

# GENESIS OF MITOCHONDRIA IN INSECT FAT BODY

W. J. LARSEN

From Case Western Reserve University, Developmental Biology Center, Cleveland, Ohio 44106

## ABSTRACT

Electron microscopy and stereological methods have been used to study the time course and mechanism of mitochondrial genesis in the adult fat body of *Calpododes ethlius*, (Lepidoptera, Hesperidae). Most of the larval mitochondria are destroyed during a phase of autolysis shortly before pupation, so that pupal and early adult fat body cells have few mitochondria. The number of mitochondria per cell increases rapidly at the end of the 1st day after the adult emerges. Characteristic partitioned mitochondria appear during the period when the number is rapidly increasing. This evidence, coupled with the results of morphometric analyses of mitochondrial diameter, volume, and surface area, confirms the view that the genesis of adult mitochondria involves the growth and division of mitochondria surviving from the larva.

## INTRODUCTION

Fat body cells of *Calpododes ethlius* undergo major functional changes during larval-adult metamorphosis. The larval fat body cell is involved in the synthesis, secretion, and uptake of protein and the accumulation of lipid reserves (Locke and Collins, 11, 12). The pupal fat body stores these reserves, and adult fat body cells are involved with their mobilization and utilization. These changes in function are accompanied by extensive cell remodeling—the process by which cell structures are destroyed and replaced as function changes during development.

Locke and Collins (11) have described how fat body mitochondria are destroyed shortly before pupation. The mitochondria are enveloped by isolation membranes, and the resulting structures fuse together with a loss of the inner membranes to become autophagic vacuoles.

This paper describes the timing and mechanism of mitochondrial replacement in the adult. By using electron microscopy and morphometric techniques, I found that the number of mitochondria per cell increases approximately 700% at the end of the 1st day after adult emergence. Many

characteristic partitioned mitochondria appear during the period of rapid mitochondrial replication. This evidence, coupled with the results of morphometric analyses of mitochondrial diameter, volume, and surface area, confirms the view that the genesis of adult mitochondria involves the growth and division of mitochondria surviving from the larva. Some of this information was presented in a preliminary report (8).

## MATERIALS AND METHODS

### *The Experimental Animal*

*Calpododes ethlius* Stoll (Lepidoptera, Hesperidae) were reared in a greenhouse on the leaves of *Canna* lilies. The life cycle lasts about 5 wk at 25°C, with five larval stadia. *Calpododes* is particularly useful since its development has been precisely timed (10, 12). All animals used in this study were raised at 25°C on a 12–12 photoperiod. Under these conditions, the pupal stadium lasts for  $174.9 \pm 3.2$  hr. 37 animals of both sexes were used, ranging in age from 36 hr before the larval-pupal ecdysis to 340 hr after adult emergence. Only females were used to obtain the quantitative results.

### Tissue Preparation for Electron Microscopy

The animals were chopped into small pieces in a fixative containing 2.5% glutaraldehyde, 2% sucrose, and 0.05 M phosphate buffer, and fixed on ice for 2 hr. After rinsing, the tissues were fixed in veronal-buffered osmium tetroxide for 2 hr, stained in block with uranyl acetate (4, 5), dehydrated in alcohol, and embedded in Araldite. Thin sections were stained for 25 min in saturated uranyl acetate in 1:1 70% ethanol: absolute methanol and for 5 min in lead citrate (15). Micrographs were taken with an RCA EMU 3F electron microscope at magnifications of 872–32,000.

### Morphometric Analysis

It was necessary to obtain an estimate of the number of mitochondria per fat body cell at various stages of development. Examination of thick sections, thin sections, and whole mounts revealed that pupal and adult fat body cells are mononucleate. This allows the ratio of the number of mitochondria per unit volume to the number of nuclei per unit volume to be used as an approximation of the number of mitochondria per cell.

The number of mitochondria and nuclei per unit volume was determined by using the formula of Weibel et al. (20, 21).

$$N_{V_i} = \frac{1}{\beta_i} \cdot \frac{N_{A_i}^{3/2}}{V_{V_i}^{1/2}} \cdot K \quad (1)$$

$N_{A_i}$  is the number of transections of specific particles per unit area,  $V_{V_i}$  is the fractional volume occupied by that population of particles,  $\beta_i$  is the shape coefficient, and  $K$  is the ratio of the third to first moment of the distribution of diameters.

$N_{A_i}$  of mitochondria ( $N_{A_m}$ ) was obtained by counting profiles of mitochondria directly from 35-mm negatives taken with the electron microscope at a magnification of 872. The film was mounted on a light box under a dissecting microscope for this purpose. The fractional volume of mitochondria was determined by superimposing a grid with 500 random dots over the 35-mm negatives, using the technique of Curtis (3).

Camera lucida drawings were made of transections of nuclei in  $1\frac{1}{2} \mu$  Araldite sections with a light microscope at a magnification of 1000. These sections were taken from the same blocks used for electron microscopy and were stained with iron hematoxylin and safranin O (16).  $N_{A_i}$  of nuclei ( $N_{A_n}$ ) was determined by counting the number of nuclear transections per unit area directly from these drawings. Nuclear fractional volume was determined by superimposing a clear grid with 500 random dots over the drawings.

