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Detoxification of cadmium

Ultrastructural study and electron-probe microanalysis of the midgut in a cadmium-resistant strain of *Drosophila melanogaster*

Sylvie Lauverjat[1], Christiane Ballan-Dufrancais[1], and Maurice Wegnez[2]

[1] Laboratoire d'Histophysiologie Fondamentale et Appliquée, Unité de Recherche Associée 6 du Centre National de la Recherche Scientifique, Université Paris 6, 12 rue Cuvier, F-75005 Paris, France
[2] Laboratoire d'Embryologie Moléculaire et Expérimentale, Unité de Recherche Associée 25 du Centre National de la Recherche Scientifique, Université Bâtiment 445, F-91405 Orsay, France

Summary. The midgut of a cadmium-resistant strain of *Drosophila melanogaster* has been studied at the ultrastructural level and by electron-probe microanalysis (EPMA). Chronic exposure to cadmium leads to a concentration of the metal in a lysosomal system developed in both anterior and posterior segments of the midgut, where it coexists with copper and sulfur. This mechanism apparently ensures a permanent cadmium detoxification and prevents cellular injury. Wild-type flies fed on a cadmium-contaminated medium manifest the same detoxification process. As a result of contamination, copper is stored along the entire length of the midgut, including a part of the middle-midgut previously named 'copper-accumulating region'. Our data demonstrate that the midgut, particularly the posterior segment, is an accumulative organ for both cadmium and copper. The involvement of the metallothionein system in the detoxification process is discussed.

Key words: Cadmium resistance — *Drosophila* midgut — Lysosomes — Electron-probe microanalysis — Metallothionein

Introduction

Different metals can accumulate in the midgut epithelium of dipteran larvae (Waterhouse and Stay 1955; Filshie et al. 1971; Tapp 1975; Martoja and Ballan-Dufrançais 1984). In *Drosophila melanogaster*, copper accumulation has been investigated in the middle midgut, a region comprised of cup-shaped and interstitial cells that contain lysosomes when larvae are reared on a copper-enriched medium (Poulson and Bowen 1952; Filshie et al. 1971). The cup-shaped cells are also called calycocytes (Deegener 1928) or cuprophilic cells (Waterhouse and Stay 1955). Investigations using elctron-probe microanalysis (EPMA) demonstrated that copper accumulates in their lysosomes (Tapp 1975; Tapp and Hockaday 1977). Thus, a short segment of the *D. melanogaster* middle midgut seems to be involved in copper bioaccumulation.

Cadmium, unlike copper, is highly toxic to animals. Lethal concentrations, in *D. melanogaster*, are two orders of magnitude lower for cadmium than for copper (Maroni and Watson 1985). Viability, defined as the proportion of individuals reaching the pupal stage, is reduced to zero when $CdCl_2$ concentrations in the medium are greater than 50 μg $Cd \cdot g^{-1}$ (Maroni and Watson 1985). A sharp decrease in fertility is rapidly observed following cadmium intoxication (Wegnez, unpublished data). Increasing cadmium concentration in the culture medium also progressively lengthens the duration of development (Vasudev and Krishnamurthy 1981).

Metallothioneins are small metal-binding proteins found in most eukaryotes and probably involved in both metal homeostasis (control of Zn^{2+} and Cu^{2+} concentrations) and metal detoxification (such as binding of Cd^{2+}) (Hamer 1986). In *Drosophila*, metallothioneins are induced in larvae, in adults and in cell lines, following either copper or cadmium treatment (Debec et al. 1985; Maroni and Watson 1985; Erraïss et al. 1989). Two metallothionein genes have been cloned in *D. melanogaster*. The gene *Mtn* codes for a protein of 40 amino acids (Lastowski-Perry et al. 1985) and the gene *Mto* for a protein of 43 amino acids (Mokdad et al. 1987). Several cadmium-resistant *D. melanogaster* strains have been isolated

Offprint requests to: S. Lauverjat

and characterized (Otto et al. 1986; Maroni et al. 1987). All of them were shown to contain duplications of the metallothionein *Mtn* gene (Maroni et al. 1987). This correlation thus strongly suggests that the resistance phenotype is due to higher levels of metallothioneins, presumably in the gut.

To approach the problem of cadmium storage at the cellular level, we undertook an ultrastructural and EPMA study of the larval midgut. Finding that the middle midgut, the site previously described as a copper-accumulating region (Poulson and Bowen 1952; Filshie et al. 1971), does not accumulate cadmium, we extended our study to the entire midgut. In this paper, we compare a cadmium-resistant strain capable of being maintained on a medium containing 50 μg $Cd \cdot g^{-1}$, with its cadmium-sensitive parental strain, unable to sustain full development on this medium. We also present data related to the accumulation of copper in the larval midgut of wild-type *Drosophila*.

Materials and methods

Drosophila *strains*. The *D. melanogaster* strains were reared on a standard yeast/agar/maize/sucrose medium according to Gans et al. (1975). This medium was shown to contain, when assayed by atomic absorption: 5.9 ± 2.7 ng $Cd \cdot g^{-1}$, 2.1 ± 0.2 μg $Cu \cdot g^{-1}$ and 3.7 ± 0.4 μg $Zn \cdot g^{-1}$.

The wild-type cadmium-sensitive strain (strain 192-1, Ivory Coast) was provided by L. Tsacas from the Laboratoire de Biologie et Génétique Evolutives in Gif-sur-Yvette. The cadmium-resistant strain (strain CdR 50) was selected in 1984 starting from a large number of 192-1 flies reared on a medium containing 50 μg $Cd \cdot g^{-1}$ ($CdCl_2$). This strain is maintained continuously on this medium.

