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Acid phosphatase activity distribution in salivary glands of triatomines (Heteroptera, Reduviidae, Triatominae)

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ABSTRACT. Acid phosphatase activity (Gömori technique) in salivary gland cells was investigated in adult insects (males and females) of four species of triatomines: *Triatoma infestans*, *Panstrongylus megistus*, *Rhodnius neglectus*, and *Rhodnius prolixus*. Binucleated cells with bulky and polyploidy nuclei were detected, with acid phosphatase activity in the heterochromatin and nucleolus, which showed the most intense response. Thus, the activity of these phosphatases during rRNA molecule transcription, possibly in the nucleolar fibrillar center, is suggested. The difference in reactivity found among salivary glands is associated with the cellular metabolism of these regions and, probably, with the biosynthesis of their different secretions. This must be essential in maintaining the hematophagy of triatomines.

Key words: Acid phosphatase, Salivary gland, Nucleolus, Triatominae, Heteroptera

INTRODUCTION

Triatomines (Heteroptera, Reduviidae, Triatominae) are hematophagous insects of medical interest, as they may act as invertebrate hosts of the hemoflagellate protozoan *Trypanosoma cruzi*, the causative agent of Chagas' disease. This is one of the major endemic parasitic diseases in Latin America, where it is a significant cause of heart disease affecting about 11 million individuals in endemic areas (Tartarotti et al., 2004). Triatomines are also hosts of *T. rangeli*, a hemoflagellate protozoan that develops in the salivary glands of these insects and is transmitted by their bite. Although not pathogenic, it causes mixed infections with *T. cruzi* (Oliveira and DeSouza, 1999).

Triatomines of the *Triatoma*, *Panstrongylus* and *Diptelogaster* genera have a salivary gland complex that is physiologically and histologically composed of three pairs of well-differentiated glands: anterior (D1), median (D2) and posterior (D3) (Barth, 1954; Lacombe, 1999). Triatomines of the *Rhodnius* genus have two close and independent salivary glands: the larger is reddish and elongated (principal gland), while the smaller is round and translucent (accessory gland) (Meirelles et al., 2003). The salivary glands of triatomines are covered by a net of small, highly branched tracheae and all cells have bulky nuclei with accentuated polyploidy.

The saliva of hematophagous insects abounds in proteins and both anticoagulant and hemolytic enzymes, which facilitate feeding (Barth, 1954; Hellmann and Hawkins, 1964, 1965; Ribeiro and Garcia, 1980; Smith et al., 1980; Ribeiro, 1987; Ribeiro et al., 1993; Ciprandi et al., 2003). Some of these enzymes are phosphatases, responsible for the hydrolysis of organic phosphate esters, whose activity has been demonstrated in the liver of rats (Siebert, 1966), nucleoli of human cells (Soriano and Love, 1971), *Allium cepa* (Sanches-Pina et al., 1978), maize roots (Deltour et al., 1981), salivary glands of *Drosophila melanogaster* (Jones and Bowen, 1993), and in gerbil (*Meriones unguiculatus*) prostate glands of both sexes (Custódio et al., 2004).

da Cruz-Landim et al. (2002) reported nuclear acid phosphatase activity in the somatic (intra-ovariolar and stromatic) and germ cells of differentiating honey bee worker ovaries, as well as in the midgut cells of metamorphosing bees. The activity was evidenced in chromatin, nucleoli or nucleoplasm, and a relationship was postulated between the intensity of the reaction and the level of transcription in bee cell chromatin.

In triatomines, especially in Malphigian tubule cell nuclei, studies of nuclear phosphatase activity have shown its action in heterochromatin and the nucleolus, and also suggest a relationship with transcription phenomena (de Azeredo-Oliveira and Mello, 1997, 1998).

As acid phosphatase activity has been detected in high-metabolism nuclei, the study of its distribution in triatomine salivary glands can be considered a differential parameter of cell metabolism.

