Formation and Involution of Striated Muscle Fibres during the Growth and Moulting Cycles of *Rhodnius prolixus* (Hemiptera)

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With two plates (figs. 1 and 2)

SUMMARY

The ventral intersegmental muscles of the abdomen in *Rhodnius* undergo a cycle of development and involution during each larval stage. They are fully developed only at the time of moulting or hatching from the egg. Within 3 or 4 days after moulting the fibrils have disappeared; but the nuclei, with a little cytoplasm containing mitochondria, survive within the highly folded muscle-sheath.

The formation of fibrils begins between 2 and 3 days after feeding. At first they are uniformly birefringent. The striations appear later, and the muscles will then contract after transection. The fibrils are 0.1-0.2 μ thick when first formed; they grow by intussusception and splitting to a thickness of about 0.8 μ.

Succinoxidase first appears in quantity in the mitochondria at the time when striation and contractility develop. It disappears within 3 days after moulting, during the involution of the fibrils. The distribution of ribonucleic acid in the developing muscle is described.

Involution results from autolysis which begins around the nuclei in the centre of the muscle. The phagocytic blood-cells play no part in the break-down.

The rich nerve-supply to the muscles persists apparently unchanged throughout the cycle of involution; and the cycle of growth occurs normally after section of the nerves.

THE blood-sucking bug *Rhodnius prolixus* Stål takes only a single meal of blood in each of its five larval stages. These large meals may exceed twelve times the weight of the unfed insect and cause enormous distension of the abdomen. In order to allow for this stretching of the body-wall, the cuticle of the abdomen is highly extensible (Wigglesworth, 1933), and at the time of feeding, as will be shown in this paper, the intersegmental muscles are wanting.

The intersegmental muscles of the abdomen play an essential role during moulting in insects: by their contraction they raise the hydrostatic pressure of the blood and so bring about the expansion of the newly formed appendages. It has been found that in *Rhodnius* these muscles are developed only in preparation for the act of moulting; after moulting they rapidly break down.

This repeated cycle of muscle formation and involution, which does not appear to have been described in other insects, affords favourable material for observing some of the histological details in the development and break-down of muscle-fibrils.

MATERIAL AND METHODS

All five larval stages of *Rhodnius* have been used, but most observations have been made on the 1st and the 4th stages. When kept at 25° C the interval between feeding and moulting is 11 days in the 1st stage and 14 days in the...
4th stage. After moulting these insects will survive several months without feeding again.

The tergites and sternites are isolated by cutting along the margins of the abdomen and removing the integument with the adherent epidermis, fat-body, and muscles. For studying in polarized light these isolated parts are fixed in neutral formol and the fat removed with xylene after dehydration in alcohol; they are then returned to water and mounted in Farrants' medium. For close study the muscles are scraped away from the cuticle with a dissecting needle and mounted in the same way.

Whole mounts of the sternites, &c., have been fixed in Carnoy's or Bouin's fixative and stained with Hansen's trioxyaematein, the muscles being isolated as above for closer study. Mitochondria can be studied in similar whole mounts, after fixation in 1% osmium tetroxide in phosphate-citrate buffer at pH 6-8, if stained with Hansen's trioxyaematein and mounted in Farrants' medium. Tangential and transverse sections of the sternites, after double embedding by Peterfi's method, have been stained with Masson's trichrome stain (modification of Foot) and other stains.

Ribonucleic acid has been followed by the use of pyronin/methyl green, and with galloycyanin at pH 1-6 (Lagerstedt, 1947); controls (both whole mounts and sections) being treated for 2 hours at 37° C with 0-1% ribonuclease (Armour) previously heated at 90° C for 10 minutes to inactivate protease and deoxyribonuclease.

The development of cytochrome oxidase and succinoxidase has been followed by the Nadi reaction and the neotetrazolium method of Shelton and Schneider (1952).

Nerve-endings have been stained with methylene blue injected into the intact animal.

