Nuclear Fusion in the Malpighian Tubes of a Blood-sucking Hemipteran

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High ploidy degrees have been cytophotometrically demonstrated in the epithelial cell nuclei of Malpighian tubes of blood-sucking reduviid hemipterans (Mello 1971, 1975). The Feulgen-DNA content of these nuclei increases as nymphae develop and arrests at the 5th nymphal instar (Mello and Raymundo 1977). Based on constancy of the totality of the Malpighian tube nuclei throughout the insect life under fully-nourished conditions, polyploidy could be proved to arise in this case by endomitosis (Mello 1978b). The maximal number of DNA endoreplications normally encountered in the nuclei of the Malpighian tubes of several reduviid species is five, irrespective of their chromosome numbers or sizes, and of the Feulgen-DNA content of their sperm line cells (Mello 1975, 1978a, Mello and Lima 1978). In the fat body cells of the reduviid, Rhodnius sp., however, high ploidy degrees appear induced by nuclear fusion, provided that insects had been reared under fasting conditions (Wigglesworth 1967).

In the present work, a feasible additional increase in ploidy degrees, promoted by nuclear fusion, was ascertained in the Malpighian tubes of blood-sucking hemipteran specimens subjected to starvation. The purpose was detecting whether nuclear fusion is a generally occurring mechanism induced by fasting conditions in these insects, and affects cells which had previously attained their normally maximal ploidy degrees by endomitosis.

Materials and methods

Malpighian tubes of 4.5-month starved adults and late 5th instar nymphae of Panstrongylus megistus Burmeister (Hemiptera, Reduviidae) were used. Fully-nourished specimens were employed as controls. Organs from at least 3 individuals were used for each experimental condition. The Malpighian tubes were fixed in acetic ethanol for 5 min, and very gently squashed in a drop of 45% acetic acid onto slides. Coverslips were removed with the dry ice technique. The material was then subjected to Feulgen reaction, hydrolysis being carried out with 4 N HCl at 26°C for 55 min. The preparations were mounted in Canada Balsam (nD = 1.54).

Feulgen-DNA values in arbitrary units were calculated for heterochromatin, euchromatin, and whole nuclei, multiplying the various areas by respective mean extinction values. The areas in \( \mu \text{m}^2 \) were obtained by means of planimetry of the
projected photographic image of the nuclei. Care was taken as not to consider for measurements nuclei disrupted during squash preparation. For cases where delimitation of the heterochromatic area was hard to define, determination of the Feulgen-DNA values was carried out only for total nuclear area. Light extinctions were determined with the multiple plug method. Microspectrophotometry was performed with a Zeiss photomicroscope equipped with a 01 photometer and an EMI 6256 photomultiplier. A Pol/Neofluar 63/0.90 objective, optovar 2, measuring diaphragm dia.=0.16 mm, field diaphragm dia.=0.3 mm, and an LD-Epiplan 16/0.30 condenser were used. The area of the specimen measured per plug point was 1.26 µm². Ten to fifty-five measurements per nucleus were carried out, depending on the heterogeneity of the stained chromatin. For normal nuclei containing one heterochromatic body, only one measurement was made on the chromocenter, due to its small size and homogeneous distribution of its stained material. The light extinction values were measured at λ=560 nm, wavelength at which the spectral absorption peaks were always detected.

The Feulgen-DNA values and nuclear areas were plotted as frequency histograms distributed in a scale of values in geometric progression. For the distribution of the Feulgen-DNA data, the scale was elaborated in such a way that between values 25 and 50, 50 and 100,100 and 200 etc. 10 intermediary classes were interspersed. This scale provides better visualization of doublings in Feulgen-DNA content and nuclear volumes (Bucher and Horisberger 1950, Hintzche 1954, Valeri 1962). For the case of the nuclear areas, the scale displayed 10 intermediary classes interspersed between values 10.00 and 15.87, 15.87 and 25.20, 25.20 and 39.99, etc. This choice was based on report by Bucher and Horisberger (1950) establishing that an increase of nuclear surface by a factor of \( \sqrt[4]{4} \) corresponds to a duplication of nuclear volume.

**Results**

The Malpighian tubes of the fully-nourished specimens of *P. megistus* display binucleate epithelial cells (Fig. 1). Their nuclei generally exhibit one small round heterochromatic mass (Fig. 2). In starved specimens nuclei looking like those of normal controls can be observed; however, fusing nuclei are also frequent (Fig. 3). The latter display a gradual loose of the well-defined boundaries which outline the heterochromatic areas (Figs. 4, 6, 7) as chromatin stainability usually changes after nuclear fusion. Intense heteropyknosis of the euchromatin develops in some of the fusing nuclei (Figs. 3, 5, 8), which is probably concerned with an eventual nuclear necrosis and cell death (Wigglesworth 1967).

