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INVESTIGATIONS INTO SEX DIMORPHISM DURING NUCLEAR INTERPHASE OF THE ARGASID TICK, *ORNITHODOROS SAVIGNYI* (AUDOUIN, 1827)

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Barr & Bertram (1949) were the first to consider the possibility of differentiating the sexes in bisexual animals by the presence or absence of a specific chromocentre during nuclear interphase. Since then several papers have been published on the phenomenon of sex dimorphism during nuclear interphase in various tissues in mammals, birds and man (Hay & Moore, 1961; Barr, 1963; Moore, 1965).

In the present article it is proposed to determine whether sex dimorphism, expressed by the sex-chromatin body, is present in the tick *Ornithodoros savignyi*. Further it is proposed to see to what extent the male and female karyotypes, as set up by Howell (1966), can be correlated with the present concept of the aetiogenesis of the sex-chromatin body.

According to Howell (1966) the chromosome number is $2n = 20$. The heterochromosomes differ but slightly, the Y-chromosme being only slightly smaller than the X-chromosome. The female tick with an XX-complement is homogametic, while the male with an XY-combination is heterogametic.

Material and Methods

Male and female ticks were collected in the Kalahari and stored in a climate-chamber at 26° to 27°C and 90 per cent R.H. For dissection they were fixed onto masking tape in a petri dish. The ganglion, salivary glands, muscles, midgut, tracheae, accessory sex glands and rectal ampulla were carefully dissected out under a binocular stereo-microscope and tissue spreads were prepared from each. Without allowing them to dry, these spreads were immediately fixed in 96 per cent ethyl alcohol for 10 to 15 min. Immediate fixation was found to be essential for efficient staining

Reduction of the DNA, which is necessary for differential staining of the sex-chromatin body, was achieved by treating the spreads with 5 N HCl at 37°C for 20 min. Thereafter they were rinsed in several changes of distilled water to remove all traces of HCl. Nuclear staining was completed by staining with Biebrich Scarlet (Biebrich scarlet—water soluble, Dr. K. Hollborn und Söhne—Leipzig, 1·0 gm; phosphotungstic acid, 0·3 gm; glacial acetic acid, 5·0 ml; 50 per cent ethyl acohol, 100·0 ml) for 2 min and rinsing briefly in 50 per cent ethyl alcohol. Counter-staining was completed with Fast-Green (Fast-Green FCF, Anachemia Ltd., Montreal, 0·5 gm; phosphomolybdic acid 0·3 gm; phosphotungstic acid 0·3 gm; glacial acetic acid 5·0 ml; 50 per cent ethyl alcohol 100·0 ml) followed by a rinse in 50 per

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cent ethyl alcohol. The spreads were then dehydrated in 70 per cent, 96 per cent and absolute ethyl alcohol, cleared in three changes of xylol and mounted in permount (Fisher Scientific Company, U.S.A.).

Other staining procedures, e.g. the thionin method (Klinger & Ludwig, 1957), Ehrlich's hematoxylin eosin and cresylecht-violet were also tried. None was as satisfactory as the modified Guard-method described above (Guard, 1959).

The microscopic investigation was undertaken with a Wild-microscope, ocular 10x, oil immersion H 100, 1·25.

Observations

A sex difference in the nuclear interphase cells could be seen only in cells of the rectal ampulla. A specific sex-chromatin body was found only in the female Sex dimorphism was not apparent in nuclei of any of the remaining tissues The thionin method was found to be unsuitable for interpreting chromocentres as sex-chromatin bodies, because it did not stain them differentially. The same applied to the hematoxylin-eosin method. The chromatin particles were so prominent and dense that no specific differentiation was possible with these two methods. Spreads treated with cresylecht-violet and hydrolysed, gave a similar nuclear picture but it was possible to recognise paranucleolar sex-chromatin bodies with difficulty

Only the modified Biebrich scarlet-Fast-Green method gave successful results. In this procedure hydrolysis with HCl, which reduced the DNA of the chromatin particles and the RNA of the nucleolus, was found to be important in producing clarity and specificity in the final results. The results thus obtained with the modified Guard method rendered the sex-chromatin body specifically and differentially stained, varying in colour from a bright red to a dark blue-red. Other nuclear and cytoplasmic components were green. Some non-specific chromocentres occasionally appeared rather dark.

The sex-chromatin body was situated near the nucleolus in 82 per cent of the female cell nuclei examined (Plates 1, 2, 3). This figure was obtained as the average of all nuclei seen in fifteen microscope fields per preparation. These fields were picked from the most suitable areas of each spread.

