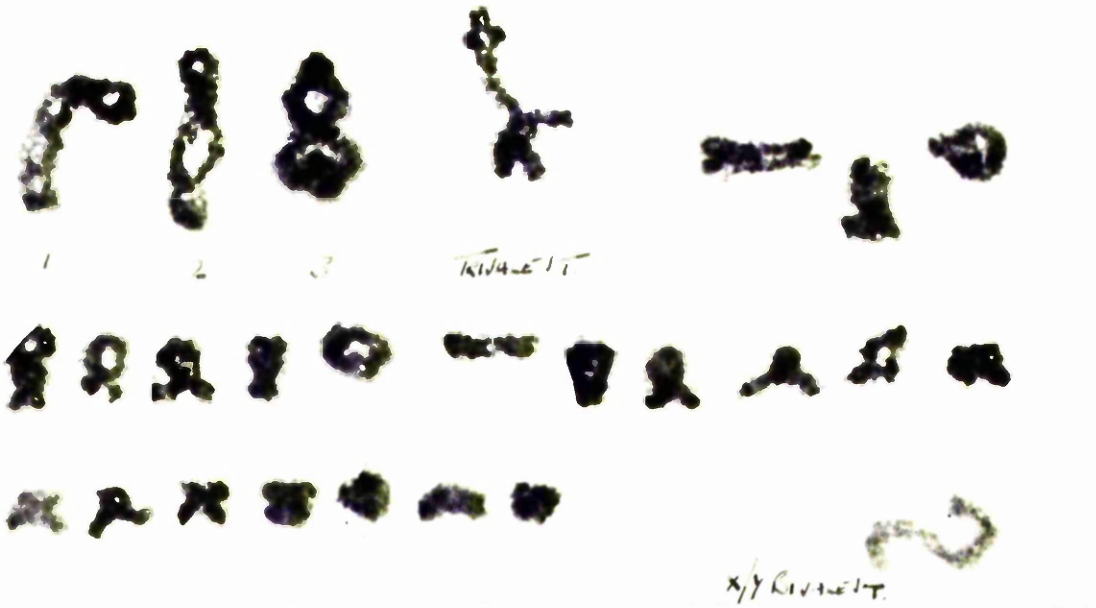
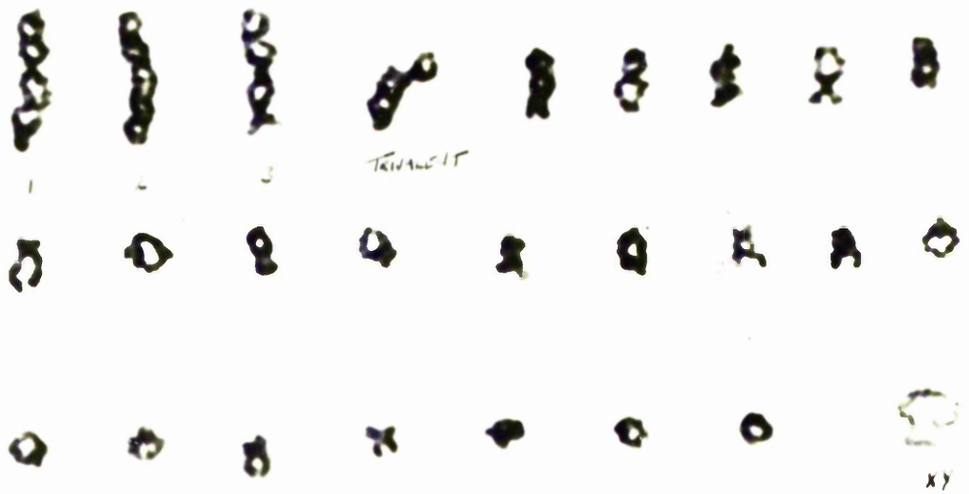


Fig. 28

Karyotype and spread of a cell at diakinesis from meiotic preparations of the left testis of Ram F_1 113.

2n = 53xyT+

Note: Twenty-five bivalents and one trivalent.


1
2
3
TRIVALENT
XY

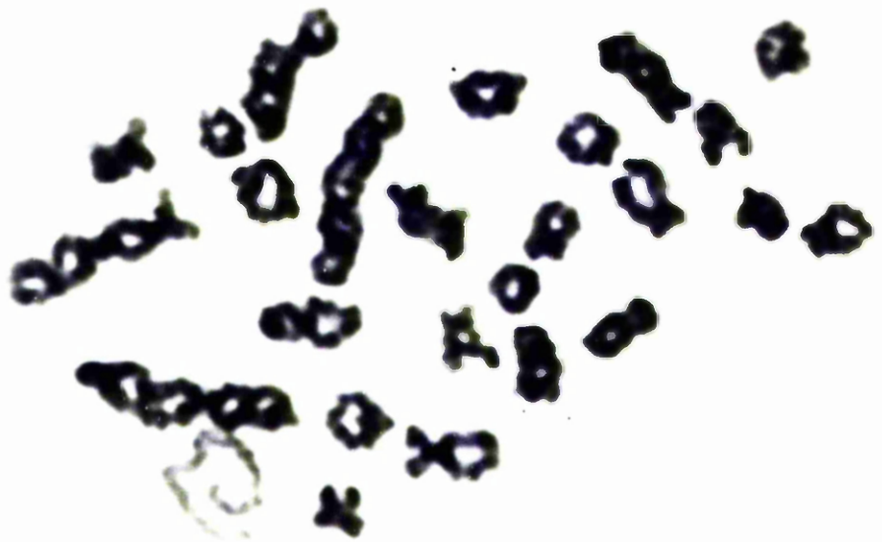


Fig. 29

Karyotype and spread of a cell at diakinesis from meiotic preparations of the left testis of Ram $F_2$200.

2n = 52xyT++

Note: Absence of a trivalent figure and the symmetric configuration of the translocation bivalent in the homozygous animal.

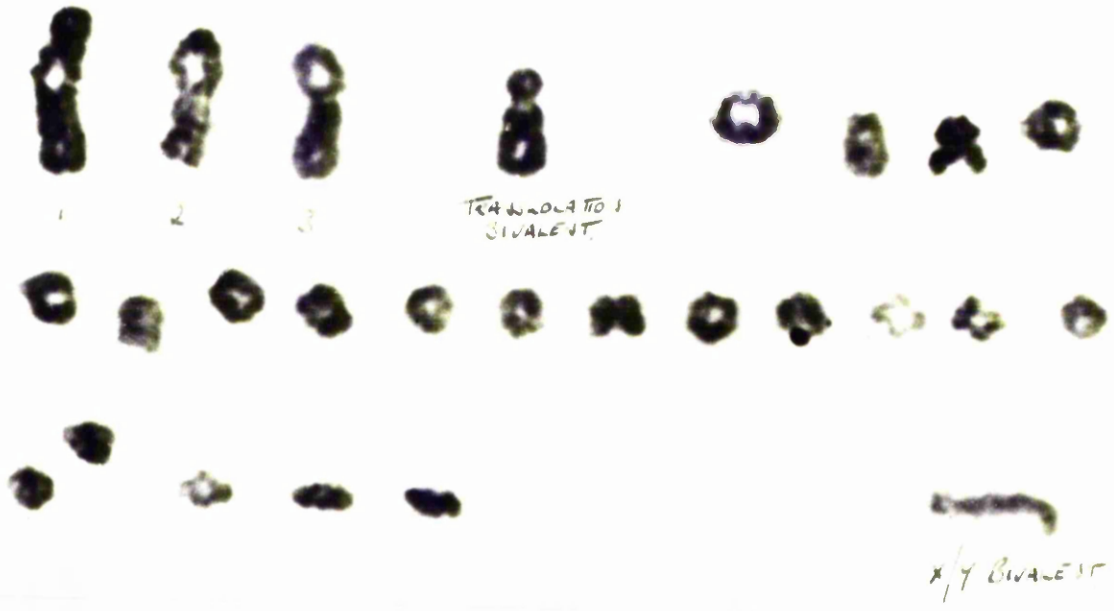
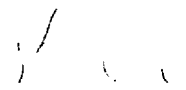
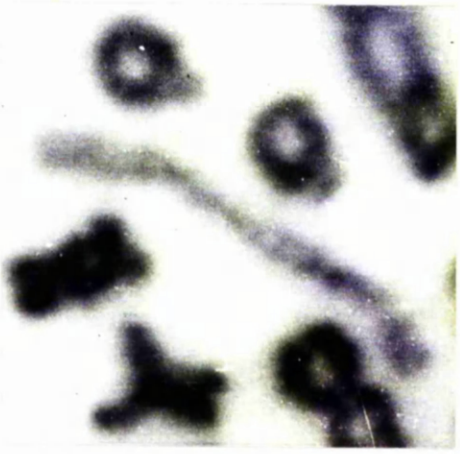

