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MORPHOLOGY AND BIOCHEMICAL COMPOSITION OF MINERALIZED GRANULES FROM THE MALPIGHIAN TUBULES OF *MUSCA AUTUMNALIS* DE GEER LARVAE (DIPTERA: MUSCIDAE)

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Abstract—*Musca autumnalis* De Geer (face fly) larvae form numerous small mineralized granules in the lumen of the anterior Malpighian tubules. The granules are spherical, ranging in diameter from 0.2 to >10 µm and are composed of many concentric thin layers. The layers appear to be covered by an organic-rich material that may help to bind adjacent layers together. Individual layers appear to be heterogeneous with some composed of small rounded particles of inorganic-rich material imbedded in a loose fibrous mat while other layers appear more solid. The granules are primarily inorganic in composition (>85% ash) with calcium, phosphorus, and magnesium contributing more than 98% of the inorganic elements. Water content is approx. 10%. Carbonate carbon and hydrogen account for 2 to 3% of the granule weight. Nitrogen and organic carbon are only minor elements primarily present in the form of amino acids which contribute only 0.4% of the total granule weight. Aspartic acid and glutamic acid comprise more than 30% of the total amino acids detected after acid hydrolysis. The properties of face fly granules and puparial cuticle are compared, together with those of mineralized granules from other species of invertebrates.

Key Word Index: Face fly, mineralized granules, Malpighian tubules, morphology, composition

INTRODUCTION

Musca autumnalis De Geer (face fly) larvae have a relatively unique mechanism for puparial cuticle stabilization where deposition of mineral salts primarily occurs instead of sclerotization that is thought to involve dehydration and cross-linking of the chitin-protein matrix by catecholic and quinonoid metabolites (Fraenkel and Hsiao, 1967; Grodowitz and Broce, 1983; Darlington *et al.*, 1983; Roseland *et al.*, 1985). The minerals are extracted from the larval diet (bovine dung) during feeding and are stored in the anterior Malpighian tubules as small granules (Grodowitz and Broce, 1983). The granules are composed mainly of calcium, phosphorus and magnesium with appreciable quantities of carbonate (Darlington *et al.*, 1983; Grodowitz and Broce, 1983).

Similar types of granules have been reported in other invertebrate species (reviewed by Brown, 1982). Waterhouse (1950) characterized mineralized granules from the Malpighian tubules of the larval blowfly, *Lucilia cuprina* L. This species does not mineralize the puparial cuticle and accordingly granules are discharged with the pupal meconium. Several other species of flies accumulate mineralized granules

in the Malpighian tubules including *Eristalis tenax* L., *Myiatropa florea* L., *Mallota eristaloides* W., *Merodon equestris* F., *Syrirta pipiens* L., *Eumerus strigatus* Flin., *Tychoptera contaminata* L., several species of Stratiomyidae, *Anastrepha striata* (Keilin, 1921), *Rhagoletis cerasi* L. (Weismann, 1938), and *Musca domestica* L. (Sohal and Lamb, 1979; Grodowitz and Broce, 1983; Elonon, 1985). Thus, the storage of minerals in small granules appears to be fairly common among the Diptera. Mineralized granules have also been characterized from many other phyla including Protozoa, Annelida, Trematoda, Cestoda, Mollusca, Gastropoda, and Cephalopoda (reviewed by Watabe *et al.*, 1976; Brown, 1982). Few of these granules have been characterized in terms of chemical composition and morphology. The best characterized granules are the calcareous corpuscles of tapeworms, especially *Taenia taeniaeformis* (Scott *et al.*, 1962; von Brand *et al.*, 1967; Neiland and von Brand, 1969; von Brand and Nylén, 1970; von Brand, 1973) and the spherules of *Pomacea paludosa* (Watabe *et al.*, 1976). These species have granules that are remarkably similar both in morphology and to a lesser extent in mineral composition (Watabe *et al.*, 1976). Hence, it appears that mineralized granule formation is common among the invertebrates with similar properties between granules from widely separated species.

Grodowitz and Broce (1983) and Elonon (1985) correlated granule disappearance from the Malpighian tubules of face fly larvae during pupariation with increasing calcium concentration in the cuticle

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and concluded that the granules are the primary source of inorganic salts for mineralization of the puparial cuticle. In this study we have characterized the morphology and chemical composition of the mineralized granules in face fly larvae. We have also compared our results with those obtained from studies on mineralized cuticle and granules present in other invertebrate species. Mechanisms of granule morphogenesis are discussed in relation to mineral utilization by the face fly.

