Transformations in the abdominal muscles of the blue blow-fly, *Calliphora erythrocephala* (Meig), during metamorphosis

**by A. C. S. CROSSLEY**

*From the Department of Zoology, University of Cambridge, and Department of Biology, University of Virginia*

**WITH FOUR PLATES**

**INTRODUCTION**

In the century that has elapsed since Weismann (1864) published his pioneer work on insect post-embryonic development, the changes in insect muscles at metamorphosis have been studied by numerous workers. Although the treatises of Breed (1903) and Perez (1910) provide an insight into earlier work, a brief survey of the early literature is included here to clarify the origin of certain terms that have come into general use, and this survey is extended to include the more recent studies pertaining to muscle metamorphosis in Diptera.

Weismann (1864), in his work on *Calliphora erythrocephala* and *Sarcophaga carnaria*, described the breakdown of larval tissues in the puparium to form a thick suspension of fatty droplets in the haemolymph. Aggregations of these droplets were said to surround themselves with a membrane, becoming ‘Kornchenkugeln’, from which materialized a mass of nuclei (unrelated to haemocyte or fat cell nuclei), which were said to subsequently differentiate into imaginal structures, including muscles.

Lowne (1870) described the birth of large bright ‘nuclei’ in the histolysing larval muscles of *Calliphora*, which rapidly became surrounded by aggregations of disintegrating muscle.

Viallanes (1883), working on *Calliphora vomitoria*, introduced the concept of phagocytosis, but believed that the phagocytes had their origins in the muscles themselves. He described the phagocytosis of muscle, leading to the formation of the structures termed ‘Kornchenkugeln’ by Weismann, but in addition showed that a second process was responsible for the breakdown of certain muscles. In this second process, which Viallanes termed ‘l’histolyse par dégénérescence’,

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1 Author’s address: Department of Biology, University of Virginia, Charlottesville, Virginia, U.S.A.
initial nuclear changes were followed by a dissolution of the myoplasm, during which the muscle retained its regular outline. This type of histolysis was the last to set in and was said to complete the destruction of the larval muscles.

Van Rees (1888), also working on *Calliphora vomitoria*, showed that haemocytes penetrated muscle fibres, engulfing cytoplasm and nuclei. These haemocytes were probably the structures that Lowne had earlier described as bright ‘nuclei’, and the phagocytes to which Viallanes had erroneously ascribed a muscular origin. The gorged haemocytes are the ‘Kornchenkugeln’ described by Weismann. Van Rees believed that all larval abdominal muscles were actively penetrated and engulfed by phagocytic haemocytes.

De Bruyne (1898), in a study of *Calliphora vomitoria* and other species, suggested that muscles initiated their own destruction, and fragmented before the intervention of phagocytic haemocytes. He also believed that certain phagocytic cells were derived from muscle tissue.

Mercier (1906) injected sterilized carmine powder into larvae of *Calliphora vomitoria* 4–5 days before metamorphosis, and showed that dye particles were ingested by haemocytes. He believed that haemocytes penetrated the muscles before any morphological signs of degeneration were visible.

Perez (1910), in a comprehensive study of metamorphosis in *Calliphora erythrocephala*, described the active penetration of muscle plasma membrane by haemocytes which bore pseudopodia, and had cytoplasm filled with basophilic granules. He supposed that fragments of tissue were sometimes only temporarily engulfed by haemocytes, thus accounting for the observation of small muscle fragments floating in the haemolymph. Gorged haemocytes Perez termed ‘sphères de granules’, and regarded as homologues of the ‘Kornchenkugeln’ described by Weismann. Perez described the breakdown of muscle fragments within the haemocytes, during which the basophilic granules originally present in the haemocytes disappeared.

Perez noted that not all abdominal muscles were engulfed by haemocytes. Some persisted, lost their fibrillar structure and cross-striation, and became apparently homogenous strands of cytoplasm with axial nuclei. Then ‘myoblasts’ were said to penetrate the sarcolemma and migrate into the muscle syncitium. These myoblasts were said to multiply inside the muscle strand by amitotic division. In some cases the imaginal muscle nuclei were said to arise from a multiple division of a larval muscle nucleus, but some larval muscle nuclei degenerated without division. The strand then expanded and redifferentiated into axial fibres, beginning at the extremities.

Authors working on other species of Diptera describe processes of abdominal hypodermal muscle metamorphosis in which phagocytosis plays a minor part, and also in which the extent of metamorphosis of larval muscle varies greatly. Table 1 summarizes these authors’ conclusions.

Kowalevsky (1887) noted that the phagocytic haemocytes bore pseudopodia and had cytoplasm filled with small round bodies. Haemocytes penetrated
### Table 1