1
2
3
TRANSLOCATION
BIVALENT
X/Y BIVALENT

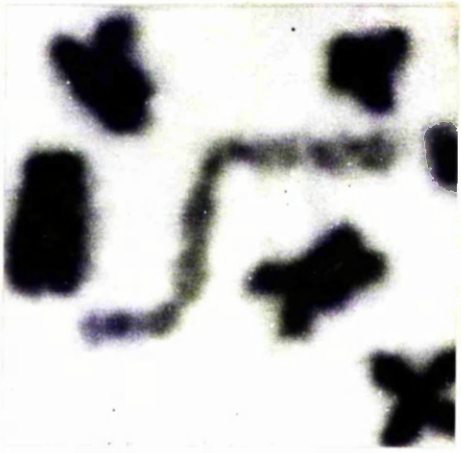
Fig. 30

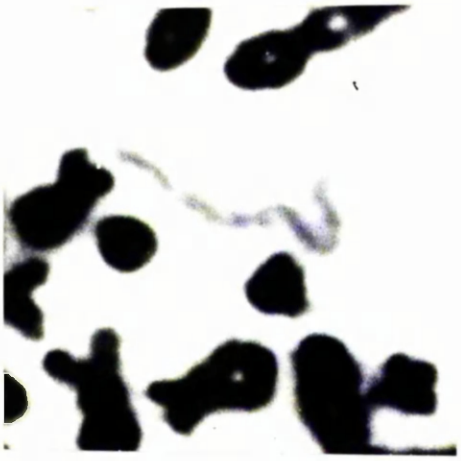


The x/y bivalent at diakinesis of male meiosis in the sheep.

Note: The elongated configuration of the x/y bivalent compared to the autosomal bivalents and its negative heteropycnosis.







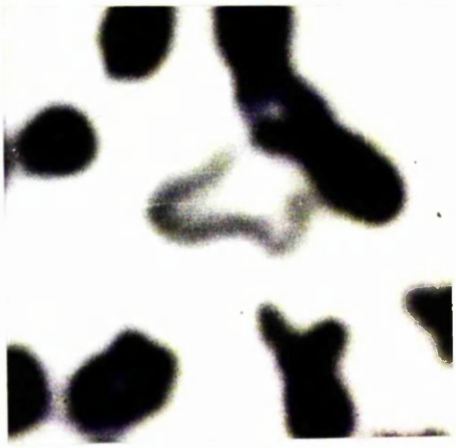


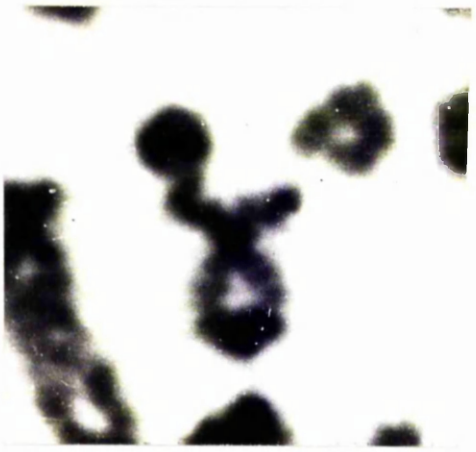
Fig. 31

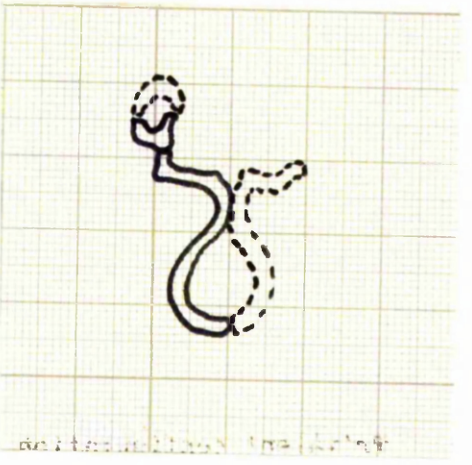
Trivalent configuration of the Massey I translocation at diakinesis and a diagramatic interpretation.

