

## Membrane-bound iron-rich granules in fat cells and midgut cells of the adult honeybee (*Apis mellifera* L.)

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(received 25 April 1988; accepted 18 May 1989)

**Summary** — Previous studies have found iron-containing granules in fat cells of adult worker honeybees. By using different preparation techniques, we collected additional data on their occurrence, origin, formation, relative composition and possible function.

In addition to fat cells, we found the same type of iron-rich granules in columnar cells of the midgut; in both cell types they are formed within the cisternae of the rough endoplasmatic reticulum. In the midgut their occurrence is limited to the period of pollen nutrition; during that period the reticulum forming the granules is arranged in a fingerprint design.

Energy-dispersive X-ray analyses of air-dried granule preparations show that, apart from Fe, P and some Ca and K which could be demonstrated on thin sections, Na, Mg, S, Cl, Mn and Zn are always present. All our results suggest that their function is similar to that of other mineralized granules, *i.e.* storage of surplus ions and toxic metals; in support of this is the fact that perorally administered Pb is accumulated in the iron rich granules as well as in the spherocrystals.

***Apis mellifera* — biomineralization — iron-rich granule — fat cell — midgut cell**

### Introduction

Many invertebrates are capable of dealing with surplus ions and toxic metals by a process of biomineralization. In several tissues — mostly involved with digestion, storage or excretion — mineralized granules are formed within the Golgi vesicles or the cisternae of the granular endoplasmatic reticulum (Jeantet *et al.*, 1977; Simkiss, 1979, 1981; Brown, 1982).

The presence of calciferous granules in midgut epithelial cells of the honeybee has been known for some time (Koehler, 1920); they clearly correspond to the spherocrystals described in other insects (Martoja *et al.*, 1984).

Iron-rich granules were described for the first time by Kuterbach *et al.* (1982) in the fat body of adult honeybees; however, these fat cells were mistakenly called cenocytes. This slip was adopted by Loper (1985), who described iron-rich granules

in the oenocytes of drones. Later, Kuterbach and Walcott (1986a,b) provided a more thorough description of the granules in the trophocytes of adult workers. They examined the possible role of these structures in the detection of the earth magnetic field by honeybees; the granules were described as membrane-bound, but "without association with any cellular organelle such as mitochondria or endoplasmatic reticulum".

Finally, similar granules were described in 1987 by Da Cunha in the fat cells of a stingless bee, *Scaptotrigona portica* Latr.

Pending a study on the ultrastructure of the fat body and the midgut in the adult honeybee, our attention was drawn by the occurrence of electron-dense, apparently mineralised structures both in the trophocytes and in the midgut columnar cells. By using different preparation techniques, we collected additional data on their occurrence, origin, formation, relative composition and possible function, which adds considerably to the knowledge of these cellular inclusions.

## Materials and Methods

### Animals

Honeybees of known age were used, both from an outside hive and from experimental cages. Adult bees less than 24-h old, which emerged in the incubator, were color-marked and joined to their colony, or kept in the laboratory in rearing cages with 50 to a cage. The animals were provided with a piece of comb foundation, drinking water, sugar syrup (1 : 1) and a mixture of comb pollen and honey (2 : 1). In some experiments where the effect of pollen nutrition on the occurrence of iron-rich granules was studied, the mixture of comb pollen and honey was omitted. The rearing cages were kept in a dark, climatized room at 30 °C.

### Histology

Dissection and preparation of the fat body was carried out as described by Raes *et al.* (1985); additional care was taken to adjust the osmolarity of the fixation buffer at 440 mOsm (Raes *et al.*, 1987). The midgut was removed from the abdomen by gently pulling the last abdominal segment. The honey stomach, the rectum and the Malpighian tubules were carefully cleared away.

For fluorescence and phase contrast microscopy, unstained paraffin sections or fresh tissue were used. In order to visualise the iron-rich granules at the light microscope level, paraffin sections were stained with the Prussian blue method according to Hutchison (1953).

The ultrastructural study was hampered by the fact that honey bee tissue show only a slight affinity for uranium and lead, resulting in very poor membrane contrast. The method adopted here is the result of much experimentation and could probably be further improved :

— Dissection in ice-cold fixative (2% gluteraldehyde and 2% paraformaldehyde in 0.1% cacodylate buffer, containing 0.12 M of sucrose and 0.05% CaCl<sub>2</sub>, and adjusted to a pH of 7.4).