The sex-chromatin body, when in a paranucleolar position, appeared either as a round or an oval cap on the nucleolus, a spherical chromatin mass just touching the nucleolus, or as a round or an ellipsoid swelling on the nucleolar surface. When the nucleolus was excentric, the round or oval sex-chromatin body often touched the nuclear membrane. In nuclei with two or more nucleoli only one sex- chromatin body was present. When near to the nuclear membrane the sex-chromatin body had a planoconvex shape, but it was rarely seen in this position. Its diameter varied between 0·5 and 1·1 μ. The sex-chromatin bodies were Feulgen-positive.

In the male cell nuclei the sex-chromatin body could not be demonstrated.

Discussion

Different explanations have been advanced for the aetiogenesis of this sex-determining component of the resting nucleus. The staining reactions, especially positive Feulgen reaction, indicate a chromosomal origin. But a possible extra-chromosomal origin, although unlikely, cannot be excluded completely at this stage. Its extra-chromosomal origin was suggested by Tobias (1954, 1958). The increase

in numbers of the sex-chromatin bodies in hyperdiploid nuclei as well as the proportionate increase of such bodies in polyploid nuclei stress its chromosomal nature (Klinger & Schwarzacher, 1958). This hypothesis is also supported by the behaviour of the chromatin body during amitosis, when during division the two halves of the sex-chromatin wander to opposite poles so that each daughter cell gets its sex-chromatin mass (Klinger, 1958). This hypothesis is further supported by the resistance shown by the sex-chromatin to hormonal influences (Peer, 1958; Caratzali, 1959; Woodruff & Lennox, 1959).

While Segal & Nelson (1957) assume an autosomal origin, Graham & Barr (1952) suggest that the sex-chromatin in female cell nuclei is formed from positive heteropycnotic parts of the two X-chromosomes, which are in close contact with one another in interphase nuclei. But Graham & Barr's hypothesis is not supported by the following observations, suggesting a possibly different origin: In the heterogametic female of *Archips fumiferana* (Smith, 1944, 1945) and in *Bombyx mori* there are distinct chromocentres in the interphase nuclei (Frizzi, 1948). Also Kosin & Ishizaki (1959) could demonstrate sex-chromatin bodies in the smooth muscle cells and cells at the base of growing feathers in the heterogametic domestic hen. From this Barr (1963) draws the conclusion that throughout the animal kingdom the nuclear chromocentre is not developed exclusively in the homogametic sex and that one chromosome of the XX-pair is able to become positively heteropycnotic and so form sex-chromatin.

Ohno, Kaplan & Kinosita (1959) found that in liver cells of the male rat all chromosomes in prophase nuclei were isopycnotic, while in the female one chromosome showed positive heteropycnosis and was considered to be an X-chromosome and responsible for the formation of the sex-chromation. It was suggested that the positively heteropycnotic chromosome is of paternal origin. Ohno & Makino (1961) observed a single heteropycnotic chromosome in prophase nuclei of a female human foetus, which they considered to be an X-chromosome. Lyon (1961) suggested, from genetic experiments in mice, that the heteropycnotic X-chromosome may be either of paternal or maternal origin and that it is genetically inactive.

The correlation demonstrated between sex-chromatin bodies and heterochromosome-complements supports the theory of a single X-origin of the sex-chromatin mass (Barr & Carr, 1960). Jacobs, Harnden, Court Brown, Goldstein, Close, MacGregor, MacLean & Strong (1960) report a case of mosaicism in a man with two different cell populations of 45 and 47 chromosomes. They interpreted these as XO and as XXX complements. The XXX complex comprised two sex-chromatin masses. The sex-chromatin bodies were formed in three-fold in the cell nuclei of a female with an XXXX and in a male with an XXXXY sex-chromosome complex (Anders, Prader, Hauschteck, Scharer, Siebenmann & Heller, 1960; Carr, Barr & Plunkett, 1961).

Ohno (1963) stresses that the sex-chromatin develops only from one X-chromosome. Each sex-chromatin body found in the somatic interphase nuclei in man and other mammals (in which either X-chromosome can be either heterochromatic or euchromatic) develops from one chromosome which is condensed along its whole length. If there is only one X-chromosome as in the somatic cells and in the spermatogonia in male organisms, it always behaves as an euchromatic chromosome. It is condensed only in meiotic cells. In female organisms, however, the components of the XX-complex behave differently. In an interphase nucleus one X-chromosome remains euchromatic while the other contracts to form the specific chromocentre. The X-chromosome in oogonia and oocytes always behaves euchromatically. Ohno (1963) suggests that the XX-chromosome complement is under autosomal control

and that an autosomal pair always keeps one X-chromosome in an euchromatic phase, while one or more X-chromosomes may undergo heteropycnosis. Autosomal control is established early in embryonic life so that normal male organisms, which later may form aneuploid tumor cells with an XXX-complement, remain sex-chromatin-negative. Two sex-chromatin bodies are developed in female organisms when the zygote shows a 2A: XXX complex. With the loss of a heterochromatic X-chromosome, a normal female organism becomes sex-chromatin-negative, as is instanced in the malignant transformation of tumor cells in which the condensed X-chromosome contributes to the formation of the 2A: XXX constitution.