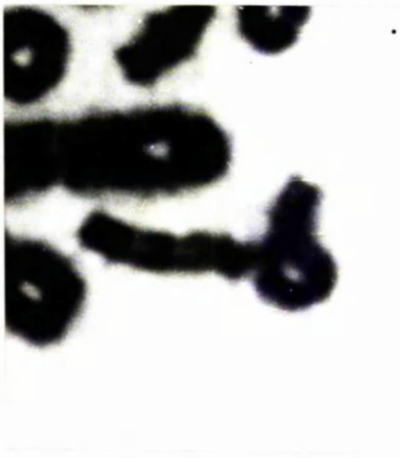
a) THREE ~~Four~~ chiasmata

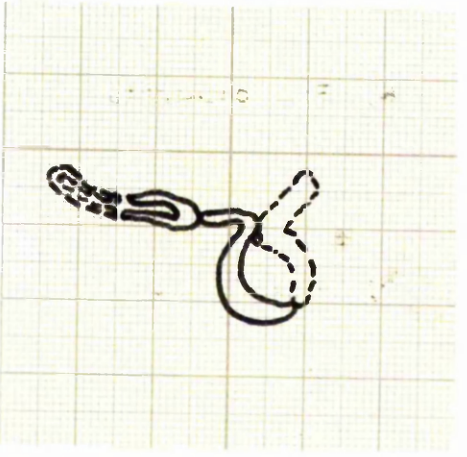
b) THREE ~~Four~~ chiasmata

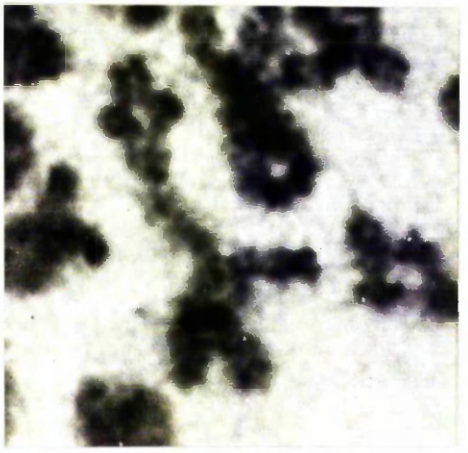
c) Two. ~~Three~~ chiasmata











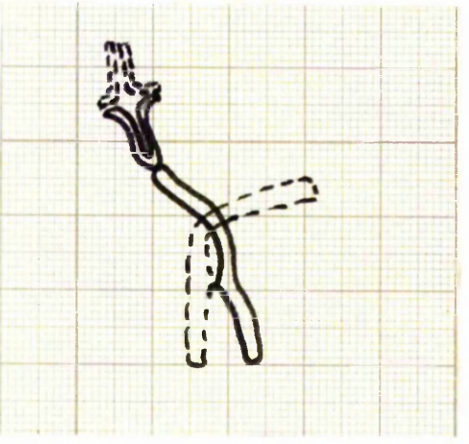


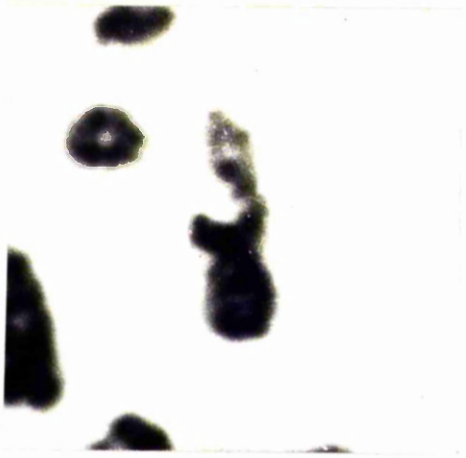
Fig. 32

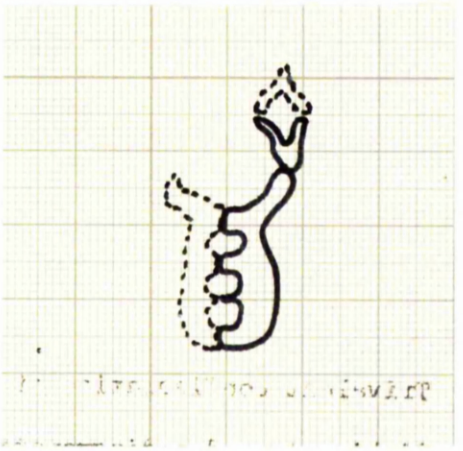
Trivalent configuration of the Massey I translocation at diakinesis and a diagramatic interpretation.

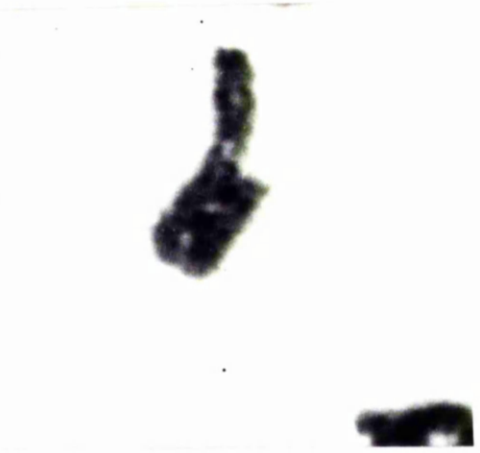
a) Six chiasmata

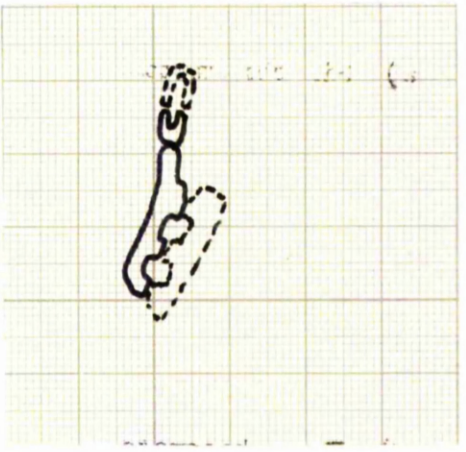
b) Five chiasmata

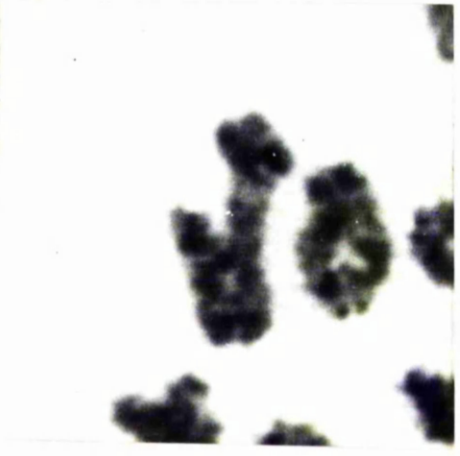
c) Four chiasmata











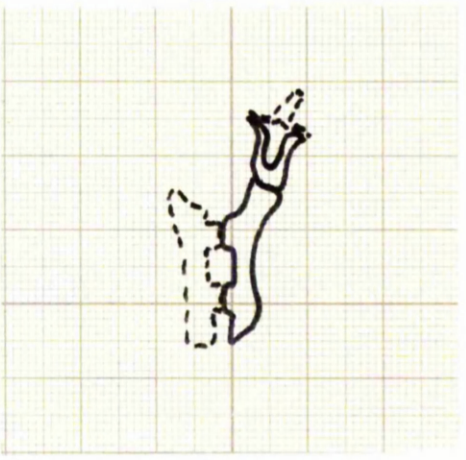


Fig. 33

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of Ram $F_1$113.

2n = 53xyT+

The cell contains the normal haploid number for the sheep of 26 autosomes and a sex chromosome. (x) No translocation chromosome is present.

This represents the type b segregation shown in Fig. 23a.

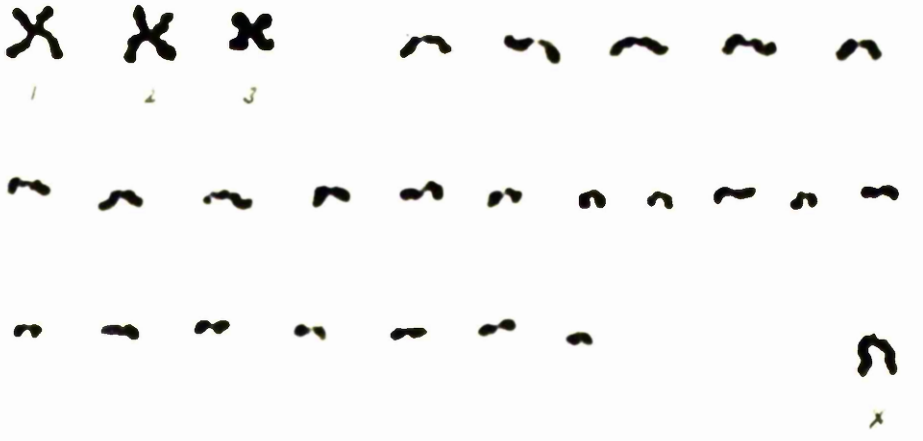

1
2
3
X

Fig. 34

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of Ram F_1 113.

2n = 53xyT+

There are 24 autosomes, a Massey I translocation chromosome and an X chromosome. The cell is a balanced translocation carrier and represents a type a segregation shown in Fig. 23a.

