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# Cytochemical Studies of the Nuclei of the Venom Glands' Cells of *Apis mellifera* (Hymenoptera, Apidae)

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**Cytochemistry studies of the nuclei of the venom glands' cells of worker bees of *Apis mellifera* indicated that there is a higher activity in the young workers while there is a predominance of degenerative characteristics in the older workers. In addition, we demonstrated that there is an occurrence of differential nuclear synthetic activities between the cells of the distal and the proximal regions of the secretory filament and of the venom reservoir. Signs of a higher nuclear activity were evidenced at the distal regions of this gland in 14-day old workers, while at the more proximal regions of the venom gland of 40-day old workers we identified the most obvious signs of degeneration. Therefore, it was evident that the process of glandular degeneration begins at the distal region of the venom gland instead of beginning at the proximal region as had been established previously. In addition, characteristics of nuclear synthetic activities were noted in the cells of the proximal region of the reservoir; these cells were thought to be non-secretory.**

**Key words: venom gland, *Apis mellifera*, worker, cytochemistry**

## I. Introduction

The venom gland of worker bees of *Apis mellifera* is located at the posterior part of the abdomen, between the rectum and the ovaries. In general, this gland consists of a long, thin, convoluted, and bifurcated tubule.

The glandular cells compose the wall of the secretory filament and the distal portion of the reservoir. To date, the main alterations in the activity of these cells have been described at the ultrastructural level and only with regard to the cytoplasmic aspect of the glandular cells [6].

The nuclei of the glandular cells have been described as large and spherical in the secretory filament of young workers, and as having become small and irregular in the older workers [3, 4, 6], thus accompanying the loss of secretory characteristics that are observed during the aging process. In the reservoir, no nuclear alterations have been shown; these nuclei have been described as very small, flat, and condensed [3, 4, 6].

In the present study, cytochemistry techniques were employed in order to establish the location of DNA, RNA, and nucleoproteins in the nuclei of the venom glands of *Apis mellifera*. The object of this work was to provide details of the structural organization of the nuclei, mainly at the stage of nuclear metabolic activity, in relation to the cellular location of those components and the age of the worker.

## II. Materials and Methods

About 200 newly emerged workers were taken from a colony during the period from September to December of 1997 and 1998. These workers were marked in the thorax with synthetic aluminum paint (Suvinil) and returned to the colony. Marked workers of 14 and 40 days of age were then collected and dissected in order to remove the venom gland.

The venom glands were fixed in acetic acid: ethanol (3:1) for five minutes, dehydrated in an ethanol series of increasing concentration (70% to 100%), and subjected to routine techniques for their inclusion in historesin from Leica laboratories.

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Histological sections, 6 μm thick, were transferred to glass slides and used for the cytochemistry analyses. For the detection of DNA and RNA, we employed the dye toluidine blue (Synth) at pH 4.0 [20]. In order to observe the nucleoli, we employed two different techniques: 1) variation of the critical eletrolytes concentration, in which the material was stained with toluidine blue, pH 4.0, and exposed to 0.05 M magnesium chloride during eight, nine, and ten minutes [15, 16]; and 2) silver nitrate (Merck) imbuing—AgNOR—[10], which reveals proteins that constitute the nuclei with the nucleolar activity [5]. The material was analyzed and photographed under a Zeiss photomicroscope.

## III. Results

The staining procedure using toluidine blue showed that the nuclei of the cells of the distal (Fig. 1A, C) and proximal (Fig. 1B, D) regions of the secretory filament of the venom gland of *A. mellifera* exhibit metachromasy, independently of the age of the workers.

Throughout the secretory filament of workers at 14 days of age (Fig. 1A, B) the nuclei appear large and spherical, and are slightly more basophilic at the distal region than at the proximal region (Fig. 1B). In workers at 40 days of age (Fig. 1C, D), although still large, the nuclei assume an elongated shape at the distal region (Fig. 1C), while they become smaller, irregular, and with low metachromasy in the cells of the proximal region (Fig. 1D). In addition, at the secretory filament, the epithelial cells that form the intercellular canaliculi and the intima that surrounds the lumen of the tubules possess small and strongly dyed nuclei in all the glands examined (Fig. 1B).