MATERIALS AND METHODS

Insects

Granules were obtained from wandering larvae reared in bovine dung at the Department of Entomology, Kansas State University. Wandering larvae were defined as those individuals which had left the larval habitat and had completely emptied their digestive tract in preparation for pupariation. This is the larval stage with the highest quantity of granules (Grodowitz and Broce, 1983). Granules were recovered from the Malpighian tubules by a technique developed by A. A. Rueda (unpublished data) in our laboratory. Minced larvae were washed while suspended on a fine mesh nylon cloth and immersed in a flowing deionized water bath. Granules accumulated in the bottom of the bath and were relatively free of extraneous insect tissues. After this initial separation, granules were further cleaned by repeated washing in deionized water and centrifugation. For each centrifugation the top layer of the pellet was removed to ensure that no insect tissues were remaining with the granules. Purified granules were lyophilized and stored at 20°C in a desiccator.

Electron microscopy

Granules were frozen in liquid nitrogen while placed between two small tool steel plates and subsequently fractured by sharply striking the upper plate with a rubber mallet. The organic material in the fractured granules was oxidized using plasma ashing at 60 W and 20 cm³ air min⁻¹ for 60 min. Since plasma ashing uses low frequency radio waves to form oxygen plasma which allows the rapid oxidation of organic material at relatively low temperatures, the technique apparently keeps the inorganic structure intact (Humphreys *et al.*, 1979; Mason, 1983). Inorganic material was removed using a modified low pH demineralization and simultaneous chromium sulfate fixation method (Sundstrom, 1966). This technique apparently preserved the organic matrix and did not allow the loss of water soluble components. For all treatments, isolated granules were prepared for scanning electron microscopy (SEM) by freeze-drying. Malpighian tubules and their contents, however, were preserved in 10% formalin for 24 hr, dehydrated in a graded ethanol series and then critical-point dried. All specimens for SEM were coated with gold-palladium and viewed with an ETEC Auto Scan U-1 electron microscope. Specimens for energy dispersive X-ray analysis (EDXA) were placed on carbon stubs and coated with carbon only; they were analyzed at 20 keV.

For transmission electron microscopy (TEM) thin sections of granules from the proximal portion of the Malpighian tubules were prepared. This portion of the Malpighian tubule was chosen because of the relative ease of sectioning naturally dissolving granules. Specimens were fixed in 2% glutaraldehyde and 1% osmium tetroxide in 0.2 M cacodylate buffer at pH 7.0, dehydrated in a graded ethanol series and subsequently infiltrated and imbedded in a low viscosity plastic (Mascorro *et al.*, 1976). Thin sections were stained with uranyl acetate and Reynold's (1963) lead citrate, and viewed with a Phillips 201 transmission electron microscope at 60 keV.

Elemental analysis

Lyophilized granules were dried under vacuum prior to ashing to eliminate weight increase due to atmospheric water uptake. Granules were then ashed, solubilized in HCl, and analyzed for mineral content by multichannel graphite furnace atomic absorption spectroscopy at the Kansas State University Emission Spectroscopy Laboratory. Total carbon, carbonate carbon, hydrogen and nitrogen were determined by Huffman Laboratories, Inc. (Wheatridge, Colorado). Water content was determined gravimetrically by heating the granules at 150°C for 18 hr.

Amino acid analysis

Isolated granules were first solubilized in 6 N HCl. Hydrolysis was carried out in sealed containers for 20 hr at 110°C *in vacuo*. Amino acids were separated by cation exchange chromatography and quantified by post column ninhydrin derivatization.

Carbohydrate analysis

Total carbohydrates (except hexosamines) were determined using a phenol-sulfuric acid method (Dubois *et al.*, 1956). One half of a milliliter of a 5% phenol solution was added to approx. 1 mg of sample, followed by 2.5 ml of concentrated sulfuric acid with subsequent mixing. The solution was placed in a water bath at 30°C for 20 min. Absorbances were measured at 490 nm using a Cary 118C spectrophotometer.

RESULTS

Morphology

Granules isolated from the distal portion of the anterior Malpighian tubules of face fly larvae were spherical and ranged in diameter from 0.2 to >10.0 µm (Fig. 1a). They almost completely filled the lumen of the distal portion of the tubules and were randomly distributed. The outer surface of the granules was relatively smooth with only minor surface irregularities. Granules in the proximal portion of the anterior Malpighian tubules were smaller ranging in diameter from <0.2 µm to only slightly greater than 2.0 µm and they did not fill the lumen as completely as was observed in the distal portion (Fig. 1b). Some of the granules were localized in between the microvilli of the tubule (Fig. 1b). Granules were composed of numerous concentric thin layers as evidenced by TEM (Fig. 1b) and SEM of fractured granules (Fig. 1c). This concentric layered organization was evidenced in thin sections by alternating electron dense and electron lucid areas (Fig. 1b). The outer layers appeared very dense as were some core regions. The electron density pattern in the transmission electron micrographs probably depended on the orientation of the plane of the section taken with the dense core region visible only in granules sectioned at or near their center (Brown, 1982). The concentric layers in fractured granules were closely packed and appeared as thin circular depressions (Fig. 1c).