F 112 SELECTED METAPHASE #3 11C-2/270

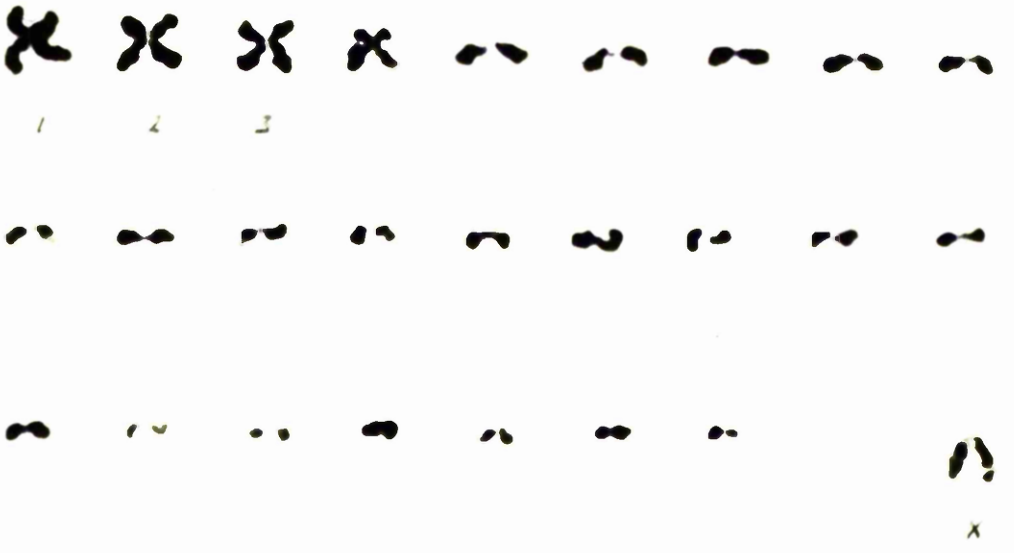


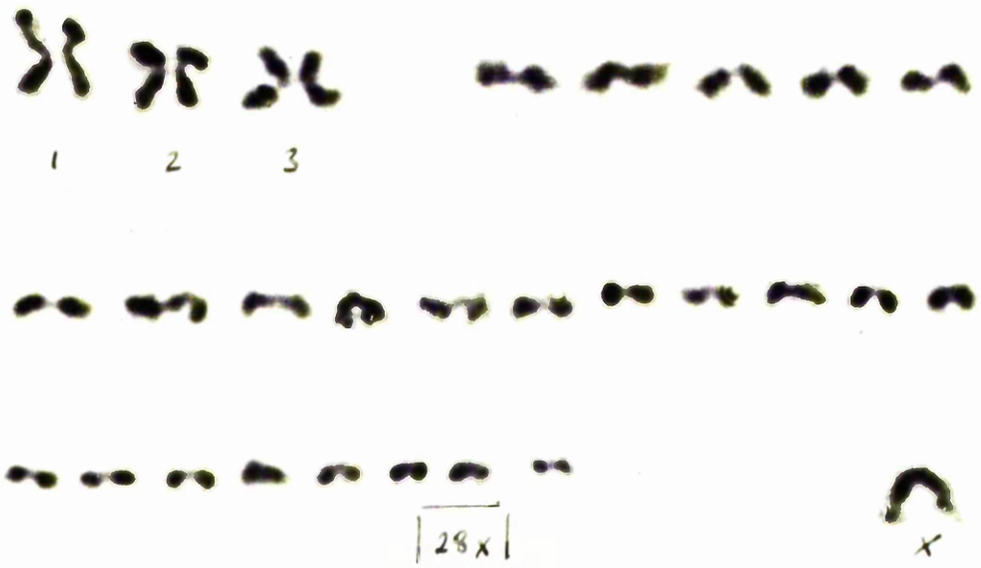
Fig. 35

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of Ram $F_2$199.

2n = 54xy

There are 27 autosomes and the X chromosome. The cell is presumed to be trisomic for one of the acrocentric autosomes.

F2 199 2nd Metaphase 48/3 750/1



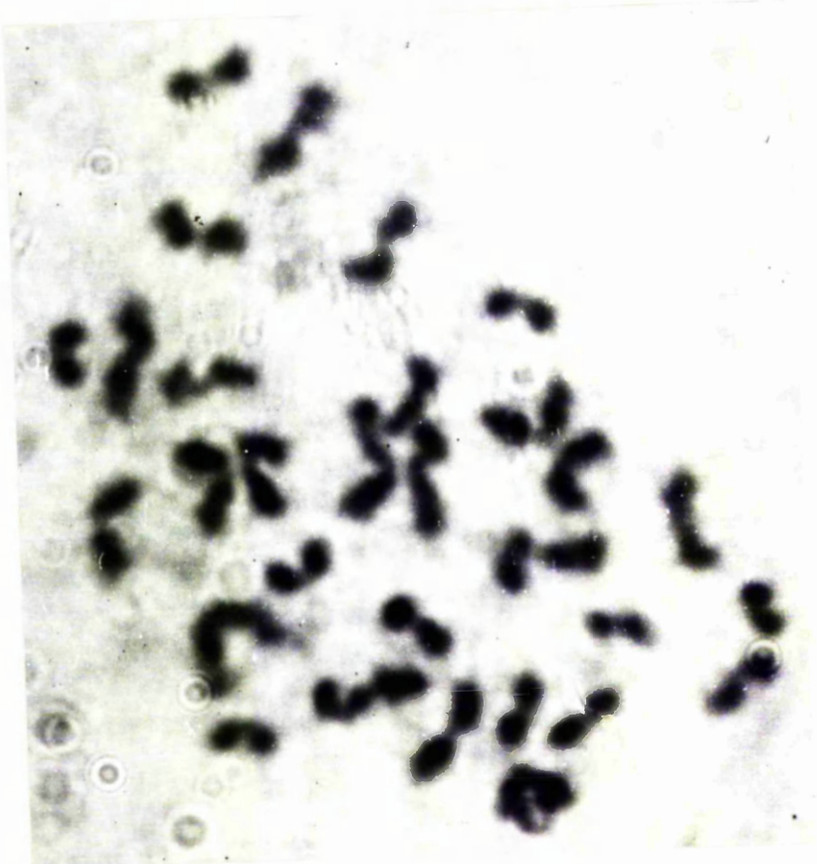


Fig. 36

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of Ram $F_2$199.

2n = 54xy

There are the normal haploid number of autosomes for the sheep of 26 but both an X and Y chromosome are present.

F2 199. 2nd Metaphase M4/2 K4Y

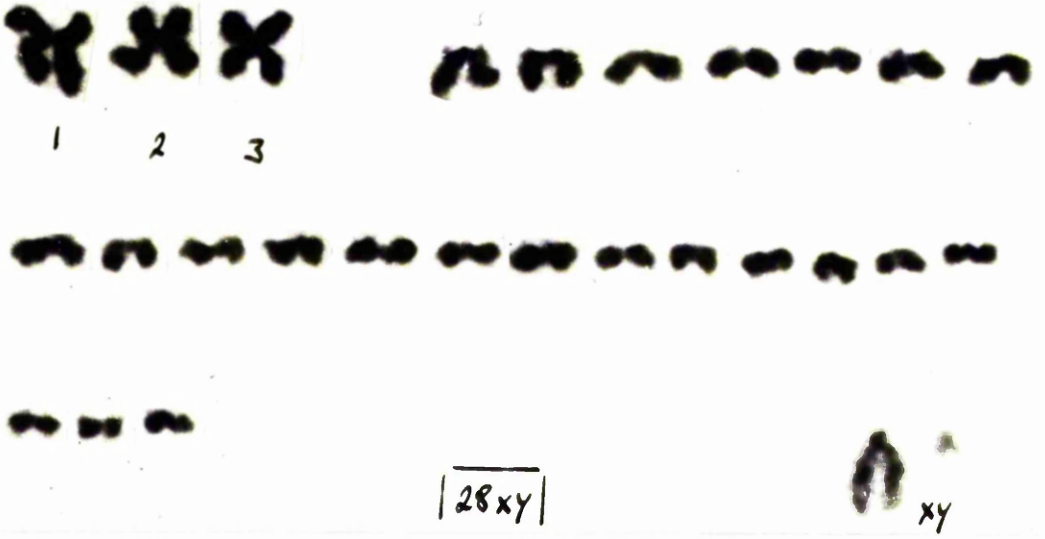


Fig. 37

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of Ram F_1 101.

2n = 53xyT+

There are 26 autosomes, including the Massey I translocation chromosome, plus the X chromosome so that

n = 27xT+

This cell is presumed to be trisomic for one of the acrocentric chromosomes involved in the Massey I translocation and represents segregation type c or e in Fig. 23a.