**Cycles of Growth in the Intersegmental Muscles**

The intersegmental muscles consist of strap-like bands running from the anterior margin of one segment to the anterior margin of the next. On the tergites (for example, in the 5th-stage larva at the time of moulting) the muscles are more or less vestigial. They are moderately developed on segment 1, vestigial on segments 2–6, slightly more developed on segment 7, and again fairly well developed on segment 8. Fig. 1, A shows these slender striated
Fig. 1

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muscles on segments 6 and 7. All disappear completely within 4 days after moulting. (In fig. 1, A, and other similar figures, the small 'Maltese crosses' which form the background are produced by the dome-shaped plaques scattered over the surface of the cuticle—the cuticle being birefringent when viewed in profile.)

The muscles of the sternites are much better developed, and only these have been studied in detail. There are nine muscle-bands on each side of each segment. (As a rare anomaly a muscle-band may extend across two segments without intermediate insertion.) When the *Rhodnius* larva hatches from the egg the sternal muscles form almost a continuous sheet (fig. 1, B); by 3 days after hatching muscle-fibres have completely disappeared (fig. 1, C). When the 1st-stage larva is fed, birefringent muscle-fibrils begin to appear within 3–4 days (fig. 1, D); they are becoming more conspicuous and striations are appearing by 5 days (fig. 1, E); and they are fully developed by 10 days (fig. 1, F). At this and later stages the muscles show gaps between them and do not form a continuous sheet as in the newly hatched insect. The 1st-stage larva moults to the 2nd stage at 11 days after feeding and the muscle-fibres disappear again within 3 or 4 days.

A similar cycle occurs after feeding in each stage. Fig. 1, G–L shows four stages in the development of the muscles when the 4th-stage larva moults to the 5th stage. Fig. 2, A shows the muscles of the 5th stage at the day of moulting. In fig. 2, B, taken 3 days later, the muscles can just be seen as 'ghosts' running across the background of 'Maltese crosses'. In fig. 2, C, at 5 days after moulting, the muscles have completely disappeared.

The nerve-supply to the sternal muscles is conspicuous. The nerves, which are formed by branches from the fused ganglionic mass in the thorax, run transversely across the middle of the bands giving off numerous branches to them (figs. 2, K; 9, p. 478).

**STATE OF THE MUSCLE IN THE RESTING PHASE**

Although the conspicuously birefringent muscle-fibrils disappear soon after moulting, the muscle-sheath and the nuclei remain. In a recent paper (Wig-
Wigglesworth (1956) it was shown that the sheaths around the muscles in *Rhodnius* appear to be of the same nature as the basement membranes, the sheath enclosing the fat-body cells, and the perilemma around the ganglia and nerves. These membranes are largely, often wholly, the product of the amoebocytes. These cells contain PAS-positive inclusions which they discharge to form or add to the connective tissue membranes, composed apparently of some neutral mucopolysaccharide.

When the muscle-fibrils undergo involution the sheath becomes too large for the muscle and is therefore thrown into longitudinal folds. This is shown in transverse section in fig. 4, A and L. These connective tissue sheaths contain submicroscopic fibres (Baccetti, 1955) and therefore when seen in profile they appear birefringent. It is not surprising, therefore, that although the involuted muscles show no conspicuous birefringence, if they are separated from the cuticle they do show a weak birefringence. This is readily demonstrated by the use of a compensator. The muscle-sheaths then appear just perceptibly bright in the addition position; they show up clearly as dark bands on a grey background in the subtraction position.

This weak birefringence might be due to a few surviving muscle-fibrils. But in sections stained with Masson's trichrome stain, in which the sheath stains strongly with the light green and the muscle-fibrils stain red with the ponceau, I have been unable to detect any muscle-fibrils at this stage. Whether the birefringence is due wholly to a fibrous membrane seen in profile, or whether (as seems probable) there is some longitudinal orientation of the fibrous component in the sheath, which might render the membrane birefringent even in surface view, has not been determined.

Inside this sheath the nuclei are shrunken and inactive, with relatively small nucleoli. They are dispersed more or less irregularly. There is very little cytoplasm; but this contains mitochondria, both globular and filamentous in form, which are most plentiful immediately around the nuclei (fig. 6, A, p. 472).

**Formation of Muscle-fibrils**

After feeding, when the muscle-sheaths are stretched, the nuclei tend to arrange themselves in longitudinal rows. In the 1st-stage larva there is only a single row of 9–12 nuclei in each muscle (fig. 3, A). In the 4th stage, each muscle usually has three or four rows of nuclei, but these do not all run the whole length of the muscle.

