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MORPHOLOGIC AND MORPHOMETRIC ASPECTS OF OENOCYTES OF *APIS MELLIFERA* QUEENS AND WORKERS IN DIFFERENT PHASES OF LIFE

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Oenocytes of adult workers and queens of Apis mellifera *L. were studied in different ages or life stages, by means of morphometric and histologic techniques. In workers, the oenocytes were found in the head, near the mandibles and in the abdomen, immersed in the parietal fat body mainly below the sterna, close to the wax glands. In queens, two populations of oenocytes different in size and localization were found within the parietal and visceral fat body, respectively. The oenocytes of workers and queens show the presence of acid lipids and acid phosphatase. The role of these cells in the castes differences is discussed.*

Key words: oenocytes – *Apis mellifera* – morphology – development – castes differences – fat body

Oenocytes are usually large ovoid cells with a dense, hyaline, and acidophilic cytoplasm. They are formed in the embryo, or derived from the larval epidermis. Although easily identified and many times observed changing in relation to the molt cycle, their metabolic function is not yet well known.

They appear scattered throughout the insect body, but mainly in the abdomen, below the epidermis or in small clusters near the spiracles, attached to tracheal trunks, or embedded in the fat body cells (Bernard & Philogene; 1967; Evans, 1967). The localization may change in different stages of the insect development.

Oenocytes may be involved in the synthesis of lipidic and lipoproteic materials which are deposited in the epicuticle in order to make the insect surface impermeable (Kramer & Wigglesworth, 1950; Wigglesworth, 1970; Dielph, 1973, 1975) or they may participate in the production of ecdysteroids that act during molting (Locke, 1969; Romer, 1974; Rinterknecht, 1985; Rinterknecht & Matz, 1983). Rinterknecht et al. (1969, 1972, 1973) also believe their role to be the elimination of toxins by insects. These functions attributed to oenocytes are in accordance with the obvious development of the smooth endoplasmic reticulum of these cells (Ksiazkiewicz-Kapraleka, 1984; Cruz-Landim, 1985).

Stoppie et al. (1981) observed that during vitellogenesis of *Sarcophaga bullata,* the oenocytes release a compound of unknown nature, that could have some role in vitellogenin production by females.

As the oenocytes function is not yet very well known, the purpose of this paper is: (a) to compare workers and queens for the localization, morphology and distribution of oenocytes throughout the body; (b) to search for differences in oenocyte number, size and content in different ages or phases of bees life, in order to investigate the possible role of these cells in this insect.

MATERIALS AND METHODS

The material used in the studies were adult workers, 0, 5, 12, 17 and 29 days old and newly emerged (QE), just mated (MQ), laying (LQ) and old (postlaying) queens (OQ). Five individuals of each age or stage were studied.

Histological techniques – The bees (workers or queens) in the desired phase were anaesthesized with ether, and had their heads, thoraxes and abdomes separated. The separated

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parts were fixed in alcoholic Bouin mixture during 24 hours and embedded in paraffin. The bee parts were them sectioned at 7 µm thickness and stained with hematoxylin and eosin for general morphological studies. Also fat bodies dissected from abdomens of workers and queens were spread on glass microscope slides previously covered with a thin layer of albumin-glycerin and stained with Sudan Black B (Junqueira & Junqueira, 1983) and Nile's Blue (Behmer et al., 1976) for lipids, and lead nitrate (Mello & Vidal, 1980) for acid phosphatase.

Morphometric techniques – The cellular and nuclear volumes, nucleus/cytoplasm (N/C) ratio and oenocyte number were calculated for workers and queens at all ages and stages studied.

Statistical analyses – The Kruskal Wallis test (Siegel, 1979) was applied for means comparison, of cellular and nuclear volume and the N/C ratio of the oenocytes. The U-Test of Mann-Whitney (Siegel, 1979) was used in order to determine if the observed differences were significant at a level of 5%.

RESULTS AND DISCUSSION

Oenocytes localization – The localization of oenocytes does not vary much in the different groups of insects. They can be found practically in all parts of the body, but seem predominate in the abdomen, connected to other cells, other tissues, or free (Bernard & Philogene, 1967). They are often located below the epidermis spread in the fat body (Evans, 1967, Pajni, 1968, Ksiazkewicz-Kapralska, 1984).

In adult workers and queens of *Apis mellifera* the abdominal oenocytes appear among the parietal fat body cells (Figs 10, 11), with an exception of newly emerged queens, where they appear also in the perivisceral fat body (Fig. 11). In the other stages of the queen life they appear more frequently in the sternites of the medial area of the gaster. In workers they are located close to the sternites where the wax glands develop (Fig. 10).

Morphology and morphometric analysis in workers – In general, the oenocyte tends to be spherical and have a well defined nucleus.

In the worker's head, these cells appear in small groups, just below the epidermis of the mandible (Fig. 9). Due to their subepidermic localization, we suppose that they act in production of compounds, that are liberated to the cuticle, specifically of the mandible articulation area.

The number of oenocytes in the head of workers in very small compared with the abdomen (Fig. 4, 9D), varying from zero to 12 in the studied specimens. Although the average number found in 0-5 days old worker was 6, the young-workers tend to have less oenocytes than the old ones. The oenocytes of the head are larger than those of abdomen, exhibiting cellular and nuclear volumes (N/C ratio) bigger than those found in the abdomen (Fig. 1, 2, 3 and Table I).