Granules were relatively insoluble in water, but they were completely solubilized during dialysis against 0.15 M EDTA buffered at pH 7. Chelation of metal cations, such as calcium and magnesium ions, leads to solubilization of both inorganic and organic components. Removal of organic-rich material by plasma ashing showed that organic material occurs between and within the concentric layers (Fig. 1d-f),

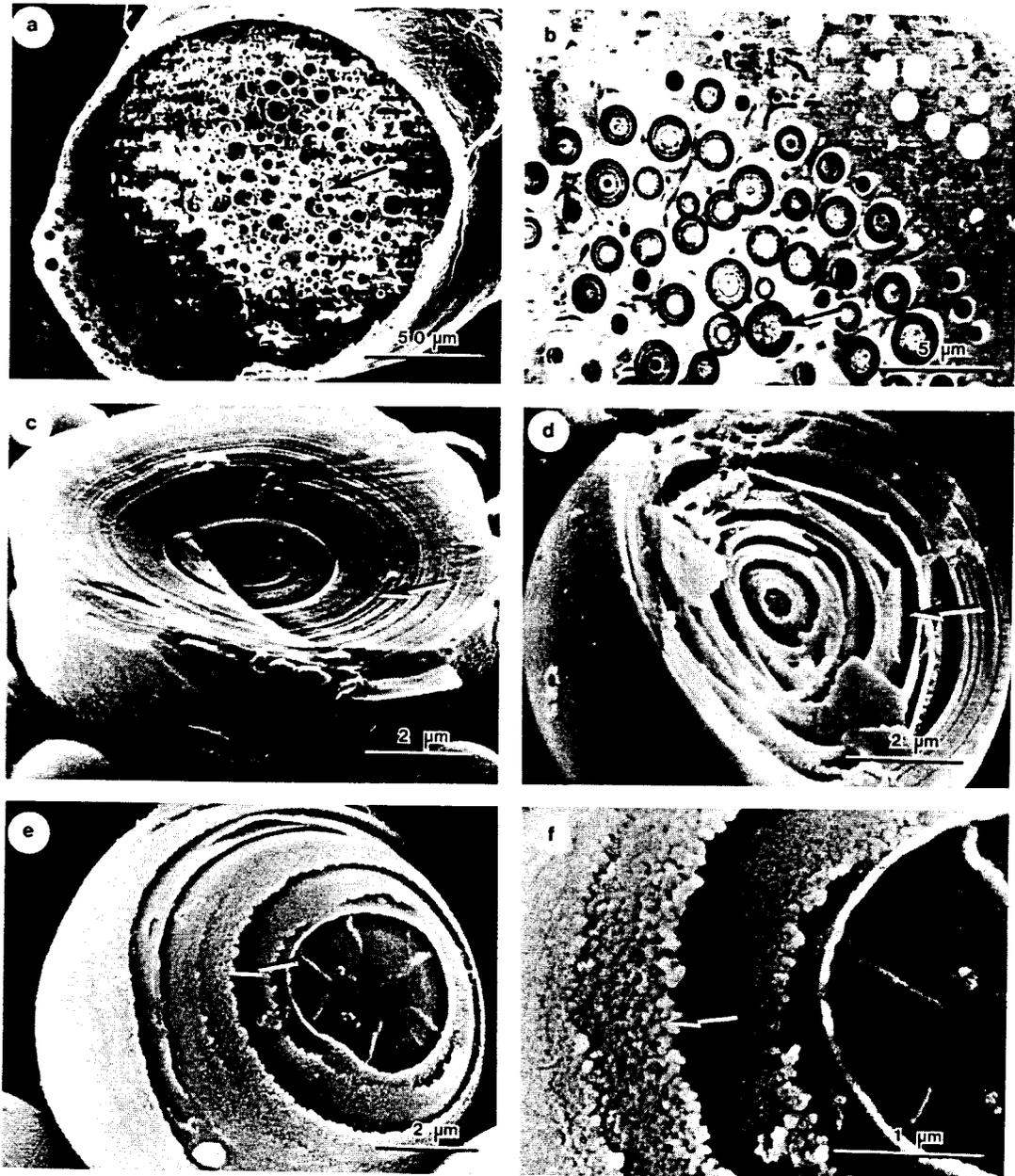


Fig. 1. Electron micrographs of *M. autumnalis* granules from Malpighian tubule. (a) SEM of distal portion of anterior Malpighian tubule showing mineralized granules (arrow) completely filling lumen. (b) TEM of proximal portion of anterior Malpighian tubules showing naturally dissolving granules (arrow). Note loss of concentric ring pattern. (c) SEM of freeze-fractured granules isolated from anterior Malpighian tubules illustrating concentric layer (arrow). (d-f) Granule in which organic-rich matrix has been removed using plasma ashing. (d) Note that organic-rich matrix has been removed from between layers. (e,f) Note the small rounded particles composing the individual layers (arrows).

