KOREAN JOURNAL OF ZOOLOGY
Vol. 16, No. 1, 43—55 (1973)

Electron Microscopical Study on Mitochondrial Changes of Flight Muscle with Aging in a Butterfly, *Pieris rapae L.*

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배추흰나비, *Pieris rapae L.* 飛筋의 年齡에 따른 미토콘드리아의 變化에 관한 電顯的硏究

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(Received December 6, 1972)

摘　　要

年齡에 따른 미토콘드리아의 構造的變化를 調査하기 위하여 電子顯微鏡을 利用하여 배추흰나비, *Pieris rapae L.*의 飛筋을 觀察하였다. 미토콘드리아는 크리스타가 잘 발달되지 않은 A型과 크리스타가 복잡하게 발달된 B型의 두種類가 存在하였다. 羽化直後의 것에서는 A型과 B型미토콘드리아가 꼭같이 存在하였고 羽化後 10日 經過한 것에서는 B型미토콘드리아에 比하여 A型이 急速히 減少하였다.

羽化後 10日이 經過된 것의 筋細胞에서는 B型미토콘드리아가 年齡의 增加에 따른 微細構造的變化를 이르켰다. 이러한 老衰된 미토콘드리아의 微細構造的 變化像은 部分的으로 그 內膜이 髓鞘狀構造를 再形成하는 것으로 나타났는데 이것은 곧 年齡에 따른 退化를 意味한다. 髓鞘狀構造를 形成하는 것은 많은 同心圓體이며 이同心圓體의 數와 크기는 年齡과 比例하면서 增加했다. 筋細胞의 細胞質에서 미토콘드리아內로 侵入한 글리코겐 粒子는 同心圓體의 中央에서도 觀察되었다.

INTRODUCTION

Flight muscular cells of insect, which have higher activities than other muscles, possess many large mitochondria (Tribe *et al.*, 1972). It is known that two to three mitochondria occur frequently between sarcomeres. The respiratory efficiency of these mitochondria has recently received much attention, particularly in relation to the problem of aging (Levenbook and Williams, 1956; Rockstein and Clark, 1964; Rockstein and Briandt, 1963; Tribe, 1966). Insect flight muscle has advantages for studying this kind of work since it is possible to examine changes in flight

performance with increasing age, and then to correlate these changes with the associated metabolic processes taking place in the intact muscle fiber.

Mitochondria of flight muscle, which generally have multicristae, vary biochemically and morphologically with age. Recent investigations including various measurements of oxidative phosphorylation (Tribe, 1967) showed that degeneration occurred in the respiratory efficiency of isolated flight muscle mitochondria as the fly aged. The observations were supported by changes in metabolic procedure during the life span of the insect, for example, higher rates of oxygen consumption were observed in the whole and in the intact muscle.

Another evidence of mitochondrial changes with age was ultrastructural degradation. Flight muscle mitochondria deteriorated morphologically from small fruit fly, *Drosophila melanogaster* (Takahashi *et al.*, 1970), and housefly, *Musca domestica* (Simon *et al.*, 1969). Particularly, Simon *et al.* (1969) reported that the flight muscle of housefly had two kinds of mitochondria: type A mitochondria degenerating by swelling due to development of vacuoles caused by a non-uniform increase in their intercristal spaces and type B by fenestration of the multicristae or the whorled cristal membrane. Inner mitochondrial membranes of flight muscle from old blowfly, *Phormia regina*, were also reported to undergo degenerative change (Sacktor and Shimada, 1972).

Remarkable changes in the ultrastructure of flight muscle mitochondria observed in a butterfly, *Pieris rapae*, are reported in the present paper.

MATERIALS AND METHODS

Butterflies (*Pieris rapae*) were reared in a cage of 50% humidity. The adult butterflies were given a sugar/water diet. Under these conditions the mean survial time of adult was 12 ± 1.0 days with a maximum of 15 days.

Flight muscles were obtained from the adults just emerged to 15-day-old. Butterflies were dissected by a medical knife with razor blade in ice-cold fixative containing 2.5% glutaraldehyde, 0.05M cacodylate buffer, pH 7.5, and 0.18M sucrose (Smith, 1966). Samples of muscles less than $1mm^3$ were kept in fixative at 0—4°C for 2 hrs, and washed several times with 0.05M cacodylate cold buffer.

The tissues were incubated for 2hrs at 37°C with 0.5% α-amylase (from E. Merck, Darmstadt) in 10mM phosphate buffer, pH 7.4, containing 20mM NaCl (Coimbra, 1967). Control tissues were incubated in buffer without α-amylase.