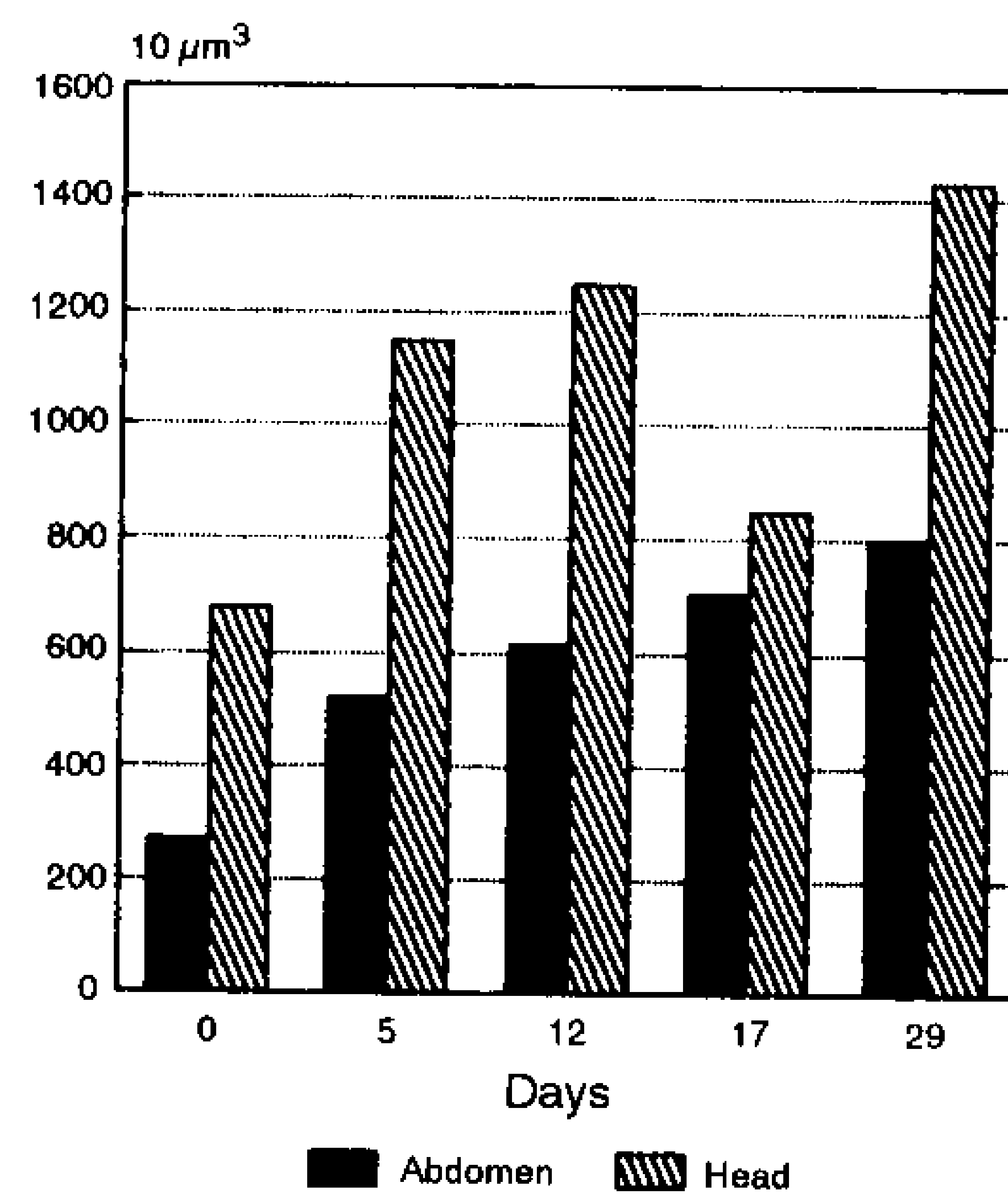

Fig. 1: distribution of cellular volumes of head and abdominal oenocytes along the worker life cycle.

In workers, just emerged and five days old, the abdominal and head oenocytes exhibited nuclei less visible than in other ages (Figs 9, 10) and the abdominal oenocytes of workers 12, 17 and 29 days old are more acidophilic (as indicate by the cytoplasm eosinophily) than those of workers newly emerged and five days old (Figs 10A, B, C, D, E).

Kramer & Wigglesworth (1950) verified that oenocytes of bees that were secreting wax are larger and have cytoplasm that stain deeply with hematoxylin (are basophilic), while in foraging bees, the cytoplasm appeared pale, with exception of the nuclear surroundings and the cells are smaller. This is not in accordance with that observed here since in 12 days old workers, the oenocytes exihibited acidophilic granular cytoplasm, with large and well defined nucleus (Figs 10C, D, E) and the morphometric evaluation shows that cellular volume does not decrease in foraging bees (Fig.

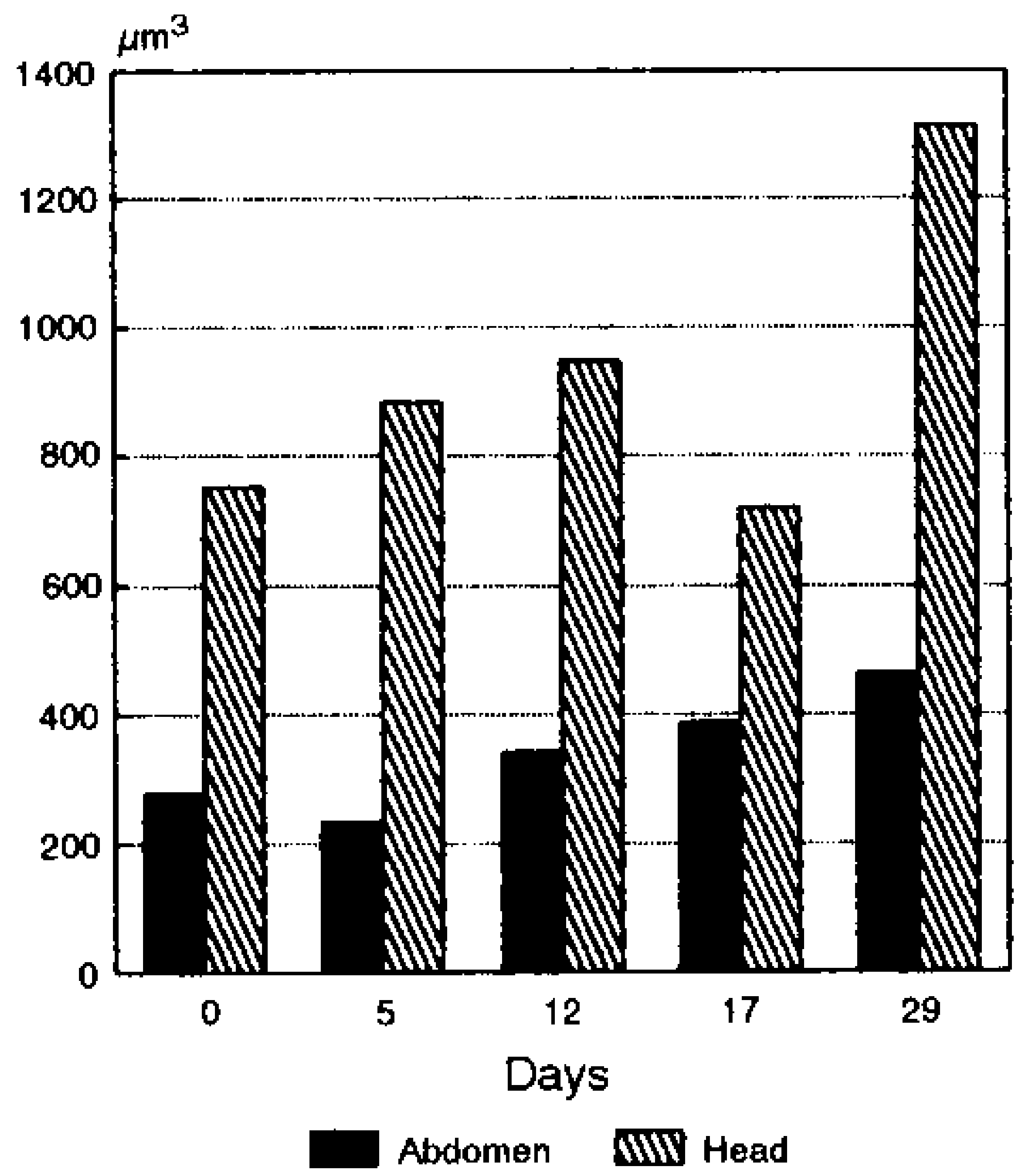

Fig. 2: distribution of nuclear volumes of head and abdominal oenocytes along the worker life cycle.

