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SHORT NOTE

The application of tissue-culture techniques to the chromosomal analysis of *Bos taurus*

R. CROSSLEY, M.B., CH.B. AND G. CLARKE

Medical Research Council, Population Genetics Research Unit, Old Road, Headington, Oxford

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Until 1960 all the chromosome counts performed on cells of this species were done on histologically prepared specimens of testicular tissue. In January 1960, Chiarelli *et al.* published the results of their experiments to grow renal tissue in culture. Their specimens were obtained from male and female calves. They confirmed that $2n = 60$ and described the chromosomes as being all acrocentric except the X chromosome, which was submetacentric.

The purpose of the work described below was to try to apply the peripheral-blood culture technique of Moorhead to bovine blood, and to attempt to grow bovine muscle cells in a tissue culture.

THE COLLECTION AND PREPARATION OF MATERIAL

By arrangement with the local abattoir it was possible to be present at the time of slaughtering several bullocks and heifers, and to collect specimens from the poleaxed beasts.

(1) The peripheral blood was collected from the severed neck veins of the poleaxed beast in a sterile jar, from which it was decanted into heparinized universal containers.

The leucocytes were then cultured by the technique described by Moorhead (1960). It was found necessary to modify the technique used for human blood cultures in the following ways:

(i) The initial concentration of heparin required was double that required for human blood and was 0·4 mg./10 ml. blood.
(ii) The amount of Difco Bacto Phytohaemaglutinin used had to be increased to 16 drops from a No. 12 needle per 10 ml. of blood. This was added immediately prior to the blood being put into the centrifuge.
(iii) The blood was centrifuged for 30 minutes at 1000 r.p.m. instead of 10 minutes.
(iv) In some cultures the cells could be harvested at 3 days, whilst in others a satisfactory yield was only available after 5 days' culture.

(2) The muscle specimen was taken simultaneously with the peripheral blood and was placed in Glaxo Medium 199. The Harnden clot culture technique (Harnden, 1960) was used for the primary culture. When the cells had grown they were treated by the technique described by Hsu & Kellogg (1960).

RESULTS

Both of the methods used yielded cells showing many mitotic figures arrested in metaphase by colchicine. The chromosomes were examined and counted. The majority of cells counted confirmed the diploid number of chromosomes to be sixty ($2n = 60$). The few cells which deviated from this number were obviously broken during preparation. The autosomes were acrocentric, but some had the appearance of true telocentric chromosomes. However, the number of these was inconstant from cell to cell, which suggests that the appearance might be due to the very small short arms being in the same axis as, and therefore hidden by, the long arms. The autosomes showed no great morphological variations which would facilitate pairing of chromosomes in the karyotype, and it was therefore decided to arrange them in an order of descending size.

The sex chromosomes, on the other hand, were both observed to be submetacentric.

The female has an XX constitution. The X chromosome is the longest in the karyotype, and the short arm represents approximately one-third of its total length.

The male, on the other hand, has an XY constitution. The Y is the smallest chromosome present and is also submetacentric. The short arm represents two-fifths of the total length of the Y chromosome. In view of these findings, more specimens were collected from several bullocks and the leucocytes were grown in culture. Many more cells were then examined and it was found that the Y chromosome was a constant feature in the cells grown from specimens from different bullocks of different breeds. Both the X and the Y chromosomes are indicated in the photomicrographs (Plates I and II).

DISCUSSION

The results confirmed that the diploid number of chromosomes in *Bos taurus* is sixty ($2n = 60$) as described by Chiarelli *et al.* (1960). The main feature of this work has centred around the sex chromosomes. Chiarelli *et al.* described the X chromosome as long and submetacentric, whilst the Y chromosome was described as a medium-sized acrocentric. Whilst the former finding is confirmed, the latter is not in agreement with our findings.

In view of this difference of opinion about the Y chromosome it was felt justifiable to publish these results together with the photomicrographs.

We would like to acknowledge the encouragement given to us by Dr B. M. Slizynski, of the Institute of Animal Genetics, Edinburgh, who kindly checked our microscopic findings.

