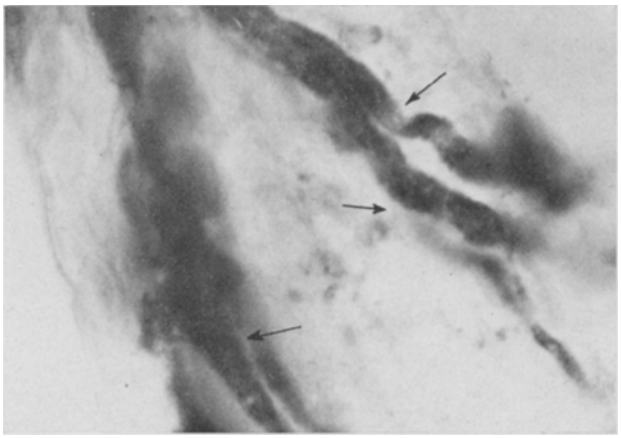
in this study. The pattern of the neurosecretory system of the bark bug *Halys dentatus* (Heteroptera: Pentatomidae) studied here resemble grossly other heteropterans. Some new features have been observed in the above species, so far as the storage and release organ of A-cell NSM, is concerned.

Although 10–12 nerve fibres only from A-cells of the brain innervate the aortal wall, a large amount of materials are generally visible in it. Whether such a large amount of material could be stored in the single-layered aortal wall[5] or its intercellular spaces[1], is a matter of further investigation, to establish any conclusion. The present observations show that the materials are neither stored in the aortal wall nor in its intercellular spaces, but remain confined to the axons (Figure), and the aortal wall simply serves as a framework for the axons. This observation is in congruence with the electron microscope study[7], which showed that the axonal endings of the neurosecretory axons are themselves the storage and release organs of the NSM in diverse groups of animals.

Further, the neurosecretory axons have been observed to divide and sub-divide at their distal ends (Figure), forming a dendrite-like pattern within the aortal wall. Earlier, JOHNSON[8] has also suggested that the neurosecretory axons in aphids divide and directly supply the tissues, they influence. However, the significance of the division of axons is not clear. It is probable that it helps to increase the storage capacity of the handful of neurones from A-cells and their area of release[9].



A portion of the aortal wall under oil immersion showing division and subdivisions of NSM-loaded axons (arrows). (PAVB, in situ.) × 800.

Zusammenfassung. Neurosekretorisches Material kann in der Wand der Aorta eines Insekts nachgewiesen werden. Das Material befindet sich dort ausschliesslich in den sich verzweigenden Axonen der NS-Zellen.

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28 October 1968.

[7] H. A. BERN, *The General Physiology of Cell Specialization* (Ed. D. MAZIA and A. TYLER; McGraw Hill, New York 1963), p. 349.
[8] B. JOHNSON, J. Insect Physiol. *9*, 727 (1963).
[9] Thanks are due to Dr. B. K. SRIVASTAVA, Agricultural Experiment Station, University of Udaipur, for providing laboratory facilities, and to Dr. G. S. DOGRA for helpful suggestions.

Sex Chromatin in an Australian Marsupial *Perameles nasuta* Geoffroy, 1804

Sex chromatin has been reported in a wide variety of female mammals, including apes, lions, cattle, sheep, pigs and man[1]. Its significance was originally recognized by BARR and BERTRAM[2] in female cat neurons. In the Virginian opossum (*Didelphis marsupialis*) and a Brazilian opossum (*Philander opossum*) Barr bodies (sex chromatin masses) are present in interphase nuclei of both males and females but those of the female are larger than those of the male[3–5].

In the Australian long-nosed bandicoot *Perameles nasuta* male and female gonadal tissue has a diploid complement of 14 chromosomes. In the adult females one of the *X* chromosomes is eliminated from most somatic tissues. In male adults the *Y* chromosome is eliminated[6–7]. However adults of both sexes have 12 autosomes and both sex chromosomes in corneal epithelium[8].

Sex chromatin has been studied extensively in ocular tissues and is easily recognized in the epithelial cells of the cornea[9]. This study was undertaken to see if a nuclear sex difference occurred in corneal epithelial cells of long-nosed bandicoots.

