MUSCLE ATTACHMENT RELATED TO CUTICLE ARCHITECTURE IN APERTYgota

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SUMMARY

In Apterygota muscles are attached to the cuticle by a series of discrete structures. The junction of the muscle and epidermal cells is demarcated by regular interdigitations of the two tissues, with desmosomes lining these processes. Within the epidermal cells, microtubules link up the desmosomes of the interdigitated region with dense material associated with cone-like depressions in the apical plasma membranes of the epidermal cells. Each of these 'conical hemidesmosomes' is situated opposite a pore canal. From within each cone, an electron-dense 'muscle attachment fibre' extends up the corresponding pore canal through the procuticle and is inserted on the epicuticle. There is no direct link between the microtubules and the muscle attachment fibres. The muscle attachment fibres are slightly elliptical in cross-section, and are twisted, this twist being in phase with the orientation of the chitin-protein microfibrils forming the lamellae of the procuticle. The attachment fibres are straight, and not helically arranged; patterns obtained in oblique sections of procuticle including these structures support the twisted ribbon model of pore canal shape. The cuticle, particularly in Thysanuran and Dipluran intersegmental membrane, displays the parabolic patterning typical of softer insect procuticle and procuticle deposition zones. The epicuticular insertion of the muscle attachment fibre is characterized by a pit in the cuticulin layer, the fibre passing through the middle of this pit.

The microtubule-associated conical hemidesmosomes appear to be cytoskeletal in function. The muscle attachment fibres are rigid structures which are not digested by the moultling fluid enzymes. New muscle attachment fibres may only become attached to the epicuticle during its formation. The structures described in regions of muscle attachment in Apterygota are similar to those recorded for other arthropods.

INTRODUCTION

The attachment of muscle to the insect cuticle provides the necessary mechanical connexions required for muscular activity. Although muscles were at first thought to attach directly to the cuticle, it has been shown that it is more general for muscles to terminate at the base of the epidermal cells (Weber, 1954; Auber, 1963; Shafiq, 1963; Lai-Fook, 1967). The epidermal cells are modified structurally in this region, appearing attenuated, whilst the cytoplasm is drawn into plasma fibres, or 'tonofibrillae' (Richards, 1951; Weber, 1954). These tonofibrillae may extend into the procuticle, and may be chitinous in this region (Wigglesworth, 1965).

The tonofibrillae were originally thought to terminate before reaching the epicuticle and are indeed shown as such by Bouligand (1962) in a copepod crustacean. It has, however, recently been demonstrated that the tonofibrillae passing through the...
procuticle do in fact extend into the epicuticle, and attach to the cuticulin layer in this region (Noble-Nesbitt, 1963a; Lai-Fook, 1967). The tonofibrillae and the cuticulin layer in this region appear electron dense in electron-microscopical studies of *Podura aquatica* (Collembola) and Lai-Fook (1967) found the tonofibrillae to be of the same electron density as the ‘dense’ layer of the epicuticle. During the preparation for the moult, the newly secreted cuticulin layer becomes attached to the old tonofibrillae at an early stage, the new insertions thus being the same as the old (Noble-Nesbitt, 1963b; Lai-Fook, 1967).

The nuclei of the epidermal cells in which tonofibrillae are present are displaced laterally by these structures (Weber, 1954). Whenever pigment is found in the region of muscle attachment, the granules are aligned between the tonofibrillae.

Further recent studies have shown in the Diptera (Auber, 1963), Hemiptera and Lepidoptera (Lai-Fook, 1967), that the tonofibrillae within the epidermal cytoplasm are composed of fields of microtubules. The microtubules link up the myo-epidermal junctions and the epidermo-cuticular junctions of the muscle attaching system. Similar findings have been recorded in the epidermal tissues in regions of muscle attachment in the Dictyoptera, Phasmida, Orthoptera and Coleoptera (A. C. Neville & B. M. Luke, unpublished observations), and in copepods (Bouligand, 1962, 1966).