— Long fixation times (between 6 and 18 h) to improve membrane contrast. Post-fixation in 1% osmium tetroxide in the same buffer.

— During dehydration 2 successive "en bloc" staining steps : first with 2% uranyl acetate in 50% ethanol; secondly in saturated lead acetate in ethanol—acetone (1 : 1) (Kushida, 1966); both steps take 1 h.

— Embedding in a mixture of Epon and Araldite (1 : 1), thus combining the good penetration of the former with the better contrast given by the latter (penetration in 3 steps, within 48 h).

— Silver and gray sections were post-stained with Reynolds lead solution and 0.8% uranyl solution in 50% methanol; alternatively, the sections were stained with alkaline bismuth (Hayat, 1981).

The last method gives a much better contrast than the first, but the coarser grain of this stain interferes with magnifications of > 30 000.

The ultrastructural studies were performed on a Philips EM 420 analytical transmission microscope.

### *Energy-dispersive X-ray analysis (EDX)*

EDX analysis was performed on 2 types of samples : 1) Thin gold sections made from tissue prepared without post fixation, excluding any contact with heavy metals. 2) Air-dried, granule preparations processed as follows :

— Both dissection and homogenising were performed in deionized water.

— The fat body or the midgut of one honeybee was homogenized in an Eppendorf tube containing 200  $\mu$ l of deionized water, with an epoxy-resin pestle. This system was especially conceived for small quantities and to minimize metal contamination. A small droplet of the homogenate was placed on a Formvar-supported single slot copper or nickel grid and air-dried. In these preparations the mineralized granules could easily be recognized and analyzed under the transmission microscope. According to Morgan (1984) this and similar preparation procedures are acceptable for the EDX analysis of relatively insoluble mineralized granules.

The analyses were performed with an EDAX-9100 system and the same microscope, at an accelerating voltage of 60 kV and a probe size of 200  $\mu$ m. All spectra were recorded for at least 60 live s.

## **Results**

### *Histology*

*Fat cells.* Initially we noticed the granules on paraffin sections of old winter bees, where they appeared as a blackish granulation in the cytoplasm, but were too small to be discerned individually. The granules are most obvious when the microscope is slightly out of focus. Their presence causes a strong PAS (Periodic acid — Schiff) coloration in the fat cell cytoplasm. In fresh preparations, under phase contrast, they can be seen as tiny black dots, whereas under UV light, they show a bright yellow autofluorescence. These results suggest that they are at least partly organic in nature.

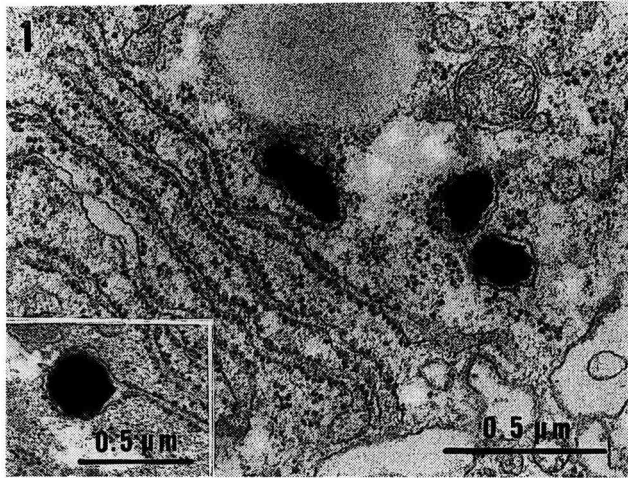
After treatment of paraffin sections with Prussian blue, the positive reaction showed that the granules were rich in iron.

On thin sections we found that the granulation corresponded with the occurrence of very electron-dense, membrane-bound granules, measuring between 400—800 nm. In fat cells of old bees, they are the predominant feature in the cytoplasm and seem to be distributed at random.

Their extreme hardness makes sectioning of the granules difficult; even with a diamond knife, the granules tend to break or to be torn from the resin.

Their form is irregular and they appear coarsely granular. From sections of tissue that had not been stained with heavy metals, it can be concluded that the granules are naturally electron-dense. However, in unstained sections, their appearance is less homogenous, suggesting the existence of a matrix which is not naturally electron-dense, but which has a strong affinity for lead and uranyl.