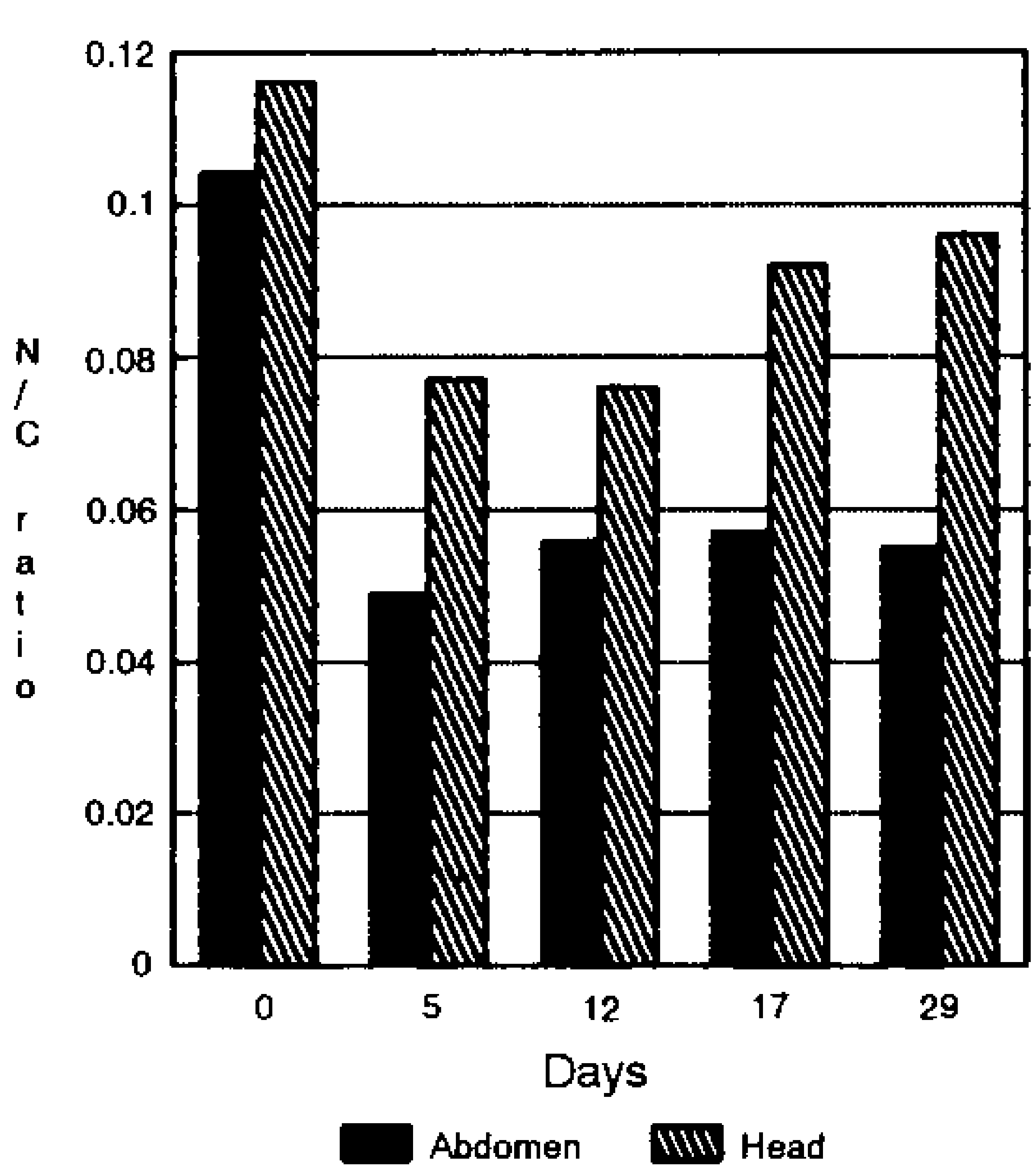

Fig. 3: nucleo/citoplasmatic ratio of worker head and abdominal oenocytes.

TABLE I

H-values obtained from Krustal Wallis test applied to cellular and nuclear volumes and N/C ratio of *Apis mellifera* worker and queen oenocytes

	Cellular volume	Nuclear volume	N/C ratio
Worker abdomen	97,86[a]	26,90[a]	42,26[a]
Worker head	77,03[a]	39,92[a]	20,71[a]
Queen	49,48[a]	50,56[a]	46,29[a]

a: significant values at 5% level.