Beyond the presence of acid phosphatase in high-metabolism organs, the lack of cytochemical studies in the literature and the medical interest in triatomines, the purpose of the present study was to investigate the distribution of acid phosphatase in salivary gland cells, with emphasis on nuclear activity, of adult triatomines (males and females) of the species *Triatoma infestans*, *Panstrongylus megistus*, *Rhodnius neglectus*, and *R. prolixus*.

MATERIAL AND METHODS

Unfixed whole salivary glands of adult, male and female *T. infestans*, *P. megistus*, *R.*

neglectus, and *R. prolixus* (Heteroptera, Reduviidae, Triatominae) were studied. Triatomines, free of contamination, were provided by the Insectary of the Araraquara Special Health Service, Araraquara, SP, Brazil, organ of the Department of Epidemiology, São Paulo Public Health School, São Paulo University, SP, Brazil. The Gömori technique (Gömori, 1950) was used to demonstrate acid phosphatase activity and salivary glands of 30 insects (15 males and 15 females) of each species were analyzed. The material was incubated in a medium containing sodium β-glycerophosphate substrate and lead nitrate. The enzyme released phosphate from the substrate resulting in the accumulation of an insoluble lead phosphate precipitate at the site of enzyme activity. This precipitate was treated with 1% ammonia sulfide solution converting it into lead sulfide which was seen through the light microscope as a dense brown granular accumulation. At the same time, a reaction control was performed using an incubation medium without substrate (sodium β-glycerophosphate). The preparations were examined with a Zeiss Jenaval photomicroscope and photographed, after which the negatives were developed and printed in specialized laboratories.

RESULTS

Acid phosphatase activity was evidenced in all pairs of salivary glands of males and females of *T. infestans* (Figure 1A-L), *P. megistus* (Figure 2A-L), *R. neglectus* (Figure 3A-F), and *R. prolixus* (Figure 3G-L).

In males (Figure 1A-F) and females (Figure 1G-L) of *T. infestans*, the most intense activity was observed in anterior salivary glands (Figure 1A,G). A predominance of binucleated cells was found in the anterior (D1) (Figure 1B,H) and median (D2) (Figure 1C,I) salivary glands, and of mononucleated cells in posterior glands (D3) (Figure 1E,F,K,L), with bulky and polyploidy nuclei. In the nucleus, the most intense activity, based on the precipitation of lead sulfide, occurred in nucleolar corpuscles. A clear association with heterochromatin was also observed, revealed by a light halo with less intense activity. A thin granulation occurred in the nucleus, which is characteristic of the presence of dispersed euchromatin. Males had larger corpuscles than females, but fewer in number.

In *P. megistus*, just as in *T. infestans*, D1 (Figure 2B,H) and D2 (Figure 2C,I) had binucleated cells, while D3 (Figure 2E,F,K,L) had mononucleated ones, with bulky and polyploidy nuclei. In both sexes, the enzyme reaction was more intense in the anterior gland (Figure 2A,G), and, in all glands, the most intense lead sulfide precipitation was observed in nucleolar corpuscles, whose size and number varied between the two sexes and among all three salivary glands. Small corpuscles occurred in D1 (Figure 2B) and D2 (Figure 2C) of males, and in all glands of females (Figure 2H,I,K,L); large ones occurred in D2 (Figure 2C) and D3 (Figure 2E,F), in males alone. In the chromatin, enzyme activity was less intense: heterochromatin staining was observed as a light halo in association with nucleolar corpuscles, and euchromatin staining was seen as a thin granulation dispersed in the nuclei.

In *R. neglectus* (Figure 3A-F), as in *R. prolixus* (Figure 3G-L), salivary glands of males (Figure 3A-C,G-I) and females (Figure 3D-F,J-L) displayed acid phosphatase activity distributed throughout the gland, the most intense activity being detected in the principal one (Figure 3A,D,G,J). Binucleated cells were observed in both salivary glands and activity was detected in both the nucleus and cytoplasm. This activity was revealed by the presence of numerous small nucleolar corpuscles, derived from the precipitation of lead sulfide.