**Larval-adult continuity of abdominal hypodermal muscles and origin of imaginal muscle nuclei**

<table>
<thead>
<tr>
<th>Author and species</th>
<th>Process of muscle breakdown and extent of continuity</th>
<th>Origin of imaginal muscle nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>D'Herculais (1875)</td>
<td>Dissolution, no continuity</td>
<td>Hypodermal histoblasts</td>
</tr>
<tr>
<td>(Syrphoidea Spp.)</td>
<td></td>
<td>(only thoracic muscles examined)</td>
</tr>
<tr>
<td>Ganin (1876) <em>Anthomyia rufipes</em> (Diptera Muscoidea)</td>
<td>Fragmentation, no continuity</td>
<td>Hypodermal histoblasts</td>
</tr>
<tr>
<td>Kowalevsky (1887)</td>
<td>Phagocytosis by haemocytes of morphologically intact muscles showing physiological change</td>
<td>Hypodermal histoblasts</td>
</tr>
<tr>
<td><em>Muscoidea Spp.</em></td>
<td></td>
<td>(abdominal and thoracic muscles)</td>
</tr>
<tr>
<td>Vaney (1902) <em>Simulia</em> (Diptera Nematocera)</td>
<td>Gradual wasting without intervention of haemocytes</td>
<td>Hypodermal histoblasts</td>
</tr>
<tr>
<td>Kellogg (1901) <em>Holorusia rubiginosa</em> (Dip. Nem.)</td>
<td>No phagocytosis, possible continuity</td>
<td>—</td>
</tr>
<tr>
<td><em>Blepharocephala capitata</em> (Dip. Nem.)</td>
<td>Phagocytosis, continuity</td>
<td>—</td>
</tr>
<tr>
<td>Miall and Hammond (1900) <em>Chironomus</em> (Diptera Nematocera)</td>
<td>Spontaneous fragmentation</td>
<td>—</td>
</tr>
<tr>
<td>Snodgrass (1924) <em>Rhagoletis pomonella</em> (Dip. Cyclo. Otidoidea)</td>
<td>Haemocytic phagocytosis follows initial changes in muscle</td>
<td>—</td>
</tr>
<tr>
<td>Hulst (1906) <em>Culex pungens</em> (Dip. Nem.)</td>
<td>Spontaneous disintegration of muscles, occasional invasion by phagocytes, no continuity</td>
<td>Hypodermal histoblasts</td>
</tr>
<tr>
<td>Robertson (1936) <em>Drosophila melanogaster</em> (D. Cyclo. Drosophiloida)</td>
<td>Internal digestion, followed by redifferentiation, no phagocytosis</td>
<td>—</td>
</tr>
<tr>
<td>Jones (1956) <em>Sarcophaga bullata</em> (Dip. Muscoidea)</td>
<td>Spontaneous fragmentation, haemocytes secondary scavengers, no continuity</td>
<td>—</td>
</tr>
<tr>
<td>Whitten (1964) <em>Sarcophaga bullata, Drosophila melanogaster</em></td>
<td>Spontaneous fragmentation, active phagocytosis by multinucleate granular haemocytes, no continuity</td>
<td>—</td>
</tr>
</tbody>
</table>
muscles which gave no morphological indication of degeneration, but which nevertheless were unable to contract, indicating a physiological change. Kellogg (1901) noted that in the two species he examined, the species having the greatest degree of change at metamorphosis showed phagocytosis of larval muscles, whereas the species showing little change at metamorphosis but with a shorter pupal period, showed no phagocytosis. Jones (1962), reviewing the literature on insect blood cells, notes that if myoblasts are to be considered as transformed haemocytes, it must be shown that they circulate before they settle down to form muscles. He also notes that, ‘during the differentiation of various organs and tissues, when haemocytes are said to transform and enter into their formation, no convincing evidence is ever given that non-circulating mesodermal cells were not already present as undifferentiated imaginal disc cells. Thus there may be no need to evoke an exogenous source for certain mesodermal tissues.’

Åkesson (1953) examined the larval haemocytes of Calliphora erythrocephala and attempted unsuccessfully to show types which were phagocytic in vitro. The type of cell responsible for phagocytosis of larval muscles in vivo was termed a ‘sphaerule cell’ and described as ‘morula like’, having highly vacuolar basophilic cytoplasm in stained preparations.

Jones (1956) later termed similar phagocytic cells in Sarcophaga ‘granular haemocytes’.

Whitten (1964) described the larval haemocytes in Sarcophaga bullata and studied especially the granular haemocytes which engulf fragments of disintegrating muscle. The formation of large multinucleate granular haemocytes by mitotic division followed by at most partial cytoplasmic division was described. The seven-fold increase in numbers of circulating granular haemocytes shortly before puparium formation, which was noted by Jones (1956), was accounted for by mitotic division. The granular haemocytes were said to develop extensive cytoplasmic processes which adhered to fragments of muscle, drew them to the main body of the haemocyte, and engulfed them with the formation of ‘spherules’. Each group of larval fragments was generally associated with a single nucleus; however, the different groups of fragments were joined by strands of cytoplasm. Some granular haemocytes were seen to remain uninucleate and were thought perhaps to achieve multinucleate condition prior to breakdown of muscles in late pupal life. Close connexions between spherules and certain developing adult tissues were noted, and a direct transfer of ‘food materials’ was hypothesized.

Van Rees (1884, 8) describes three pairs of imaginal discs for each segment of the abdomen in Calliphora; the second dorsal discs were small, and soon united with other discs when expansion began.

Kowalevsky (1887) describes two pairs in each abdominal segment (Muscoidea), Ganin (1876) describes two pairs in each abdominal segment (Muscoidea), Snodgrass (1924) describes two pairs in each abdominal segment (Otidoidea), Robertson (1936) describes two pairs in each abdominal segment (Drosophiloidea).
Very few anatomical studies of muscles of dipterous larvae exist, and this makes the study of the pupal physiology of individual muscles impracticable and has prevented a detailed comparative study of different muscles in the same species.

D’Herculais (1875), working on syrphid flies, provided the first anatomical plan of the muscles of a cyclorrhaphous larva, and compared this with the plan of Lyonet (1762) for Sphinx ligustri (Lepidoptera).

Hewitt (1914) gave a relatively complete diagram of the anatomy of larval muscles of Musca.

From this review of the literature it is apparent that a detailed re-examination of the metamorphosis of specific abdominal hypodermal muscles in Calliphora might be worthwhile. Such investigation should be based on a preliminary anatomical study and should be designed to clarify the processes of muscle breakdown and imaginal histogenesis, with particular reference to the origins of the phagocytic and imaginal nuclear elements.

**MATERIALS AND METHODS**

*Calliphora erythrocephala* larvae were reared at 25°C. on excess quantities of lean meat and allowed to pupate in sawdust.

For anatomical study larval muscles were fixed in extended condition by rapid passage of the entire larva through water maintained at 80°C., or by fixative of etherized larvae in chilled Carnoy’s fluid. Unstained, cleared preparations were examined with polarizing optics. Other preparations were stained with Hansen’s trioxyhaematin.