The materials were rinsed with 0.1M phosphate buffer, pH 7.4 and transferred to cold 2% osmium tetroxide in 0.1M phosphate buffer, pH 7.4, for 2 hrs. The dehydration was done in an ethanol series and pure acetone. The materials were embedded in Epon 812 (Luft, 1961). Section were cut using Porter-Blum MT-2

Ultramicrotome and mounted on 200-mesh copper grids. The specimens were stained with uranyl acetate and lead citrate (Reynolds, 1963) and then the grids were examined in the Hitachi HS-7S electron microscope (50KV).

RESULTS

In all specimens two distinct types of mitochondria were observed. The first type, type A, has simple folded cristae and light matrix, while the second type, type B, possesses complex multicristae and electron-dense matrix. The total area of cristal membranes in the type B mitochondria is far greater per unit of mitochondrial volume than in the type A.

In just newly emerged butterflies (Figs. 1, 2, 3) both type A (Figs. 2, 3) and B mitochondria (Fig. 1) are found arranged between the myofibrils. Myofibrils are usually small, having a mean diameter of 1.2μ. The mitochondria are 1.22μ in average diameter, and sometimes up to 3.6μ in length (Fig. 2), and not always aligned in parallel with the myofibrillar striations. Aggregations of glycogen particles are prominently located in relatively small spaces between the myofibrils (Figs. 1, 2). Profiles of sarcoplasmic reticulum and T-system elements can also be seen between myofibrils.

One day after emergence (Figs. 4, 5, 6) the interfibrillar sarcoplasm is tightly packed with the type B mitochondria (Figs. 5, 6) and sarcoplasmic reticulum(Fig. 6). Internal structures of the type B mitochondria are characterized by the presence of myelin-like cristae. The mean diameter of mitochondria is 1.32μ, while a few more than 2.6μ in length.

In ten-day-old adult (Figs. 7, 8, 9, 10) the type B mitochondria increased, while the type A rapidly decreased. The type A mitochondria were hardly seen on all sections, but the type B underwent the degenerative changes. Membranous whorls in the mitochondria were evident (Figs. 7, 8, 9, 10). At higher magnifications the whorls appear as myelin-like structures. Concentric rings may be either loosely (Fig. 9) or tightly packed (Fig. 10). It also seems to represent a reorganization of the inner mitochondrial membrane into myelin-like whorl (Figs. 9, 10). From the flight muscle incubated in buffer solution without α-amylase (Fig. 10), the particles, resembling glycogen granules, are frequently seen in the center of whorls, while the flight muscle mitochondria incubated with α-amylase were empty in the center of whorl instead of glycogen rosettes (Fig. 9).

Examinations from 10- to 15-day-old adult the sections revealed mitochondria in different phases of ultrastructural degeneration. The whorls of mitochondria increase in size and number. These myelin-like structures of the type B mitochondria were eventually replaced by normal cristae conformation (Fig. 8).

DISCUSSION

Rockstein and Bhatnagar (1965) recorded mitochondria in different sizes ranging from 1 to 6μ in diameter. Simon *et al.* (1969) also reported that in spite of size variation only two types of mitochondria could be morphologically distinguished in flight muscle of house flies, *Musca demestica*, viz. type A with simple folded cristae and light matrix, and type B with complex multicristal and dense matrix. The presence of two types of mitochondria reported by Simon *et al.* (1968) is confirmed in the present study on *Pieris*. In ten-day-old butterflies the B type mitochondria undergo ultrastructural changes which represent morphological deterioration with age. However, fenestration of the cristae in the type B and vesiculation in the type A were not found. Inner membranes of only the type B form the whorls, myelin-like structures, with age. The degenerative changes of the type B in flight muscle from *Pieris* are characterized by formation of inner mitochondrial cristae into myelin-like whorls. Concentric rings, which form the whorl of mitochondria, increase in size and number with age.

Membranous whorls in mitochondria have been found in spinal ganglion neuroblasts (Pannese, 1966). Pannese (1966) has suggested that the whorls may be related to the formation of new mitochondria. This hypothesis is not supported by our observations on the flight muscle in *Pieris*.

Although the time course of the development and degeneration of both types of mitochondria is independent of size of cage in which the adult butterflies are kept, the type B mitochondria, which possess complex multicristae and dense matrix, appear to be greater in number in the butterflies for which was prepared longer flight path. The above mentioned results suggest that the morphology of the flght muscle mitochondria indicates the age and the use of flight muscle.