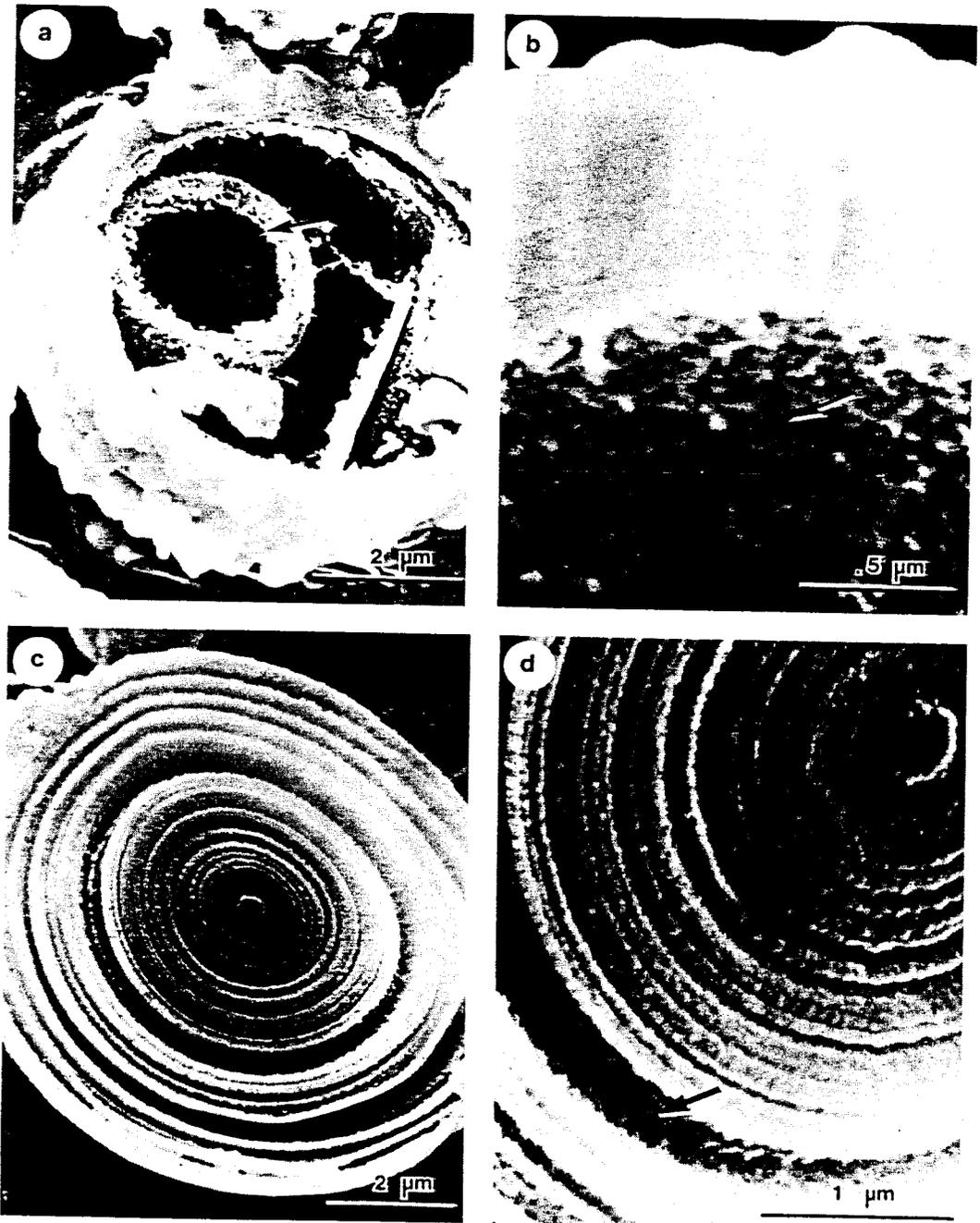


Fig. 2. Scanning electron micrographs of *M. autumnalis* granules from Malpighian tubules after chromium sulfate treatment. (a,b) Organic matrix of individual layers showing fibrous mat structure (arrows). (c,d) Organic matrix envelopes and connecting fibrous material (arrow) between layers.