For histological studies, dissections of larval and pupal hypodermal regions were performed under fixative on polyethylene tables 2 × 15 × 25 mm., and the pinned preparations were stained and dehydrated in situ to yield a flat whole mount. Sectioned material was also prepared for comparative purposes at all stages. The following procedures gave satisfactory results:

(a) Fixation in Osmium buffered with veronal acetate at pH 7.0 and made slightly hypotonic to the blood by the addition of sucrose, coupled with ethyl gallate visualization.

(b) Alcoholic Bouin fixation coupled with staining in Hansen’s trioxyhaematin.

(c) Carnoy’s fixation coupled with Feulgen’s staining method, counterstained with fast green.

(b) and (c) were suitable for whole mounts.

Preparations for sectioning were either double-embedded in Agar-Ester wax (Wigglesworth, 1959) or embedded in Carbowax (Riopel & Spurr, 1962).

Fresh preparations were examined using phase-contrast microscopy, and fresh haemolymph droplets were examined in immersion oil (Rizki, 1953).

Tissues were prepared for the electron microscope by dissection and fixation in chilled 1 per cent. osmium tetroxide, buffered at pH 7.3 with veronal acetate to which sucrose was added to give a final molarity of 0.45. The tissues were
embedded in Araldite, sectioned, and stained either with lead alone (Reynolds, 1963), or with uranyl acetate and lead.

OBSERVATIONS

Anatomy of muscles of the larval abdomen

Text-figs. 1A, B, C should be superimposed to show the musculature of one half of the fifth abdominal segment. In order to facilitate identification during changes brought about by metamorphosis, the muscles have been numbered. Text-fig. 1C shows superficial muscles running closest to the cuticle, numbered 18–29. Text-fig. 1B shows the intermediate muscles, numbered 9–17. The left-hand portions of the diagrams show the ventral muscles. An approximation to the natural disposition of the muscles is obtained by curving the diagram so as to form half a cylinder. With the exception of the anal region, each larval abdominal segment possesses muscles similar to those shown in the diagram for segment 5. The muscles of the left side were mirror images of those of the right side, so that the three diagrams enable the reader to reconstruct the musculature of the entire abdomen apart from the anal region. In some cases the muscles described can be homologized with Hewitt's nomenclature for *Musca* (Hewitt, 1914, Fig. 48).

<table>
<thead>
<tr>
<th>Muscles in <em>Calliphora</em></th>
<th>Muscles in <em>Musca</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 1, 2, 3, 4</td>
<td>Internal dorso-lateral oblique recti muscles</td>
</tr>
<tr>
<td>(b) 5</td>
<td>Internal lateral oblique muscle</td>
</tr>
<tr>
<td>(c) 6, 7, 12, 13</td>
<td>Longitudinal ventro-lateral muscles</td>
</tr>
<tr>
<td>(d) 8</td>
<td>Lateral intersegmental muscle</td>
</tr>
<tr>
<td>(e) 9, 10, 11, 14</td>
<td>External dorso-lateral oblique recti muscles</td>
</tr>
<tr>
<td>(f) 15, 16, 17</td>
<td>Ventral oblique muscles</td>
</tr>
<tr>
<td>(g) 21, 22, 23, 24</td>
<td>Lateral muscles</td>
</tr>
<tr>
<td>(h) 26</td>
<td>? Ventral-lateral oblique muscle</td>
</tr>
<tr>
<td>(i) 25</td>
<td>Is figured but not named</td>
</tr>
</tbody>
</table>

Muscles 18, 19, 20, 27, 28 and 29 are not described for *Musca* by Hewitt.

A functional appraisal of the larval musculature is not within the scope of this paper, but it should be noted that division of the muscles into ‘turgor muscles’ or ‘locomotor muscles’ (Barth, 1937) is apparently not possible. All the abdominal hypodermal muscles appeared to contract violently when the cuticle was punctured, and muscles 1–4 were observed undergoing rhythmic waves of contraction along their length, indicating a combined locomotor and turgor function. No hypodermal muscles were thrown into passive folds when the cuticle was punctured.

Anatomical changes during metamorphosis

The metamorphosis of the larval abdominal hypodermal muscles was found to involve two major phases of histolysis. The first or ‘prepupal’ histolysis phase
Muscle changes during metamorphosis of Calliphora

TEXT-FIG. 1. Hypodermal musculature of the right side of the fifth abdominal segment.

A, B, C: Full grown larva; A—deep muscles, B—intermediate muscles, C—superficial muscles.

D: 24 hr. after puparium formation.
E: Newly emerged adult. For explanation of numbering see the text.
began soon after the larva rounded up to form the puparium, and lasted for about 10 hr. Thereafter there were no anatomical changes until the onset of the second or ‘pupal’ histolysis phase, which began at the onset of phanerocephalic pupal life, at about 25 hr. after formation of the puparium.

Most of the larval abdominal hypodermal muscles were broken down during the prepupal histolysis phase, such muscles are suffixed A in Text-figs. 1A, B, C for segment 5. Text-fig. 1D shows the muscles which are retained after this phase. It seems likely that the muscles which remain have one or more of the following functions:

(i) Eversion of the head at the end of the cryptocephalic pupal stage.
(ii) Regulation of the form of the pupa during early imaginal differentiation.
(iii) Transformation into imaginal muscles.

The muscles that disappeared during the pupal histolysis phase must therefore function as (i) or (ii), (muscles 10, 13, 21–5 and 27 suffixed B in Text-fig. 1); whereas the remainder, suffixed C, undergo metamorphosis to imaginal muscles. The imaginal abdominal hypodermal musculature is shown in Text-fig. 1E.