In order to determine the origin of these granules, we studied fat cells of young honeybees. In 24-h-old bees, the granules are relatively rare, but they can already be found in every fat cell. At this time they measure between 100—150 nm. New granules are formed within the cisternae of the rough endoplasmic reticulum (RER), close to both the plasma membrane and the nucleus. Young granules bud from the RER with their membrane still carrying some ribosomes (Fig. 1, inset). They increase by the accumulation of coarsely granular material which is most obvious on unstained sections; sometimes, fusion of 2 small granules can be seen. The largest or mature granules in older animals have lost their ribosomes and are at this point randomly distributed in the cytoplasm.

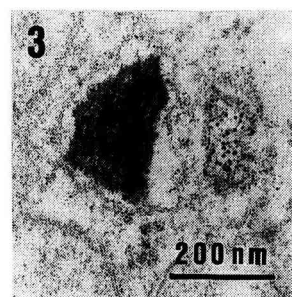
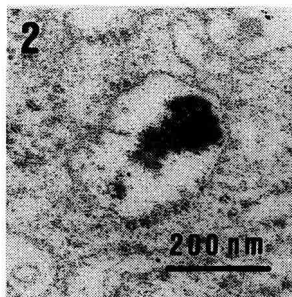


**Fig. 1.** Iron-rich granules in fat cell of 24-h old honeybees, before (inset) and after their isolation from the cisternae of the RER.

In animals reared without pollen, granules are very rare and their ultrastructure differs from that described in pollen-fed bees. They are considerably less electron-dense and show a finely granular material which is very similar to ferritin (Figs. 2, 3). In addition, the electron-dense material does not fill the vesicle formed by the RER as in the pollen-fed bees.

*Midgut epithelial cells.* In midgut epithelial cells we found the same type of

granules as in the fat cells. However, in the former the granules only occur during a limited period of the animal's life. By means of the Prussian blue stain, we followed the distribution, appearance and disappearance of the iron-rich granules in the midgut epithelium on longitudinal paraffin sections. In a first experiment the animals were reared in cages as described; they were studied after 1, 4, 8, 10, 14, 18, 20 days. In a second experiment, pollen nutrition was stopped on the 4th day; we studied the midgut



**Figs. 2, 3.** Electron-dense granular material with ferritin-like ultrastructure in RER vesicles of fat cells of 18-day-old bees reared without pollen (fat cells).

tissue on the 5th, 6th, 7th and the 8th day respectively.

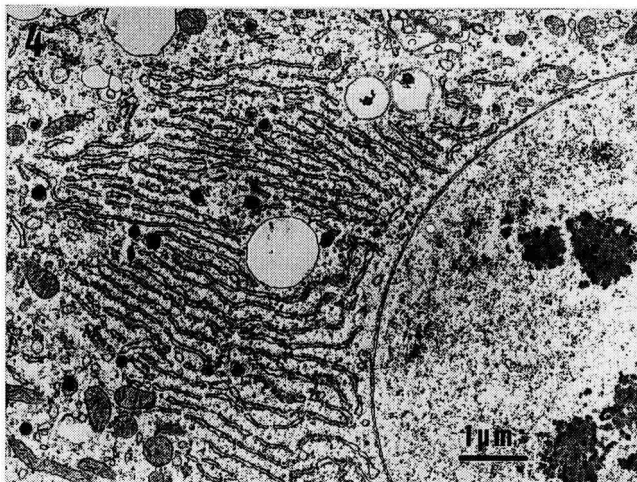
In control bees fed pollen, the granules cannot be found on day 1; they are very abundant on day 4. Their formation does not occur in the most apical and caudal parts of the midgut which are free of iron-rich granules. They can be seen in all the columnar cells but not in the crypt cells. From the 10th day onwards, their occurrence decreases gradually. Between the 10th and the 20th day they no longer occur in the young epithelial cells and the area where they are found becomes restricted to the most central part of the middle region of the midgut. On the 18th day they are generally absent and when they still occur, it is only in the oldest cells, which are ready to be shed.

When pollen feeding is terminated on the 4th day, the amount of granules on the 5th day is the same as on the 4th day. On the 6th day the occurrence of granules shifts gradually to the older epithelial cells. By the 8th day, all the granules have disappeared from the epithelium.