The present study, then, is an attempt to correlate the structure of the muscle insertion in the Apterygota with that already reported for some Crustacea, and for several exopterygote and endopterygote orders. Three orders of apterygote insects are dealt with, the Thysanura, the Diplura, and the Collembola. A member of the last-mentioned order, *Podura*, has already been studied in this respect by Noble-Nesbitt (1963a, b), but the present findings warrant a new description of the muscle attachments in this order.

The study also attempts to establish the relationship between the epithelial microtubules and the cuticular tonofibrillae, the latter in relation to the pore canals and the molecular architecture of the procuticle, which has undergone drastic revision during the past few years (Bouligand, 1965; Neville, Thomas & Zelazny, 1968).

**Materials and Methods**

**Materials**

A single species of Collembola, *Entomobrya* sp., was collected amongst decaying vegetation in the Johannesburg district (family Entomobryidae). Three species of Thysanura were studied. *Ctenolepisma longicaudata* Esch., the common South African ‘fishmoth’, was collected amongst old books and other undisturbed corners of the department; *Monachina cf. schultzei* Silvestri was collected in the Kalahari Gemsbok National Park (Gordonia District, Cape Province). Both *Ctenolepisma* and *Monachina* belong to the family Lepismatidae. The third Thysanuran, *Machiloides spinipes* (family Machilidae) was obtained from the type locality, the Townbush Forest in the hills north-west of Pietermaritzburg in Natal. A single species of Dipluran, possibly *Anisocampa* sp. (family Campodeidae), was collected in the Woodbush Forest in the North Eastern Transvaal, near Magoebaskloof.
Light microscopy

Thick Araldite sections (0.5-1.0 μ) for light microscopy were stained in toluidine blue-pyronin B solution for exactly 30 sec on a hot plate at 60 °C, and rinsed in cold tap water (Ito & Winchester, 1963).

Electron microscopy

Specimens were anaesthetized with ether, and cut into blocks no more than 1 mm square in cold 5% glutaraldehyde-sodium cacodylate fixative, at a pH between 6.9 and 7.1. The blocks of tissue were then placed in fresh fixative at 4 °C, and fixed for an hour at this temperature.

Collembola were also fixed using the method of Noble-Nesbitt (1963a). This method gave best fixation. After immersing whole specimens in absolute alcohol for a few seconds, the specimens were fixed whole for 24 h at 4 °C in 5% glutaraldehyde-sodium cacodylate buffered fixative. The alcohol immersion removed adhering air bubbles, and prevented the collembolans from floating in fixative.

All blocks, or whole animals, were subsequently washed, with several changes, in a buffer solution of 0.33M sucrose in 0.1M sodium cacodylate, at room temperature, for 2 h.

Post-fixation was carried out in a 1% osmium tetroxide solution in 0.05M sodium cacodylate for 1 h at room temperature, and the tissue then dehydrated through a series of alcohols, cleared in propylene oxide (two changes of 15 min each) and finally embedded in Araldite (Glauert & Glauert, 1958).

Light straw to silver-grey sections were mounted on uncoated 300-mesh grids and double stained in lead citrate and uranyl acetate (Reynolds, 1963). All grids were examined in a Siemens Elmiskop IA at 80 kV.

RESULTS

Light-microscopical studies of thick Araldite sections stained with toluidine blue-pyronin B revealed the following: where the muscle cell and the epidermal cell came into contact, a dark wavy intervening layer was to be seen. Within the epidermal cells, cell inclusions, such as nuclei and pigment granules, were arranged in such a manner that it appeared as though filamentous structures passed amongst them. These linear structures were not resolved, but this region of the epidermal cell stained darkly. Within the cuticle, dark strand-like structures were seen directly opposite muscle insertions. Usually these were obscured by the dense-staining cuticle. Even with the light microscope, it can be seen that specialized structures occur in the cuticle at regions of muscle insertion.