Metal contaminations. Wild-type flies (192-1) were allowed to lay eggs on metal-contaminated medium [20 μg $Cd \cdot g^{-1}$ ($CdCl_2$) or 1 mg $Cu \cdot g^{-1}$ ($CuSO_4$). Third instar larvae were collected for all analyses.

Ultrastructural study. The midgut samples were fixed 1 h at 4° C in 2% glutaraldehyde, 0.1% paraformaldehyde, 0.2 M sodium cacodylate, pH 7.3, then post-fixed in 2% osmium tetroxide. Epon araldite was used for embedding after ethanol dehydration. Ultrastuctural examination of osmium-tetroxide-treated but unstained material was performed on a Philips 300 electron microscope at 80 kV.

Electron probe microanalysis (EPMA). The same fixation used for ultrastuctural studies was employed, but without the osmium tetroxide treatment. Ultrathin sections (80–100 nm) were collected on titanium grids (Fullam) overlaid with formvar and carbon-coated. EPMA was carried out with a Cameca MBX (Camebax) equipped with a transmission electron microscope and two wavelength-dispersive spectrometers. The probe diameter (about 500 nm) was produced with a beam intensity of 150 nA at 45 kV. A crystal of pentaerythritol was used for Cd and S and a crystal of lithium fluoride for Zn and Cu (Ballan-Dufrançais et al. 1985). Signal measurement was automatically recorded for 100-s periods. Calculated intensities (N_c) for each element where then determined as $N_c = N_p - N_b$, where N_p is the mean of two values expressing the number of impulsions obtained for the peak line, and N_b the mean value between the left and right background. The calculated intensities were retained only when the values were significant (confidence interval $N_c \pm 2\sigma$ with a standard deviation σ estimated as $\sigma = \sqrt{(N_p + N_b)}$.

Results

Comparative structure of the midgut in wild-type and CdR 50, D. melanogaster *larvae*

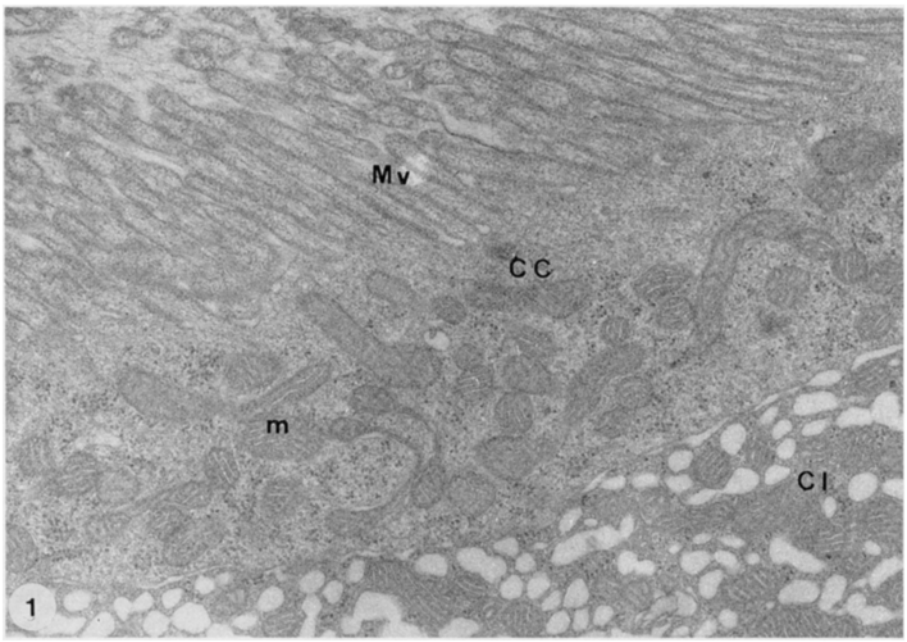

Fig. 1. CdR 50 strain-middle midgut. Magnification ×16000. Cup-shaped cells *(CC)* and interstitial cells *(CI)* are practically devoid of lysosomes. *Mv*, microvilli of a cup-shaped cell; *m*, mitochondria. (Original magnification ×16000; reduced to 80%)

Table 1. EPMA of elements in lysosomes of *Drosophila* larval midgut segments

Strain	Midgut: segments analyzed	Number of lysosomes analyzed	Cd	Cu	Zn	S
Wild type	Anterior	11	ns	ns–200	ns	40–300
(6 ng Cd·g^{-1})	Middle	3	ns	ns–30	ns	ns–40
as control	Posterior	14	ns	ns–100	ns	40–250
CdR 50	Anterior	15	100–1500	100–300	ns–60	100–1500
(50 μg Cd·g^{-1})	Middle	3	ns	ns–120	ns	200–600
	Posterior	34	100–1600	100–3200	ns–60	100–1500
Wild type	Anterior	4	ns	ns–150	ns	80–500
(20 μg Cd·g^{-1})	Middle	2	ns	ns–40	ns	ns–50
	Posterior	16	ns–1200	ns–700	ns–40	80–1500
Wild type	Middle	4	ns	150–200	ns–40	200–300
(1 mg Cu·g^{-1})	Posterior	11	ns	300–1000[a]	ns–60[a]	100–300[a]

Two strains (wild type and CdR 50) and control wild type intoxicated by cadmium and copper are compared. Extreme values of calculated intensities are expressed in counts/100 s; ns = no significant values; [a] = values restricted to the peripheral area of the lysosomes

In this section we compare wild-type larvae grown on the normal medium, i.e. containing approximately 6 ng Cd·g^{-1}, and cadmium-resistant larvae grown on a medium containing 50 μg Cd·g^{-1}.