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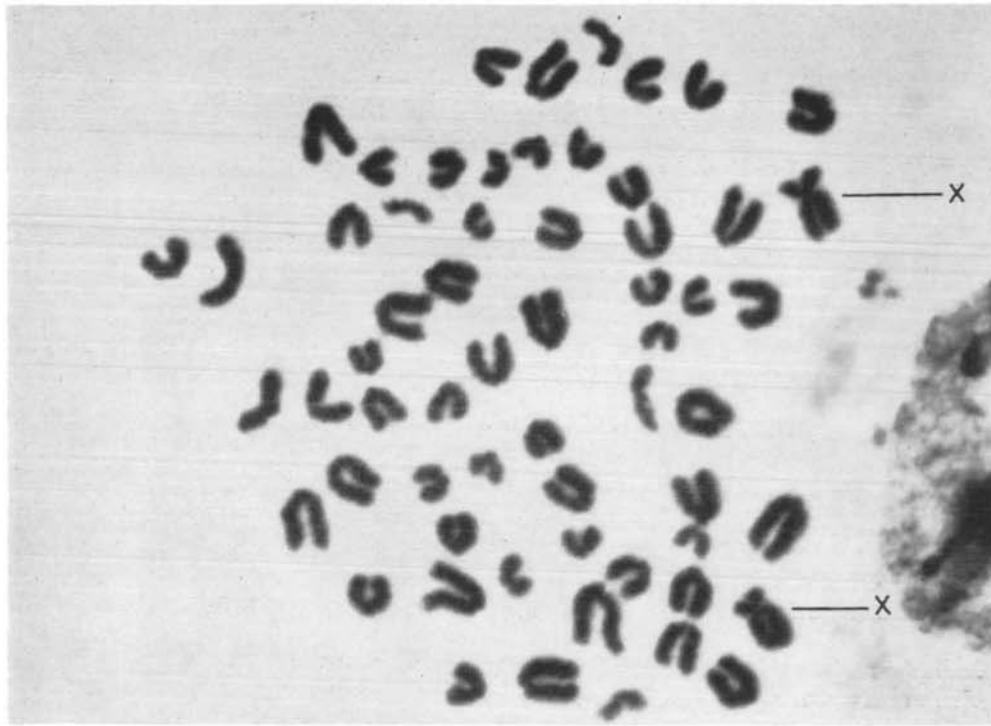

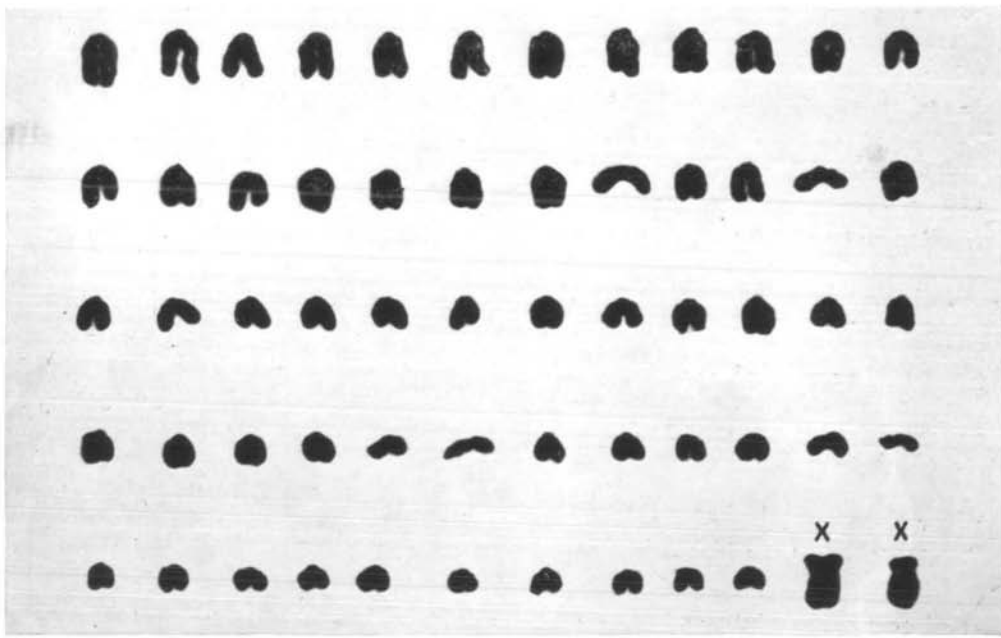

Fig. 1. *Bos taurus:* chromosomes of a female leucocyte shown in the metaphase of mitosis. Both X chromosomes are marked.

