size of testes of *Aedes aegypti* L. treated with apholate. These controversial results may be due to specificity in the action of chemicals and need confirmation by further experiments. Reduction in the testis size and collapse of the testicular envelope might be correlated with deterioration of testicular contents as observed by Schwartz[4] in *H. pusio*. In contrast to our findings, hempa and hemel did not produce any abnormalitites in ovaries of *Achoea janta* L. even at very high doses, while tepa and metepa significantly reduced the size of the ovary[11]. Morgan[12], working with house flies, reported extensive damage to the ovary after treatment with hempa. According to our findings reduction in size of the ovary may be due to the direct effect of the chemical on cell division in the follicle cells as observed by Beattie[13], in *Lucilia cuprina* Wied. Secondly, reduction in ovary size may be due to damage of nurse cells which feed ova, and degeneration of follicle cells, which in turn leads to inhibition of yolk formation as evidenced by Nath and Sharma[14] who studied the effects of apholate and tepa on *Locusta migratoria migratorioides* Reiche & Farmaire.

Table 2. Effect of indole-3-acetic acid on the size of ovaries (mm) of *Dacus dorsalis* Hendel

Post-treatment days	0.3% concentration	0.5% concentration	Control
8	0.435a	0.429a	0.45
12	0.762a	0.595b**	0.743
16	1.570a	1.598a	1.629

Student's t-tests were performed to determine the significance of the difference between IAA treated values and control values and among the different concentrations. Figures in the same row with same letter are not statistically significantly different from one another at 5% level. * $p < 0.05$; ** $p < 0.01$.

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2 I. Keiser, L.F. Steiner and H. Kamasaki, J. Econ. Ent. *58*, 682 (1965).
3 G.C. La Brecque, in: Pest control; biological, physical and selected chemicals methods, p.214. Eds W.W. Kilgore and R.L. Dautt. Academic Press, London 1960.
4 P.H. Schwartz, J. Invert. Path. *7*, 148 (1965).
5 B.J. Smittle, J.B. Schmitt and G.S. Burden, J. Econ. Ent. *59*, 1419 (1966).
6 N. Dutt and S.S. Ghatak, J. ent. Res. *3*, 60 (1979).
7 K.K. Grover, M.K.K. Pillai and C.M.S. Dass, J. med. Ent. *9*, 451 (1972).
8 S.C. Saxena and V. Aditya, Cytologia *34*, 148 (1969b).
9 B.V.R. Madhukar, M.K.K. Pillai, H.C. Agarwal and V.C. Shah, Ent. exp. Appl. *13*, 275 (1970).
10 K.S. Rai, Biol. Bull. *127*, 119 (1964).
11 K. Sukumar and M.B. Naidu, Indian J. exp. Biol. *15*, 708 (1977).
12 P.B. Morgan, Ann. ent. Soc. Am. *60*, 812 (1967).
13 G.A.C. Beattie, Aust. J. Zool. *27*, 349 (1979).
14 V. Nath and G.P. Sharma, Final Res. Prog. Rep., U.S. PL480 Res. Proj. A7-ENT-117 (1977).

Centromeric heterochromatin in the karyotype of the male Greater Kudu *(Tragelaphus strepsiceros)*

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Summary. The chromosomes of a male Kudu *(Tragelaphus strepsiceros)* have been studied by C-banding and H^3 thymidine labelling. It is suggested that heterochromatin may have accumulated on the 14th pair of autosomes before its translocation to the Y-chromosome.

The chromosomes of the Kudu *(Tragelaphus strepsiceros)* were described by Wallace and Fairall[1] who found in the male an extra-large Y-chromosome, a slightly smaller, unpaired autosome and at meiosis a large quadrivalent, which they took to be an end to end association of the X- and Y-chromosomes with an autosomal bivalent. This demonstrated the association of the Y-chromosome with one homologue of an autosomal pair. Wurster et al.[2] discussed the observations by Schmid et al.[3] of the aggregation of heterochromatin on the sex chromosomes in species with extra-large sex chromosomes. They proposed the translocation of this material from autosomes to sex chromosomes during Robertsonian fusions which reduced the chromosome numbers. 2 species with almost complete and complete reduction, the Sitatunga *(Tragelaphus spekei)* and the Blackbuck *(Antilope cervicapra)* have 29 and 30 pairs respectively, with fundamental numbers of 58 and 60. Their late-labelling heterochromatin is mostly on the extra-large sex chromosomes. Wurster[4] demonstrated by measurements and autoradiographic studies that the original large acrocentric X-chromosome of the Sitatunga underwent inversion, breakage and fusion with the homologue of the autosome to which the Y is attached. Our study concerns the C-banding and autoradiographic characteristics of the male Kudu ($2n = 31$), another species with extra-large sex chromosomes and almost complete reduction; but only the Y-chromosome is translocated to one homologue of an autosomal pair, which is complete and unattached in the female Kudu ($2n = 32^{1,5}$).