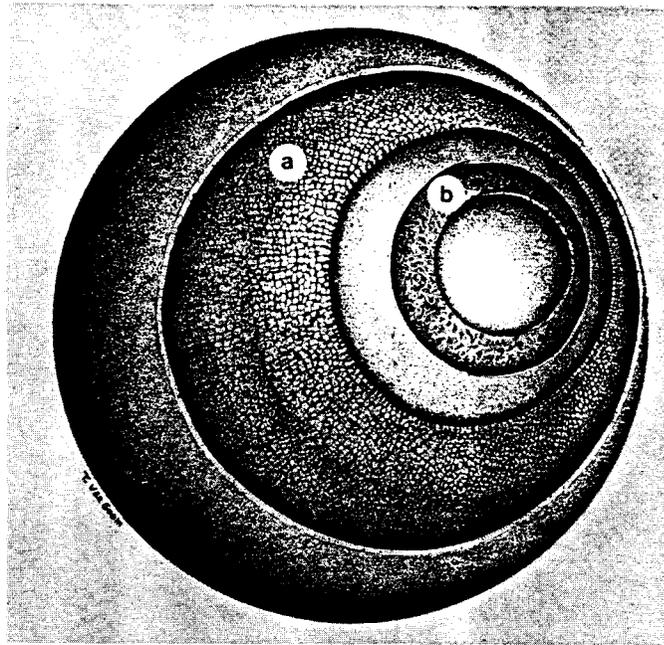


Fig. 4. Three dimensional reconstruction illustrating the proposed morphology of the concentric layered mineralized granules from *M. autumnalis*. Note that layer (a) represents the particulate aggregates with organic-rich material depleted while layer (b) represents the fibrous "organic matrix" with inorganic-rich material depleted. Refer to Fig. 1e and Fig. 2a,c for micrographs from which model was constructed.

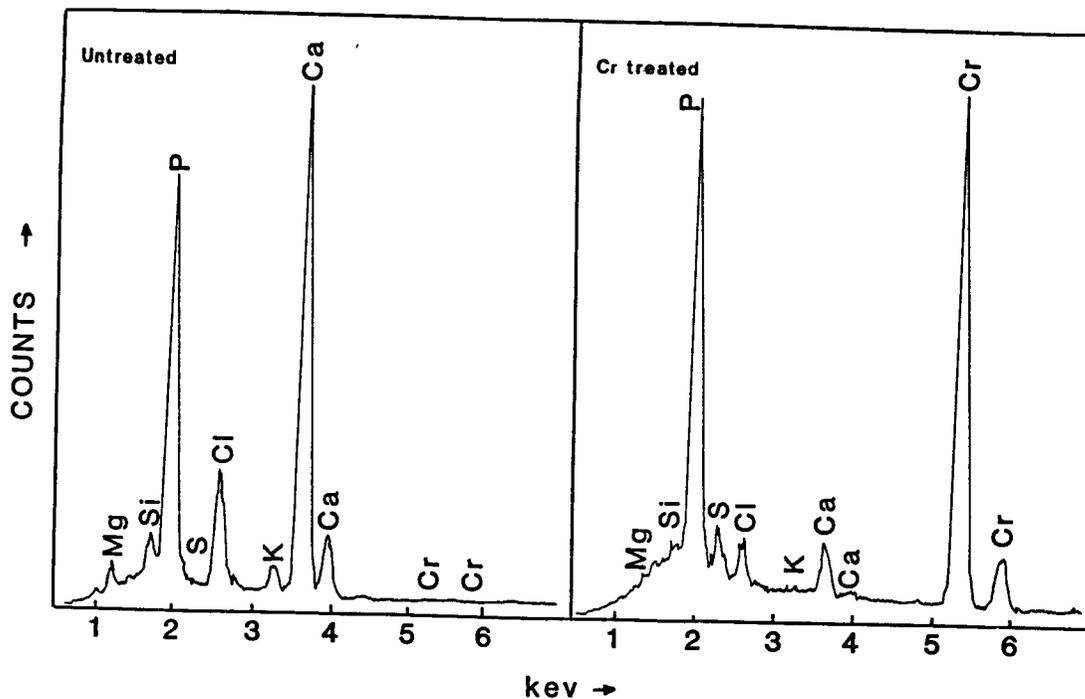


Fig. 3. Energy dispersive X-ray analysis of *M. autumnalis* granules with no treatment (left) and demineralized with chromium sulfate (right). Note relative absence of calcium in chromium sulfate treated granules and high concentrations of chromium.

since the layers separate after plasma ashing treatment (Fig. 1d,e). Soaking the granules in dilute sodium hypochlorite, a solution used to oxidize organic components, did not change the surface morphology. The layers were heterogenous with some being essentially solid (Fig. 1d), while others were composed of appreciable quantities of organic-rich components since substantial material was removed within the layers by plasma ashing leaving small rounded particles comprising the layers (Fig. 1e,f).

The hypothesis that the organic-rich material occupied the area around these subparticles was supported by microscopic analysis of demineralized granules (Fig. 2a-d). After removal of the inorganic components using a low pH fixation with chromium sulfate, the organic-rich matrix appeared as fibrous material organized into a loose mat (Fig. 2a,b) that is distributed throughout the granule, being present on the surface of the granule, within the mineral-rich layers (Fig. 2b) and also between the layers as indicated by the fibrous material covering and connecting the individual concentric layers (Fig. 2c, d). Some inorganic components were also removed from the space within the fibrous mat.

