J.K.A.U.: Sci., vol. 3, pp. 113-153 (1411 A.H. / 1991 A.D.)

Mitochondrial and Myofibrellar Development in the Flight Muscle of *Locusta migratoria* L. (Orthoptera: Acrididae)

ALI A. AL-ROBAI
Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT. The fine structure of the median indirect dorsal longitudinal flight muscles have been examined throughout the first week of *Locusta migratoria* adult life. During this period, muscle colour changed from white to reddish-brown and the banding pattern, characteristic of mature adult flight muscles, was established. Associated with these changes, there was an increase in myofibril size and the mean number of myosin filaments per myofibril; no significant change was observed in the actin: myosin ratio. There were indications that the number of myofibrils per muscle fibre increased by "longitudinal splitting" of the myofibrils existing in the first four days of adult life. A marked increased in mitochondrial size and complexity was noted with increasing age. The increase in mitochondrial size is probably attributed, partly, to fusion of adjacent mitochondria which become gradually arranged in straight columns between the myofibrils by the 5*th* day of adult life. The developmental changes observed in the fine structure of myofibrils and mitochondria are important in explaining the improvement in flight performance noted in the developing adult locusts.

Introduction

Muscle development has received a considerable interest in the last 25 years. Goldspink[1] suggested that one reason for this is that the muscle affords a good example for tissue differentiation and growth. This is because they are, apparently, programmed to produce considerable quantities of specialized proteins in order to carry out its contractile function. Pringle[2] reported that the form of the arthropod body and, in particular, the structure of the locomotor appendages is quite different

from that of a vertebrate, although such muscles appear at the first sight to be similar both in structure and physiology[3]. Flight muscles, for example are analogous to the muscles of higher vertebrates and also man[4,5]. Many researchers, therefore, have considered the insect flight muscle as a suitable object for the study of developmental changes; such as the cases of *Drosophila melanogaster*[6,7], *Hyalophora cecropia*[8], *Apis mellifera*[9], *Lucilia cuprina*[10], Leptinotarsa decemlineata[11], *Homorocoryphus nitidulus*[12], *Schistocerca gregaria*[13], *Attagenus megatoma*[14], *Calliphora erythrocephala*[15,16], *Musca domestica*[17], and *Manduca sexta*[18]. The general conclusion is that, as development proceeds, there is a marked increase in the size of the myofibrils and mitochondria, and that the density of the mitochondrial cristae increases.

The major development of *Locusta migratoria* flight muscle takes place during the *5th* instar and the early part of adult life[19]. The precursors of the flight muscles are formed early in development, probably during embryogenesis[20]. Similarly, Tiegs[21] has shown that, in *Chortoictes terminifera,* flight muscle was present as an undeveloped muscle in the *1st* instar larva. In *Schistocerca gregaria,* the future adult pterothoracic musculature is already present at the eclosion from the egg[22]. In *Locusta,* Hill and Goldsworthy[23] showed that the weight of the flight muscle increased more than 2-fold during the *4th* larval istar and about 16-fold in the *5th* instar. On the basis of weight, Brosemer *et al.*[24] and Bucher[19] have described four phases in the growth of *Locusta* flight muscle in the final larval instar and the *1st* week of adult life: "Larval growth", "the moulting interval", "the phase of differentiation" and "the phase of duplication". The latter two phases occur in the adult insect during which the dry weight of the flight muscle increased by some 300%[25].

The present study has been carried out to determine the developmental changes in the fine structure of mitochondria and myofibrils of *Locusta migratoria* which occur during the early few days of adult life.

Material and Methods

Stock Animals

A population of *Locusta migratoria,* R and F phase gregaria was supplied by Philip Harris Biological Ltd and was maintained at a temperature of 30 ± 0.5°C and 60 ± 5% relative humidity (Durhan University, England).

A population was prepared for sampling by removing all *5th* instar insects at 9 a.m. It was examined at 24-hour intervals (9 a.m. each day) and all *5th* instar insects were removed. Thus, the time of ecdysis must lie between the time the insect was removed and the previous inspection. The midpoint at this period was taken as the time of ecdysis and the insect aged as 12 hours ± 12 hours (1st day of *5th* instar) at this time. When adult locusts were required, the procedure was the same except that all adult locusts were initially removed from the population.

Electron Microscopy

Adult male locusts, aged 1-6 days, were killed by twisting their heads 90° in one direction and then 90° in the opposite direction. This broke the "neck" membrane but allowed the gut to remain attached to the head. The posterior tip of the abdomen was then severed and the gut drawn out (still attached to the head). The thorax was then open ventrally and pinned out on a cork board, prior to the application of ice-cold (0-4°C) fixative, to reduce muscle contraction during fixation. The musculature was covered with 2.5% glutaraldehyde fixative in 0.1 M sodium cacodylate buffer (pH 7.3) and contain 0.32 M sucrose. After 15 minutes, the median dorsal longitudinal indirect flight muscles of both meso- and meta-thoraces were dissected out and placed in a fresh cold fixative at 0-4°C overnight. The tissue was then washed in several changes of cold 0.1 M sodium cacodylate buffer (pH 7.3) for total of 6-8 hours followed by post-fixation for 2 hours in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.3) at room temperature (Ca. 20°C). Following dehydration through a graded series of ethanol solution, the muscles were passed through propylene oxide and embedded in Epon 812 epoxy resin. Sections were cut on a Reichert NK ultratome, mounted on uncoated copper grids and stained in uranyl acetate followed by lead citrate[26]. They were then examined in an AEI 801 electron microscope.

Estimation of Mitochondrial Size

Two different methods were used to measure mitochondrial size :

(i) Mitochondrial size was determined by measuring their maximum and minimum diameters as seen in electron micrographs of transverse sections through flight muscles. Similar methods have been used in measuring mitochondrial size in *Calliphora erythrocephala* flight muscles[16] and *Clopodes ethlius* fat body[27]. Only well-fixed mitochondria, with distinct double membranes and cristae, were measured. Mitochondrial size was expressed as the mean profile diameter [*i.e.* (maximum + minimum diameter)/2].

(ii) Mitochondrial size was estimated by tracing mitochondrial profiles from electron micrographs of transverse sections through flight muscle onto standard paper. The tracings were then cut out and weighed; a similar method to that used by Forbes *et al.*[28]. The results were expressed in arbitrary units. This method was also applied to sections through mitochondrial pellets.

Estimation of Myofibril Size

Myofibril size was estimated using essentially the same method described in (ii) above.

Tracings from electron micrographs of transverse sections through flight muscles were cut out, weighed and the myofibril size expressed in arbitrary units.

Sarcomere lengths were determined from measurements made on electron micrographs of longitudinal sections through flight muscles.

Relative Composition of Flight Muscle

The relative volumes of the various organelles (excluding nuclei) present in *Locusta* flight muscle (viz. myofibrils, mitochondria and sarcoplasmic reticulum plus the T-system) and the tracheoles present within the muscle fibres were estimated using essentially the same method as (ii) above. The results were expressed in terms of the percentage of total muscle volume.

Results

No difference in either the histological or the fine structural appearance of the muscle of the meso- or meta-thoraces. Similar results have been observed in the flight muscle of *Homorocoryphus nitidulus*[12]. Consequently, the results presented will not distinguish between dorsal longitudinal flight muscles from those two divisions of the thorax.

The flight muscles of newly emerged adult *Locusta* are relatively small and characteristically "white" in appearance. As growth and development proceed, the colour changes gradually to reddish-brown. Associated with this change in colour, there was an increase in the mass of the flight musculature.

Examination of light micrographs (Fig. 1) of transverse sections through the median dorsal longitudinal indirect flight muscles of *Locusta* shows that the shape and size of each individual fibre profile vary considerably even within the same bundle of fibres. Tracheae are clearly seen running between the fibres. In high power light micrographs (Fig. 2), whilst it appears that the majority of myofibrils are less regular, some seem to be arranged in a more-or-less radial fashion. The peripheral position of the nuclei and their presence under the cell membrane is clearly seen in Fig. 1 and 2. This represents the normal position of these organelles in the vast majority of muscle cells examined. However, occasionally one nucleus was encountered being positioned within the muscle fibre (Fig. 3). In the early stages of muscle development, nuclei are situated in the middle of the myoblast, and as growth and development proceed, the increase in muscle cell components tends to push the nuclei to a peripheral position[1]. The above observation would seem to suggest, therefore, that all muscle differentiation is not complete at this stage (3-day old adult).

Myofibrils

The myofibrils and mitochondria represent the bulk of the muscle fibre with the nuclei situated in a peripheral position. The arrangement of the myofibrils is irregular (Fig. 4) and, as described, as closed-packed[19]. In longitudinal sections of muscle fibres from 6-day old adult animals (Fig. 5) the myofibrils are usually in almost perfect parallel register, as witnessed by the relatively straight rows of Z-bands, which divide the myofibrils into sarcomeres. These sarcomeres exhibit the band pattern typical of striated muscles. Distinctive I-bands can be seen on either side of the Z-band: the majority of the sarcomere length being represented by the A-band (Fig. 5).

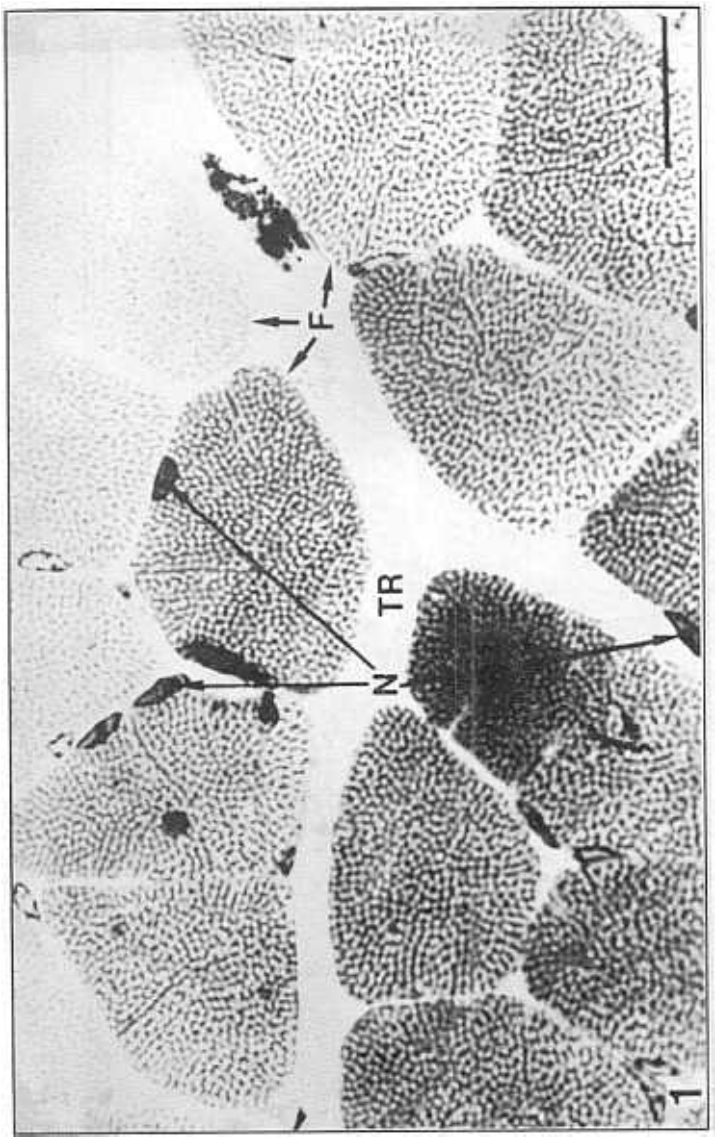


FIG. 1. —A photomicrograph of transverse section through a part of the median dorsal longitudinal indirect flight muscle of a 6-day old locust. Note the peripheral position of the nuclei (N). The shape and size of the muscle fibres (F). Invagination of the surface cell membrane can be seen (arrowheads). TR : trachea. Scale 25 μm.

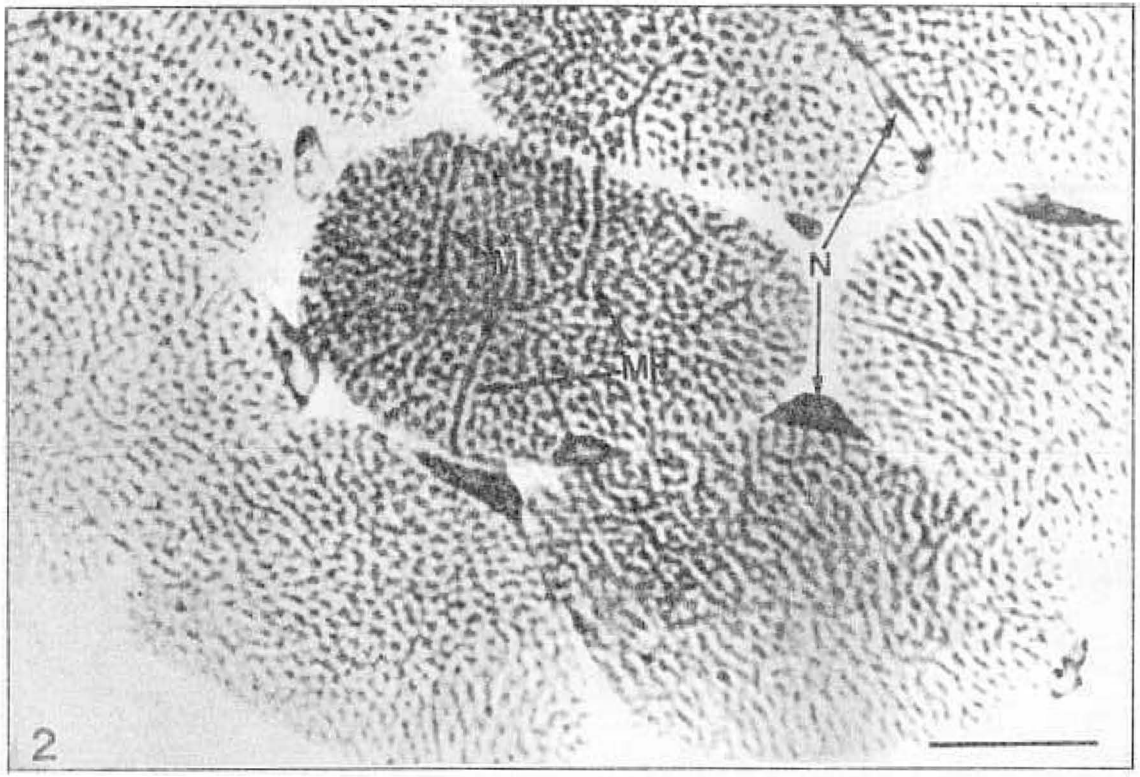


FIG. 2. A high power photomicrograph of a transverse section through a part of the median dorsal longitudinal flight muscle from an animal aged 6 days. Note that myofibrils (MF) are arranged in a more-or-less radial fashion. M : mitochondria; N : nucleus. Scale 20 μm.