Animals and methods. Tissues were obtained post mortem from an adult male Kudu *(Tragelaphus strepsiceros)* at Marwell Park Zoo, Winchester, Hampshire, England.

Chromosome spreads were prepared from the monolayer cultures of kidney fibroblasts by a modification of the method of Tucker et al.[6] and C-banding was done by a modification of the method of Sumner[7]. H^3-thymidine pulse labelling was carried out by the treatment of 4 monolayer cultures with culture medium containing methyl ^3H-thymidine for 30 min. Photographic prints were enlarged approximately $\times 4000$ and linear measurements of the H^3-thymidine labelled chromosomes X, Y and 14 from 5 cells were made using vernier calipers. An image analyzing computer (Quantimet 720) was used to measure total chromosome and centromeric C-band areas on karyotypes prepared from 5 C-banded cells ranked according to Wallace and Fairall[8]. In the X-chromosomes the 2 strong proximal C-bands were also measured.

Results. The C-bands (fig. 1, a and b) and the H^3-thymidine labelling patterns (fig. 2) define 2 distinct regions which show the partial homology of the Y-chromosome and the

Table 1. Length measurements of the Y-chromosome and autosome 14 of the male Kudu: regions defined by H^3 thymidine labelling

Cell No.	Y-chromosome		Chromosome 14			'Original' Y segment (Y-14)
	Total	Short arms	Total	Proximal segment	Distal segment	
1182	2.044	0.562	1.382	0.806	0.576	0.662
1193	1.982	0.683	1.262	0.634	0.628	0.720
1184	2.088	0.608	1.470	0.832	0.638	0.618
1190	2.530	0.754	1.521	0.589	0.932	1.009
1189	1.544	0.614	1.022	0.316	0.706	0.522
Mean	2.038	0.644	1.331	0.635	0.696	0.706
SE	±0.157	±0.034	±0.089	±0.093	±0.063	±0.082

acrocentric autosome 14. The short arm of the Y-chromosome and the distal region of autosome 14 are composed of euchromatin which is in the synthetic phase at 10 h and 8 h before metaphase: they are 0.630 (±SE=0.034) and 0.696 (±SE=0.063) units of length respectively (table 1) and the difference between them is not significant ($p>0.4$). The long arm of the Y-chromosome and the proximal region of chromosome 14 are both heterochromatic and they label at 4 and 6 h before metaphase. The length of this region in chromosome 14 is 0.635 (±SE=0.093) units and like its homologous region in the Y-chromosome it has 3 equally spaced, narrow, dark bands on a generally dense ground. The position of the centromere is different; in chromosome 14 it is telomeric at the heterochromatic end (fig. 1, a and b), while in the Y-chromosome it lies between the heterochromatin and the euchromatin. The remainder of the long arm is assumed to be the 'original' Y-chromosome translocated to the homologue of chromosome 14; it is estimated as 0.706 (±SE=0.082) units, or 29.6% of the whole Y-chromosome. It is a dark, apparently heterochromatic region with one (sometimes two) darker narrow bands separated by a broad, lighter band. A fairly close adhesion of the sister chromatids is observed in some cases in the proximal, heterochromatic regions of autosome 14 and of the long arm of the Y-chromosome.

The Quantimet measurements of chromosome area showed that centromeric heterochromatin is relatively sparse in the paired autosomes of the complement where it forms 0.9% of the total chromatin (table 2), but it takes up considerable proportions of the extra-large X- and Y-chromosomes