Conclusion

Considering the various theories mentioned above in connection with the aetiogenesis of the sex-chromatin body and following the single X-origin of the sex-chromatin body and Ohno's train of thought and comparing them with the results obtained by the modified Guard method, one is justified in concluding that these results coincide with Howell's (1966) interpretation of the male and female karyotypes of *Ornithodoros savignyi* and that, here also, the sex-chromatin body is formed by the one heteropycnotic X of the female. Howell (1966) proved the female tampan to be homogametic with an XX-complex, and the male heterogametic with an XY-combination.

Summary

1. Sex dimorphism, in agreement with the normal karyotype, was shown to exist in interphase nuclei of the argasid tick *Orhithodoros savignyi*.

2. Sex-chromatin bodies were suitably demonstrated only in nuclei of the rectal sac. Other tissues proved to be completely unsuitable.

3. An adapted method, giving excellent differential staining of the sex-chromatin bodies, is described.

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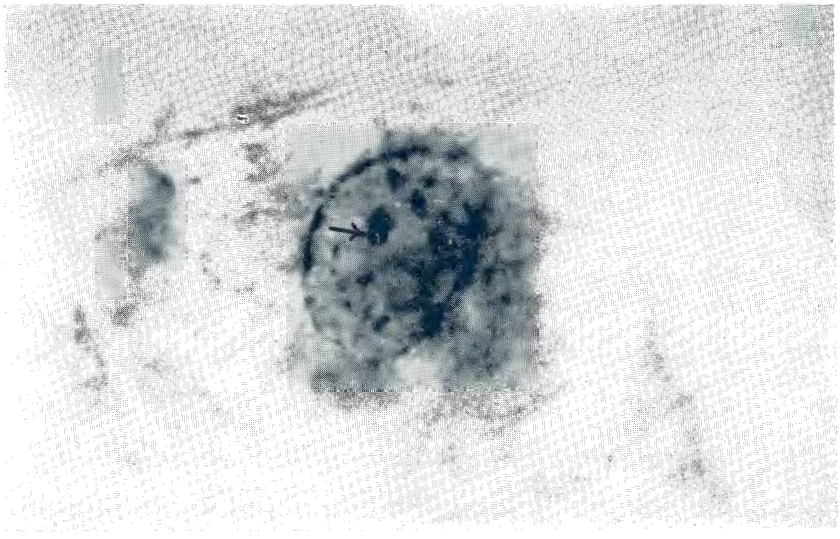

PLATE. 1.—Sex-chromatin body in paranucleolar position of interphase nuclei of rectal ampulla (arrow). (Magnification 1200×)

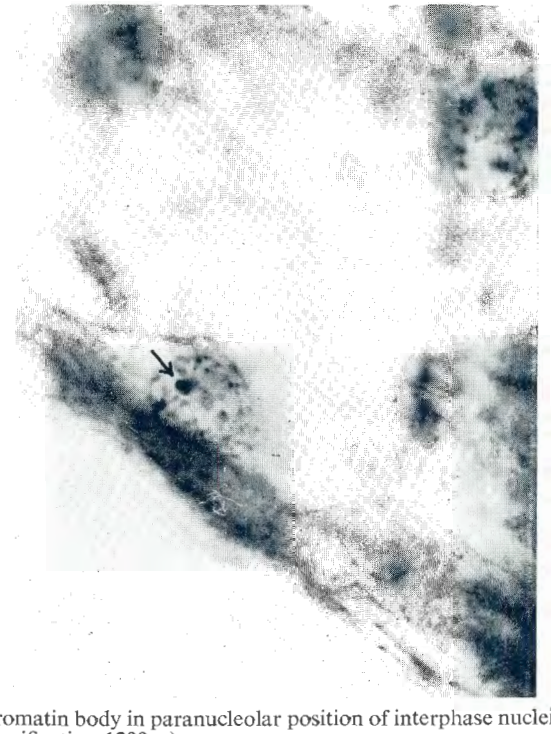

PLATE. 2.—Sex-chromatin body in paranucleolar position of interphase nuclei of rectal ampulla (arrow). (Magnification 1200×)

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