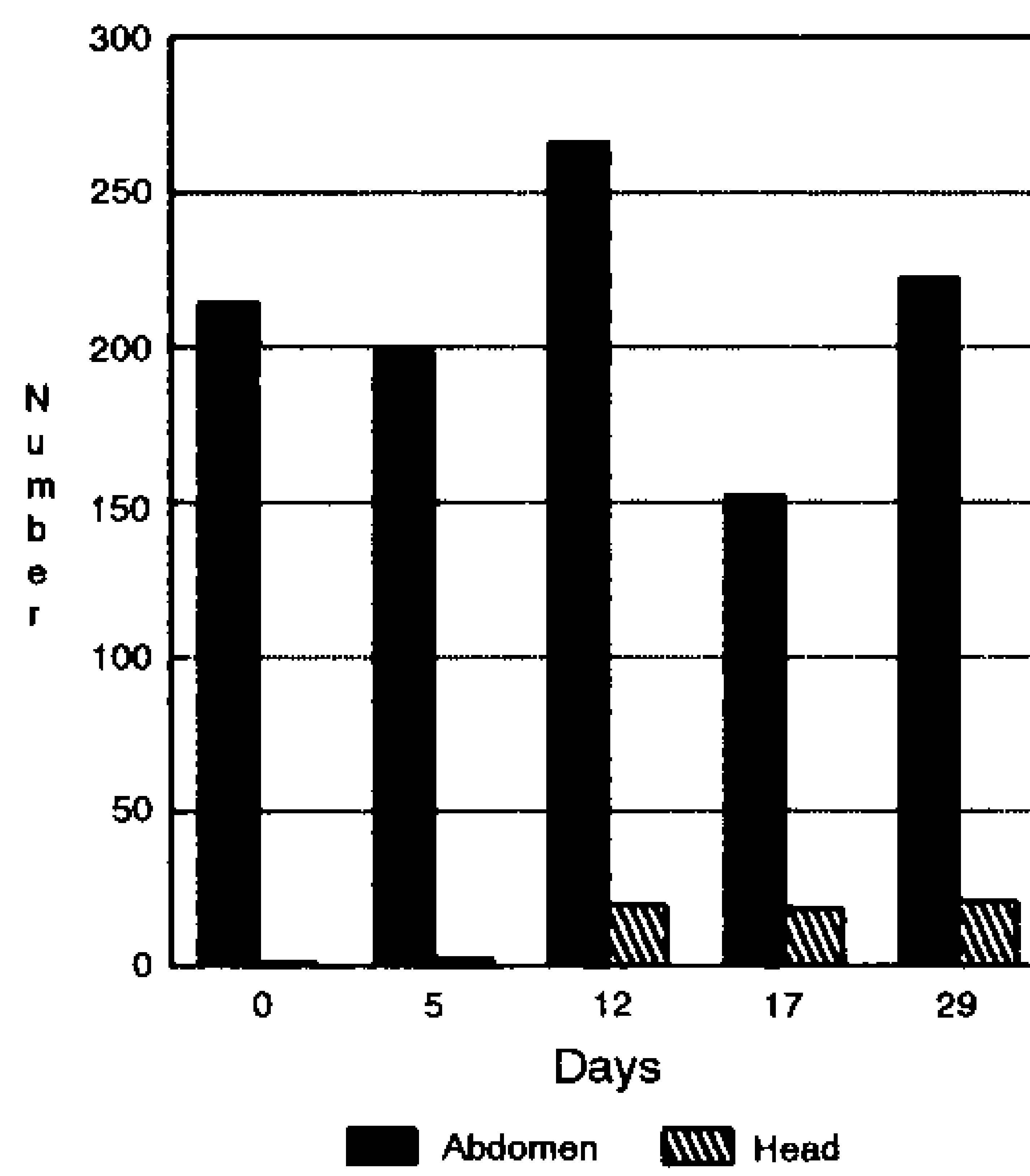

Fig. 4: distributions of head and abdominal oenocyte numbers along the worker life cycle.

1). In addition, the N/C ratio shows that cellular activity does not change with age (Fig. 3), and differences on means among ages were not significant (Table II). Besides, results of wax epithelium height mesures showed that it has two peaks of activity (Table II), one in 5 days old workers and another in 17 days old ones (Table III), while the oenocytes size remain stable during all period.

Therefore the cycle of worker's wax gland development is not followed by size changes or oenocytes.

Morphologic and morphometric analysis in queens – In newly emerged queens two populations of oenocytes may be distinguished ac-

TABLE II

Z-values obtained from U-Mann Withney-test applied to cellular and nuclear volumes N/C index; and U-values obtained to the number of *Apis mellifera* workers head oenocytes, at different ages (in days)

	Number	Cellular volume	Nuclear volume	N/C index
0 ; 5	6,00	−5,96[a]	−1,78[a]	−3,77[a]
0 ;12	0,00[a]	−6,42[a]	−2,49[a]	−4,01[a]
0 ;17	4,00	−1,58	−0,79	−2,44[a]
0 ; 29	0,00[a]	−6,41[a]	−4,93[a]	−2,29[a]
5 ;12	0,00[a]	−0,53	−0,77	−0,02
5 ;17	5,00	−3,99[a]	−2,09[a]	−1,07
5 ; 29	0,00[a]	−2,63[a]	−3,62[a]	−1,83[a]
12 ;17	9,00	−4,49[a]	−2,98[a]	−1,24
12 ; 29	12,50	−2,17[a]	−3,11[a]	−1,90[a]
17 ;29	9,00	−5,05[a]	−5,09[a]	−0,49

a: significant values at 5% level.

cording to their localization and size. The smallest oenocytes are parietal and found in small groups immersed in the fat body (Fig. 11A). The larger ones appear as isolated cells scattered in the perivisceral fat body. Their nuclei are large and well defined (Fig. 11B). Two distinct populations of oenocytes, on the basis of size or differentiation degree were also observed in larvae of *Locusta migratoria* (Rinterknecht et al., 1973).

In older queens only one population of oenocytes is present in the parietal fat body mainly ventrally located (Figs 11C, D, E).

The abdominal oenocytes of queens are less acidophilic than those of workers. Furthermore, the perinuclear cytoplasm in all stages, except in newly emerged, have more affinity to eosin than the rest (Figs 11C, D, E).

The statistical results show that the changes exhibited by queen oenocytes along the different stages (Figs 5, 6, 7) are significantly different. Statistical differences do not exist, only between the N/C ration of mated and old queens, which probably may be related to the drop in oenocyte activity in old queens. The results indicate that not only the oenocytes grow in size during the life of queen but that their activity changes in different phases. The number of oenocytes (Fig. 8D) in different phases of the queen life also varied significantly indicating that the requirement of the queen for products made in oenocytes is not only reached by the growth of existing cells, but also requires a differentiation of new cells.

Oenocytes contents – In larvae of *Tenebrio molitor* the oenocytes show a weak response to the staining with Sudan Black. Only after special treatment to isolate lipids from proteins the response was stronger, indicating that the lipids were linked to proteins (Romer, 1980). Similar results were obtained by Wigglesworth (1970) and Cruz-Landim (1985) when studying larval oenocytes of *Rhodnius* and bees respectively.

The above results are not in agreement with those obtained in oenocytes of *A. mellifera* workers, where in 12 days old bees they showed a very strong reaction to Sudan Black B, even without any previous treatment. The intensity of staining by Sudan Black B drops little by little between 17 and 29 days (Table III). The results of Nile Blue treatment indicate that these cells contain acid lipids, that could be participating in wax synthesis.

The abdominal oenocytes of queens differently from workers shown weak reaction to Sudan Black B (Table III). The lipids in these oenocytes are also acids, as shown by the answer to the Nile Blue stain. The differences between the oenocytes of queens and workers indicate that these cells may have different functions in both castes.