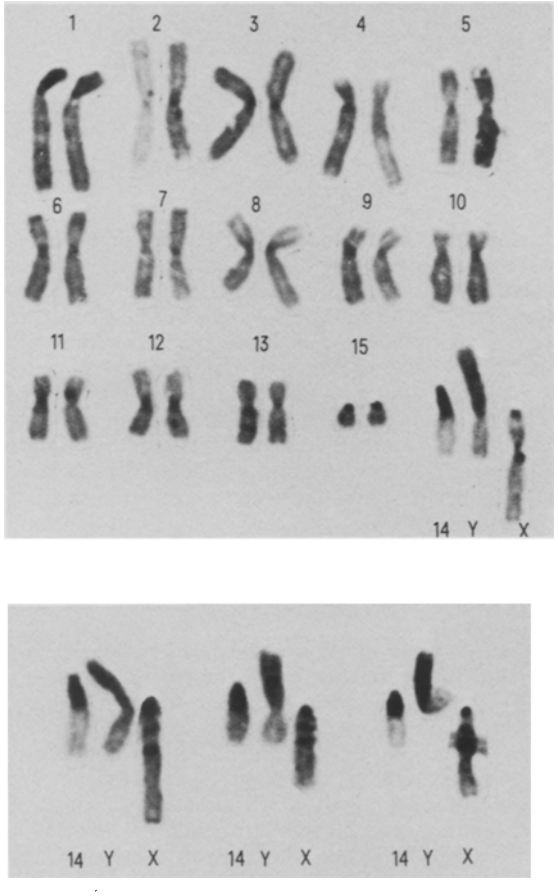

Figure 1. C-banding in the male Greater Kudu *(Tragelaphus strepsiceros)*. *a* Karyotype; *b* C-banded chromosomes No. 14, Y and X from 3 different cells.

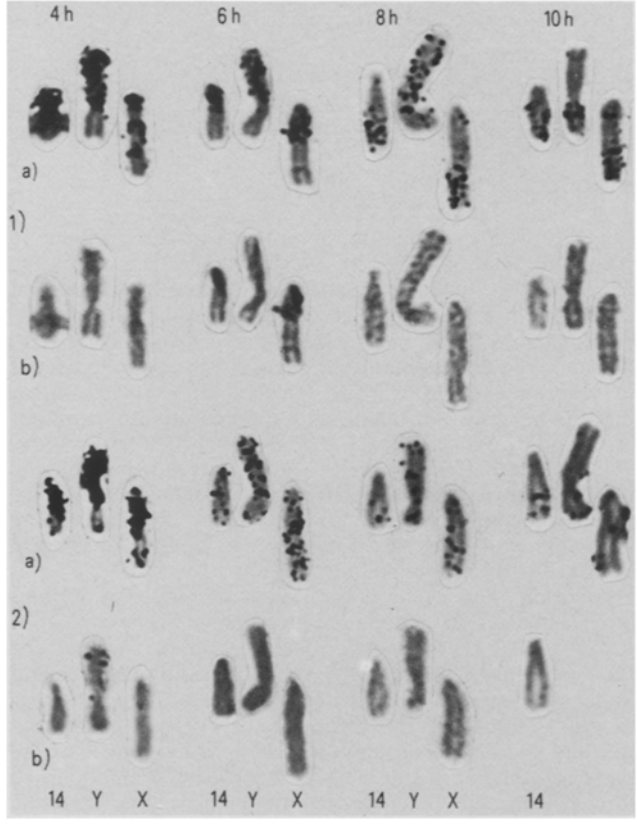

Figure 2. Autoradiographs of H^3-thymidine flash labelled No. 14, Y and X chromosomes from 8 cells of the male Greater Kudu *(Tragelaphus strepsiceros)* at 4, 6, 8 and 10 h before metaphase. *1a* and *2a*, labelled chromosomes; *1b* and *2b*, the same chromosomes after clearing (X and Y of cell 2b at 10 h were damaged during removal of the silver grains).

Table 2. The distribution of centromeric heterochromatin in the karyotype of the male Kudu

	Mean areas/cell				Centromeric heterochromatin
	Total chromatin	SE	Centromeric heterochromatin	SE	Mean total chromatin (%)
Autosomes	540377.6	±30478.1	5365.7	±940.0	0.9
Sex chromosome complex 14+Y+X	60701.6	± 1928.0	17691.0	± 335.0	2.9
All chromosomes	601079.2	±32393.9	23056.7	±1065.1	3.8

Units of measurement are picture-points on the Quantimet image analyzer.

Table 3. The distribution of centromeric heterochromatin in the autosomes and sex chromosome complex of the male Kudu

Chromosomes	Total area (Mean of pair)	SE	Area centromeric heterochromatin (Mean of pair)	SE	Heterochromatin / Total chromatin (%)
Autosome					
1	30051.6	±1457.26	394.9	± 69.55	1.31
2	26751.7	±1297.78	153.0	± 60.48	0.57
3	25917.7	± 852.33	72.4	± 69.41	0.28
4	22742.3	±1505.44	26.8	± 17.34	0.12
5	21809.0	±1571.19	137.2	± 75.37	0.63
6	20000.9	± 616.53	195.6	± 83.79	0.98
7	19455.3	±1568.10	100.2	± 57.14	0.29
8	20399.6	±1553.74	176.9	± 83.89	0.87
9	18427.5	±1920.38	568.6	±171.71	3.09
10	16748.1	±1644.67	56.2	± 13.23	0.34
11	14814.5	±1684.19	131.8	± 55.99	0.89
12	14114.4	± 744.93	156.6	± 37.86	1.11
13	13726.1	± 752.94	92.6	± 50.37	0.67
15	5230.0	± 454.53	420.1	± 91.17	8.03
Sex chromosome complex					
14	15555.0	±1264.15	4955.0	±146.81	31.85
Y	22654.0	± 382.33	10120.0	±441.25	44.67
X	22492.0	±1113.14	2616.0	±190.37	11.63