The structures of the muscle-epidermal cell junction

Where the two tissues come into contact, they interdigitate. In the insects studied, the distinct basement membrane of the two tissues is lacking, although the intercellular spaces found in this region are filled with some form of connective tissue (Figs. 2, 3).
The dense cytoplasmic plaques of the desmosomes and hemidesmosomes in this region are about 200 Å in thickness, the intercellular space between two opposing outer leaflets of the triple-layered unit membrane at the desmosomes varying between

Fig. 1. Schematic representation of muscle attachment to cuticle in Apterygota

A. General view.

B. Detail of region indicated in A. The muscle attachment fibre traverses the procuticle within a pore canal into a pit-like depression in the cuticulin layer of the epicuticle. The attachment fibres is not connected directly with the cuticulin layer.

C. Similar area to B, but in oblique section.

D. Region at base of cuticle as indicated in A. Muscle attachment fibres can be seen originating in conical hemidesmosomes. Microtubules are inserted into the dense material of these hemidesmosomes. The inner leaflet of the plasma membrane appears to be thicker than the outer leaflet.

E. Base of epidermal cell, indicated in A at junction with muscle cell, showing part of a desmosome. Microtubules originate in the dense plaque associated with the interdigitations found in this region. The inner leaflets of the plasma membranes of both the epidermal and muscle cells appear thicker than the outer leaflets. A dense region of the intercellular matrix is found between the two hemidesmosomal components of the desmosome.
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200 and 300 Å (Figs. 2, 3). The material of the intercellular space appears to be oriented parallel to the adjacent plasma membranes (Fig. 3). The outer leaflets of the plasma membranes are less electron dense than the inner leaflets. The thickness of the triple unit plasma membrane is about 70 Å, the inner electron-dense leaflet being about 30 Å, the outer leaflet about 20 Å, and the intervening electron lucent space being approximately 20 Å in thickness. These sheet-like desmosomes completely cover the contact invaginations of the epidermal cells and the muscle cells, extending for many microns when the invaginations are seen in cross or longitudinal section (Fig. 3).

The intersegmental muscle myofilament arrangement tallies well with that described for Periplaneta intersegmental muscle (Smith, 1966). Around each myosin filament of about 190 Å in diameter, 12 actin filaments (about 70 Å in diameter) are arranged in a regular fashion (Fig. 4). Some of the myosin filaments appear to have a less dense core (Fig. 2).

The muscle cells appear to terminate against the base of the epidermal cells in regions of the Z-line. Due to this fact, only actin filaments appear to insert into the dense material of the muscle cell hemidesmosomes, or into the muscle cell side of the desmosomes.

Modifications within the epidermal cells in regions of muscle attachment

The most conspicuous characteristic of the epidermal cells at the point of attachment of muscle is the large number of microtubules in the cytoplasm (Figs. 2, 5, 11, 12). The microtubules arise in the dense material of the sheet-like desmosomes on the epidermal cell side of the muscle cell-epidermal cell junction. These microtubules (forming the epidermal portion of the tonofibrillae) exclude all other cytoplasmic organelles in passing to the cuticle, with the exception of the ribosomes, and the occasional small mitochondrion. The nuclei of the epidermal cells are often rodlike, if present in these areas, with the long axis of the nucleus parallel to the direction of the tonofibrillae, and wedged between them.

The microtubules of the epidermal cells vary in diameter from 210 to 230 Å, and appear in all respects similar to those found in many other organisms. The microtubules give the impression of being tubular due to the less dense nature of the core, whilst the walls have a greater electron density. The microtubule walls have a thickness of about 50 Å, and the remaining non-dense core is about 120 Å in diameter (Fig. 3). Unlike the condition found by Gupta & Berridge (1966) and Bassot & Martoja (1966), where the microtubules often seemed to have a hexagonal packing when viewed in cross-section, the microtubules were packed in an irregular fashion in the apterygote epidermis (Figs. 2, 3). When sectioned longitudinally, the microtubules invariably run normal to the surface of the cuticle, i.e. parallel to the myofilaments of the attached muscle.

The substructure of the microtubules can be seen in Fig. 3. Granular subunits, about 40 Å in diameter, make up the dense cortical portion of the microtubule. It is difficult to resolve the actual number, but it may well be in the region of 13, as
suggested for the filamentous strands making up the microtubule in Heliozoa (Tilney & Porter, 1967).

