

## CHANGES IN THE NUCLEAR PHENOTYPES OF *Triatoma infestans* KLUG, INDUCED BY THERMAL SHOCKS\*

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### ABSTRACT

The nuclear phenotypes of Malpighian tubule epithelial cells of 4th instar nymphs of *Triatoma infestans* Klug were subjected to thermal shocks at 0°C and 40°C for one and 12 hours and were analyzed just after the stress, and three and 30 days later. In addition to the phenotypes usually described for control specimens, pyknotic, giant and vacuolized nuclei, as well as nuclei exhibiting an apparent unravelling of chromocenter chromatin were detected in the test specimens. The decrease in number of cell nuclei, which occurred at shorter or longer intervals after the thermal shocks, were assumed to have been caused not only by degeneration of the cells bearing vacuolized and pyknotic nuclei, but also by nuclear fusion. It was assumed that the giant nuclei, which appeared predominantly in the specimens subjected to the hyperthermal shock, and the presence of *hsp*, played a role in the insect's unchanged survival under this specific experimental condition. Although many of the nuclear characteristics found in the specimens subjected to thermal shock have also been observed with other stress agents, the heat shock was much more effective in eliciting the apparent chromocenter heterochromatin unravelling response in *T. infestans*. Based on the types and frequencies of nuclei observed after the hypothermal shock and on the drop in insect survival caused by this treatment, it seems that, if cryoprotectors are present in *T. infestans*, they are not efficient enough to protect the insect from a relatively prolonged hypothermal shock.

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## INTRODUCTION

The nuclear phenotypes of cells from organs such as the Malpighian tubules of the blood-sucking hemipteran, *Triatoma infestans* Klug, have been largely described visually (Mello, 1971, 1975) or with image analysis (Mello, 1978). Under stress conditions such as starvation or treatment with heavy metals, these phenotypes may undergo marked changes (Kubrusly, 1984; Andrade and Mello, 1987; Mello, 1989). The stressor-elicited effects at the nuclear level generally affect the chromatin packing states and sometimes induce nuclear fusion. In addition they promote various degrees of degeneration in parts of the nuclear population (Kubrusly, 1984; Mello, 1983, 1989; Andrade and Mello, 1987).

Although changes in nuclear phenotypes have not been investigated in *T. infestans* specimens subjected to heat shock, other changes resulting from environmental stress may occur under these conditions. In other cell systems a response typically seen with heat shock is observed after treatment with stressors such as amino acid analogs, arsenite or heavy metals (Hightower *et al.*, 1985; Schlesinger, 1985).

In *T. infestans*, both survival and molting incidence are affected by thermal shock depending upon the temperature and the duration of the treatment (Rodrigues *et al.*, 1991). A shock of 0°C for 12 hr, for instance, has been found to elicit a very deleterious effect on insect survival and shocks of 40 and 0°C lasting as little as one hr affect the hormonal balance which controls molting. However, as mentioned before, no studies have been published on the nuclear phenotypes occurring under these circumstances.

The present investigation was thus undertaken to study the nuclear phenotypes of Malpighian tubule cells of *T. infestans* which were subjected to hypo- and hyperthermal shock conditions. The conditions used have been reported to elicit changes in the insect's survival and molting incidence (Rodrigues *et al.*, 1991). The objective was to establish to what extent changes in nuclear images may be associated with changes in the insect's biology due to thermal stress.

## MATERIALS AND METHODS

Fully-nourished 4th instar nymphs of *T. infestans* Klug (Hemiptera, Reduviidae) reared in the laboratory of SUCEN (Mogi-Guaçu, SP) were used. Experimental specimens were subjected to stress at temperatures of 0°C and 40°C for 1 hr and 12 hr. These temperatures were chosen because of operational convenience and the need to use temperature extremes in relation to the control (Rodrigues *et al.*, 1991). Temperatures higher than 40°C were avoided since a previous test indicated that the insects could barely tolerate them and temperatures lower than 0°C were also avoided since there is no report on the existence of cryoprotectors in the hemolymph of *T. infestans*.