Middle midgut

Two types of epithelial cells are of special interest: the cuprophilic or cup-shaped cells, and the interstitial cells characterized by plasma-membrane infoldings that reach the microvillar border, forming a labyrinth.

Wild-type strain. Rough endoplasmic reticulum and numerous free ribosomes are evenly distributed within both types of cells. Few Golgi bodies are recognizable. Lysosomes containing cytoplasmic components in varying stages of degeneration are rare and small (1 μm) in cuprophilic cells, and even rarer in interstitial cells. They are devoid of cadmium, but contain some copper and sulfur (Table 1). Large numbers of mitochondria are present, particularly in the interstitial cells. Some deposits of glycogen are located at the basal pole of these latter cells.

CdR 50 strain. No ultrastructural alterations have been observed, even in mitochondria (Fig. 1). The distribution and the number of lysosomes in the cytoplasm is the same as in controls. As shown in Table 1, EPMA data also are similar. Surprisingly, no cadmium could be detected.

Anterior and posterior midgut

The anterior midgut corresponds to only an eight of the entire midgut length, while the posterior midgut constitutes two-thirds of the organ.

Wild-type strain. The cells of both segments are very similar. They are large, have a centrally-located nucleus, and in some cases extend into the central lumen. Basal plasma membranes exhibit a large number of infoldings. Rough endoplasmic reticulum is well developed, especially in the posterior segment (Fig. 2). The Golgi bodies produce small and dense secretory granules (0.5 μm), particularly in the posterior midgut. Mitochondria are concentrated at the apical pole of the cells. Lipid inclusions, located in the same area, are very abundant in some cells but can be absent in adjoining cells. They are more abundant in the posterior than in the anterior midgut. Some cells contain deposits of glycogen at the basal pole. In both segments, lysosomes are sometimes filled with lipid globules. They contain some copper and sulfur, but neither cadmium nor zinc (Table 1).

CdR 50 strain. No major cytological alterations have been noticed in either of the two segments,

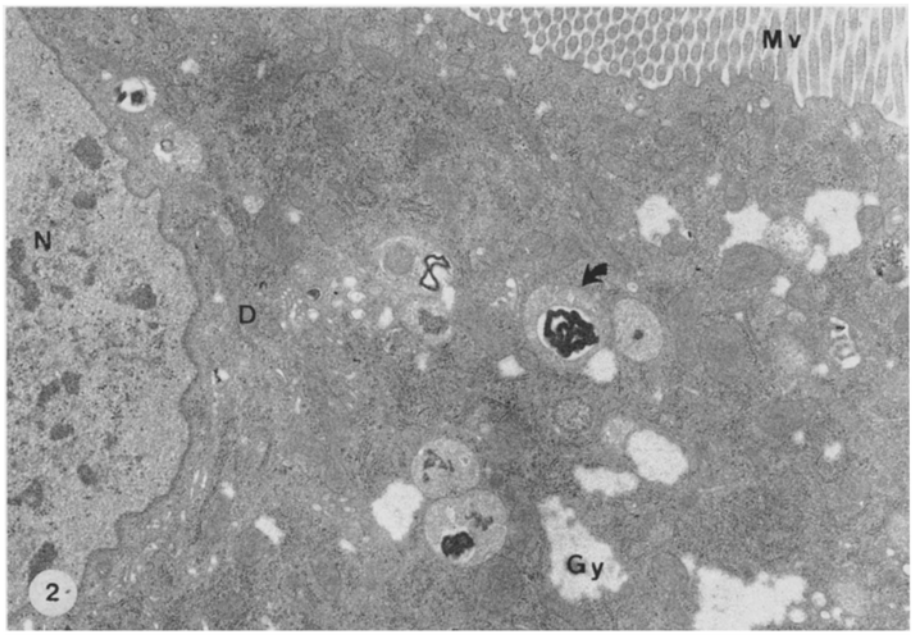

Fig. 2. Wild-type strain-posterior midgut. Magnification ×6800. Lysosomes *(arrow)* are numerous. *N*, nucleus; *Mv*, microvilli; *Gy*, glycogen; *D*, dictyosome. (Original magnification ×6800; reduced to 80%)

