THE CHROMOSOMES OF SHEEP × GOAT HYBRIDS*

By P. L. T. Ilbery,† G. Alexander,‡ and D. Williams‡

Hybrid foetuses carried by female goats after mating with male sheep usually die within 2 months of conception (see Alexander, Williams, and Bailey 1967). Since the chromosome number of 54 in the sheep (Makino 1943; Borland 1964; see Plate 1, Fig. 2, for illustration) differs from the 60 in the goat (Makino 1943; Basrur and Coubrough 1964), it is possible that death of these hybrids is due to the development of cell lines with abnormal numbers of chromosomes resulting from mitotic non-disjunction. Berry (1938) could find no evidence of chromosomal non-disjunction in sheep × goat hybrids. However, his display of chromosomes was considerably less clear than those produced by modern methods, and this led to the study by Buttle and Hancock (1966). They reported variations of 53–58 chromosomes per cell in cultures of a minced hybrid embryo, and concluded that it was not possible, on the available evidence, to reject the possibility that death was due to chromosomal non-disjunction; only 5 of 14 cells contained 57 chromosomes. Subsequently, Hancock and Jacobs (1966), using uncultured cells, found fewer departures from the expected number of 57 chromosomes in the hybrid embryos than in the previous study, and fewer departures (10 in 135 cells) in the hybrids, than in sheep and goat embryos (6 in 30 and 6 in 23 cells, respectively).

In the present paper we present confirmatory evidence, based on both cultured and uncultured cells, that the chromosome number of the hybrid is consistently 57, as originally concluded by Berry (1938), despite his unsophisticated methods, and recently by Hancock and Jacobs (1966). A brief description of a chromosomal abnormality that appeared after prolonged culture is also included.

Source of Material

Details of the mating of the goats and of the collection of the foetuses are presented elsewhere, together with a description of the foetuses (Alexander, Williams, and Bailey 1967).

Preparation of Chromosomal Displays

(i) *Direct Processing.*—Samples of liver were taken from the individuals in two sets of twin hybrid foetuses, newly delivered at 59 and 61 days after the mothers (Nos. 28 and 49 respectively) had been mated; liver samples in the second set were

* Manuscript received June 13, 1967.

† Department of Preventive Medicine, University of Sydney.

‡ Division of Animal Physiology, CSIRO, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

pooled. Following the method of Ilbery and Ahmad (1965), the mothers had been injected intravenously, 2 hr before Caesarian section, with 10 mg of the spindle poison, vinblastine sulphate (Velbe, Lilly). Chromosome displays were then prepared by the method summarized as follows: the foetal liver was cut into small pieces in 3·8% sodium citrate for transport to the laboratory. The tissue was teased out into a fine suspension 1 hr later and the cells were gently centrifuged down and resuspended in 0·7% citrate. After 15 min under these hypotonic conditions at 37°C, the cells were again gently centrifuged down and washed several times in a mixture of absolute ethanol and glacial acetic acid (3 : 1 v/v), before being resuspended in this fixative. A drop of the suspension was then placed on a clean dry microscope slide and air-dried at room temperature. Finally, the slides were stained in lacto-acetic orcein (Welshons and Russell 1959).

(ii) *Skin Culture.*—Skin samples were also taken from one foetus of goat 28, and were cultured by the method of Ilbery and Williams (1967). In brief, the samples were placed in Medium 199 [described by Paul (1960) and supplied by Commonwealth Serum Laboratories, Melbourne] and 4 hr later were transferred into clots of chicken plasma and chicken embryo extract, in Carrel flasks. Incubation proceeded at 37°C in a medium containing 80% Medium 199, 20% foetal calf serum, 200 units/ml of penicillin and of streptomycin, and 50 units/ml of Mycostatin (Squibb). Subsequently, fungal infections were controlled by varying the concentrations of Mycostatin between 50 and 1000 units/ml. Tissue growth appeared in the first Carrel flask after 5 days. Following removal of the medium, a subculture was made by adding 1·5 ml of 0·5% trypsin (Difco 1 : 500) in phosphate-buffered saline (pH 7·6) for 12 min. The trypsin solution was transferred to a small centrifuge tube containing 0·8 ml foetal calf serum at 0°C. Following centrifugation at 400 r.p.m. for 5 min, the cells were washed twice in the incubation medium and allowed to settle down further in Carrel flasks. Subcultures were made at intervals of 4–10 days. When cytological sampling was desired, Colcemid (Ciba) was added to the medium at the rate of 2·5 μg/ml, 4 hr before trypsinization. Cells were centrifuged down, swollen in hypotonic citrate, air-dried, fixed, and stained as above.