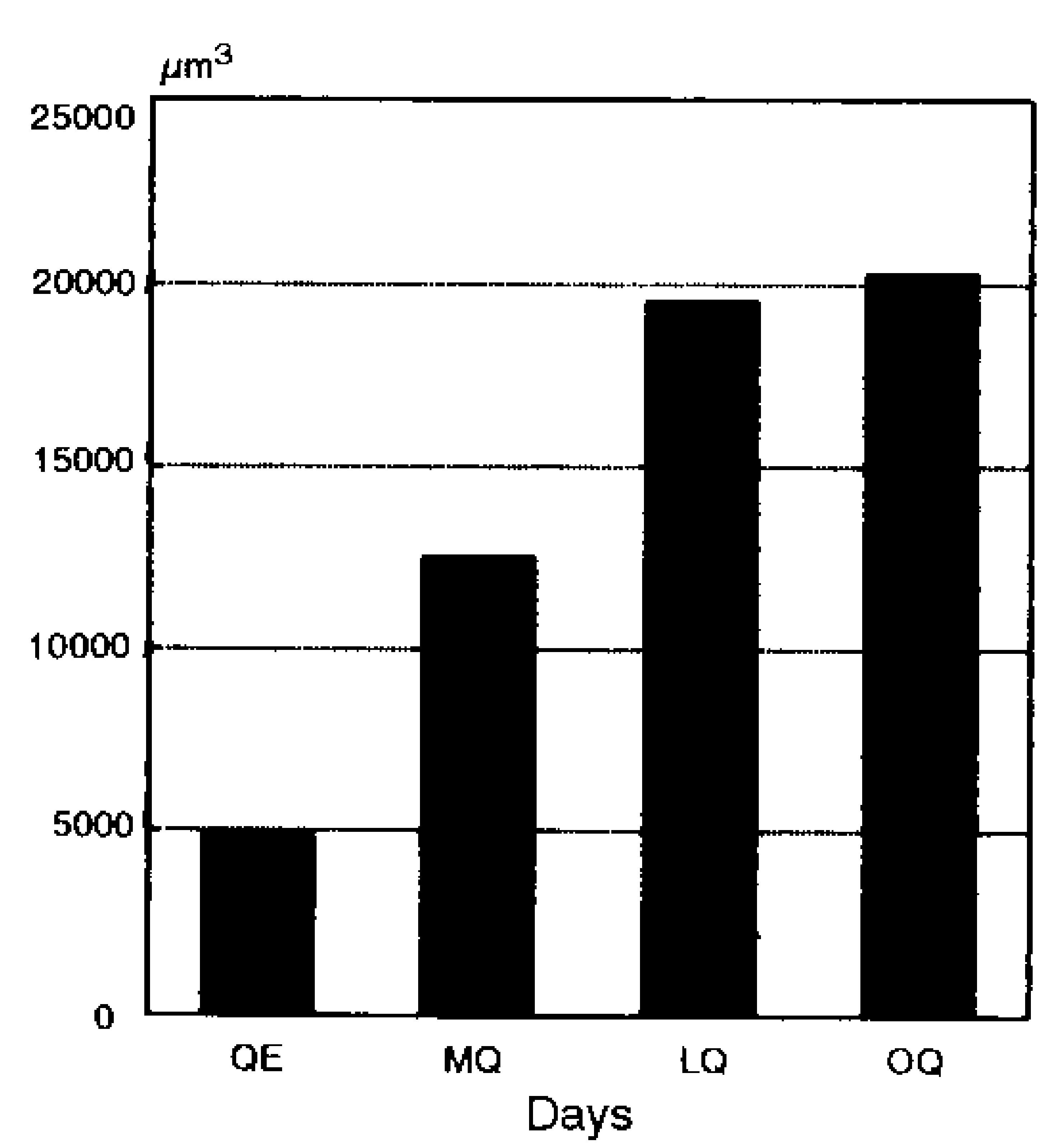

Fig. 5: distribution of cellular volumes of abdominal oenocytes in different stages of queen activity.

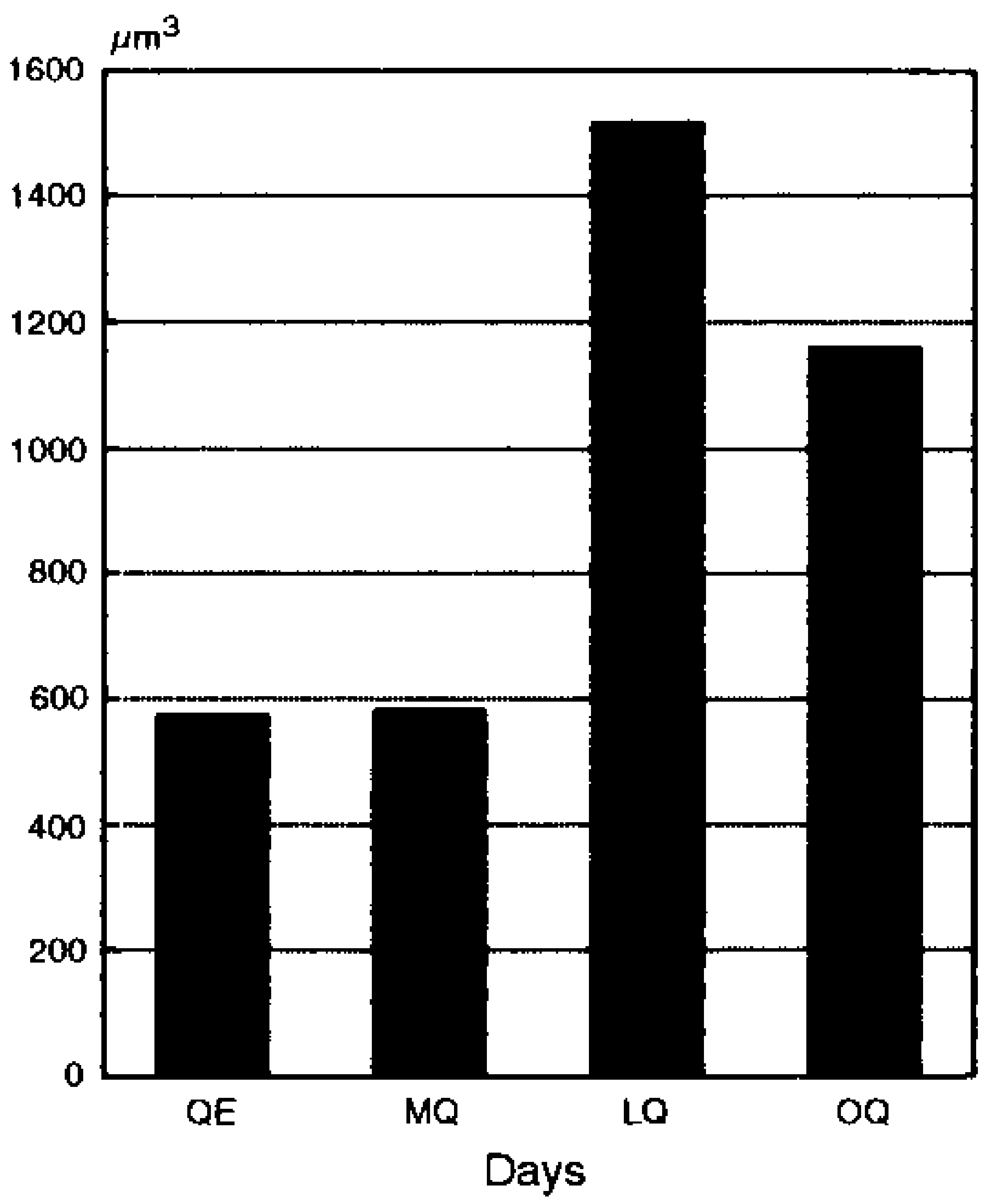

Fig. 6: distribution of nuclear volumes of abdominal oenocytes in different stages of queen activity.