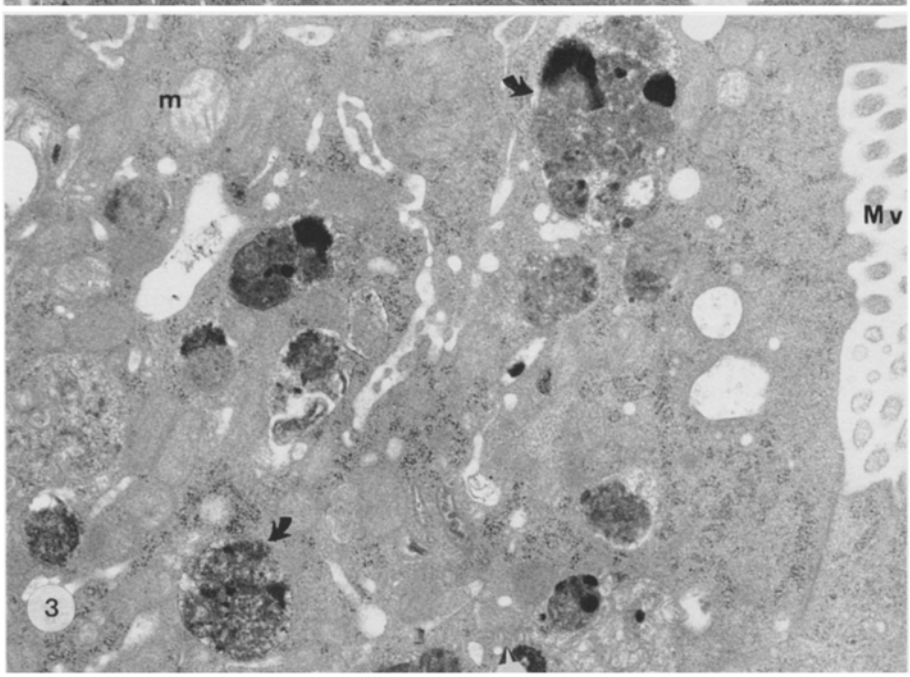

Fig. 3. CdR 50 strain-anterior midgut. Magnification ×16000. No cytological alterations are noticed. Lysosomes are conspicuous and contain Cd, Cu and S. (Original magnification ×16000; reduced to 80%)

except for the absence of glycogen. Mitochondria are undamaged and are normally distributed within the cells (Fig. 3). The Golgi apparatus is present. Lysosomes are present in greater quantity than in control larvae and occur either throughout the cytoplasm or only at the apical region of the cells (Table 2). They vary in size and shape (Fig. 4). Some lysosomes contain cytoplasmic components easily recognizable (rough endoplasmic reticulum, glycogen, lipids, mitochondria), but most of them are deeply transformed. Some contain an electron-dense structure (1 μm) in a granular matrix while others, much larger (6 μm), contain fibrous material arranged in tightly packed lamellae as well as dense inclusions. Some lysosomes are entirely granular with a crystallized area. Cadmium, copper, zinc and sulfur are detected mostly in the granular lysosomes (Table 1). Cytoplasmic extrusions, occurring in both anterior and posterior segments of the midgut, are filled with lysosomes (3–5 μm) that contain high amounts of cadmium.

Table 2. Estimation of quantities of lysosomes in the three *Drosophila* larval midgut segments

Strain	Anterior midgut	Middle midgut		Posterior midgut
		Cup-shaped cells	Interstitial cells	
Wild type (6 ng Cd·g^{-1})	***	*	*	***
CdR 50 (50 μg Cd·g^{-1})	****	*	*	****
Wild type (20 μg Cd·g^{-1})	***	*	*	***
Wild type (1 mg Cu·g^{-1})	****	****	***	****

Different metal intoxication conditions are compared for the two strains (wild type and CDR 50). * = very rare lysosomes, *** = abundant lysosomes, **** = very abundant lysosomes

Structure of the midgut in wild-type larvae reared on a medium supplemented with cadmium

When wild-type larvae are grown on a medium containing 20 μg Cd·g^{-1}, there is a considerable prolongation of developmental processes. Nevertheless, the third instar larvae show a midgut which is very similar to the midgut from CdR 50 larvae reared continuously on a medium containing 50 μg Cd·g^{-1}. In the middle midgut, cuprophilic cells and interstitial cells are practically devoid of lysosomes and those rare lysosomes never contain any detectable cadmium (Fig. 5c). In contrast, numerous lysosomes are present in anterior and posterior segments of the midgut (Fig. 5a). They contain electron-dense granules (0.5 μm) or are entirely granular (Fig. 5b). The storage of cadmium as well as the accumulation of copper, zinc and sulfur are accomplished as in the CdR 50 strain (Table 1) particularly in the posterior midgut. Cytoplasmic areas are filled with dilated cisternae of rough endoplasmic reticulum which occasionally bulge into the lumen.

Structure of the midgut in wild-type larvae reared on a medium supplemented with copper

The significantly higher concentration of cadmium in the lysosomes of the anterior and posterior midgut relative to the lysosomes of the middle midgut led us to reconsider the specificity of the cuprophilic cells in sequestering copper. The only visible effect of copper on the midgut ultrastructure was a large increase in the number and size of lysosomes which are distributed in cells all along the midgut. These organelles have a distinctive ultrastructure when compared to those described in the CdR 50 strain. Whatever the considered region, lysosomes are filled with a fine granular material (Fig. 6a, b, c). Their sizes range over 0.2–3 μm. The smaller ones are very dense with no internal structure recognizable. In the biggest lysosomes, the matrix contains some reticulum elements and dense microgranules (Fig. 6c). In these large lysosomes, a dense precipitate is visible inside cisternae of endoplasmic reticulum or is found surrounding the lysosome. In the posterior midgut, lysosomes contain rosettes of glycogen or myelinic figures.

The very compact structure of lysosomes in the anterior and posterior midgut made sectioning of samples prepared for EPMA difficult. This in fact led to a partial disruption of the lysosomal matrix. EPMA, thus, has been restricted to the preserved part of the lysosomes, i.e. the peripheral area. Quantitative data are thus highly underevaluated. As shown in Table 1, whatever the segment, copper, zinc and sulfur concentrations appear similar to those obtained with the CdR 50 strain continuously reared on a cadmium-contaminated medium. It is to be noticed that no cadmium was detected.