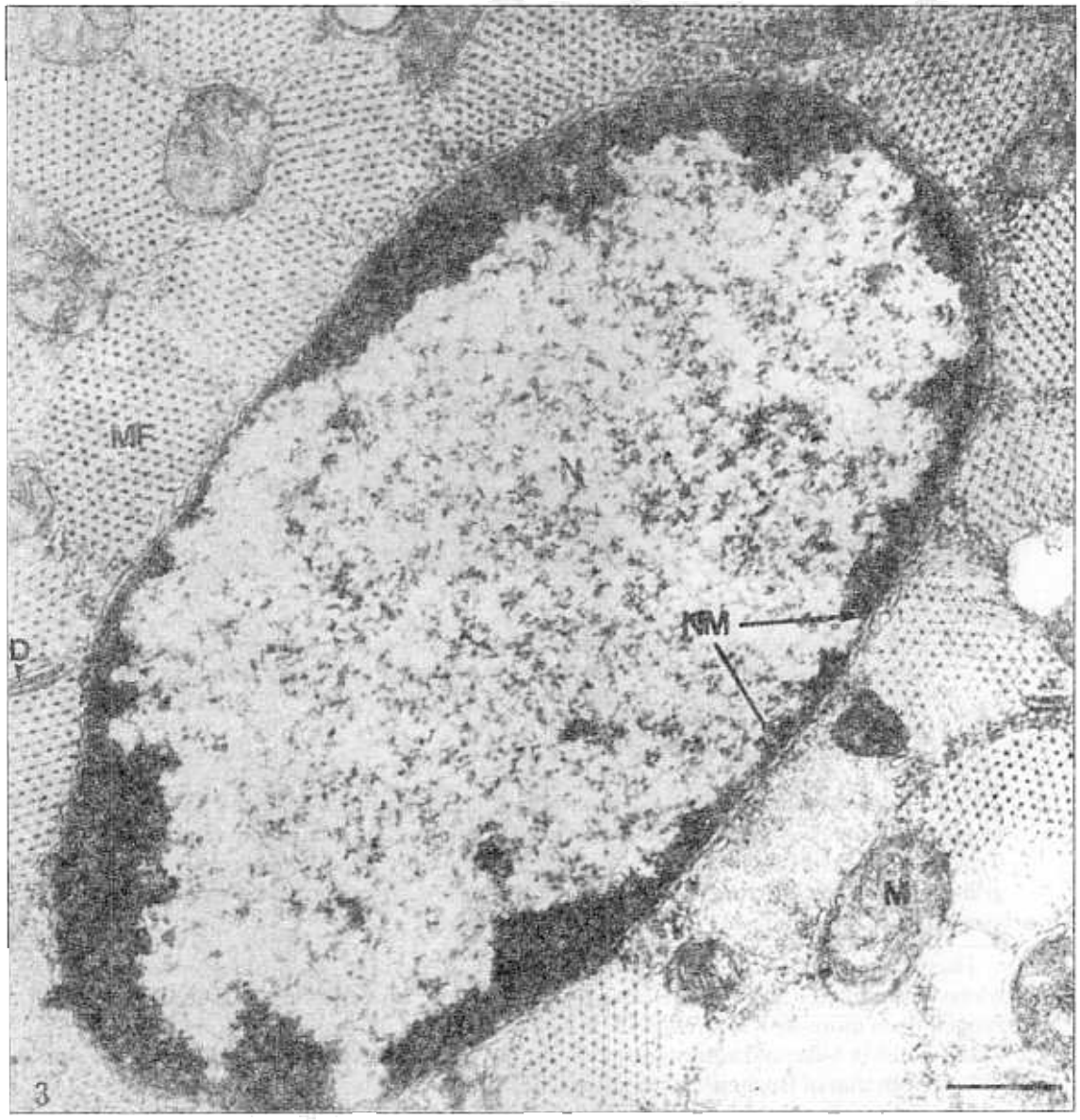


FIG. 3. An electron micrograph of a transverse section through a portion of a muscle fibre from an animal aged 3 days. Note the position of the nucleus (N) in the middle of the muscle fibre. M : mitochondrion; MF : myofibril; D : dyad; NM : nuclear membrane. Scale 0.5 μm.

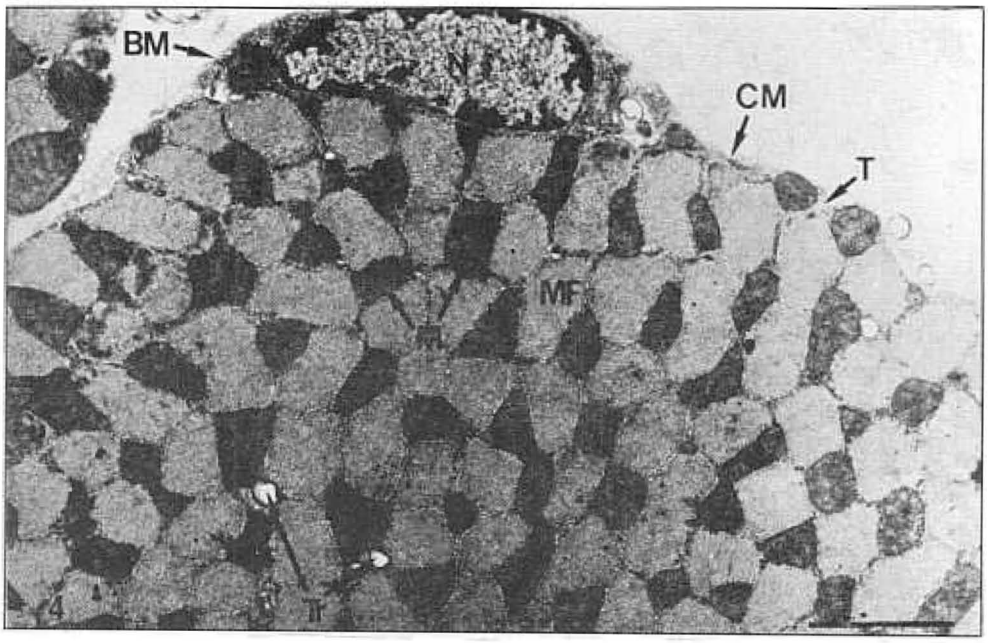


FIG. 4. A transverse section through a muscle fibre from a 6-day old locust. Note the variation in size and shape of the myofibrils (MF) and mitochondria (M). A layer of amorphous material forms the basement membrane (BM) of the muscle fibre and one nucleus (N) is seen lying immediately beneath the cell membrane (CM). Invagination of the cell membrane can be seen to give rise to the T-system (T). Tr : tracheole, Scale 2.5 μm.

There is a light H-band in the A-band, within which one can see a localized scattering of small granules. Similar granules have been observed in the flight muscles of *Tenebrio molitor*[29], *Aeshna* sp.[30] and *Homorocoryphus nitidulus*[12]. These granules are thought to represent the M-line, which is normally absent from insect synchronous flight muscle.

There was considerable variation in the shape of the myofibrillar profiles as seen in transverse sections. This was so for all ages studied (cf. Figs. 6, 7 and 8). The shapes ranged from more-or-less circular to oblong in the first 4 days following the final ecdysis, whilst in 6-day old adults most myofibrils were polygonal (Fig. 4). This finding differs from that of Bucher[19], who reported that the variation in myofibrillar profile shape disappears after the final ecdysis. By the 3*rd* day of adult life, there was a tendency for some of the peripheral myofibrils to be somewhat larger in size than the more centrally placed ones (Fig. 7).

Examination of Fig. 9, 10, 11 and 13, clearly indicates that "longitudinal splitting" took place in the flight muscle of 1-4 days old adult *Locusta*. Furthermore, this pro-

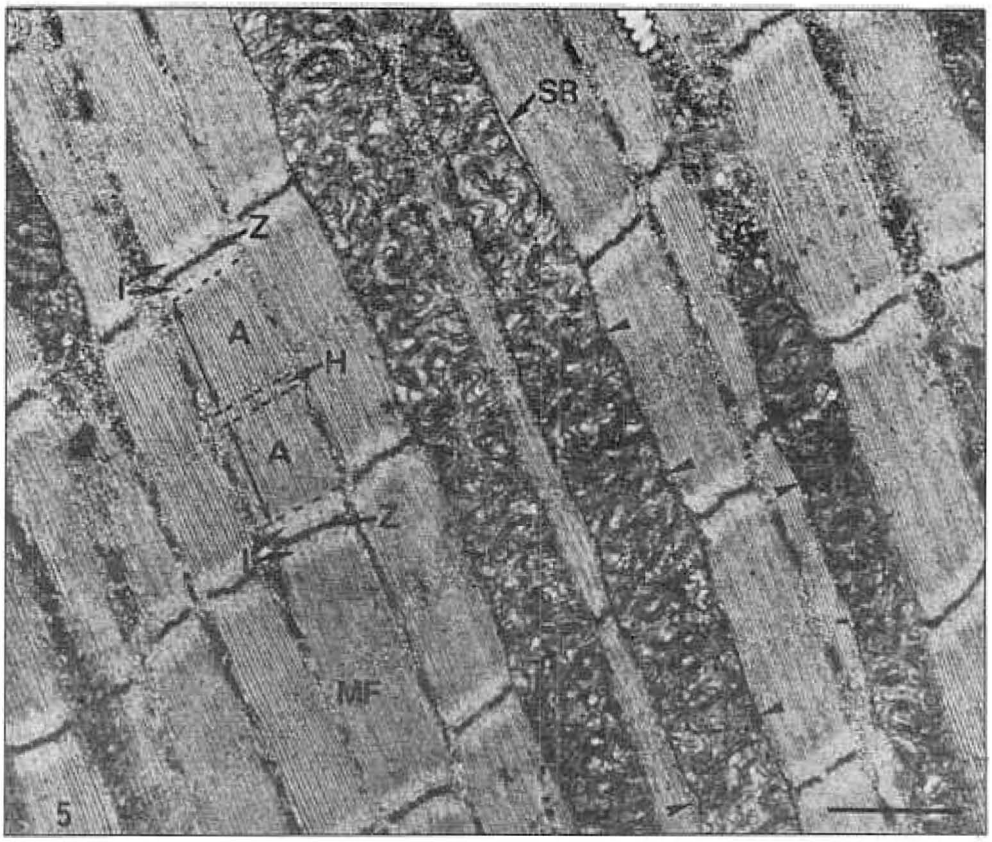


FIG. 5. A longitudinal section through the muscle fibre from a 6-day old locust. The sarcomere is delimited by Z-bands. The I- and A-bands are clearly defined at this stage. Dyads (arrow-heads) can be seen normally in identation of adjacent mitochondria midway between the H-band, which is characterized by a localized scattering of small granules at the centre of the A-band, and the I-band. Note also that individual mitochondria extend more than 4 sarcomere lengths. M : mitochondrion; MF : myofibril; SR : sarcoplasmic reticulum; G : glycogen. Scale 1.5 μm.

cess appears to be more or less complete by the 4*th* day of adult life. However, "myofibrillar splitting" was occasionally observed in 4-day old locusts where it appeared to be restricted to the more peripheral myofibrils. This is shown in Fig. 13 where a dyadic junction is present in the middle of a myofibril.

The typical hexagonal arrangement, with 6 actin filaments around each myosin, is clearly seen in transverse sections of 5 and 6-day old locusts (Fig. 12). Each thin filament is equidistant between adjacent pairs of thick filaments. A similar arrangement has been described elsewhere in insect flight muscle, for example, in *Periplaneta*

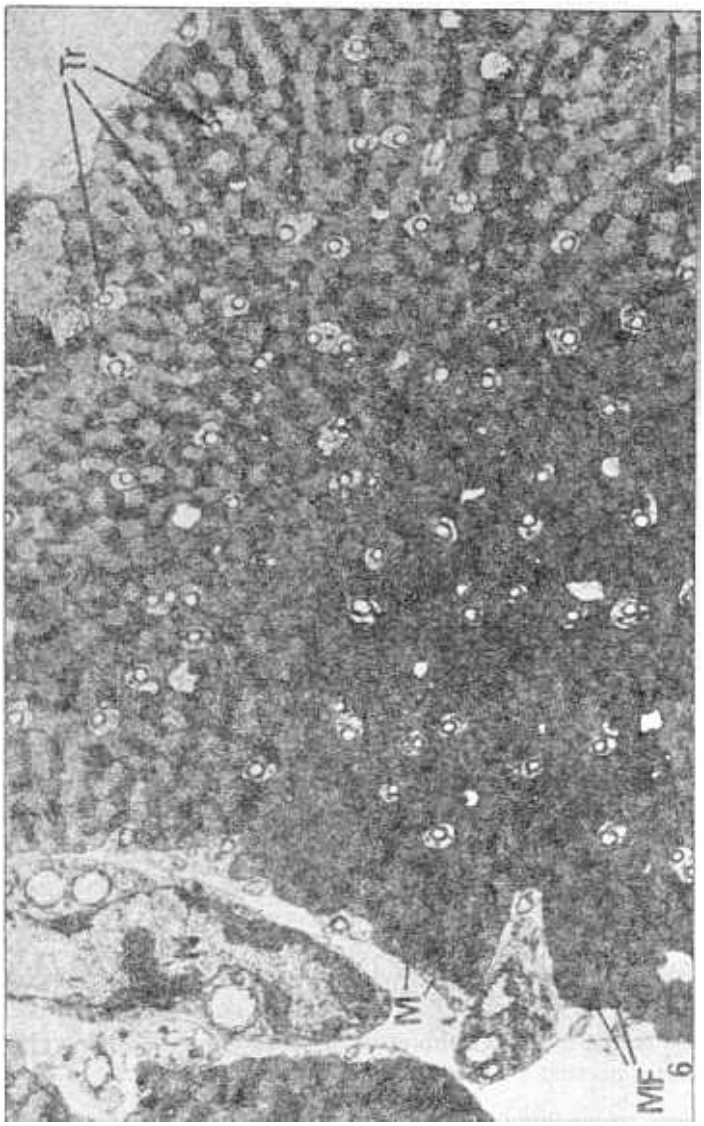


FIG 6. Low power electron micrograph of a transverse section through a muscle fibre of one day old adult locust showing the typical appearance of flight muscle at this age. Note the presence of numerous tracheoles (Tr) and that the cytoplasm of tracheoblast occupies a large area of the total muscle volume. Numerous small mitochondria (M) are seen in close association. MF : myofibril; N : nucleus. Scale 4 μm.

