PROGRAMMED CELL DEATH—I. CYTOLOGY OF DEGENERATION IN THE INTERSEGMENTAL MUSCLES OF THE PERNYI SILKMOTH*

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Abstract—In the silkworm, *Antheraea pernyi*, the intersegmental muscles of the abdomen degenerate within 48 hr after the ecdysis of the moth from the old pupal cuticle. The process of breakdown was studied by light and electron microscopy. At about the 5th hour after ecdysis, the first trace of degeneration is seen as scattered areas of erosion of the myofibrils, accompanied by a slight contraction of the mitochondria. By the 10th hour after ecdysis, one can detect larger areas of degeneration and vacuolization, and an apparent extrusion of sarcoplasm. Electron micrographs at the 10th hour show degenerative myelin figures, shrunken mitochondria, and deposits of lipid near the mitochondria. Meanwhile, small, darkly staining bodies, presumed to be lysosomes, undergo swelling and apparent dissolution; in their vicinity, the myofilaments are eroded and destroyed. By the 15th hour after ecdysis, the muscle is flaccid, non-contractile, and reduced in volume by about one-third; the myofibrils and cross-striations become disorganized and disappear, except for the persistent Z-bands, to which remnants of the myofilaments are attached. The nuclei are pycnotic, the mitochondria greatly shrunken and degenerate, and the lysosome-like bodies rarely visible. The muscle has completely disappeared by the 48th hour. Meanwhile, the pre-synaptic portion of the neuromuscular junctions remains intact. The breakdown of the muscles can be accounted for by cytolysis brought about by what we interpret to be the rupture of lysosomes in the apparently viable tissues. This event is followed by the erosion and eventual loss of the myofibrils and by the degeneration of the intracellular organelles.

INTRODUCTION

METAMORPHOSIS is an orderly and predictable pattern of birth and death at the cellular level. At the outset of metamorphosis, many of the specialized tissues and organs of the larva break down, their substance being reinvested in the formation of specialized pupal structures. Later, the process repeats itself as the pupa is transformed into the imago. Further breakdown may take place after the completion of adult development to furnish energy-rich substrates or to provide materials for

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the maturation of the gonads. At each stage the breakdown is highly selective in that it spares all those tissues and organs which are needed for further significant functions.

These happenings are obviously prerequisite for the reconstruction of the organism during successive stages in metamorphosis. In the absence of any nutritional intake, the pupa must be constructed from the materials already present in the mature larva; the adult, from materials in the pupa.

As pointed out previously (WILLIAMS, 1961), there can be little doubt that the death of specific cells and tissues is a part of the 'construction manual' for the insect as a whole. The cells that will die have been programmed to do so. Therefore, their individual deaths represent the decoding and acting-out of a fresh, albeit final, bit of genetic information.

The orderly patterning of cell death is by no means limited to insects. GLUCKS-MANN (1951) cites over seventy instances in which cell death occurs as a normal developmental event. Well-known examples in the vertebrates include the resorption of the tail and opercular epidermis of tadpoles (WEBER, 1957) and of the right Müllerian duct of female chick embryos (BRACHET et al., 1958; DECROLY-BRIERS and BRACHET, 1959; HAMILTON, 1960). In the case of the chick embryo, SAUNDERS and his collaborators (1957, 1962) have emphasized the key role which cell death plays in shaping the contours of the wings and legs.

The literature pertaining to insects is replete with references to cell death and tissue dissolution. BODENSTEIN (1943) has described the destruction of the salivary glands in the Blatteria. The breakdown of the prothoracic glands has been studied in Coleoptera (SRIVASTAVA, 1960), Lepidoptera (REHM, 1951) and Hemiptera (WIGGLESWORTH, 1955). In the higher Diptera and Hymenoptera virtually all the larval tissues except the Malpighian tubules are swept away to nourish the imaginal disks which form the pupa and adult (PÉREZ, 1910; TIEGS, 1922; OERTEL, 1930; ROBERTSON, 1936; STAY, 1953; BISHOP, 1958). The degeneration of the flight muscles of adult insects has been a recurrent object of study in such diverse forms as queen ants (JANET, 1907), parasitic flies (MERCIER, 1920, 1924, 1928; MERCIER and POISSON, 1923), mosquitoes (HOCKING, 1954), aphids (JOHNSON, 1953, 1957, 1959), locusts (WIESEND, 1957), and termites (FEYTAUD, 1912). The breakdown of the intersegmental muscles of larvae has been described in the Lepidoptera (BLAUSTEIN, 1935), Coleoptera (MURRAY and TIEGS, 1935), Diptera (HULST, 1906; PÉREZ, 1910; ROUBAUD, 1932; EVANS, 1936; ROBERTSON, 1936), Hymenoptera (TERRE, 1899), and Hemiptera (WIGGLESWORTH, 1956). Very recently, STEGWEE and his co-workers have described physiological and cytological studies of the temporary resorption of the flight muscles of diapausing potato beetles (STEGWEE et al., 1963; STEGWEE, 1964).