According to Weibel et al. (21),  $K$  may be disre-

garded for most practical purposes or an arbitrary value of 1.02–1.1 may be used for most particle populations. Since  $K$  could not be determined for nuclei because of their complex shape, it was disregarded in the computations of numbers of mitochondria and nuclei.

All profiles of mitochondria observed in adult fat body were circular or slightly oval. The proportion of oval profiles to circular profiles in any preparation was so low that these mitochondria were assumed to be spherical. The value of the shape coefficient ( $\beta$ ) for spheres is 1.382 (20). This value was used in the computation of numbers of mitochondria per unit volume.

Because the shape of fat body nuclei is complex an indirect method was used to calculate the shape coefficient for nuclei.  $N_{A_n}^{3/2}/V_{V_n}^{1/2}$  was determined by counting transections of nuclei and estimating fractional volume as described above.  $N_{V_n}$  was determined by direct counts of Feulgen-stained nuclei in a known volume of fat body in a whole-mount preparation.  $N_{V_n}$  was found to be  $33,539 \mp 3,581$  nuclei per mm.<sup>3</sup> Formula 1 was then solved for  $\beta_n$  by using this value. The shape coefficient for nuclei was calculated as 2.1. This calculation was carried out on an animal at the midpoint of the series studied. Observations of Feulgen-stained whole mounts of other animals spanning this series indicated that the size and shape of nuclei do not change during this period. Stability of nuclear size and shape is also supported by the fact that there is no fat body cell division or incorporation of thymidine-<sup>3</sup>H into fat body nuclei during this period.<sup>1</sup> Errors arising from variations in swelling or contraction during tissue preparation were assumed to be negligible since sectioned tissue and whole mounts were fixed in the same way.

The mean diameter of mitochondrial profiles was obtained by measuring profiles of mitochondria from prints of final magnification 9,500 with a Vernier caliper. Only well fixed mitochondria with definitive double membranes and cristae were measured (1). 100–200 mitochondria of each animal were measured.

The total volume of mitochondria per cell for each animal was computed by using the formula of Weibel et al. (21).

$$V_m = V_{V_m} \cdot V \quad (2)$$

$V_m$  is the total volume of mitochondria per cell,  $V_{V_m}$  is the fractional volume of mitochondria, and  $V$  is the mean volume of a cell which is equal to  $1/N_{V_n}$ .

The total surface area of outer mitochondrial membrane per cell was calculated with the following formula (21).

$$S_{om} \tau_{OT} = S_{V_{om}} \cdot V \quad (3)$$

<sup>1</sup> T. R. Johnson, personal communication.

$S_{om\tau\tau}$  is the total surface area of outer mitochondrial membrane per cell,  $S_{v_{om}}$  is defined as the surface density of outer mitochondria membrane, and  $V$  is the mean volume of a cell.  $S_{v_{om}}$  was determined by placing a grid of lines of known length  $L_T$  over the 35-mm negatives and counting the number of intersections ( $N_{om}$ ) with outer mitochondrial membranes (21).

$$S_{v_{om}} = \frac{2 \cdot N_{om}}{L_T} \quad (4)$$

Statistical analysis was carried out by using the procedures of Weibel et al. (21) and Curtis (3). Two-stage sampling was used to determine numbers and fractional volumes of mitochondria and nuclei and surface area of outer mitochondrial membrane. Four random fields from each of five randomly chosen sections were analyzed for both mitochondria and nuclei. The size of each sample field for the determination of number and fractional volume of mitochondria and the surface density of outer mitochondrial membrane was 0.0011 mm<sup>2</sup>. The size of each sample field for the determination of nuclear number and fractional volume was 0.0061 mm<sup>2</sup>. 15,885 profiles of mitochondria and 627 nuclear profiles were counted in the 15 animals used to obtain the quantitative results.

## RESULTS

### *Changes in the Number of Mitochondria during Metamorphosis*

This study confirmed the observation by Locke and Collins (11) that most fat body mitochondria are destroyed in autophagic vacuoles about 30 hr before the larval-pupal ecdysis. The remaining mitochondria are localized in thin strands of cytoplasm surrounding protein storage granules, autophagic vacuoles, and lipid droplets. These cytoplasmic inclusions occupy most of the volume of the cell at this time. During the pupal stadium, until about 2 days before adult emergence, there are few mitochondria. By 30 hr after emergence, however, the number of mitochondria increases several times. Determinations of the number of mitochondria per cell by morphometric techniques indicate that the greatest increase in number occurs between approximately 22 and 34 hr after emergence of the adult (Fig. 1 a).