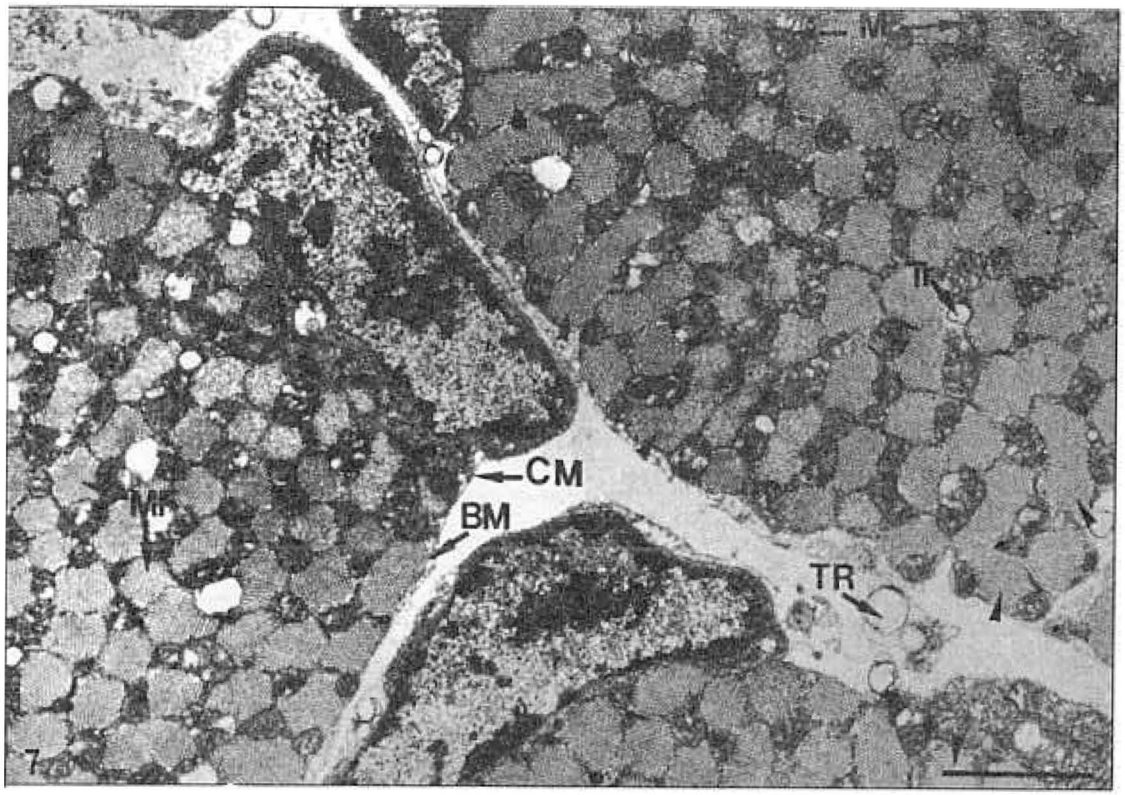


FIG. 7. An electron micrograph of a transverse section through a muscle fibre from a 3-day old adult locust. The peripheral region of three fibres is shown. Note the variation in size and shape between the peripheral (arrow-heads) and more centrally placed myofibrils (MF). The latter tend to be smaller in size and more circular in outline. Mitochondrial shape ranges from oval to circular. N : nucleus; M : mitochondrion; Tr : tracheole; CM : cell membrane; BM : basement membrane; TR : trachea. Scale 2.5 μ.

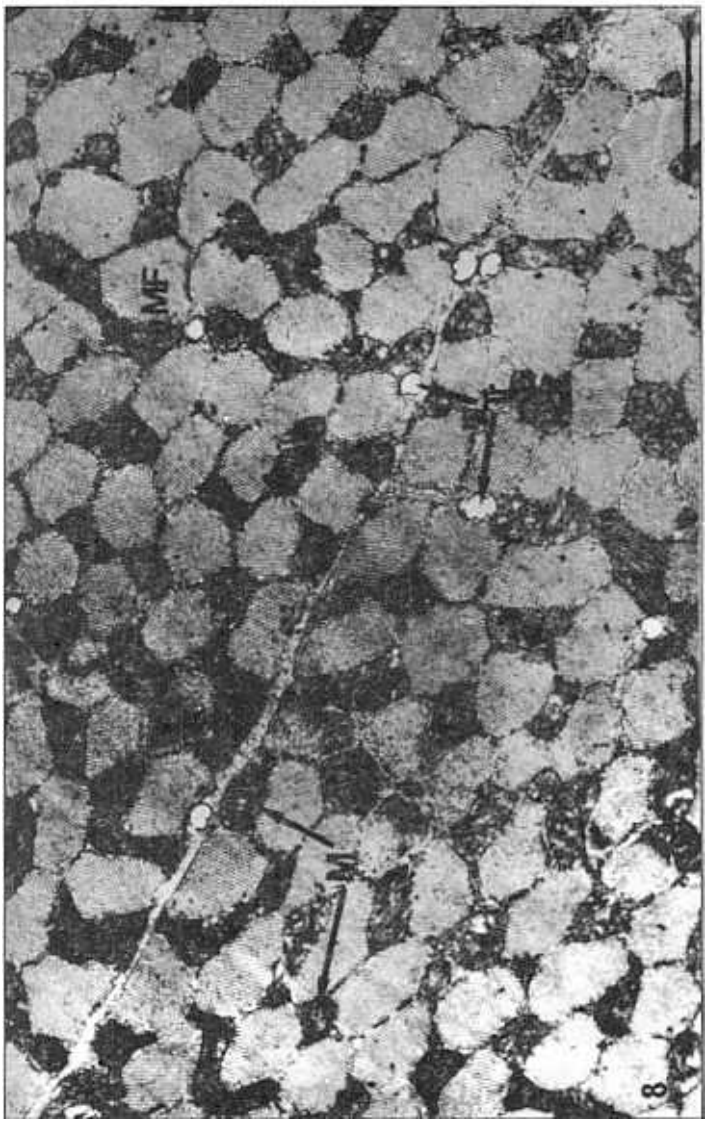


FIG. 8. An electron micrograph of the peripheral region of two muscle fibres from a 5-day old adult locust. Note the enormous increase in size and change in shape of the mitochondria (M) and myofibrils (MF). The tracheoles (Tr) occupy a small area of the total muscle volume. Scale 2.5 μm.

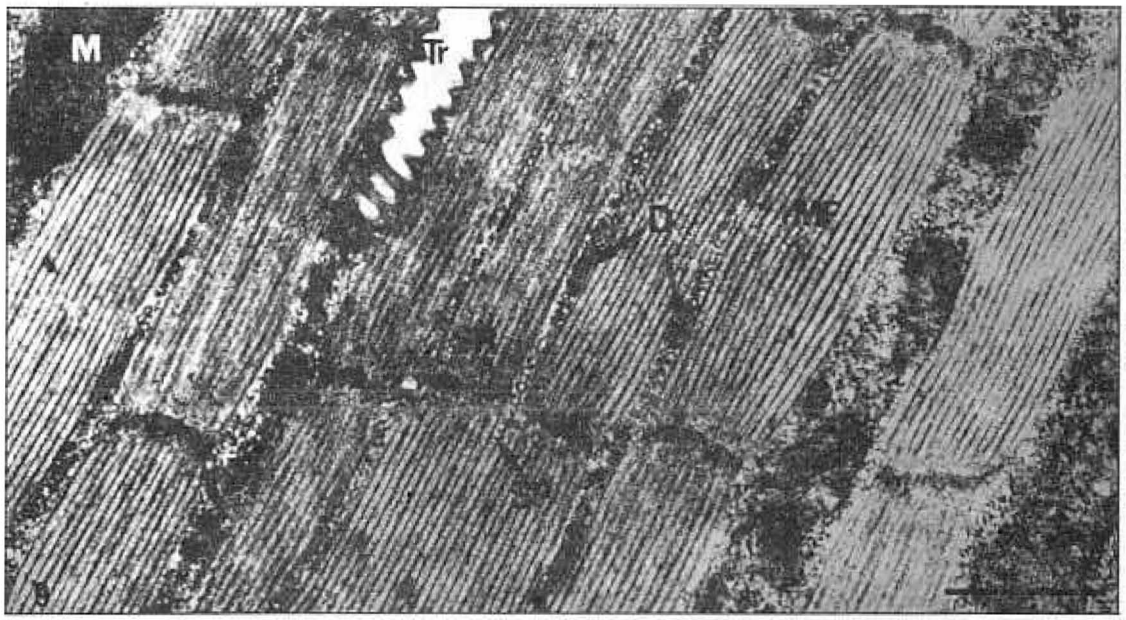


FIG. 9. A longitudinal section through a muscle fibre of a 3-day old adult locust. Note the indication of splitting (SP) of a single myofibril (MF). The normal banding pattern is clearly seen. SR : sarcoplasmic reticulum; M : mitochondria; D : dyed; Tr : tracheole; Z : Z-band. Scale 1 μm.

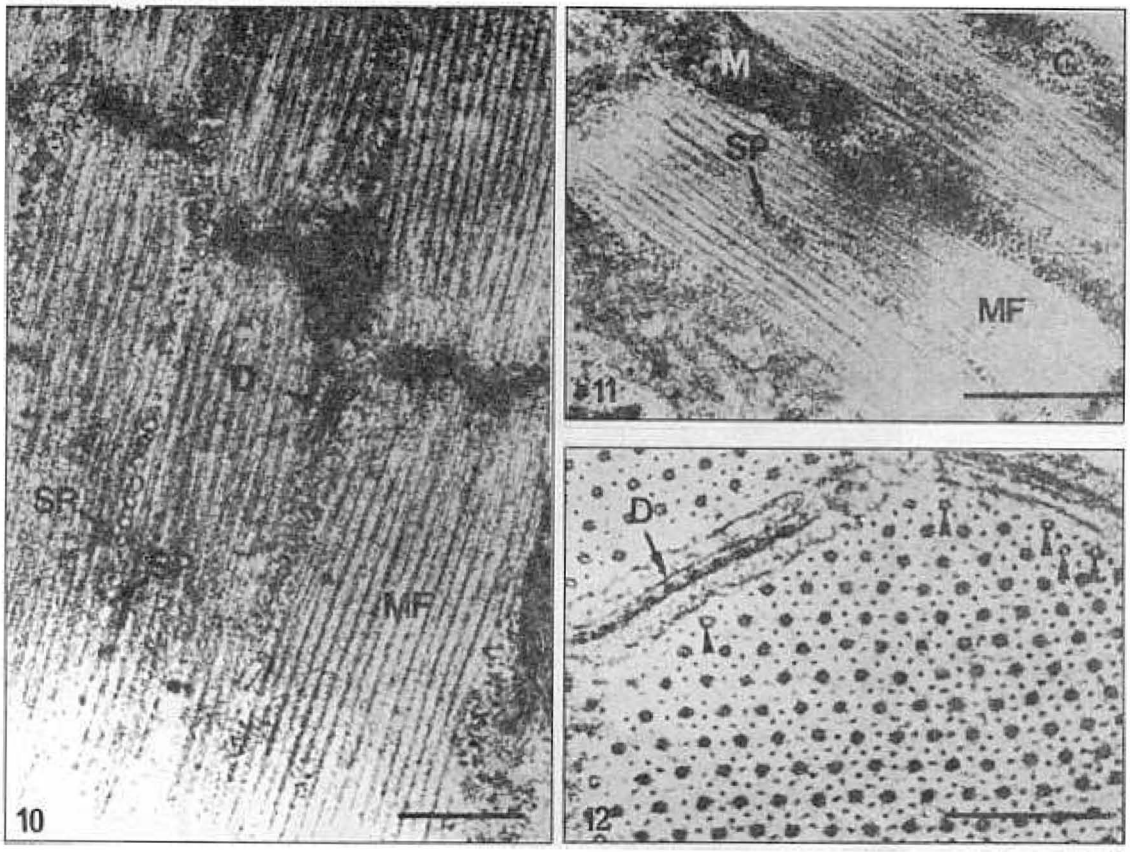


FIG. 10. A longitudinal section through a muscle fibre of a 1-day old adult locust. Myofibrillar splitting (SP) as indicated in figure 9 is apparent. Note the position occupied by the dyad (D) and its oblique arrangement in relation to the myofibril (MF); SR : sarcoplasmic reticulum. Scale 0.5 μm.

FIG. 11. A longitudinal section through a muscle fibre of a 1-day old adult locust. Note once again that myofibrillar splitting (SP) is taking place in single myofibril. M : mitochondrion; MF : myofibril; G : glycogen. Scale 0.8μm.

FIG. 12. A transverse section through a myofibril of a 5-day old adult locust at the level of the A-band. Note the presence of actin (thin) and myosin (thick) filaments. The peripheral myosin filaments appear to have a less dense core (arrow-heads). D : dyad. Scale 0.25μm.

americana[31] and *Neoconocephalus*[32]. However, occasionally, 6-9 actin filaments were observed around each myosin filament. Examination of Fig. 14, 15, 16 and 17 indicates that following the increase in the number of myofibrils, there is an increase in the number of actin and myosin filaments per myofibril as the later increase in size.

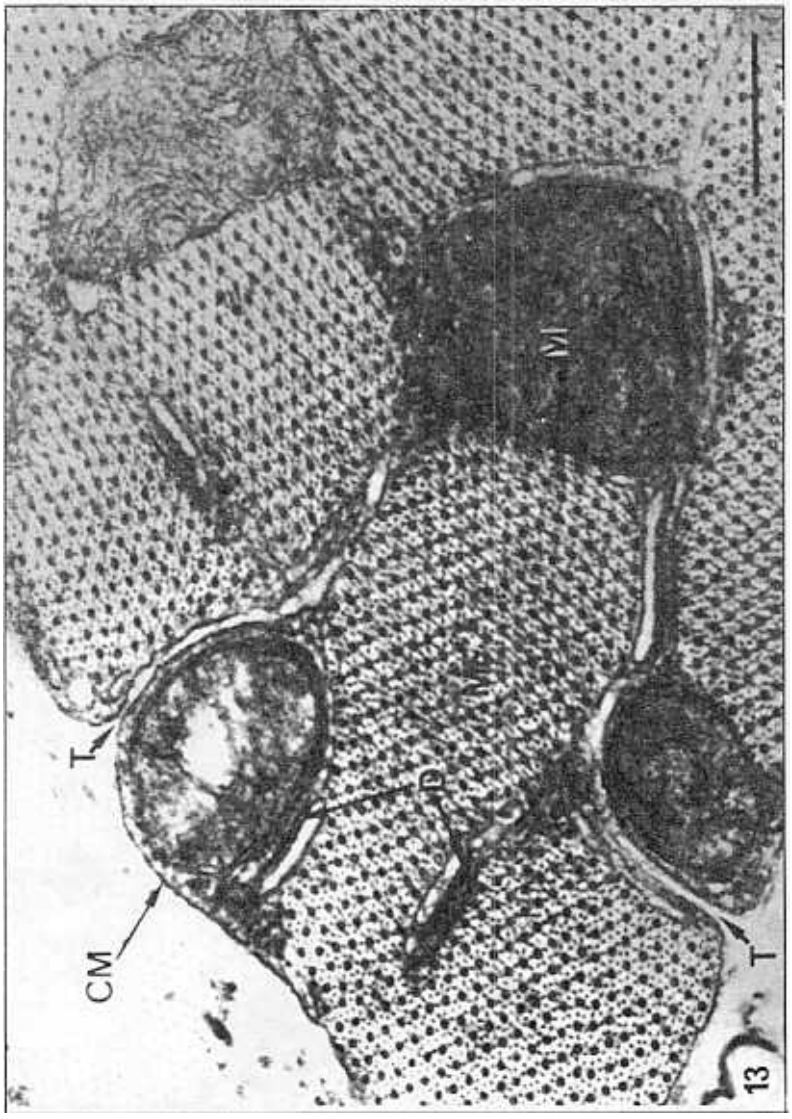
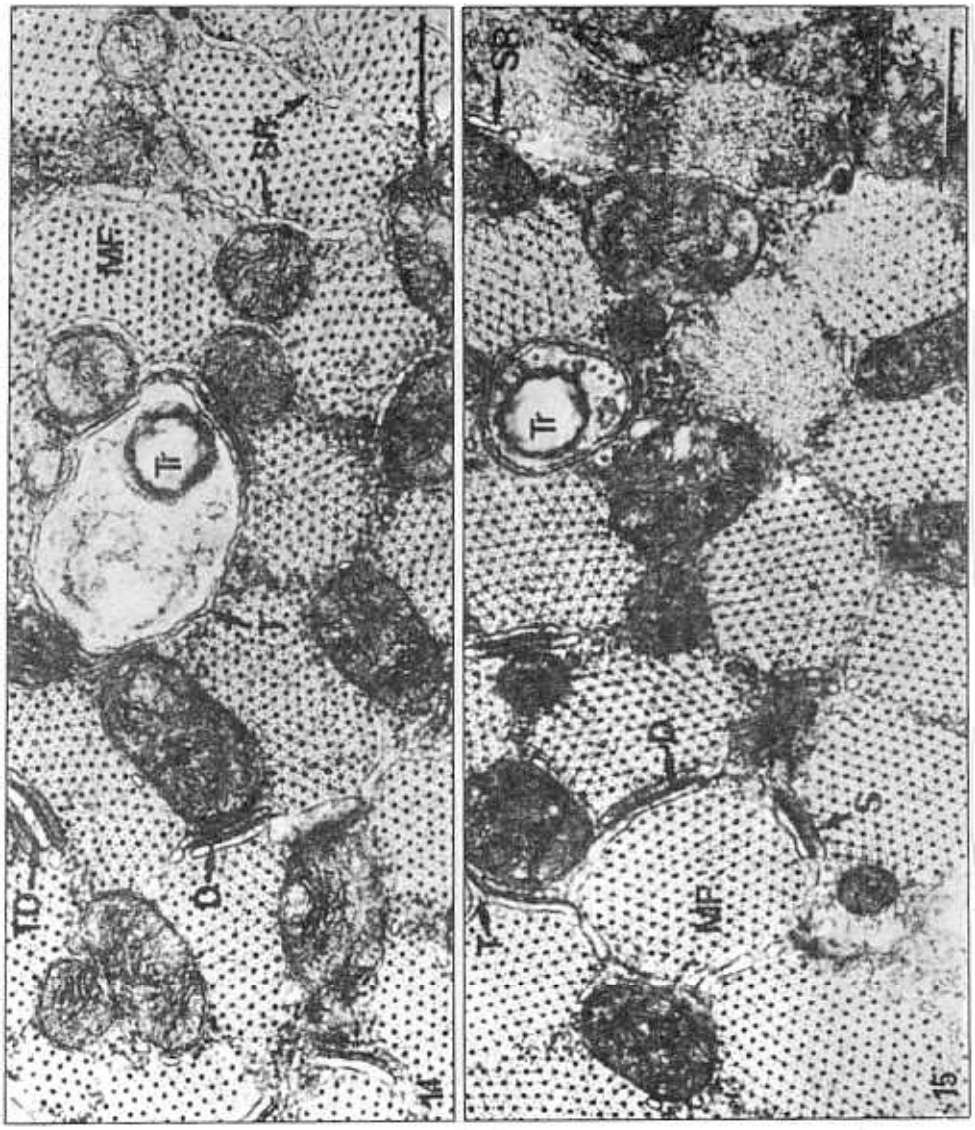