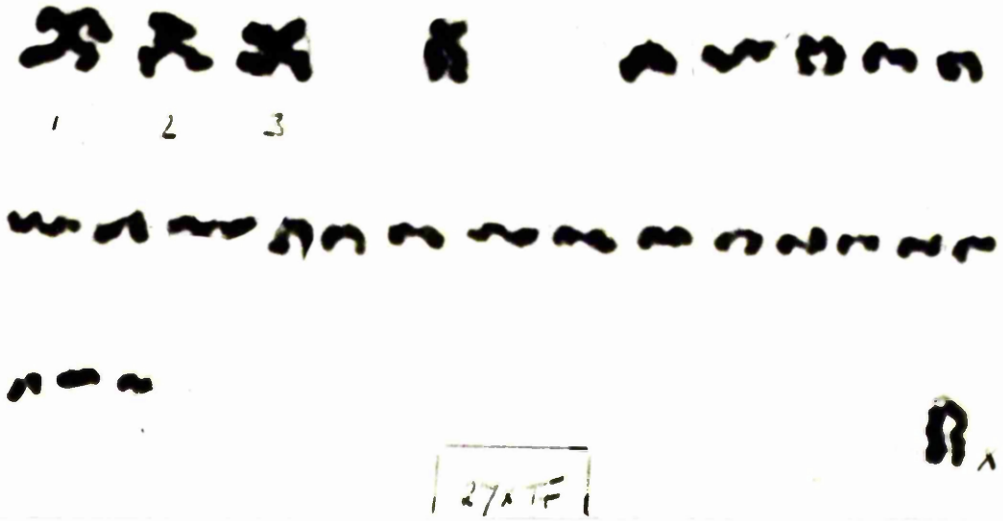

1
2
3

Fig. 38

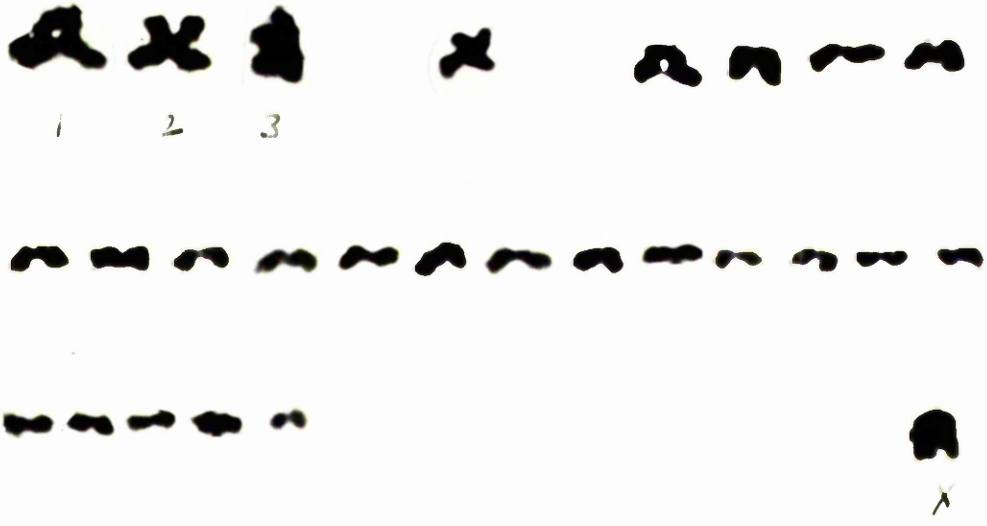
Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of Ram $F_1$49.

2n = 53xyT+

There are 26 autosomes, including the Massey I translocation chromosome, plus the X chromosome so that

n = 27xT+

This cell is presumed to be trisomic for one of the acrocentric chromosomes involved in the Massey I translocation and represents segregation type c or e in Fig. 23a.


1
2
3
X

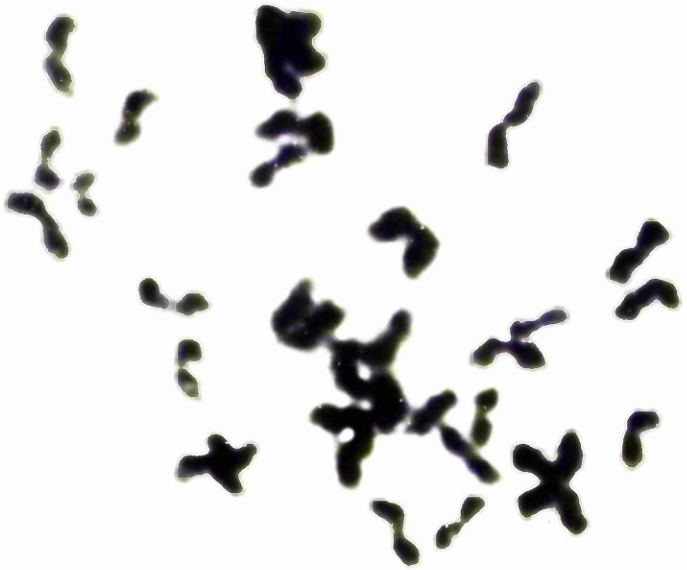


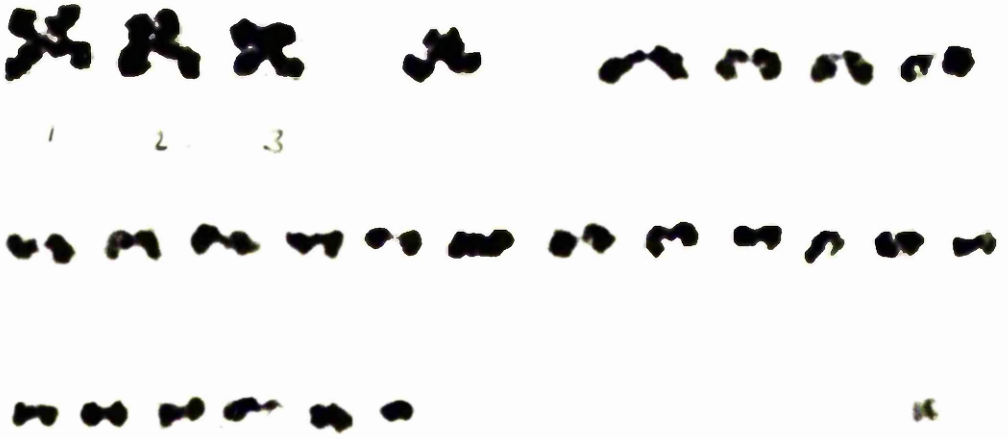
Fig. 39

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of ram $F_1$101.
2n = 53xyT+.

There are 27 autosomes, including the Massey I translocation chromosome, and a Y chromosome so that n = 27yT+.

This cell is presumed to be trisomic for one of the acrocentric chromosomes involved in the Massey I translocation and represents segregation type c or e in Fig. 23a.

F1 101 M 2nd Metaphase 3/2 47. 8 378.



24 TF

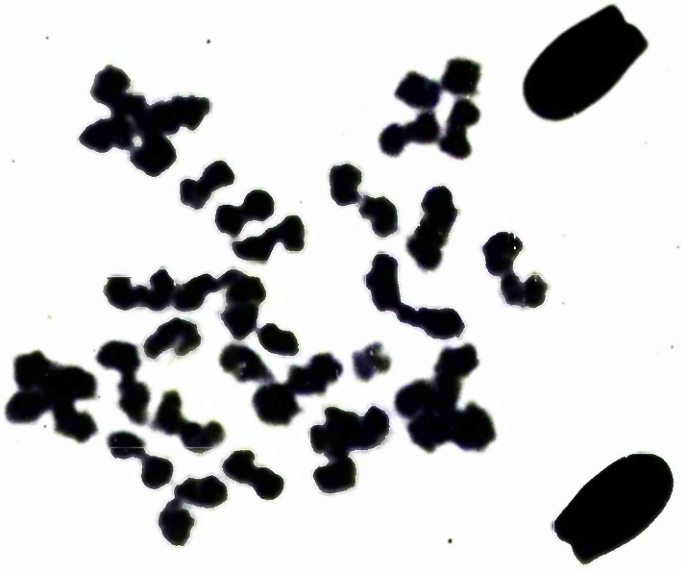
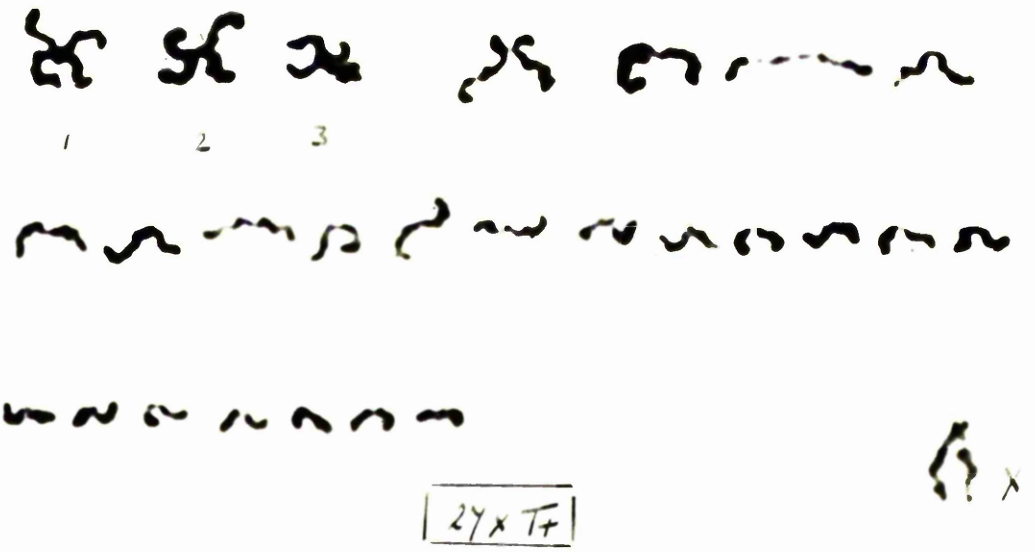