Some of the microtubules, when sectioned transversely, appear to have a slightly denser axis (Fig. 3). This has been termed the 'axial density' by Gupta & Berridge (1966), or 'structure axiale' by Bassot & Martoja (1966), and has also been observed by Tilney & Porter (1967). It cannot be seen in longitudinal sections of the microtubules.

**The structures of the epidermal cell-cuticle junction**

The microtubules do not extend into the cuticle. In all cases, they terminate at the apical plasma membrane, from where an electron-dense fibre passes to the epicuticle. This extension of the tonofibrilla in the cuticle is called the 'muscle attachment fibre' in this discussion as it was found to be a distinct structure. It differs both in electron density and in dimensions from the pore canal filaments and other filamentous structures seen within the pore canals. It is most likely homologous to the tonofibrilla recorded by Noble-Nesbitt (1963a, b), and undoubtedly serves the same function.

The inner surface of the apical cell membrane of the epidermal cells is studded with 'conical hemidesmosomes'. These conical hemidesmosomes each consist of a single involution of the apical plasma membrane, these involutions being lined on the epidermal cell side of the plasma membrane with electron-dense material (Figs. 1, 5). In cross-section these hemidesmosomes appear as circular profiles, with an internal diameter of between 500 and 1000 Å (Fig. 5). The diameter varies in relation to the plane of sectioning, being greatest at the base, i.e. at the cuticle; and is smallest at the apex of the cone, i.e. furthest away from the cuticle. These conical hemidesmosomes are not as deep as the 'sockets' recorded by Lai-Fook (1967) in Hemiptera and Lepidoptera.

The microtubules traversing the epidermal cells are attached to the dense material of the cones from the apex to the base. The interior of the cone contains a dense amorphous granular strand which passes from the base of the cuticle to the epicuticle. One, or sometimes two, of these 'muscle attachment fibres' originate in each conical hemidesmosome (Figs. 1, 5).

The apical plasma membranes lie well back from the attachment fibres, being separated by spaces about 100 Å wide. In this region, also, the outer leaflet of the triple unit membrane is less distinct and thinner than the inner leaflet.

**Modifications within the procuticle**

The base of the muscle attachment fibre has a diameter of between 300 and 500 Å, and tapers off slightly as it passes through the procuticle, finally reaching a thickness of about 250 Å at the level of the cuticulin layer. Cross-sections of attachment fibres are generally slightly elliptical. The attachment fibres traverse the procuticle to the epicuticular surface through pore canals (Figs. 5, 6). Exceptions to this were occasionally seen. In the pre-exuvial cuticle of *Machiloides*, although pore canal filaments were seen within pore canals, many muscle attachment fibres traversed the procuticle.
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not within pore canals, a situation similar to that observed by Lai-Fook (1967) in Calpodes. Oblique sections of procuticle in regions of muscle attachment yield patterns of muscle attachment fibres similar to those of twisted pore canals (Neville et al. 1968). This would indicate that the muscle attachment fibres, like the pore canals themselves, are twisted through 180° per cuticular lamella. The attachment fibres within the conical hemidesmosomes are randomly oriented, their elliptical cross-sections displaying no regular orientation (Fig. 5).

In Machiloides and Monachina, more than a single attachment fibre may pass up each pore canal. This may be correlated with the number of these fibres found in each conical hemidesmosome, as it is probable that each conical hemidesmosome may supply a single pore canal. During the deposition of the pre-exuvial cuticle, these structures may be found together with pore canal filaments (about 150 Å in diameter) within the same pore canals. The pore canals are about 1000 Å in diameter, and although containing granular material near the epidermal cells, are generally filled with a light uniform substance in the outer procuticle.