Histological changes during metamorphosis

Prepupal phase

During the prepupal histolysis phase muscles 5–7, 9, 11, 14–20, 27 and 29 disappear. Muscle 16, one of the ventral oblique muscles described by Hewitt, was chosen for detailed study because of its accessibility. Text-fig. 2A, B, C, trace the changes in this muscle, and Plate 1 shows its condition 4 hr. after the larva had rounded up to form the puparium. At this stage a large number of phagocytic haemocytes are present in the vicinity of the muscle, but the muscle plasma membrane is intact and surrounded by a thick basement membrane. It will be noted that, even at this early stage, many large vacuoles are appearing in the peripheral cytoplasm. These large vacuoles are not present in the larval muscle, and are the first morphological indication of the onset of muscle degeneration. Other changes, observed with the light microscope, included a blistering of the sarcolemma, and a separation of the muscle fibrils with the production of elongated vacuoles in the myoplasm. These changes often occurred when there were no haemocytes in the immediate vicinity, but numbers of haemocytes congregating in the vicinity of the muscle insertions made it impossible to exclude the possibility of a preliminary haemocyte-muscle interaction elsewhere on the same muscle (Text-fig. 2B).

Five to six hours after puparium formation, haemocytes invaded the peripheral cytoplasm of muscle 16, beginning near the insertions of the muscle on the cuticle (Text-fig. 2C). Peripheral sarcoplasm and nuclei were engulfed first, but during this process the myoplasm began to fragment. Fragments larger than 50 μ in maximum dimension were not normally ingested by haemocytes; however, numerous haemocytes often surrounded a single large fragment of muscle,
Text-fig. 2. Transformations of abdominal hypodermal muscles.

A: Muscle 16, in full-grown larva.
B: Muscle 16, 4 hr. after puparium formation.
C: Muscle 16, 7 hr. after puparium formation.
D: Muscle 8, 30 hr. after puparium formation.
E: Muscle 8, 60 hr. after puparium formation.
F: Muscle 1, 60 hr. after puparium formation.
G: Muscle 1, 72 hr. after puparium formation.
H: Muscle 1, 120 hr. after puparium formation.
appearing to co-operate in some way in the subdivision of the fragment. The forma-
tion of multinucleate haemocytes by mitosis followed by at most partial cytoplasmic
division was not observed. Indeed, mitosis was never observed in the fully
differentiated phagocytic haemocyte. However, it is quite possible that fusion of
plasma membranes of adjacent haemocytes occurs, with the formation of a giant
syncytial haemocyte. Such a fusion has yet to be confirmed in electron micro-
graphs, and is probably at most a temporary condition, since few, if any, multi-
nucleate haemocytes are found in the haemolymph of pupae 15 hr. after pupar-
ium formation.

The origin and structure of the haemocytes involved in the prepupal phase of
muscle breakdown, the type F haemocytes, have been considered in some detail
in an earlier paper (Crossley, 1964). Part of such a haemocyte is shown in Plate 2,
Fig. B. The cytoplasmic membrane is extended into long processes, which appear
to be involved in active cytosis, and the cytoplasm is filled with vacuoles which in
turn are filled with myelin-like aggregations of concentric membranes, endo-
plasmic reticulum, ribosomes and mitochondria (not seen in this field). These
vacuoles give the strongly positive acid phosphatase reaction typical of lysosomes
(Essner & Novikoff, 1961). Type F haemocytes were capable of engulfing
sarcoplasm, myoplasm and nuclei with the formation of the structures termed
‘Kornchenkugeln’ by Weismann (1864). ‘Kornchenkugeln’ at first remained in a
mass outlining the engulfed muscle, but later became irregularly distributed
throughout the haemolymph. By 12 hr. after puparium formation nearly all
muscle fragments appeared to be associated with a single haemocyte nucleus.

Eventually, about half way through the phanerocephalic pupal period, the
‘Kornchenkugeln’ ruptured, releasing minute fragments into the haemolymph.

It is not altogether certain that all the hydrolytic enzymes involved in muscle
breakdown have their origin in the haemocytes. However, electron micrographs
of muscle cytoplasm before haemocyte invasion show few lysosome-like vacuoles,
(Plate 1) in contrast to the cytoplasm of the invading haemocyte (Plate 2, Fig. B).
Nevertheless the changes in the muscle which precede haemocyte invasion, and
the differing behaviour of adjacent muscles in the same haemocyte environment,
both indicate that the initial signal for muscle breakdown has its origin in the
muscle itself.

Pupal histolysis phase

Muscles that disappear during the phanerocephalic pupal stage, suffixed B
in Text-fig. 1, are numbered 8, 10, 13, 21–4, 25 and 26, of which number 8, the
lateral intersegmental muscle of Hewitt, is the most easily studied in detail.

In the larva and early pupa, the condition of muscle 8 differed little from that of
muscle 16 (shown in Plate 1, and Text-fig. 2A), except that the nuclei were
embedded in a particularly thick layer of peripheral sarcoplasm and situated
only on the anterior and posterior faces of the muscle.

Muscle 8 remained unchanged throughout the prepupal histolysis phase, and
PLATE 1
Part of abdominal hypodermal muscle 16, 4 hr. after puparium formation. Double stained with uranyl acetate and lead. × 18,000. B.M. thick basement membrane; VAC, large vacuoles; MY, myelin-like aggregations of concentric membranes; MI, mitochondria in association with myelin-like membranes; L.NUC, nucleus.

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(Facing page 98)
FIG. A. Part of abdominal hypodermal muscle number 1, 70 hr. after puparium formation. Stained with lead. × 30,000. B.M., diffuse basement membrane; T, remains of the T-canal system.

FIG. B. Part of a type F phagocytic haemocyte from a newly formed puparium. Stained with lead. × 300,000. Note the long extensions of the cell membrane (EXT.), some apparently sequestering volumes of haemolymph. MY, myelin-like aggregations of membranes contained in lysosomal structures.

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Muscle changes during metamorphosis of Calliphora

the muscle contracted at the time of eversion of the head, at 20–35 hr. after puparium formation, and remained in a contracted state until the onset of histolysis.