### *Changes in the Diameter of Mitochondria*

Measurements of the diameter of mitochondrial profiles indicate that fat body mitochondria

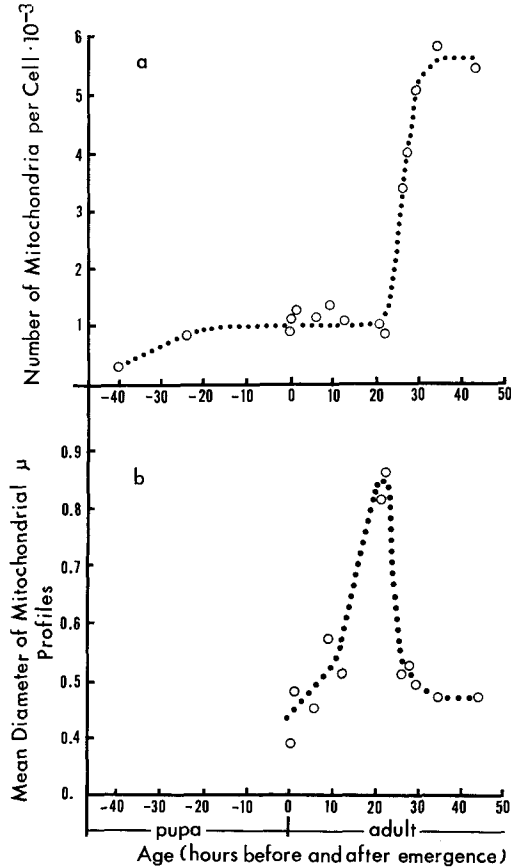


FIGURE 1 a Time course of mitochondrial replication during early adult development. During the period between 22 and 34 hr after adult emergence, fat body mitochondria rapidly increase in number.

FIGURE 1 b Changes in the mean diameter of fat body mitochondria during the 1st day of adult development. Mitochondria increase in size between emergence and 22 hr after emergence, but rapidly decrease in size during the period when mitochondria are increasing in number.

grow rapidly during the first 22 hr after adult emergence (Fig. 1 b). The mean diameter of mitochondria decreases rapidly, however, between 22 and 34 hr after emergence. The series of selected histograms in Fig. 2 illustrates the loss of smaller size classes and an increase in the proportion of larger mitochondria during the first 21 hr after emergence. During the next 9–13 hr, the trend is reversed, and by 34 hr after emergence the size distribution is similar to that shortly after emergence. Histograms of diameters of fat body mitochondria of six additional animals were consistent with these trends.

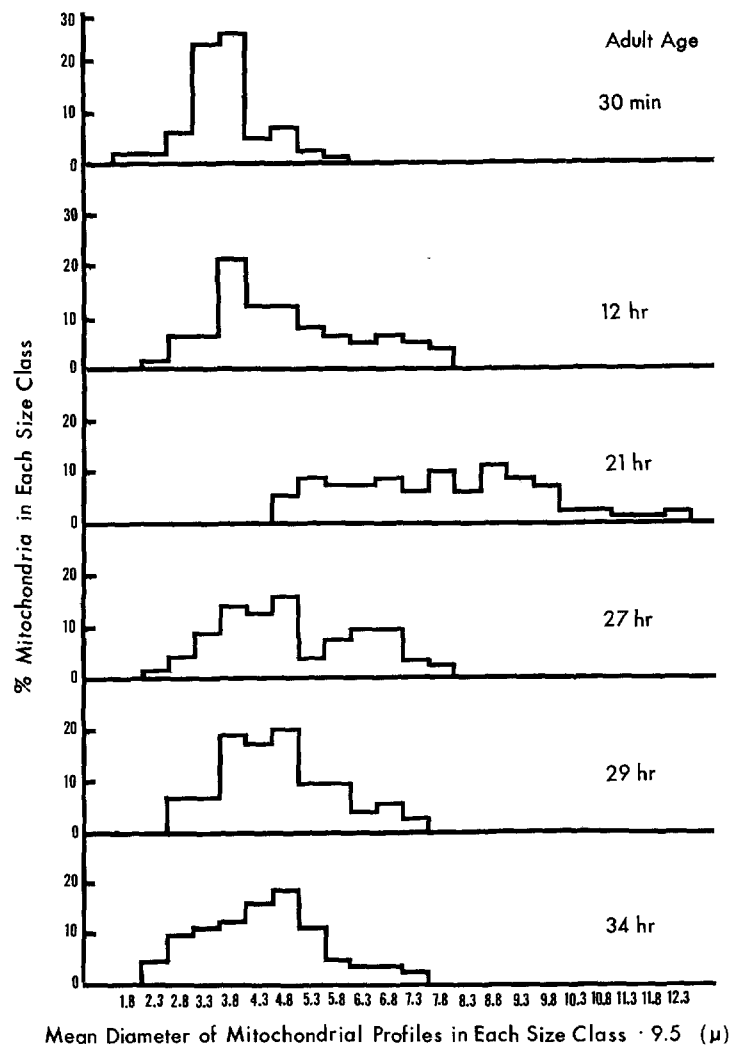


FIGURE 2 Histograms showing the frequency distribution of mitochondrial diameters in fat body of six female adults. This series illustrates the loss of smaller size classes and a concomitant increase in the number of larger mitochondria during the first 21 hr after adult emergence, and a reversal of that trend during the next 9-13 hr.

