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FINE STRUCTURE OF DEGENERATING AND REGENERATING FLIGHT MUSCLES IN A BARK BEETLE, *IPS CONFUSUS*

I. DEGENERATION

N. M. G. BHAKTHAN, J. H. BORDEN AND K. K. NAIR
Pestology Centre, Department of Biological Sciences,
Simon Fraser University, Burnaby 2, British Columbia, Canada

SUMMARY

The flight muscles of the bark beetle *Ips confusus* undergo a pronounced degeneration within 4 days of introducing beetles into the bark of pine logs. Numerous lysosomes develop between the myofibrils, and the fibrils become greatly reduced in size. In female beetles many of the mitochondria and most of the myofilaments disappear from the muscle, and apart from lysosomes, tracheoles and a few fine granules, little structural organization remains in the fibres. The muscles of males degenerate to a lesser extent and, unlike those of females, contain numerous lipid globules in the degenerating condition. The significance of flight muscle degeneration as a possible prerequisite for reproduction in females is discussed. In males, flight muscle degeneration may have behavioural significance in confining the flightless insect to the host tree for repetitive mating and gallery maintenance.

INTRODUCTION

The phenomenon of flight muscle degeneration associated with reproduction has been observed in many insects (Hocking, 1952, 1954; Johnson, 1953; Chapman, 1956; Reid, 1958; Atkins & Farris, 1962; Chudakova & Bocharova-Messner, 1968). The California five-spined ips, *Ips confusus* (LeConte) (Coleoptera: Scolytidae) shows the typical scolytid pattern of cyclic de- and regeneration of the flight muscles during the reproductive phase (Borden & Slater, 1969). This paper reports the sequential changes in fine structure of *I. confusus* flight muscles during their degeneration.

MATERIALS AND METHODS

The beetles were obtained from a stock culture maintained in caged logs of *Pinus ponderosa* Laws. Adult beetles were collected after emerging from logs in which they had developed. Each of 24 males was introduced into a preformed entrance hole punched in the bark of fresh pine logs. After 24 h, 1 female/male was allowed to join and mate with a male in the newly constructed nuptial chamber in the phloem tissue. Beetles were excised from the log at 24-h intervals. The flight muscles of beetles excised 24, 48, 72 and 96 h after introduction and those of control beetles freshly emerged from host logs were dissected out in cold 3 % glutaraldehyde. After 30 min they were post-fixed for 2 h in 2 % osmium tetroxide in veronal buffer at pH 7·4. After dehydration through grades of ethanol the tissue was embedded in Araldite. Sections showing grey to silver interference colours were cut with a diamond knife on a Reichert ultra-

microtome and picked up on 200-mesh uncoated grids. The sections were stained for 15 min in uranyl acetate, followed by lead citrate for 10 min, and examined and photographed in an RCA EMU-3H electron microscope.

RESULTS

The dorsoventral flight muscles are fully developed in the freshly emerged beetle. The myofibrils are well developed with all the typical bands present in a fibrillar flight muscle (Fig. 1). The space between the myofibrils is filled with large mitochondria with closely packed cristae. The nuclei possess dense chromatin profiles on their peripheries. Tracheoles are often found near the mitochondria. Numerous granules are present in the highly restricted sarcoplasm. The size (10–15 nm) and the structural configuration of most of these granules are similar to those of ribosomes. Nevertheless, the dense larger granules may be glycogen granules. A detailed study to distinguish between these granules, by cytochemistry and enzyme digestion, will be reported later. The T-system tubules are represented by small vesicles. Occasionally mitochondria with a denser matrix are also present.