Material and methods. 5 females and 6 males of the long-nosed bandicoot *Perameles nasuta* Geoffroy trapped in the Sydney district of New South Wales were killed with an overdose of ether. Both eyes were removed from all animals and fixed immediately in 95% ethanol. Slides of corneal epithelial cells were prepared by a modification of the technique of FREGDA[10]. 12–24 preparations were examined from each animal. Preparations were made permanent according to CONGER and FAIRCHILD[11].

[1] K. L. MOORE, in *The Sex Chromatin* (Ed. K. L. MOORE; W. B. Saunders Co., Philadelphia/London 1966), p. 16.
[2] M. L. BARR and E. G. BERTRAM, Nature *163*, 676 (1949).
[3] M. A. GRAHAM and M. L. BARR, Archs Anat. microsc. Morph. exp. *48*, 111 (1959).
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[9] C. PEDLER and N. ASHTON, Br. J. Ophthal. *39*, 362 (1955).
[10] K. FREDGA, Hereditas *51*, 269 (1964).
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The following criteria were adhered to in the examination of all preparations. (1) Cells of the following categories were excluded from the data: (a) the many degenerative cells normally found in this tissue; (b) disrupted and folded cells; (c) cells containing numerous heterochromatic bodies. (2) Only cells with a single heterochromatic mass situated on the periphery of the nucleus were considered sex chromatin positive[12]. Coded slides selected at random were examined for sex chromatin.

Results and discussion. Every preparation of corneal epithelium contained numbers of cells showing a densely staining chromatin mass on the periphery of the nucleus. Figures 1 and 2 show this in both male and female cells. The sex chromatin body was frequently V-shaped with the apex of the V towards the centre of the nucleus; see Figure 3. Occasionally it could be seen as a bipartite structure as in Figure 4. There appeared to be no gross size difference between the male and female sex chromatin masses (Figures 3 and 4) as was described for the American and Brazilian opossums[3-5]. The coded slides selected at random contained sex chromatin bodies.

In their study of ocular tissue PEDLAR and ASHTON[9] observed that cells containing a sex chromatin mass tended to be grouped together. This feature was also noted in the bandicoot preparations.

No frequency of sex chromatin was estimated due to the nature of this continuously regenerative tissue. The number of degenerate cells, in the preparations made, varied according to the extent of regeneration at the moment of death of each individual animal. In the coded slides examined preparations were equally favourable for finding sex chromatin positive cells. This eliminated the possibility that this male sex chromatin mass occurred at a lower frequency than the female one.

There appears to be no recorded information on sex chromatin in any Australian marsupial species. In the red kangaroo (*Megaleia rufa*), the eastern grey kangaroo (*Macropus giganteus*), the tammar (*Macropus eugenii*), the fat-tailed marsupial mouse (*Sminthopsis crassicaudata*) and the brush-tailed possum (*Trichosurus vulpecula*) hair roots have been examined for sex chromatin[13]. In all

[12] M. M. GRUMBACH and M. L. BARR, Rec. Progr. Hormone Res. *14*, 255 (1958).

[13] S. M. WALTON, unpublished observations.

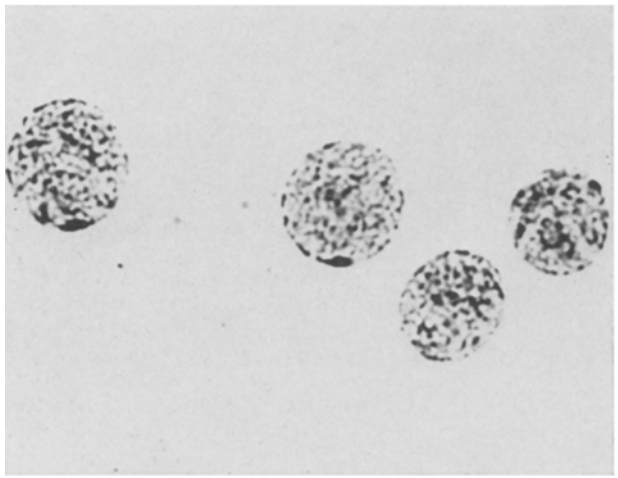

Fig. 1. Interphase nuclei of corneal epithelial cells from a male bandicoot, 2 of which show a sex chromatin mass on the periphery of the nucleus. × 600.