At the EM level, the RER in the cells close to the regenerative crypts is arranged in lamellar stacks around the nucleus in 4-day-old bees (Fig. 4) : in these cisternae the granules are formed the same way as in the fat cells. However, this lamellar arrangement was not found in the fat body cells. In 18-day-old bees the granules have become extremely rare. At that age the RER has lost its well-ordered appearance and the cisternae have become swollen and vesiculated.

#### *EDX analysis*

Qualitative EDX analyses of granules in thin sections of unstained, unosmicated material show large peaks for Fe and P, and smaller peaks for Ca and K (Fig. 5) (the Cu peak is an artefact from the copper grid; it disappears when nickel grids are used; the Si peak is an artifact from the detector). Analyses of several granule-free areas in the cytoplasm show a small peak for P and only trace amounts of Ca and K. These results are not influenced by the location of the granules or by the age of the animal.



**Fig. 4.** Typically ordered RER with iron-rich granules in midgut columnar cell of 4-day-old pollen fed bee.

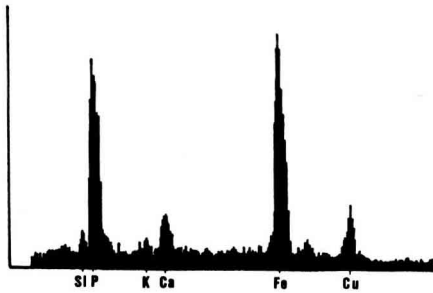


Fig. 5. EDX spectrum of iron-rich granule in thin section of unstained, unosmicated tissue (fat body).

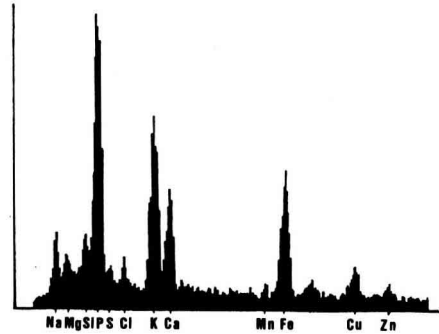


Fig. 6. EDX spectrum of iron-rich granule in air-dried granule preparation (fat body).

On osmicated sections, the granules show only trace amounts of osmium; thus they are not osmiophilous.

In air-dried granule preparations of fat body tissue, the granules can very easily be identified under the transmission electron microscope due to their electron density. In midgut tissue this identity must be confirmed by the typical high iron peak in the EDX spectrum because confusion with the so-called "calciferous granules" is possible. Typical spectra of the granules in air-dried preparations (Fig. 6) show several elements which cannot be detected on thin sections. The granules, irrespective of their origin, are mainly composed of P, Fe, Ca and K. Smaller peaks for Na, Mg, S, Cl, Mn and Zn are always present; again, the Cu- and Si peaks are artefacts.

## Discussion and Conclusion

Fat cells and midgut epithelial cells from adult honeybees contain electron-dense mineralized granules which are formed within the cisternae of the rough

endoplasmic reticulum. After their isolation from the reticulum they appear surrounded by a ribosome-covered membrane and are dispersed throughout the cytoplasm. They increase in size, probably until they have lost their ribosomes.

Their place of origin, PAS positive reaction and autofluorescence indicate that they are partly composed of organic matter. This would agree with one of the characteristics of mineralized granules (Gouranton, 1968; Martoja *et al.*, 1984). At this point the nature of this organic matrix has not been elucidated.

EDX analyses of granules on thin sections yield the same results as described by Kuterbach and Walcott (1986a) : 2 large peaks for P and Fe, and a smaller peak for Ca; in some cases we also found traces of K and Na. However, it is known that the preparation procedures necessary for thin resin sections involve serious extraction of both organic and inorganic material (Brown, 1982; Mason and Simkiss, 1982). This is reconfirmed by the comparison of the analytical results of thin sections with those of air-dried granule preparations. This extremely simple preparation method provides a

considerably better conservation of the mineral components of the granules. Apparently the granules are composed of a wide variety of elements, which is characteristic of mineralized granules (Ballan-Dufrançais, 1970; Sohal *et al.*, 1976). Their occurrence in trophocytes, high iron content, formation within the RER and the absence of concentric strata differentiate them from the well-known spherocrystals.