The architecture of the procuticle in Apterygota is similar to that recorded in many other electron-microscopical studies of higher insects and Crustacea. The typical parabolic patterning of soft cuticle was observed in the arthrodial membranes, and deposition zones, of Thysanura and Diplura; Noble-Nesbitt (1963 a) observed similar patterning in the setal pads of Podura (Collembola). This patterning was first recorded in insects by Locke (1960) and has been suitably explained by Bouligand (1965). Parabolic patterning in regions of muscle attachment was confined to the deposition zone, being obscured in the outer procuticle, possibly due to some form of impregnation.

The epicuticular insertion

On reaching the epicuticle, the attachment fibres are approximately 250 Å in diameter, and appear to be attached to the cuticulin layer of the epicuticle. Noble-Nesbitt (1963 a, b) reports similar findings in Podura aquatica, and Lai-Fook (1967) in Rhodnius and Calpodes.

The dense attachment fibre does not merge with the cuticulin layer. This can be seen both in oblique transverse section (Fig. 9), where the fibre is surrounded by a clear disk, which is itself enclosed by cuticulin, and in sections normal to the surface of the integument, where a similar clear space about 100 Å across, flanks the muscle attachment fibre on both sides (Figs. 1, 8 and 9). The cuticulin layer is folded into small pits, into which the attachment fibres penetrate. Nowhere in the pit, which may be up to 1000 Å in depth, is the fibre attached directly to the cuticulin layer, always being separated by the clear region.

The pits in the cuticulin layer do not seem to be lined by an oriented lipid layer (Locke, 1961, 1964, 1966), although it is possible that this layer completely covers over the pits after the moult, when the old strands have broken away. The pits in the cuticulin layer possibly originate from a displacement of the normal deposition resulting from the obstruction caused by the attachment fibres. The stage of deposition
of the cuticulin layer in this region has, however, not been studied in the Apterygota up to date.

The clear region may be some form of cementing substance, providing the necessary anchorage for the muscles. It is also possible that the muscle attachment fibres are attached firmly to the pore canals in the procuticle by a similar binding substance.

Muscle attachment during the moult

Two species of Thysanura, Machiloides spinipes (Machilidae) and Monachina cf. schultzei (Lepismatidae), as well as a single species of Collembola, Entomobrya sp., were fixed at a stage when the epidermal cells were extremely active in preparation for the moult. All three species are similar in their muscle attachment structures. Ribosomes, which were observed during the intermoult closely associated with the microtubules, were seen aggregated into polyribosomal clumps during apolysis (Fig. 12). (Apolysis is the term coined by Jenkin & Hinton (1966) for the separation of the epidermal cells from the old cuticle prior to the deposition of the new cuticle.) These polyribosomes are most likely active in the protein synthesis of new cuticular material and of new microtubule subunits. The microtubules are less linear during apolysis, indicating that there may be a slight reduction in muscular tension during this stage (Fig. 12). Figures 10 and 11 show some of the features seen at the point of muscle attachment in Entomobrya during the moult preparatory phase. The retraction of the epidermal cells from the old cuticle at muscle attachments is very slight, probably being prevented by the rigid muscle attachment fibres.

The attachment fibres become attached to the new cuticulin layer as soon as it is deposited, and as the new pre-exuvial cuticle increases in thickness, so must new material be added to the fibres, to allow the increase in length necessary (compare Noble-Nesbitt, 1963b).

During the digestion of the old cuticle after the formation of the new epicuticle, the muscle attachment fibres remain relatively consolidated (Figs. 8, 10). The fibres are even resistant to digestion by the moulting fluid enzymes when surrounded by procuticle undergoing complete digestion, and are shed with the remains of the old cuticle (Fig. 8) (Wolfe, 1954; Noble-Nesbitt, 1963b). They are most conspicuous during apolysis, when they can be seen stretched across the exuvial space (Figs. 8, 10), linking up the old cuticle with the new. Fibres can be followed from the new pre-exuvial cuticle, across the new epicuticle, and into the exuvial space (Fig. 10). The muscle attachment fibres exposed to the action of the moulting fluid enzymes do appear, however, to have a slightly less ordered structure, being more diffuse and irregular (Fig. 8).