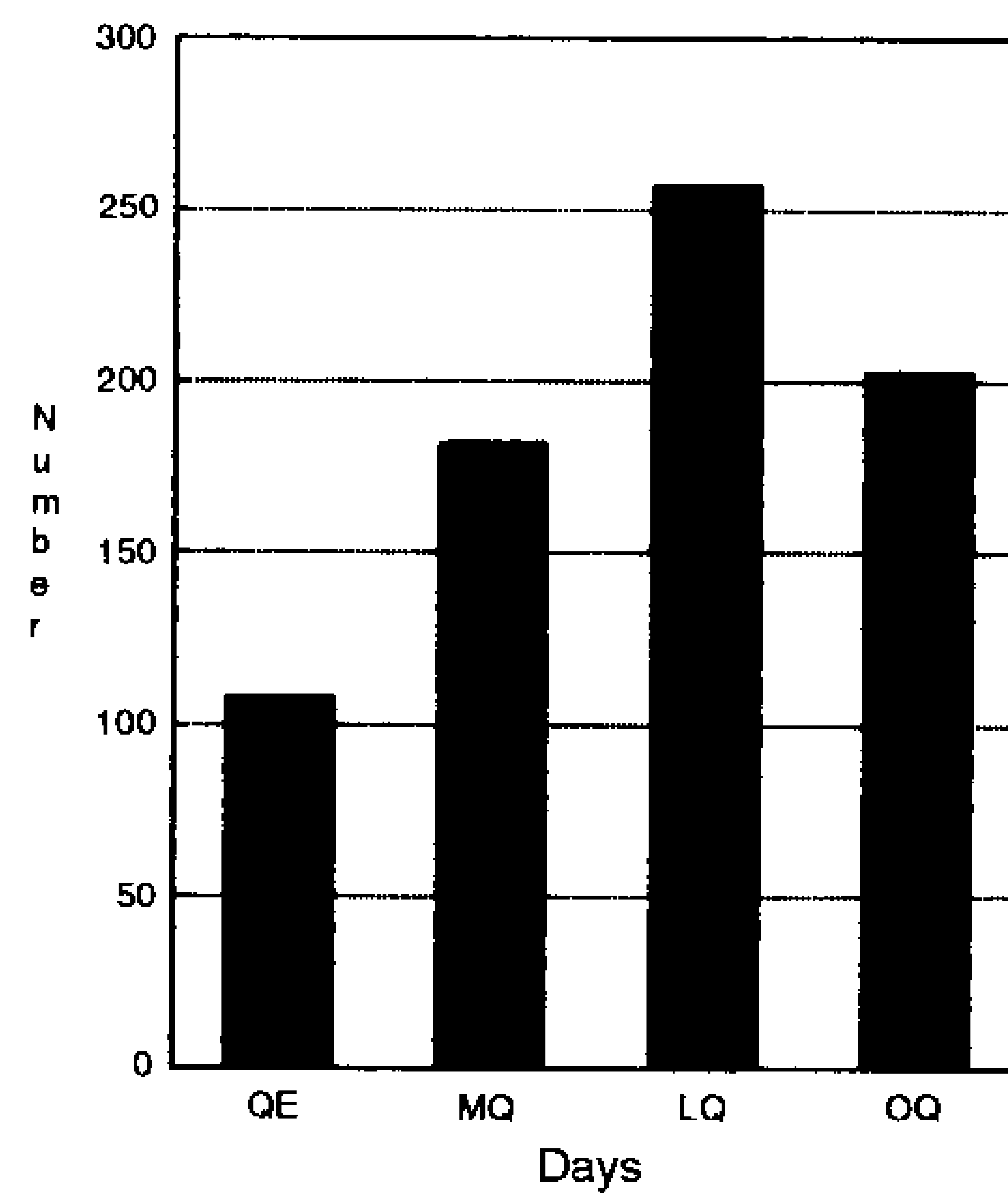

Fig. 8: distribution of abdominal oenocytes numbers in different stages of queens activity.

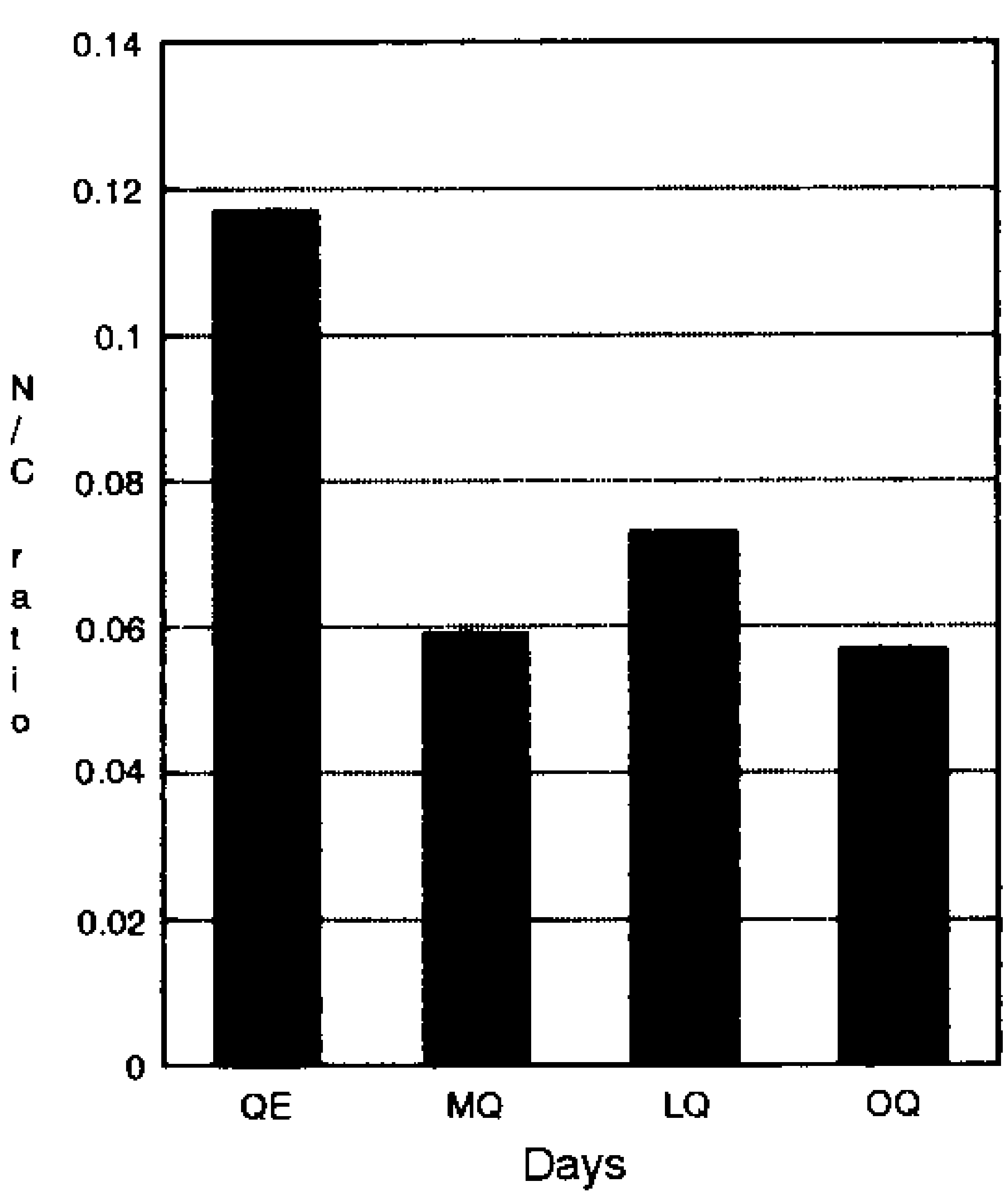

Fig. 7: nucleo/citoplasmatic ratio of queen abdominal oenocytes.

The oenocytes of young workers of *A. mellifera* shows the presence of lysosomes and positive reaction to acid phosphatase (Cruz-Landim, 1985). In the present research acid phosphatase was observed in oenocytes of all studied ages and stages of workers and queens. In workers the greater amounts were verified in 5 and 29 days old beens (Table III). Comparatively a larger amount of acid phosphatase was detected in queens than in the workers (Table III).

The presence of acid phosphatase is frequently related to lysosome presence, cellular organelles that act in the regulation of several cellular functions through intracellular digestions. The acid phosphatase constancy in oenocytes during the entire queen's life, seems to be in accordance with the permanent activity of oenocytes in this caste, a fact that does not seem to occur in workers.

