Iron-Containing Cells in the Honey Bee (*Apis mellifera*)

Abstract. *Honey bees are sensitive to earth strength magnetic fields and are reported to contain magnetite (Fe_3O_4) in their abdomens. We report bands of cells around each abdominal segment that contain numerous electron-opaque, iron-containing granules. The iron is principally in the form of hydrous iron oxides.*

There is behavioral evidence that organisms as diverse as bacteria (*1, 2*), homing pigeons (*3–5*), and honey bees (*6, 7*) are sensitive to earth strength magnetic fields. In magnetotactic bacteria the response to magnetic fields is based on intracytoplasmic magnetite (Fe_3O_4) particles that impart a permanent magnetic dipole moment to these prokaryotes (*1, 2*). The sensory systems that detect magnetic fields in homing pigeons and honey bees are still unknown. However, reports of magnetite in both homing pigeons (*8*) and honey bees (*9*) as well as in other organisms (*10, 11*) suggest that this iron oxide could also be the basis of magnetic field detection in eukaryotes. Since magnetite in honey bees is reported to be localized in the abdomen (*9*), we have histologically examined tissues of the honey bee abdomen and looked specifically for those cells that contain iron and for connections between these cells and the central nervous system, a requirement for a sensory receptor.

We have found bands of cells in each abdominal segment of the honey bee that contain numerous iron-rich granules. We have localized the cells and the granules by both light and electron microscopy. For light microscopy we stained with Prussian blue, a reaction in which iron forms a blue precipitate in the presence of acidic potassium ferrocyanide. For electron microscopy we relied on electron opaqueness coupled with x-ray microanalysis to identify the iron granules. Using Mössbauer spectroscopy we identified the iron granules as consisting principally of hydrous iron oxides.

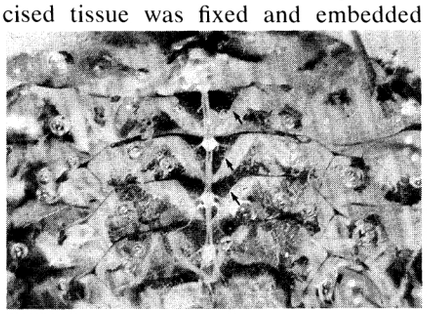
For gross examination of the honey bee abdomen (Fig. 1), adult foraging workers were pinned on dental wax, and their abdomens were cut and pinned open with stainless steel minutien pins. The abdominal contents were fixed and stained in situ (*12*). The dissected abdomens were then washed in distilled water and examined with a Zeiss dissecting microscope. The stained cells (Fig. 1) occur in a band under the epidermis in each abdominal segment. There is a higher concentration of cells in the ventral abdomen under each segmental ganglion. Sheets of this tissue were removed; examination revealed that the iron-positive staining cells form a reticular network within which is another population of smaller spherical nonstaining cells. In fresh, unstained tissue these two cell types are easily distinguished, and the iron-containing cells are seen to have granules of a yellowish color.

Examination of dissected whole abdomens stained with methylene blue shows that at each segmental ganglion a small nerve branch enters into the iron-positive tissue layer and ramifies throughout it. Mechanical movement of this small nerve trunk causes movement of this tissue layer but no other observable structures. Thus, it seems that this tissue is supplied with an efferent or afferent nerve supply (or both).

For light microscopy tissue was fixed and embedded in plastic. Sections (3 μm thick) were mounted on slides and stained for iron with the acidic potassium ferricyanide. The sectioned material (Fig. 2) shows the two cell types that were observed in the whole mounts. The smaller cells never show any sign of a positive staining for iron, while the larger cells always show a granular staining pattern.