After the thermal shocks the insects were returned to control conditions. Controls were maintained at 30°C and 80% relative humidity. These are the conditions which have been used for rearing this insect species in the SUCEN laboratory of Mogi-Guaçu (SP) since 1980 (Rodrigues *et al.*, 1991).

The specimens used were fed hen's blood, a procedure which was maintained throughout the experiment.

Malpighian tubule preparations were obtained immediately after the insects were subjected to the thermal shock and also 3 and 30 days later. The organs from three specimens were used for each experimental condition and control.

Whole Malpighian tubules were mounted on slides, immediately fixed in acetic ethanol for one min, rinsed in 70% ethanol for five min and air dried. The material was then subjected to the Feulgen reaction, with hydrolysis in 4M HCl at 25°C for one hr and five min. The stained preparations were rinsed in sulfurous and distilled water, rapidly dehydrated in an ethanol series, cleared in xylene and mounted in Canada balsam ( $n_D = 1.54$ ).

A Zeiss light microscope was used to count the total number of Malpighian tubule epithelial cell nuclei per specimen, to identify the different nuclear phenotypes and to evaluate their frequencies in each specimen.

## RESULTS

The nuclear phenotypes found in the control, which have been previously reported to be present under normal physiological conditions (Mello, 1971), were also observed in the insects subjected to the thermal shocks. These phenotypes exhibited well-characterized, single-, bi- and multichromocentered nuclei (Figures 1A-C). Pyknotic, giant and vacuolized nuclei, as well as nuclei containing faintly stained chromocenters, characterized the phenotypes found exclusively or predominantly in the test specimens (Figures 1D-I). The faintly stained chromocenters are hypothesized to be due to unravelling of the heterochromatin bodies.

All of these phenotypes were detected irrespective of the temperature used for the thermal shock, although giant nuclei were predominantly present in the specimens subjected to the hyperthermal shock.

Tables I and II report the total number of nuclei evaluated and the frequency at which the different nuclear phenotypes appeared per specimen under the various experimental conditions. In view of the wide variability found for these values, the results were listed individually in order to facilitate comparison.

The most common phenotypes observed in the controls were also the most frequent in the test specimens (Tables I and II). Single-chromocentered nuclei made up