Cells with small and metachromatic nuclei compose the wall of the venom reservoir. Nevertheless, there is an evident loss of metachromasy from the 14th (Fig. 1E, F) to the 40th day of age (Fig. 1G, H). In the venom glands of workers with 40 days of age, the nuclei of the cells of the reservoir appear more dilated and irregular (Fig. 1G, H) than in the glands of 14-day old workers (Fig. 1E, F).

The results obtained using the technique of variation of the critical electrolyte concentration showed that in the cells of the distal and proximal regions of the secretory filament of the venom glands of workers at the 14th (Fig. 1I, J) and 40th (Fig. 1K, L) day of age, the critical electrolyte concentration (CEC) point of the DNA is reached after the first time range used in this study: 8 minutes. At that point, in which the nuclei lose metachromasy, numerous nucleoli appear more evident at the distal portion of the secretory filament of workers at the 14th (Fig. 1I) and 40th (Fig. 1K) day of age than at the proximal region (Fig. 1J, L) for workers at the 14th and 40th day of age, respectively.

The results obtained with the technique of AgNOR show that in the cells of the secretory filament (Fig. 2A–D) as well as in those that form the wall of the reservoir (Fig. 2E–H) there is an imbuing of the nucleoli by the silver nitrate; thus, they appear very evident. However, in workers at 14 day of age, the nucleoli appear more numerous and evident in the cells of the distal region of the secretory filament (Fig. 2A) than at the proximal region (Fig. 2B). In the venom glands of 40-day old workers, these aspects become less evident but it is still possible to note differences between the distal (Fig. 2C) and the proximal regions (Fig. 2D); in the latter, the nucleoli are smaller in the glandular secretory portion as well as in the reservoir.

## IV. Discussion

The cytochemistry analyses performed on the venom gland of worker bees of *Apis mellifera* allowed the characterization of the nuclei from the distal and proximal regions of the reservoir and of the secretory filament. In general, at these glandular portions the nuclei are large and more spherical in workers with 14 days of age, which perform tasks inside the colony [9]. The nuclei become irregular at the proximal region of the gland of workers with 40 days of age, which spend most of the day outside the colony and execute tasks related to foraging [9].

The small nuclei of the cells that compose the reservoir's wall presented a shape that varied from spherical to flattened, depending on the age of the worker and on the region of the reservoir.

The present study demonstrated differences in the metachromatic characteristics of the nuclei of the secretory filament stained with toluidine blue, which presents a flat structural conformation and thus allows the phenomenon of metachromasy resulting in a blue violet stain. A specific loss of metachromasy at the proximal region during the period from 14 to 40 days of age was observed. This fact suggests that, at 40 days, a higher number of phosphate groups of the nuclear DNA are blocked by bonds with basic proteins, thus showing a higher chromatin condensation and a reduction in nuclear activity [19]. The same authors used the toluidine blue stain on testicle cells of the amphibian *Hyla ranki* and showed varying degrees of nuclear basophily in relation to chromatin condensation in cells undergoing cytodifferentiation. According to them, immature spermatids showed a deep blue stain while the mature spermatozoids displayed light green coloration. Other results describing the variations in intensity of the staining reaction with toluidine blue [13–16] have also been attributed to the progressive blocking of the phosphate groups of the DNA by amino groups of basic proteins.