FIG. 13. A transverse section through a muscle fibre of a 4-day old adult locust. Note the presence of dyadic junctions in the middle of two peripheral myofibrils. MF : myofibril; M : mitochondrion; D : dyad; CM : cell membrane; T : T-system. Scale 0.4 μm.


MF
BF
Tf
TD
D
14
Sc
Tf
D
MP
S
15

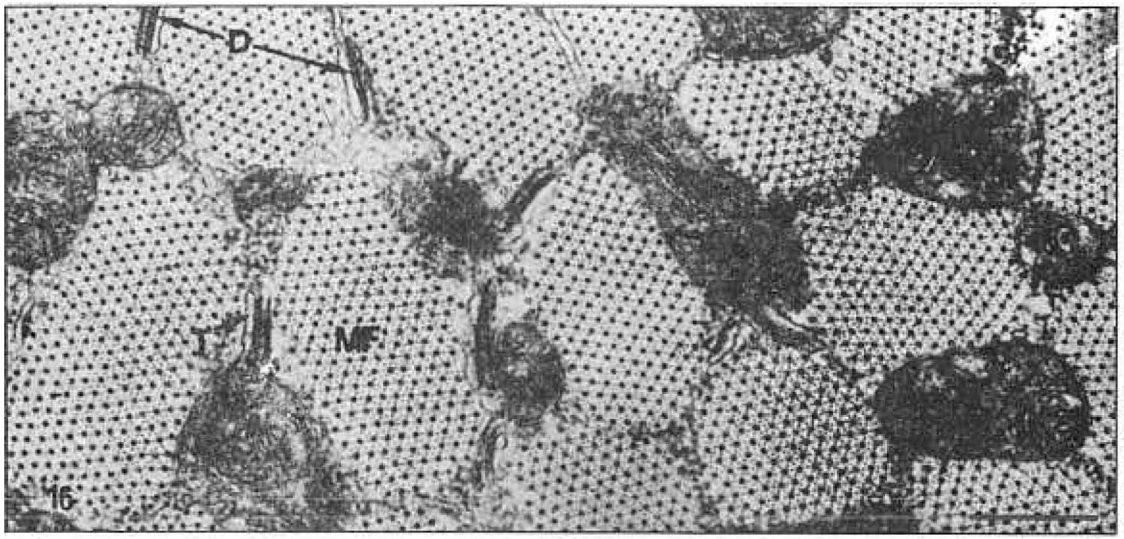


FIG. 14. A transverse section through a muscle fibre from a 1-day old locust showing its typical appearance. Mitochondria (M); myofibril (MF), dyad (D), triad (TD) and sarcoplasmic reticulum (SR). Note the size and shape of the mitochondria and the presence of relatively few cristae. Tracheolar cell (Tr) situated in a dilation of the T-system (T). Scale 0.5 μm.

FIG. 15. A transverse section through a muscle fibre from an animal aged 2 days illustrating the typical appearance, size and shape of the muscle components. It can be seen that the mitochondria (M) still contain relatively few cristae. Tr : tracheole; SR : sarcoplasmic reticulum; S : non-fenestrated sarcoplasmic reticulum; T : T-system; MF : myofibril; M : mitochondria. Scale 0.5 μm.

FIG. 16. A transverse section through a muscle fibre from an animal aged 3 days showing the typical appearance, size and shape of the muscle components at this age. Note the increase in myofibril (MF) size, myosin number and the variation in mitochondrial (M) size. D : dyad, SR : sarcoplasmic reticulum; T : T-system. Scale 0.5μm.

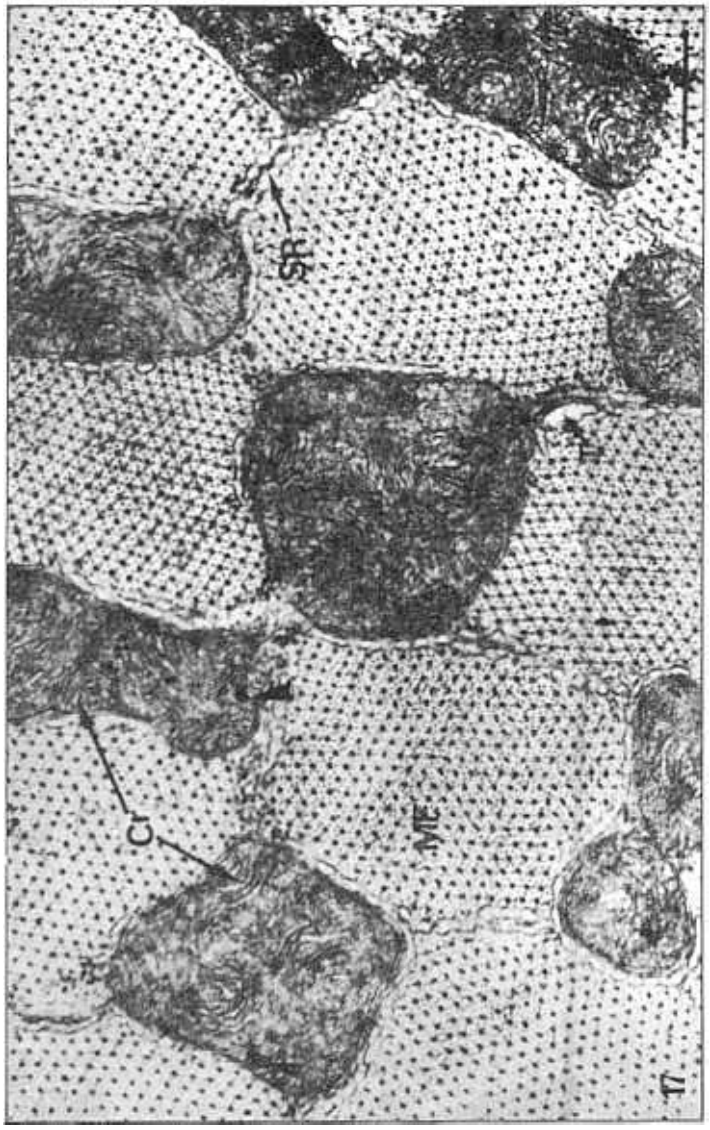


FIG. 17. A transverse section through a muscle fibre from a 5-day old adult locust. The myofibrils (MF) and mitochondria (M) show a further increase in size. Note the increase in mitochondrial size and the number of cristae (Cr). A number of electron dense granules (arrow-heads) are visible in the mitochondria. The sarcoplasmic reticulum (SR) is abundant and completely encircles each myofibril; in some cases, two sheets of SR separate the myofibrils. D : dyad; T : T-system. Scale 0.5μm.

This observation is confirmed by counting the number of myosin filaments per myofibril, the actin to myosin ratio and by measuring myofibrillar profile size at different ages (Fig. 18). There was a 4-fold increase in myofibrillar size and the number of myosin filaments per myofibril over the first 6 days of adult life. This indicates a close relationship betwen the number of myosin filaments per myofibril and myofibrillar size. The proportion of total muscle volume occupied by the myofibrils increased with age (Table 1); being 52.26 ± 1.5% and 59.66 ± 2.31% ($P > 0.001$) in 1-day old and 6-day old adult, respectively.

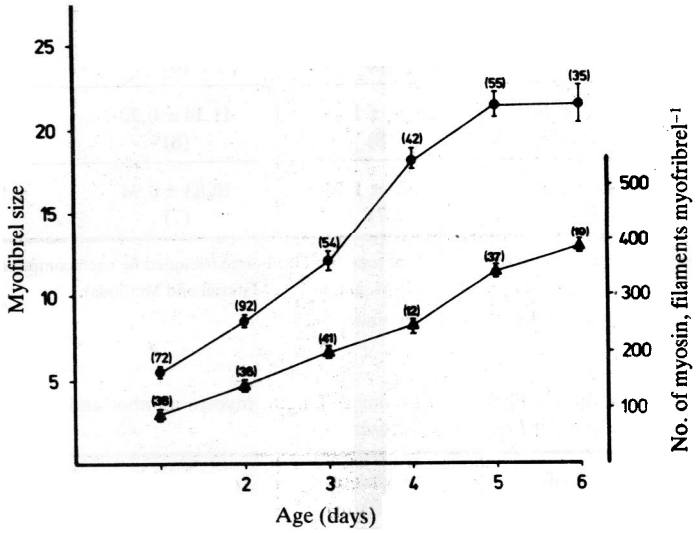


FIG. 18. Effect of age on the myofibrillar size and the number of myosin filaments per myofibril (data taken from Table 2).
• myofibril size,
Δ myosin number
The figures in parentheses indicate the number of myofibrils measured or the number of myofibrils from which the myosin filaments were counted.

In longitudinal sections of muscle from one day adult *Locusta* (Fig. 19) the cross-striations are poorly defined and the Z-bands are irregularly arranged. Similar observations have been noted in larval flight muscle of *Manduca sexta*[18]. In *Locusta*, adjacent to the Z-band is a lighter region which represents the early appearance of the I-band (Fig. 19). During this stage, the H-band is difficult to recognize. Similar observations have been reported in *Leptinotarsa decemlineata*[11]. Gradually, these fetaures undergo developmental changes and the typical structural pattern of *Locusta* flight muscle is established by day 6 of adult life (cf. Fig. 19, 20, 9 and 5). Over the period studied there was a significant increase in the average sarcomere length (see Table 2).

TABLE 1. The effect of age on the relative proportion of the flight muscle components of *Locusta migratoria*.

Age in days	Myofibril	Mitochondria	Sarcoplasmic reticulum	Tracheoles
1	52.26 ± 1.51* (5)	24.66 ± 1.38 (5)	15.98 ± 0.93 (5)	7.15 ± 1.43 (5)
2	59.54 ± 2.13 (6)	22.87 ± 2.25 (6)	13.71 ± 1.22 (6)	3.50 ± 1.01 (6)
3	64.76 ± 1.66 (6)	22.04 ± 1.76 (6)	12.36 ± 0.78 (6)	1.53 ± 0.04 (6)
5	61.55 ± 1.43 (8)	26.40 ± 1.46 (8)	11.14 ± 0.70 (8)	1.46 ± 0.92 (8)
6	59.99 ± 2.31* (7)	28.52 ± 1.70 (7)	10.83 ± 0.94 (7)	0.96 ± 0.61 (7)

The data represent the mean percentage ± S.E.M. of total muscle volume occupied by each component and were obtained by weighing component profiles traced from E.M. graphs (see Material and Methods).

The figures in parentheses indicate the number of determinations.

*$p > 0.001$

TABLE 2. Effect of age on myofibril size, sarcomere length, myosin number and actin/myosin ratio of flight muscle of adult *Locusta migratoria*.

Age	Myofibril⊕ size	Sarcomere length (μm)	Myosin number per myofibril	Actin / myosin ratio
1	5.69 ± 0.20 (72)	2.84 ± 0.04 (82)	95 ± 3 (38)	3.28 ± 0.12* (7)
2	8.49 ± 0.23 (92)	2.99 ± 0.01 (51)	141 ± 4 (36)	–
3	11.20 ± 0.39 (54)	3.06 ± 0.04 (30)	200 ± 6 (41)	2.97 ± 0.13* (7)
4	18.29 ± 0.42 (42)	2.91 ± 0.22 (44)	248 ± 10 (12)	–
5	21.65 ± 0.65 (55)	3.18 ± 0.04 (69)	346 ± 9 (37)	–
6	21.54 ± 1.16 (35)	3.19 ± 0.02 (82)	391 ± 12 (19)	2.91 ± 0.14* (7)

⊕ Determined by tracing myofibrillar profiles from electron micrographs onto standard paper. These were then cutout, weighed and expressed in arbitrary units.

*Not significantly different.

The figures in parentheses indicate the number of determinations.

