THE ORIGIN AND FATE OF MICROBODIES
IN THE FAT BODY OF AN INSECT

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ABSTRACT

The structure and life history of insect microbodies are described during the development of the fat body from the 4th to 5th larval molt through the 5th to pupal molt. The mature microbodies are flattened spheres about 1.1 X 0.9 µ, with a depression on one side where a dense mass connects the limiting membrane to the core of coiled tubules. They contain catalase and urate oxidase. The precise synchrony of development of insect cells during the molt/intermolt cycle makes it easy to study the life history of particular organelles. Phases of growth are correlated with the hormonal milieu. Mature 4th stage microbodies decrease in size before ecdysis to the 5th stage when they atrophy at the same time as the new 5th stage generation arises. The 5th stage microbodies form as diverticula of the RER and, grow while confronted by RER cisternae. The mature microbodies decrease in size when the fat body engages in massive larval syntheses. At the end of the 5th larval stage, the microbodies are invested by isolation membranes and destroyed before pupation. There are thus two mechanisms for microbody destruction: atrophy of the 4th stage organelles and isolation with autophagy at the end of the 5th stage.

INTRODUCTION

While studying the synthesis and sequestration of blood proteins by insect fat body (5, 23-24, 25), we noticed that granules with a characteristic dense core were always present. The cores appeared to be made of coiled tubules giving a pattern identical with that of the lipid-water micelles secreted by the honeybee wax gland (18). The granules did not seem to be related to the Golgi complex and secretion, or pinocytosis and the multivesicular bodies. Further work has shown that they are microbodies. Microbodies have been characterized mainly as a result of studies on vertebrate liver and kidney (e.g., 1, 10). They are unit-membrane-bounded vesicles containing catalase and a variety of oxidases such as urate oxidase (7, 15). The fat body of insects has always been compared functionally to the liver of vertebrates. The comparison becomes even more apt with the discovery of microbodies and the localization of urate oxidase and catalase.

The precise timing of development makes Calpodes tissues very suitable for studying the genesis and fate of particular organelles. The life history of several tissues has been studied intensively in the 5th stage larva which lasts for 192 ± 13 hr at 22°C (22). The 5th stage is divided into three phases of development by the critical periods for the operation of the brain thoracotropic hormone on the prothoracic glands at M + 66 hr (i.e., by 66 hr after ecdysis from the 4th stage the prothoracic glands no longer need the brain to induce pupation), and by the critical period for the operation of the prothoracic glands upon the tissues at M + 156 hr (i.e., by 156 hr after ecdysis from the 4th stage the tissues are independent of the prothoracic glands in their progress toward pupation). During the first phase from M + 0 to M + 66 hr, the fat body acquires a complement of organelles appropriate for the larval syntheses taking place during the second phase.
At M + 156, there is a switch from larval syntheses to overt changes related to pupation, including the sequential autophagy of specific larval organelles. This paper is concerned with the origin and fate of microbodies in Calpodes fat body during these three phases. It provides a temporal and morphological framework for further studies on the function and development of this kind of microbody.

MATERIALS AND METHODS

Tissue Preparation for Light and Electron Microscopy

Larvae of Calpodes ethlius were reared on cannily plants in a greenhouse at about 30°C. Fourth stage larvae were transferred to a 22°C incubator where they were timed for ec dysis to the 5th stage. More than 150 larvae were observed in order to get the information on development during the 192 hr of the 5th stadium.

Larvae were fixed by injecting them with cold 2.5-6% glutaraldehyde buffered with 0.05 M phosphate at pH 7.2 containing 2-4% sucrose and stored at 0°C in a similar buffer with 10% sucrose. Storage was kept short to avoid extraction. For electron microscopy, tissues were postfixed in 1% osmium tetroxide buffered at pH 7.2 with cacodylate or phosphate containing 4.5% sucrose, dehydrated in alcohols, and embedded in Araldite.

Fixation in absolute acetone at 0°C was required for the light microscope localization of urate oxidase since the enzyme is destroyed by traces of aldehydes. Acetone also prevented oxidation of 3-amino-9-ethylcarbazole by mitochondrial cytochrome oxidase (4). Fixed tissues were stored in 100% acetone at -16°C.