1st-stage larva. Fig. 3, D–L shows the development of fibrils in the 1st-stage larva. By 2 or 3 days after feeding, birefringent fibrils are beginning to appear on each side of the row of nuclei. By 4 days the fibrils are increasing in number and in polarized light each muscle now appears in the form of two birefringent threads, one on each side of the isotropic core occupied by the nuclei (fig. 1, D); there is no striation at this stage. By 5 days (fig. 1, E) the muscles are thickening and traces of dark banding are beginning to appear, particularly at the periphery of the fibres. During the next few days the muscles increase rapidly in thickness as more fibrils are laid down, and striation becomes con-
spicuous and regular (fig. 3, H–K). By 10 days the muscles are fully formed (fig. 1, F); the isotropic core is still detectable but is less obvious. Moulting occurs during the night between the 10th and 11th days.

![Diagram](image)

**Fig. 3.** Changes in the abdominal muscles of the 1st-stage larva after hatching and after feeding. A, immediately after hatching (compare fig. 1, B). B, 2 days after hatching. C, 4 days after hatching (compare fig. 1, C). D, 14 days after hatching immediately after feeding. E, 2 days after feeding. F, 4 days after feeding (compare fig. 1, D). G, 5 days after feeding (compare fig. 1, E). H, 6 days after feeding. J, 7 days after feeding. K, 9 days after feeding (compare fig. 1, F). L, 11 days after feeding (newly moulted 2nd-stage larva).

**4th-stage larva.** The formation of muscle-fibrils has been followed in the 4th-stage larva in whole mounts, and in transverse and tangential sections of the abdomen. At 2 days after feeding (fig. 4, B) the nuclei have enlarged and the cytoplasm within the sheath has increased, but as a rule no fibrils can be detected. By 3 days a large number of fine fibrils (estimated to be between 0.1 and 0.2 μ in thickness) have separated out in the cytoplasm (fig. 4, C). These fibrils often form a single layer disposed as a sheath around the core of nuclei, each fibril being separated from its neighbours by a space about equal to its own diameter (fig. 2, D).
Thereafter the muscles increase progressively in thickness (fig. 4, D-G). The individual fibrils appear to grow by intussusception, so that by 6–8 days they are perhaps 0·3–0·4 μ wide (fig. 2, E). These enlarged fibrils are quite widely spaced in the cytoplasm, they often appear flattened, and in the sections they may bend over at their cut ends (fig. 5, A). These cut ends may spread out slightly in a fan-like manner, and can then be seen to split into finer fibrils.

As this process continues the fibrils come to form the bulk of the muscle, and it becomes increasingly difficult to distinguish the individual fibrils in sections. But they are still more or less ribbon-like in general form, and although the fibril bundles are arranged in groups (Cohnheim's fields), if they are observed in cross-sections and the microscope is focused up and down, it is clear that the large fibrils are still splitting and anastomosing, often with fibrils in adjacent bundles, so that a given Cohnheim field changes its outline with the focus (see Tiegs, 1955).
When the fibrils are first formed, at 3–4 days after feeding, it is impossible to detect any striation in polarized light (fig. 1, G and H). A somewhat irregular striation begins to appear at 5 days, and thereafter becomes increasingly conspicuous as the muscle grows in thickness. At the same time the striation in adjacent fibrils becomes progressively better aligned; and in the fully formed muscle at 12–13 days after feeding the striations are usually continuous right across the muscle (fig. 1, L).

In whole muscles fixed in Carnoy’s or Bouin’s fixative and stained with Hansen’s trioxyhaematein, striation becomes visible at just the same time as in polarized light. It takes the form of a rather indefinite dark band in the isotropic region. The telophragma (Z line) does not stain. The development of the telophragma is most readily followed in preparations fixed in osmium tetroxide and stained with Hansen’s trioxyhaematein. It first appears, at 5–6 days after feeding, in the form of minute black points irregularly dispersed throughout the muscle. At 7 days these points are becoming aligned across the fibre and can be recognized as the telophragma. By 9 days the structure is fully formed and appears as a sharp black line extending across adjacent fibrils, with a very narrow zone of diffuse black staining on each side of it. The distance between successive Z lines before moult ing occurs is about 12 to 13 μ. After moult ing, when the muscles contract somewhat, it is about 9 to 10 μ.
Immediately after feeding, as we have seen (fig. 6, A), the mitochondria are irregularly distributed, but most plentiful around the nuclei. By 2 days after feeding they are more numerous, there are many elongated forms and many of these are becoming orientated in the long axis of the muscle. At 3 days this change is more evident: the plentiful mitochondria in the axial core of cytoplasm between the nuclei are often rounded and quite irregularly arranged.