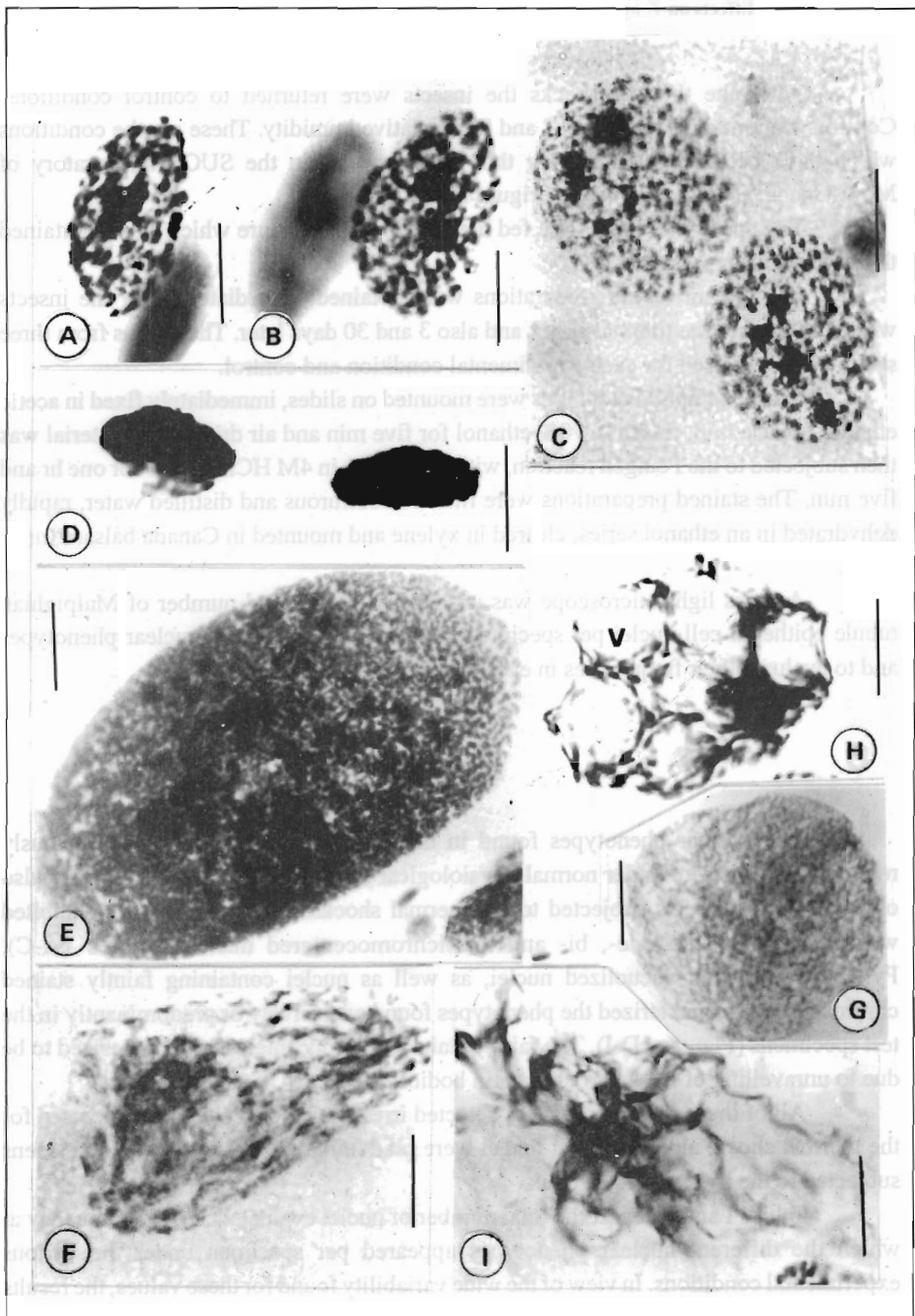


Figure 1 - Nuclear phenotypes of Malpighian tubule epithelial cells of 4th instar nymphs of *T. infestans* subjected to thermal shocks. A-C, Single- (1), bi- (2) and multichromocentered (3) nuclei. D, Pyknotic nuclei. E, Giant nucleus. F and G, Nuclei exhibiting different levels of chromatin staining loss assumed to be associated with heterochromatin unravelling. H and I, Degenerating nuclei. v - vacuolization. Bars equal 10  $\mu$ m.

Table I - Relative frequencies of the nuclear phenotypes of the Malpighian tubule epithelial cells of *T. infestans* specimens various periods (t) after a hypothermic shock at 0°C.