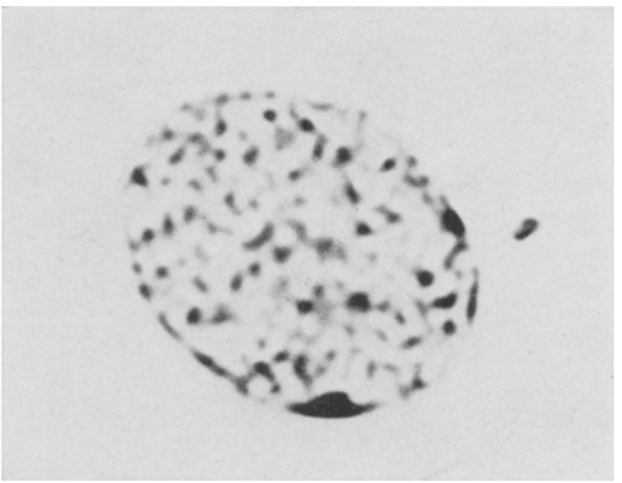

Fig. 3. A male cell enlarged to show the typical shape of the sex chromatin. × 3100.

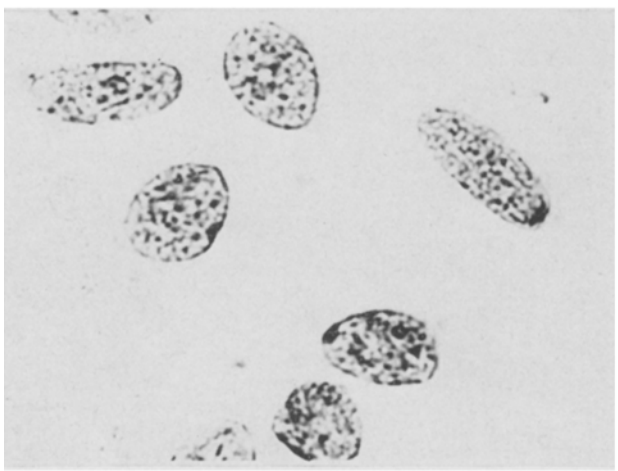

Fig. 2. Interphase nuclei of female cells. A sex chromatin mass can be seen in 2 cells. × 600.

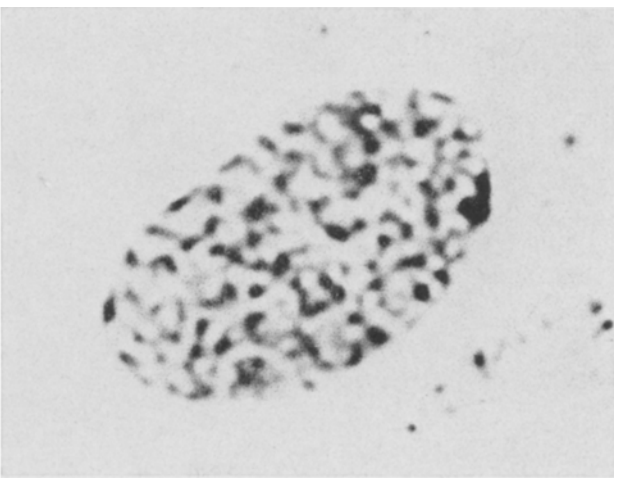

Fig. 4. An enlarged female cell in which the sex chromatin can be seen as a bipartite structure. × 3100.

these the presence of many heterochromatic bodies in interphase nuclei made sex chromatin diagnosis impossible. This same difficulty was encountered in some rat tissues that contained numerous chromocentres[14].

The current concept that sex chromatin represents the inactive X in female interphase nuclei does not explain the presence of a sex chromatin body in any male cells. It is known to occur at a low but variable frequency in apparently normal human males[12].

OHNO et al.[4] interpret the chromatin mass in the male Virginian opossum as representing the Y chromosome. The size difference between male and female chromatin masses is attributed to the length of the X and Y chromosomes, the X being twice as long as the Y.

In *P. nasuta* there is the same size relation between the X and Y chromosomes[7], but not, as mentioned earlier between the male and female sex chromatin masses. If one accepts the interpretation that the male sex chromatin in the marsupials studied so far is the Y chromosome, then in *P. nasuta* the extent of heterochromatization of the X and Y chromosomes in interphase nuclei is equivalent.