Elsewhere the mitochondria lie between the newly forming fibrils and many of them are compressed into an elongated form (fig. 6, B). This state of affairs continues until 6 days after feeding; but thereafter the fibrils are becoming so numerous and densely packed that it has not been possible to observe the mitochondria. Fig. 6, C shows the mitochondria in a fully formed muscle from the base of the leg. They lie irregularly distributed between the fibrils, very variable in form and size, but many of them greatly elongated. In addition, there is a concentration of rather small mitochondria ('J granules') on each side of the Z line (compare fig. 2, 1).

In sections of the fully developed muscles stained with Masson's trichrome stain, the continuous fibrils staining with the ponceau are crossed by a deep-purple-staining telophragma which, as usual, runs across the whole muscle and is attached to the muscle-sheath. On each side of this there is a relatively pale region, and then a long zone (the A disk) in which a blue-grey coloration may obscure the red staining of the fibril (fig. 5, B). A very similar arrangement
is seen in fibrils stained with bromothymol blue to show protein density (Mazia, Brewer, and Alfert, 1953) (fig. 5, c); in this figure the anastomosis of adjacent fibrils can be seen (compare Edwards and Ruska, 1955); they are now about 0.8 \mu across. Thus in their fully formed state these muscles show a fairly elaborate degree of striation of standard type.

**Changes in the Muscle-Nuclei and Distribution of Ribonucleic Acid (RNA)**

The changes in the muscle-nuclei under the influence of the moulting hormone will be described elsewhere. It may be said here that in the resting insect these nuclei are small, with a relatively inconspicuous nucleolus and a nuclear membrane which is pale staining and (in fixed preparations) often crumpled. The cytoplasm around is almost non-existent. Within 6 hours after feeding the nuclei are becoming active and the nucleoli enlarging. By 24 hours after feeding these changes are conspicuous: the nucleus is now tense and vesicular, the nucleolus is greatly enlarged and rich in RNA, and so is the bounding membrane of the nucleus. The cytoplasm is beginning to increase in amount and, particularly in the neighbourhood of the nuclei, it contains much RNA. These changes can be seen in the muscles of the 1st-stage larva in fig. 3, e. Mitosis in the muscle-nuclei begins 2 or 3 days after feeding; it is coming to an end (in the 4th-stage larva) by 7 days.

At the 3rd or 4th day after feeding, when the fibrils are beginning to separate out, the nuclei are very large and the nucleoli and the cytoplasm of the nuclear core contain abundant RNA. In addition, there are elongated deposits and granules of RNA between the newly formed fibrils. The fibrils themselves show no nucleic acid staining—they appear as glassy rods between the RNA deposits (fig. 7, a and b).

During the 5th and 6th days, when the striation is beginning to appear, these interfibrillar deposits of nucleic acid are becoming aggregated at the level of the I bands. Fig. 7, c shows a section of a muscle at 8 days, stained with pyronin and methyl green. The dark staining in this figure is due wholly to RNA; it takes the form of granules between the fibrils; some of these granules are readily seen with the light microscope, but there are others so fine as to merge gradually into an apparently diffuse staining. There are still massive deposits of nucleic acid in the cytoplasmic core between the nuclei and there are occasional granules between the fibrils outside the I bands.

After treatment with ribonuclease these bands and the other deposits staining with pyronin and gallocyanin are completely removed and the nucleoli no longer stain with these dyes; but that makes no detectable difference to the isotropy of the I bands and these will still stain (though not so strongly as before) with Hansen's trioxyaematein. This staining with haematoxylin reveals no distinct structural difference in the I band, but it sometimes gives the impression that there is a fusiform thickening of the fibril at this level with material staining with haematoxylin.
A comparison of the preparations stained for ribonucleic acid (fig. 7) with those showing the mitochondria (fig. 6) suggests that the mitochondria are probably the source of many of the granular deposits of RNA, but that in addition, there is a large amount of RNA in the nuclear core of the muscle and in the I bands which appears diffuse in the light microscope and is probably associated with cytoplasmic reticulum.