Discussion

Numerous metals are known to be stored in the insect midgut cells (in Martoja and Ballan-Dufrançais 1984). Previous studies on copper accumulation in the *Drosophila* midgut focused on the middle midgut, which is composed of very particular cells, namely the cup-shaped cells. The conclusion of these investigations was that the cup-shaped cells accumulate copper following an intoxication with this metal. The cells, for this reason, were named 'cuprophilic' cells (Poulson and Bowen 1952; Filshie et al. 1971; Tapp 1975; Tapp and Hockaday 1977). In this paper, we confirm

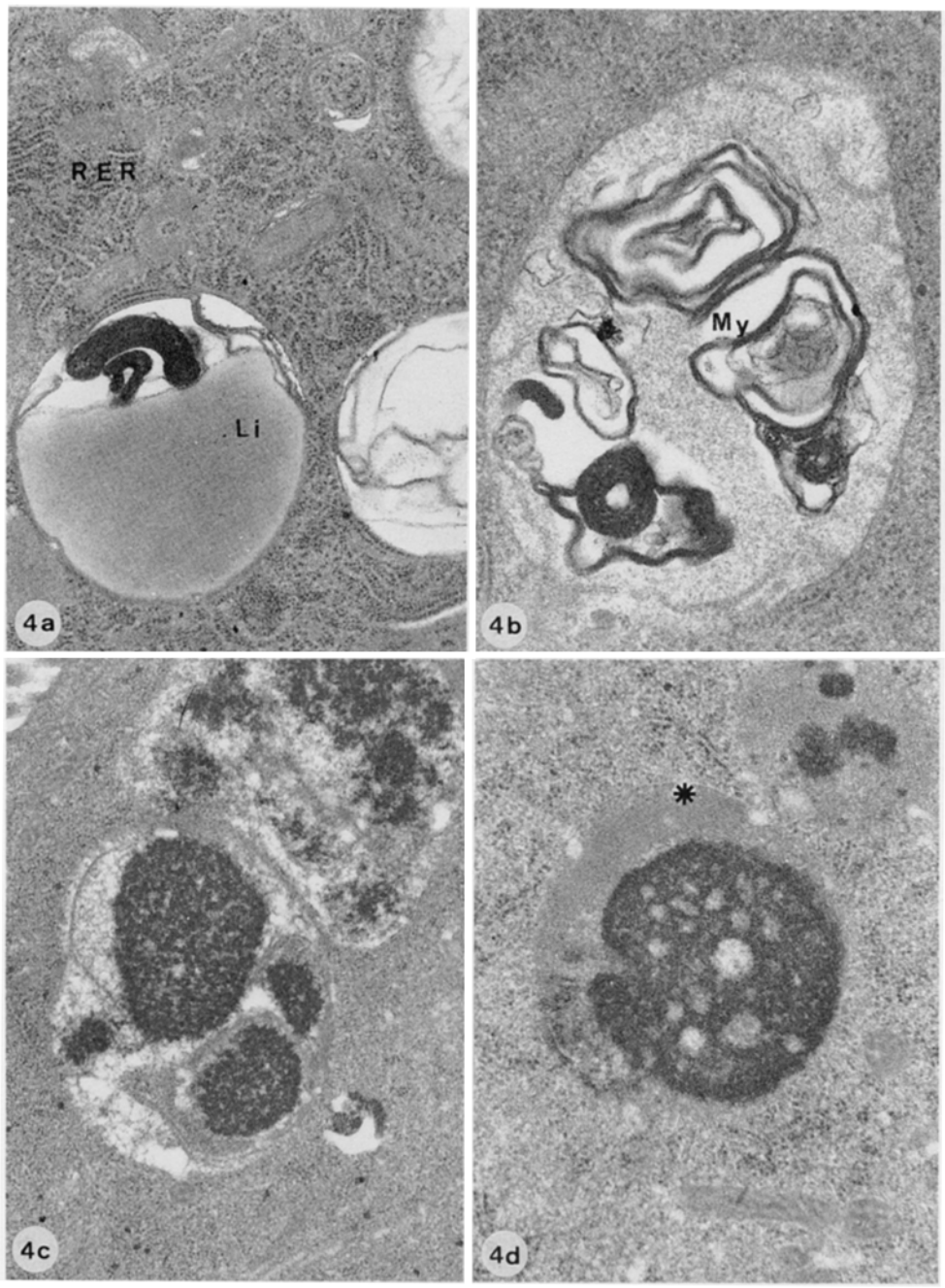

Fig. 4. CdR 50 strain: different aspects of lysosomes. **(a, b, c)** Posterior midgut; magnification ×16000, ×27000 and ×16000, respectively; **(d)** anterior midgut; magnification ×16000. RER, rough endoplasmic reticulum; Li, lipidic globule; my, myelinic strata; * crystallized area in a granular lysosome. (Original magnifications: **a:** ×16000; **b:** 27000; **c:** 16000; **d:** 16000; reduced to 80%)

that the cup-shaped cells trap copper in their lysosomes, but we also demonstrate that copper accumulation is not restricted to these cells of the middle midgut. In fact, most of the ingested copper enters the anterior and posterior midgut cells. More striking results were obtained with cadmium, that was almost exclusively found in lysosomes of the anterior and posterior midgut compartments. These two segments, which account for more than two-thirds of the length of the *Drosophila* midgut, thus seem to play a crucial role in metal detoxification. We discuss these observations with reference to the metallothionein system of *Drosophila*.