Fig. 40

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of ram $F_1$101. 2n = 53xyT+.

There are 27 autosomes, including the Massey I translocation chromosome and an X chromosome so that n = 27xT+.

This cell is presumed to be trisomic for one of the acrocentric chromosomes involved in the Massey I translocation and represents segregation type c or e in Fig. 23a.



27 x T+

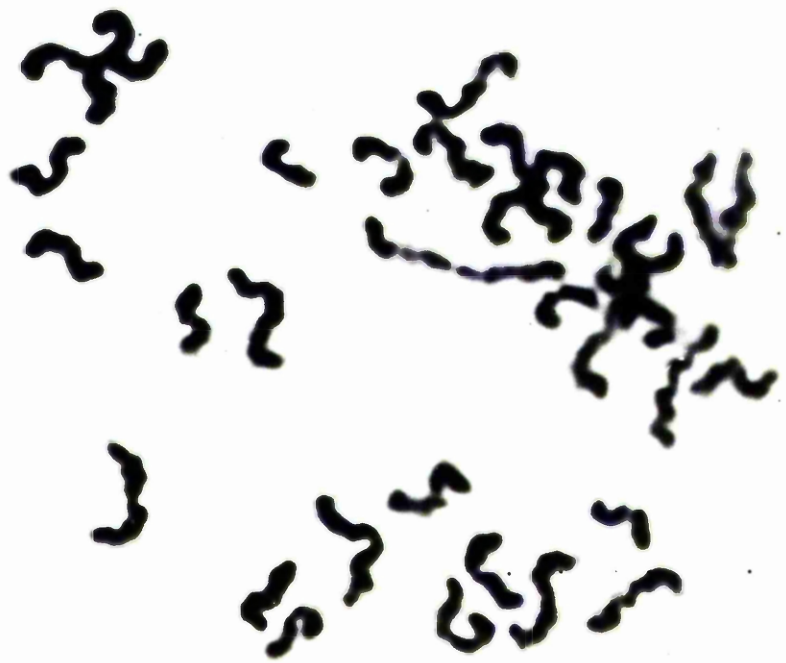


Fig. 41

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of ram $F_1$101.

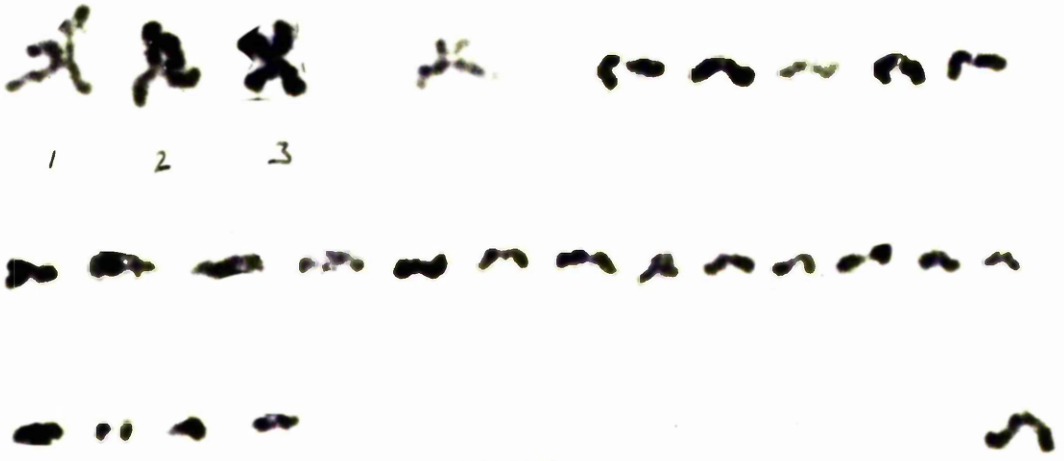
2n = 53xyT+.

There are 27 autosomes, including the Massey I translocation chromosome and an X chromosome so that

n = 27xT+.

This cell is presumed to be trisomic for one of the chromosomes involved in the Massey I translocation and represents segregation type c or e in Fig. 23a.

F, 101 M 2nd METAPHASE 3/5 112·3/40·5



27 F, X.

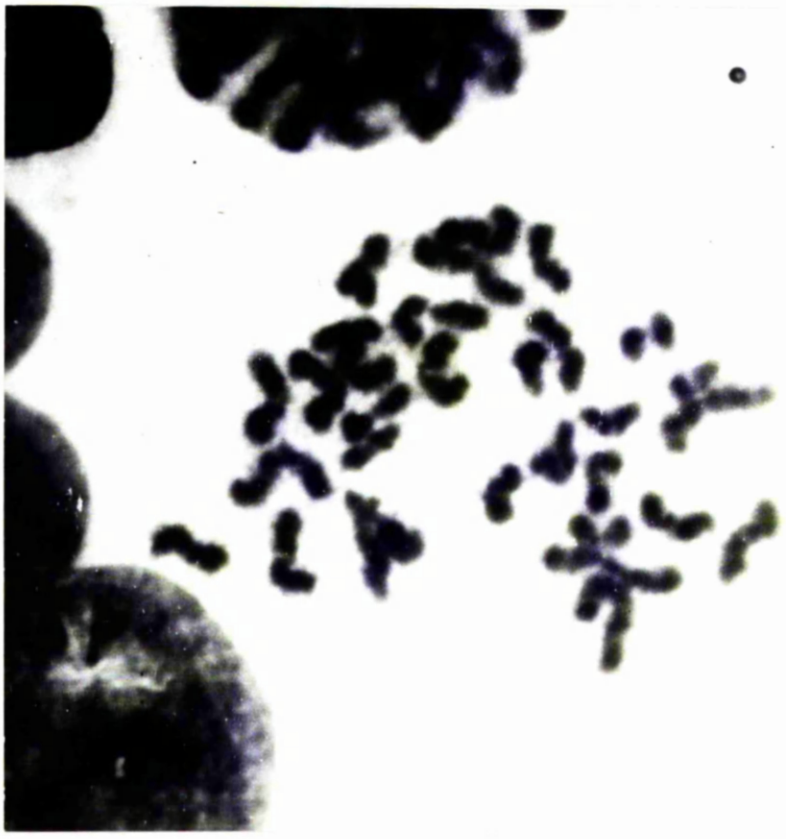


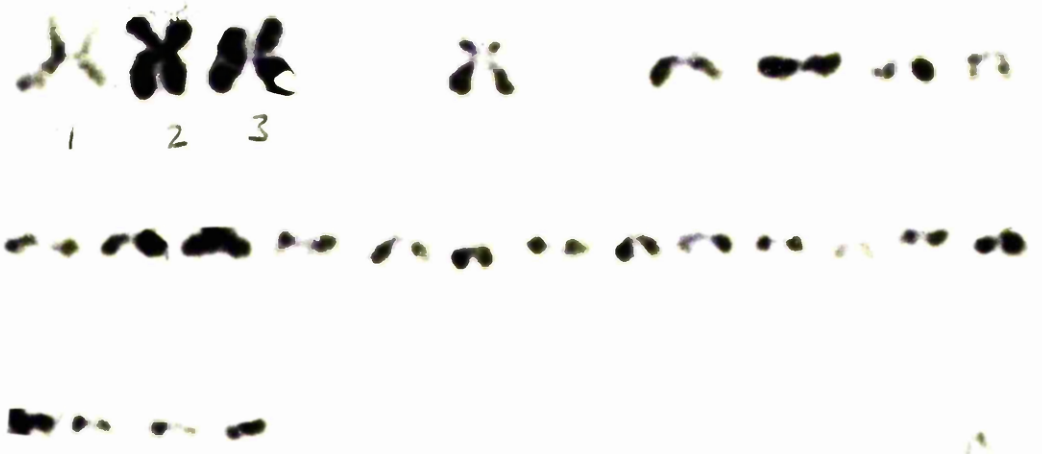
Fig. 42

Karyotype and spread of a cell at second metaphase from meiotic preparations of the left testis of ram $F_2$200.
2n = 52xyT++.