If there exists an untanned chitin/protein matrix within the fibres, then one would expect it to be removed by the enzymes of the moulting fluid. Any tanned cuticular, or cuticulin, component would not be affected by these enzymes. The less ordered nature of the muscle attachment fibres in the exuvial space may result in a weakening of these ‘tendons’, allowing them to be broken off from the new cuticle during emergence.
**DISCUSSION**

A notable feature at the interdigitated surface between the muscle and epidermal cells is the presence of sheet-like desmosomes. These desmosomes were seen in all three groups of Apterygota studied, and are to be expected, as epidermal cells at regions of muscle attachment undergo greater stress than elsewhere. These sheet-like desmosomes would have a skeletal function, and possess no significant structural differences from similar structures described by Bouligand (1962), Auber (1963), Shafiq (1963), and Lai-Fook (1967). The interdigitations would provide a greater surface area for desmosomal contact, as noted by Lai-Fook (1967).

The tonofibrillae described by the light microscopists were assumed to serve in strengthening the attachments of the muscle to the cuticle, providing firmer attachment than unmodified epidermal cells alone (see review by Richards, 1951). The tonofibrillae were seen to exhibit different chemical properties in the epidermal cytoplasm and in the procuticle. In the region of the epidermis the tonofibrillae appear basophilic, whilst they are acidophilic in the procuticle (Richards, 1951).

The present electron-microscopical study reveals that this chemical dissimilarity is supported by ultrastructural differences, the tonofibrillae in the procuticle consisting of amorphous fibres, here called 'muscle attachment fibres'. The cytoplasmic region of the tonofibril of the light microscopists consists of bundles of microtubules.

The former region of the tonofibril of the light microscopists, here called the muscle attachment fibre, has itself been termed the tonofibril in earlier electron-microscopical studies (Bouligand, 1962, 1966; Noble-Nesbitt, 1963a, b; Auber, 1963; Lai-Fook, 1967).

The muscle attachment fibres do not appear hollow in any of the apterygotes studied, disproving Noble-Nesbitt's idea that the tonofibrillae are inward hollow extensions of the cuticulin layer, which, at the end proximal to the muscle, form Y-shaped cones which receive bundles of myofilaments. The fibres are discrete structures separated from the cuticulin layer by a 100-Å space.

Another point which arises is whether the muscle attachment fibres are contractile. Richards (1951) and Wolfe (1954) maintain that the tonofibrillae (in the classical sense) are not contractile. A non-contractile structure would yield the greatest rigidity. Noble-Nesbitt (1963b) points out that during the preparation for the moult, the tonofibrillae (the portion in the procuticle) must be able to stretch to allow for the deposition of the new exuvial cuticle. The cuticle at points of muscle attachment does not move away from the epidermis to as great an extent as in other areas during apolysis. This would indicate that the fibres are still functioning. If the fibres are at all stretchable, one would expect them to be so during apolysis. This increase in length need only be very slight for a new epicuticle to be deposited.

One, or sometimes two, muscle attachment fibres travel up within a single pore canal. The pore canals have a greater density in regions of muscle insertion and do not branch. All pore canals in a region of muscle insertion possess attachment fibres, although other filaments may also be present within these pore canals.

The attachment fibres run a straight course through the procuticle, and therefore
cannot be helical. This linear configuration lends support to the twisted ribbon model of pore canal shape, as seen in locusts (Neville & Luke, 1968).

In oblique sections of Calpodes larval procuticle (figs. 7 and 14 in Lai-Fook, 1967), the muscle attachment fibres appear oriented in patterns corresponding to the parabolae produced by the chitin-protein microfibril orientation. The long axes of the elliptical cross-sections of these attachment fibres are arranged parallel to the general direction of the nearest surrounding microfibrillae. To obtain such a pattern, the elliptical muscle attachment fibres would have to be twisted, in phase with the change in microfibril orientation in the successive sheets of microfibrils deposited (see Neville et al. 1968). It would appear then that the muscle attachment fibres are first twisted by the microfibril crystallization in the deposition zone, and subsequently pore canals form around them in the procuticle proper. Even in the deposition zone, where parabolic patterns are visible, less electron-dense areas around the attachment fibres may be seen, the precursors of the distinct pore canals seen in the outer procuticle. Further support for the suggestion that the attachment fibres are twisted by the microfibril crystallization lies in the observation that the portions of the fibres within the conical hemidesmosomes are randomly oriented when seen in cross-section, their flattened elliptical shapes lying in random directions (Fig. 5).