Experimental conditions	Specimen code	Total no. of nuclei	Nuclear characterization						
			Normal phenotypes (%) (chromocenter no.)			Altered phenotypes (%)			
			1	2	> 2	Giant nuclei	Unpacked chromocenters	Vacuolization	Pyknosis
Control	1	12113	85.98	2.27	11.48	0.12	0.03	0.12	-
	2	11564	81.11	2.33	16.13	-	0.30	0.12	-
Shock: 1 hr, t <sub>0</sub>	3	11916	88.12	1.98	9.65	-	0.17	0.08	-
	h4	9951	93.27	0.33	0.45	-	2.78	3.17	-
	h5	10553	94.57	1.54	2.55	-	0.16	1.18	-
	h6	7389	78.35	1.03	15.71	-	1.22	3.69	-
	h7	9714	96.04	0.71	0.25	-	0.20	2.80	-
	h8	9721	94.24	1.08	1.35	0.03	1.14	2.16	-
Shock: 1 hr, t <sub>30d</sub>	h9	8316	96.68	0.97	1.12	-	0.07	1.15	-
	h10	3931	85.75	1.91	5.57	4.71	-	0.64	1.42
	h11	7752	96.62	1.23	0.23	0.67	0.27	0.98	-
	h12	7957	96.71	0.23	0.21	0.35	0.13	2.21	0.16
	h13	9803	85.37	9.44	1.75	-	2.98	0.46	-
Shock: 12 hr, t <sub>0</sub>	h14	9710	95.60	0.56	1.85	-	1.85	0.13	-
	h15	10038	67.61	5.30	21.86	-	2.18	3.05	-
	h16	8852	96.05	1.10	1.05	-	0.48	1.32	-
	h17	8787	95.64	1.18	1.02	-	0.41	1.74	-
	h18	9501	95.45	1.12	2.00	-	0.14	1.29	-
Shock: 12 hr, t <sub>30d</sub>	h19	5654	19.68	0.56	73.12	-	0.11	6.53	-
	h20	5483	31.86	0.66	60.64	-	0.07	6.77	-
	h21	7204	39.52	2.80	53.32	-	0.31	4.05	-

d = days; - = zero.

Table II - Relative frequencies of the nuclear phenotypes of the Malpighian tubule epithelial cells of *T. infestans* specimens various periods (t) after a heat shock at 40°C.

Experimental conditions	Specimen code	Total no. of nuclei	Nuclear characterization						
			Normal phenotypes (%) (chromocenter no.)			Altered phenotypes (%)			
			1	2	> 2	Giant nuclei	Unpacked chromocenters	Vacuolization	Pyknosis
Control	1	12113	85.98	2.27	11.48	0.12	0.03	0.12	-
	2	11564	81.11	2.33	16.13	-	0.30	0.12	-
	3	11916	88.12	1.98	9.65	-	0.17	0.08	-
Shock: 1 hr, t <sub>0</sub>	h4	5296	75.83	0.47	3.68	0.15	16.50	3.36	-
	h5	7788	74.47	3.20	18.95	-	0.74	2.63	-
	h6	8233	93.78	1.72	2.34	0.02	0.36	1.76	-
	h7	6027	92.57	0.18	3.00	0.78	1.68	1.79	-
	h8	4205	49.20	0.81	43.14	0.07	0.62	6.16	-
Shock: 1 hr, t <sub>30d</sub>	h9	6204	93.10	1.77	2.48	0.16	0.61	1.87	-
	h10	5442	84.45	0.51	13.60	-	0.07	1.25	0.11
	h11	6008	84.94	0.42	13.48	-	0.09	1.00	0.07
	h12	5084	84.62	0.71	12.71	0.51	0.03	1.42	-
	h13	2984	52.14	0.40	6.64	3.22	33.24	4.16	0.20
Shock: 12 hr, t <sub>0</sub>	h14	5850	77.33	0.26	2.55	-	17.14	2.65	0.07
	h15	9143	95.10	1.24	2.54	-	0.44	0.68	-
	h16	5312	59.96	1.60	34.20	0.47	2.18	1.58	-
	h17	7501	90.61	2.23	1.68	0.68	3.35	1.45	-
	h18	6734	83.25	0.38	12.15	0.48	1.90	1.84	-
Shock: 12 hr, t <sub>30d</sub>	h19	4566	85.87	0.42	2.23	8.52	0.11	2.85	-
	h20	5662	78.66	0.85	10.81	5.78	0.39	3.51	-
	h21	5998	84.29	0.72	10.84	2.67	-	1.48	-

d = days; - = zero.

the majority of these nuclei, except for the insects analyzed 30 days after the 12 hr hypothermal shock (Table I).

Vacuolization occurred in all of the specimens examined and even within the control group, although to a much lesser extent (Tables I and II).