Between 20 and 30 hr. after puparium formation the sarcoplasm became vacuolated and the nuclear DNA readily became heavily stained. Many ‘Kornchenkugeln’ and haemocytes were present in the haemolymph, but the haemocytes showed no tendency to congregate around these muscles. By 30 hr. the fibrils appeared to be subdividing longitudinally into fibrillae (Text-fig. 2D), and the muscle plasma membrane gradually became more irregular until at about 50 hr. after puparium formation it ruptured in many places and the sarcoplasm was seen flowing out. The cross-striation (both Z and dark bands) had already disappeared and the nucleus had become pycnotic by this stage. No aggregation of haemocytes around the muscle was noted until it had fragmented, and their rôle was clearly that of scavengers (Text-fig. 2E). Every trace of muscles suffixed B in Text-fig. 1 was lost by 65 hr. after puparium formation.

Metamorphosis of larval abdominal hypodermal muscles into imaginal muscles

Muscles suffixed C in Text-fig. 1, numbers 1–4, 12 and 28 survived both pre-pupal and pupal histolysis phases. Muscles 1–4 ran in wide bands across single segments to form, together, a long muscle running down the side of the larva, Hewitt’s internal dorsolateral oblique recti muscles. Text-fig. 2A, which shows the larval condition of muscle 16, could equally represent the condition of muscle 1 15 hr. after puparium formation. Muscle 1, in common with other muscles suffixed B or C, was actively contractile at the time of eversion of the head. After head eversion, muscles 1–4 remained contracted, with the sarcolemma heavily folded, enclosing the nuclei in blisters of cytoplasm running at right angles to the contractile material (similar to muscle 8 shown in Text-fig. 2D). During the phanerocephalic pupal stage, cracks appeared between fibres, and the fibrillae also showed signs of separation. In addition, the cross-striation began to disappear. By 35 hr. after puparium formation the Z and dark bands were no longer distinguishable in phase contrast or stained preparations, and each fibre was divided into many bundles of fibrillae. The contractile material at this stage looked identical with that of histolysing muscle; however, muscles 1–4 did not fragment, instead the fibrillar structure slowly disappeared, leaving apparently undifferentiated, finely granular, vacuolated muscle matrices (Text-fig. 2F).

At 70 hr. after puparium formation, osmium-ethyl gallate preparations revealed that the muscle matrix contained many small vacuoles and osmiophilic granules. At the electron microscope level the osmiophilia was seen to be due to lipoprotein lamellae and droplets of fat, whilst the vacuoles were mainly resolved as mitochondria (Plate 2, Fig. A). It will be noted that the thick basement membrane surrounding the larval muscle has become diffuse, and that the only trace of the contractile origin of the tissue is the T-canal system. Larval muscle nuclei remained at this stage, and were all in excess of 15 μ in maximum dimension.
In the full-grown larva, muscle 2 had an average diameter of 300 \( \mu \) when relaxed, but by 65 hr. after puparium formation the same muscle had a diameter of only 100 \( \mu \) or less. In part this thinning process was the result of elongation of the muscle as development proceeded, because the insertions became further apart to encompass the wider imaginal anterior abdominal segments. However, there was no doubt that the muscle matrix did decrease in total volume between 30 and 100 hr. after formation of the puparium. This is evidenced by the fact that the muscle nuclei became on average more closely spaced; the mean distance separating larval nuclei, 65 \( \mu \), was reduced by 50 hr. to 25 \( \mu \). The implied decrease in volume must be accounted for by a loss of muscle matrix material into the haemolymph. In this connexion it should be noted that the muscle during this period was surrounded by ‘Kornchenkugeln’, haemocytes, fat cells and many droplets, all close to the muscle surface. It is possible that some of these droplets could have been extruded from the muscle, and electron microscopic examination of muscle fixed 50 hr. after puparium formation revealed a very irregular plasma membrane, apparently blebbing material into the haemolymph. The irregularity of the sarcolemma and the proximity of many type F haemocytes also made it difficult to exclude the possibility of phagocytosis of fragments of extruded muscle matrix. However, there was no evidence of disruption of the sarcolemma and there was certainly no invasion of phagocytic haemocytes into the interior of the muscle matrix.

**Invasion of myoblasts**

Between 40 and 60 hr. after puparium formation numerous small cells of overall dimension 5 \( \mu \), with nuclei 4 \( \mu \) in diameter, appeared in the haemolymph, and by 65 hr. were found distributed throughout the abdominal haemolymph, with a concentration in the vicinity of the larval muscle matrices. These cells were first noted by Perez (1910) and he termed them ‘myoblasts’. Myoblasts were seen pressed to the boundary of the matrix, often sinking into a concavity in the matrix boundary, but not constricted or distorted (Text-fig. 2G and Plate 3, Fig. A). Quite suddenly the larval muscle matrices were seen to contain myoblast cell nuclei deep in their cytoplasm, in amongst larval muscle nuclei (Text-fig. 2H). The invasion process took place between 70 and 90 hr. after puparium formation. In any individual pupa most of the muscles were seen either with a number of myoblast nuclei in their matrices, or with none, indicating that once some condition was realized, many myoblasts invaded. As myoblast cells passed into the matrix, their cell membranes became disrupted and their cytoplasm apparently merged with that of the matrix, leaving the nuclei in syncytial condition within the muscle plasma membrane. As the nuclei invaded they appeared to expand slightly, to about 5 \( \mu \) in diameter and, possibly as a result of this increase in nuclear volume, the DNA appeared less dense.

Fresh preparations of the pupal haemolymph containing muscle matrices surrounded by invading myoblasts were examined using phase-contrast optics.
PLATE 3

Fig. A. A myoblast cell in contact with muscle matrix, 70 hr. after puparium formation. Lead staining. × 40,000. MY, myoblast cytoplasm with large numbers of ribosomes; M-NUC, myoblast nucleus; MA, muscle matrix, with few ribosomes.

Fig. B. Myoblast nucleus (M-NUC) shortly after invasion of muscle matrix (MA). This preparation is double stained with uranyl acetate and lead, to increase contrast. × 30,000. VAC, vacuoles containing granules having the dimensions of ribosomes.