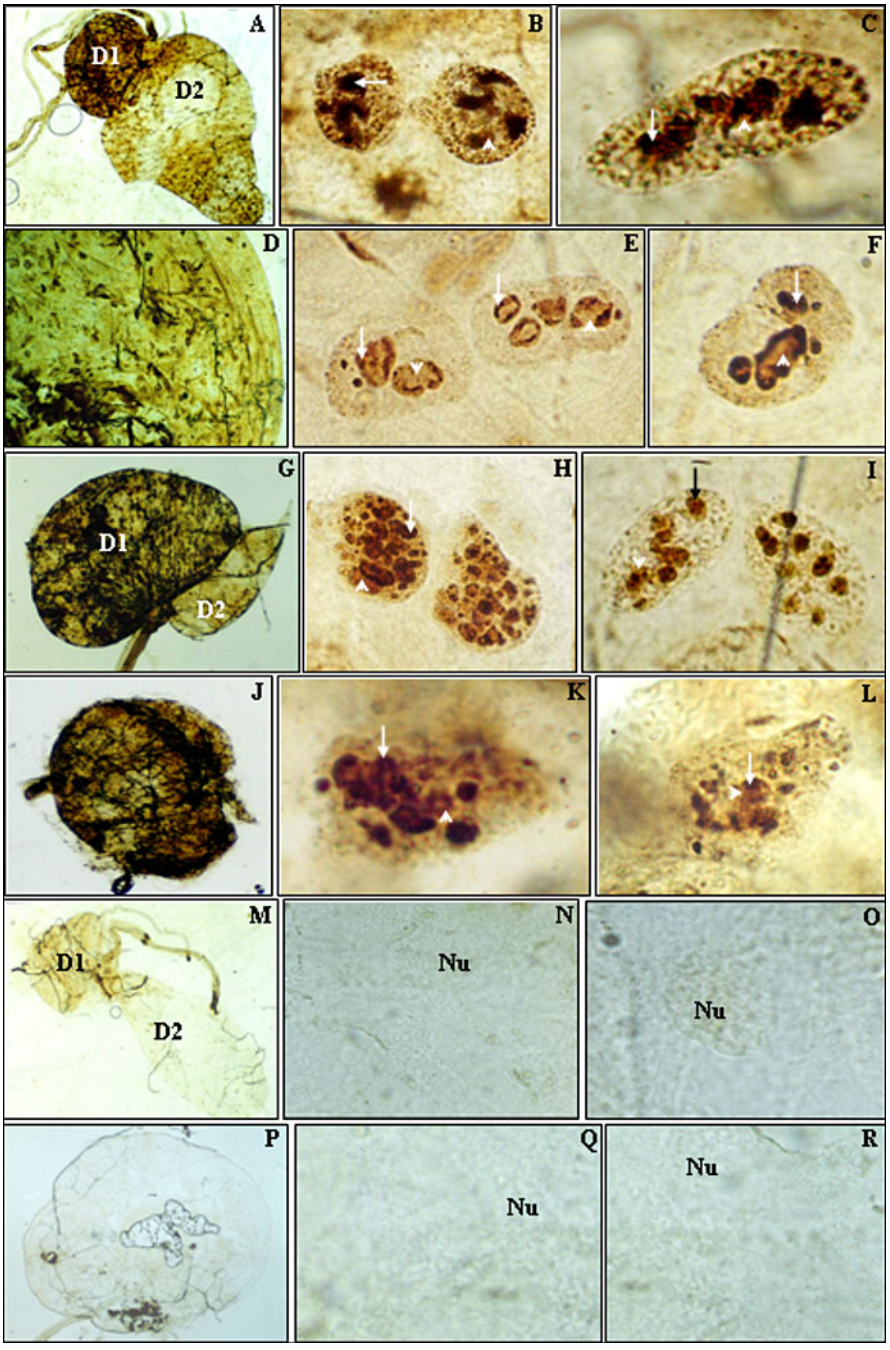

Figure 1. A-L. Salivary gland complex of adult *Triatoma infestans* submitted to cytochemical reaction for acid phosphatase (males (**A-F**) and females (**G-L**)). **A,G.** General view of anterior (D1) and median (D2) salivary glands; **D,J.** General view of posterior gland; **B,C,E,F,K,L.** Detail of the nuclei from anterior (**B,H**), median (**C,I**) and posterior salivary glands (**E,F,K,L**). Notice nucleolar corpuscles (arrows) in association with heterochromatin (arrowheads) with lead sulfide precipitate. **M-R.** Salivary gland complex of adult *T. infestans* (males), submitted to cytochemical reaction controls for acid phosphatase. **M.** General view of anterior (D1) and median (D2) salivary glands; **P.** General view of posterior gland; **N,O,Q,R.** Detail of the nuclei from anterior (**N**), median (**O**) and posterior salivary glands (**Q,R**). Notice the absence of activity in the nucleus (Nu). Magnification: **A,G,J,M,P** = 43X; **D** = 148X; **B,C,E,F,H,I,K,L,N,O,Q,R** = 1344X.