There are 26 autosomes, including the Massey I translocation chromosome and an X chromosome so that
n = 26xT+.

This cell is a balanced translocation carrier.

F_2 200 2ND METAPHASE M2/5 Kbw/4.



126x Tf

X

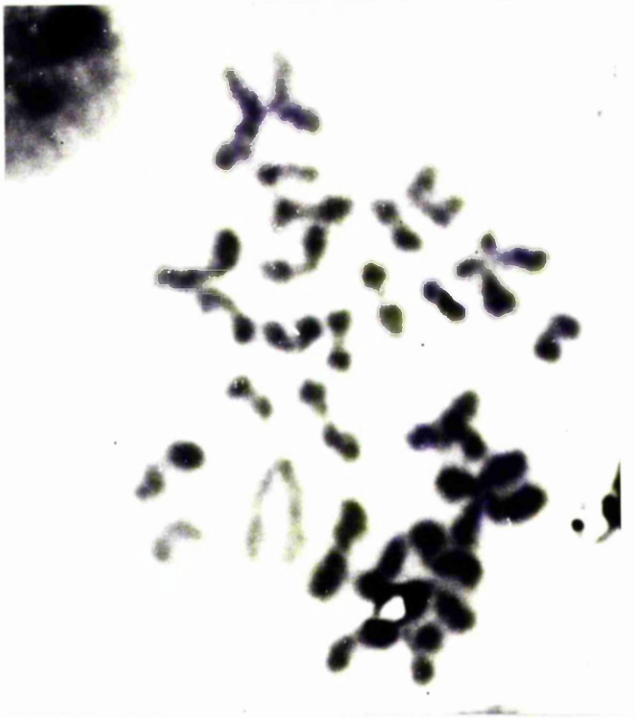
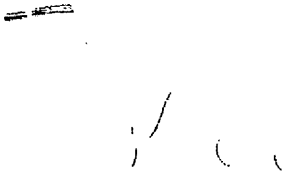


Fig. 43



Right gonad of ram $F_2$200 showing the adhesion of the tunica vaginalis to the cauda epididymis.

The scale at the bottom is a centimetre rule.

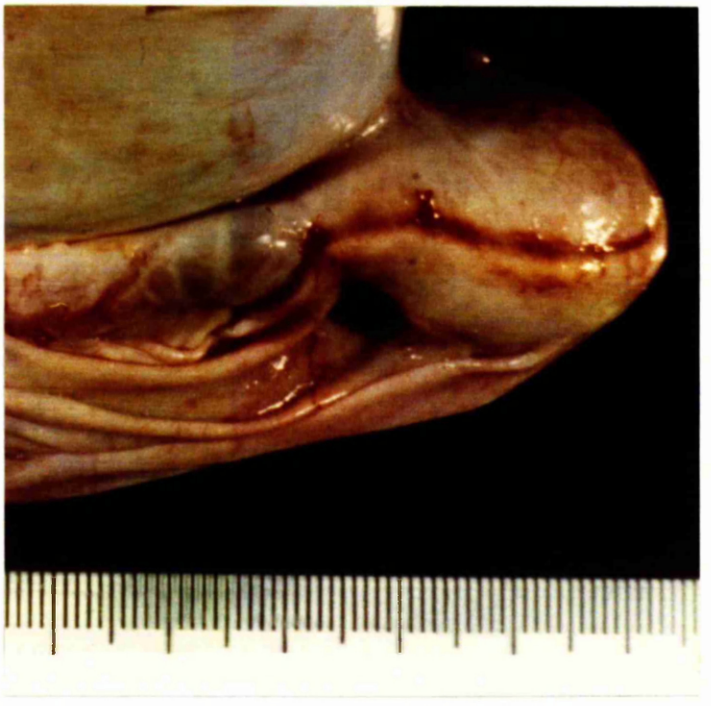
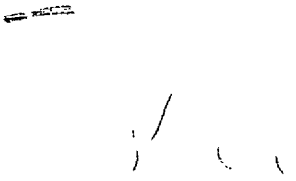


Fig. 44



Karyotype and spread of a cell at mitotic metaphase from a leucocyte culture from ram 6169. 2n = 53xyT+.

The air dried preparations have been treated with trypsin and stained with giemsa to produce G-bands.

6169

TRYPSIN BANDING

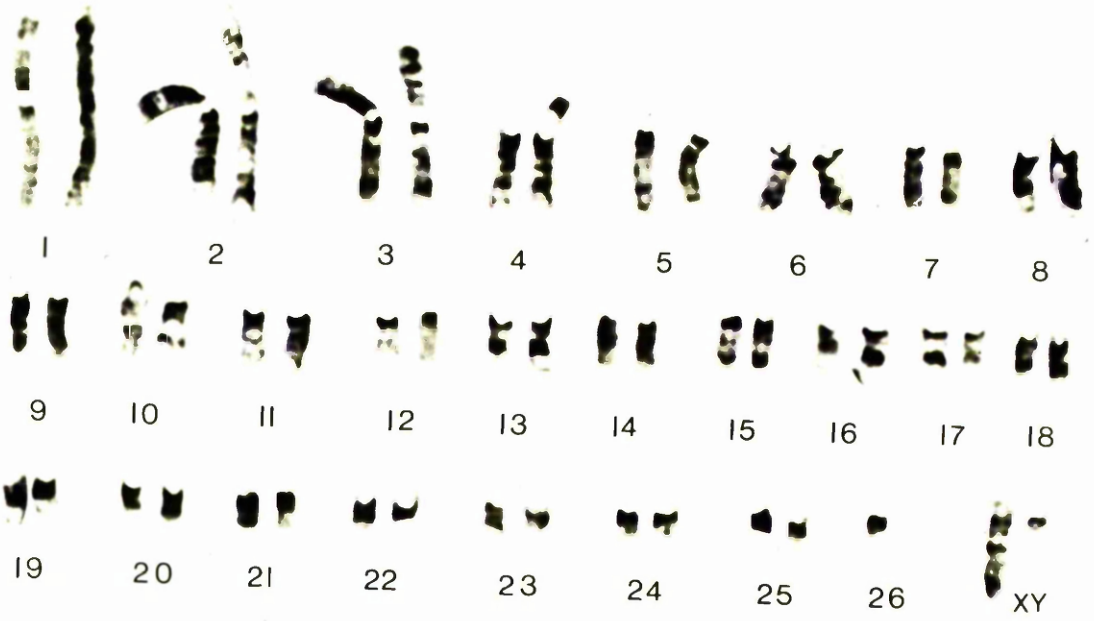


Fig. 45

G - BANDS. SHEEP CHROMOSOMES.