SUMMARY

The flight muscles in *Pieris rapae* have been examined to study ultrastructural changes in mitochondria with aging. All the mitochondria of flight muscle from the butterfly are recognized as type A which has the simple folded cristae and light matrix, and type B which possesses the complex multicristae and dense matrix. In just newly emerged butterflies both A and B type mitochondria are almost equally present. About ten days after emergence the type A mitochondria rapidly decrease, compared with the type B.

In ten-day-old butterflies the type B mitochondria vary in ultrastructure with age. Ultrastructural changes of these aged mitochondria are supposed to occur, in part, by reorganization of inner membranes into myelin-like structures which

represent the phase of degeneration of the B type with age. Age-dependent increase in size and number of concentric rings in myelin-like whorl are also found. Glycogen particles penetrated from the cytoplasmic matrix of the muscle cell into the mitochondrial matrix to be in the center of their concentric rings.

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EXPLANATIONS OF FIGURES

Abbreviations on Plates

A; type A mitochondria
B; type B mitochondria
G; glycogen particle
M; mitochondria
MF; myofibril
SR; Sarcoplasmic reticulum
T; Tracheole
TS; T-system element

Figs. 1, 2, 3. Longitudinal and transverse sections of flight muscles in the early adult just after emergence.

Fig. 1. Type B mitochondria are seen. Mitochondria appear lined up between the myofibrils. Note the complex multicristae surrounded by dense matrix which characterize type B. Mitochondria are surrounded with a large amount of glycogen. Bar line indicates 1.0μ. ×19,400.

Fig. 2. Type A mitochondria possess simple folded cristae and light matrix. Mitochondria are large up to 3.6μ in length. Bar line indicates 1.0μ. ×19,000.

Fig. 3. Interfibrillar sarcoplasm tightly occupied with type A mitochondria, T-system tubules, and sarcoplasmic reticulum. Mitochondria have very simple folded cristae and light matrix which characterize type B. Bar line indicates 0.5μ. ×56,000.

Figs. 4, 5, 6. Longitudinal and transverse sections from the one-day-old butterflies.

Fig. 4. Type B mitochondria is characterized by the bizarre shape and pattern of the cristae. Mitochondrial cristae resemble the myelin-like figure. The tracheole is seen near the mitochondria. Bar line indicates 0.25μ. ×48,460.

Fig. 5. Several large mitochondria of type B appear lined up between myofibrils. Note the presence of the tracheole between mitochondria. Bar line indicates 1μ. ×19,200.

Fig. 6. Interfibrilar spaces are tighlty packed with mitochondria and sarcoplasmic reticulum. Tracheole is evident. Bar line indicates 0.5μ. ×27,000.

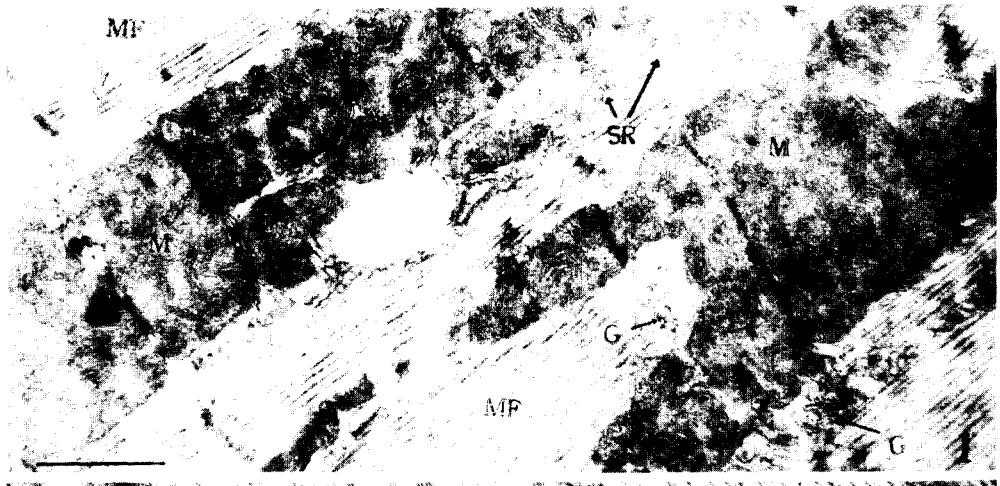
Figs. 7, 8, 9, 10. Longitudinal sections from the ten-day-old butterfly. Type B mitochondria are hardly seen.

Fig. 7. Inner mitochondrial cristae are transforming into myelin-like figure. Many of mitochondria are type B. Glycogen paricles and sarcoplasmic reticulum are seen. Bar line indicates 0.5μ. ×27,150.

Fig. 8. Type B mitochondrion is almost changed into myelin-like structure which represents the ultrastructural degeneration and is replaced by cristae. Bar line indicates 0.5μ. ×38,800.