Muscle attachment fibres are not formed by an aggregation of microtubules passing through the apical plasma membrane as suggested by Bouligand (1962, 1966), but are instead most likely structures formed from a secretion passed through the plasma membrane in the region of the conical hemidesmosomes.

One of the most characteristic features of regions of muscle attachment is the vast number of microtubules. The microtubules correspond to the cytoplasmic portion of the tonofibril of Wolfe (1954), Stuart & Edwards (1958), and Noble-Nesbitt (1963a), to the tonofilaments of Bouligand (1962) and Auber (1963), and to the tendinal fibres of Shafiq (1963); Lai-Fook (1967) correctly identifies these structures as typical microtubules.

Various functions have been proposed for the microtubules recorded in many organisms, and amongst these, cytoskeletal support seems relevant in the present discussion. The cytoplasmic tonofibrillae have for many years been compared with the tendons of vertebrates (Richards, 1951; Weber, 1954; Lai-Fook, 1967), whilst Gupta & Berridge (1966) have recently proposed a similar function for the groups of microtubules seen in the terminal cell of the blowfly rectum. The microtubules most likely lengthen during the preparation for the moult, as the epidermal cells may increase many times in thickness during this period. This swelling is a sign of metabolic activity, having been reported in the Thysanura, in the firebrat Thermobia (Watson, 1964), and is a general phenomenon in arthropods.

Hemidesmosomal cones, recorded in the apical plasma membrane of the epidermis in regions of muscle attachment, are not unique to the Apterygota. Similar structures have been recorded in the cuticle of copepod crustacea (Bouligand, 1962); in the foregut and hindgut epithelia of termites (Noirot-Timothée & Noirot, 1966); in the ejaculatory duct of male Locusta migratoria (Bassot & Martoja, 1966); and in dipteran epidermis (Auber, 1963; Gupta & Berridge, 1966). The last mentioned
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case is of internal cuticle, where muscles are attached to the junctional cells of the rectal papillae of Calliphora. Satir & Stuart (1965) have described a more specialized microtubule-associated structure, which they called a 'junctional organelle', in the sternal gland of Zootermopsis (Isoptera). This hemidesmosomal structure, although skeletal in function, is not associated with cuticle.

Each hemidesmosomal cone in the Apterygota lies adjacent to a single pore canal, with a muscle attachment fibre travelling from the inside of the cone into the pore canal (Fig. 1).

As suggested by Lai-Fook (1967) the plasma membrane of the hemidesmosomal cone provides a surface for the secretion of the muscle attachment fibre. The mechanical presence of these fibres may initiate the formation of pore canals around them. Pore canals in regions where no muscles are attached do not have hemidesmosomes associated with their bases.

As can be seen in Figs. 2 and 3, plasma membranes are distinct at muscle-epidermal cell junctions. This is contrary to Stuart & Edwards (1958) who state that the cell border at the point of apposition in Musca domestica breaks down. The present findings in Apterygota agree with those of more recent workers in this field.

New muscle attachments, such as those which form at the imaginal moult in many insects, may only become attached to the epicuticle if they form when the pre-exuvial imaginal cuticle is being deposited. Muscle attachments formed later are assumed to be attached to the procuticle only. This would probably be the case in the attachment of later developed wing muscles to later deposited procuticular lamellae in locust apodemes. In this case no epicuticular insertion would exist. Muscle insertions that persist through a moult would be the same as before the moult. The new muscle attachment fibres in these regions would be essentially newly secreted extensions of the old ones.