Units of measurement are picture-points on the Quantimet image analyzer.

together with the unpaired homologue of chromosome 14 (X, 11.63%; Y, 44.67%; 14, 31.85%; table 3): 3.8% of the chromatin in the cell is centromeric heterochromatin, and 2.9%, which is about ¾ of it, is carried on these 3 chromosomes (table 2). Table 3 shows the distribution of centromeric heterochromatin in the autosomes and the sex chromosome complex. The biarmed autosomes contain little C-banding chromatin, with the exception of numbers 1, 9 and 12 which have 1.31,3.09 and 1.11% respectively, while the small acrocentric pair 15 is unique in having 8.03% centromeric heterochromatin. Other C-bands appeared inconsistently in the biarmed autosomes where measurements suggested that they may contain an extra 2 or 3% of C-banding material.

Discussion. The euchromatic region of autosome 14 and approximately 30% of the Y-chromosome are homologous in size and H^3-thymidine labelling characteristics. The heterochromatic regions have similar banding patterns and label synchronously, but the centromeric position is exceptional. In the Y-chromosome it lies between the euchromatin and the heterochromatin; in 14 it is telocentric at the heterochromatic end. It is likely that the euchromatic piece broke from the heterochromatic segment and inverted, before its attachment to the 'original' Y-chromosome. The latter translocation would be assisted by exposure of the broken end of the heterochromatic segment, while one latent centromere, probably from the 'original' Y, persists in the translocation products[9–11]. Occasional adhesion of sister chromatids in 14 and in the long arm of the Y-chromosome may reflect additional, latent centromeres, which accumulated during base-pairing dependent exchanges between similarly orientated satellite DNAs[10]: similar observations were made by Nakagome et al. in their report of a centromeric translocation. In this way the centromeric heterochromatin, which is relatively depleted on the autosomal pairs 1–13 and 15 (0.9%) may have aggregated on the 14th pair, before translocation of the Y-chromosome. The present observations therefore support the hypothesis of Wurster et al.[3] and Schmid et al.[4] and indicate that the autosomal translocations of heterochromatin to chromosome 14 preceded that of the Y-chromosome. Together these autosomes and the sex chromosomes carry heterochromatin equivalent to 2.9% of the total area of chromatin in the complement, or about ¾ of the total heterochromatin.

The relatively high proportion of centromeric heterochromatin on the sole surviving pair of acrocentric chromosomes (15) supports the theory that the transfer occurred during the Robertsonian translocations which reduced the chromosome numbers. Work on the content of heterochromatin in the karyotype of the domestic sheep (Dain and Dott, in preparation) shows that this species, in contrast to the Kudu, carries nearly all its centromeric heterochromatin, which is about 8% of the whole, on its autosomes. The interstitial C-bands which appeared inconsistantly in the autosomes of the Kudu may also contain highly repetetive sequences of DNA; in which case the total content would be about 6 or 7%.

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1 C. Wallace and N. Fairall, S. Afr. J. med. Sci. *32*, 61 (1967).
2 D.H. Wurster, K. Benirschke and H. Noelke, Chromosoma *23*, 317 (1968).
3 W. Schmid, D.W. Smith and K. Theiler, Arch. Klaus-Stift. Vererb.-Forsch. *40*, 35 (1965).
4 D.H. Wurster, Cytogenetics *11*, 197 (1972).
5 T.C. Hsu and K. Benirschke, *Tragelaphus strepsiceros* (Greater Kudu). Atlas of Mammalian Chromosomes *6*, Folio 297 (1971).
6 E.M. Tucker, A.R. Dain and R.M. Moor, J. Reprod. Fert. *54*, 77 (1978).
7 A.T. Sumner, Exp. Cell Res. *75*, 304 (1972).
8 C. Wallace, and N. Fairall, Mammalian Chromosomes Newslett. *10*, 11 (1969).
9 T.C. Hsu, S. Pathak and T.R. Chen, Cytogenet. Cell Genet. *15*, 41 (1975).
10 G.P. Holmquist and B. Dancis, Proc. natl Acad. Sci. USA *76*, 4566 (1980).
11 Y. Nakagome, F. Teramura, K. Kataoka and F. Hosono, Clin. Genet *9*, 621 (1976).