The nucleoli, evidenced by the AgNOR technique, appeared clearer and in higher numbers in workers at 14 days of age; the nucleoli become smaller and less visible in 40-day old workers. This indicates a reduction in the process of rRNA synthesis in relation to age and, consequently, a diminution in the rate protein synthesis that is more accentuated at the proximal region than at the distal region of the secretory filament. According to some authors [8], the variation in the shape of the nucleolus is related to variations in its transcriptional activities. In this way, the more active stage of the glandular cells of the bees studied occurred in workers at 14 days of age. The larger nucleolar size in these bees

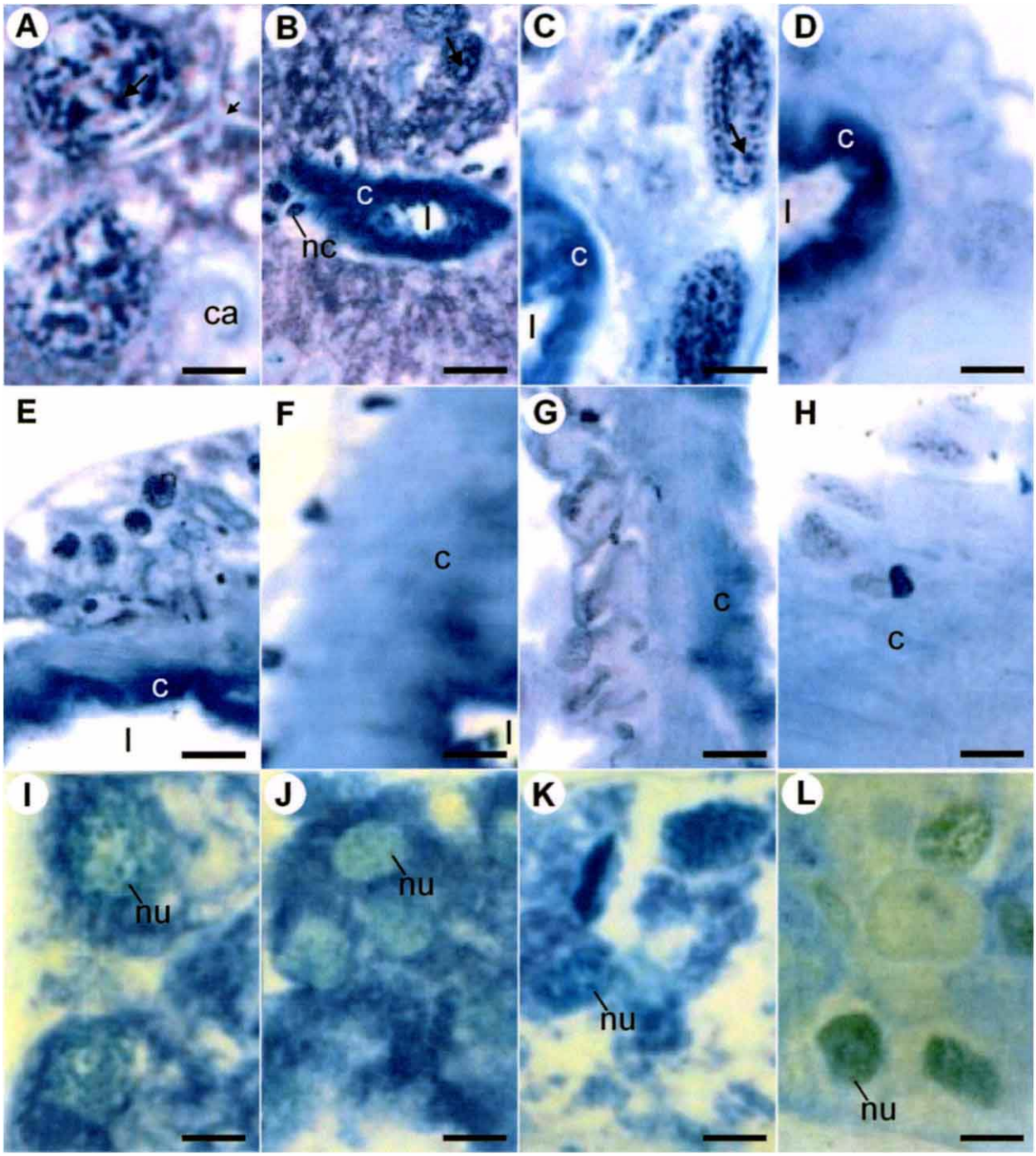


**Fig. 1.** Secretory filament and venom reservoir of the venom glands of worker bees of *Apis meliffera* L. at 14 and 40 days of age. 1. Toluidine blue stain. Secretory filament: (**A**) Distal region (14 days), (**B**) Proximal region (14 days), (**C**) Distal region (40 days), (**D**) Proximal region (40 days); Venom reservoir: (**E**) Distal region (14 days), (**F**) Proximal region (14 days), (**G**) Distal region (40 days), and (**H**) Proximal region (40 days). 2. Variation of the critical electrolyte concentration. Secretory filament. (**I**) Distal region (14 days), (**J**) Proximal region (14 days), (**K**) Distal region (40 days) and (**L**) Proximal region (40 days). Large arrows, chromatinic granule; small arrows, intercellular space; c, cuticle; ca, canaliculum; l, lumen; nc, canaliculum cell; nu, nucleoli. Bars=0.5 μm (**A, C, I, J, K, L**), 10 μm (**B, D, E, F, G, H**).

strengthens this hypothesis, since in cells with a high rate of ribosome production, the nucleoli are large and complex, while in most cells with a low rate of ribosome production the nucleoli are small and possess a simple structure [18].

In the case of the reservoir, the basophilic response of the nuclei suggested the occurrence of chromatin condensation in 40-day old worker bees but it did not show evidence of differences among the nuclei of the distal and proximal