In the fat cells the number of granules increases considerably as the animal grows older; in accordance with Kuterbach and Walcott (1986b), we found that this increase takes place primarily in the young adult. However, we cannot agree with the authors' conclusion that the number of granules would be limited by "a finite number of sites within the cell available for granule formation", rather than by the amount of iron in the diet. The underlying experiments are unconvincing because the difference between the "iron-deficient" group and the "iron-enriched" group was insignificant: both groups received pollen, which is a much richer iron source than the iron supplied in the sugar syrup of the latter group.

The period in which the number of granules increases the most coincides with the period in which the animals feed on pollen. In old winter bees, who feed on pollen much longer, the amount of iron-rich granules is markedly higher than in old summer bees. Moreover, when we kept bees in experimental cages without pollen, very few granules were formed. We therefore suggest that the number of granules is limited by the amount of pollen consumed, and most of all by their iron content.

At the low iron diet (without pollen), we found that the fine structure of the granules was different. Normally, even on gray sections, it was impossible to discern

any ordered substructure in the granules; without pollen, their ultrastructure revealed the occurrence of finer granules, resembling ferritin. While it cannot be excluded that in normal granules the finer substructure may be masked by their high mineral content, it is also possible that normally the iron is bound to an amorphous matrix like haemosiderin or apoferritin. The latter would follow a theory developed by Locke and Leung (1984) while studying the occurrence of ferritin in *Calpodes* larvae. These authors report for the first time the occurrence of ferritin within the cisternae of the ER in several tissues of this insect. At a normal low iron intake, ferritin molecules are formed; in contrast, when the animals are loaded with unnaturally high iron concentrations, ferritin is replaced by a fluffy amorphous material. The authors interpret this material as apoferritin which has gained affinity for iron before assuming its shell structure. It is not impossible that in an insect with a high dietary iron intake like the honeybee, the latter system is the rule rather than the exception.

To our knowledge, iron-rich granules similar to those described here have not yet been found in midgut cells of insects. In the columnar cells of adult honeybees they appear only during a limited period of adult life, and their occurrence is restricted to the middle half of the midgut. In 4-day-old bees, the RER in which they are formed shows a whirl-like organisation, not unlike that described by Staubli *et al.* (1966) in midgut cells of mosquitoes. This has been interpreted as an adaptation to the temporal need to digest a protein-rich food. This is particularly interesting, because, as in blood-sucking insects, the midgut of honeybees must only temporarily digest protein-rich food (pollen); afterwards, both insects are nectar feeders.

In contrast to the fat cells, the midgut epithelial cells are constantly renewed, and thus some days after the granules are formed they are eliminated from the tissue. Two days after the premature ending of the pollen nutrition in our second experiment, granules can no longer be seen in the younger columnar cells where normally they are most actively synthesized. This indicates that no new granules are formed past this period and that their formation is closely linked to pollen nutrition.

Neither at the light microscope level nor at the ultrastructural level have we ever seen the extrusion of individual granules from the cells; their elimination seems bound to the expulsion of old, spent cells which are filled with spherocrystals, iron-rich granules and secondary lysosomes. Therefore, the results of this experiment indicate that the renewal of the midgut epithelium in adult honeybees takes about 4 days.

We cannot exclude the possibility that the iron-rich granules have a function related to the detection of earth magnetism as suggested by Loper (1985) and by Kuterbach and Walcott (1986a). However, we think it more probable that, like the typical spherocrystals which are also numerous in the midgut epithelium, the iron-rich granules serve in eliminating surplus ions by a process of biomineralization. As the midgut spherocrystals contain only traces of iron, their affinity for this element is apparently too small to deal with the high concentrations present in pollen nutrition. A further intensification of the iron elimination system described by Locke and Leung (1984) would therefore form a useful adaptation.

Apart from iron, the granules contain several other elements and it is interesting to note that after peroral administration of lead chloride, they store

this heavy metal just like the spherocrystals (Raes *et al.*, 1988). In this context we are now engaged in a study on the detoxification of heavy metals by honeybees, and their potential use as biomonitors for this form of pollution.

### Acknowledgments

We are grateful to Mrs. U. Rzeznik for skillful technical assistance. This research has been partly supported by grant No. 2.0015.88 from the FKFO (Belgian Fund for Collective Fundamental Research).