Sections were cut on a Huxley ultramicrotome and double stained in uranyl acetate (23) and lead citrate (31) with or without in-block staining in uranyl acetate (11). Unstained sections were used for the demonstration of catalase. Electron micrographs were taken on an RCA EMU-3F microscope with double condenser, operated at 100 kv, with 25-35 µm objective apertures.

Urate Oxidase Histochernistry

Urate oxidase was demonstrated on acetone-fixed fat body by the method of Graham and Karnovsky (14). A good reaction was obtained by using 1/10 of the urate concentration they recommended after incubation for 2-12 hr at 22°C. 2,6,8-trichloropurine (26) gave 100% inhibition of the reaction when the ratio of inhibitor to urate was greater than 50-100:1.

Catalase Cytochemistry

Catalase was demonstrated on glutaraldehyde-fixed fat body by procedures modified from those developed for vertebrate tissues (9, 12, 13, 27, 28). Reactions were carried out at 22°C, the temperature at which the larvae are reared, rather than mammalian blood heat. Despite its greater solubility, the tetra HCl salt of diaminobenzidine gave a much fainter reaction product than the free base. Gentle agitation on a rotary shaker facilitated penetration of the reaction mixture and consistently gave uniform results. Since insect tissues are only one or two cells thick, whole animals can be incubated in reaction mixtures with good penetration and mounted for light microscopy without sectioning. The chief variables tested were pH (6-9), incubation time (12-16 hr), buffer, (0.05 M Tris, borate, diethanolamine), and hydrogen peroxide concentration (0 and 0.06%-0.12%). The chief problem for light microscopy is to separate the reaction in mitochondria from that in microbodies. High pH favored the reaction in the microbodies. With 0.06% hydrogen peroxide at pH 9, the mitochondrial reaction was abolished by adding KCN to the reaction mixture to make a final concentration of 5 X 10^-4 M. Unfortunately, this strength KCN reduced some of the activity of the catalase. 0.03 M 3-amino-1,2,4-triazole added to the reaction mixture caused almost complete inhibition of the catalase. A more satisfactory separation between the catalase and mitochondrial reactions was obtained by increasing the hydrogen peroxide strength to 0.12%. This completely inhibited the reaction in the mitochondria while enhancing that in the microbodies. After this treatment, 0.06 M 3-amino-1,2,4-triazole caused almost complete inhibition of the reaction in the microbodies. In the absence of hydrogen peroxide, there is a slight increase in microbody density after incubation for several hours. The most favorable conditions for demonstrating microbody catalase were found to be 12 hr of incubation at 22°C in 0.03% diaminobenzidine free base at pH 9 (0.05 M diethanolamine buffer) with 0.12% H2O2. For electron microscopy tissues were postfixed in osmium tetroxide after the reaction and embedded as usual.

Measurements of Microbody Growth

About 350 microbodies were measured on electron micrographs of fat body of all ages during the 5th stage to get a rough but objective estimate of changes in size. The long and short axes were measured on those profiles showing the core and a sharp unit membrane. Although the measurements show much scatter, the differences at different stages are considerable and reinforce the subjective impressions obtained only from scanning.
RESULTS

The Structure of the Microbodies

In the 5th stage larvae, the fat body cells form longitudinal sheets floating freely in the blood. During the phase of active larval syntheses (M + 66 - 156 hr), they contain abundant mitochondria, RER, and Golgi complexes with secretory vesicles discharging to the blood, and multivesicular bodies with pinocytic vesicles. The cells enlarge as lipid and glycogen reserves are laid down. There are also characteristic oval-to-spherical bodies about 1 µ across with a depression and dense core on one side (Fig. 1). The dense core is made up of coiled tubules together with dense material close to the depressed membrane (Figs. 2 and 3). The tubules may appear loose or densely packed like a honeycomb, but

Figure 1 The fat body towards the end of the growth phase (M + 49). The microbodies (mi) are large and numerous. The ER is still sparse, but there are numerous free ribosomes. The Golgi complexes (gc) are small and without large secretory vesicles. The RER cisternae are not inflated as they are at the time when the microbodies are forming. Stained in lead citrate and uranyl acetate. ×18,000.
they are always intertwined like hanks of rope, never straight regular crystals. The dense material closer to the membrane may have a substructure but it is too fine for satisfactory resolution in most micrographs. These bodies have a general similarity to some microbodies although differing in detail from any described previously (15).