We also examined this tissue in the transmission electron microscope. Excised tissue was fixed and embedded conventionally. Silver sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Ultrastructurally one can distinguish the two cell types. The smaller, spherical cells have an abundant endoplasmic reticulum and contain many vesicles. These appear to be the fat cells described by Snodgrass (*13*). The other cells (Fig. 3a) are larger, more irregular in shape, and also have extensive rough endoplasmic reticulum free ribosomes, and many vesicles. These cells also contain many small (0.1 to 0.9 μm) round, very electron opaque particles. At high magnification (Fig. 3b) these particles do not seem to be membrane bound, and they do not have any obvious substructure.

The electron opaque particles in thick (1500 to 2000 Å) sections of unosmicated tissue embedded in Epon 812 were studied by electron diffraction and energy-dispersive x-ray microanalysis. These analyses were done on a JEOL 200CX operating at 200-kV accelerating voltage. The x-ray analytical system utilizes an energy dispersive Si(Li) detector. Selected area diffraction patterns showed only the weak diffuse rings from the Epon. No evidence of crystalline material was observed in either the selected area or microdiffraction modes. On the basis of the size of the particles, the section thickness, and the quantity of iron in the particles as suggested by the x-ray analysis (see below), a diffraction pattern would be expected if significant crystal-



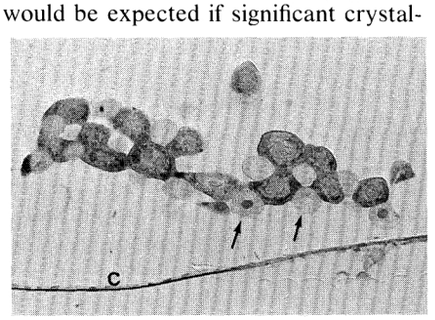


Fig. 1 (left). Honey bee abdomen pinned open and stained for iron. Arrows show large groups of iron-staining (dark) cells under segmental ganglion. Fig. 2 (right). Light photomicrograph of a section of the ventral portion of an abdominal segment containing oenocytes and fat cells (arrows). The oenocytes are characterized by a granular staining pattern; *c*, cuticle.

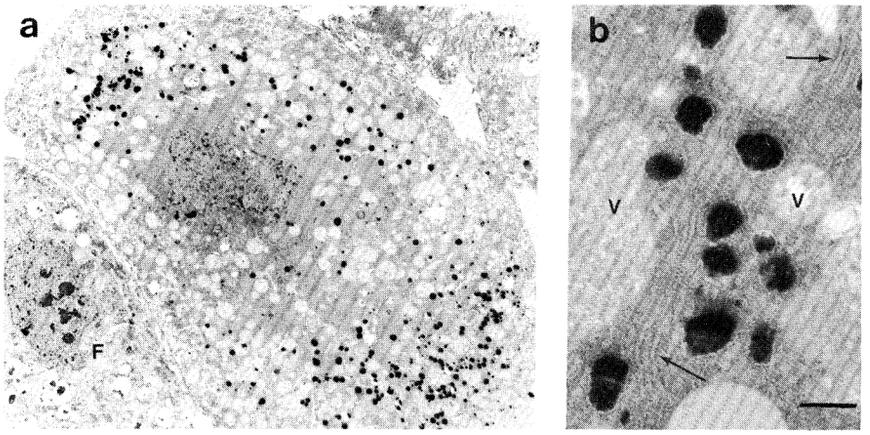


Fig. 3. (a) Low magnification transmission electron micrograph of iron-containing cell and neighboring fat cell (*F*). Dark granules are perinuclear iron containing granules. Many vesicles can also be seen (× 1,320). (b) High-magnification transmission electron micrograph showing an area containing iron granules, vesicles (*V*), and rough endoplasmic reticulum (arrows); scale bar, = 0.5 μm (× 19,200).

line structure existed.

X-ray spectra were obtained in the STEM mode with a 200-Å spot counting for 200 seconds with the use of a low background graphite specimen holder with a specimen tilt of 40°. The spectra from an electron opaque granule (Fig. 4a) show a large quantity of iron present as compared to an immediately adjacent area devoid of granules (Fig. 4b). Spectra taken in the CTEM mode with a 0.1-μm spot gave the same results. These particles also contain calcium and phosphorus as seen in Fig. 4a.