Within 24 h of introduction of the female beetle into the male nuptial chamber, numerous membrane-limited, electron-dense bodies, presumably lysosomes, appear between the myofibrils and mitochondria (Fig. 2). The mitochondrial matrix becomes less dense and there is an increase in the space between the cristae. Many ribosome-like granules appear inside the myofibrils, mostly along the line of disintegrating actin filaments. By 48 h these granules increase in number and the myofilaments become thinner and discontinuous. The M-band disappears completely and the Z-line becomes diffused and less coherent. The nuclei, however, appear normal. The mitochondrial cristae become discontinuous, resulting in further increases in space between the cristae. A few granules appear in many mitochondria. After 72 h (Fig. 3) most of the mitochondria have disappeared. The myofibrils show a reduced number of filaments still held together at the Z-line. Lysosome-like bodies and intracellular tracheoles become more conspicuous as many of the mitochondria and most of the myofilaments disappear from the muscle.

Similar degenerative changes are evident in the flight muscles of male beetles. However, after 72 h in the log (Fig. 4), the extent of degeneration is less than that observed in the 72-h females. Another difference in males is the presence of numerous lipid globules in the degenerating flight muscle.

In females after 96 h the myofibrils are devoid of most of the myofilaments and almost all the mitochondria have disappeared (Figs. 5, 6). Apart from lysosomes, tracheoles and a few fine granules (Figs. 5–7) there is little structural organization left in the muscle fibres. When compared with females after 96 h the flight muscles of males are considerably less degenerate (Fig. 8).

DISCUSSION

The degeneration of muscular tissue in insects can be divided into 2 categories: reversible and irreversible. Larval and pupal abdominal muscles of some insects

degenerate irreversibly (Lockshin & Williams, 1965*a*, *b*; Crossley, 1965, 1968), whereas in some the degeneration of flight muscle is reversible (Reid, 1958, 1962; Atkins & Farris, 1962; Stegwee, Kimmel, De Boer & Henstra, 1963; De Kort, 1969; Borden & Slater, 1969). The breakdown of the abdominal muscles in blowfly larvae (Crossley, 1968) is by autolysis and phagocytosis, whereas in silkmoths (Lockshin & Williams, 1965*a*) it is mainly by the lytic activity of lysosomal enzymes. In *I. confusus* females, degeneration of the flight muscles is evident within 24 h, with the appearance of numerous lysosomes and granulation of the myofilaments. At no stage in the process of degeneration did we observe phagocytosis in the region of the flight muscle. At the end of 96 h most of the muscle material, including mitochondria, had disappeared from the muscle. The nuclei and the sarcolemma appeared not to be affected by the lytic process. During the same period the male flight muscle also degenerated but not to the same extent as the female flight muscle.

Two important questions arise. What factors control or regulate the degenerative process; and what is the role of the metabolites released from the degenerating muscles. Flight muscles of the Colorado potato beetle degenerate during diapause mainly due to the inactivity of the retrocerebral complex of the endocrine system (Stegwee *et al.* 1963). Active retrocerebral complexes from non-diapausing beetles implanted into diapausing ones induced premature regeneration of the flight muscle. Thus, juvenile hormone apparently broke diapause and stimulated regeneration of the flight muscle. According to Lockshin & Williams (1964, 1965*a*, *b*, *c*, *d*) the intersegmental muscles of silkmoths are programmed to die by a neurohormonal mechanism which activates the lysosomal enzymes to initiate muscle breakdown. In blowfly larvae invasion of muscles by phagocytes can be induced by crustecdysone, an analogue of the insect moulting hormone (Crossley, 1968). Borden & Slater (1968) showed that topical application of a synthetic juvenile hormone on adult *I. confusus* induced 30% (males) or 50% (females) reduction in flight muscle volume. Thus, the factors which initiate muscle degeneration differ in insects and the effectiveness of these factors (nervous and/or hormonal) depends upon the physiological condition and the stage of the insects.