**Résumé — Granules riches en fer associés à la membrane dans les cellules adipeuses et les cellules de l'intestin moyen chez l'abeille adulte (*Apis mellifica* L.).** Kuterbach *et al.* (1982) et Kuterbach et Walcott (1986a, b) ont décrit chez l'abeille des granules riches en fer dans les cellules adipeuses. Nous vous présentons ici des données complémentaires sur la présence, l'origine, la formation, la composition relative et la fonction possible de ces granules.

Les abeilles, d'âge connu, proviennent d'une ruche de plein air et de cagettes expérimentales. Pour la microscopie en fluorescence et en contraste de phase, nous avons utilisé des coupes paraffinées non colorées ou du tissu frais. Les granules riches en fer ont été mis en évidence sur les coupes paraffinées par la méthode du bleu de Prusse. A cause du faible contraste membranaire des tissus de l'abeille il a fallu mettre au point une technique spéciale de préparation de ces tissus pour la microscopie électronique à transmission. La composition relative des

granules a été étudiée par l'analyse par diffraction aux rayons X (EDX) sur les coupes fines réalisées dans du tissu préparé sans fixation ou coloré avec des métaux lourds et sur des préparations de granules séchés à l'air. Les analyses ont été faites avec un système EDAX-9100 sur un microscope analytique à transmission Philips EM 420.

Les concrétions minéralisées se forment dans les cellules adipeuses et les cellules épithéliales de l'intestin moyen des abeilles adultes; elles sont riches en fer, naturellement opaque aux électrons et possèdent probablement une matrice organique.

Un spectre EDX typique de granules séchés à l'air a montré plusieurs éléments qui n'ont pu être détectés sur les coupes fines. Outre les principaux pics correspondant à P, Fe, Ca et K, des pics plus petites pour le Na, Mg, S, Cl, Mn et Zn étaient toujours présents.

Des granules riches en fer se forment à l'intérieur des cisternae du reticulum endoplasmique rugueux. Après avoir bourgeonné, à partir du reticulum, ils restent entourés d'une membrane couverte de ribosomes qui leur permet de grandir. Dans les cellules adipeuses, ils se forment soit contre la membrane cellulaire, soit contre le noyau; leur nombre s'accroît principalement durant la période où les abeilles se nourrissent de pollen. Chez les abeilles plus vieilles, les granules ont perdu leurs ribosomes et sont alors distribués au hasard dans le cytoplasme. Le même type de granules se forme dans les cellules columnaires. Dans les cellules jeunes, le RER à partir duquel elles se forment se présente de façon typique. Tandis que dans les cellules adipeuses les granules sont accumulés durant toute la vie de l'insecte, dans l'intestin moyen, ils disparaissent avec les cellules épithéliales utilisées. Ils

sont très abondants le 4<sup>e</sup> jour mais leur nombre décroît à partir du 10<sup>e</sup> jour, lorsque les abeilles arrêtent leur alimentation pollinique. Lorsque l'on supprime l'alimentation pollinique le 4<sup>e</sup> jour, les granules disparaissent de l'épithélium de l'intestin moyen avant le 8<sup>e</sup> jour fournissant ainsi une indication sur la durée de vie des cellules columnaires. Chez les abeilles élevées sans pollen, les granules sont très rares et leur ultrastructure différente : ils sont moins opaques aux électrons et présentent de fins granules semblables à de la ferritine.

Nous proposons l'hypothèse suivante : comme les sphéro cristaux typiques, également nombreux dans l'intestin moyen, les granules riches en fer servent à éliminer les ions en surplus, par un processus de biominéralisation. Leur formation semble être liée à la nutrition pollinique. Il s'agit probablement d'une adaptation particulière à la forte teneur en fer de cette source de protéine.

***Apis mellifera* — biominéralisation — granule riche en fer — cellule adipeuse — cellule épithéliale de l'intestin**

**Zusammenfassung — An Membranen gebundene eisenhaltige Granula in den Zellen des Fettkörpers und des Mitteldarmes der erwachsenen Honigbiene (*Apis mellifera* L.).** Eisenhaltige Granula in Fettzellen wurden bei Honigbienen von Kuterbach *et al.* (1982) und von Kuterbach und Walcott (1986a, b) beschrieben. In dieser Arbeit legen wir zusätzliche Daten über Vorkommen, Ursprung, Bildung, anteilmäßige Zusammensetzung und mögliche Funktion dieser Granula vor.