Of special interest to the present study is KUWANA's (1936) finding that the intersegmental muscles of the abdomen of Bombyx mori break down shortly after the emergence of the adult moth—a phenomenon which was subsequently rediscovered in saturniids by FINLAYSON (1956) and WILLIAMS (unpublished observations). In brief, within 15 hr after the escape of the adult moth from its pupal

exuviae, the intersegmental muscles are flaccid and poorly contractile. By the end of 48 hr, they have completely disappeared.

It is this particular phenomenon which is the focus of the present series of communications. We propose to show that the breakdown of the muscles is compounded of a sequence of events—an endocrine potentiation, a biochemical preparation, and an ultimate activating signal administered by the central nervous system. The present communication addresses itself to the breakdown of the muscle, itself, as studied by light and electron microscopy.

MATERIALS AND METHODS

The experiments were performed on female *Antheraea pernyi*. The cocoons were purchased from dealers and stored at 2–3°C. Pupae were removed from their cocoons, placed at 25°C and 60% relative humidity, and allowed to develop into adults. The moment of complete escape from the pupal exuviae was noted to within 0·5 hr at 25°C.

In the preparation of the muscles, the abdomen was removed, cut along the dorsal midline, and pinned in a spread and slightly extended position to plasticine under insect Ringer (EPHRUSSI and BEADLE, 1936). The viscera and overlying fat body were removed. The central nerve cord was stimulated electrically; if the muscles failed to contract, one or two fibres were mechanically stimulated and, finally, cut with microscissors in order to test their contractility. The saline was then removed, and ice-cold saline added to chill the preparation. After 2 min this was drained and ice-cold fixative added.

The histological techniques of FEDER (1960, and personal communication) were employed. The pinned preparation was placed in an ice bucket in a fume-hood. One or 2 ml of ice-cold 10% acrolein in xylene was poured directly onto the preparation. After 5 min, the entire abdomen was placed in 5 ml of ice-cold acrolein in xylene and kept at 0–2°C overnight. The entire preparation was dehydrated by exposure to several changes of equal parts of methanol and methoxyethanol; 100% ethanol; and 100% n-propanol, over a total period of 36 hr. The initial solution was used at 0°C and the others at −20°C. The dehydrated preparation was returned to room temperature. The dorsal muscles were isolated and embedded in glycol methacrylate and then later polymerized at 60°C.

Sections were cut at 1·5 μ on a dry glass knife in a Porter-Blum microtome. Each section was picked up with forceps and spread on a drop of water on glass slides. The preparations were air-dried and stained directly with haematoxylin-eosin or toluidine blue-acid fuchsin.

Tissue prepared for electron microscopy was processed in the following manner. Approximately 1 ml of phosphate-buffered osmium (MILLONIG, 1961a) at 0°C was dropped onto the cleaned specimens mentioned above. After 5 min, fibres of the dorsal band were dissected free and cut into small pieces. They were placed in the ice-cold osmium solution for 2 hr, then dehydrated and embedded in Epon according to the procedure of LUFT (1961). Silver or gold sections were cut on a diamond knife in a Porter-Blum microtome and stained with 1% uranyl acetate

and lead hydroxide (KARNOVSKY, 1961; MILLONIG, 1961b). Sections were collected on uncoated copper grids and examined with a Siemens Elmiskop I electron microscope. For study with the compound microscope, additional sections from the same blocks were cut at 1 μ and stained with Azur II and methylene blue (RICHARDSON et al., 1960).

RESULTS

General morphology

The muscles in the diapausing pupa consist of three paired, segmented bands extending from the anterior margin of the 3rd or 4th abdominal segment through the posterior margin of the 6th. Anterior and posterior extensions of these bands are present in the larva but degenerate shortly after pupation (FINLAYSON, 1956, 1960). In A. pernyi, the widest pair of longitudinal bands is dorsal to the spiracles. Each of these contains approximately 110 individual fibres arranged in a double layer. Two bands lie ventral to the spiracle on each side: the wide lateral band containing approximately ninety fibres, and the narrow ventral band consisting of approximately seventeen fibres, the latter just lateral to the nerve cord. The muscles can produce a tension of about 13 g and resist elongation by a hydrostatic pressure of 0·9 atm.