In *I. confusus*, like many other bark beetles, flight muscle degeneration appears to be a prerequisite for reproduction. As the phloem tissue of the bark is mostly carbohydrates, the proteins and lipids required for egg yolk formation must be synthesized by the insect. The fat body is the chief organ for synthesis, interconversion and release of the metabolites. However, in *I. confusus* females the fat body does not have the store of protein bodies found in other insects (unpublished observation). It is known that, in insects, lipids, carbohydrates and proteins are synthesized in the fat body prior to yolk deposition (Telfer, 1965; Gilbert, 1967*a*, *b*; Wyatt, 1967, 1968; Engelmann, 1968). Therefore it appears logical that, as the flight muscles are not functional while female *I. confusus* are inside the bark, their proteins and other metabolites can be advantageously utilized for the formation of yolk materials in the fat body. This view is supported by the observation that, in a house cricket, implantation of active corpus allatum induced flight muscle volume reduction and subsequent yolk deposition in the eggs, even in immature females (Chudakova & Bocharova-Messner, 1968). We

have observed that female *I. confusus* did not lay eggs until 2–3 days after entering the nuptial chamber to join the male. These observations suggest that the flight muscle degeneration may be a prerequisite for yolk formation and egg maturation in female ips.

The considerable reduction in *I. confusus* flight muscle volume due to degeneration also provides room for the developing ovaries. This was also observed by Atkins & Farris (1962) in a different bark beetle. The above reasons may elucidate the significance of flight muscle degeneration in the female. In the male, however, it may have behavioural significance in confining the male in a flightless condition to the host for the repetitive mating (B. A. Barr, personal communication) and gallery maintenance necessary during construction of the egg galleries and oviposition by the female (Chapman, 1958; Borden, 1967). The fat body in the male becomes loaded with metabolites during muscle degeneration (unpublished observation), whereas in the female there is a high turnover of the metabolites as they are synthesized and released during egg maturation.

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Fig. 1. Longitudinal section of the flight muscle of an adult female beetle freshly emerged from brood log. Note the well-developed structural characteristics. The larger granules appear to be glycogen, the smaller ones ribosomes. (*gr*, granules; *m*, mitochondria; *n*, nucleus; *t*, tracheole.)