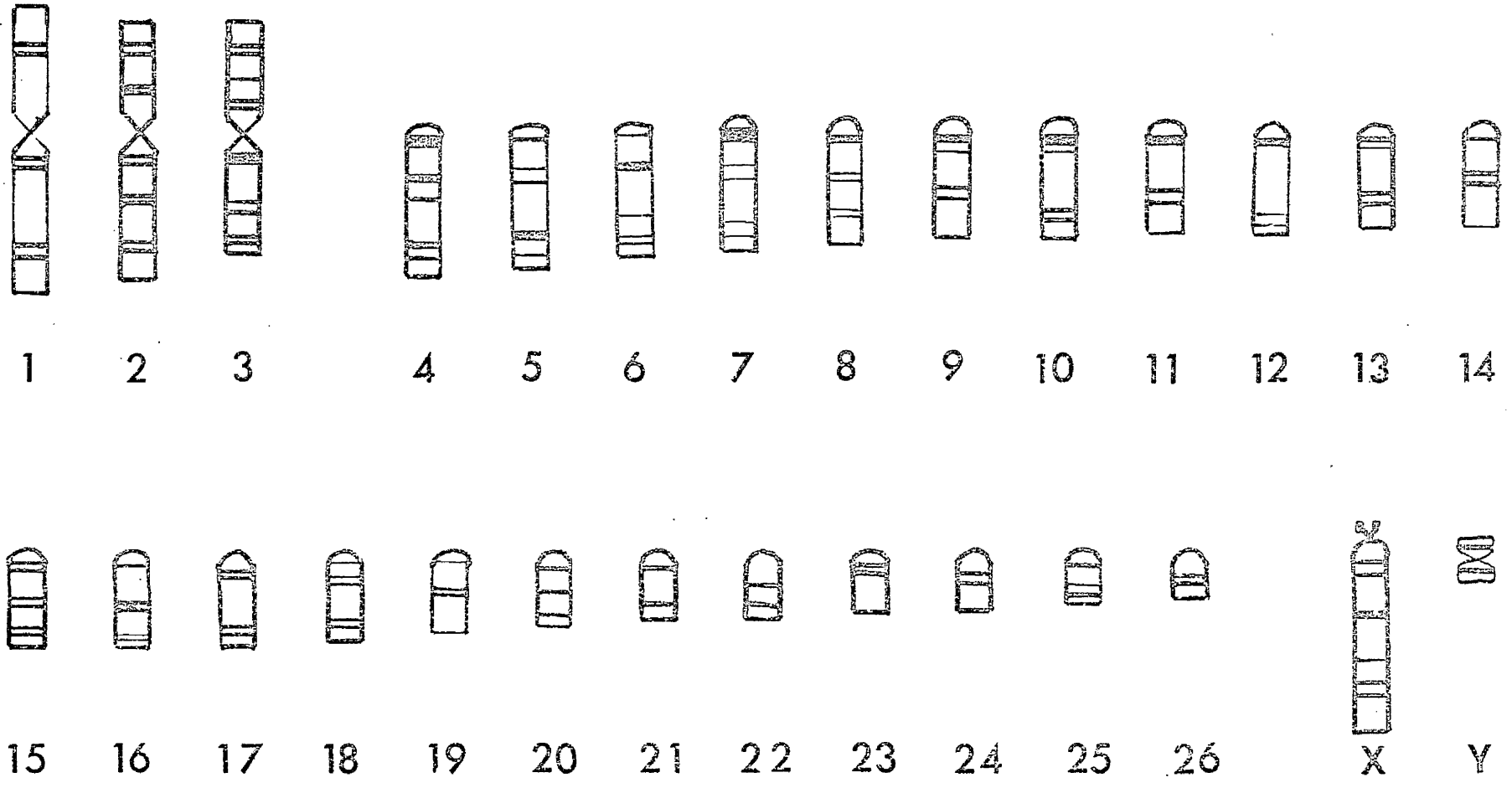


Fig. 46

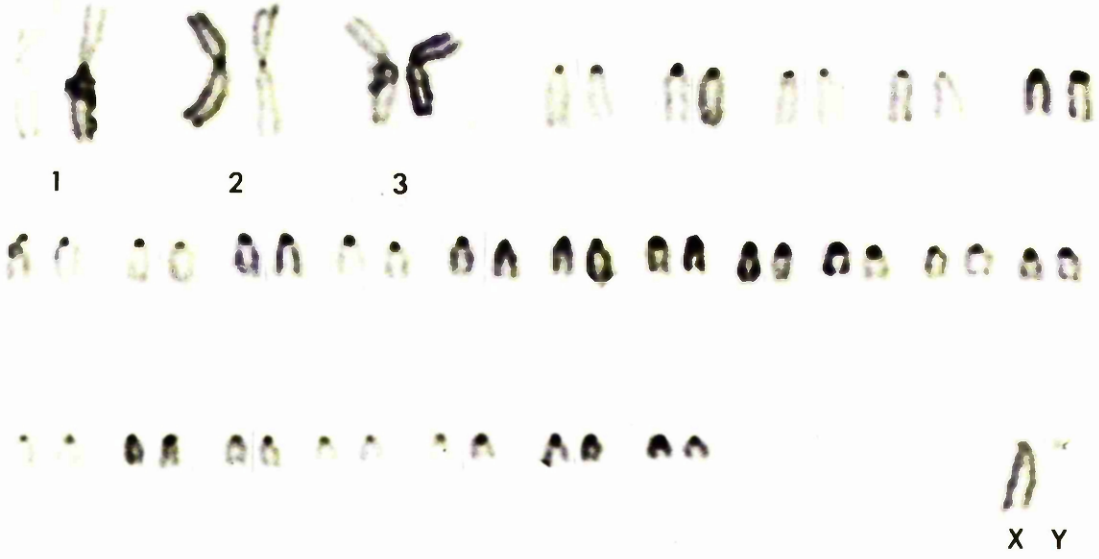
a) Karyotype of a cell at mitotic metaphase from a leucocyte culture from ram $F_2$199, treated with HCl and BaOH to produce C - bands.

Note: The absence of centromeric heterochromatin on the X and Y chromosome.

b) Comparison of C - banding in chromosomes 1,2,3 and X in two different cells. In the top line the chromosomes are lightly stained. In the bottom line they are heavily stained.

Note: Chromosome No. 2 is markedly heavier stained than Nos. 1 and 3.

C Banding.



C Banding.

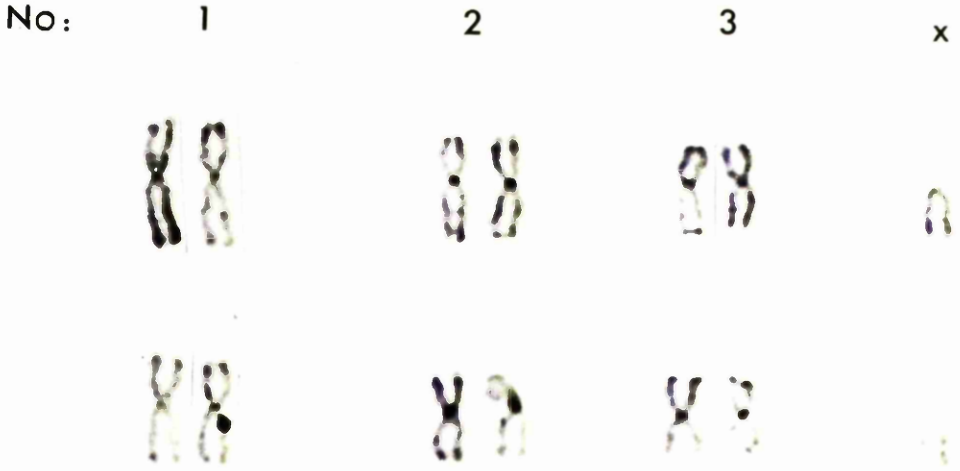


Fig. 47

C - banding of the Massey I translocation.

a) Three pairs of Massey I translocation chromosomes from three different cells of ram $F_2$200. 2n = 52xyT++.

b) Karyotype of a cell from ram $F_2$200 with C - banding.

C Banding.

Massey 1 Translocation.