The Localization of Catalase

Observations were made on tissue from larvae of all ages. Fat body was reacted with DAB and 0.06% H$_2$O$_2$ at pH 9 to determine the distribution of catalase. The mitochondrial reaction was inhibited with $5 \times 10^{-4}$ M KCN and the catalase

Figures 2 and 3  Microbodies from insect fat body. Fig. 2 (M + 49 hr), section through the core of tubules and the dense mass between the core and the surrounding unit membrane. Fig. 3 (M + 83 hr), mature microbody with core and matrix. Stained with lead citrate and uranyl acetate. $\times 56,000$. 

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FIGURES 4-6 Catalase in microbodies. Light micrographs of unstained epoxy sections from fat body incubated with DAB + 0.06\% $\text{H}_2\text{O}_2$ at pH 9. Larva aged M + 24 hr. Fig. 4, complete medium, both microbodies and mitochondria appear dense. Fig. 5, complete medium + $5 \times 10^{-4}$ m KCN, only the microbodies can be distinguished. Fig. 6, complete medium + $5 \times 10^{-4}$ m KCN and $0.03 \, \text{m} \, 3$-amino-1,2,4 triazole. Mitochondria and microbodies inhibited. $\times 1,800$. 

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Figures 7–9  Catalase in microbodies. Unstained sections of fat body. Fig. 7, (M + 24 hr) DAB medium with 0.06% H$_2$O$_2$ at pH 9.0; microbody matrix and mitochondrial cristae both dense. Fig. 8, (M + 24 hr) the same medium + 0.03 M 3-amino-1,2,4-triazole; microbodies inhibited, mitochondrial cristae unaffected. ×19,000. Fig. 9, (M + 60 hr) low-power survey, DAB medium with 0.12% H$_2$O$_2$ at pH 9.0. Microbodies appear very dense. 1, lipid droplet. ×8,000.
reaction with 0.03 M 3-amino-1,2,4-triazole. Figs. 4-6 show the result with light microscopy. In uninhibited material (Fig. 4), the reaction product is intense and localized in many small granules. Most of these are presumed to be mitochondria since the reaction is abolished in the presence of KCN. There are always a small number of granules which continue to react in the presence of KCN (Fig. 5). These are presumed to be microbodies since they fail to react in the presence of aminotriazole (Fig. 6). Electron microscopy confirmed these results and showed catalase activity in the matrix of the microbodies. Fig. 7 shows reaction product in both the cristae of the mitochondria and the matrix of the microbodies after incubation in the reaction mixture without inhibitors. The reaction product reported in the outer mitochondrial membrane in rat tissues (28) was not seen. Aminotriazole inhibits the reaction in the microbodies without affecting that in the mitochondria. In another series, the reaction in the microbodies was distinguished from

**Figures 10-12** Catalase in microbodies. Unstained sections of fat body incubated in DAB + 0.127% H₂O₂ at pH 9.0. Larva aged M = 60 hr. Fig. 10, complete medium, microbody matrix dense; mitochondrial reaction inhibited at high H₂O₂ concentration. Fig. 11, complete medium + 0.06 M 3-amino-1,2,4-triazole. Catalase reaction greatly reduced in the microbodies. Fig. 12, medium without H₂O₂. Microbody shows very little reaction with DAB alone. 1, lipid droplet. ×32,000.
that in the mitochondria by increasing the strength of hydrogen peroxide in the reaction mixture to 0.12%. Figs. 9 and 10 show the reaction localized in microbodies and absent in mitochondria. Control tissues incubated with 0.06 M 3-amino 1,2,4-triazole (Fig. 11) have the same density as tissues incubated without hydrogen peroxide (Fig. 12).