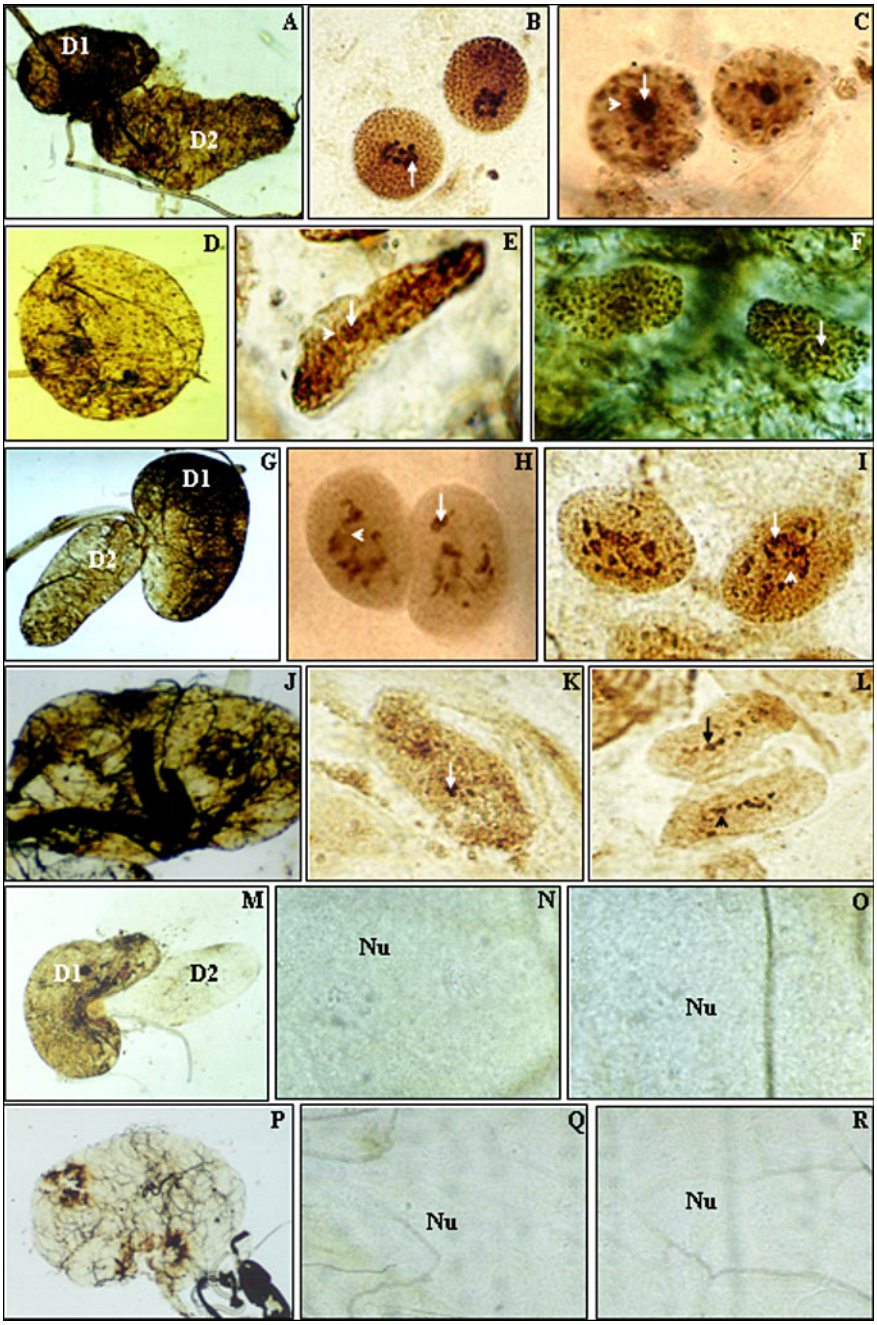

Figure 2. A-L. Salivary gland complex of adult *Panstrongylus megistus* submitted to cytochemical reaction for acid phosphatase (males (**A-F**) and females (**G-L**)). **A,G.** General view of anterior (D1) and median (D2) salivary glands; **D,J.** General view of posterior gland; **B,C,E,F,K,L.** Detail of the nuclei from anterior (**B,H**), median (**C,I**) and posterior salivary glands (**E,F,K,L**). Notice nucleolar corpuscles (arrows) in association with heterochromatin (arrowheads) with lead sulfide precipitate. **M-R.** Salivary gland complex of adult *P. megistus* (males) submitted to cytochemical reaction controls for acid phosphatase. **M.** General view of anterior (D1) and median (D2) salivary glands; **P.** General view of posterior gland; **N,O,Q,R.** Detail of the nuclei from anterior (**N**), median (**O**) and posterior salivary glands (**Q,R**). Notice the absence of activity in the nucleus (Nu). Magnification: **A,D,G,M,P** = 43X; **J** = 50X; **B,C,E,F,H,I,K,L,N,O,Q,R** = 1344X.