It is now becoming clear that muscle attachment in the classes of arthropods may well be very uniform in ultrastructure. Muscle attachment in Pterygote insects (Auber, 1963; Lai-Fook, 1967), in copepod crustacea (Bouligand, 1962, 1966), and in Symphyla (personal observations), together with the present findings in Apterygota insects, support this.

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REFERENCES


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For legends see next page.
Fig. 2. Transverse section of interdigitations at muscle-epidermal cell junction in *Monachina*. The two cells are linked by sheet-like desmosomes (*des*) in some areas, whilst in others they are separated by a wider intercellular space (*ics*) containing slightly fibrous material. Actin filaments (*a*) are inserted on the muscle side of the desmosome, microtubules (*mt*) on the epidermal side. (*epid*, epidermal cell; *mus*, muscle cell.) × 80,000.

Fig. 3. Higher magnification of region indicated in Fig. 2, indicating the ultrastructure of the unit membrane in desmosomal regions. The outer leaflets (horizontal arrows) of the plasma membranes are thinner than the inner leaflets (vertical arrows). The microtubules terminate in the dense material of the desmosomes (*des*), and appear to have a granular substructure when seen in cross-section. Axial densities (double shafted arrows) are seen within several of the microtubules. × 147,000.

Fig. 4. Transverse section of a small region of a sarcomere of intersegmental muscle close to the termination of the muscle cell against the base of the epidermis in *Monachina*. The 12 actin (*a*) filaments surrounding each myosin (*m*) filament are characteristic of insect intersegmental muscle. × 102,000.

Fig. 5. Tangential section, nearly parallel to the surface of the sclerite cuticle, of *Monachina*. Muscle attachment fibres (*ma*) can be seen within pore canals (*pc*) in the cuticle, within the deposition zone (*dep*), and within hemidesmosomal cones (*hd*). These elliptical attachment fibres are randomly oriented within the hemidesmosomes (circle). × 31,000.

Fig. 6. Muscle attachment fibres within the sclerite procuticle of *Monachina*. The fibres run within distinct pore canals and being elliptical twisted structures, appear densest and thinnest in the interlaminar regions (vertical arrows), and appear least dense and thickest in the laminar regions (horizontal arrows). × 47,000.
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Fig. 7. Oblique section through intersegmental membrane of Monacha. The procuticle (pro) gives the parabolic artifact effect explained by the Bouligand model for chitin-protein microfibril orientation. (epic, epicuticle; epid, epidermis.) × 42,000.

Fig. 8. Muscle attachment to sclerite cuticle in Machiloides during deposition of new pre-exuvial cuticle. The old cuticle has become separated from the new, but muscle attachment fibres (ma) within the exuvial space (ex) are still attached to the old cuticle. Newly secreted extensions of these fibres (ma2) lie within the pore canals of the new procuticle, and pass through pits in the cuticulin layer (arrows). (c, cuticulin layer; ol, oriented lipid layer.) × 100,000.

Fig. 9. Oblique section of the intermoult cuticle of Ctenolepisma, showing the epicuticular insertion of an attachment fibre (arrow). Muscle attachment fibres (ma) can also be seen within the underlying procuticle. (c, cuticulin layer.) × 68,000.
Muscle attachment to cuticle in Apterygota
Fig. 10. Cuticle of *Entomobrya* in preparation for the moult. The epidermis has retracted from the old cuticle (cut) and a new epicuticle (epi) has been deposited. The muscle cell (mus) is attached to the base of the epidermal cell in a region corresponding to the Z-line. × 18000.

Fig. 11. Area indicated in Fig. 10. The muscle attachment fibre (ma) has been shifted laterally during fixation and therefore does not lie directly opposite its corresponding hemidesmosome. Desmosomes (des) are clearly visible at the muscle-epidermal cell junction. Microtubules (mt) extend from the desmosomes to the conical hemidesmosomes (hd) at the base of the new epicuticle. × 26400.

Fig. 12. Area of microtubules in an epidermal cell of *Monachina* during preparation for a moult. The microtubules do not appear as 'taut' as during the intermoult period, and polyribosomal clumps can be seen amongst them. × 32000.
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