We concluded from these experiments that insect fat body has vesicles containing catalase (or perhaps some other heme protein with peroxidatic activity [32]). We tentatively classify these vesicles as microbodies or peroxisomes (7).

Urate Oxidase in the Fat Body

In many vertebrate microbodies the core has been correlated with urate oxidase (15). The involvement of the fat body in urate metabolism suggested that its microbodies might also contain urate oxidase, perhaps as part of their core.

Various techniques were tried for the localization of urate oxidase in the fat body. Adult Calliphora Malpighian tubules were used as control test material since they contain microbody-like granules (2) and urate oxidase (3). In Calliphora Malpighian tubules, good localization of urate oxidase was obtained in granules of the same size and distribution as the microbodies. Calpodes larvae of all ages were reacted, and the distribution of urate oxidase was compared with that of catalase demonstrated for both light and electron microscopy. In one series the larvae were halved, part was reacted for urate oxidase and the other half for catalase. In Calpodes the fat body was the only tissue which consistently reacted positively for urate oxidase. The reaction product is sharply localized in granules of about the same size and spatial distribution as the microbodies. The reaction was inhibited by 2,6,8-trichloropurine, a competitive inhibitor of urate oxidase. The size and number of granules containing urate oxidase also paralleled the changes in number and size of catalase-containing granules described below (Figs. 13–15). We concluded that urate oxidase is probably located in the microbodies of the fat body.

The Origin and Growth of the Microbodies

From M + 0 to M + 66, the fat body prepares for later syntheses. For example, ribosomes and RER develop and the nuclei become polyplloid (22). It seemed likely that the microbodies might also arise during this phase of preparation.

A series of larvae of all ages during the 5th stadium were fixed and reacted for catalase. Light microscopy showed that catalase-containing granules are smallest and fewer in number during the first 24 hr. There is an abrupt loss of catalase after the second critical period at M + 156 hr. It seemed from this that the microbodies might form shortly after ecysis and be lost as the fat body switches to activities related to pupation.

A series of fat bodies of various ages throughout the stadium was prepared for electron microscopy. There are some large microbodies surviving from the 4th stage into the 5th. From about M + 8 to M + 20 hr small irregular vesicles appear, some having connections with the RER, others being partially enveloped in RER cisternae (Figs. 16–21). Some of these small diverticula from the ER are early stage microbodies since (a) they contain cores of tubules, (b) the DAB reaction shows them to contain catalase, and (c) there are no other secretory or phagic vacuoles at this time that could be confused with microbodies. The Golgi complexes, for example, are little more than distended RER cisternae with a few microvesicular blebs.

After the first 24 hr, the small irregular microbodies become rarer but many profiles still show confronting cisternae of the RER. This relationship is not seen during the central phase of active syntheses when the RER is associated with Golgi complexes having several saccules and numerous large secretory vesicles. It seems likely that the microbodies not only arise as pockets from the RER but also grow afterwards by the addition of material from nearby cisternae. Attempts were made to follow catalase from the cisternae into the microbodies. Although there are occasional dense masses in the cisternae, they cannot be certainly identified as catalase. The technique itself is probably sensitive enough to show up catalase in the cisternae if it is concentrated enough to show up catalase in the cisternae if it is concentrated to the same degree as it is in the microbodies, since in epidermal cells peroxidase can be detected in the RER, Golgi complexes, and secretory vesicles (21).