Cytology of the intersegmental muscles in diapausing pupae

The muscles at this stage are well preserved and show the typical organization of cross-striated muscle. The conspicuous sarcolemma—identified in electron micrographs as the basement and plasma membranes—is somewhat buckled in the slightly contracted fibre shown in Fig. 1. Each fibre is about 300 μ in diameter and consists mainly of longitudinal elements (fibrils), about 5 μ in diameter. The sarcomeres, which show conspicuous Z-bands, are about 3 μ long. The sarcoplasm contains numerous oval nuclei, approximately 10–12 μ in long diameter, in which the chromatin tends to be clumped just internal to the nuclear membrane. The nucleoli are inconspicuous. Ovoid or rod-like mitochondria (sarcosomes), 2–3 μ long, may be detected between the fibrils.

Muscles immediately after adult ecdysis

Figure 2 shows a typical muscle fibre at this stage. Its diameter of 120–170 μ is substantially reduced from the condition in the pupa. There is little shrinkage and the fibrils, with their conspicuous Z-bands, are tightly packed. The sarcolemma is thinner and less distinct than previously. The nuclei are lobate and have enlarged by about 40–100 per cent. Nucleoli are clearly seen and the clumps of basophilic material are now scattered throughout the nuclei. The mitochondria are far larger and more elongate than in the pupa.

Figure 8, an electron micrograph, demonstrates that the mitochondria are also markedly convoluted. The largest proved to be 8 μ long—a threefold increase. Narrow regions of glycogen-containing sarcoplasm lie between the myofibrils
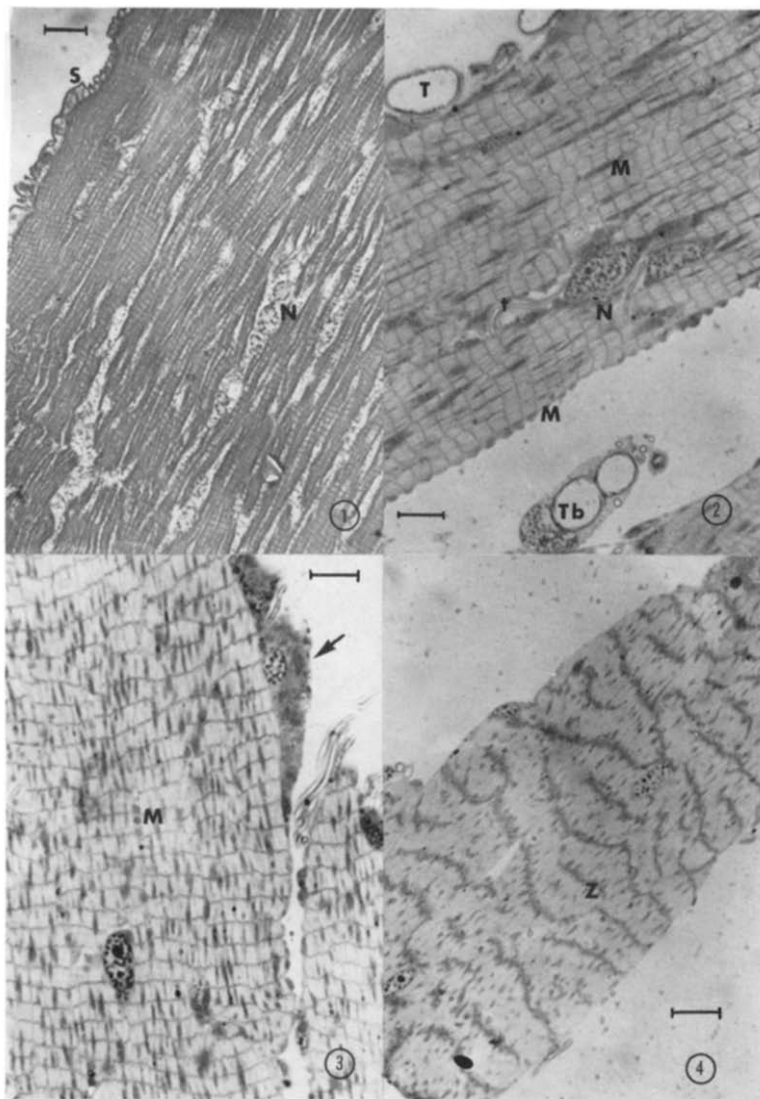
FIG. 1. Section, 1·5μ thick, of a muscle fibre of a diapausing pupa. × 40 objective. The thick sarcolemma sheath (S) is buckled. Mitochondria are barely visible where fibre has separated during dehydration. Note small amount of chromatin in nuclei (N). Acrolein fixation, glycol methacrylate embedding. Scale indication = 20 μ. N.B. In the case of all figures, unless otherwise indicated, all sections are longitudinal sections of dorsal intersegmental muscles of *Antheraea pernyi*.