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A: Cross-sectional diagram of early phanerocephalic pupa, 24 hr. after puparium formation. The third pair of histoblasts is in the plane of another section.

B: Cross-sectional diagram of pupa, 60 hr. after puparium formation.

C: Drawings of living myoblasts, seen in phase-contrast preparations of pupal tissues obtained 70 hr. after puparium formation.

FR: Free-floating myoblast.
CO: Myoblast making contact with muscle matrix.
R.U.: Myoblast rounded up in contact with muscle matrix.
L.NUC: Part of a larval muscle nucleus.
Free-floating myoblasts were revealed as bipolar cells with long, filamentous, usually paired, tail-like extensions of the cell membrane (Text-fig. 3C). These cells, when initially making contact with muscle matrices, formed a shallow concavity and often remained poised at right angles to the matrix before slowly falling and eventually rounding up, with withdrawal of the tail-like extensions.

Electron micrographs of the invasion process are shown in Plate 3. Plate 3, Fig. A, shows part of an intact myoblast cell rounded up in contact with the muscle matrix. It should be noted that the myoblast cytoplasm contains many more ribosomes than an equivalent region of muscle matrix. Plate 3, Fig. B, shows a recently invaded myoblast immediately beneath the matrix plasma membrane, before migration into the interior of the matrix. This preparation has been stained with uranyl acetate to improve the contrast of the nucleic acid material. It is interesting to note that the cytoplasm of the myoblast cell may not be incorporated into the muscle matrix, since areas of matric surrounding myoblast nuclei do not contain larger numbers of ribosomes that the rest of the matrix. Furthermore, vacuoles containing large numbers of granules of the same dimensions as ribosomes appear to be detached in the vicinity of invading myoblasts (Plate 3, Fig. B). Certainly there is no evidence that the myoblast nucleus deep within the muscle matrix retains a discrete volume of myoblast cytoplasm bounded by a plasma membrane. Plate 4 shows two myoblast nuclei and part of a larval muscle nucleus within the muscle matrix.

Careful light and electron microscope examinations revealed no undifferentiated mesoblast cells pre-existing in the muscle matrix before the myoblast invasion phase. Further, there was no indication of migration of any nuclei from the imaginal epidermis to the muscle matrix. All imaginal muscle nuclei were, in the muscles examined, apparently derived from myoblasts invading from the haemolymph.

By 90 hr. after puparium formation the imaginal epidermis, newly formed from imaginal histoblast cells, had folded inwards at the insertions of the larval muscle matrices, and the link between the matrices and the epidermis and cuticle had become tenuous, as in Text-fig. 2H. By 150 hr. the larval muscle nuclei within the matrix were all pycnotic, and the DNA formed a dense core within a large nuclear vacuole. The pycnotic nuclei lost their vesicular shape, became elongated and finally disintegrated in the central region of the matrix. At 200 hr. after puparium formation they appeared as diffuse granular columns of DNA and all traces of individual nuclei were lost. There was no evidence to suggest that the nuclei divided in any way to form smaller nuclei, or other structures; all larval hypodermal muscle nuclei disintegrated.

By 96 hr. after puparium formation each muscle matrix had a large complement of myoblast nuclei, but the population of morphologically undifferentiated cells in the haemolymph had been greatly reduced, and concentrations around the muscle matrices were no longer seen. The myoblast nuclei within the matrices became arranged in short broken chains along the length of the muscle matrix.
Matrix of muscle 1, 96 hr. after puparium formation. Double stained with uranyl acetate and lead. × 20,000. M–NUC, myoblast nuclei; L–NUC, larval muscle nucleus.

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Careful examination of many hundreds of muscles rapidly fixed in liquid propane or hot Carnoy’s fluid, followed by staining using Feulgen’s technique, provided no evidence for mitotic or non-mitotic nuclear cleavages of myoblast nuclei within the muscle matrix, although morphologically undifferentiated presumptive myoblast cells were repeatedly found dividing in the haemolymph.

In order to ascertain the origin of myoblast cells, the disposition of all groups of imaginal cells in the abdomen was plotted during larval development.

**Origin and nature of imaginal muscle nuclei**

The larval complement of imaginal cells in the anterior seven segments of the abdomen is confined to three pairs of compact histoblasts in each segment. These cells undergo infrequent mitotic divisions during larval and cryptocephalic pupal life. The arrangements of the histoblasts in the 24-hr. (early phanerocephalic) pupa is shown in Text-fig. 3A. Although the size of individual histoblasts was slightly variable, their anatomical arrangement was identical in the first seven abdominal segments. The picture in the anal region was complicated by the presence of developing rudimentary genitalia. Each histoblast was found to be composed of 50–150 ovoid to fusiform cells of length 5–6μ, nucleus 4–4.5μ. No cells of this size were found floating in the haemolymph away from the histoblasts, nor were any cells or nuclei of these dimensions present inside, or concentrated near to the abdominal muscles at this stage.

The mitotic division rate in the hypodermal histoblasts increased suddenly about 30 hr. after puparium formation, and by 60 hr. the histoblast cells had spread out to form a complete imaginal abdominal epidermis, with the concomitant breakdown of the larval epidermis, which passed inwards to be engulfed by type F haemocytes, forming more ‘Kornchenkugeln’.

Not all the hypodermal histoblast cells expanded to form imaginal epidermis, however; a number of groups of multiplying cells sank inwards from the site of epidermis formation. The groups of cells were segmentally arranged, and each abdominal segment possessed three pairs, as shown in Text-fig. 3B. The anatomical arrangement of these groups of cells was not identical to that of the histoblasts, but it seems likely that each group was derived from an imaginal disc, and had migrated to its new position during the expansion of the imaginal hypoderm. Although the larval or cryptocephalic pupal histoblast was not composed of identical cells, no distinct subdivision into two types of cell, foreshadowing the epidermal cell–myoblast differentiation was apparent.