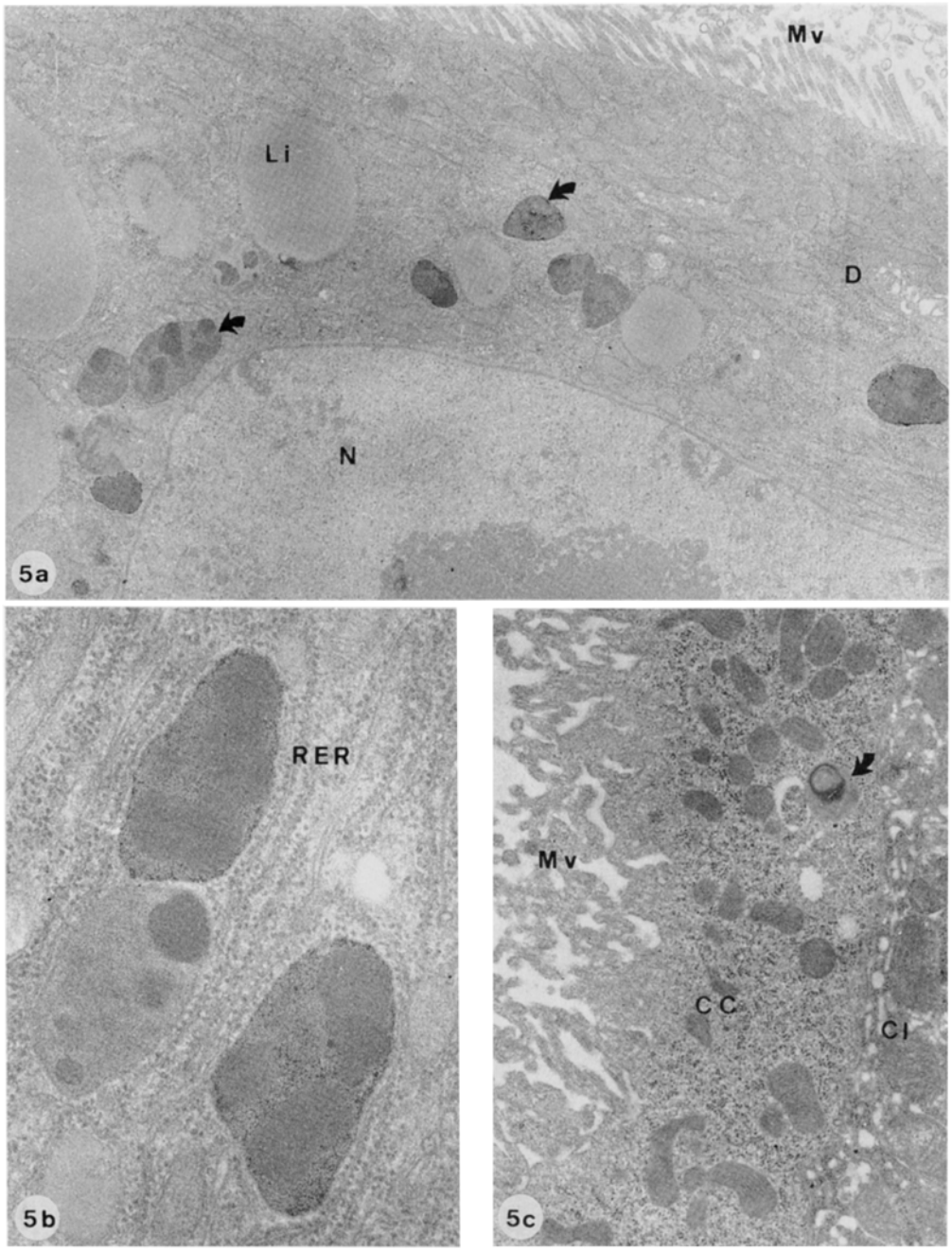

Fig. 5. Wild-type strain reared on a medium supplemented with cadmium. **(a)** Anterior midgut; magnification ×6800. Lysosomes are numerous *(arrows)*. Cytological features show no alterations. **(b)** Posterior midgut; magnification ×27000. Detail of lysosomes in which Cd, Cu and S are detected. Note the granular content. **(c)** Middle midgut; magnification ×10000. Cup-shaped cells as well as interstitial cells are very poor in lysosomes *(arrow)*. (Original magnifications: **a:** 6800; **b:** 27000; **c:** 10000; reduced to 80%)

Ultrastructural features of cadmium-contaminated midgut cells

The midgut cells in both cadmium-resistant (CdR 50) and cadmium-sensitive (wild-type) larvae fed with cadmium do not show great ultrastructural alterations. Mitochondria, for example, are normal and not swollen as usually occurs in other invertebrates exposed to cadmium (Seidman et al. 1986). Nevertheless, a noticeable difference between cadmium-reared (CdR 50) and control (wild-type) larvae was found for glycogen. This