FIG. 2. Section of a muscle fibre fixed immediately after ecdysis. Oil immersion objective. Nuclei (N) have one or two nucleoli. Mitochondria (M) are flattened and elongate except where they lie between the sarcolemma and the myoplasm. Note closely oppressed trachaea (T) and the tracheoles (t) penetrating the muscle. A tracheoblast (Tb) is at the bottom of the picture. Osmium, Epon. Scale indication = 10 μ.

FIG. 3. Muscle fibre fixed 5 hr after ecdysis. Oil immersion objective. Mitochondria (M) are either elongate or ovoid. The arrow indicates a neuromuscular junction. Osmium, Epon. Scale indication = 10 μ.

FIG. 4. Slightly oblique section of muscle fibre 10 hr after ecdysis. Oil immersion objective. Note disorganization of Z-bands (Z). Osmium, Epon. Scale indication = 10 μ.

Fig. 5. Section of muscle fibre fixed 10 hr after ecdysis. Oil immersion objective. Note enlarged sarcoplasmic region at periphery of mucle (arrow). This extends a considerable distance along the fibre and is evidently not a neuromuscular junction. Note also disruption of Z-bands. Osmium, Epon. Scale indication = 10 μ.

Fig. 6. Muscle fibre fixed 15 hr after ecdysis. Oil immersion objective. The nuclei range from nearly normal to highly pycnotic (large arrow). Nucleoli are very large. The mitochondria (M) are round and condensed. Numerous small dark bodies (small arrows) may be seen. A blood cell lies near the lower edge of the photograph. Osmium, Epon. Scale indication = 10 μ.

Fig. 7. Oblique section of muscle fibre fixed 25 hr after ecdysis. Little or no residual organization remains. Regions staining with haematoxylin (N) are remains of nuclei. Phagocytes (small arrow) invade the tissue. The large arrow points to a remnant of a neuromuscular junction. Acrolein, glycol methacrylate. Scale indication = 20 μ.

Fig. 8. Electron micrograph of muscle fibre of a moth immediately after ecdysis. A complex neuromuscular junction, with synaptic vesicles (V) in the axon of the nerve, is seen at the upper left of the photograph. Several osmiophilic, finger-like objects lie in the post-synaptic region (arrow). In the upper right, a tracheoblast lies apposed to the fibre. The latter is bounded by a 'sarcolemma' consisting of a basement membrane (B) and a plasma membrane (C). The nuclei contain numerous chromatin patches. A number of irregularly shaped mitochondria (M) are seen. The longitudinal myofilaments and Z-bands are prominent. A lysosome-like object (L) lies near the nucleus. Scale indication = 1 μ.

