ULTRASTRUCTURE AND HISTOCHEMISTRY OF THE MINERAL CONCRETIONS IN THE MIDGUT OF BEES (HYMENOPTERA: APIDAE)

by

CARMINDA DA CRUZ-LANDIM and JOSÉ EDUARDO SERRÃO*

(Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista, Caixa Postal 199, 13506-900 Rio Claro, SP, Brasil)

ABSTRACT

Mineral concretions in the digestive cells of bees were examined under transmission electron microscope and histochemically. Ultrastructure shows two types of mineral deposits: 1) mineral concretions which are organized in granules with a striking concentrically layered organization of opaque and clear zones and 2) electron dense granules which appear inside small vacuoles (0.4-0.7 µm). These two structures are present in the apex of the digestive cells of the posterior midgut. Histochemical data reveal that mineral concretions are composed of calcium, iron and uric acid or its salts while calcium determination gives a positive reaction for electron dense granules. Morphological and chemical similarities between the mineral concretions of bees and those described for other insects suggest that they have an important physiological role regulating the composition of the internal environment and to avoid intoxication. Since concretions and granules are structurally distinct, it is suggested that they are functionally different.

KEY WORDS: bee, histochemistry, midgut, mineral concretions, spherites.

INTRODUCTION

Intracytoplasmatic mineral concretions are very often present in insect tissues, specially in Malpighian tubules (BERKALOFF, 1960; WIGGLESWORTH & SALPETER, 1962) and midgut (GOURANTON, 1968; GOODCHILD, 1969; JEANTET, 1971; CRUZ-LANDIM, 1971; HUMBERT, 1974). The mineral concretions are also named spherocrystals or spherites and are composed of a collection of metals in the form of phosphates, carbonates or chlorides (MARTOJA & BALLAN-DUFRAÎS, 1984).

*To whom correspondence should be addressed.

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Mineral concretions have been attributed the function of regulating the composition of the internal environment and detoxification when insects are contaminated with toxic metals (JEANTET, 1971; MARTOJA & BALLAN-DUFRAÎNAIS, 1984).

The present report refers to mineral concretions observed ultrastructurally and histochemically in adult workers of honey bee, Apis mellifera and stingless bee Melipona quadrifasciata anthidioides.

MATERIALS AND METHODS

Experiments were conducted with 5 adult workers of Apis mellifera L. and Melipona quadrifasciata anthidioides Lep.

Light Microscopy

Digestive tracts from the workers were transferred to buffered saline solution for insects and the midgut was isolated. The pieces were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After dehydration, they were embedded in histo resin and sections cut at 6 μm thickness. The following histochemical procedures were carried out to reveal mineral elements: 1) detection of calcium: silver substitution of Von Kossa (PEARSE, 1960), 2) detection of iron: potassium ferrocyanide solution of CHAYEN et al. (1973) and 3) detection of uric acid and urates: hexamine-silver of Gomori (PEARSE, 1960).

Transmission Electron Microscopy (TEM)

Digestive tracts from adult workers were removed as described. The pieces were fixed in 2.5% glutaraldehyde in 0.1 M Na cacodylate buffer at pH 7.2, washed twice in the buffer, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a series of increasing concentrations of ethyl alcohol, and embedded in Epon-Araldite resin, following usual procedures. Thin sections cut with glass knives were stained with uranyl acetate and lead citrate, and examined in a Zeiss EM9S2 electron microscope.

Detection of calcium

The midgut was removed and to detect calcium the silver substitution of Von Kossa modified for electron microscopy was used. The substitution was made by passing 2.5% glutaraldehyde fixed midgut through 2.5% AgNO₃ at light. The silver excess was washed out by 5% sodium hyposulphite. After the treatment, the pieces were post fixed in 1% osmium tetroxide and processed for TEM as usual.