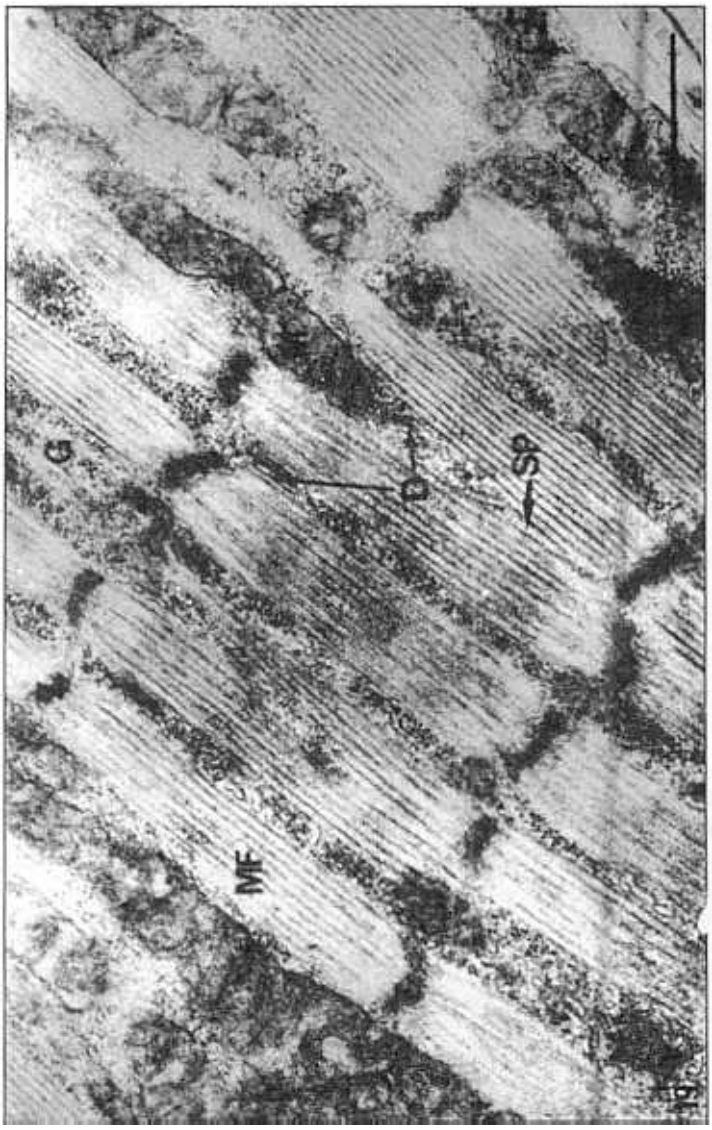


FIG. 19. A longitudinal section through the muscle fibre of a 1-day old adult locust. Note the poorly developed banding pattern of the sarcomere and the irregular Z-bands. Also noticeable is the lighter developing I-bands. The splitting (SP) of a single myofibril is also seen at this age. M : mitochondrion; MF : myofibril; D : dyad; G : glycogen. Scale 1 μm.

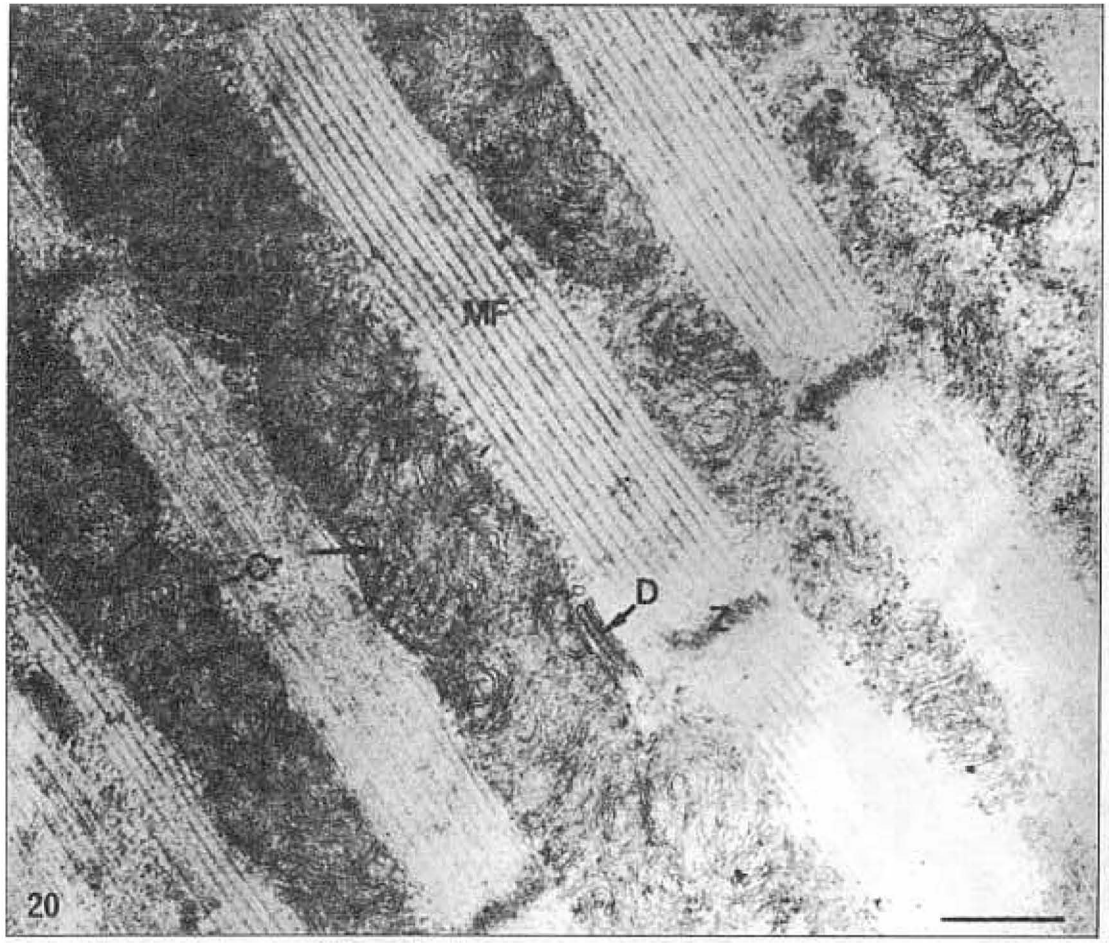


FIG. 20. A longitudinal section through the muscle fibre of a 2-day old adult locust. Note that the mitochondria (M) are more-or-less arranged in columns between the myofibrils (MF), the Z-bands are more regular and so are the I-bands. D : dyad; Cr : cristae; Scale 0.5 μm.

Mitochondria

Mitochondria are abundant in muscle fibres at all ages studied and are packed between the myofibrils (Fig. 14, 15, 16 and 17). The mitochondrial arrangement is clearly shown in longitudinal sections where they appear as columns of different sizes (Fig. 21 and 22). As reported for *Homorocoryphus*[12], there is no obvious alignment with the striation pattern of myofibrils. This is in contrast to the finding of Smith[33] who reported that in the fibrillar flight muscle of wasp, *Polistes* sp., the mitochondria are aligned with each half sarcomere. Michejda[8] reported that, in *Hyalophora cecropia* flight muscle, there is a single row of mitochondria situated between adjacent myofibrils in the proportion of three mitochondria per sarcomere.

The main changes observed in the mitochondria, associated with development, involved increased size, number and density of cristae. Transverse sections through flight muscles of 1-day old adult locusts are characterized by the presence of numerous small elliptical or circularly shaped mitochondria packed between the myofibrils (Fig. 6 and 14). They contain cristae and their matrices are relatively large (Fig. 23). In longitudinal sections (Fig. 24), the mitochondria are seen between the myofibrils and their non-overlapping arrangement in columns is incomplete. It can be also seen that, at this stage, there is more than one mitochondrion between the adjacent myofibrils. By the 3*rd* day of adult life, the mitochondria are arranged in columns with little or no indications of overlapping (Fig. 22). However, examination of transverse sections from this stage indicates that aggregated mitochondria occur in the peripheral regions of the fibres suggesting that the development of single mitochondrial column between myofibrils is not complete at this stage (Fig. 25). This phenomenon disappears as growth proceeds and by the 5*th* and 6*th* day of adult life, the mitochondria exhibit marked changes in their internal structure (Fig. 5). At this stage, single mitochondrial columns become so compressed between adjacent myofibrils that they appear polygonal in transverse sections (Fig. 4). In addition, the number of mitochondria per sarcomere length changed with age; up to three mitochondria per sarcomere length can be seen in 1-3-day old adult (Figs. 22 and 26), but by the 5*th* day of adult life single giant mitochondria are observed extending over the length of two or more sarcomeres (Fig. 27). This elongation is continued, such that in the 6-day old locusts, a single mitochondrion may extend the length of up to 5 sarcomeres (Fig. 5).

The results shown in Table 3 summarize the change in mitochondrial size with development. The mean mitochondrial diameter *in situ* showed little change during the first 4 days of adult life. However, by day 6, the mean diameter was approximately double that of newly ecdysed adult. A similar result was obtained when mitochondrial mean size was estimated by tracing their profiles onto standard paper, followed by their being cut out and weighed. The overall increase in size was even more apparent by this method. Again little change was noted over 1-3 days of adult life, but by the 4*th* day an increase in size was apparent whilst by day 6 there had been a 4.4 fold increase in mitochondrial size compared with newly ecdysed insect. Figure 28 shows the variation in mitochondrial size at various ages. The trend towards increasing mitochondrial size with age, is again clearly visible.

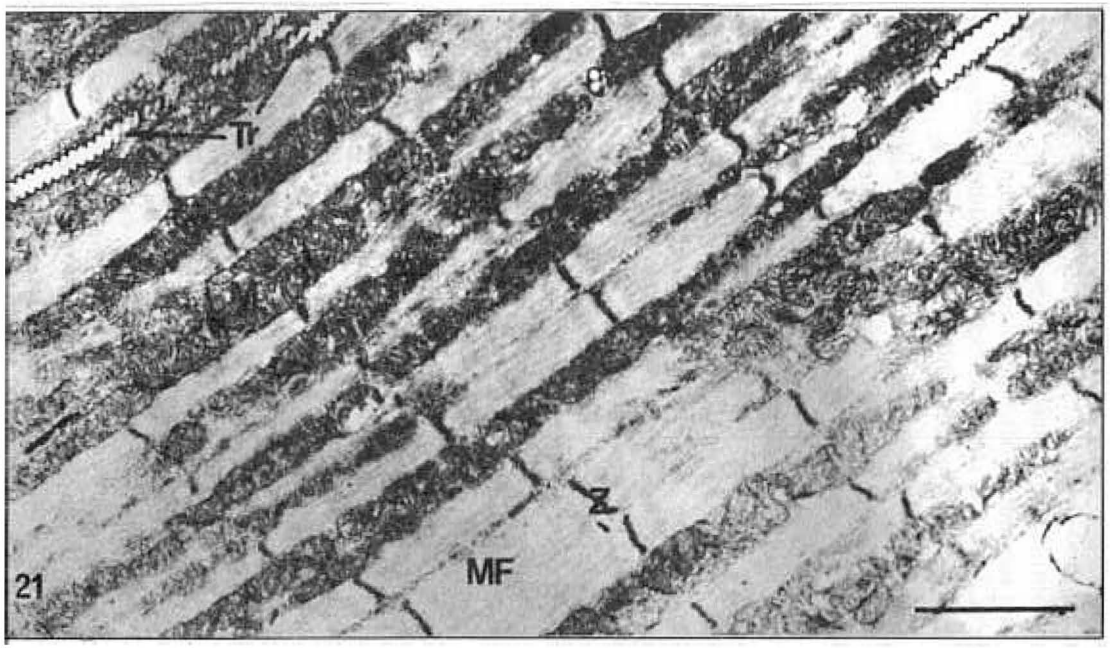


FIG. 21. A longitudinal section through a muscle fibre of a 2-day old adult locust. Note the columns of mitochondria of different sizes between the myofibrils. M : mitochondrion; MF : myofibril; Z : Z-band; D : dyad; Tr : tracheole. Scale 2.5 μm.

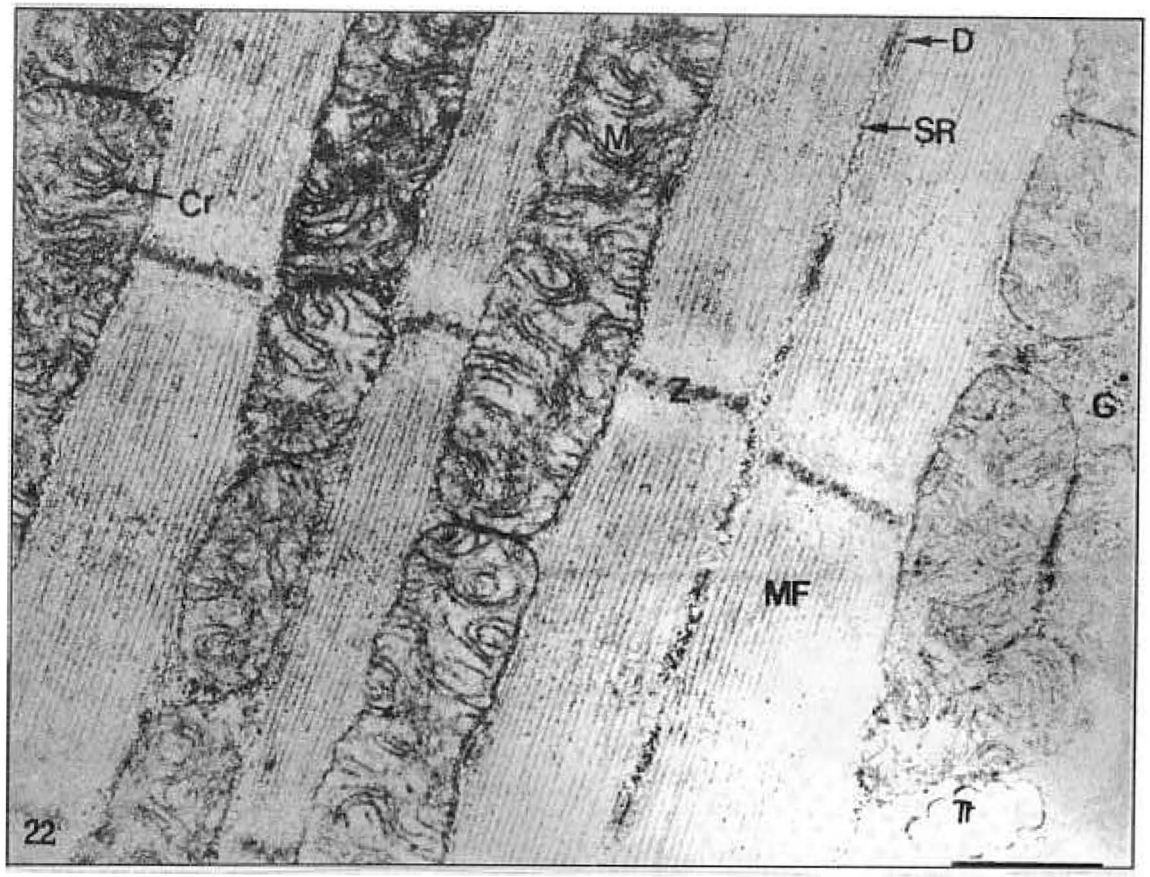


FIG. 22. A longitudinal section through a muscle fibre of a 5-day old adult locust. More than two mitochondria (M) are present per sarcomere. Note also that the sarcoplasmic reticulum (SR) at this age separates each myofibril (MF) completely (two sheets of it in some cases). Cr : cristae; D : dyad; G : glycogen; Tr : tracheole. Scale 1μm.