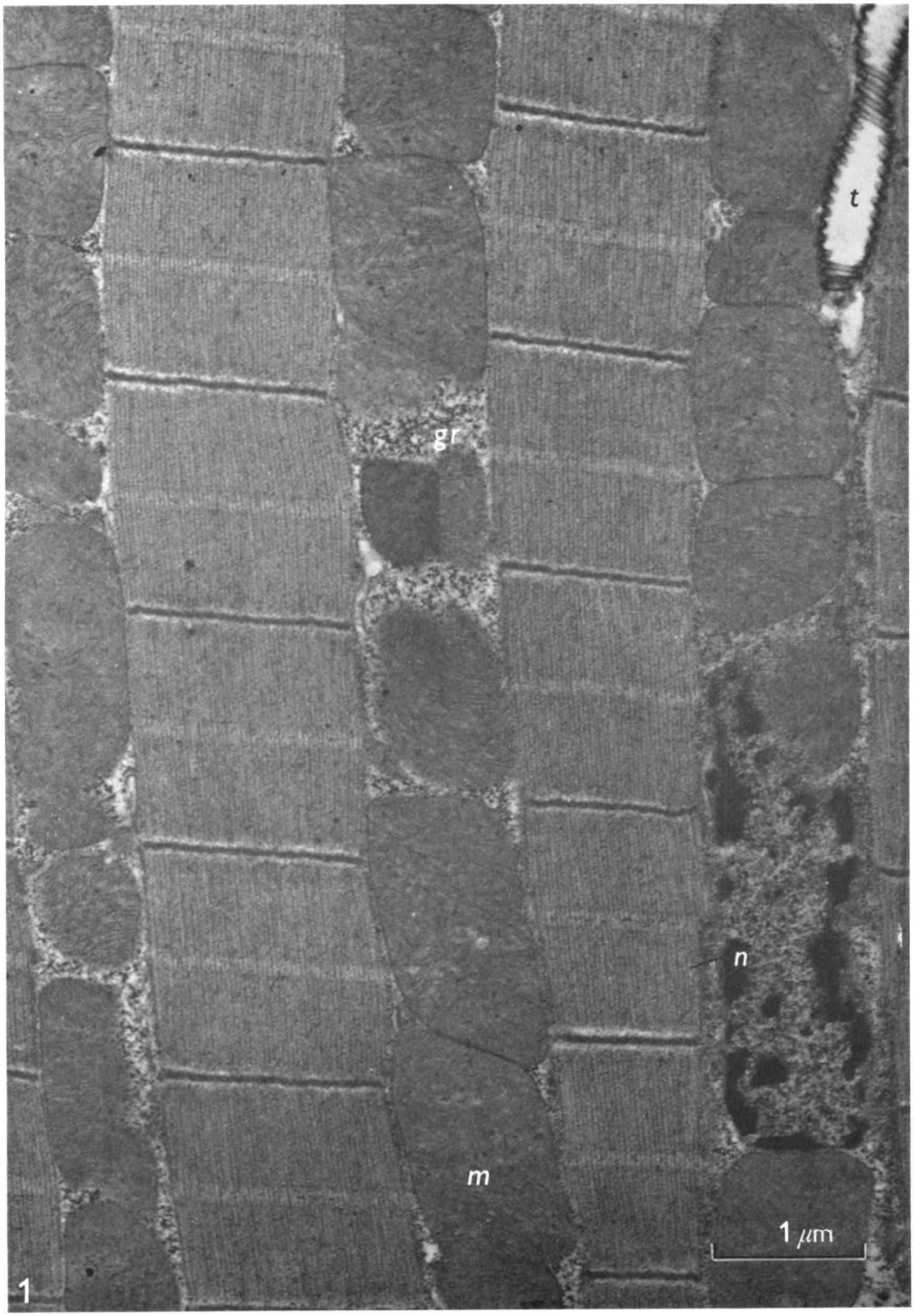
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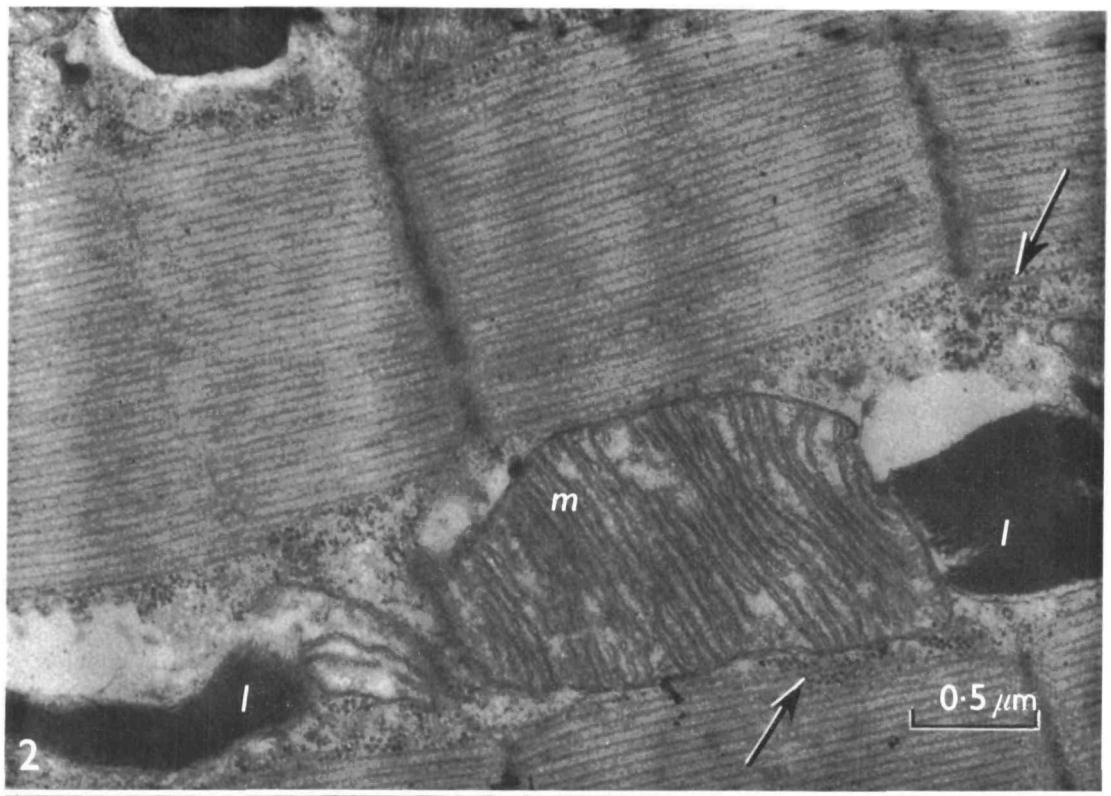
Fig. 2. Longitudinal section of the flight muscle of a female 24 h after introduction into the bark. Dense, membrane-limited lysosome-like bodies (*l*) have appeared. The space between the cristae of the mitochondrion (*m*) has increased. Many ribosome-like granules (arrows) appear between the filaments.