*Changes in the Volume and Outer Surface Area of Mitochondria*

The total volume of mitochondria per cell increases until about 22 hr after emergence and does not increase significantly after this time (Fig. 3 a). This increase takes place before the period when mitochondria are increasing in number. The total outer membrane surface area increases before and during the period when mitochondria are increasing in number (Fig. 3 b).

*Changes in the Morphology of Mitochondria during Development*

The mitochondria of larval fat body are filamentous or rod-shaped (Fig. 4). Mitochondria of late pupae and adults are generally spherical, although some oval mitochondrial profiles may be seen at this time (Fig. 5). Electron micrographs of fat body from animals (both sexes) of ages between 23 and 27 hr after emergence have many unusual mitochondrial profiles. These are

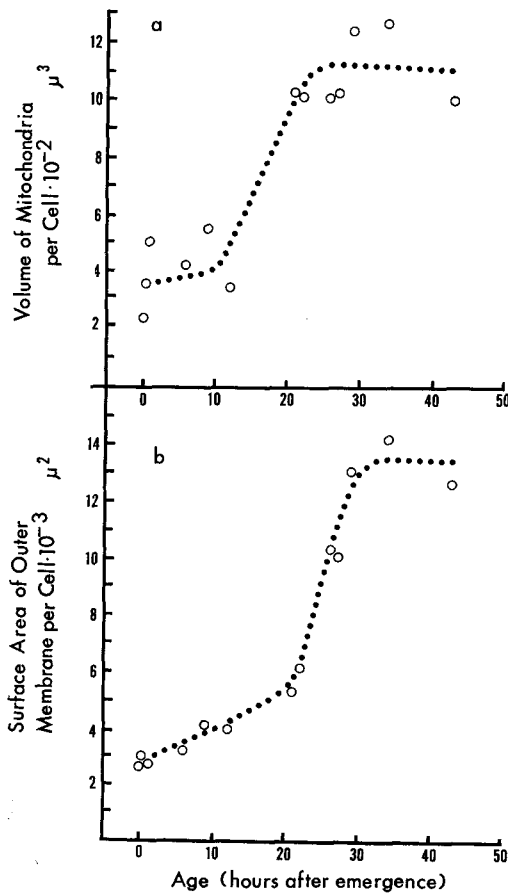


FIGURE 3 *a* Changes in the total volume of mitochondria per cell. Total mitochondrial volume rapidly increases sometime during the period between 12 and 21 hr after emergence. During the period of mitochondrial replication between 22 and 34 hr after emergence, there is no additional increase in total volume.

FIGURE 3 *b* Changes in the total mitochondrial outer membrane surface area per cell. The total amount of outer mitochondrial membrane per cell increases during the period of replication as well as the period of mitochondrial enlargement.

regular oval profiles with one straight transecting crista (Fig. 6). This crista is continuous with the inner mitochondrial membrane and may have some branches along its length. Transecting cristae almost always bisect the mitochondrion into nearly equal halves. Many bisected mitochondria are constricted at the site of the straight, transecting crista (Fig. 7). Tandler et al. (17) have referred to these profiles as partition figures and

to the straight, transecting crista as a partition. This terminology will be used in this paper.

Some mitochondria may be transected into more than two parts. Mitochondria which appear to have only one partition may actually be parts of a single mitochondrion with two or more partitions as shown by serial sections. One mitochondrion appeared to be transected into four parts by both transverse and longitudinal partitions (Fig. 8).

Partitioned mitochondria are numerous in the fat body of adults between 23 and 27 hr after emergence (Fig. 9). It was not unusual to see as many as four to eight partition figures in an area as small as  $20 \mu^2$ . The relative proportion of these structures to spherical mitochondria is difficult to determine since only a few orientations of the partitioned mitochondrion with respect to the plane of section will reveal the partition.

Spherical mitochondria or partitioned mitochondria were not found in specialized associations with other membrane-bounded structures including the nucleus, Golgi complex, or endoplasmic reticulum. Spherical and partitioned mitochondria, however, were often found clustered together. Partition figures were never seen in fat body of pupae or adults younger than 23 hr or older than 27 hr.

#### DISCUSSION

Partitioned mitochondria of the type described in this investigation have been reported in other studies. In some cases, it has been shown that partition figures appear at a time when mitochondria are increasing in number, and it has been suggested that they may represent dividing mitochondria. See Tandler et al. (17) for a review of these studies.

In many of these investigations, the replication of mitochondria was experimentally induced. LaFontaine and Allard (7) stimulated an increase in the number of mitochondria in rat liver by feeding rats the azo dye 2-Me-DAB. Tandler et al. (17) induced the replication of mitochondria in liver cells of riboflavin-deficient mice by injecting riboflavin. Onishi (13, 14) bled rats and observed degeneration and subsequent replication of cardiac mitochondria. Wigglesworth (22) stimulated mitochondria to replicate in the fat body of *Rhodnius* by feeding severely starved animals.