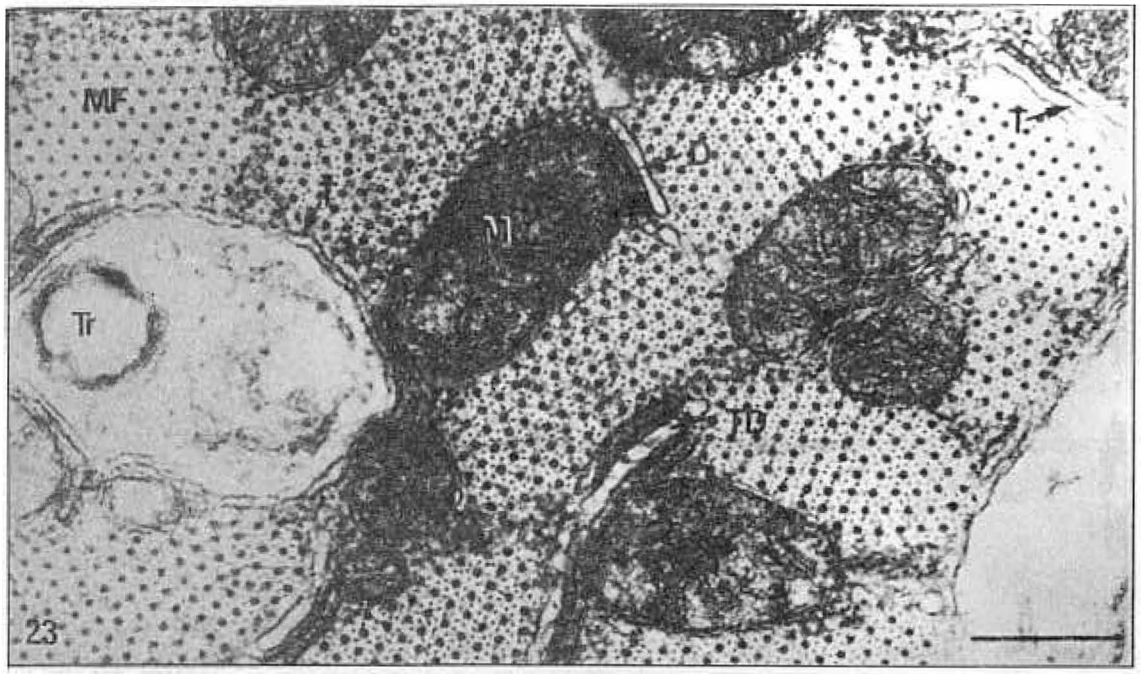


FIG. 23. A transverse section through a muscle fibre of a 1-day old adult locust. Note that the mitochondria (M) contain few cristae and some of the mitochondria appear to be in close contact with the adjacent ones. Note also the close association between a dyad (D) and a triad (TD), and a mitochondria. MF : myofibril; T : T-system; Tr : tracheole. Scale 0.4μm.

FIG. 24. A longitudinal section through a muscle fibre of a 1-day old adult locust. At this early stage of development, incomplete columns of mitochondria (M) are clearly seen between the myofibrils (MF). Note the presence of dyads (D) between two adjacent myofibrils. SR : sarcoplasmic reticulum; SP : splitting; T : tracheole; G : glycogen. Scale 1 μm.

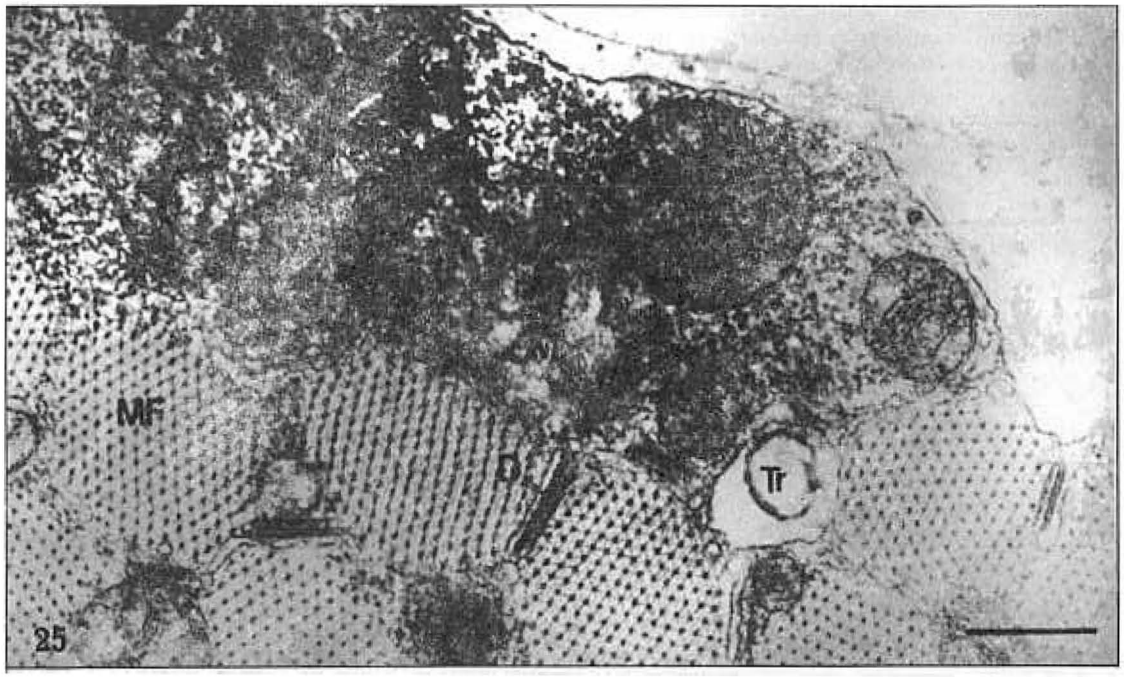


FIG. 25. A transverse section through a muscle fibre of a 3-day old adult locust. Note the aggregation of mitochondria (M) in the peripheral region of the fibre. D : dyad; MR : myofibril; Tr : tracheole. Scale 0.5 μm.

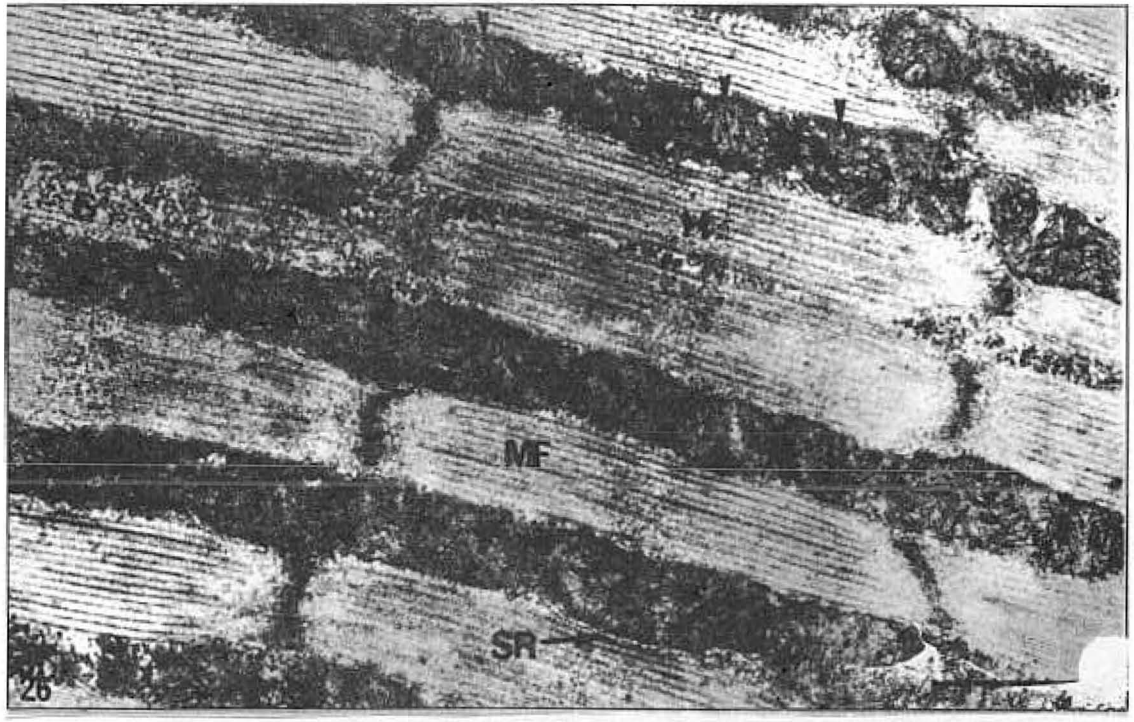
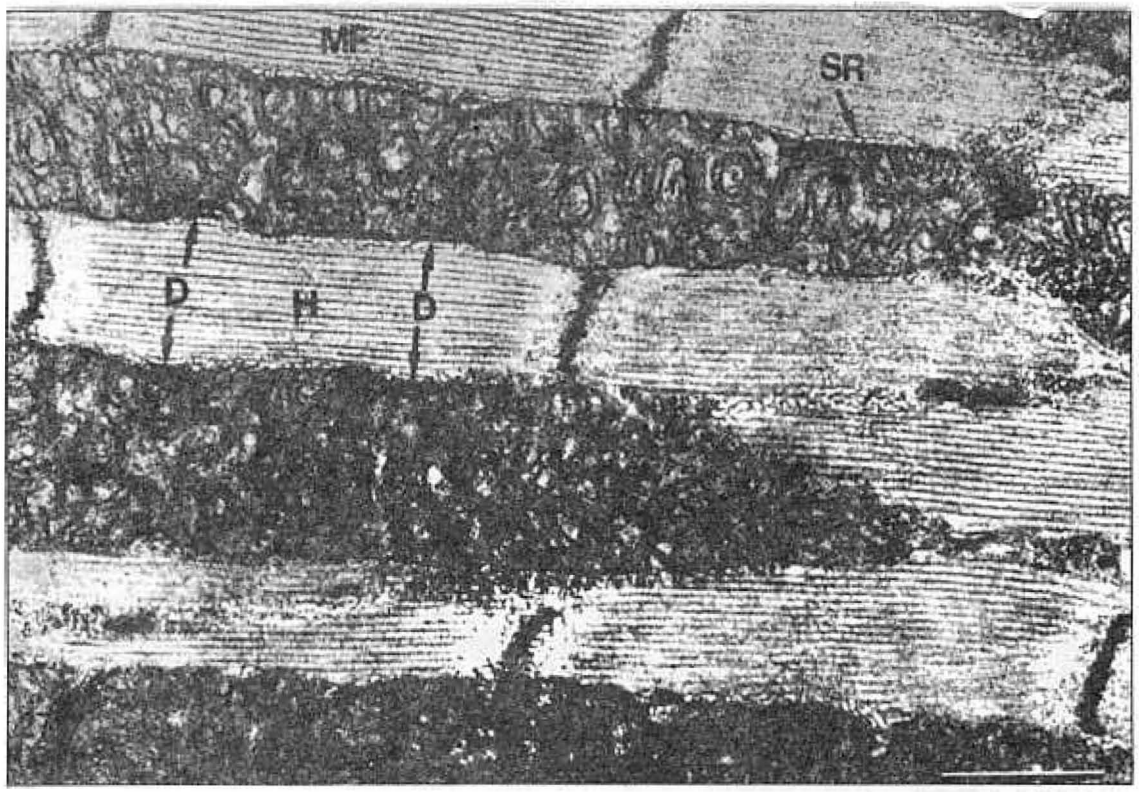


FIG. 26. A longitudinal section through a muscle fibre of a 3-day old adult locust. The number of mitochondria (M), per sarcomere, varies and it can be seen that there are up to three mitochondria per sarcomere length (arrow-heads) at this age. Z : Z-band; H : H-band; MF : myofibrils; SR : sarcoplasmic reticulum; G :glycogen. Scale 1 μm.



IG. 27. A longitudinal section through a muscle fibre of a 5-day old adult locust. Note that a single mitochondrion (M) extends for more than two sarcomere lengths whilst others extend less than one sarcomere length. Note also the dayd (d) is midway between the I- and H-band. Sarcoplasmic reticulum (SR) is also seen between the mitochondria (M) and the myofibril (MF). G : glycogen; H-band; Z : Z-band. Scale 1μm.

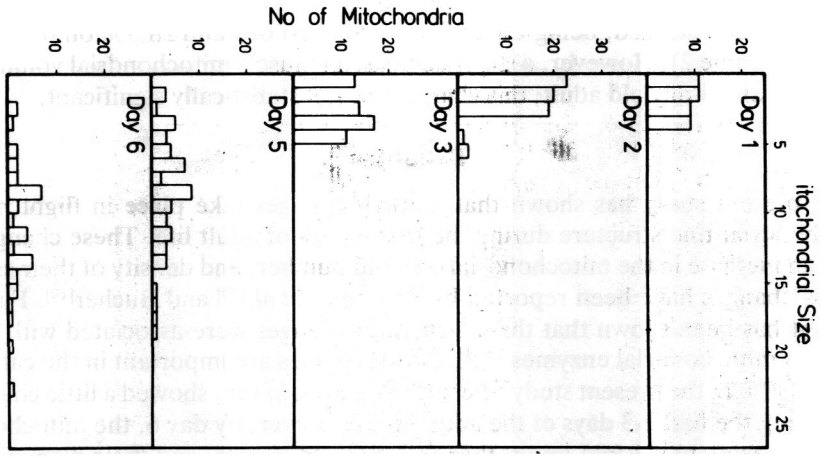


FIG. 28. Histograms showing size distribution of flight muscle mitochondria from *Locusta migratoria* at different ages. The data were obtained by tracing the mitochondria from electron micrographs. These were then cut out and weighed. The results were expressed in arbitrary units.

TABLE 3. Effect of age on mitochondrial size *in situ*.

Age in days	[1]Mitochondrial size (in arbitrary units)	[2]Mitochondrial diameter *in situ*		
	in situ	maximum (μm)	minimum (μm)	mean
1	2.62 ± 0.17 (42)	0.50 ± 0.02 (80)	0.36 ± 0.01 (80)	0.43 ± 0.01 (80)
2	2.28 ± 0.15 (71)	0.49 ± 0.02 (86)	0.34 ± 0.01 (86)	0.42 ± 0.01 (86)
3	2.96 ± 0.18 (80)	0.57 ± 0.01 (87)	0.43 ± 0.01 (87)	0.51 ± 0.01 (87)
4	6.23 ± 0.42 (50)	0.55 ± 0.02 (38)	0.36 ± 0.01 (38)	0.46 ± 0.02 (38)
5	8.96 ± 0.55 (55)	1.05 ± 0.01 (59)	0.68 ± 0.01 (59)	0.86 ± 0.02 (59)
6	11.50 ± 0.83 (95)	1.22 ± 0.03 (95)	0.75 ± 0.05 (95)	0.99 ± 0.02 (95)

The results are expressed as mean ± S.E.M. The figures in parentheses indicate the number of determinations.

[1]Determined by tracing mitochondrial profiles from E.M. graphs onto standard paper etc. (see Material and Methods).

[2]Determined by direct measurement from E.M. graphs.

The proportion of total muscle volume, occupied by mitochondria, changed a little over the period studied, being Ca 24.7% on the first day and 28.5% on the 6*th* day adult life (Table 2). However, a slight decrease in muscle mitochondrial volume was noted in 2 and 3-day old adult; this change was not statistically significant.