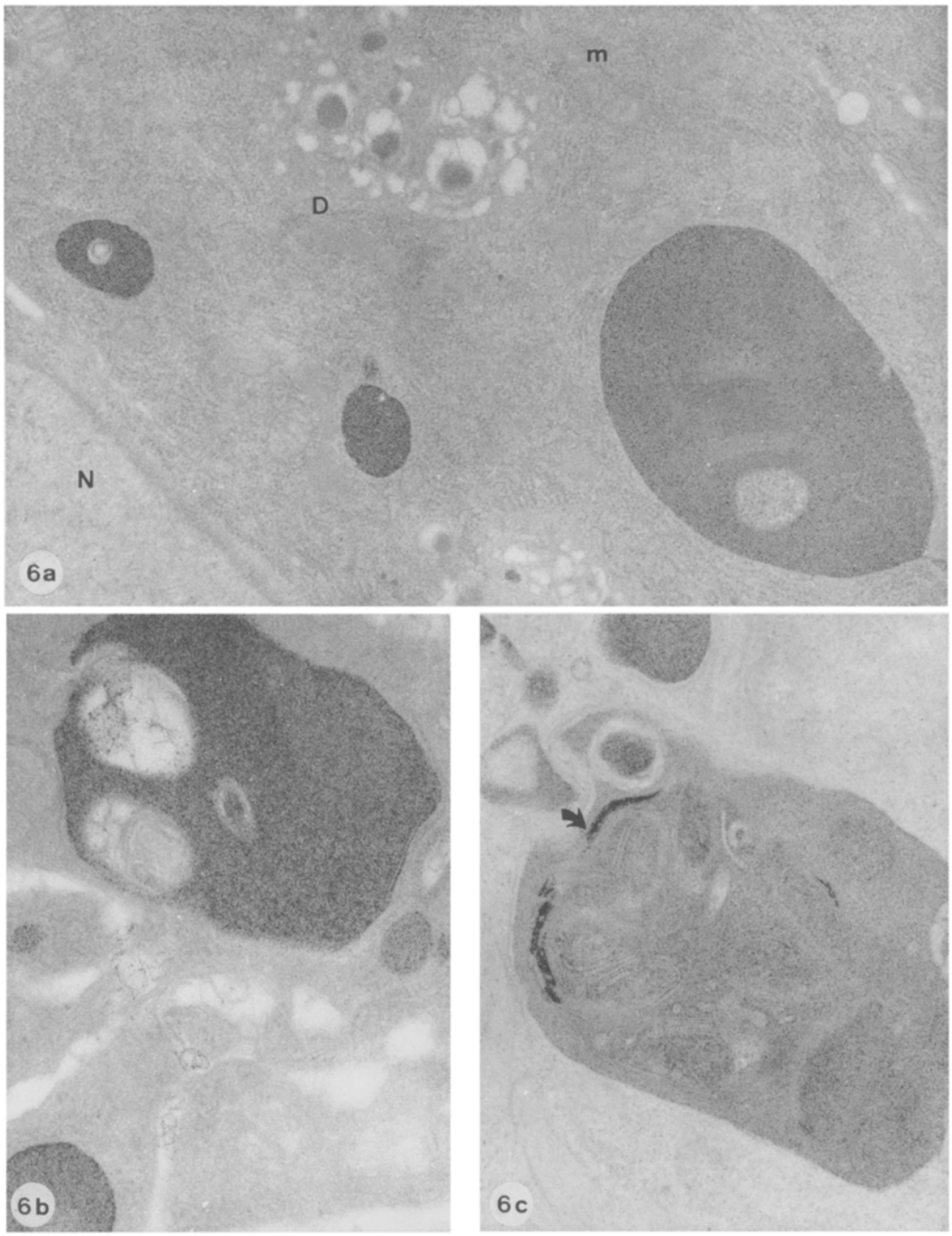

Fig. 6. Wild-type strain reared on a medium supplemented with copper. **(a)** Posterior midgut; magnification × 16000. Conspicuous lysosomes in which Cu and S are detected. These are scattered throughout cells which otherwise show no alterations. **(b)** Anterior midgut; magnification × 33000. A lysosome rich in granular content. **(c)** Middle midgut; magnification × 42000. Large lysosome in a cup-shaped cell showing an heterogeneous structure with dense microgranules *(arrow)*. (Original magnifications: **a:** 16000; **b:** 33000; **c:** 42000; reduced to 80%)

cellular constituent is present in anterior and posterior midgut control larval cells, but is no longer present in cadmium-treated larvae. It is already known that cadmium enhances intracellular glycogen mobilization in rat liver, probably through the activation of glycogen phosphorylase (Singhal et al. 1974). Glycogen disappearance may also be related to the observation that glucose incorporation into lipid is stimulated in rat adipocytes exposed to cadmium (Yamamoto et al. 1986). However, it is to be noted that the number of lipid globules is not increased in the CdR 50 midgut cells.

The most important morphological difference found between cadmium-reared larvae (CdR 50 and wild-type) and control larvae grown on normal medium is at the level of the number and of the size of lysosomes present in the anterior and posterior midgut (Table 2). Significant variations were observed when comparing different cells. Some cadmium-contaminated cells look like control cells, i.e. they contain very few lysosomes, while others are filled with these organelles. Cadmium, as well as copper and sulfur, are accumulated in these lysosomes, which also contain traces of zinc (Table 1). Some apocrine extrusions also contain lysosomes displaying high cadmium levels. These results are consistent with the fact that the intestinal epithelium retains 95% of the cadmium present in the diet (Maroni and Watson 1985). The mesenteron, in *Drosophila* as well as in other insects, could act as a barrier against toxic metals (Ballan-Dufrançais et al. 1971; Jeantet et al. 1977).

Specificity of the midgut compartments in accumulating metals

The 'cuprophilic' cells, in both CdR 50 and wild-type larvae reared on a medium contaminated with cadmium, contain very few lysosomes, with no EPMA-detectable cadmium and only traces of copper (Tables 1 and 2). As discussed previously, the anterior and the posterior midgut of these larvae contain numerous lysosomes which, on the other hand, concentrate cadmium. This led us to question the specificity of the so-called 'cuprophilic' cells in sequestering copper. The middle midgut of copper-contaminated larvae contains numerous lysosomes with relative concentrations of copper and sulfur above the control values. Nevertheless, the other parts of the midgut contain numerous lysosomes which concentrate much more copper and sulfur than do the cuprophilic cells (Tables 1 and 2). Bioaccumulation of copper and cadmium in *Drosophila* thus primarily occurs in the anterior and posterior midgut, the latter being the most important because of its length. Similarly, accumulation of cadmium in *Chironomus thummi* larvae was found to be restricted to the posterior midgut epithelium (Seidman et al. 1986).