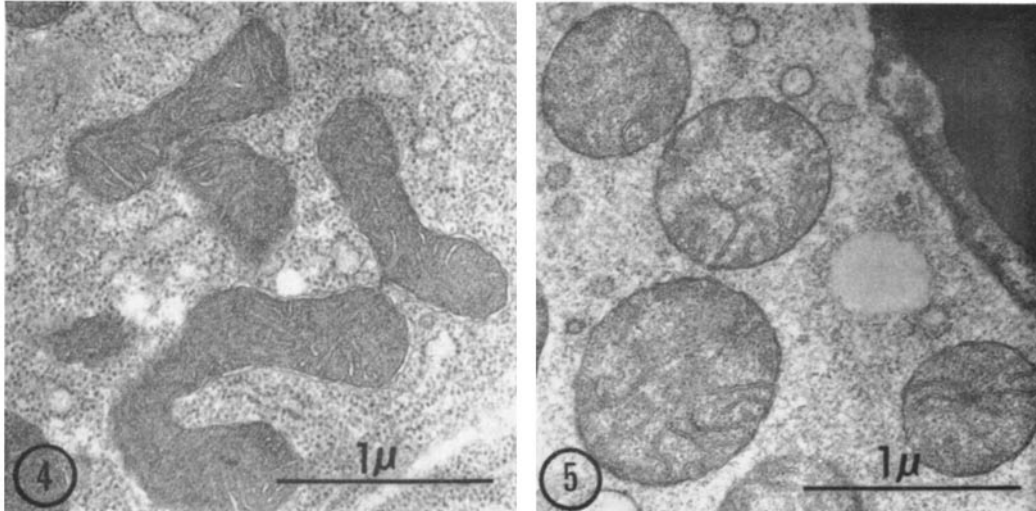


FIGURE 4 Larval fat body mitochondria. These mitochondria are generally filamentous or rod-shaped.  $\times 29,000$ .

FIGURE 5 Adult fat body mitochondria. At this stage, mitochondria are almost perfectly spherical.  $\times 29,000$ .



FIGURE 6 A fat body mitochondrion of a 27 hr old male adult. This organelle possesses a straight transecting crista (between arrowheads), which is characteristic of a large proportion of mitochondria from fat body of adults between 23 and 28 hr old. This crista is continuous with the inner mitochondrial membrane and separates the matrix into two compartments.  $\times 66,000$ .

In the present study, it is shown that mitochondria divide in *Calpodex* fat body during normal development. The two most significant observations which support this view are (a) large numbers of partition figures appear only at the time when mitochondria are rapidly increasing in number, and (b) the size of mitochondria decreases during this period. These two observations rule out the possibility that partition figures represent stages in mitochondrial fusion. In addition, the presence of partitioned mitochondria with two to three partitions and the clustering of some partitioned mitochondria suggest that these grouped mitochondria represent a clone.

It is presumed that the growth and division of mitochondria in the fat body is necessary for the increased energy requirements of the adult butterfly. The change in morphology of mitochondria from filamentous larval type to spherical adult mitochondria may reflect a shift of functional emphasis of the fat body. Mitochondria in developing insect flight muscle have been reported to go through a phase of growth which is associated with a change in morphology and their enzymatic differentiation (2, 6, 9, 19). No partitioned mitochondria, however, have been observed in this tissue.

Fig. 10 illustrates a possible mechanism of division as determined from numerous electron



FIGURE 7 Two fat body mitochondria of a 27 hr old male adult. These mitochondria are constricted at the site of the straight transecting crista. This crista may have short branches (arrow). The outer membrane never penetrates between the membranes of the straight transecting crista.  $\times 81,000$ .

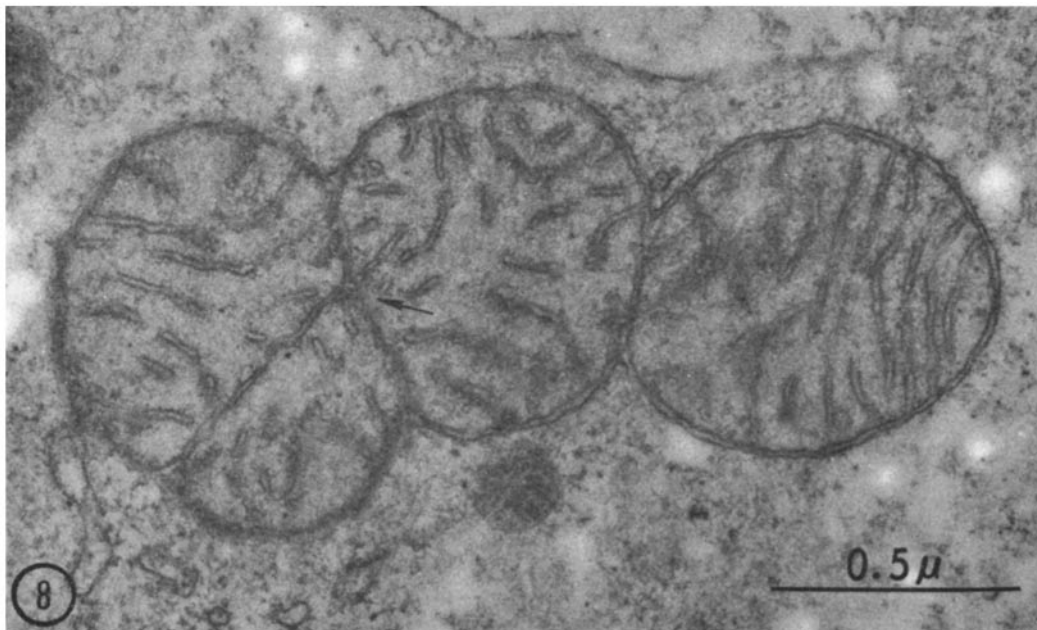


FIGURE 8 A mitochondrion with two transverse partitions and one longitudinal partition. The matrix of this mitochondrion appears to be separated into four compartments. Several short cristae appear to originate at the intersection of the longitudinal partition and one of the transverse partitions (arrow).  $\times 69,000$ .