Fig. 9. Electron micrograph of muscle fibre from a moth immediately after ecdysis. Numerous lysosome-like organelles (L) are present. One of the dark bodies (arrow) is elongate, like the 'fingers' seen in the preceding figure. Note the inflated cisterns of sarcoplasmic reticulum (R), some of which contain lipid inclusions (P). Small regions of glycogen granules (G) are scattered throughout the fibres. Scale indication = 1 μ.
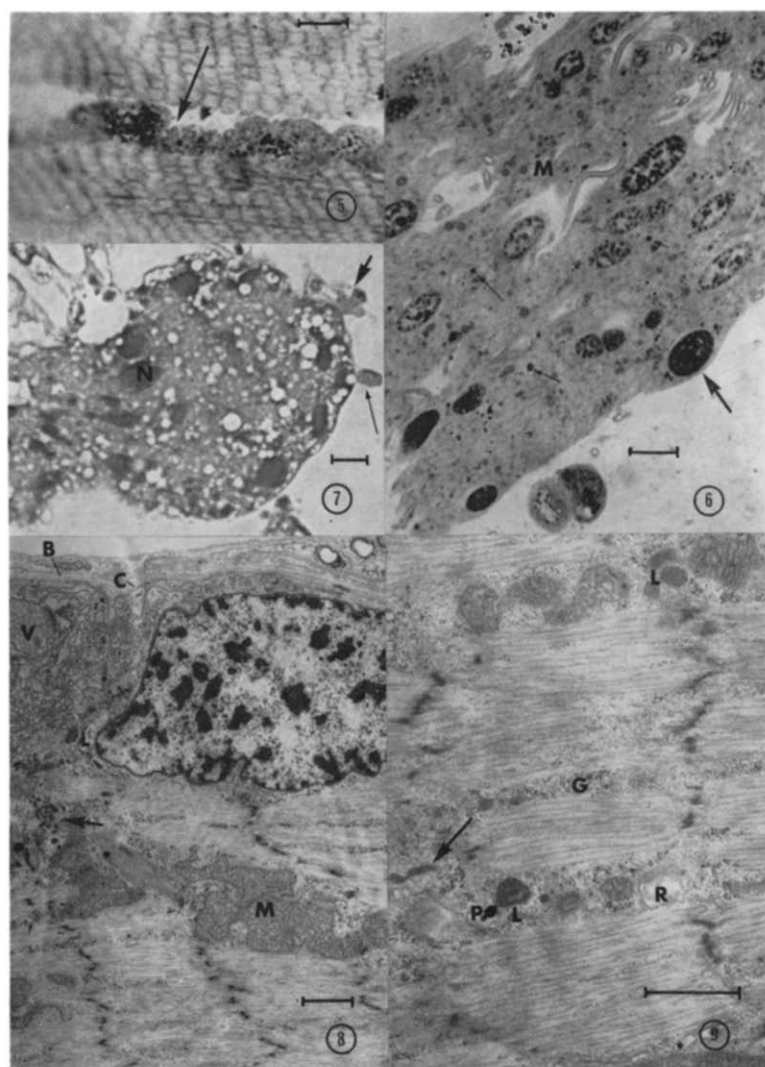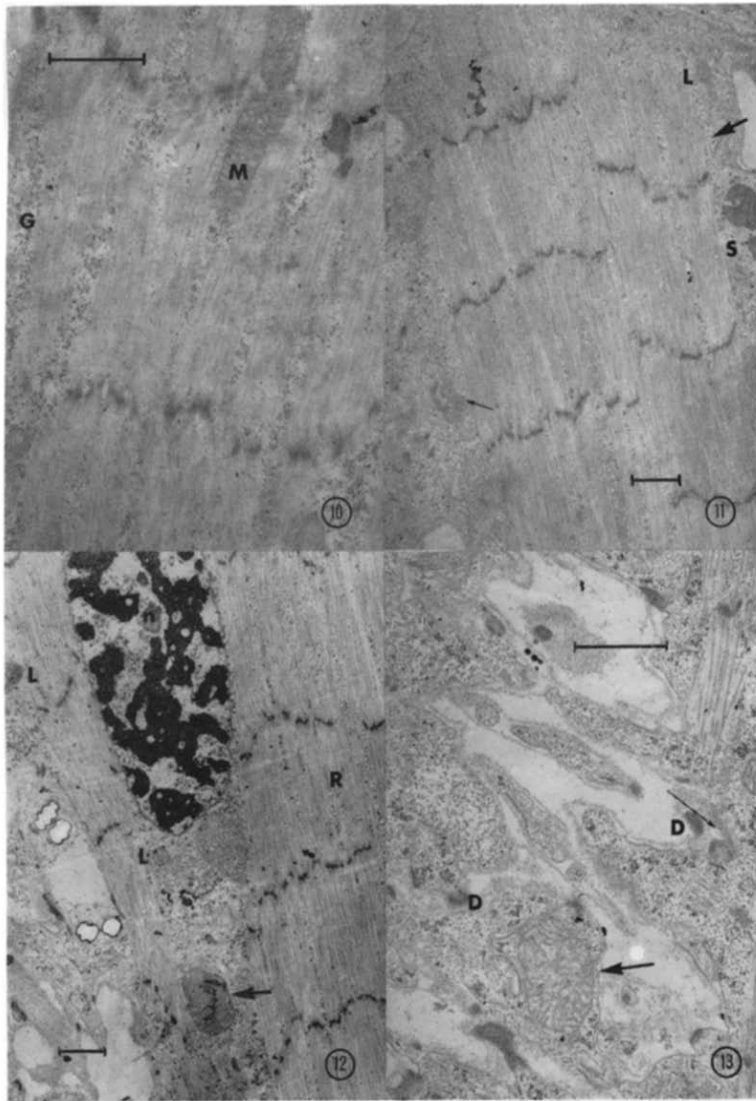
FIG. 10. Electron micrograph of muscle fibre 5 hr after ecdysis. Mitochondria (M) and glycogen granules (G) are prominent. Scale indication = 1 μ.

FIG. 11. Electron micrograph 10 hr after ecdysis. Stellate bodies (S) are seen next to mitochondria. Lysosome-like objects (L) and degenerating mitochondria (small arrow) are frequent. Some filaments lie freely in the increasing region of non-filamentous sarcoplasm (large arrow). Scale indiction = 1 μ.

FIG. 12. Electron micrograph of muscle fibre 10 hr after ecdysis. Note vacuolation of the large amount of chromatin in the nucleus. The paler object (n) is a part of a nucleolus. Lysosome-like particles (L) are common. A large convoluted object (arrow), possibly a mitochondrion, is present. The basement membrane is highly infolded. The interior of the fibre shows increased numbers of glycogen granules and an occasional disoriented myofilament. Sarcoplasmic reticulum retains normal appearance in the A band region. Scale indication = 1 μ.