The enormous enlargement of the nucleoli, and the deposits of RNA between the nuclei, persist until the day before molting (figs. 3, K; 4, C). All this time the fibrils appear to be increasing in number. But on the day of molting the nucleoli are markedly reduced (figs. 3, L; 4, H), the nuclei are smaller, and the conspicuous deposits of RNA have disappeared from the cytoplasm between them.

**Involution of the Muscles**

*1st-stage larva.* Fig. 3, A shows the appearance of a sternal muscle of a 1st-stage larva on the day of hatching from the egg. By 2 days after hatching (fig. 3, B) striated fibrils persist only at the periphery of the muscle. By 4 days (fig. 3, C) all trace of fibrils has disappeared.

*5th-stage larva.* Fig. 8, A shows a portion of a sternal muscle in the 5th-stage larva immediately after molting, with the striations aligned right across the muscle. At 1 day after molting there is almost no change, though perhaps the striations do not stain quite so strongly.

At 2 days after molting, striation is still evident in stained preparations and
in polarized light. The muscles are becoming reduced in thickness and the birefringence is weaker. Vacuoles of varying size are appearing in the muscle core between the nuclei (figs. 4, j; 8, b). Droplets of fat are present in the muscle at this stage.

At 3 days, fibrils and striations are still distinct in polarized light, but in stained preparations the banding is becoming faint. The fibres are now highly vacuolated, most of the muscles being reduced to vacuolated sacs with irregularly dispersed nuclei connected by cytoplasmic strands enclosing the vacuoles.

At 4 days, the weak birefringence that persists throughout most of the muscle is probably due solely to the folds that are now developing in the muscle-sheath (p. 468). The greater part of the muscle contains nothing but vacuoles (fig. 8, c). A few striated fibrils still remain along the margins of the muscles near their insertions (fig. 4, k).

At 10 days after moulting the muscles are reduced once more to highly folded sheaths containing irregular strings of nuclei and very little cytoplasm. No muscle-fibrils remain (figs. 4, l; 8, d).

In some insects the phagocytic amoebocytes in the blood are said to play an active part in removing the tissue debris that results from the autolysis of muscles during metamorphosis. They are not concerned in the process of involution of the abdominal muscles in Rhodnius. They are excluded from the muscle by the fibrous sheath, and there is no accumulation of these cells in the neighbourhood of the autolysing muscles.

**DEVELOPMENT OF SUCCINOXIDASE**

The neotetrazolium method of Shelton and Schneider (1952) was applied to a series of 4th-stage larvae at intervals after feeding. The freshly dissected sternites were incubated in the neotetrazolium mixture at 37° C for 45 minutes, rinsed in Ringer's solution, mounted in glycerine jelly, and
examined at once. Controls carried out on the same material in the absence of succinate were always negative.

There is no reaction when the intact muscles in the fully developed state are incubated in this mixture: it is necessary to cut through the muscle-sheath. Fig. 2, F shows the absence of any reaction in the sternal muscles on the left side, where the muscles are intact, but an intense positive reaction in the contracted stumps of the cut muscles on the right side.

The thoracic muscles and the dorso-ventral muscles of the abdomen always show a strong positive reaction. But the reaction is practically negative in the cut muscles of the abdominal sternites during the first few days after feeding. On the 5th day a few scattered purple granules may appear in some of the muscles. On the 6th day the reaction is quite definite. From then onwards it increases in intensity up to the day of moulting.

As the reaction develops the purple granules of the formazan show a characteristic distribution. They occur in rows between the fibrils, just like the mitochondria; and they are much more abundant in the isotropic disk, so that the striation of the muscle shows up conspicuously (fig. 2, G, H; compare fig. 5, D). There is also a faint pink staining of the substance of the isotropic disk—which suggests the presence of lipid material in this region. In fact the granules show just the same distribution as the mitochondria (figs. 2, L; 6, c). Rutenberg, Wolman, and Seligman (1953) describe a similar distribution of granules between the fibrils in mammalian muscle, but the concentration in the anisotropic disks was not so evident as in *Rhodnius* muscle.