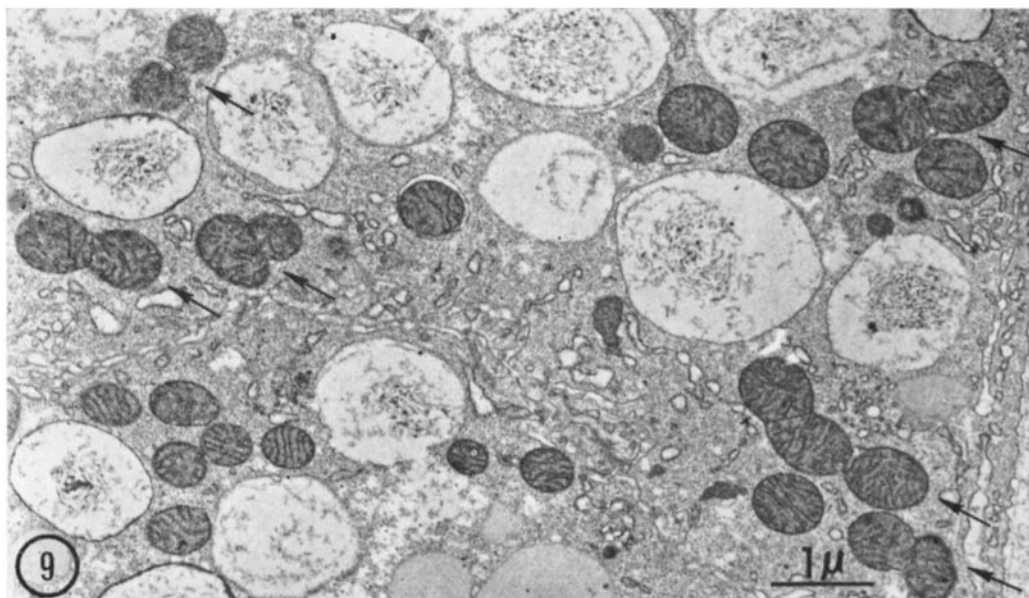


FIGURE 9 A low-power electron micrograph of fat body from a 26 hr old female adult. Partition figures are numerous in fat body of adults between 23 and 28 hr old (arrows). All other mitochondrial profiles are circular or slightly oval.  $\times 14,000$ .



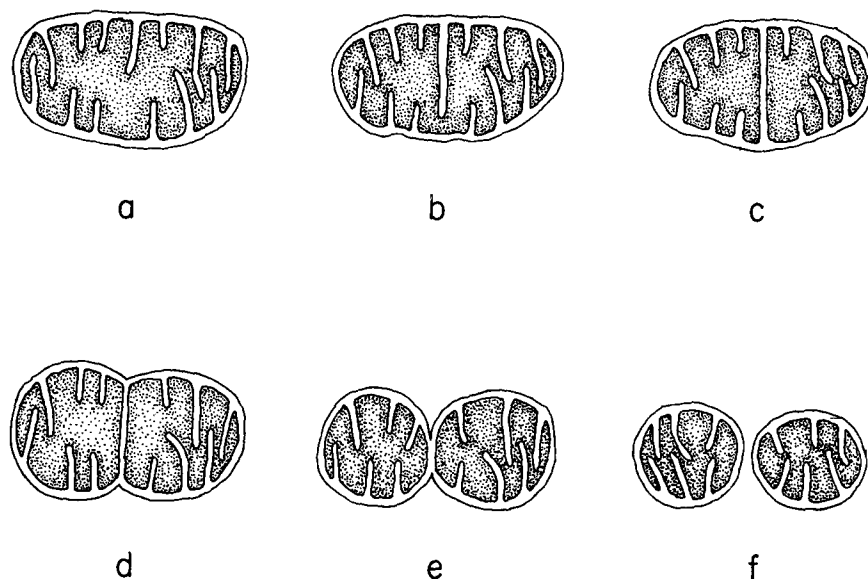


FIGURE 10 Diagram of possible sequence of events leading to mitochondrial division: (a) The mitochondrion elongates slightly. (b) One centrally placed crista begins to grow across the mitochondrion. (c) This centrally placed crista fuses with the inner membrane around its entire periphery, thus separating the matrix into two distinct compartments. (d) A constriction forms at the site of the straight transecting crista or partition. (e) The constriction tightens uniformly around the mitochondrion. (f) Continuing construction results in fission of the mitochondrion.