FIG. 13. Electron micrograph of the edges of two fibres 10 hr after ecdysis. The sarcoplasm is deeply infolded and appears to be pinching off material. A mito-chondrion (large arrow) is nearly completely separated from the fibre. The infolding (small arrow) penetrates deep into the fibre. Desmosomes (D) appear to be disinte-grating. Scale indication = 1 μ.
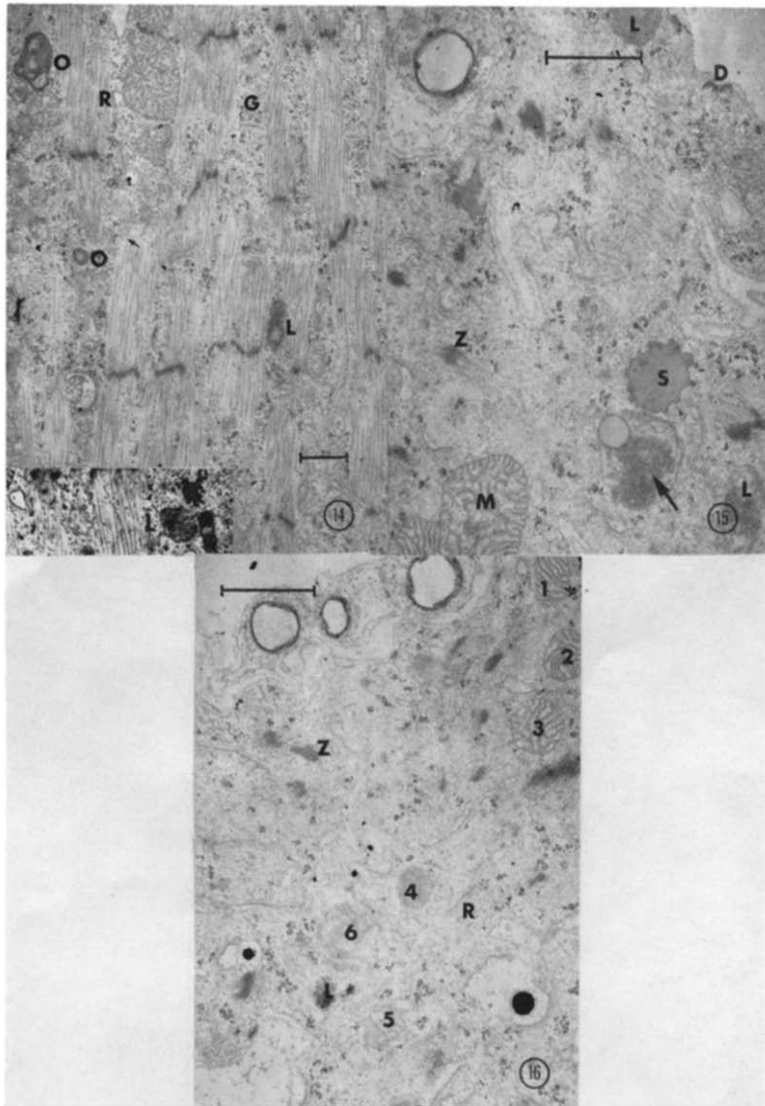
Fig. 14. Electron micrograph 10 hr after ecdysis. Degeneration is well under way. Many myofilaments have disappeared and are replaced by glycogen granules (G) and myelin figures (O). The sarcoplasmic reticulum (R) shows cisternae. Lysosome-like bodies (L) are more diffuse. Several mitochondria appear to be shrunken but otherwise normal. Myofilaments are adrift in the cytoplasm (arrow). Scale indication = 1 μ.

Fig. 15. Electron micrograph 15 hr after ecdysis. Large, diffuse lysosomes (L) and degenerating mitochondria (M) are present. One stellate body (S) lies next to an object (arrow) with a vacuole which may be a degenerating mitochondrion. A few remnants of Z bands with a few attached filaments are seen. A desmosome (D) remains intact. Scale indication = 1 μ.

Fig. 16. Electron micrograph of a muscle fibre from a moth 15 hr after ecdysis. The orientation can be determined from the remnants of the Z-bands (Z) and the position of the sarcoplasmic reticulum (R). A possible sequence of mitochondrial degeneration is indicated by the numbers 1 through 6. The last three may correspond to lysosomal remnants. The figure marked L is clearly related to the lysosome-like bodies. Scale indication = 1 μ.

(Figs. 8 and 9). On each fibre the peripheral sarcoplasm is approximately 0·25 μ thick and contains oval, osmiophilic, membrane-limited organelles 0·3 μ in length. These lysosome-like objects are also found between the fibrils in the interior of the fibre (Fig. 9, L), along with larger structures with eccentric vacuoles (Fig. 8). Droplets of lipid-containing material are occasionally encountered within enlarged regions of sarcoplasmic reticulum (Fig. 9, P).