The cytophotometric and karyometric data are also indicative of nuclear and, eventually, cell fusions in the Malpighian tubes of the starved insects. Figures 9 and 10 show data for adult specimens. Fifth instar nymphae depicted similar results. The Feulgen-DNA contents of whole nuclei of the normal controls and of the unfusing nuclei of the starved insects do not practically differ (Fig. 9). (The same was valid for their hetero- and euchromatic zones in separate.) Nuclear Feulgen-DNA values pertaining to 32C and 64C classes were found in both
cases. Determination and delimitation of these classes were carried out by comparing the profiles of the Feulgen-DNA frequency histograms raised in this work with those obtained with the two-wavelength cytophotometry for Feulgen-stained Malpighian tube and spermatid nuclei of same species (Mello 1975). On the other hand, most of the fusing nuclei of the starved specimens pertain to the 128 C Feulgen-DNA class; some nuclei are also distributed within the 64 C and 256 C classes. 64 C and 128 C nuclei probably arise by fusion of two 32 C and 64 C nuclei, respectively. This would be favoured by the fact that same ploidy degrees occur in both nuclei of the binucleate epithelial cells of the Malpighian tubes of P. megistus under normal nourishment conditions (Mello 1978—unpublished data). The nuclei classified as pertaining to the 256 C interval may have arisen by fusion of nuclei previously fused. Cell fusion, in this case, must have occurred.
Whether fusion of 128 C×64 C nuclei also exists, it could not be determined. If this kind of fusion had occurred, Feulgen-DNA values pertaining to the 256 C class would be shifted to the left side of the interval distribution. That was not the case, which may have been due to the analysis of few 256 C nuclei. In fact, their number was very small in the studied insects. The three 256 C nuclei

Figs. 4–8. Feulgen-stained fusing nuclei of the Malpighian tubes of starved P. megistus.. Heteropyknosis of the euchromatin is shown in Figs. 5 and 8. 4, 5, 6, 8=adults. 7=5 th nymphal instar. 4.×970, 5.×1390, 6.×1390, 7.×1240, 8.×1130. Bar=10 μm.
observed in starved *P. megistus* specimens were characterized by compactness and heteropyknosis of most of their euchromatin.

The distributions of values for the nuclear areas of the fully-nourished controls and of the unfusing nuclei of the starved specimens also do not differ (Fig. 10).

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**Fig. 9.** Frequency histograms of the nuclear Feulgen-DNA content of Malpighian tube epithelial cells of *P. megistus* subjected to fully-nourished (A) and fasting (B and C) regimens. A and B = unfusing nuclei; C = fusing nuclei; f = frequency.

**Fig. 10.** Frequency histograms of the nuclear areas of Malpighian tube epithelial cells of *P. megistus* subjected to fully-nourished (A) and starving (B and C) regimens. A and B = unfusing nuclei; C = fusing nuclei; f = frequency.
These nuclear areas appear distributed within classes which were named X and 2X. The nuclear areas of the fusing nuclei, on the other hand, were found to be distributed in the 2X, 4X, 8X, and 16X classes. The fusing nuclei which display areas pertaining to the X interval are those classified as pertaining to the 32C Feulgen-DNA class. The doubling intervals encountered for the nuclear areas generally agree with those for Feulgen-DNA contents of same nuclei. The only exceptions are the nuclei classified as pertaining to the 256C Feulgen-DNA class, the areas of which are situated in the 16X class, instead of the 8X one, and some 128C nuclei, which display areas pertaining to the 8X class.

**Discussion**

Visual, cytophotometric, and karyometric data indicate that nuclear fusion occurs in the Malpighian tubes of starved *P. megistus*, increasing the ploidy degrees previously attained by endomitosis (Mello 1975, 1978b). Based on results concerning Feulgen-DNA values and nuclear areas, it is assumed that cell fusion may also exist. Therefore, the phenomenon of cell and nuclear fusion described for the fat body cells of *Rhodnius sp.* (Wigglesworth 1967) is suggested to be of widespread occurrence for other organs of blood-sucking reduviids subjected to starvation. Even cells like those of adult and late nymphal Malpighian tubes, which had previously attained their maximal ploidy degrees by normal DNA endoreplication phenomenon are affected. The process of nuclear and cell fusion in the blood-sucking reduviids with starvation probably comes about because of the extremely low rate of their metabolism (Wigglesworth 1967).

The fact that the increase in nuclear size exceeds the threshold value expected from the number of doublings on Feulgen-DNA contents in 256C and some 128C fusing nuclei is maybe related to increase in nuclear hydrating levels (Pal-kovits and Fischer 1968), or inclusion of cytoplasmic matter in the nuclei during their fusion, or both. Considering that an extensive heteropyknosis is generally exhibited by the euchromatin of these nuclei, an increase in nuclear products such as RNA and non-histone proteins, typically indicating intense transcriptional activity, does not appear valid to be assumed.

Nuclear fusion, as described in this work, may contribute to reported finding of cytoplasmic “concretions” inside the nuclei of virus-affected Malpighian tube cells of *P. megistus* (Dolder and Mello 1978). Under prolonged viral infection conditions the reduviids do not feed blood and acquire cell ultrastructural features resembling those of non-infected specimens subjected to starvation (Mello *et al.* 1980).

**Summary**

When fifth instar nymphae or adults of the reduviid, *Panstrongylus megistus*, are subjected to a 4.5-month starvation period, nuclear fusion occurs in some of the epithelial cells of their Malpighian tubes. The affected nuclei had previously
attained their maximal ploidy degrees by endomitosis. Nuclear fusion was demonstrated visually or with cytophotometric and karyometric procedures. Fusing nuclei pertaining to 64 C, 128 C, and 256 C Feulgen-DNA classes were found in the starved specimens, whereas unfusing nuclei pertaining to 32 C and 64 C classes were detected in normal controls and fasted insects. The doublings on nuclear areas in the fusing nuclei generally agree with those on Feulgen-DNA data. Exceptions showing nuclear sizes exceeding those expected from Feulgen-DNA contents are maybe concerned with an increase in nuclear hydrating levels and/or inclusion of cytoplasmic matter in the nuclei during their fusion.

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