The numbers of these small imaginal cells increased as the result of mitotic divisions forming sub-groups, until by 70 hr. after puparium formation they were dispersed throughout the periphery of the abdomen, and all segmental arrangement was lost. Many of these cells were found concentrated in the vicinity of muscle matrices and some were seen partially sinking into a concavity in the matrix boundary. These observations leave little doubt that the invading myoblasts are derived from histoblast cells.
In the meantime many similar imaginal cells had concentrated posteriorly in the region of developing genitalia, and in the thorax in the region of developing flight muscles. However, no migration of cells from these regions to other parts of the pupa was noted, and it is believed that their contribution to the reservoir of myoblast cells can be neglected.

Initiation of muscle transformation

It has already been noted that the haemocytes alone cannot initiate muscle breakdown. However, it is possible that two systems affecting muscle metabolism, namely the tracheal and nervous systems, could play a part in the breakdown process. It is extremely unlikely, however, that a selective termination of the oxygen supply to individual muscles could initiate muscle transformation, since branches of the same trachea serve adjacent muscles, which disappear or transform at different histolytic phases. Nevertheless, the possibility that muscle breakdown could be the result of cessation of nervous impulses must be considered. In certain Saturniid moths, Finlayson (1960) noted that denervation influenced the degeneration of certain abdominal muscles. Fortunately a simple experiment to test the effect of denervation can be devised as a result of the observation of Fraenkel (1936), that temporary light ligation of the larva of Calliphora severs the nerve trunks connecting the brain with the hypodermal muscles. Accordingly, eighty crop-empty, resting, third instar larvae were tightly ligatured at the sixth apparent larval segment, then immediately released. After the operation it was noted that the hypodermal muscles in the segments behind the point of ligation were completely paralysed, whereas the hypodermal muscles in front of the point of ligation continued to contract.

Development proceeded, and a partially expanded puparium was formed about 48 hr. later. Thereafter muscular transformations occurred in the precise pattern established for normal insects, with normal timing and without any distinction between normal innervated muscles and experimentally denervated muscles. It seems unlikely that regeneration of the severed nerves could occur between the time of puparium formation, when the muscles clearly remained denervated, and the time of onset of the prepupal histolysis phase some 4 hr. later. Some credence should therefore be given to the hypothesis that muscular transformations in Calliphora are initiated by a system in the muscle itself, although such a system could be reacting to humoral changes.

DISCUSSION

It is clear that in Calliphora, haemocytes play a major rôle in the breakdown of larval muscles. The evidence that the invasion of haemocytes takes place only after preliminary changes in the muscle have taken place, is of two types. Firstly there is histological evidence; and secondly, there is evidence for clear differentiation of muscles into reactive and unreactive types at the onset of histolysis.

Histological examinations have shown that, in Calliphora, changes in the
Muscle changes during metamorphosis of Calliphora

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The observation of Hulst (1906) that separation of myofibrillae often preceded haemocyte invasion in *Calliphora*, is extended to *Calliphora*.

That the production of sarcolytes (muscle fragments) can be independent of haemocyte action, as de Bruyne (1898) found for *Calliphora*, Evans (1936) found for *Lucilia* and Robertson (1936) found for *Drosophila*, is confirmed; hence no need arises to consider the temporary phagocytosis theory of Perez (1910). That histological changes are advanced before haemocyte invasion begins is clearly shown in muscles at the pupal histolysis phase. Here the autolytic changes are probably those described as 'histolyse par dégénérescence' by Viallanes (1882), and analogous to the 'selbstandige Degeneration' of Schmidt (1928) and the 'wasting away' of Miall & Hammond (1900).

In *Calliphora*, during this phase, the haemocytes only enter the muscles in the last stages of disintegration. The suggestion that haemocytes enter entirely normal muscles in *Calliphora*, originally made by Van Rees (1888) and supported by Mercier (1906) and Perez (1910), thus seems to the present author to be unlikely.

Any theory of humoral control of muscle breakdown must include notice of a change in competence on the part of the muscle to react to humoral factors since the present work shows clearly that histologically identical adjacent muscles differ in behaviour from one to the next when surrounded by haemocytes. Similarly, the mechanism controlling muscle breakdown cannot act purely through changes in the haemocytes. Rizki (1962), working on *Drosophila*, noted that encapsulation haemocytes were a system responsive to stimulation emitted from fat masses.

There was no separation in the tracheal supply to disintegrating muscles, since such muscles were often served by tracheae which also supplied muscles which survived to adult life.

It is possible that the innervation of surviving muscles may differ from that of histolyzing muscles, but in the present work experimental denervation did not affect the muscle transformation pattern. Wigglesworth (1956) found that, in *Rhodnius*, denervation did not affect the normal cycle of growth and involution in the intersegmental muscles. Nor did obvious muscle degeneration result from denervation in larvae of *Lymantria* (Kopeć, 1923) or *Galleria* (Finlayson, 1956), and little change was noticed after similar experiments on *Periplaneta* (Roeder & Weiant, 1949). However, Finlayson (1960) provides evidence that in certain Saturniid moths, experimental denervation causes degeneration of affected muscles at metamorphosis, and concludes that innervation is one of the factors influencing maintenance of muscle in these species.

The theory of the origin of phagocytic cells in larval muscular material, which was implied by the work of Lowne (1870), developed by Viallanes (1882), who described the formation of 'cellules musculaires' in *Calliphora*, integrated into a general phagocytic theory by Metchnikoff (1883), and thence further extended to
differentiate between ‘sarcoclasts’ and ‘myoclasts’ by de Bruyne (1898), is contradicted by the present work. There is no evidence for the formation of phagocytic cells controlled by larval muscle nuclei. All the disintegrating muscle nuclei, together with the rest of the muscular remnants, are engulfed by phagocytic haemocytes. In Calliphora, groups of sarcolytes were rarely found unaccompanied by haemocyte nuclei, in contrast to the situation described for Rhagoletis by Snodgrass (1924). The origin of the phagocytic haemocytes is discussed in another paper (Crossley, 1964).