The myoneural junctions are filled with synaptic vesicles while the junctions themselves are complexly folded. Elongate dense vesicles are found in the post-synaptic region (Fig. 8, arrow).

Muscles 5 hr after ecdysis

The compound microscope fails to detect any noteworthy changes during the first 5 hr after ecdysis (Fig. 3). But in electron micrographs one can observe definite changes in the muscle ultrastructure at this time (Fig. 10). The mitochondria are rod-shaped rather than convoluted and are somewhat smaller than 5 hr earlier. The myofibrils are diminished in diameter, thereby enlarging the peripheral sarcoplasm to approximately 0·5 μ. Moreover, localized concentrations of concentric or lamellar membranes are seen for the first time.

Muscles 10 hr after ecdysis

Within the first 10 hr after ecdysis the muscle fibres decrease 40 per cent in cross-sectional area. The fibre structure, as seen in Figs. 4 and 5, is less compact than at ecdysis. The oval nuclei stain intensely with haematoxylin or toluidin blue. Striations are occasionally indistinct, and small vacuoles are encountered. In osmium-fixed sections, the Z-bands are disorderly and occasionally interrupted. Wide sarcoplasmic regions border some of the fibres (Fig. 5).

At the higher magnification of the electron microscope (Figs. 11 through 14), the interruption of the Z-bands can be accounted for in terms of the histolytic changes in the fibre. More than half of the fibre area is occupied by disrupted areas which are filling with glycogen granules. Myofilaments lie askew and disoriented in the fibrils. Fibrils have disappeared from the outer margins of the fibre, and the width of the peripheral sarcoplasm, though variable, has greatly increased. In many regions the basement and plasma membranes are highly infolded, so that the peripheral sarcoplasm appears to be pinching off from the fiber. The plasma membrane is locally disrupted (Figs. 11 and 13). The mitochondria, now oval in shape, are one-third their length at ecdysis. Many show degenerative changes.

Stellate, lipid-containing structures are associated with the shrinking mitochondria. The lysosome-like bodies, always associated with regions of degeneration, are larger but less numerous than previously. Their membranes are disrupted, and their boundaries are indefinite. Diffuse regions of heavy staining may represent a later stage in their evolution (Figs. 11, 12, and 14). Numerous membrane-limited structures are present: multivesicular bodies, concentric lipoprotein lamellae of different sizes and shapes (myelin figures), and a few, small, membrane-limited particles. Swollen vesicles or sarcoplasmic reticulum are seen, as are droplets of

lipid within cisternae. A few nuclei stain intensely with uranyl acetate. The chromatin is in places diffuse and slightly vacuolate (Fig. 12).

Muscles 15 hr after ecdysis

Muscle fibres which present the histological picture illustrated in Fig. 6 are totally flaccid and non-contractile. Their cross-sectional area is half that in the freshly emerged adult. Phagocytes are occasionally seen near the muscle surface, though they are not particularly associated with degenerating areas. Striations are diffuse or absent. Nuclei are often heavily stained and pycnotic. The muscle contains vacuoles of various sizes. The mitochondria, round and contracted to a diameter of 2 μ, are fewer in number than at any previous time. As illustrated in Figs. 15 and 16, the cristae of the mitochondria are ordered in a more parallel fashion, and occasionally terminate in diffuse regions. A possible sequential progression of mitochondrial degeneration is indicated in Fig. 16.

Almost no fibrillar structure remains. A few slender filaments trail off the isolated Z-bands and are seen infrequently throughout the body of the fibre. The glycogen granules are now scattered and often clumped. The sarcoplasmic reticulum is now dispersed between the remaining Z-bands but is otherwise similar to that seen before the onset of degeneration.

The stellate lipid bodies associated with the mitochondria are larger and more numerous than they were at 10 hr. A few membranous bodies remain but are disorganized and difficult to classify. Likewise, the small, osmiophilic, lysosome-like bodies are diffuse and frequently almost unrecognizable. Two synapses were encountered and looked normal, though the dense post-synaptic vesicles were not observed in the degenerate fibre.

Muscles more than 15 hr after ecdysis

Material at this late stage was examined only by light microscopy. As illustrated in Fig. 7, phagocytes are present throughout the degenerating tissue, which gradually loses all its residual organization. Twenty-five hours after ecdysis, the muscle is a structureless, acidophilic mass, while the nuclei have become disorganized regions of basophilia. By the 50th hour, the site of the muscles may be identified only by tracing the nerves to a hyaline sheath—the remnant of the basement membrane and the now fluid-filled tracheoles.