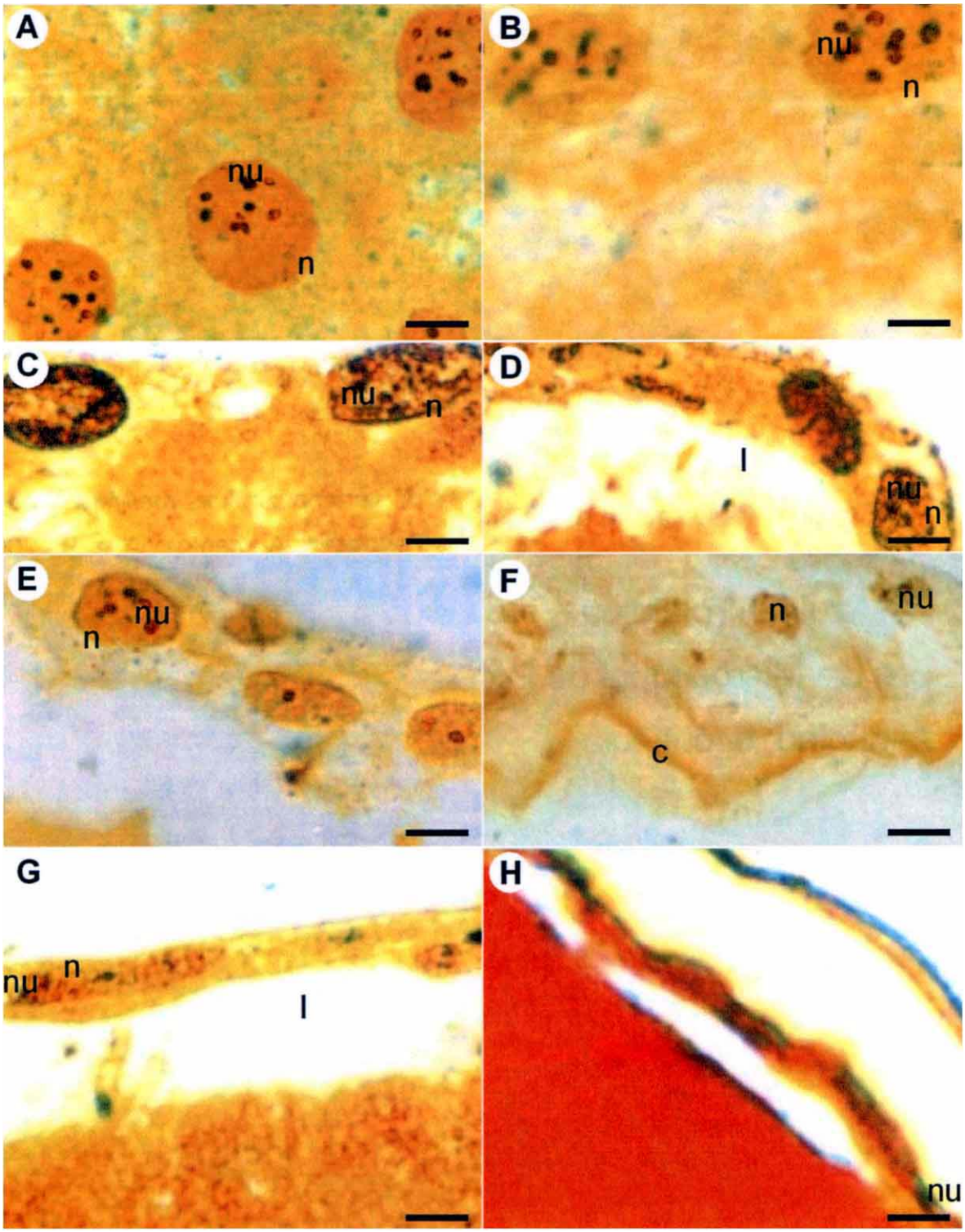


**Fig. 2.** Secretory filament and venom reservoir of the venom glands of worker bees of *Apis meliffera* L. at 14 and 40 days of age. AgNOR Technique. Secretory filament: (**A**) Distal region (14 days), (**B**) Proximal region (14 days), (**C**) Distal region (40 days), (**D**) Proximal region (40 days); Venom reservoir: (**E**) Distal region (14 days), (**F**) Proximal region (14 days), (**G**) Distal region (40 days) and (**H**) Proximal region (40 days). nu, nucleoli; c, cuticle; l, lumen; n, nuclei. Bars=0.5 μm (**A–E**), 10 μm (**F–H**).

regions at each age range studied. Such results suggest that, at least in the 14-day old workers, the nuclei of those two regions showed evidence of secretory activity. This observation contradicts the results of several other researchers [2, 6, 7, 11], according to whom only the cells of the distal region of the reservoir display secretory characteristics. In the cells

of the reservoir, the nucleoli that predominated in the workers at 14 days of age were also good indicators of the occurrence of synthetic activities. This observation was confirmed by the results obtained with the technique of variation in CEC.

Therefore, the techniques employed revealed nuclear changes related to the aging process of the workers, providing evidence of the existence of a differential synthesis among young and old workers, with this process being more intense in the younger ones. This observation agrees with the results obtained by other authors [2, 7, 17], according to whom the period of glandular activity is during the 5th and the 15th days after the emergence of the adult, and with the process of cellular degeneration of the venom gland beginning at the 20th day of age during summer or at the 30th day of age during winter [2, 7].

The process of degeneration, based on the nuclear and nucleolar modifications found, can be considered to be more advanced at the proximal region of the gland of worker bees at 40 days of age. Such modifications correspond to the loss of metachromasy and to the diminution of the nuclear size as well as the lower visibility, definition, and number of the nucleoli. Therefore, this work confirms the morphological results we obtained [1], which established that the degeneration process of the venom gland commences at the proximal region at the age of 40 days, thus contradicting the propositions made by other authors who suggested that this process starts at the distal glandular region and ends in the proximal region [7]. It was also shown in this study that there is a differential metabolic activity between the nuclei of the distal and proximal regions of workers with the same age, with the ones in the distal region being more active than in the proximal region at both 14 and 40 days of age.

Finally, besides indicating a differential activity between the cells of the proximal and the distal regions of the secretory filament, our data also extend this function to the cells of the proximal region of the reservoir. The first studies concerning the venom gland of *Apis mellifera* proposed that only the distal region of the secretory filament had a secretory function [12]. Later works showed that the whole glandular filament and the distal portion of the reservoir had a secretory function [3, 4, 6].