Our results strengthen the idea that the actual role of the middle midgut in insects is related to nutritional physiology rather than to detoxification physiology. It is known that a low pH characterizes the middle midgut in higher dipterae and thus probably in *Drosophila* (Anderson and Harvey 1966; Terra et al. 1988). This strongly suggests that the role of the cells in this segment is to acidify the lumen content. The presence of copper in the cup-shaped cells could thus result from a saturation of the anterior and posterior midgut compartments in the case of acute copper intoxication. This could explain why other authors, using copper concentrations identical to ours, also detected copper in the cup-shaped cells (Filshie et al. 1971; Tapp 1975; Tapp and Hockaday 1977). However, it should be noted that, in these studies, the anterior and posterior midgut was not analyzed. We think therefore that naming the middle midgut, as Filshie et al. (1971) do, 'a copper-accumulating region', is incorrect. Accordingly, we consider that the so-called 'cuprophilic' cells should better be named cup-shaped cells, a term that does not presume their function. All these data, in our opinion, favor the hypothesis that the anterior and the posterior midgut are the segments of the digestive tract involved in the metal detoxification processes.

Involvement of metallothioneins in detoxification processes

Accumulation of toxic metallic ions bound to metallothioneins in the cytoplasm does not constitute a very efficient way for the cells to resist chronic cadmium or copper contamination. Lysosomes, in this context, should play a crucial role in sequestering these metals. The nature of the molecular structures binding metals inside lysosomes is difficult to ascertain. It has been inferred from many experiments that polymerized metallothioneins could bind mercury in the ileum lysosomes of the insect *Blatella germanica* (Ballan-Dufrançais et al. 1980; Jeantet et al. 1980; Bouquegneau et al. 1985) as well as in other invertebrates (Viarengo et al. 1984). Direct evidence for the presence of metallothioneins in lysosomes has been found in dogs affected by an inherited disease resulting in the accumulation of large quantities of copper (Johnson et al. 1981). In these animals, hepatocytes display dense copper-rich lysosomes which clearly contain metallothioneins (Freedman et al. 1986; Sternlieb 1987). There is as yet no direct evidence for the presence of metallothioneins inside *Drosophila* midgut lysosomes. A significant correlative increase of both cadmium/copper and sulfur in lysosomes is observed in all of our metal-contamination experiments, which could suggest the presence of metallothioneins in lysosomes (Tables 1 and 2).

Duplications of metallothionein genes, and correlative resistance phenotypes, have been characterized in mammalian cadmium-resistant cell lines (Beach and Palmiter 1981; Gick and McCarty 1982; Crawford et al. 1985). Several cadmium-resistant *Drosophila* strains have been characterized (Maroni et al. 1987). All of them possess a duplication of the *Drosophila Mtn* metallothionein gene (Otto et al. 1986; Maroni et al. 1987). The cadmium-resistant *Drosophila* strain (CdR 50) used in this study is also characterized by a duplication of the *Mtn* gene, and by a single copy of the *Mto* gene (Silar and Wegnez, unpublished observations). However, the cadmium-sensitive parental strain, used as a control strain in this work, also possesses the *Mtn* duplication (Silar and Wegnez, unpublished observations). The CdR 50 strain nonetheless synthesizes higher amounts of metallothioneins than the control strain after cadmium induction (Eraïss and Wegnez, unpublished results).

In cadmium-reared larvae (CdR 50 and wild-type), a marked EPMA-detectable metal increase within lysosomes has been detected not only for cadmium but also for copper (Tables 1 and 2). It is to be noted that there was no copper addition to the diet. This lysosomal copper augmentation could be related to a cytoplasmic displacement of the metal or to an increase in copper uptake. Similar observations, i.e. modifications of the overall metal pattern following poisoning with a single element, have already been reported for metals bound to metallothioneins (Brady 1982; Webb and Cain 1982; Funk et al. 1987). As shown in Table 1, no zinc was detected in control larvae and was found only as traces in cadmium-reared larvae. In parallel with this finding, we note that zinc does not induce *Mtn* nor *Mto* genes in *Drosophila* (Maroni et al. 1986; Mokdad and Wegnez, unpublished observations). These results agree with the hypothesis that metallothioneins are the molecules binding metals inside lysosomes. These metals, i.e. cadmium, the inducer, and copper, which is known to bind with strong affinity to metallothioneins (Kägi and Kojima 1987) would be 'extracted' from the medium following metallothionein synthesis. Surprisingly, we found no differences in our analyses of the different parts of the midgut (ultrastructure, metal levels) when comparing the CdR 50 larvae (50 μg $Cd \cdot g^{-1}$) and the control larvae fed with cadmium (20 μg $Cd \cdot g^{-1}$). Although it is likely that the metallothionein system is involved in some way in conferring the cadmium-resistant phenotype in the CdR 50 strain, more data are needed to obtain a clear understanding of the phenomenon. Further analysis of the metallothionein genes in the cadmium-resistant and cadmium-sensitive strains, and of their expression, should give new insight into the detoxification processes occurring in *Drosophila*.

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