DISCUSSION

The intersegmental muscles are the motive power of ecdysis. On these occasions, their vigorous contraction generates an internal turgor which forces haemolymph into the thorax and thereby assists in the rupture of the old cuticle. Then, after the ecdysis of the adult, the turgor propels haemolymph into the wings to expand them. Once these functions have been served, the muscles commonly degenerate. Thus, 10 hr after the ecdysis of A. pernyi, the muscles in question are unresponsive to efferent nerve stimuli; by the 15th hour, they are unresponsive even to direct stimulation.

The breakdown of the muscles can be accounted for by cytolysis brought about by what we interpret to be the rupture of lysosomes in the apparently viable tissue. This event is followed by the erosion and eventual loss of the myofibrils and by the degeneration of the intracellular organelles. We shall now consider these happenings in further detail.

Activation of lysosomes

Numerous membrane-limited osmiophilic bodies, approximately 0.3μ in diameter, are seen in the muscles of the freshly emerged moth. These organelles resemble structures seen in vertebrate tissues which have been considered (PELLE-GRINO and FRANZINI, 1963) and demonstrated (ESSNER and NOVIKOFF, 1960, 1961) to be lysosomes. The latter swell, the limiting membrane ruptures, and the contents present a diffuse area which remains identifiable until 15 hr after ecdysis. Biochemical studies (LOCKSHIN and WILLIAMS, in preparation) indicate that the organelles in question are, indeed, lysosomes.

The manner in which the lysosomes are activated is reminiscent of their behaviour in denervated rat muscle (PELLEGRINO and FRANZINI, 1963) and in induced toxic conditions, such as carbon tetrachloride poisoning (BEAUFAY and DE DUVE, 1957; BEAUFAY et al., 1959) or anoxia (DE DUVE and BEAUFAY, 1959), where they rupture and release their enzymes to the cytoplasm. It differs from the situation in rat liver (ASHFORD and PORTER, 1962), tadpole tail muscle (FRANZINI, personal communication), and differentiating embryonic limbs (JURAND, 1964) where the lysosomes engulf and destroy other cell organelles without breaking down. The appearance of the insect lysosomes in the electron microscope suggests that either the limiting membrane is dissolved or that it bursts under osmotic pressure. In either case, the mechanism causing their activation is unexplained.

Erosion of myofibrils

Destruction of the muscle protein, characterized by the disappearance of myofilaments and their replacement by glycogen granules, spreads from the vicinity of the lysosomes. It is particularly evident at the periphery of the fibre. The Z-bands resist attack.

Approximately 10 hr after ecdysis, the periphery of the muscle infolds markedly and appears to discard large droplets into the haemocoele. This material, which retains staining properties similar to those of the muscle, is apparently engulfed by phagocytes.

Other cytoplasmic organelles

The various cell organelles undergo degenerative changes concurrently with that of the myofilaments. The most striking alteration was the shrinkage of the mitochondria to one-quarter their length at ecdysis, accompanied by the formation of a star-shaped deposit of lipoprotein. STEGWEE and his co-workers, who observed similar formations in the flight muscle of hibernating *Leptinotarsa* (STEGWEE et al., 1963), interpreted these objects as degenerate mitochondria. Our evidence suggests that they are more probably material jettisoned by the mitochondria.

Pleiomorphic lipoprotein lamellae, such as those formed during the first 10 hr after ecdysis, are characteristic of degenerating tissue (WEISS, 1955; FRIEDMANN, 1958, WADDINGTON and OKADA, 1960; DAVID and KETTLER, 1961). They evidently represent a concentration of membranes and other fairly stable elements, but they are eventually destroyed as well.

The nuclei, notably sensitive to other forms of cytoplasmic alteration, are resistant to the changes going on within the cytoplasm. Only after degeneration is well advanced do large numbers of nuclei become pycnotic, and some appear to be unaffected even 20 hr after ecdysis. In *Rhodnius* and *Leptinotarsa* (WIGGLES-WORTH, 1956; STEGWEE *et al.*, 1963) in which certain muscles are resorbed and later regenerate, the nuclei are similarly undisturbed by the marked cytoplasmic changes which take place. The muscles of *Antheraea*, however, are incapable of regeneration, even if the moth is induced to moult (LOCKSHIN and WILLIAMS, 1964).

In view of the controlling influence of the nervous system in flight muscle formation (WILLIAMS and SCHNEIDERMAN, 1952; NÜESCH, 1953) and in long-term maintenance of intersegmental muscle (FINLAYSON, 1956), it is of interest that the pre-synaptic region of the neuro-muscular junction does not degenerate, even though all recognizable postsynaptic structures disappear.

The intersegmental muscles, in brief, are destroyed through the intervention of lysosomes. Their activation results in the digestion of the myofilaments, contraction and destruction of the mitochondria, and deposition of lipoprotein lamellae throughout the fibre. The controlling mechanisms which precede this event are described in the following publications in this series.

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