Fig. 3. Flight muscle of the female 72 h after introduction into the bark. Note the reduction in myofibrils. The few that are remaining are still held together at the Z-lines (*Z*). Tracheoles (*t*) and lysosomes (*l*) are more conspicuous. Only a few mitochondria are retained.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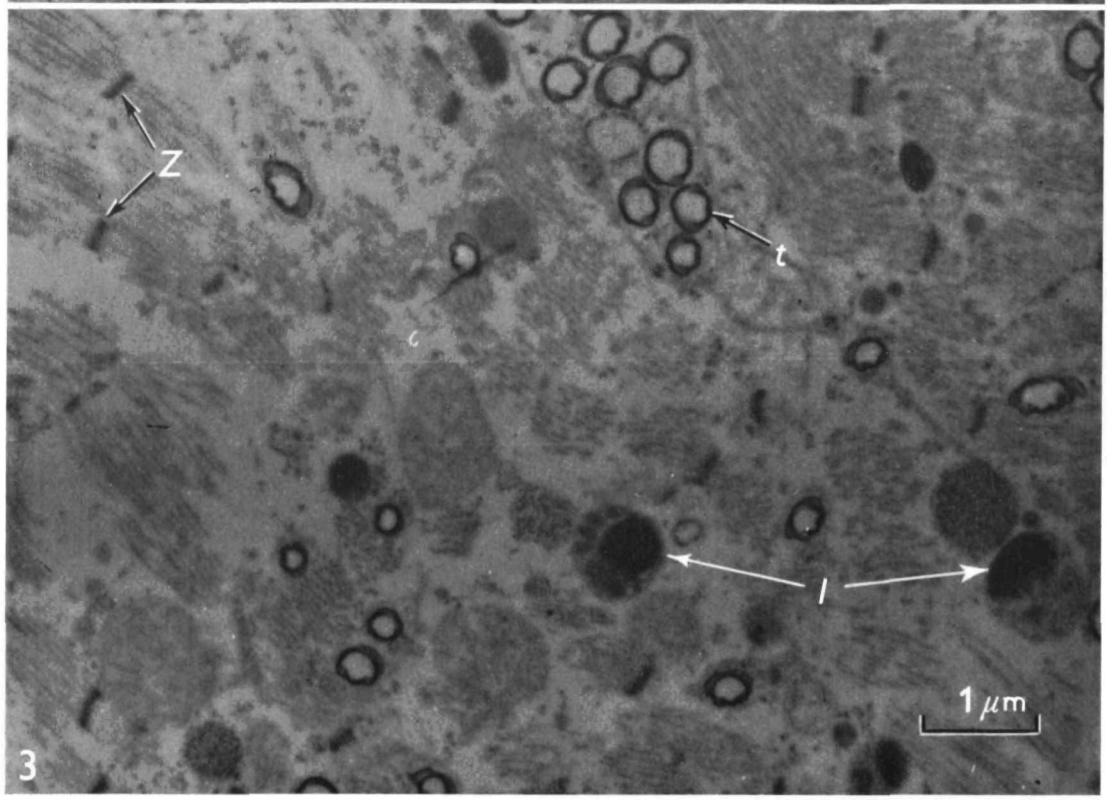
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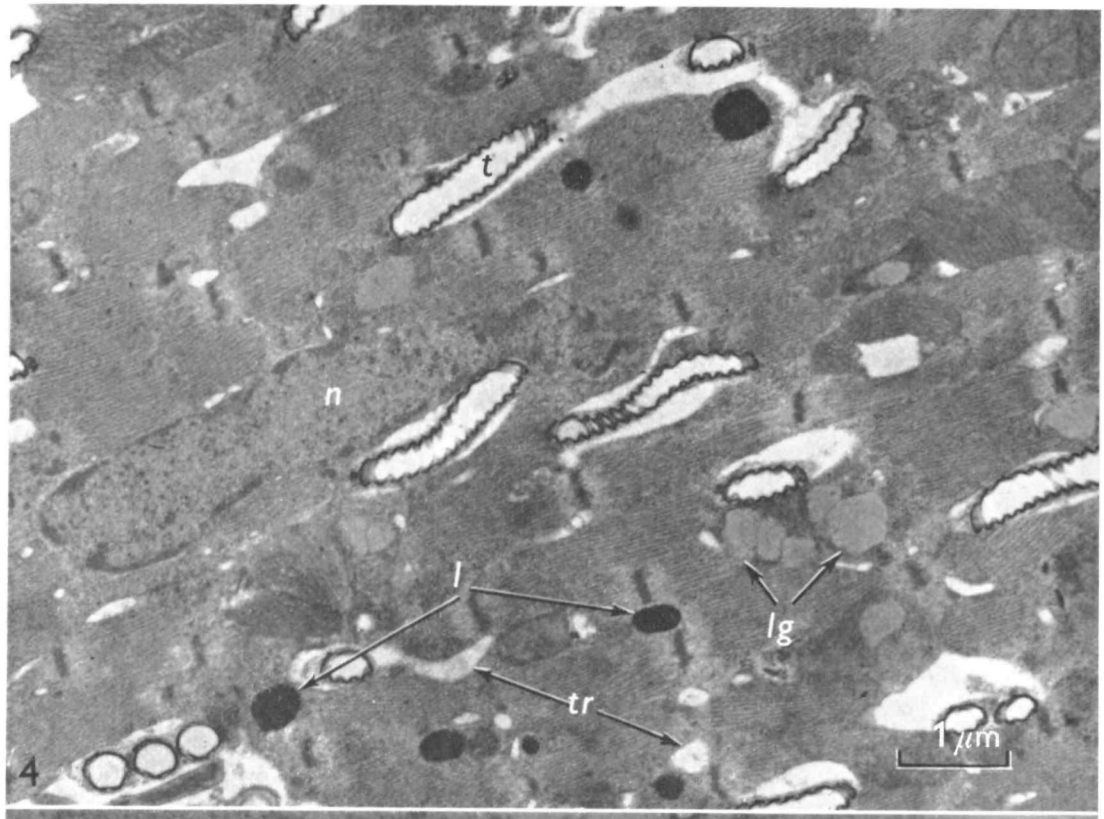
Fig. 4. Longitudinal section of the flight muscle of the male 72 h after introduction into the bark. Degenerative changes, though evident, are not as extensive as in the female (Fig. 3). The nuclei (*n*) appear unaffected. Vacuolization of the transverse reticulum (*tr*) is conspicuous. Many lipid-like globules (*lg*) and lysosomes (*l*) are evident. (*t*, tracheole.)