Results

The karyotype was determined in 11 uncultured foetal liver cells (1, 2, and 8 cells from the respective samples), and in 10 foetal skin cells examined on the 67th day of culture. All cells contained 57 chromosomes including three prominent metacentric chromosomes (Plate 1, Fig. 1).

During the fourth month of subculture of skin fibroblasts, polyploid cells made their appearance. When subculturing for the last time (at 172 days) before storage in liquid nitrogen, 34 cells were examined: all were again diploid, 21 with normally appearing chromosomes, but a new line of cells containing 56 chromosomes had appeared in which a small metacentric chromosome was present (13 cells). This chromosome appeared to involve centric fusion of two of the small acrocentric chromosomes (Plate 1, Fig. 3).

CHROMOSOMES OF SHEEP × GOAT HYBRIDS

Fig. 1.—Chromosome set and karyotype of a cultured fibroblast from a female hybrid, showing a modal number of 57 chromosomes. The three metacentric chromosomes derived from the sheep are displayed in the first line. The remainder of the acrocentric chromosomes are represented in three classes—large, medium, and small.

Fig. 2.—Chromosome set and karyotype of a cultured leucocyte from a female sheep included for comparison; there are six large metacentric chromosomes. In the goat there are 60 acrocentric chromosomes and no metacentrics.

Fig. 3.—Chromosome set and karyotype of a cultured fibroblast from a female hybrid showing an abmodal number of 56 chromosomes with the presence of an extra metacentric.

Discussion

Our results from the direct and culture methods agree with the findings of Berry (1938) and Hancock and Jacobs (1966) that there is no evidence of non-disjunction in the sheep × goat hybrid, and that its cells contain 57 chromosomes, a number midway between 54 in the sheep and 60 in the goat. However, we agree with Buttle and Hancock (1966) that hybrid cells contain three large metacentric chromosomes, contributed by the sire, rather than two as indicated by Berry (1938). The range of chromosome numbers about the modal value of 56 in the hybrid reported by Buttle and Hancock is probably due to their culture conditions; abnormalities were found in the present study, but only after prolonged culture. It appears necessary, therefore, to look beyond cytological anomalies for the explanation of death of the foetal hybrids.

The appearance of the small metacentric chromosome *in vitro* recalls that Soller, Wysoki, and Padeh (1966) recorded a family of goats with 59 chromosomes. The one metacentric in these goats appeared to result from centric fusion of two of the large acrocentrics. Centric chromosome fusion, or translocation of Robertsonian type, has been observed by Gustavsson (1966) in domestic cattle.

References

Alexander, G., Williams, D., and Bailey, L. F. (1967).—Natural immunization in pregnant goats against red blood cells of their sheep × goat hybrid foetuses. *Aust. J. biol. Sci.* **20**, 1217.

Basrur, P. K., and Coubrough, R. I. (1964).—Anatomical and cytological sex of a Saanen goat. *Cytogenetics* **3**, 414.

Berry, R. O. (1938).—Comparative studies on the chromosome numbers in sheep, goats and sheep–goat hybrids. *J. Hered.* **29**, 343.

Borland, R. (1964).—The chromosomes of domestic sheep. *J. Hered.* **55**, 61.

Buttle, H. L., and Hancock, J. L. (1966).—The chromosomes of goats, sheep, and their hybrids. *Res. Vet. Sci.* **7**, 230.

Gustavsson, I. (1966).—Chromosome abnormality in cattle. *Nature, Lond.* **211**, 865.

Hancock, J. L., and Jacobs, Patricia, A. (1966).—The chromosomes of goat × sheep hybrids. *J. Reprod. Fert.* **12**, 591.

Ilbery, P. L. T., and Ahmad, A. (1965).—An extra small acrocentric chromosome in a case of acute monocytic leukaemia. *Med. J. Aust.* **2**, 330.

Ilbery, P. L. T., and Williams, D. (1967).—Evidence of the freemartin condition in the goat. *Cytogenetics* **6**, 276.

Makino, S. (1943).—The chromosome complexes in goat (*Capra hircus*) and sheep (*Ovis aries*) and their relationship. *Cytologia* **13**, 39.

Paul, J. (1960).—"Cell and Tissue Culture." 2nd Ed. (E. and S. Livingstone, Ltd.: Edinburgh.)

Soller, M., Wysoki, M., and Padeh, B. (1966).—A chromosomal abnormality in phenotypically normal Saanen goats. *Cytogenetics* **5**, 88.

Welshons, W. J., and Russell, L. B. (1959).—The *Y*-chromosome as the bearer of male-determining factors in the mouse. *Proc. natn. Acad. Sci. U.S.A.* **45**, 560.