The highest frequency of giant nuclei was found in the insects analyzed 30 days after the 12 hr heat shock (Table II). This phenotype was rarely detected in the controls ( $\approx 0.1\%$ ) (Table I).

Nuclei exhibiting an apparent unravelling of the chromocenter were found in the insects under all experimental conditions, but more frequently in the specimens analyzed soon after the heat shock (Tables I and II).

Pyknotic nuclei were detected only in the specimens analyzed 30 days after the 1 hr hypothermal shock and, to a very low extent, in specimens analyzed just after the 12 hr heat shock or 30 days after the 1 hr heat shock (Tables I and II).

The total number of epithelial cell nuclei found in the Malpighian tubules was generally decreased, but more so after the heat shock. In the specimens subjected to the hypothermal shock, the number of the cell nuclei appeared to decrease gradually with time after the shock (Table I). However, the number of cell nuclei varied widely among individuals in response to the hyperthermal shock. A drastic decrease often occurred immediately after the shock (Table II).

## DISCUSSION

Significant alterations affecting part of the nuclear population occurred in the Malpighian tubule epithelial cells of *T. infestans* after thermal shock. The nuclear phenotypes which were observed predominantly or exclusively in the specimens subjected to the hypo- and hyperthermal shocks have also been reported for *T. infestans* nymphs subjected to other stress conditions, such as the action of heavy metals, gamma radiation and long periods of starvation (Mello, 1983, 1989; Krubrusly, 1984; Álvarez-García, 1988). These observations support the idea that different stressors can elicit the heat shock response (Hightower *et al.*, 1985; Schlesinger, 1985).

The decrease in number of epithelial cell nuclei, which occurred 30 days after the thermal shocks, is assumed to have been partly due to degeneration of the cells bearing vacuolized and pyknotic nuclei. This idea is supported by the occurrence of a general decrease in the number of vacuolized nuclei with an increase in post-shock time. However, specimens subjected to heat shock for 12 hr were an exception. The number of vacuolized nuclei observed in these specimens increased during the 30-day period following the shock treatment. This may have been due to an intensification of the deleterious effect of heat shock when delivered over a longer period of time.

The finding that pyknotic nuclei were only detected in those specimens analyzed 30 days after the insects received a thermal shock of one hr also favors the idea of a long-term degenerative effect of the stress conditions.

However, nuclear degeneration was not the only factor responsible for the decrease in number of cell nuclei after the thermal shocks. Nuclear fusion certainly played a significant role in this phenomenon. In fact, the presence of giant nuclei, which in *T. infestans* results from fusion of several nuclei (Mello, 1989), was increased after the 12 hr heat shock. In addition, many nuclei may well represent the fusion of only the two nuclei of the same binucleate cell, a fact that may not be perceived visually because the nuclear size was not that of a giant nucleus.

If the presence of giant nuclei represents the existence of a mechanism for cell survival in blood-sucking hemipterans under unfavorable conditions (Wigglesworth, 1967), then their presence in this set of experiments may also explain the unchanged survival rate of the *T. infestans* specimens subjected to heat shock (Rodrigues *et al.*, 1991). This hypothesis is also supported by the observation that the survival rate was greatly reduced among the specimens that were subjected to the hypothermal shock for 12 hr and were devoid of giant nuclei (Rodrigues *et al.*, 1991). In fact, only a hypothermal shock of 12 hr was strongly effective in terms of inducing a drop in survival of 4th instar nymphs of *T. infestans* (Rodrigues *et al.*, 1991).

In addition to the mechanism which generates giant nuclei, the presence of *hsp* certainly played a part in the insect survival rate under the hyperthermal conditions used (Southgate *et al.*, 1985). On the other hand, if cryoprotectors are present in *T. infestans*, they are not efficient in protecting the insect from a relatively long hypothermal shock.

The individual variations observed in response to the thermal shocks resembled those found in *T. infestans* specimens subjected to treatment with heavy metals (Kubrusly, 1984). This suggests that there are specimens which are more resistant to the deleterious action of stressors, including thermal shocks.