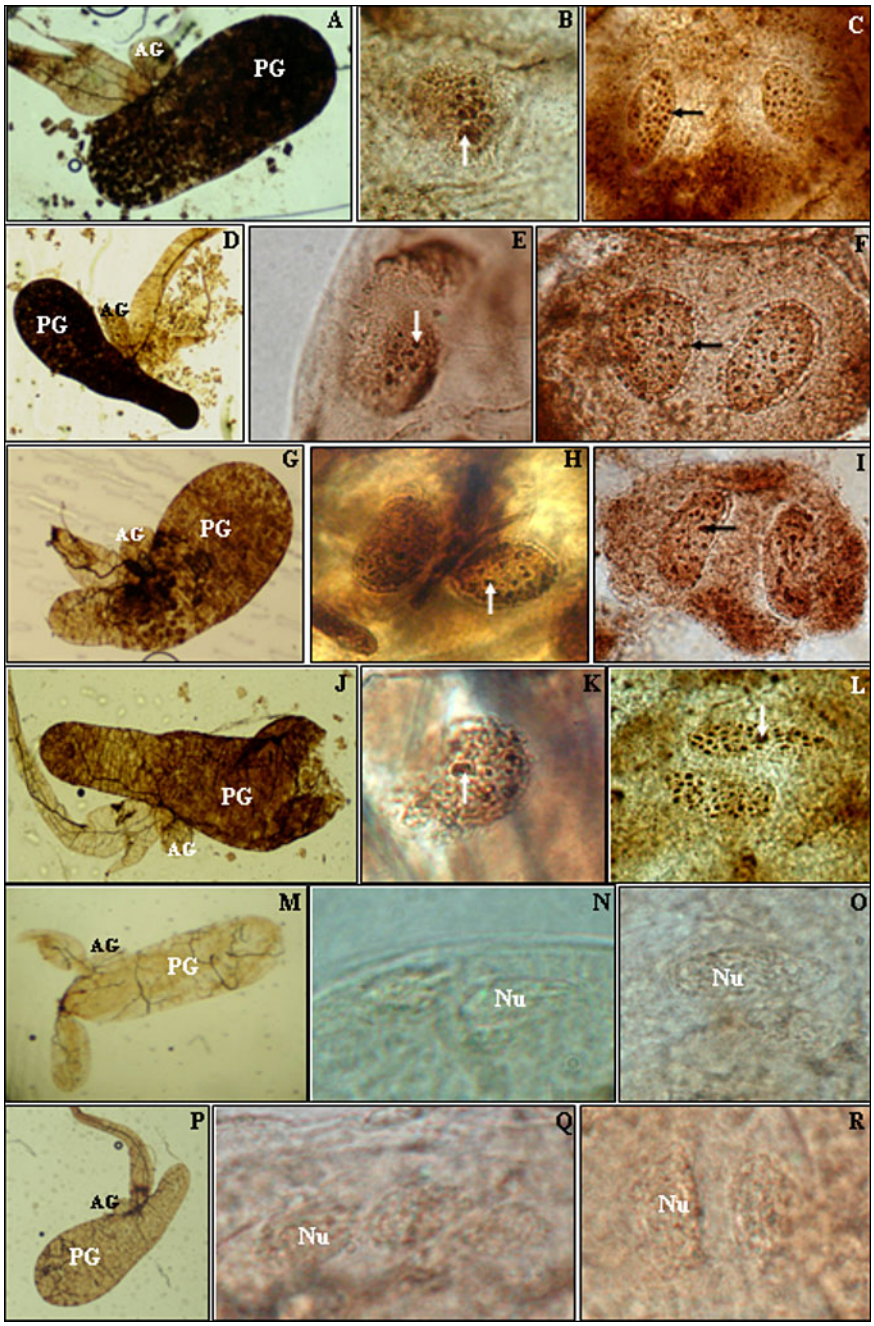

Figure 3. A-L. Salivary gland complex of adult *Rhodnius neglectus* (**A-F**) and *R. prolixus* (**G-L**) submitted to cytochemical reaction for acid phosphatase (males (**A-C,G-I**) and females (**D-F,J-L**)). **A,D,G,J.** General view of principal (PG) and accessory (AG) salivary glands; **B,C,H,I,E,F,K,L.** Detail of the nuclei from principal (**B,C,H,I**) and accessory glands (**E,F,K,L**). Notice nucleolar corpuscles (arrows) in association with heterochromatin (arrowheads) with lead sulfide precipitate. **M-R.** Salivary gland complex of adult *Rhodnius neglectus* (**M-O**) and *R. prolixus* (**P-R**) (males) submitted to cytochemical reaction controls for acid phosphatase. **M,P.** General view of principal (PG) and accessory (AG) salivary glands; **N,O,Q,R.** Detail of the nuclei from principal (**N-Q**) and accessory glands (**O-R**). Notice the absence of activity in the nucleus (Nu). Magnification: **A,D,G,J,M,P** = 43X; **J** = 50X; **B,C,E,F,H,I,K,L,N,O,Q,R** = 1344X.

A total absence of enzyme activity was observed in both cytoplasm and nucleus of salivary glands submitted to the control technique (incubation medium without sodium β-glycerophosphate), in both sexes of the four species of triatomines studied [*T. infestans* (Figure 1M-R), *P. megistus* (Figure 2M-R), *R. neglectus* (Figure 3M-O), and *R. prolixus* (Figure 3P-R)].

DISCUSSION

Acid phosphatase activity, phosphatase activity at a pH of 4.5-6.0, was detected in the salivary gland cells of adult insects (males and females) of the species *T. infestans*, *P. megistus*, *R. prolixus*, and *R. neglectus* (Heteroptera, Reduviidae, Triatominae).