To characterize the chemical state of the iron in the granules, ^{57}Fe Mössbauer spectroscopic measurements at 4.2, 160, and 300 K were made on bands of iron-rich cells that had been excised from honey bees and placed in distilled water and lyophilized. The Mössbauer spectrum (data not shown) of 180 mg of lyophilized cells pooled from 300 bees

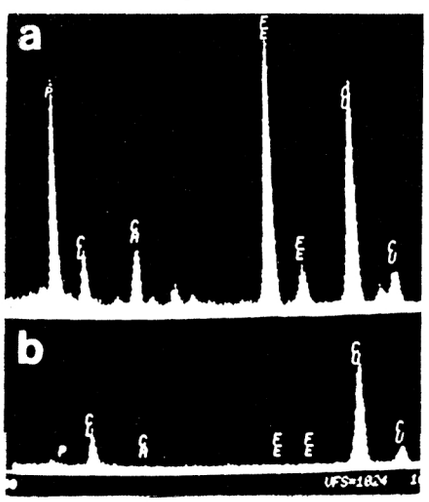


Fig. 4. (a) Energy dispersive x-ray spectrum of electron dense granules. STEM mode with a spot size of 200 Å at 200-kV accelerating voltage was used. Counts were for 200 seconds with a background of 20 to 40 counts. (b) Energy dispersive x-ray spectra of adjacent cell area devoid of granules; same conditions as (a).

consists of a broadened quadrupole doublet at 160 and 300 K, based on a computerized, least squares fit to the data; the quadrupole splitting and isomer shift at 160 K are 0.65 mm/sec and 0.46 mm/sec (relative to iron metal at 300 K), respectively. At 4.2 K the spectrum shows evidence of nonhomogenous magnetic hyperfine splitting. These spectral characteristics indicate densely packed ferric iron with oxygen coordination similar to iron in biological iron storage materials including ferritin and others that contain amorphous iron oxides (*14*). In particular, hydrous iron oxide granules associated with calcium and phosphorus are known in the marine invertebrate *Molpadia intermedia* (*15*).

The iron-rich granules occupy approximately 7 percent of the 3×10^{-8} cm^3 volume of the cells which contain them. Passage of trypsinized cells through a Coulter counter which selected for the large cells gives approximately 11,300 of these cells per bee. Thus the estimated volume of the granules is 2.5×10^{-5} ml per bee or 7.2×10^{-5} g, if the density is 3.0. Gould *et al.* (*9*) measured an average induced magnetic moment of about 2×10^{-6} E.M.U. per bee corresponding to at least 2.2×10^{-8} g of Fe_3O_4. Since hydrous iron oxides are typically paramagnetic at room temperature (*16, 17*) they would not contribute to the remanent magnetism of the bee. However, only 0.33 percent of the hydrous iron oxide present would have to be reduced to magnetite to account for the measured magnetic moment. Hydrous iron oxides are precursors to Fe_3O_4 formation in chitons and magnetotactic bacteria (*2, 18*). This small quantity of Fe_3O_4 would not be detected with any of our analytical techniques.

We believe that these iron-containing cells are the oenocytes of the honey bee as described by Snodgrass (*13*). These cells have been studied in the larvae of other insects [*Gryllus bimacultaus* (*19*) and *Calpodes ethilius* (*20*)] where they seem to be involved in steroid synthesis during development. Their function in the adult is at present unknown. There are no reports, however, that these cells contain either dense granules or iron in any insect except in our studies of adult bees.

In summary, the oenocytes of the adult foraging honey bee form an innervated sheet of tissue around each abdominal segment. Each cell of the sheet contains many electron-opaque iron-containing granules in the cytoplasm.

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