Fig. 2. *Bos taurus:* karyotype of a female leucocyte constructed from the cell shown in Fig. 1.

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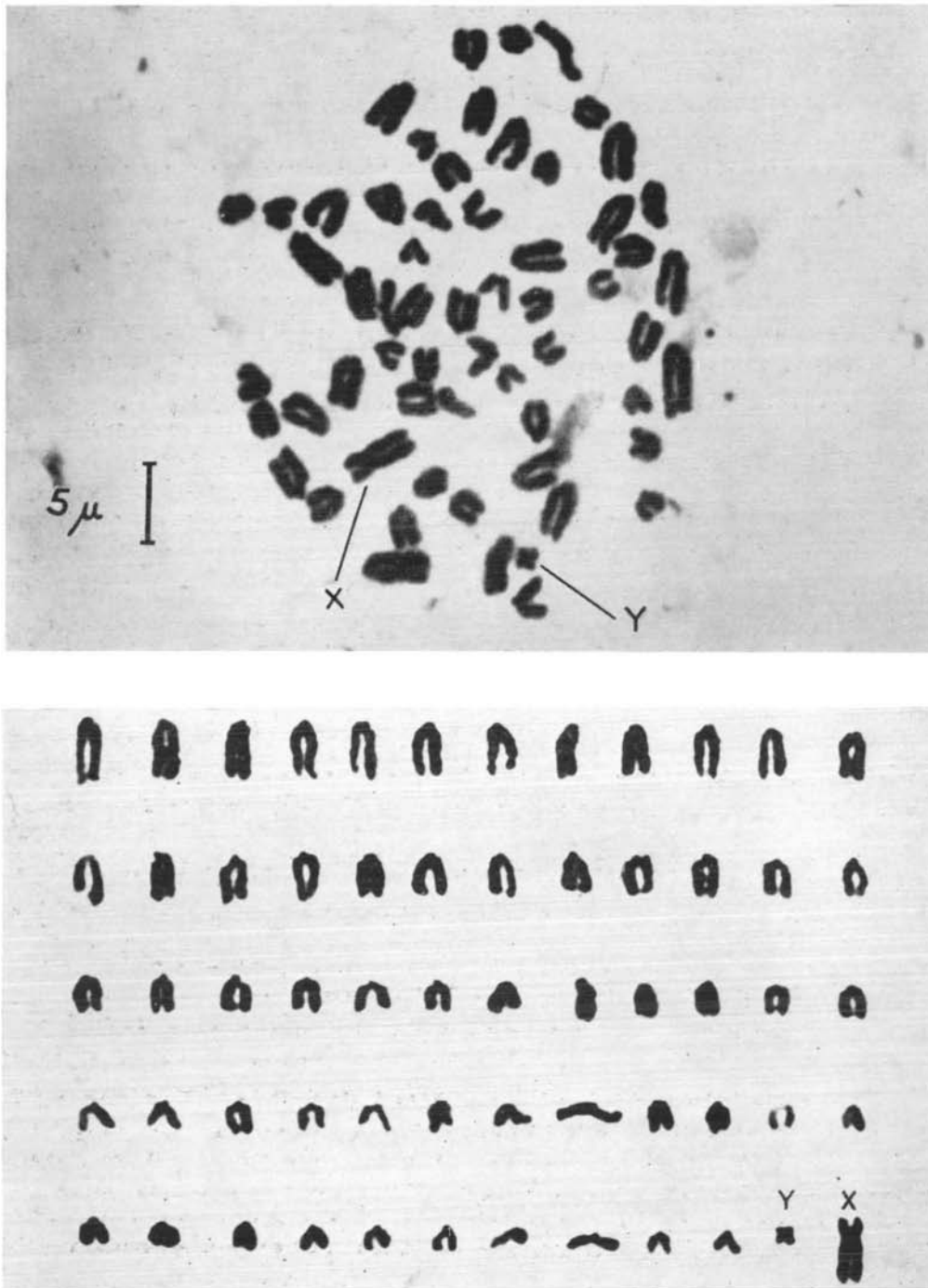

Fig. 3. *Bos taurus:* chromosomes of a male leucocyte shown in the metaphase of mitosis. The X and Y chromosomes are marked.

Fig. 4. *Bos taurus:* karyotype of a male leucocyte constructed from the cell shown in Fig. 3.

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