Discussion

The present study has shown that marked changes take place in flight muscle mitochondrial fine structure during the first 6 days of adult life. These changes include an increase in the mitochondrial size and number, and density of their cristae. Similar changes have been reported by Brosemer *et al.*[24] and Bucher[19]. Furthermore, it has been shown that these structural changes were associated with an increase in mitochondrial enzymes[24,34]; those enzymes are important in the catabolic pathways[35]. In the present study, the mitochondria, *in situ,* showed a little change in size during the first 2-3 days of the adult life. However, by day 6, the mitochondria were approximately 4-fold larger than in 1-day old insects, and their diameter had been more-or-less doubled. Mitochondria constituted Ca. 28.5% of the total muscle volume in 6-day old locusts compared with Ca. 24.7% in 1-day old adult. These values compare well with those of Brosemer *et al.*[24] and Bucher[19] who reported that, in *Locusta,* the relative volume, occupied by the mitochondria, increased from 6% to 23% between the 8*th* day before and the 1*st* day after the final ecdysis. By the 8*th* day after the final ecdysis, the mitochondria attained their maximum relative volume of 30%. Bucher[19] demonstrated that in *Locusta,* the total flight muscle mass increased by a factor of 10 during the entire developmental period. Thus, the total increase in the muscle chondriome is about 50-fold. Similarly, Richard *et al.*[13] reported that, in *Schistocerca gregaria,* the mass of the flight muscle increased 13-fold between the beginning of the 5*th* instar and the onset of sexual maturity, and that the increase in mitochondrial volume was comparable with that of *Locusta.* It is interesting to note that, in mature flight muscle of different insect species, there are variations in the proportion of the total muscle volume occupied by mitochondria. For example, mitochondria occupy 44% of the total flight muscle in *Neoconocephalus robustus*[32,36], 28.5% in the present material and 30%[19] in *Locusta migratoria,* 40% in *Aeshna*[30] and 30% in *Leptinotarsa decemlineata*[11]. In general, it seems that, in synchronous flight muscle, the more active the flight muscle is, the more mitochondria it contains[37].

Similar changes, to those described above, have been reported elsewhere, for example, in *Phormia regina*[38] in *Musca domestica*[39,40] and *Calliphora erythrocephala*[16]. Levenbrook and Williams[41] reported that, in *Phormia regina,* the wing-beat frequency is maximum by about the 7*th* day of adult life, and that the number of mitochondria is independent of age (6.7×10^8/thoracic flight muscle). However, this improved flight performance was associated with a 3-fold increase in mitochondrial dry weight in the first week of adult life.

Several researchers have reported the presence of mitochondria of differing fine structure in the flight muscles of insects. For example, Gregory *et al.*[10] have de-

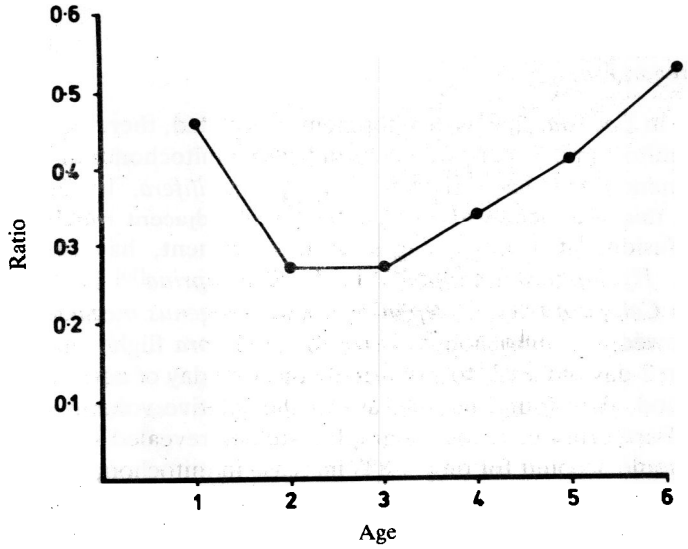
scribed two types of mitochondria in the flight muscle of *Lucilia cuprina;* one type present in the pupa, in which both tubular and lamellar cristae were observed and a second type, found in the adult, in which only lamellar cristae occur. Elder[32] has reported two distinct mitochondrial configurations (normal and vesiculated) in the very fast contracting synchronous flight muscle in mature *Neoconocephalus robustus.* Similarly, Simon *et al.*[42] described two types of mitochondria (Type A and B) in *Musca domestica* flight muscle. In contrast, only one mitochondrial type was observed in 6-day old adult in the present study. These possessed characteristically dense matrices, numerous densely packed cristae and are not too different from type B mitochondria described by Simon *et al.*[42]. Type A mitochondria which are characterised by less dense matrices and a few cristae were only observed in 1-day old adult *Locusta.* Similar observations have been reported in *Homorocoryphus nitidulus*[12] and in *Attagenus megatoma*[14].

At no time in the present study, on adult flight muscle, were any structures observed which might be taken to represent incomplete mitochondria, or mitochondria precursors. The observations are consistent with those reported by Bucher[19] that the increase in the chondriome, in *Locusta* flight muscle, is mainly due to the growth of existing mitochondria. However, Brosemer *et al.*[24] and Richard *et al.*[13] have reported mitochondrial division in developing flight muscle of *Locusta migratoria* and *5th* instar *Schistocerca gregaria.* Similarly, De Kort[11] reported a significant increase in mitochondrial numbers in the flight muscles of *Leptinotarsa decemlineata* during the first few days after the final ecdysis. He concluded that this was due to mitochondrial division. The latter finding has also been reported in the fat body of *Calpodes ethlius*[27]. However, no signs of mitochondrial division were observed in the present study. It must be concluded, therefore, that if mitochondrial division takes place in the flight muscle of *Locusta,* it does so prior to the final ecdysis, as in *Schistocerca gregaria*[13].

It was noted, in *Locusta,* that as development proceeded, there was a decrease in the number of mitochondria per sarcomere indicating mitochondrial elongation. A similar phenomenon has been reported in *Apis mellifera,* by Herold[9], who suggested that this was achieved by the fusion of adjacent mitchondria. Such mitochondria fusion, at various stages of development, has been suggested elsewhere, *e.g. Hyalophora cecropia*[8], in *Lucilia cuprina*[10] in *Musca domestica*[17,40,43,44], in *Calliphora erythrocephala*[16], and *Attagenus megata*[14]. Tribe and Ashhurst[16] showed that mitochondrial size in *Calliphora* flight muscle increased from 1.66 μm in 2-day old adult to 2.28 μm by the *10th* day of adult life. However, during this period, they found no increase in the relative volume of the muscle mitochondria. Furthermore, autoradiographic studies revealed that newly synthesized protein could account for only 4-8% increase in mitochondrial size, whilst a 25% size increase was noted in electron microscopial and Coulter Counter studies. They concluded that these observations were best explained by mitochondrial fusion. A similar conclusion was recorded by Sohal[17] with *Musca domestica.* He reported that whilst the number of mitochondria was reduced by more than 44% bet-

ween the 1*st* and 9*th* day of adult life, the mean area of individual mitochondrial profiles increased by 143% and the relative area of the sarcoplasm occupied by mitochondria increased by 43%. Moreover, Sohal[17] described the formation of highly elongated and irregularly shaped mitochondria, which are often referred to as "giant" mitochondria by side-to-side and end-to-end, as well as oblique fusion. On the basis of the present study, it appears that in *Locusta*, end-to-end fusion is the major mechanism by which mitochondrial elongation is affected. The structural mechanism of fusion has been described by Sohal[17]. Initially joining of the outer mitochondrial membranes into a single thick membrane occurs, followed by the development of regularly patterned cristae at the site of fusion. The nature of the factor(s) causing mitochondrial fusion is unknown. However, Tandler *et al.*[45] have suggested that mitochondrial fusion may be related to the intracellular distribution of membrane components such as phospholipids.

The change in the ratio of mitochondrial size to myofibrillar size suggests that the growth of these organelles is not constant (Fig. 29). The ratio decreased from 0.45 on day 1 to 0.27 on day 3 of adult life and then increased to a value of 0.53 by day 6 of adult life. This latter value is similar to that reported by Michejda[8] in *Hyalophora cecropia*. When the relative proportions of total muscle volume occupied by mitochondria and myofibrils (see table 1) were compared, a similar patterned emerged, i.e. an initial decrease in the ratio followed by an increase. On this basis, one can deduce that myofibrillar growth proceeds mitochondrial enlargement, but the latter subsequently restores the original relationship between the contractile machinery and the organelles supplying the energy for contraction.



29. Effect of age on the ratio between mitochondrial size and myofibrillar size in flight muscle of *Locusta migratoria*. The ratios were calculated from the mean values shown in Tables 2 and 3 for mitochondrial and myofibrillar size, respectively.

Richard *et al.*[13] have shown that the actin and myosin fractions constitute the major protein present in the flight muscle of *Schistocerca gregaria*. Furthermore, the relative proportion of these proteins increase from 50.9% of the total muscle protein in 1-day old adults to 59.5% by the 4*th* day of adult life before stabilizing at this level. Bucher[19] has reported that there is a substantial increase in the number of myofibrils in individual muscle fibers (from Ca. 30 to 1000) during the "phase of duplication" from the 3*rd* - 8*th* day following the final ecdysis. This observation is consistent with the fact that "longitudinal splitting" of myofibrils was observed in the present study during the first 4 days of adult life. Goldspink[46] was the first to provide electron micrographs as evidence for proliferation of muscle fibre by myofibrillar splitting. He suggested that this mechanism explained the substantial increase in the number of myofibrils per muscle fibre in post-natal mouse development. Since this time myofibrillar splitting has been reported in insect and crustacean muscles[3]. Goldspink[46,47] proposed a mechanism to explain "longitudinal splitting" and suggest the advantage of such a splitting process in allowing the sarcoplasmic reticulum and T-system to develop at the same rate as the contractile apparatus and permitting the mitochondria to become interspersed between the myofibrils. The latter is important in that it ensures the availability of energy for mechanical activity. Goldspink[46] found that splitting myofibrils were about twice the size of non-splitting myofibrils. He concluded that, in mouse muscle development, this was the mechanism whereby myofibrils increase in number within a muscle rather than "new" myofibrils arising by *de novo* synthesis as has been reported in embryonic chick[48] and in the early stages of development in *Drosophila melanogaster*[6] and *Calliphora erythrocephala*[49]. Thus "longitudinal splitting" of myofibrils would result in the production of smaller myofibrils. From the physiological standpoint, the latter is very important in fast acting synchronous flight muscle, because the larger the myofibrils size, the greater is the distance between the central myofilaments and the surrounding sarcoplasmic reticulum[32,50-52]. In other words, the Ca^{2+} would need a longer time to diffuse to central myofilaments to trigger the contraction. This agrees with the fact that small myofibrils are reported to be mechanically more active[5,32]. In contrast, in asynchronous flight muscle, myofibrillar splitting is not found beyond the early stages of formation in spite of a large addition of myosin filaments. Consequently the myofibrils grow to a very large size[49].

The present study showed that in the first 4 days after the final ecdysis there was a 3.2 fold increase in myofibril size. A clear levelling off was seen around the 4*th* - 5*th* day. This increase in myofibrils size is also reflected in an increase in the number of myosin filaments per myofibril. Similarly, Bursell *et al.*[53] reported a marked correspondence between the increase in myofibril volume and the estimated contractile protein during the growth of *Glossina morsitans*. They found that the bulk of the contractile protein was synthesised by the 8*th* day of adult life. Working with the same species, Anderson and Finlayson[54] confirmed this result by showing that the number of myosin filaments per myofibril increased during the same period.

Auber[49] found that in *Calliphora erythrocephala*, the number of thick filaments visible in a cross-section of a single myofibril of a dorsal longitudinal muscle fibre in-

creased from 669-1186 at the time of ecdysis to a maximum of around 2000 after 10 days of adult life. In the present study, whilst the number of myosin filaments increased with age, no statistically significant changes in actin:myosin ratio (Ca. 3:1) could be demonstrated over the 6-day period studied. It would appear, therefore, that both actin and myosin filaments increase at a more-or-less equivalent rate. This is in contrast to the finding of Valvossori *et al.*[55] who reported that in the dragonfly *Aeschna mixta* the actin:myosin filaments ratio is quite high initially (Ca. 4-4.5:1) but rapidly drops to the final 3:1 ratio when the myofibrils are well developed. Similar changes in the number of actins around each myosin have been reported between the "slow larval" and "fast adult" of *Manduca sexta* dorsal longitudinal muscle[18]. The ratio of 3:1 between actin and myosin observed in *Locusta* flight muscle is of widespread occurrence in flight muscle of both synchronous and asynchronous types, although several exceptions have been noted in synchronous flight muscle of certain species[3,5,56,57].

The average sarcomere length increased from 2.34 μm in 1 day old adult locusts to 3.19 μm in 6-day adult locusts ($P > 0.001$). This was accompanied by the appearance of a well defined I-band. Similar increases have been reported in vertebrate muscles[58] and in invertebrate muscles[6,49,59,60]. Two explanations of increasing sarcomere length have been suggested: (i) the deposition of new protein sub-units to the end of the myofilaments; (ii) the results of the sliding filament mechanism. Auber[59] has measured the sarcomere length in *Calliphora erythrocephala* during development, and reported that, whilst the sarcomere length increased from 2 μm on the *6th* day before to 3.2 μm at ecdysis, no significant change in I-band size was noted at any state. He concluded, therefore, that some increase in myofilaments length was taking place by deposition of new protein. However, Goldspink[58] has shown that in mice, the sarcomeres which increase from 2.3 μm in new born animals to 2.8 μm in the adult do so by the sliding-myofilament mechanism rather than by a change in myofilament length. Whilst it is difficult to reach the final conclusion about the method of sarcomere elongation in *Locusta,* on the basis of the present study, the fact that the I-band increases in size with increasing sarcomere length, suggests that the sliding-myofilament explanation is more appropriate here.

It is perhaps significant, therefore, that sarcoplasmic reticulum (SR) protein increases approximately 4-fold between the *9th* day of 5th instar and *7th* day of adult life as more-or-less did the specific activity of the Ca^{2+}-transporting ATPase (Al-Robai, in preparation). The importance of free Ca^{2+} concentration in the metabolic activity of insect flight muscle has been highlighted by Sacktor[61]. It is clear, therefore, that the development of SR is an important factor in the function of flight muscle and may explain the improvement in flight performance noted in developing adult locust[19,62]. The result reported in the present study may help in understanding the developmental changes of skeletal muscle in higher vertabrates. In addition, one may suggest that the *Locusta migratoria,* which is an agriculture pest, can be effectively control during the early days of adult life. This is because the maturation of organs, such as the component of flight muscle, pass through some weak points and it is

possible that the effect of synthetic chemical or some natural product may prove to be effective during these stages.

Conclusion

The present study is concerned with the growth and developmet of flight muscles in *Locusta* with particular reference to those systems involved in energy supply and utilization. The data obtained from morphometric studies revealed that both mitochondria and myofibrils increased in size, and that the relative proportion of mitochondria and myofibrils changed with increasing age. The mechanisms by which mitococho ndria and myofibrils increase in size have been discussed in detail. It was concluded that the increase in mitochondrial size was probably due largely to fusion of small adjacent mitochondria. The developmental changes were accompanied by an increase in the number and density of the cristae per mitochondrion.