In conclusion since both male and female corneal epithelial cells of *P. nasuta* contain sex chromatin, this tissue cannot be used for nuclear sexing in this animal. In addition due to its sex chromosome anomaly any general conclusions regarding the sex chromatin situation in other Australian marsupials cannot be inferred from this study[15].

Résumé. La chromatine sexuelle des cellules de l'epithelium corneal de mâles et femelles adultes du Marsupial australien *Parmeles nasuta* Geoffroy a été étudiée. Les cellules des mâles comme celles des femelles manifestent l'existence de la chromatine sexuelle dans le noyau interphasique. Il n'y a pas de différence de taille entre chromatine sexuelle mâle et femelle.

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School of Zoology, University of New South Wales, Kensington (N.S.W., Australia), 20 January 1969.

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[15] The author wishes to express her gratitude to Professor G. B. SHARMAN, for his helpfulness in reading the manuscript.

A γ-Globin-Chain Mutation in the Hemoglobin 1 (Hb1) of the Domestic Chicken

In a previous work[1] we have shown, in agreement with other authors[2,3], that chicken hemoglobin (Hb) is not homogeneous, but is composed of 2 fractions, easily detectable by electrophoretic or chromatographic techniques. We have called Hb1 the minor fraction, and Hb2 the major one. The 2 hemoglobins seemed to have one globin chain in common, α, and 2 different counterpart globin chains, β, and γ: thus the molecular 'formula' of Hb1 is $\alpha_2\gamma_2$, and that of Hb2 is $\alpha_2\beta_2$.

We have recently described the occasional finding of a 'mutant' Hb in the domestic chicken (breed Arbor Acres and Vantress)[4]. By routine starch gel analysis, a certain number of chickens were found to have 3 Hb fractions instead of 2. Crossing experiment revealed that this pattern corresponds to a heterozygous condition; in fact we could obtain the segregation of 2 homozygous groups of chickens, one group with the 'normal' Hb pattern, and the other with the 'mutant' Hb fraction. The present study was undertaken for the purpose of identifying the mutation responsible for the appearance of the new hemoglobin type.

Materials and methods. The heterozygous cocks and hens were isolated and their eggs collected and incubated. The homozygous 'normal' and 'mutant' chickens were identified by analysing the Hb pattern of the hatched chickens[6]. The analysis was carried out by starch gel electrophoresis at pH 8.6, as previously reported[1].

The purification of the Hb types present in 'normal' and in 'mutant' chickens, was achieved by chromatography on Amberlite CG-50 columns[1]. The globin chain types present in the total lysate, and in purified Hb fractions, were studied by starch gel electrophoresis in 6 M urea at pH 3.6 as previously reported[1]. A new buffer system was devised for a better characterization of the 2 γ globin chains. Its composition was the following: 0.2 M succinic acid brought with solid *Tris* to pH 5.4, diluted 50 times for the gels and 6 times for the buffer reservoirs.

Results and discussion. The electrophoretic pattern of the hemoglobins under investigation is presented in Figure 1. The migration of Hb2 is the same in the 3 conditions considered, while the electrophoretic mobility of the Hb1 fractions differ in 'normal' and in 'mutant' homozygotes. In the 'heterozygous' condition, both Hb1 types are present; in this last case, the relative distribution of the Hb fractions, as determined by analysis of the starch gel with a Chromoscan, is Hb1 'mutant' = 25%, Hb1 'normal' = 25%, Hb2 = 50%.

The hemoglobins from 'normal' and 'mutant' chickens have been purified on Amberlite CG-50 columns. As previously reported[1], this resin allows the separation of Hb1 from Hb2; moreover Hb1 can be separated into 2 fractions, which we have called Hb1 and Hb1a: despite their different chromatographic behaviour, the 2 fractions have been shown to have the same electrophoretic mobility

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[6] The crossing experiments, using heterozygous cocks and hens, gave a total of 314 hatched chickens, distributed as follows: Homozygous 'normal' = 100, heterozygous = 155, and homozygous 'mutant' = 59. No explanation can be given up to now, for the unexpectedly low number of the homozygous 'mutant' chickens, as the post-hatching development of the 'mutants' seems to be normal.