In *T. infestans* and *P. megistus*, the enzyme reaction was more intense in the anterior salivary gland (D1), and in *Rhodnius* (*R. neglectus* and *R. prolixus*), in the principal gland. The difference in reactivity found between the glands is associated with the cellular metabolism of these regions and, probably, with the biosynthesis of their different secretions.

An increase in nuclear mass was observed by the presence of bulky and polyploidy nuclei in all salivary glands, besides binucleated cells in D1 and D2 in *T. infestans* and *P. megistus*, and in all glands of *Rhodnius* (*R. neglectus* and *R. prolixus*). This is because these organs are highly active and, in order to accelerate and regulate cellular regeneration after saliva secretion, depend on bulky nuclei with extensive surfaces (Barth, 1954). This intense salivary gland activity can be corroborated by the presence of numerous nucleolar corpuscles.

In the salivary gland cells of triatomines, granules derived from the precipitation of lead sulfide, indicative of phosphatase activity, were observed in chromatin (euchromatin and heterochromatin) and nucleolar corpuscles; in this case, the enzyme reaction was more intense. This corroborates previous studies where the same granules that were shown to be positive for acid phosphatase activity were also revealed by silver impregnation and by the critical electrolyte concentration variant technique (Lima-Oliveira, 1997; Anhê, 2005).

However, effective staining for nuclear acid phosphatase activity is controversial. While some researchers do not hesitate to assume that there may be authentic enzyme sites in the nucleus (Love et al., 1969; Soriano and Love, 1971; Deltour et al., 1981), others believe there may be a particular affinity between chromatin and lead ions (Deane, 1963; Remy et al., 1975) or a lysosomal phosphatase diffusion into the nucleus (Anastasia-Sawicki and MacIntyre, 1976; Washitani and Sato, 1976).

On the other hand, the validity of the nuclear presence of acid phosphatase is emphatically advocated by Deltour et al. (1981), who worked with maize root cells and studied the presence of these enzymes at the biochemical level as well as at the cytochemical and electron microscopy levels. These authors suggested that nuclear transcription rate, acid phosphatase activity level and the accumulation of nuclear inorganic phosphate are all correlated. Intense acid phosphatase activity was found in those nuclei showing an elevated metabolism.

As for nuclear acid phosphatase activity such as that observed in the present study, the literature indicates that it has been detected in some other systems. Siebert (1966) found this activity in rat cells at a biochemical level. Ultrastructurally, acid phosphatase has been reported in the nucleoli of human cells (Soriano and Love, 1971) and the nucleoli of *Allium cepa* (Sanches-Pina et al., 1978). In the latter study, activity was observed near the nucleolus organizer region and in the fibrillar component. High-acid phosphatase activity was found in nuclei with an el-

evated metabolism, and the authors suggested a relationship between nuclear transcription rate, acid phosphatase activity level and nuclear organic phosphate accumulation.

Acid phosphatase activity in the nuclei of triatomine cells has also been detected in the Malpighian tubules of *T. infestans* (de Azeredo-Oliveira and Mello, 1997, 1998) using light and electron microscopy. According to the authors, much of the enzyme activity occurred in the heterochromatin and nucleolus, and this may be related to transcription phenomena in these nucleolar regions. Enzyme activity varied according to tubule region. The localization, shape and number of heterochromatic bodies and nucleolar morphology also varied.

The nuclear enzyme activity found in the salivary glands of *T. infestans*, *P. megistus*, *R. prolixus*, and *R. neglectus* corroborated previous findings obtained in the Malpighian tubules of *T. infestans* (de Azeredo-Oliveira and Mello, 1997, 1998), human cells (Soriano and Love, 1971), *Allium cepa* (Sanches-Pina et al., 1978), and somatic and germ cells of differentiating honey bee worker ovaries (da Cruz-Landin et al., 2002). Although the organs studied were different, a role for acid phosphatase in the nuclear matrix could be assumed, as the process was detected in both the nucleolus and chromatin. Thus, phosphatase activity during rRNA transcription, possibly in the nucleolar fibrillar center, is suggested.

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