Myofibrils size and number of myosin filaments, within each individual myofibril, increased approximately 4-fold, in *Locusta,* over the period studied. On the basis of the present study, it was concluded that the increase in the number and size of myofibrils was probably the result of "longitudinal splitting" and the addition of contractile protein (actin and myosin) into the peripheral region of the new myofibrils. The relative proportion (by volume) of myofibrils to mitochondria was more than 2:1 at all ages studied; being maximal on the 3*rd* day of adult life. It can be concluded that, the relative proportion of myofibrils and mitochondria referred to above support the general view that mitochondrial formation is strictly coordinated with the formation of other cellular constituents in flight muscle of *Locusta migratoria.*

Acknowledgement

This work is part of study submitted to the Faculty of Science of Durham University for the degree of Doctor of Philosophy. The study was carried out under the supervision of Dr. J.H. Anstee (Zoology Department). I am indebted to King Abdulaziz University for providing me with financial support.

References

[1] **Goldspink, G.,** Development of muscle, *In:* **Goldspink, G. (ed.),** *Differentiation and growth of cell in vertebrate tissues,* Chapman and Hall, London, pp. 69-99 (1974).

[2] **Pringle, J.W.S.,** Locomotion: Flight. *In:* **Rockstein, M. (ed.),** *The Physiology of Insecta,* 2nd ed., Academic Press, New York, **3:** 433-476 (1974).

[3] **Huddart, H.,** *The comparative structure and function of muscle,* Pergamon Press, Oxford, 397 p. (1975).

[4] **Chesky, J.,** Comparative aspects of declining muscle function, *Aging* **6:** 179-210 (1978).

[5] **Elder, H.Y.,** Muscle structure. *In:* **Usherwood, P.N.R. (ed.),** *Insect Muscle,* Academic Press, London, New York, 1-73 (1975).

[6] **Shafiq, S.A.,** Electron microscopic studies on the indirect flight muscles of *Drosophila melanogaster.* I. Structure of the myofibrils, *J. Cell Biol.* **17:** 351-362 (1963).

[7] **Shafiq, S.A.,** An electron microscopical study of the innervation and sarcoplasmic reticulum of the fibrillar flight muscle of *Drosophila melanogaster, Q.J. Micr. Sci.* **105** (1): 1-6 (1964).

[8] **Michejda, J.**, Physiology and structure of flight muscle sarcosomes in silkworm, *Hyalophora cecropia L., Bull. Soc. Amis. Sci. Lett. Poznan. Ser. D. Liv.* **4:** 61-102 (1964).
[9] **Herold, R.C.**, Development and ultrastructural changes of sarcosomes during honey-bee flight muscle development, *Devl. Biol.* **12:** 269-286 (1965).
[10] **Gregory, D.W., Lennie, R.W.** and **Birt, L.M.**, An electron microscopic study of flight muscle development in the blowfly *Lucilia cuprina, JL. Roy. Microsc. Soc.* **88:** 151-175 (1968).
[11] **De Kort, C.A.D.**, Hormones and the structural and biochemical properties of the flight muscles in the Colorado beetle, *Meded. Landbouwhogeschool Wageningen* **69** (2): 1-63 (1969).
[12] **Anstee, J.H.**, An ultrastructural study of Tettigoniid muscle in relation to function. *J. Insect. Physiol.* **17:** 1983-1994 (1971).
[13] **Richard, C. Tanguay, R.** and **Chaudhary, K.D.**, Nucleic acid and protein variations in relation to ultrastructural changes in the developing thoracic muscle of the African desert locust, *Schistocerca gregaria* Forsk, *Cytobios* **3:** 145-160 (1971).
[14] **Butler, L.** and **Nath, S.**, Post-emergence changes in ultrastructure of flight and leg muscle of the Black Carpet Beetle, *Ann. Ent. Soc. Am.* **65:** 247-254 (1972).
[15] **Ashhurst, D.E.**, The fibrillar flight muscles of giant water-bugs: An electron microscope study, *J. Cell Sci.* **2:** 435-444 (1967).
[16] **Tribe, M.A.** and **Ashhurst, D.E.**, Biochemical and structural variation in the flight muscle mitochondria of ageing blowflies, *Calliphora erythrocephala, J. Cell Sci.* **10:** 443-469 (1972).
[17] **Sohal, R.S.**, Ageing changes in insect flight muscle, *Geront.* **22:** 317-333 (1976).
[18] **Rheuben, M.B.** and **Kammer, A.E.**, Comparison of slow larval and fast adult muscle innervated by the same motor neuron, *J. Exp. Biol.* **84:** 103-118 (1980).
[19] **Bucher, T.**, Formation of the specific structural and enzymic pattern of the insect flight muscle, *Biochem. Soc. Symp.* **25:** 15-28 (1965).
[20] **Van Marrewijk, W.J.A., Schrikker, A.E.M.** and **Beenakkers, A.M. Th.**, Contents of nucleic and amino acids and rate of protein synthesis in developing flight muscles of *Locusta migratoria, Comp. Biochem.* **65B:** 251-257 (1980).
[21] **Tiegs, O.W.**, The flight muscle of insect – their anatomy and histology; with some observation on the structure of striated muscle in general, *Phil. Trans. Roy. Soc., Lond.* **B238:** 221-348 (1955).
[22] **Bernays, E.A.**, The muscles of newly hatched *Schistocerca gregaria* larvae and their possible functions in hatching, digging and ecdysial movements (Insecta:Acrididae), *J. Zool.* **166:** 141-158 (1972).
[23] **Hill, L.** and **Goldsworthy, G.J.**, Growth, feeding activity and the utilization of reserves in larvae of *Locusta, J. Insect Physiol.* **14:** 1085-1098 (1968).
[24] **Brosemer, R.W., Vogell, W.** and **Bucher, T.**, Morphologische und enzymatische Muster bei der Entwicklung indirekter flugmuskeln von *Locusta migratoria, Biochem. Z.* **338:** 854-910 (1963).
[25] **Hill, L., Luntz, A.J.** and **Steele, P.A.**, The relationship between somatic growth, ovarian growth and feeding activity in the adult locust. *J. Insect Physiol.* **14:** 1-20 (1968).
[26] **Reynolds, E.S.**, The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell. Biol.* **17:** 208-212 (1963).
[27] **Larsen, W.J.**, Genesis of mitochondria in fat body of an insect, *J. Cell. Biol.* **47:** 373-383 (1970).
[28] **Forbes, M.S., Rubio, R.** and **Spereiakis, N.**, Tubular systems of limulus myocardial cells investigated by use of electron-opaque tracers and hypertonicity, *J. Uttrastruct. Res.* **39:** 580-597 (1972).
[29] **Smith, D.S.**, Innervation of the fibrillar flight muscle of an insect: *Tenebrio molitor* (Coleoptera), *J. Biophys. Biochem. Cytol.* **3:** 447-466 (1960).
[30] **Smith, D.S.**, The organisation of flight muscle in a dragonfly, *Aeschna* sp. (Odonata), *J. Biophys. Biochem. Cytol.* **11:** 119-145 (1961).
[31] **Hagopian, M.** and **Spiro, D.**, The sarcoplasmic reticulum and its association with the T-system in an insect, *J. Cell. Biol.* **32:** 535-545 (1967).
[32] **Elder, H.Y.**, High frequency muscles used in sound production by a Katydid II. Ultrastructure of the signing muscles, *Biol. Bull.* **141:** 434-448 (1971).
[33] **Smith, D.S.**, Cytological studies on some insect muscle (with special reference to the sarco-plasmic reticulum), *Revue Can. Biol.* **21:** 279-301 (1962).

[34] **Beenakkers, A.M. Th.**, Fatty acid oxidation in insect muscle, *Acta Physiol. Pharmacol. neerl.* **12:** 332-335 (1963).

[35] **Beenakkers, A.M. Th., Van Den Broek, A.Th. M.** and **De Ronde, Th. J.A.**, Development of catabolic pathways in insect flight muscles. A comparative study, *J. Insect Physiol.* **21:** 849-859 (1975).

[36] **Josephson, R.K.** and **Elder, H.Y.**, Rapidly contracting muscles used in sound production by a Katydid, *Biol. Bull.* **135:** 409 (1968).

[37] **Hoyle, G.**, Comparative aspects of muscle fibres, *Ann. Physiol. Rev.* **31:** 43-84 (1969).

[38] **Watanabe, M.I.** and **Williams, C.M.**, Mitochondria in the flight muscles of insects. II. Effect of the medium on the size, form and organization of isolated sarcosomes, *J. Gen. Physiol.* **37:** 71-90 (1953).

[39] **Rockstein, M.** and **Bhatnagar, P.L.**, Age changes in size and numbers of the giant mitochondria in the flight muscle of the common housefly, *Musca domestica, J. Insect. Physiol.* **11:** 481-491 (1965).

[40] **Sohal, R.S., McCarthy, J.L.** and **Allison, V.F.**, The formation of "giant" mitochondria in the fibrillar flight muscles of the housefly, *Musca domestica, J. Ultrastruct. Res.* **39:** 484-495 (1972).

[41] **Levenbrook, L.** and **Williams, C.M.**, Mitochondria in flight muscles of insect. III. Mitochondrial cytochrome C in relation to ageing and wing beat frequency of fies, *J. Gen. Physiol.* **39:** 497-512 (1956).

[42] **Simon, J., Bhatnagar, P.L.** and **Milburn, N.S.**, An electron microscope study of changes in mitochondria of flight muscle of ageing houseflies *(Musca domestica), J. Insect Physiol.* **15:** 135-140 (1969).

[43] **Sohal, R.S.** and **Allison, V.F.**, Age-related changes in the fine structure of the flight muscle in the housefly, *Expl. Gerontol.* **6:** 167-172 (1971).

[44] **Weeb, S.** and **Tribe, M.A.**, Are there major degenerative changes in the flight muscle of ageing Diptera, *Expl. Gerontol.* **9:** 43-49 (1974).

[45] **Tandler, B., Erlandson, R.A.** and **Wynder, E.L.**, Riboflavin and mouse hepatic cell structure and function. I. Ultrastructure alteration in simple deficiency, *Am. J. Path.* **52:** 69-95 (1968).

[46] **Goldspink, G.**, The proliferation of myofibrils during muscle fibre growth, *J. Cell Sci.* **6:** 593-603 (1970).

[47] ————. Changes in striated muscle fibre during contraction and growth with particular reference to myofibril splitting, *J. Cell Sci.* **9:** 123-137 (1971).

[48] **Fischman, D.A.**, An electron microscope study of myofibril formation in embryonic chick skeletal muscle, *J. Cell Biol.* **32:** 557-575 (1967).

[49] **Auber, J.**, *La myofibrillogenese du muscle strie.* I. Insectes, *J. Microscopie* **8:** 197-233 (1969).

[50] **Fahrenbach, W.H.**, The sarcoplasmic reticulum of striated muscle of a cyclopoid copepod, *J. Cell Biol.* **17:** 629-640 (1963).

[51] **Auber, J.**, Distribution of the two kinds of myofilaments in insect muscles, *Amer. Zool.* **7:** 451-456 (1967).

[52] **Ebashi, S.** and **Endo, M.**, Calcium ion and muscle contraction, *Prog. Biophys. Mol. Biol.* **18:** 123-184 (1968).

[53] **Bursell, F., Slack, E.** and **Kuwengwa, T.**, *Aspects of the development of flight musculature in the tsetse fly, Glossina moristans. Second Tsetse fly breeding symposium*, Bristol, pp. 319-320 (1971).

[54] **Anderson, M.** and **Finlayson, L.H.**, Ultrastructural changes during growth of the flight muscles in the adult tsetse fly, *Glossina austeni, J. Insect. Physiol.* **19:** 1989-1997 (1973).

[55] **Valvassori, R., De Egulleor, M.** and **Lanzavecchia, G.**, Flight muscle differentiation in nymphs of a dragonfly *Anax imperator, Tissue and Cell* **10:** 167-178 (1978).

[56] **Smith, D.S.**, The organization and function of the sarcoplasmic reticulum and T-system of muscle cells, *Prog. Biophys. Mol. Biol.* **16:** 107-142 (1966).

[57] **Pringle, J.W.S.**, Arthropod muscle, *In:* **Bourne, G.H. (ed.)**, *The structure and function of muscle*, 2*nd* Ed. **1**, part 1, Academic Press, New York and London, pp. 491-541 (1972).

[58] **Goldspink, G.**, Sarcomere length during the post-natal growth of mammalian muscle fibres, *J. Cell Sci.* **3:** 539-548 (1968).

[59] **Aronson, J.**, Sarcomere size in developing muscles of a trasonamid mite, *J. Biophys. Biochem. Cytol.* **11:** 147-156 (1961).

[60] **Auber, J.,** L'accroissement eu longueur des myofibrilles et al formation de nouveaux sarcomeres on cours du development des muscles chez *Calliphora erythrocephala, C.R. Acad. Sci.,* Paris **261:** 4845 (1965).

[61] **Sacktor, B.,** Biochemistry of insect flight. Part I, Utilization of fuels by muscle. *In:* **Candy, D.J.** and **Kilpy, B.A. (ed.)** *Insect Biochemistry and Function,* Wiley, New York, 1-88 (1975).

[62] **Gewecke, M.** and **Kutsch, W.,** Development of flight behaviour in maturing adults of *Locusta migratoria:* 1. **Flight performance and wing-stroke parameters,** *J. Insect Physiol.* **29:** 249-253 (1979).

نمو الميتوكندريا والليفات العضلية بعضلات الطيران في الجراد الرحال *Locusta migratoria* L. (مستقيمة الأجنحة : أكريديدي)

علي بن أحمد الرباعي
قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز
جـــدة ، المملكة العربية السعودية

المستخلص . تمت دراسة التركيب الدقيق لعضلات الطيران الظهرية الطولية وغير المباشرة أثناء الأسبوع الأول في الجراد الرحال البالغ . لوحظ أن لون هذه العضلات يتغير من البيضاء إلى الحمراء البنية . ويتم تأسيس نموذج التخطيط المميز لعضلات الطيران في الحشرة البالغة . يرافق التغيرات السابقة زيادة في حجم الليفات ومتوسط عدد خيوط المايوسين لكل ليفة عضلية ، ولم يلاحظ تغير مهم في نسبة خيوط المايوسين : والأكتين . وجد بكل وضوح حدوث « انفصال طولي » في بعض الليفات العضلية في الأربعة الأيام الأولى بعد الانسلاخ الأخير ، وهذا يفسر زيادة أعداد الليفات العضلية أثناء النمو . وجدت زيادة في أحجام الميتوكندريا وتعقيد في تركيبها الداخلي وتعزي هذه الزيادة (جزئيا) إلى حدوث « التحام » بين الميتوكندريا المتجاورة ، وتصبح الميتوكندريا منتظمة على هيئة أعمدة بين الليفات العضلية أثناء الخمسة الأيام الأولى من عمر الحشرة البالغة . التغيرات النهائية التي شوهدت في التركيب الدقيق للميتوكندريا والليفات العضلية مهمة في شرح زيادة فعالية الطيران في الجراد الرحال أثناء النمو .