EDXA analysis revealed that calcium, phosphorus, chlorine, magnesium, potassium and silicon were present in native granules (Fig. 3). Chromium sulfate treated granules contained high levels of chromium and phosphorus and relatively little calcium. The chromium concentration was similar to that of calcium in untreated granules. Thus, chromium sulfate fixation caused chromium deposition as well as the depletion of calcium, magnesium, chlorine and potassium.

Elemental analysis

Isolated granules were composed mainly of inorganic material as indicated by the high percentage of ash (>85%, Table 1). The rest of the granule weight was primarily water ($11.9 \pm 1.5\%$) together with minor quantities of organic material (see next section). The primary inorganic elements were calcium, phosphorus and magnesium which accounted for >98% of the inorganic material analysed and >48% of the total weight of the granule. Calcium was the most abundant inorganic element, being 3 and 9-fold higher than phosphorus and magnesium, respectively. Minor quantities of manganese and potassium were also detected with both occurring in excess of 1.0 mg g^{-1} . In addition, there were trace quantities of zinc and copper present ($<1.0 \text{ mg g}^{-1}$).

Table 1. Ash content (%) and inorganic and organic elemental composition (mg/g) of granules from the larval Malpighian tubule and of puparial cuticle of *Musca autumnalis* (mean \pm standard error)

	Granule	Puparial cuticle*
Per cent ash	87.7 ± 1.2	62.8
Calcium	340.0 ± 20.0	184.7 ± 20.0
Phosphorus	113.0 ± 6.7	99.3 ± 20.0
Magnesium	35.3 ± 0.8	1.5 ± 0.9
Potassium	1.5 ± 0.1	8.9 ± 0.4
Zinc	0.8 ± 0.1	2.4 ± 0.7
Copper	0.2 ± 0.1	0.6 ± 0.1
Cobalt	<0.1	<0.1
Iron	<0.1	0.6 ± 0.1
Carbonate carbon	1.5 ± 1.2	77.9 ± 0.8
Hydrogen	22.6 ± 0.5	33.1 ± 0.1
Nitrogen	1.8 ± 0.2	15.0 ± 0.8
Total carbohydrate	<2.0	6.0 ± 1.0

*Roseland *et al.* (1985).

Table 2. Amino acid composition of granules and puparial cuticle from *Musca autumnalis*, and granules from *Pomacea paludosa* (Gastropoda), and larval *Taenia taeniaeformis* (Cestoda)*

	<i>Musca autumnalis</i>		<i>P. paludosa</i> †		<i>T. taeniaeformis</i> ‡
	Granules		Cuticle§, res./1000	ACG¶, res./1000	
	nmol/mg ± SE	res./1000			res./1000
Aspartic	3.73 ± 0.23	132	82	137	187
Threonine	1.69 ± 0.17	60	46	63	77
Serine	1.20 ± 0.09	42	41	76	51
Glutamic	4.92 ± 0.33	174	97	125	124
Proline	0.42 ± 0.02	15	52	66	12
Glycine	1.85 ± 0.17	65	342	102	155
Alanine	2.33 ± 0.12	82	70	79	102
Cystine	0.45 ± 0.11	16	ND	30	7
Valine	2.48 ± 0.31	87	61	68	55
Methionine	0.49 ± 0.13	17	5	13	ND
Isoleucine	0.72 ± 0.05	25	28	38	40
Leucine	0.98 ± 0.05	35	32	60	40
Tyrosine	1.30 ± 0.21	46	26	23	14
Phenylalanine	1.05 ± 0.12	37	20	46	34
Histidine	2.29 ± 0.06	81	47	8	18
Lysine	1.36 ± 0.11	48	31	46	33
Arginine	1.09 ± 0.07	38	19	28	51
Ammonia	NA	NA	NA	101	120
Glucosamine	+	+	+	+	+

*NA = not available; ND = not determined; + = present.

†Watabe *et al.* (1976).

‡von Brand and Nylén (1970).

§Roseland *et al.* (1985).

¶ACGE = albumen-capsule gland complex.

Carbonate carbon and hydrogen were the fourth and fifth most abundant elements in the granules and were present in excess of 20 mg g⁻¹ which represents >5.0% of the total weight of the granule. Nitrogen and organic carbon levels were considerably lower. They were detected in quantities of less than 2 mg g⁻¹ of the granule weight.

Amino acid analysis

Minor quantities of amino acids were detected in isolated granules after acid hydrolysis (Table 2), and altogether these represented only about 0.4% of the total weight of the granules. All of the commonly occurring amino acids were found with the two most abundant being aspartic acid and glutamic acid and/or their amides. These amino acids made up over 30% of the total amino acids detected. The next most abundant amino acids were valine, alanine and histidine. These three represented over 25% of the total amino acids detected. Hence, only five amino acids accounted for >50% of those found. The remaining amino acids were found in minor levels of <1.4 nmol mg⁻¹ of the granule weight.