The different localization of oenocytes may be due to the fact the oenocytes eliminate the products they secrete directly into the haemolymph, and the place of it use must be reached throughout circulation. Since the haemolymph circulation in insect, may not be an efficient way for product distribution, the oenocytes tend to locate close to the points where their products will be useful, which is different in the castes or life phases.

Conclusions – From our observations it may be concluded that: (1) In workers the oenocytes are found in head and abdomen. In the head, they are found in small groups next to epidermis of the mandibles insertion. In the abdomen they are placed in the parietal fat body, being more frequent next to the wax glands of workers. (2) In newly emerged queens there

TABLE III

Content tests in *Apis mellifera* workers and queens oenocytes

	Workers					Queens			
	0	5	12	17	29	QE	QM	LQ	OQ
Sudan Black B	++	+	+++	+	–	–	+	+	–
Nilo's Blue	++	+	+++	+++	+++	+		+	++
Acid Phosphatase	+	+++	++	++	+++	+	–	+	++

–: negative; +: poorly stained; ++: positive; +++: strongly positive.

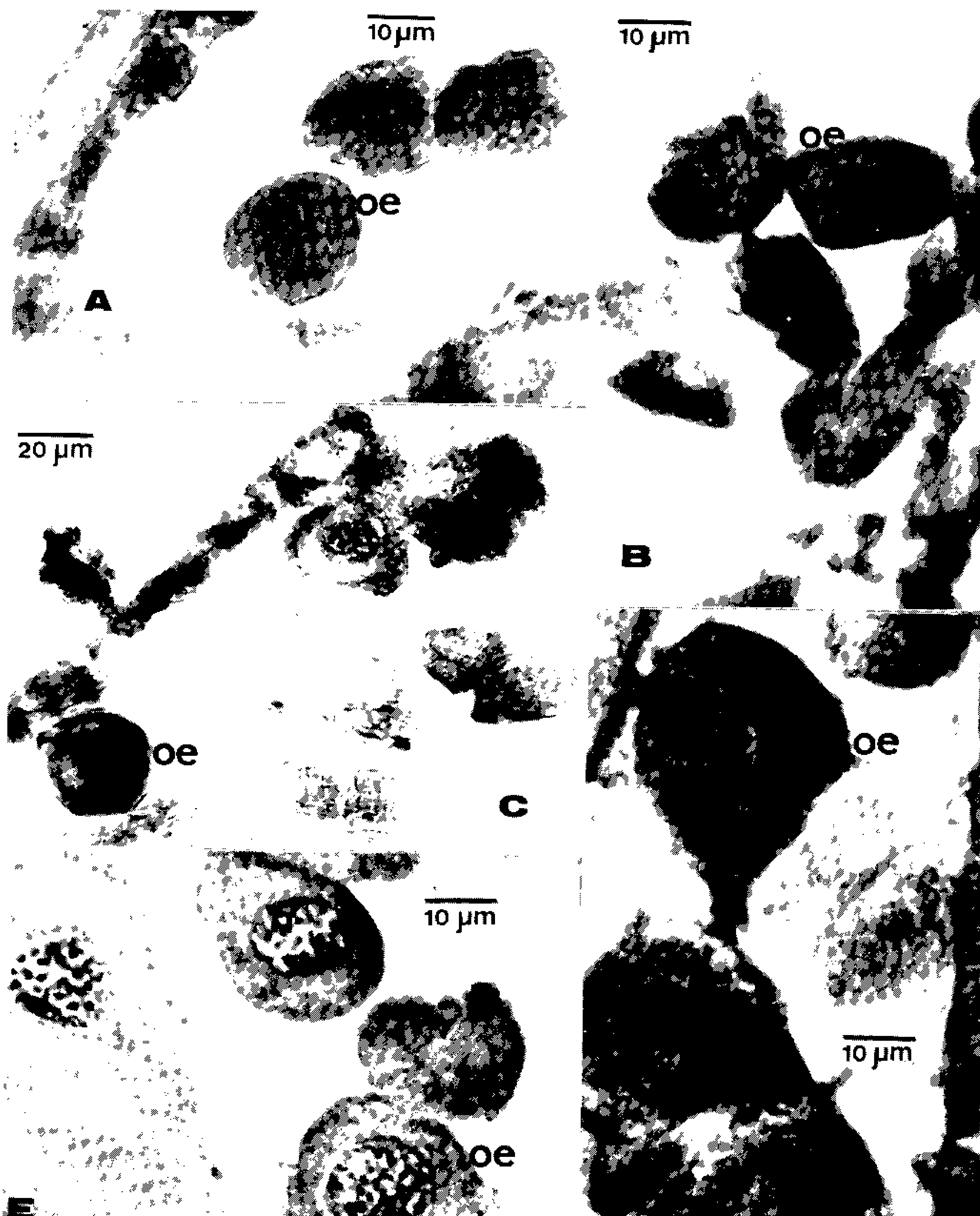

Fig. 9: head oenocytes from *Apis mellifera* workers. A: just emerged. B: 5 days old. C: 12 days old. D: 17 days old. E: 29 days old. n: nucleus. oe: oenocytes.