micrographs. Elongation of the mitochondrion may occur shortly before or during the formation of the partition as indicated in Fig. 10 *a*. This is based on the observation that at this time most fat body mitochondria are spherical and all partitioned mitochondria are slightly elongate. As illustrated in Fig. 10 *b*, the partition may be formed by the outward growth of one crista. This crista then fuses with the inner membrane on the opposite side of the mitochondrion. This is based on the observation of some profiles with a straight transverse crista connected to only one side of the mitochondrion. Tandler et al. (17) have also observed this in mouse liver mitochondria which are very large with relatively short cristae. Occasionally, they observed a large mitochondrion with one very long crista connected to the inner membrane on one side of the mitochondrion. A similar mitochondrion was observed by Vogel and Kemper (18) in an extracellular culture of mitochondria from the common meadow mushroom. Since some mitochondria in fat body possess a partition but show no signs of constriction, it is suggested that constriction follows the formation of the partition (Fig. 10 *c, d*). Continuing con-

striction finally results in division of the mitochondrion.

Electron microscopy has failed to indicate any other possible source of the new mitochondria. Mitochondria are never found in intimate association with other membranes or organelles, although this does not preclude the possibility that other cellular structures may play some role in the genesis of new mitochondria. Tandler et al. (17) have reviewed other mechanisms of mitochondrial replication in various cells.

Additional parameters of mitochondrial replication have been revealed by computing the total volume and total outer surface area of mitochondria per cell (Fig. 3 *a, b*). These results suggest that the synthesis or incorporation and assembly of some or all matrix components occurs before the period of replication since the most significant increase in volume takes place before 21 hr after emergence. Total outer membrane surface area, however, increases before and during the period of division. Synthesis or incorporation and assembly of matrix components seem to be separated in time from the period of division, while outer membrane assembly

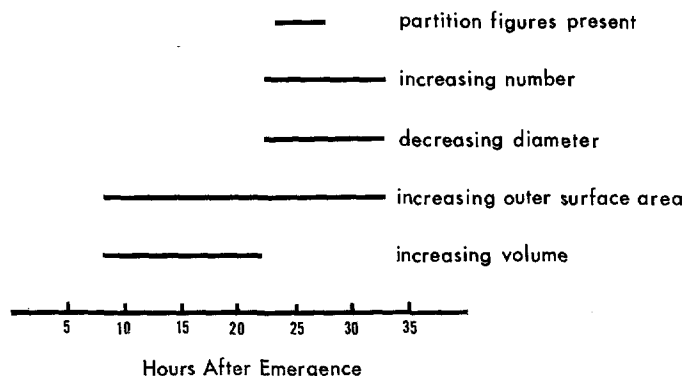


FIGURE 11 A summary of some parameters of growth and division of adult fat body mitochondria. Mitochondria grow rapidly during the period between approximately 10 and 22 hr after adult emergence. Outer mitochondrial membrane is added during growth *and* division. Partition figures are present only when mitochondria are increasing in number.

occurs during growth and division. This interpretation is summarized in Fig. 11.

One additional calculation was carried out to substantiate the results determined by morphometric analysis. This calculation is based on the assumption that (a) the adult mitochondria can be treated as spheres, (b) the volume of mitochondria per cell remains constant during the period of mitochondrial replication, and (c) mitochondria replicate by division. If these assumptions are correct, the following equation is justified.

$$\frac{4}{3}\pi\bar{r}_1^3 \cdot n_1 = \frac{4}{3}\pi\bar{r}_2^3 \cdot n_2 \quad (5)$$

where  $\bar{r}_1$  is the measured mean radius of mitochondrial profiles before replication begins,  $\bar{r}_2$  is the measured mean radius of mitochondrial profiles during or after division,  $n_1$  is the number of mitochondria per cell before replication, and  $n_2$  is the number of mitochondria per cell during or after the period of replication. The equation can be solved for  $n_2/n_1$ , and the proportional increase in the number of mitochondria per cell can be determined. If  $n_2/n_1$  determined in this manner is similar to the proportional increase determined independently by morphometric analysis, then additional confidence can be placed in the data already presented. The proportional increase in numbers of mitochondria per cell ( $n_2/n_1$ ) was determined for periods between 22 hr after emergence and 26–43 hr after emergence by using equation 5. The proportional increase

TABLE I

*Comparison of Percentage Increase in Numbers of Mitochondria per Cell during Period of Mitochondrial Replication Determined by Morphometric Analysis and by Calculations Using Equation 5*

The proportional increases in number of mitochondria per cell for periods between 22 hr after emergence and 26–43 hr after emergence determined independently by these two methods are similar.

Period after emergence when increase in no. of mitochondria occurs	Increase in no. of mitochondria per cell determined by morphometric analysis	Increase in no. of mitochondria per cell determined using equation 5 ( $n_2/n_1$ )
hr	%	%
22–26	402	478
22–27	474	454
22–29	598	541
22–34	693	611
22–43	645	611

determined in this way was then compared to the proportional increase determined by morphometric analysis (Table I). It can readily be seen that there is good correspondence between the percentage increases in number of mitochondria per cell determined independently by these two methods. These data also suggest that two to three generations of mitochondria are produced during the period of rapid replication between 22 and 34 hr after emergence.