Immuno-electron microscopic localization of fibronectin on rat mast cells*

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Summary. Rabbit anti-rat plasma fibronectin (pFN) causes histamine release from rat mast cells in the presence of complement. Fibronectin (FN) on rat mast cells, as shown by immuno-electron microscopy, is principally localized on cell folds, so they may play a role of attachment in the matrix of connective tissue.

Cell surface FN is distributed on many cell types and one of its main functions is to mediate cellular adhesion to the substratum (see Pearlstein et al.[1] for a review). Rat mast cells also have FN molecules on their surfaces. It has been shown[2] that: a) anti-rat pFN serum releases histamine from rat mast cells, b) mast cells adhere to collagen-coated dishes and some of them flatten and elongate on the dishes, c) this adhesion is inhibited by anti-pFN serum treated at 56 °C for 30 min, and d) immunofluorescence microscopic study shows the presence of FN on mast cells. In the present study, complement-dependent histamine release from rat mast cells by rabbit anti-rat pFN serum is described and localization of FN is shown by immuno-electron microscopy.

Materials and methods. Purification of rat pFN and preparation of rabbit anti-rat pFN serum have been described previously[2]. Purified pFN showed a single band by SDS-polyacrylamide gel electrophoresis, and the antiserum formed a single precipitation arc against purified pFN and rat plasma by immunoelectrophoresis. The antiserum preabsorbed by the purified pFN lost histamine releasing activity from mast cells as well as a binding activity on these cells. Rat peritoneal cells were collected after the injection of physiological solution (PS: NaCl 154 mM, KCl 2.7 mM, $CaCl_2$ 0.9 mM and 10% Sörensen phosphate buffer, pH 7.2). Peritoneal cells from a few rats were pooled for each experiment and the isolation procedure for mast cells[2] was omitted in the experiment using immuno-electron microscopy. Isolated mast cells were suspended in 0.9 ml Hanks' solution containing 0.05% bovine serum albumin and incubated with 0.1 ml rabbit anti-pFN serum at 37 °C for 15 min. These cells were centrifuged at 2000 × g for 15 min and histamine in the supernatant and the precipitate was determined[3]. Histamine release was expressed as a percentage of total histamine content. For immuno-electron microscopy, collected peritoneal cells were washed and fixed with 0.125% glutaraldehyde in PS for 2 h in an ice bath. They were washed with PS 3 times for a total of more than 30 min, then incubated with rabbit anti-rat pFN serum diluted 1:40 for 30 min at room temperature. After washing, ferritin-conjugated goat anti-rabbit IgG (Miles-Yeda, Ltd) diluted 1:100–200 was used for detection of bound antibodies. The cells were refixed with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), post-fixed with 1% OsO_4 in 0.05 M phosphate buffer (pH 7.4) in suspension, collected in agar[4], dehydrated with an acetone series and embedded in Epon. As a control, normal rabbit serum was used instead of antiserum. PS was used as a washing solution throughout the experiment unless otherwise stated. Unstained thin sections (60 nm thick) were viewed with a JEOL 100-CX electron microscope.

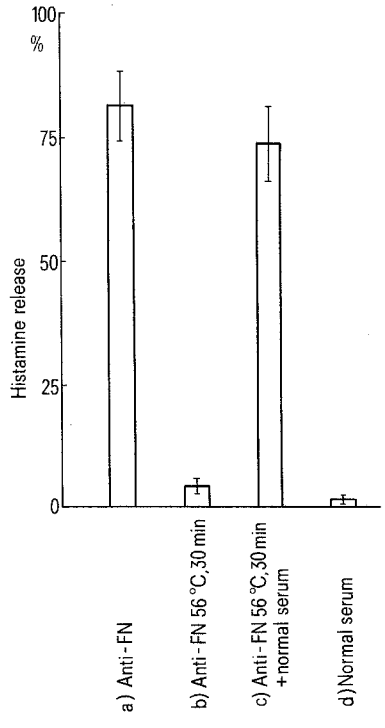

Figure 1. Histamine release (%, mean value ± SE) from isolated rat mast cells by *a* rabbit anti-rat pFN serum, *b* rabbit anti-rat pFN serum treated at 56 °C for 30 min, *c* rabbit anti-rat pFN serum treated at 56 °C for 30 min and normal rabbit serum and *d* normal rabbit serum.