The Nadi reaction has exactly the same distribution as the reduction of neotetrazolium. It is much more evanescent, but when examined at the right moment the muscles again show rows of interfibrillar blue granules concentrated in the I bands.

During the involution of the sternal muscles, the reaction remains intense in the cut muscles at 1 day after moulting. By 2 days after moulting the granules are still related with the striation, but the intensity of the reaction is reduced to about half. At 3 days after moulting, the reaction is almost completely negative, though a faint pink staining of the I bands remains.

**Development of Contractility**

When the fully developed sternal muscles are cut through, they rapidly contract down to stumps not more than 10–15% of their initial length. The development of this property has been studied by removing the sternites, cutting across the cuticle and muscles in the middle of each segment on one side of the mid-line, and examining at once in Ringer’s solution with polarized light.

In the involuted state the muscles do not contract after cutting. The same is true during the first 3 days after feeding. On the 4th day, when the birefringent fibrils are becoming quite conspicuous, the ends of the cut muscles still remain almost up to the margin of the cut cuticle. At the most they contract down to 90–95% of their initial length. On the 5th day, when striations are
beginning to appear, the amount of contraction is extremely variable: some of the muscle bands may contract to 40% of their length, others contract only to about 60%. Thereafter the contractility increases progressively: on the 7th day, most muscles contract to about 40% of the original length, a few contract down to 33%. During the 8th, 9th, and 10th days most muscles contract down to about 33%; at 12 days they contract at once to less than 25%; and in the fully developed muscle at 14 days they contract rapidly to 10-15% of the initial length.

Thus, spontaneous contraction of the cut muscles does not occur until the isotropic bands first appear in the fibrils at the 5th day after feeding. As the muscles thicken, contraction becomes more intense.

At the time of moulting, as we have seen, the muscles shorten considerably; but when cut they still contract down to about 30–40% of their initial length. One day after moulting they contract to about 50–60%. At 2 days, not quite so much—say 60–70%. At 3 days after moulting, when, as we have seen, the muscles are filled with vacuoles, though striation persists in the fibrils which remain, the cut muscles show slight irregular twitching but no shortening.

INNERVATION OF MUSCLES

The abdominal ganglia in *Rhodnius* are fused with the ganglia in the thorax. They give off a fan-like array of nerves to the abdomen, and these in turn give rise to branches which run transversely across the middle of the sternites at right angles to the sternal muscles (fig. 2, K). In the neighbourhood of each muscle these nerves give off small branches which ramify all over the surface of the muscle and, as is usual in insects, they end in clusters of fine branches in every part of the muscle; there is no sign of an end plate (compare Meyer, 1955).

The transverse nerves to the muscles, and the branches which ramify on the muscle-sheath, are enclosed, as usual, in a perilemma with a cellular perineurium beneath. The perineurium is always rich in succinoxidase, so that in preparations in which the neotetrazolium method is applied to the intact muscles, the course of the nerve-branches is marked out by the purple granules which accumulate in the perineurium (fig. 2, j).

Fig. 9, A shows the middle portion of a sternal muscle in the fully developed state in a newly moulted 5th-stage larva, with the rich nerve-supply stained with methylene blue. Fig. 9, B shows a similar muscle 10 days after moulting, when the muscle-fibrils have completely degenerated. The muscle-sheath is collapsed and shrunken but the nerves persist apparently unchanged.

It was shown by Kopeč (1923) that the thoracic muscles in the adult *Lymantria* do not develop if they are deprived of their innervation by removal of the thoracic ganglia in the young pupa (compare Nüesch (1952) on *Platysamia*). It was therefore of interest to see whether the innervation of the sternal muscles in *Rhodnius* is necessary for the formation of fibrils. Fourth-stage larvae at 24 hours after feeding were injected with 1 μg of the moultling hormone 'ecdyson' of Butenandt and Karlson (1954) (for the gift of which I am
The sternal muscles show some distortion but otherwise they are well developed and show the normal striation. Clearly these muscles are not dependent on their nerve-supply for the deposition of fibrils.