Although Weismann (1864), and Viallanes (1882), working on Calliphora, and D’Herculais (1875), Ganin (1876) and Evans (1936), working on other Diptera, denied any continuity of muscles from the larval to the imaginal abdomen, the present work confirms the beliefs of Van Rees (1888) and Perez (1910) that in Calliphora such a continuity does exist. Strong evidence is presented on anatomical grounds, and this is supported by tracing the histological changes in individual abdominal muscles throughout metamorphosis. The work of Perez (1910) on the abdominal muscles in general is extended to define the changes in specific muscles.

In Calliphora no larval abdominal muscles pass unchanged into the imago in the way that Vaney (1902) describes for Chironomus.

The invasion of imaginal cells into reduced larval muscles from the blood, originally described by Perez (1910) but doubted by Snodgrass (1924) and Jones (1962), is confirmed by the present work. There is no evidence that imaginal abdominal muscle nuclei ever had their origin in the nuclei of larval muscle as Lowne (1870), Van Rees (1884), de Bruyne (1898) and Perez (1910), all suggest may sometimes happen in Calliphora, and Schmidt (1928) and Snodgrass (1924) suggest for other Diptera.

The present author believes that all the larval abdominal hypodermal muscle nuclei disintegrate during metamorphosis, and that all the imaginal abdominal hypodermal muscle nuclei are derived from cells having their origin in histoblast tissue. It is shown that there are six hypodermal histoblasts in each abdominal segment of Calliphora, confirming the observation of Van Rees (1884). It is also shown that there are six sites of multiplication of presumptive myoblasts in each abdominal segment, but that these sites are not in every case close to the hypodermal histoblasts. Definitive proof of the origin of an individual myoblast in an individual histoblast is not, however, provided by these observations. Further experiments are also needed to answer the question; do the imaginal embryonic cells circulate freely in the haemolymph, or do they migrate directionally to specific, or to the nearest, muscle matrix?

The invasion of dedifferentiated abdominal hypodermal muscle matrices by histoblast nuclei, followed by the pycnosis of the original larval muscle nuclei, and the redifferentiation of imaginal muscle, would seem to involve two possibilities unusual in insect post-embryonic development. Firstly, the myoblast nucleus apparently relinquishes interaction with one volume of cytoplasm,
and initiates interaction with another. Secondly, as was noted in an earlier paper (Crossley, 1964), not all histoblast nuclei become incorporated into muscle as myoblasts. Certain cells, apparently derived from the same histoblasts as the myoblasts, differentiate into haemocytes, whilst others differentiate into fat body cells. Thus the possibility arises that the differentiation pathway of a histoblast entering a muscle matrix may be determined by the matrix environment. An interesting parallel in early embryonic development comes to mind. Strasburger (1934) showed that in Calliphora, following fertilization and cleavage, the cleavage nuclei migrate from the inside of the egg to the cortical layer, where at first they form a syncytium. After a definite time-lapse cell membranes separating the nuclei arise. Furthermore, in the mosaic egg of Calliphora, the region of the cortex in which the cleavage nucleus comes to reside determines the differentiation pathway of that nucleus (Pauli, 1927).

Final resolution of the problems of histoblast differentiation must probably await refinement of techniques of insect tissue culture.

**SUMMARY**

1. A re-examination of the changes at metamorphosis of certain abdominal hypodermal muscles in Calliphora is described.
2. Methods of preparation of insect hypodermal muscles for anatomical, histological and electron microscopical studies are outlined.
3. The anatomical changes in the abdominal hypodermal muscles were traced from larval to adult instars. Two major phases of histolysis occur. Certain larval muscles transform to adult muscles.
4. Histological changes have been followed with various light and electron microscopical techniques. The relative importance of haemocyte phagocytosis and autolysis are considered for different groups of muscles.
5. Evidence indicating that free-floating myoblasts, almost certainly derived from abdominal histoblast tissues, can invade modified larval muscles is presented, and electron micrographs of the invasion process are given.
6. Redifferentiation of imaginal muscles associated with myoblast nuclei is described.
7. Experimental denervation did not affect muscle histolysis.
8. The results are compared with those reported in the earlier literature. Certain similarities of metamorphic and early embryonic development are pointed out.

**RÉSUMÉ**

*Modifications des muscles abdominaux pendant la métamorphose de la mouche bleue de la viande, Calliphora erythrocephala (Meig.)*

1. L'auteur rend compte du réexamen des changements qui surviennent, pendant la métamorphose, au niveau de certains muscles abdominaux chez Calliphora.
2. Les techniques de préparation des muscles hypodermiques d’Insecte en vue d’études anatomiques, histologiques et ultra-structurales sont exposées.

3. Les modifications anatomiques des muscles hypodermiques abdominaux ont été suivies depuis le stade larvaire jusqu’à l’état adulte. Deux phases essentielles d’histolyse se produisent. Certains muscles larvaires se transforment directement en muscles adultes.

4. Les transformations histologiques ont été analysées au moyen de diverses techniques relevant de la microscopie ordinaire ou électronique. L’importance relative de la phagocytose hémocytaire et de l’auto-lyse a été appréciée pour différents groupes de muscles.

5. L’auteur présente des documents montrant que des muscles larvaires modifiés sont envahis par des myoblastes libres qui dérivent presque certainement des tissus histoblastiques abdominaux, et des micrographies électroniques illustrent le processus d’invasion.

6. La redifférentiation des muscles imaginaux est décrite, en association avec des noyaux myoblastiques.

7. La dénervation expérimentale n’affecte pas le processus d’histolyse musculaire.

8. Ces résultats sont comparés avec ceux qui ont été publiés antérieurement. Certaines analogies entre l’évolution métamorphique et le développement embryonnaire precoce sont soulignées.

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