Fig. 9. Mitochondria from flight muscle sample incubated with 0.5% α-amylase are empty in the center of concentric rings. Glycogen particles in the center of whorl are digested. Bar line indicates 0.25μ. ×62,600.

Fig. 10. Type B mitochondrion possesses the glycogen rosette in the center of myelin-like whorl in the section incubated with buffer without α-amylass. Bar line indicates 0.5μ. ×43,950.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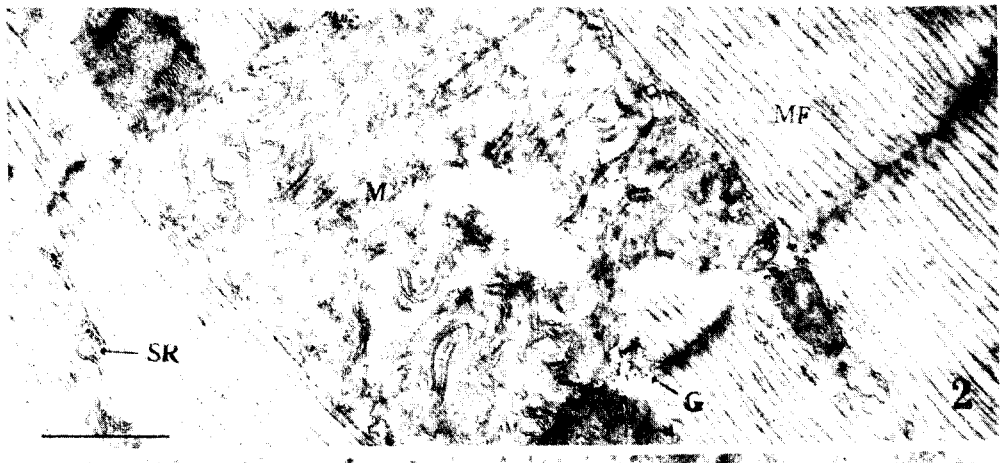
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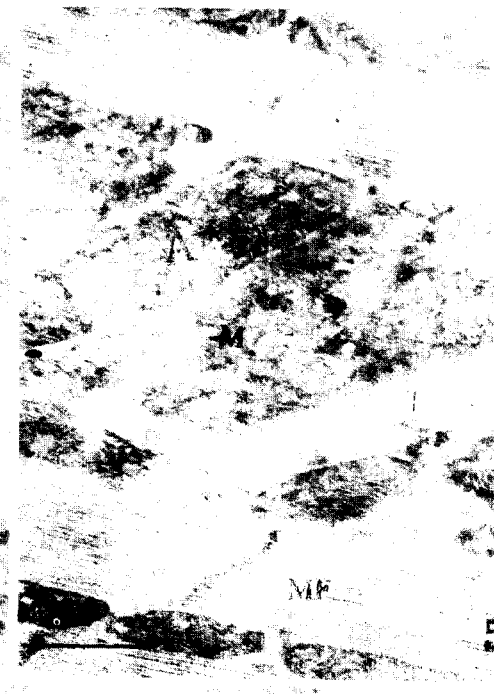