Fig. 5. Longitudinal section of the flight muscle of the female 96 h after introduction into the bark. The filaments are now greatly reduced. The Z-line (*Z*), though incoherent, can still be seen as a dense plate holding the remaining filaments. The nucleus (*n*) is oval, with a distinct nucleolus (*nu*). Lysosomes (*l*) are still present. Numerous granules have accumulated in the enlarged vesicles of the reticulum (*tr*). (*t*, tracheole.)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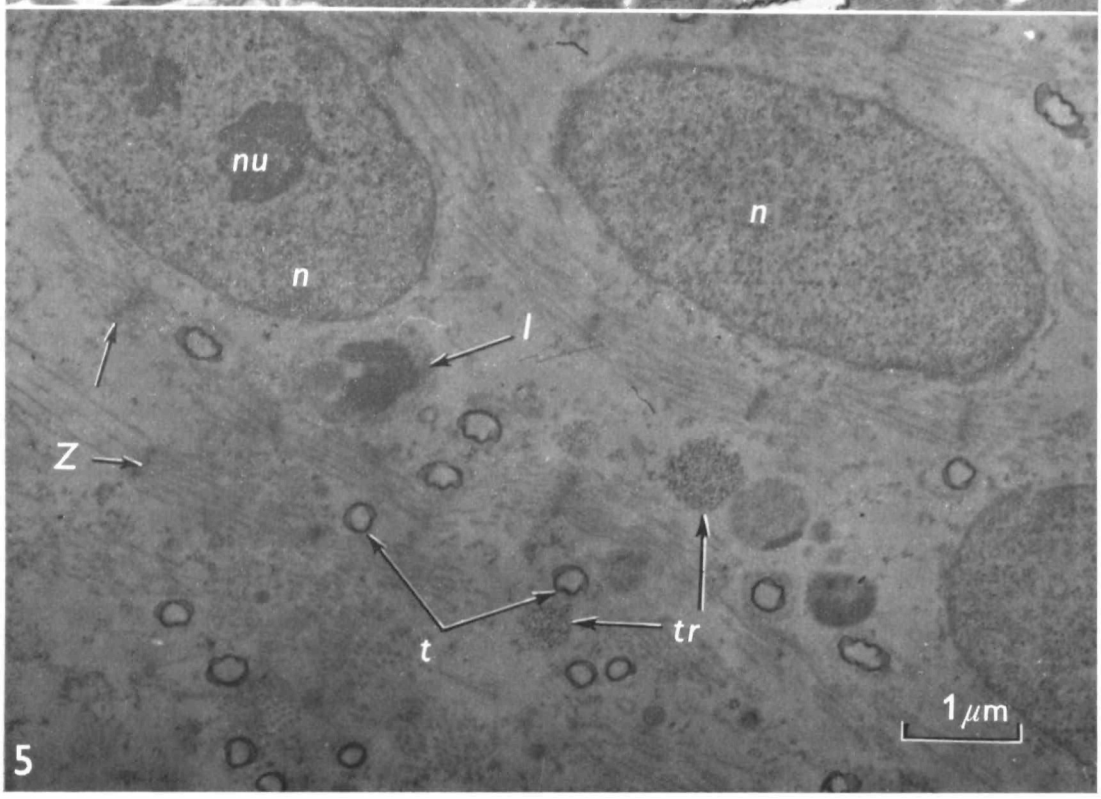
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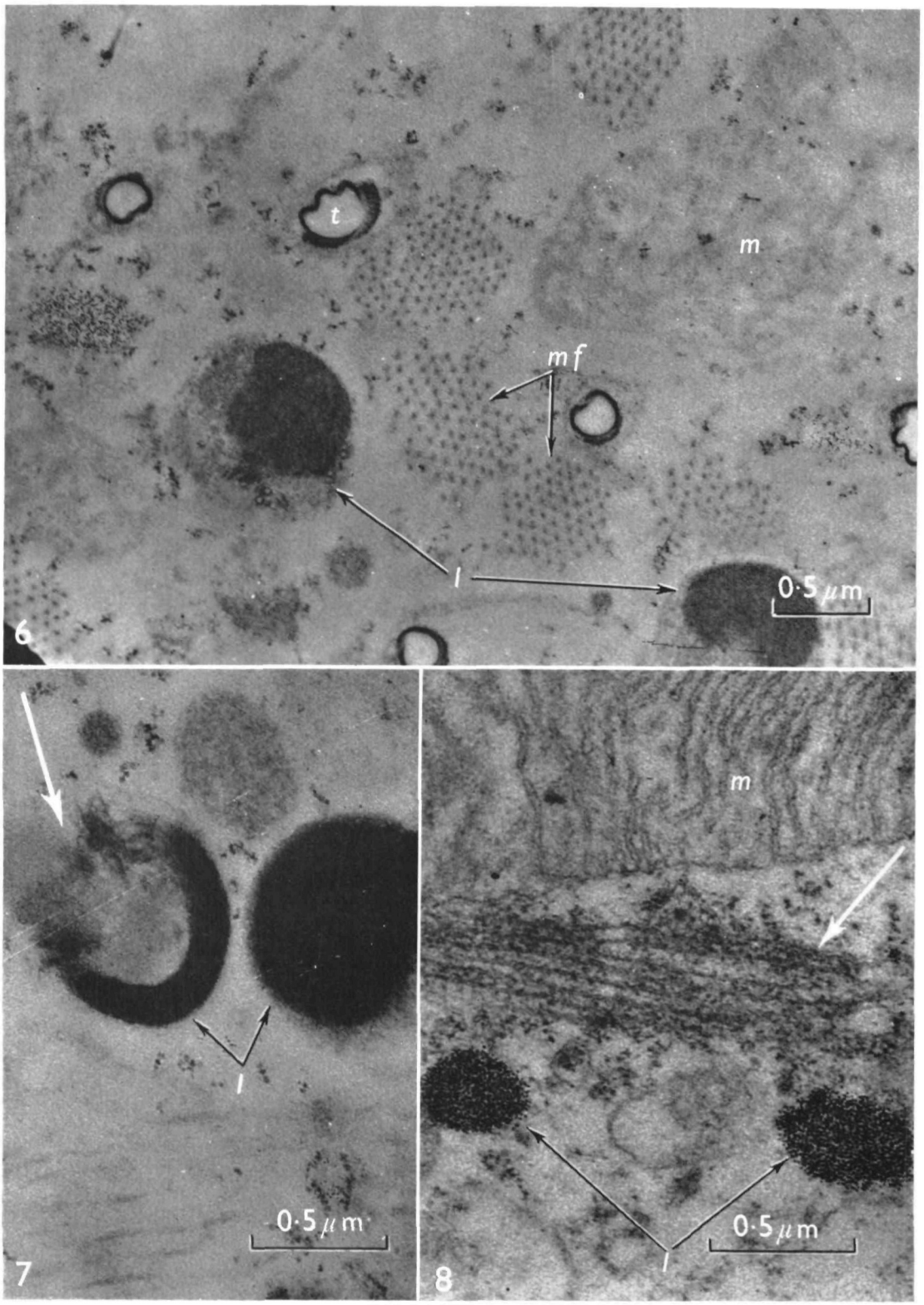
Fig. 6. Transverse section of the 96-h female flight muscle. Many of the fibrils have completely degenerated and those that persist have only a few myofilaments (*mf*). Many lysosomes (*l*) are still present. (*m*, mitochondrion; *t*, tracheole.)

Fig. 7. Higher magnification of the 96-h female flight muscle showing a lysosome (*l*) in the process of lysis (arrow). Remnants of the filaments are seen below the lysosomes.

Fig. 8. Longitudinal section of the flight muscle of the male 96 h after introduction into the bark. Many filaments have disappeared. The remaining ones show signs of granulation (arrow). (*l*, lysosome; *m*, mitochondrion.)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