## V. References


1. Abreu, R. M. M., Silva de Moraes, R. L. M. and Malaspina, O. (2000) Histological aspects and protein content of the venom glands of *Apis mellifera* L. workers: Effect of electrical shocks in Summer and Winter. *The Journal of Venomous Animals and Toxins* 6; 87–98.
2. Autrum, H. and Kneitz, H. (1959) Die Giftsekretion in der Giftdruse der Honigbiene in Abhangigkeit vom Lebensalter. *Biol. ZBL.* 78; 598–602.
3. Bridges, A. R. and Owen, M. D. (1976) Fine structure of the honey bee (*Apis mellifera* L.) venom gland and reservoir—A system for the secretion and storage of a naturally produced toxin. *Microscopial Society of Canada* 4; 50.
4. Bridges, A. R. and Owen, M. D. (1984) The morphology of the honey bee (*Apis mellifera* L.) venom gland and reservoir. *J. Morphol.* 181; 69–86.
5. Cassab-Hassan, P. M. (1977) Efeito do estresse nos núcleos das células dos túbulos de Malpighi de triatomíneos. Dissertação de Mestrado em Genética, Instituto de Biociências, UNESP, São José do Rio Preto, São Paulo.
6. Cruz-Landim, C. and Kitajima, E. W. (1966) Ultraestrutura do aparelho venenífero de *Apis* (Hymenoptera, Apidae). *Mem. Inst. Butantan Simp. Internac.* 33; 701–710.
7. Cruz-Landim, C., Baldissera, S. and Beig, D. (1967) Degeneração da glândula de veneno de *Apis* durante o verão e inverno. *Rev. Bras. Biol.* 27; 355–361.
8. Fakan, S. and Hernandez-Verdum, D. (1986) The nucleolus and the nucleolar organizer regions. *Biol. Cell.* 56; 189–206.
9. Free, J. B. (1977) A organização social das abelhas (*Apis*). EDUSP, São Paulo, pp. 10.
10. Howell, W. M. and Black, D. A. (1980) Controlled silver staining of nucleolus organizer regions with protective colloidal developer: I. Step method. *Experientia* 36; 104–105.
11. Kaiser, E. and Michl, H. (1958) Die Biochemie der Tierischen Gifte. Frang Deuticke, Wien.
12. Kerr, W. E. and Lello, E. (1962) Sting glands in stingless bees a vestigial character (Hymenoptera, Apidae). *J. N. Y Entomol. Soc.* 70; 190–214.
13. Lison, L. (1960) Histochimie et Cytochimie Animals—Principles et Methods. 1., 3a ed., Gauthier-Villars, Paris.
14. Mello, M. L. S. (1987) Nuclear cytochemistry and polarization microscopy of the spermatozoa of *Triatoma infestans* Klug. *Z. Mikrosk. Anat. Forsch Leipzig.* 101; 245–250.
15. Mello, M. L. S. and Vidal, B. C. (1973) Linear dichoism and anomalus dispersions of birefringence on sperm heads. *Acta Histochemica.* 45; 109–114.
16. Mello, M. L. S. Vidal, B. C. Dantas, M. M. and Monteiro, A. L. P. (1993) Discrimination of the nucleolus by a crytical eletrolyte concentration method. *Acta Histochem. Cytochem.* 26; 1–3.
17. Nogueira, R. H. F. (1976) Estudo e seleção para redução do tamanho da glândula ácida de *Apis mellifera* (abelhas africanizadas) (Hymenoptera, Apidae). Dissertação de Mestrado. Faculdade de Medicina de Ribeirão Preto, São Paulo.
18. Schwarzacher, H. G. and Wachtler, F. (1993) The nucleolus. *Anat. Embryol.* 188; 515–536.
19. Taboga, S. R. and Dolder, M. A. H. (1994) Basic nuclear protein substitution during spermiogenesis in *Hyla ranki* (Amphibia, Anura, Hylidae). *Ciência e Cultura* 46; 161–163.
20. Vidal, B. C. and Mello, M. L. S. (1987) Biologia Celular, São Paulo, 347 p.