Carbohydrate analysis

Small quantities of carbohydrates were detected in isolated granules (Tables 1 and 2). Low levels of glucosamine were present but could not be quantified because of resolution difficulties with tyrosine and phenylalanine during ion exchange chromatography. Pentoses, hexoses or uronic acids accounted for less than 0.2% of the total dry weight of the granule. Carbohydrates do not appear to be a major constituent of the face fly granule.

Mass balance calculation indicated that ca 97% of the ash components are accounted for by these analyses. It is believed that ca 30% of the calcium carbonate decomposes during the ashing procedure

and that value was adjusted accordingly. The material not accounted for was probably sodium which is present as indicated by EDXA (data not shown). The non-ash components (amino acids and water) accounted for ca 100% of the weight lost by ashing and this represented about 12% of the total granule weight.

DISCUSSION

Granules isolated from the Malpighian tubules in face fly larvae have a highly complex structure (Fig. 4). They are composed primarily of inorganic material mostly calcium, phosphorus, magnesium, and carbonate carbon, with lesser quantities of hydrogen, nitrogen and organic carbon. Oxygen is presumed to be present in substantial quantities. Water is present in a significant quantity slightly greater than 10%. Carbonate is an important constituent of the granules comprising approx. 14% of the total weight. Ca:P ratios in *ortho* phosphate salts vary from 0.5 to 1.5, however, the granules exhibit a higher Ca:P ratio of 2.3. The type of mineral salt in the granules is uncertain but the major salt could be either a calcium *ortho* phosphate (CaHPO₄) or some form of amorphous calcium phosphate (ACP; Brecevic and Furedi-Milhofer, 1972).

Morphologically the granules are spherical and are composed of numerous concentric layers (Fig. 4). Within each layer the inorganic-rich material appears to be organized into smaller granular particles with no distinct distribution. The inorganic-rich material appears not in a crystalline form but is amorphous as demonstrated by X-ray diffraction (Grodowitz and Broce, 1983). The organic-rich material has a loose fibrous structure that surrounds the inorganic-rich particulate aggregates and also apparently covers each layer as an envelope that helps bind the layers

together, since the layers separate and begin to disintegrate after removal of the organic material. The main components of the organic-rich matrix are amino acids with minor quantities of carbohydrates. The major amino acids are aspartic acid and glutamic acid and/or their amides which are probably part of a small protein(s) or polypeptide(s) with an apparent molecular weight of a few thousand daltons (unpublished data). Glucosamine and other unidentified carbohydrates (probably neutral hexoses) are present at low levels of less than 0.2% of the total weight of the granule. There appears to be an intimate association between the inorganic and organic components of the granules which can be visualized only after selectively removing certain constituents by plasma ashing or demineralization.

Our results demonstrate similar biochemical compositions for the larval "salts" of face fly as reported previously by Darlington *et al.* (1983). Those researchers reported the same major inorganic elements. However, they measured 1.4 and >2.0 times higher phosphorus and magnesium levels, respectively. Those dissimilarities may be due to the isolation procedures used or to inherent variation in the composition of the granules due to rearing conditions or other factors. A nearly 2-fold higher carbonate content than that reported by Darlington *et al.* (1983) was found in the present work. This difference may be due to the different analytical techniques used.

Since the granules are the primary storage reservoir for minerals to be used in puparial cuticular stabilization by the face fly (Elonen, 1985), we have compared the biochemical components of both granule and puparial cuticle. Data on the mineralized cuticle were obtained from Roseland *et al.* (1985). Most of the mineralized puparial cuticle is also inorganic in nature, but it is approx. 1.4 times lower than that observed for the isolated granules (Table 1). Significantly lower calcium (1.8-fold), phosphorus (1.1-fold), magnesium (1.2-fold), and carbonate (1.5-fold) occur in the cuticle. However, certain elements are substantially higher in the cuticle including potassium and zinc. Potassium levels are more than 5-fold higher while zinc levels are 3-fold greater. Zinc is only a trace element in the mineralized granules.

The percentage of organic components is higher in the cuticle than in the granules. For example, organic carbon and nitrogen levels are 50 and 8 times higher, respectively, in the cuticle. Cuticular nitrogen for the most part comes from amino acids which account for 9.9% of the puparial cuticle weight as compared to only 0.4% of the total weight of granules. While the major amino acids in the granules are aspartic acid and glutamic acid or their amide derivatives, glycine is the predominant amino acid in the cuticle. However, glutamic acid and aspartic acid are the next two most abundant cuticular amino acids and account for 17% of the total amino acids as compared to >30% of the granule's amino acids. Hence, relatively higher levels of inorganic components are present in the granules while higher levels of organic material occur in puparial cuticle. This is expected since larval cuticle is largely protein and chitin prior to mineralization.