Für die Versuche wurden Honigbienen bekannten Alters aus freiliegenden

Völkern so wie aus Versuchskäfigen benutzt. Für Untersuchungen mit dem Fluoreszenz- und Phasenkontrast-Mikroskop wurden ungefärbte Paraffinschnitte oder frisches Gewebe benutzt. Die eisenhaltigen Granula wurden in Paraffinschnitten mit der Preußisch-Blau-Methode sichtbar gemacht.

Wegen des schwachen Kontrastes der Membranen des Bienengewebes mußte für TEM-Untersuchungen dieses Gewebes eine besondere Technik entwickelt werden.

Für die Untersuchung der relativen Zusammensetzung der Granula wurden EDX-Analysen der Dünnschnitte von Geweben benutzt, die ohne Fixierung oder Färbung mit Schwermetallen präpariert waren, oder luftgetrocknete Granula-Präparate. Die Analysen wurden mit einem EDAX-9100-System an einem Philips EM 420 analytischen Transmissions-Mikroskop durchgeführt.

Die mineralisierten Konkretionen werden sowohl in Fettzellen wie in Epithelzellen des Mitteldarms der erwachsenen Honigbiene gebildet. Sie haben einen hohen Eisengehalt, sind elektronendicht und besitzen wahrscheinlich eine organische Matrix. Ein typisches EDX-Spektrum von luftgetrockneten Granula zeigte mehrere Elemente, die an Dünnschnitten nicht gefunden werden konnten. Außer den Hauptpeaks für P, Fe, Ca und K waren immer auch kleinere Peaks für Na, Mg, S, Cl, Mn und Zn vorhanden.

Die eisenhaltigen Granula werden innerhalb der Zisternen des RER gebildet. Sobald sie sich aus dem Retikulum als Knospen entwickelt haben, bleiben sie von einer Ribosomen-bedeckten Membran eingeschlossen, wodurch eine Größenzunahme ermöglicht wird. In Fettzellen werden sie entweder an der

Zellmembran oder am Kern gebildet; ihre Zahl steigt besonders während der Periode, in der sich die Tiere von Pollen ernähren. Bei älteren Bienen haben sie ihre Ribosomen verloren und die Granula sind dann zufällig im Zytoplasma verteilt.

Derselbe Typ von Granula wird in den säulenförmigen Zellen in der Zentralregion des Mitteldarms gebildet. In jungen Zellen ist das RER, aus dem sie sich bilden, in einer sehr typischen Weise ausgebildet. Während die Granula in den Fettzellen während des ganzen Lebens des Tieres erhalten bleiben, werden sie im Mitteldarm zusammen mit den Epithelzellen abgestoßen; am 4. Tag sind sie sehr reichlich, aber nach dem 10. Tag — wenn die Biene die Pollenaufnahme beendet — nimmt ihre Zahl ab. Wird die Pollenernährung am 4. Tag unterbrochen, dann sind die Granula am 8. Tag aus den Epithelzellen des Mitteldarms verschwunden, wodurch auch ein Hinweis auf die Lebensdauer der säulenförmigen Zellen gegeben wird. In pollenfrei aufgezogenen Bienen sind die Granula sehr selten und deren Struktur ist verschieden: Sie sind weniger elektronendicht und zeigen feine Ferritin-ähnliche Körnchen.

Wir vermuten, daß die eisenhaltigen Granula ähnlich wie die typischen, zahlreichen Sphärokristalle des Mitteldarms dazu dienen, überschüssige Ionen durch einen Prozeß der Biomineralisation auszuscheiden; ihre Bildung scheint an die Pollenernährung gekoppelt zu sein und stellt wahrscheinlich eine Anpassung an den hohen Eisengehalt dieser Proteinquelle dar.