In summary, it has been shown that partitioned mitochondria are present at a time when mitochondria are decreasing in size and increasing in number. This evidence suggests that mitochondrial genesis involves the division of pre-existing mitochondria. The replacement of filamentous mitochondria with spherical mitochondria may indicate that larval and adult mitochondria are functionally different.

The author wishes to thank Dr. M. Locke, Dr. Bernard Tandler, and Dr. J. L. Oschman for their encouragement and critical reading of the manuscript. Thanks are also due to Mr. T. R. Johnson for his advice and comments during the course of this work. This investigation was supported by a grant from the United States Public Health Service (GM 09960) awarded to M. Locke.

Received for publication 5 January 1970, and in revised form 12 May 1970.

#### BIBLIOGRAPHY

1. BAUDHIN, P., and J. BERTHET. 1967. Electron microscopic examination of subcellular fractions. II. Quantitative analysis of the mitochondrial population isolated from rat liver. *J. Cell Biol.* 35:631.
2. BROSEMER, R. W., W. VOGEL, and T. BUCHER. 1963. Morphologische und Enzymatische Muster bei der Entwicklung indirekter Flugmuskeln von *Locusta migratoria*. *Biochem. Z.* 338:854.
3. CURTIS, A. G. S. 1960. Area and volume measurements by random sampling methods. *Med. Biol. Illus.* 10:261.
4. FARQUHAR, M. G., and G. E. PALADE. 1965. Cell junctions in amphibian skin. *J. Cell Biol.* 26:263.
5. FRIEND, D. S., and M. G. FARQUHAR. 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* 35:357.
6. GREGORY, D. W., R. W. LENNIE, and L. M. BIRT. 1967. An electron microscope study of flight muscle development in the blowfly *Lucilia cuprina*. *J. Roy Microsc. Soc.* 88:151.
7. LAFONTAINE, J. G., and C. ALLARD. 1964. A light and electron microscopic study of the morphological changes induced in rat liver cells by the azo dye 2-Me-DAB. *J. Cell Biol.* 22:143.
8. LARSEN, W. J. 1968. An ultrastructural study of mitochondrial genesis in the adult fat body of an insect. *J. Cell Biol.* 39:154a (Abstr.).
9. LENNIE, R. W., D. W. GREGORY, and L. M. BIRT. 1967. Changes in the nucleic acid content and structure of the thoracic mitochondria during development of the blowfly, *Lucilia cuprina*. *J. Insect Physiol.* 13:1745.
10. LOCKE, M. 1970. The molt/intermolt cycle in the epidermis and other tissues of an insect, *Calpodes ethlius* (Lepidoptera, Hesperidae). *Tissue and Cell.* 2:197.
11. LOCKE, M., and J. V. COLLINS. 1965. The structure and formation of protein granules in the fat body of an insect. *J. Cell Biol.* 26:857.
12. LOCKE, M., and J. V. COLLINS. 1968. Protein uptake into multivesicular bodies and storage granules in the fat body of an insect. *J. Cell Biol.* 36:543.
13. ONISHI, S. 1967. Mitochondrienvermehrung der Herzmuskelzelle nach Aderlass. *Naturwissenschaften.* 54:568.
14. ONISHI, S. 1967. Die Feinstruktur des Herzmuskels nach Aderlass bei der Ratte. Zugleich ein Beitrag zur Teilung und Vermehrung von Herzmuskelmitochondrien. *Beitr. Pathol. Anat. Allg. Pathol.* 136:96.
15. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208.
16. SCHANTZ, A., and A. SCHECHTER. 1965. Iron-haematoxylin and safranin O as a polychrome stain for Epon sections. *Stain Technol.* 40:279.
17. TANDLER, B., R. A. ERLANDSON, A. L. SMITH and E. L. WYNDER. 1969. Riboflavin and mouse hepatic cell structure and function. II. Division of mitochondria during recovery from simple deficiency. *J. Cell Biol.* 41:477.
18. VOGEL, F. S., and L. KEMPER. 1965. Structural and functional characteristics of deoxyribonucleic acid-rich mitochondria of the common meadow mushroom, *Agaricus campestris*. II. Extracellular cultures. *Lab. Invest.* 14:1868.
19. WALKER, A. C., and L. M. BIRT. 1969. Development of respiratory activity and oxidative phosphorylation in flight muscle mitochondria of the blowfly, *Lucilia cuprina*. *J. Insect Physiol.* 15:305.
20. WEIBEL, E. R., and D. M. GOMEZ. 1962. A principle for counting tissue structures on random sections. *J. Appl. Physiol.* 17:343.
21. WEIBEL, E. R., G. S. KISTLER, and W. F. SCHERLE. 1966. Practical stereological methods for morphometric cytology. *J. Cell Biol.* 30:23.
22. WIGGLESWORTH, V. B. 1967. Cytological changes in the fat body of *Rhodnius* during starvation, feeding and oxygen want. *J. Cell Sci.* 2:243.