Some similarities exist between the granules isolated from the face fly and those isolated from other

invertebrate species such as *Taenia taeniaeformis* (Cestoda) and *Pomacea paludosa* (Gastropoda). Both non-insect species have granules that are roughly spherical and composed of concentric layers (von Brand and Nylen, 1970). *T. taeniaeformis* granules have envelopes of organic material surrounding each concentric layer. An outer more prominent layer covers the entire granule and is composed of two distinct sublayers in close proximity to one another. Histochemical evidence indicates that the non-insect granules are composed of mucopolysaccharides with minor quantities of lipids (von Brand and Nylen, 1970; von Brand, 1973). Small differences are found in the organic matrix of *P. paludosa* granules. In addition to the concentric coverings there are also radially projecting membranes connecting adjacent layers forming a "net-like" organic matrix that is present throughout the granule. This appears to be similar to the organic matrix observed in face fly granules. While *P. paludosa* granules probably contain mucopolysaccharides (demonstrated qualitatively by histochemical techniques), there are no significant quantities of lipids (Watabe *et al.*, 1976).

The amino acid composition of non-insect granules is similar to that of face fly. The most abundant acids are again aspartic acid and glutamic acid (Table 2). These occur in remarkably similar concentrations in all types of granules. Major differences are demonstrated in inorganic components for *P. paludosa* granules. Those are composed of amorphous calcium carbonate and do not contain the high phosphate levels present in face fly and *T. taeniaeformis* granules.

Several researchers have noted that the surface molecules of organic matrixes may be extremely important for the nucleation and subsequent deposition of mineral salts (Easthoe, 1968; Lowenstam and Weiner, 1983; Weiner *et al.*, 1983). It has been suggested that these surface molecules are anionic in nature usually protein or proteoglycans containing relatively high concentrations of dicarboxyl amino acids such as aspartic acid and/or glutamic acid (Meenakshi *et al.*, 1971; Hackman, 1974; Weiner and Hood, 1975; Weiner, 1984; Weiner, 1985; Addadi and Weiner, 1985). This arrangement may be the case for face fly granule formation since the organic components occur at such low levels relative to the mineral components. An anionic protein rich in dicarboxylic acid residues may act as a collector, carrier or template for mineral salt deposition into face fly granules. Because face fly granules are amorphous, the organic matrix, which consists of small quantities of amino acids having high concentrations of aspartic acid and glutamic acid, may act as a nucleation site and not as a template for crystal orientation. It has been shown that proteins with similar amino acid compositions to those found in face fly granules have cation binding activity (Dedman *et al.*, 1978; Weiner, 1984; Addadi and Weiner, 1985; Weiner and Hood, 1975). Once initial mineralization occurs at a nucleation site, general mineral growth may proceed in an amorphous manner.

Amorphous calcium orthophosphate typically forms as small rounded particles *in vitro* (Termine and Posner, 1970; Termine *et al.*, 1970). The rounded

inorganic particles that compose the individual layers of granules may be aggregation centers of amorphous calcium phosphate that are associated with the initial precipitation of inorganic salts out of solution (Termine *et al.*, 1970; Termine and Posner, 1970). The organic matrix sheet or envelope separating and binding the individual layers may act as a volume constraint limiting overall mineral deposition to a layered structure.

The presence of amorphous minerals in face fly granules may be related to their availability for utilization in cuticle morphogenesis. Grodowitz and Broce (1983) suggested and Elonen (1985) demonstrated that these granules are the primary mineral storage site for later puparial cuticle mineralization. Mann (1983) indicated that an amorphous salt is more soluble than its corresponding crystalline state and that the amorphous form may act as a reservoir of mineral ions which can be rapidly mobilized. *P. paludosa* granules also occur in an amorphous state and the minerals are utilized for rapid shell regeneration (Watabe *et al.*, 1976). Elonen (1985) demonstrated that the solubility of face fly granules is greater than that of crystalline calcium phosphate.

Face fly granules have a highly complex structure that includes intimate interactions between relatively large quantities of amorphous inorganic material and small concentrations of organic components. The organic-rich matrix may act as a primary nucleation site for mineral growth and also as a volume constraining structure. The maintenance of an amorphous state of a mineral salt may be associated with its greater solubility relative to that of a crystalline state so stored ions can be rapidly solubilized and transported for subsequent deposition in the puparial cuticle. The concentric layered nature of granules found in the face fly and many other species suggests that this type of structural framework is important in most mineralized granules. The reason for this common construction is unclear, but may be attributed to a mechanism of periodic deposition correlated to behavioral and physiological rhythms.

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