The occurrence in controls of nuclear phenotypes which were more frequent in the test specimens may have been due to the effect of some other stressing agents such as viral infection (Mello *et al.*, 1980) or fortuitous starvation (Mello, 1989) during insect rearing in the laboratory. It is worth mentioning that these phenotypes have not been observed in the past in specimens from the same laboratory, especially in the period of time before the laboratory changed the control temperature conditions for rearing *T. infestans* populations (Rodrigues *et al.*, 1991). This may mean that the temperature presently used in the SUCEN laboratory at Mogi-Guaçu, which was chosen because of improved oviposition and egg hatching, is not ideal for other physiological properties.

Although the single-chromocentered nucleus phenotype predominated, multichromocentered nuclei were also quite frequent in some specimens. It is known that up to the 3rd nymphal instar single-chromocentered nuclei are practically the only

phenotype which occurs in the Malpighian tubules of *T. infestans* (Mello, 1971, 1975). From this stage on some nuclei appear with two or several chromocenters. In some specimens from different laboratories, most of the Malpighian tubule nuclei are multichromocentered and the reason for this has not as yet been explained. It has been assumed that multichromocentered nuclei result from single-chromocentered nuclei undergoing chromocenter fragmentation in association with an expression of silent genes in the chromocenter heterochromatin (Mello, 1980). Thus, the presence of a larger percentage of multichromocentered nuclei in specimens subjected to the hypothermal shock for 12 hr may represent an attempt of gene expression in response to the stress situation.

The apparent unravelling of the chromocenter heterochromatin in the specimens subjected to the thermal shocks resembled that which was found in the same species under other stress conditions (Kubrusly, 1984; Andrade and Mello, 1987; Mello, 1989). However, it was much more evident after the heat shock, suggesting that this stress condition may be more effective in promoting heterochromatin unpacking. Whether chromatin unravelling in chromocenter heterochromatin of *T. infestans* represents an attempt to activate silent genes is a matter for future investigation. Although *hsp* genes have been reported to occur in heterochromatin areas of other cell systems, their expression after the stress has not as yet been demonstrated (Southgate *et al.*, 1985).

No special nuclear phenotypical characteristics associated with the change in the insect molting process due to thermal shock (Rodrigues *et al.*, 1991) were detected.

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### RESUMO

Os fenótipos nucleares de células epiteliais dos túbulos de Malpighi de ninfas de 4º estágio de *Triatoma infestans* Klug submetidas a choques de temperatura a 0°C e 40°C por 1 e 12 horas foram investigados imediatamente após o estresse e 3 e 30 dias após o mesmo. Além dos fenótipos usualmente descritos para os espécimes controle, núcleos picnóticos, gigantes e vacuolizados, bem como núcleos com uma aparente descompactação da cromatina do cromocentro foram detectados nos espécimes-teste. Admite-se que um decréscimo no número de núcleos a curto ou longo prazo após os choques de temperatura seja contribuído não só pela degeneração das células que apresentam núcleos vacuolizados e picnóticos, mas também por fusão

nuclear. Supõe-se que os núcleos gigantes, que apareceram com predominância nos espécimes submetidos ao choque hipertérmico e, ao mesmo tempo, a presença de *hsp*, desempenhem papel na manutenção da sobrevivência do inseto nesta condição experimental específica. Embora muitas das características nucleares encontradas nos espécimes submetidos aos choques de temperatura tenham sido também descritas após ação de outros agentes estressantes, o choque hipertérmico foi o mais efetivo na promoção da aparente descompactação da heterocromatina do cromocentro. Com base nos tipos e frequências dos núcleos encontrados após choque hipertérmico e queda na sobrevivência do inseto sob tal circunstância, considera-se que, se existem substâncias crioprotetoras em *T. infestans*, estas não seriam eficientes para proteger o inseto de um choque hipotérmico relativamente longo.

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