Rough quantitation of the size of the microbodies at different stages confirms that they arise during the first 24 hr and grow until just after the first critical period (Fig. 22). The long and short
Figures 13-15. Urate oxidase in the fat body. Light micrographs of whole mounts of acetone-fixed fat body reacted for urate oxidase. During different phases of development, the reaction product is localized in granules of about the same size and distribution as the microbodies (see Figs. 33 and 34). Fig. 13, (M + 0) the fat body still contains many microbodies from the 4th stage. Fig. 14, (M + 18) granules containing urate oxidase are rare or absent. There are almost no microbodies resolvable by light microscopy at this time. Fig. 15, (M + 30) there are numerous granules containing urate oxidase; by this time, the new 5th stage generation of microbodies are resolvable by light microscopy. X 1,600.
Figures 16-21 The formation of the 5th stage microbodies. Profiles of various microbodies during their formation from about \( M + 11 \) to \( M + 24 \) (see Figs. 33 and 34). Cores are first seen in small vesicles with a loose unit membrane which may show continuity with the RER. Some slightly bigger vesicles have a confronting cisterna (cc) of the RER over a large part of their surface, a relation which may persist up to about \( M + 66 \). Stained in uranyl acetate and lead citrate. \( \times 56,000 \).
axes of microbodies were measured from micrographs of fat body from larvae of all ages. Although there is considerable scatter, the measurements show a decline in size during the first 24 hr as the new 5th stage microbodies arise and outnumber the larger survivors from the 4th stage. From about M + 24 hr and for the rest of the growth phase of the fat body there is a progressive increase in size. These results are summarized in Figs. 33 and 34.

The distribution of urate oxidase was also followed during the stadium. Acetone-fixed fat body reacted for urate oxidase showed least reaction early in the stadium, being completely negative from M + 10 to M + 22 hr (Figs. 13-15). There was a consistently high level during the synthetic phase and an abrupt loss of activity at M + 152 to M + 156 hr. Thus, the temporal distribution of urate oxidase parallels that of catalase.

In another experiment, young 5th instar larvae aged M + 0 to M + 30 hr were halved. One piece was fixed in acetone and reacted for urate oxidase, and the other was fixed in glutaraldehyde and prepared for electron microscopy. Larvae with few or no microbody cores showed least urate oxidase or gave no reaction at all. There is a correspondence between failure to detect urate oxidase and larvae without microbodies or with microbodies with minute cores just forming.

**The Destruction of 5th Stage Microbodies by Isolation and Autophagy**

Both catalase and urate oxidase are lost abruptly from the fat body at the time that it switches to
activities related to pupation. Electron microscopy shows that the time when these enzymes are lost corresponds to the envelopment of microbodies by isolation membranes and their destruction in autophagic vacuoles in exactly the same manner in which mitochondria are destroyed a few hours later (23). Figs. 23-26 show what is presumed to be the sequence of envelopment of microbodies. A small bleb flattens at the surface of the microbody. It enlarges until the microbody is completely isolated by two unit membranes and the space between them. Several of these isolation bodies come together with a fusion of their outer membranes to form an autophagic vacuole. The relation between isolated organelles and primary lysosomes will be considered in a later paper.

Mitochondria outnumber microbodies by more than 10 to 1. Nevertheless, the earliest stages of autophagy preceding pupation involve the selective envelopment of microbodies by isolation membranes, with few or no mitochondria being destroyed. In the presence of abundant mitochondria, isolation membranes are only associated with microbodies. The isolation membranes may even touch nearby mitochondria, but they still envelop only the microbodies.

A few hours later, the situation changes and many mitochondria are isolated and incorporated in autophagic vacuoles. There appears to be a sequence of organelle-specific isolation and autophagy.

The Decrease in Size of Microbodies during the Phase of Active Larval Syntheses

The microbodies at the end of the phase of active larval syntheses, for example those being enveloped for autophagy from about M + 152 to M + 156 hr, never seemed as large as those at the beginning of the synthetic phase. Measurements of the microbodies confirmed this impression. Microbodies at 150-156 hr are significantly smaller than at M + 85 hr (P < 0.01, Fig. 20). There is a progressive decrease in size during the phase of active larval syntheses. This effect was also noticeable in the 4th stage microbodies. Mid 4th stage microbodies are much larger than those surviving into the 5th stage.