**DISCUSSION**

In many insects there are striking changes in the muscular system, with the dissolution of existing muscles and the formation of new ones, during development of the pupa or the adult. But repeated cycles of development and involution in the same muscles do not seem to have been described before. It may be that this phenomenon is widespread in insects. Sternal muscles are well developed in all segments of the abdomen in the newly hatched bed-bug *Cimex*; those on the anterior segments show no obvious change, but the muscles on segments 6, 7, and 8 disappear within 3 or 4 days. In *Dysdercus* (Pyrrhocoridae) sternal muscles are vestigial in the abdomen except in segments 5, 6, and 7, where they are well developed in the newly moulted 5th-
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stage larva. One day after moulting they are becoming much less birefringent and have usually disappeared by 3 or 4 days after moult ing.

The formation of the muscles in the embryo of *Rhodnius* has not been studied; but throughout subsequent growth the nuclei of the intersegmental muscles are cut off from the cells of the body-cavity by the muscle-sheath. They increase in number by mitosis during each moult ing cycle; they are not replenished by 'mesenchyme cells' entering from outside, as described by Debaisieux (1954) in Crustacea.

The fibrils appear to 'crystallize out' as continuous threads between the longitudinally disposed mitochondria. In the earliest stage at which clearly stained fibrils have been seen (at 3 days after feeding), they measure only 0.1–0.2 μ in diameter. Thereafter they grow, as has been shown often enough in vertebrates (see Schmidt, 1937), by intussusception and longitudinal splitting. The fully formed fibril has a diameter of about 0.8 μ. As in embryonic muscle-cells (Vincent, 1955) the nuclei have greatly hypertrophied nucleoli throughout the period when new fibrils are being formed.

When they first appear the fibrils are uniformly birefringent; the periodic isotropic regions are added later. That has been described in vertebrates by Schmidt (1937), Le Gros Clark (1946), and others. The Z line seems to appear after the isotropic banding has formed—but that point has not been sufficiently studied.

There is no information about the factors which initiate the break-down of the muscle-fibrils after moulting. It is independent of the nerve-supply and is presumably controlled by humoral means—as is the break-down of certain organs at the conclusion of metamorphosis (Wigglesworth, 1955a). The process of break-down is a pure autolysis which starts around the nuclei in the core of the fibres and spreads outwards. That is in agreement with the observations of E. Schmidt (1919) on *Psychoda*. In the break-down of many insect muscles at metamorphosis the phagocytic blood-cells play a more or less important part (Lange, 1932; Blaustein, 1935). In *Rhodnius* they seem to play no part at all and do not even collect around the outside of the autolyzing muscles.

The development of contractility has been studied only by the crude method of noting the spontaneous shortening after section. It coincides with the appearance of striation. The distribution of succinoxidase, which is concentrated in the mitochondria (sarcosomes), agrees with the biochemical findings of Watanabe and Williams (1951) and the histochemical observations of Wachstein and Meisel (1955). It is characteristic of insect muscles that besides the scattered interfibrillar mitochondria there is a concentration of small globular mitochondria (the 'J granules') on each side of the Z line (Jordan, 1933). As a result, the *Rhodnius* muscles treated by the neotetrazolium method show well-marked banding in the I disks. It is perhaps worth noting that even in the resting or involuted state the muscles contain plenty of mitochondria; but the neotetrazolium test does not become positive until some 5 days after feeding, when contractility is beginning to develop.

Gerendas and Matoltsy (1948) (quoted by Perry, 1955) have ascribed the
relative isotropy of the I band to the negative birefringence of a nucleoprotein contained in this region. The sternal muscles in *Rhodnius* certainly show well-marked bands of ribonucleic acid in the I disks; but the removal of this with ribonuclease makes no difference to the appearance of the fibres in polarized light. Much of the ribonucleic acid is concentrated in the mitochondria, which have the same distribution. It is impossible to say at the moment how much of the nucleic acid in the bands is in the mitochondria and how much is in the cytoplasmic reticulum.

During the cycles of muscle-growth and involution the nerve-supply appears to remain unchanged, apart from an equivalent amount of growth. It would be interesting to know just how the nerve-endings are connected to the contractile fibres. No end-plates have been detected in insect muscles.

It is hoped to continue the study of these muscles with the electron microscope, for this material should provide a favourable opportunity to observe the first appearance of muscle-fibrils and their subsequent differentiation; and the great length of the sarcomeres (10–12 μ) may provide opportunities for obtaining further information on their fine structure.

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