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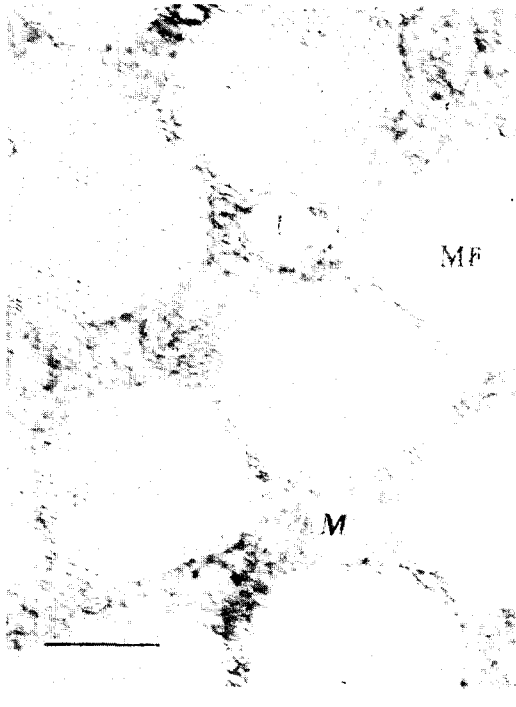
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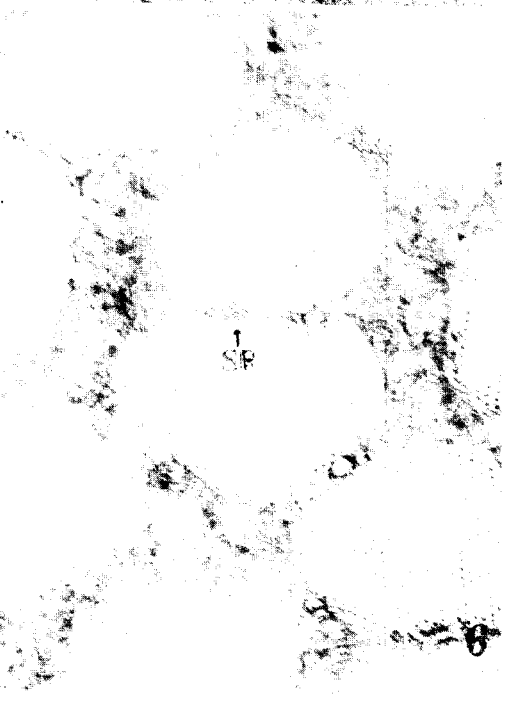
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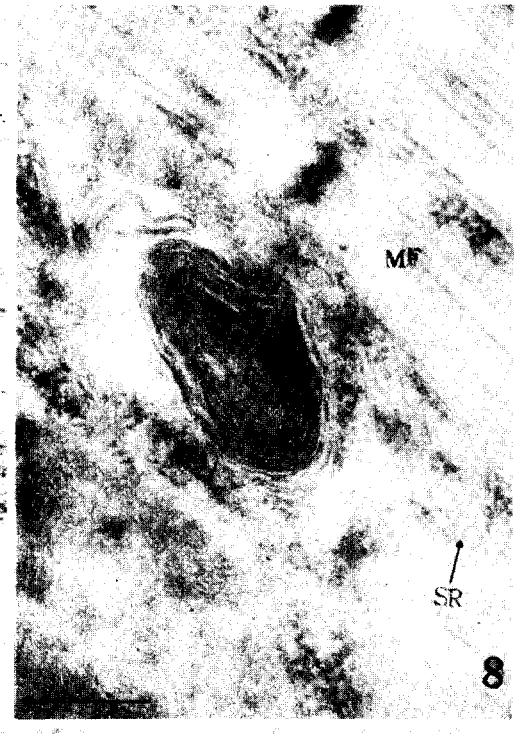
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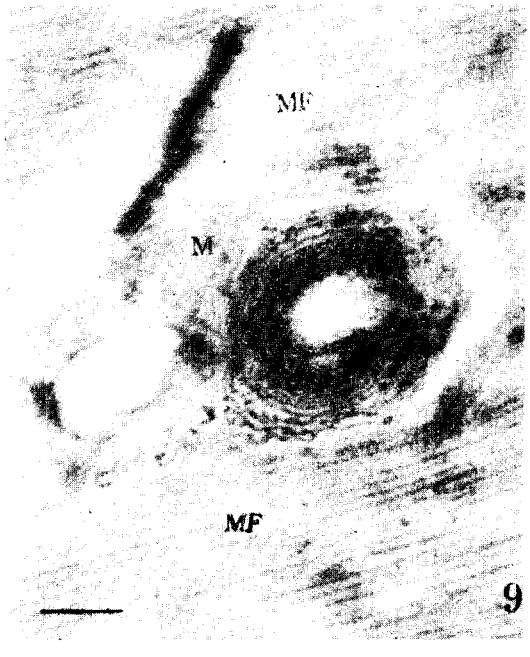
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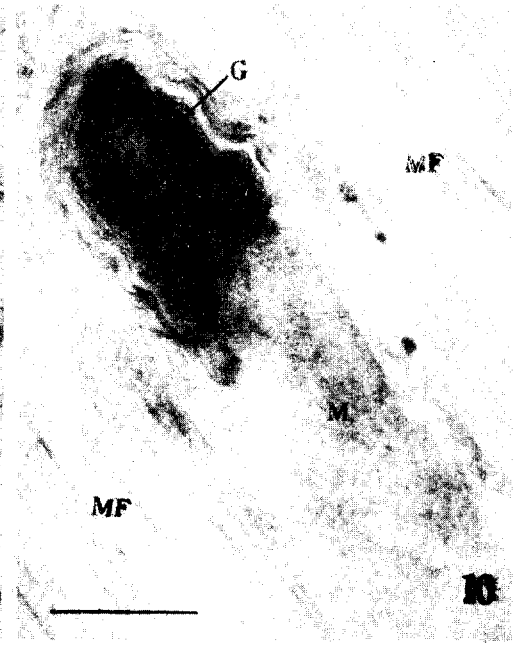
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