The Atrophy of 4th Stage Microbodies

During the first few hours of the 5th stage, many microbodies remain from the 4th stage. There is then a period from about M + 10 to M + 22 hr when light microscopy often shows no urate oxidase, when granules containing catalase are small or absent and when electron microscopy shows no microbodies or only small ones of the new generation. There is a time when fully formed microbodies are absent, so that we must account for the loss of the 4th stage microbodies. An intensive search of many larvae failed to show isolation membranes and autophagy of these microbodies, but did disclose structures which could be interpreted as microbodies atrophying without isolation from the rest of the cell. These structures are presumed to be atrophying microbodies since (a) they are the only microbody-like structures present when 4th stage microbodies with a typical structure disappear, (b) the structures are only present at this time, and (c) structures of intermediate morphology have been found which contain both cores and the myelin figures characteristic of disintegration. Figs. 27-32 show part of the presumed sequence of atrophy. At M + 0, fairly normal-appearing microbodies are usually present. In the next few hours, the core is reduced and vesicles similar to microbodies but lacking the core can be seen. This is followed by the appearance of myelin figures in the microbodies, sometimes in those still containing small cores. The myelin increases, and little trace of microbody structure remains until the vesicles finally become smaller and are lost. These observations leave little doubt that microbodies may be lost by a process other than isolation and autophagy. The life history of 4th and 5th stage microbodies is summarized in Fig. 33.

DISCUSSION

Formation and Growth

Fig. 34 summarizes the life history of 5th stage microbodies. The origin from diverticula of the RER agrees with observations on rat liver microbodies arising during embryogenesis (33). The suggestion that, during the growth phase, microbody enzymes may be added from the RER by occasional continuity with confronting cisternae also agrees with observations on mammal liver (6). There are many parallels between liver development and the fat body at molting, but the fat body differs in the precise synchrony of events and in their repetition. For example, rat liver microbodies are presumed to turn over randomly with a half-life of about $3^{1/2}$ days (29). On the other hand, fat body microbodies are one of
Figures 23-26. Organelle specific isolation of 5th stage microbodies when the fat body prepares for pupation (see Figs. 33 and 34). Profiles of microbodies from fat body at M + 151 to M + 158. Isolation membranes (im) form an envelope which appears to grow around the microbodies and not other organelles at this time. The isolated microbodies then fuse together into autophagic vacules as has previously been described for mitochondria and RER (23). In Figs. 23 and 25, the isolation membranes are as close to mitochondria as to the microbodies, although they only envelop the latter. A few hours later, the mitochondria are preferentially enveloped. Fig. 23, isolation membrane beginning to envelop a microbody. X64,000. Fig. 24, partially enveloped microbody. X44,000. Fig. 25, completely enveloped microbody. Microvesicles (mv) are frequently seen near and in association with the outer isolation membrane. X39,000. Fig. 26, completely enveloped microbody. X55,000. Stained in uranyl acetate and lead citrate.
FIGURES 27–32 Atrophy of 4th stage microbodies at the beginning of the 5th stage. Profiles of 4th stage microbodies surviving into the 5th stage from about M + 1 to M + 21 (see Fig. 33). Cores become progressively smaller and fainter (Figs. 27–29), until only traces remain (Figs. 30–32). Myelin forms appear and may overlap with the last clear vestiges of the cores (Fig. 29). The myelin becomes more extensive (Fig. 30), and clear areas develop (Figs. 31 and 32) as atrophy proceeds. Vesicles in this atrophic sequence are medium sized and never have the loose membrane margins associated with RER as in the forming microbodies (see Figs. 16–21). Figs. 27–30, 32: ×36,000. Fig. 31, ×30,000. Stained in uranyl acetate and lead citrate.
several cell structures with a synchronized life history (Fig. 33) which we may correlate with the hormonal milieu. The number of fat body cells is determined by divisions before the 4th to 5th ecdysis (22). From M + 0 to M + 66, the cells become polyploid, there is a phase of RNA synthesis as new ribosomes and ER are formed, and the mitochondria undergo division comparable to that in the newly emerged adult fat body (17). The origin and growth of the 5th stage microbodies is part of this phase of cell preparation for participation in the massive intermolt larval syntheses which take place from M + 66 to M + 156 hr. The secretion of blood protein is one of the most important of these intermolt syntheses by the microbodies (25). It is interesting that the fat body is only active in blood protein synthesis after the microbodies have formed. There is presumably a temporal separation of the syntheses of different proteins into the cisternae of the RER, first the microbody proteins, later the blood proteins. The form of the Golgi complex reflects the two stages. Large secretory vesicles of blood protein only appear in the Golgi complex after about M + 66 hr. If microbody function involves loss of constituent enzymes, a progressive involvement of the RER in blood protein synthesis after about M + 66 would account for the microbodies becoming smaller after that time.