***Apis mellifera* — Biomineralisation — eisenhaltige Granula — Fettzell — Epithelzelle des Mitteldarms**

References

Ballan-Dufrançais C. (1970) Données cytophysiologiques sur un organe excréteur particulier d'un insecte : *Blatella germanica* (L.). *Z. Zellforsch. Mikrosk. Anat.* 109, 336-355

Brown B.E. (1982) The form and function of metal-containing "granules" in invertebrate tissue. *Biol. Rev.* 57, 621-667

Da Cunha M.A.S. (1987) Iron containing cells in the stingless bee *Scaptotrigona postica* Latr. (Hym., Apidea). In : *Chemistry and Biology of Social Insects* (J. Eder & H. Rembold, eds), p. 91

Gouranton J. (1968) Composition, structure et mode de formation des concrétions minérales dans l'intestin moyen des Homoptères Cercopides. *J. Cell Biol.* 37, 316-328

Hayat M.A. (1981) *Principles and Techniques of Electron Microscopy. Biological Applications* (A.E. Kent, ed), p. 413

Hutchison H.E. (1953) The significance of stainable iron in sternal marrow sections. *Blood* 8, 236-248

Jeauntet A.Y., Ballan-Dufrançais C. & Marjota R. (1977) Insect resistance to mineral pollution. Importance of spherocrystals in ionic regulation. *Rev. Ecol. Sol.* 14, 563-582

Koehler A. (1981) Über die Einschlüsse der Epithelzellen des Bienendarmes und die damit in Beziehung stehenden Probleme der Verdauung. *Z. Angew. Entomol.* 7 (1) 68-91

Kushida H. (1966) Staining of thin sections with lead acetate. *J. Electron. Microsc.* 15, 93

Kuterbach D.A. & Walcott B. (1986a) Iron-containing cells in the honeybee (*Apis mellifera*). I. Adult morphology and physiology. *J. Exp. Biol.* 126, 375-387

Kuterbach D.A. & Walcott B. (1986b) Iron-containing cells in the honeybee (*Apis mellifera*). II. Accumulation during development. *J. Exp. Biol.* 126, 389-401

Kuterbach D.A., Walcott B., Reeder R.J. & Frankel R.B. (1982) Iron-containing cells in the honeybee (*Apis mellifera*). *Science* 218, 695-697

Locke M. & Leung H. (1984) The induction and distribution of an insect ferritin — a new function for the endoplasmatic reticulum. *Tissue Cell* 16, 739-766

Loper G.M. (1985) Influence of age on the fluctuation of iron in the oenocytes of honeybee (*Apis mellifera*) drones. *Apidologie* 16, 181-184

Martoja R. & Ballan-Dufrançais C. (1984) The ultrastructure of the digestive and excretory organs. In : *Insect Ultrastructure 2* (R.C. King & Akai, eds), Plenum Press, pp. 199-268

Mason A.Z. & Simkiss K. (1982) Sites of mineral deposition in metal-accumulating cells. *Exp. Cell Res.* 139, 383-391

Morgan A.J. (1984) The localisation of heavy metals in the tissue of terrestrial invertebrates by electron micropobe X-ray analysis. In : *Scanning Electron Microscopy IV* (O'Hare, ed.), Chicago, pp. 1847-1865

Raes H., Bohyn W. & Jacobs F. (1988) Etude de la détoxication du plomb de l'abeille (*Apis mellifera* L.). *Actes 4 Colloq. Insectes Sociaux*, pp. 95-101

Raes H., Jacobs F. & Mastyn E. (1985) A preliminary qualitative and quantitative study of the microscopic structure of the dorsal fat body in adult honeybees (*Apis mellifera*), including a technique for the preparation of whole sections. *Apidologie* 16, 275-290

Raes H., De Coster W. & Bohyn W. (1987) Light and electron microscopic study of oenocytes in adult honeybees with particular emphasis on the fixative osmolarity. In : *Chemistry and Biology of Social Insects* (J. Eder & H. Rembold, eds.), p. 89

Simkiss K. (1979) Metal ions in cells. *Endeavour* NS3, 2-6

Simkiss K. (1981) Cellular discrimination processes in metal accumulating cells. *J. Exp. Biol.* 94, 317-327

Sohal R.S., Peters P.D. & Hall T.A. (1976) Fine structure and X-ray microanalysis of mineralised concretions in the Malpighian tubules of the house fly, *Musca domestica*. *Tissue Cell* 8, 447-458

Staubli W., Freyvogel T.A. & Suter J. (1966) Structural modification of the endoplasmatic reticulum of midgut epithelial cells of mosquitoes in relation to blood intake. *J. Microsc.* 5, 189-204