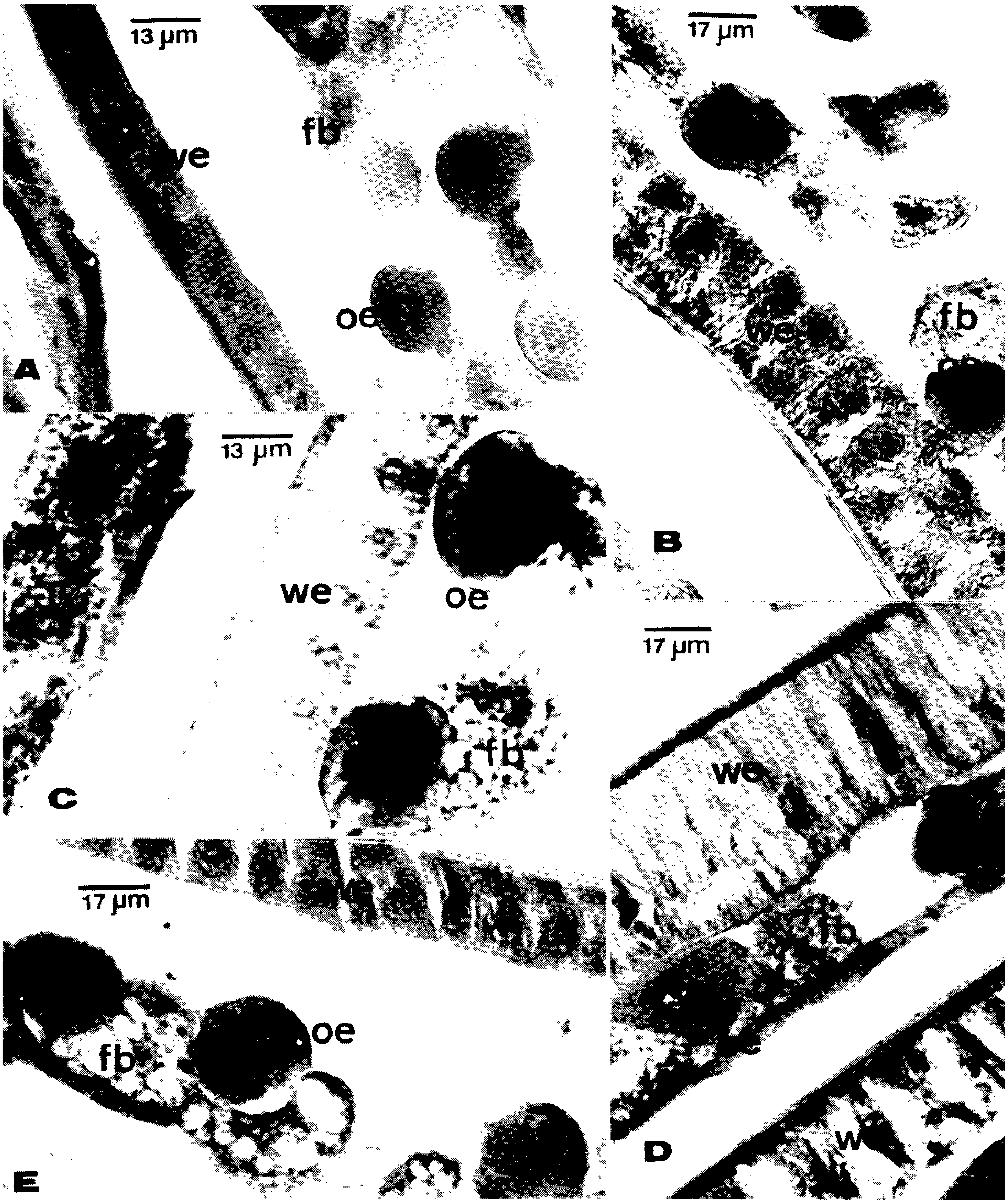

Fig. 10: abdominal oenocytes in *Apis mellifera* workers. A: just emerged. B: 5 days old. C: 12 days old. D: 17 days old. E: 29 days old. n: nucleus. oe: oenocytes. we: wax epithelium. fb: fat body.

are two different types of oenocytes. The small ones appear in small groups scattered in the parietal fat body, while the larger ones appear isolated, distributed in the perivisceral fat body. Only the oenocytes immersed in the parietal fat body, remain during egg-laying and in old queen. (3) The statistic results of the morphometric parameters studied suggest that in workers these cells have a constant activity in all ages studied, while in queens the values obtained for the abdominal oenocytes vary significantly, possibly in relation with the function that these cells fulfil in different phases of their life. (4) The presence of acid phosphatase is in accordance with the cellular activity, in queens as in workers. (5) The oenocyte number or size variation was found unrelated to the worker wax gland development, however

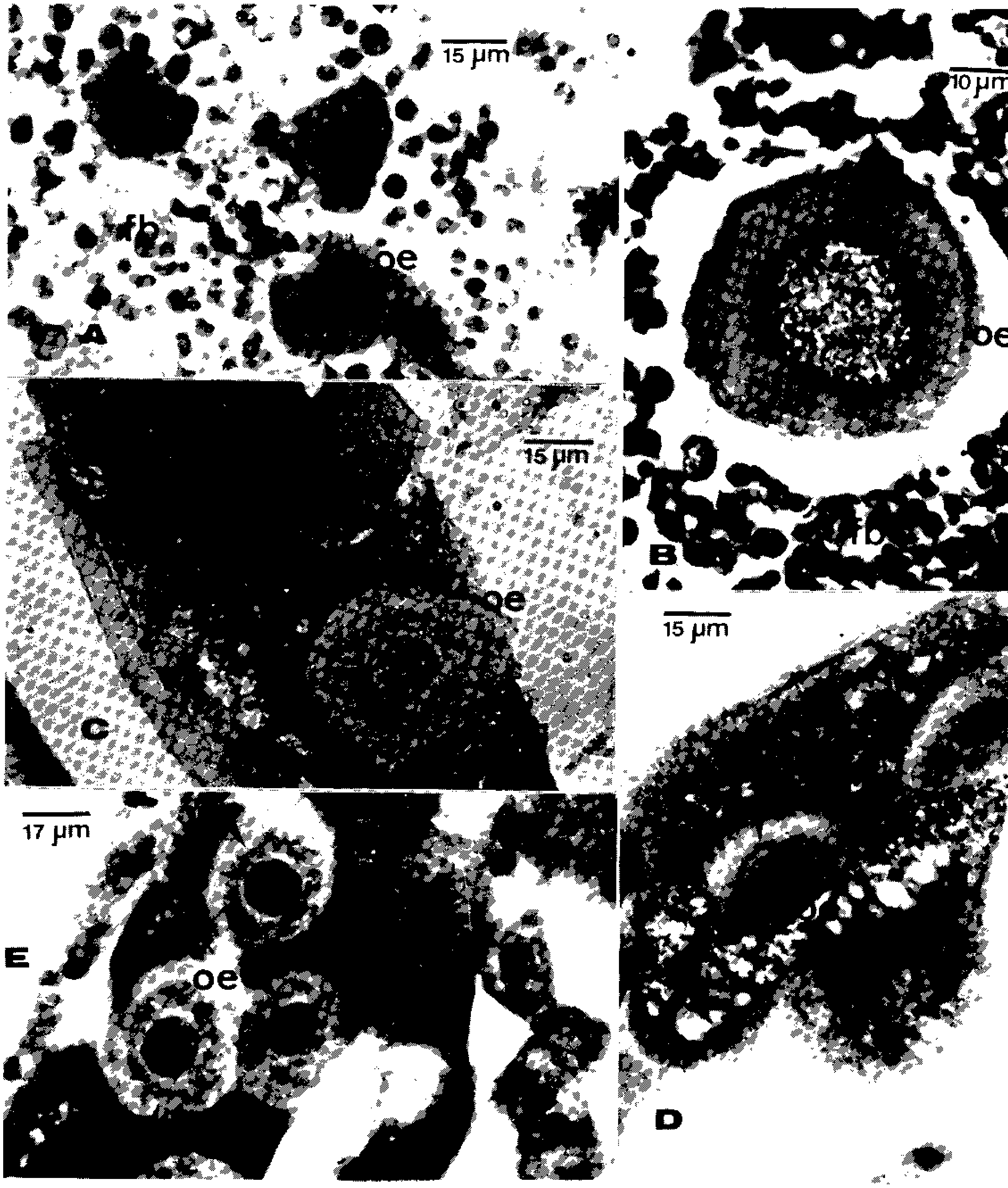

Fig. 11: abdominal oenocytes from *Apis mellifera* queen. A: newly emerged small oenocytes. B: newly emerged large oenocytes. C: mated queen. D: laying queen. E: old queen. n: nucleus. oe: oenocytes. fb: fat body. The arrow shows the more stained perinuclear region.

there is a major amount of lipids in the oenocytes of the workers that are in the wax secretion period, and less amount in foragers, indicating that these cells activity my be different during the period of wax production.

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