**Destruction**

The precision with which isolation membranes can select particular organelles for the first step in autophagy is well illustrated by the changes in fat body microbodies at the 5th to pupal molt. Isolation membranes are a general feature of cell remodeling in insects and have been seen in changing epidermal cells (19) and in oenocytes (20) as well as the fat body (23). It came as a surprise to find that the fat body does not use this mechanism for removing organelles after the 4th
to 5th molt. A possible explanation of the need for both atrophy and an isolation mechanism for organelle destruction may be in the functions of the ER and Golgi complexes. The fat body can undoubtedly engage in the synchronous formation of qualitatively different proteins. Multivesicular bodies containing lytic enzymes form at the same time as secretory vesicles of blood protein, for example, but these processes involve multiplicity of Golgi complex function whereas microbody formation involves the RER directly. At the 5th to pupal molt, isolation and autophagy involve the synthesis of lytic enzymes and their transport in primary lysosomes from the Golgi complex to the isolated organelle. At the 4th to 5th molt, an overlap between destruction of old microbodies and the formation of new ones may be necessary in order for a larval fat body cell to have functional microbodies nearly all the time. Microbody destruction not involving primary lysosomes then becomes essential unless the RER can synthesize lytic enzymes concurrently with microbody enzymes. The atrophy of microbodies may be an organelle loss without the involvement of primary lysosomes. The life history of the microbodies is paralleled by that of the mitochondria. Many mitochondria show division figures similar to those in adult fat body (17) at about the same time as the 5th stage microbodies arise, but others atrophy. At the end of the 5th stage, just after and overlapping with the isolation of microbodies, most of the 5th stage generation of mitochondria are enveloped for autophagy in preparation for pupation. The life histories of both microbodies and mitochondria show that the mode of organelle destruction varies with factors other than the type of organelle.

The destruction of 5th stage microbodies at pupation correlates with obvious changes in fat body function. There are no such obvious changes

**Figure 34** Summary of the life history of the 5th stage microbodies. Atrophy of the 4th stage microbodies takes place concurrently with the formation of the 5th stage microbodies.
from the 4th to 5th stage, and the atrophy and loss of 4th stage microbodies also came as a surprise. The life span of the microbodies is presumably limited, perhaps depending upon the replenishment of their enzymes by occasional fusion with the confronting RER cisternae that are only observed from M + 24 to M + 66 hr.

Function

Morphological studies from our laboratory have shown microbody-like structures in insect tissues ([21] *Calpodes* oenocytes; [2] *Calliphora* Malpighian tubules), but this is the first report of a correlation with some of the enzymes characteristic of microbodies. Although it used to be said that insects are uricotelic, urate oxidase has since been found to be widely distributed (30) and allantoin, allantoic acid, urea, and ammonia have all been identified in insect excreta. Almost any tissue may store and mobilize uric acid, but particularly the fat body and Malpighian tubules, where urate oxidase has been recorded most commonly. *Calpodes larvae* excrete mainly allantoic acid together with some uric acid and are probably representative of Lepidoptera (5). The fat body presumably functions in converting urate to allantoic acid for excretion by the Malpighian tubules. In the Diptera, on the other hand, microbodies and urate oxidase are present in the Malpighian tubules (e.g., adult *Drosophila*; adult *Calliphora*, [3]). The variations in urate oxidase distribution between fat body and Malpighian tubules in different insect groups may be rather like that between the liver and kidney among mammals.

Evolution

In using the term microbody to describe the structures found in insects, it should be kept in mind that only analogy may be implied. In this sense microbodies have a wide distribution in yeasts, green plants, and protozoa (15) as well as vertebrate tissues. The similarities between different microbodies are most obvious between analogous organs like the fat body and liver. For microbodies to be homologous organelles, for example as one presumes mitochondria to be, it would be necessary to show that the lineal an-

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