POUL VAGN JENSEN

STRUCTURE AND METAMORPHOSIS OF THE LARVAL HEART OF *CALLIPHORA ERYTHROCEPHALA*

Det Kongelige Danske Videnskabernes Selskab
Biologiske Skrifter 20, 2

Kommissionær: Munksgaard
København 1973

DET KONGELIGE DANSKE VIDENSKABERNES SELSKAB udgiver følgende publikationsrækker:

THE ROYAL DANISH ACADEMY OF SCIENCES AND LETTERS *issues the following series of publications:*

	Bibliographical Abbreviation
Oversigt over Selskabets Virksomhed (8°) *(Annual in Danish)*	Overs. Dan. Vid. Selsk.
Historisk-filosofiske Meddelelser (8°) Historisk-filosofiske Skrifter (4°) *(History, Philology, Philosophy, Archeology, Art History)*	Hist. Filos. Medd. Dan. Vid. Selsk. Hist. Filos. Skr. Dan. Vid. Selsk.
Matematisk-fysiske Meddelelser (8°) Matematisk-fysiske Skrifter (4°) *(Mathematics, Physics, Chemistry, Astronomy, Geology)*	Mat. Fys. Medd. Dan. Vid. Selsk. Mat. Fys. Skr. Dan. Vid. Selsk.
Biologiske Meddelelser (8°) Biologiske Skrifter (4°) *(Botany, Zoology, General Biology)*	Biol. Medd. Dan. Vid. Selsk. Biol. Skr. Dan. Vid. Selsk.

Selskabets sekretariat og postadresse: Dantes Plads 5, 1556 København V.

The address of the secretariate of the Academy is:

Det Kongelige Danske Videnskabernes Selskab,
Dantes Plads 5, 1556 København V, Denmark.

Selskabets kommissionær: MUNKSGAARD's Forlag, Nørre Søgade 35,
1370 København K.

The publications are sold by the agent of the Academy:

MUNKSGAARD, *Publishers,*
35 Nörre Sögade, 1370 København K, Denmark.

POUL VAGN JENSEN

STRUCTURE AND METAMORPHOSIS OF THE LARVAL HEART OF *CALLIPHORA ERYTHROCEPHALA*

Det Kongelige Danske Videnskabernes Selskab
Biologiske Skrifter **20, 2**

Kommissionær: Munksgaard
København 1973

BIANCO LUNOS BOGTRYKKERI A/S
PRINTED IN DENMARK
ISBN 87 7304 018 5

I. Synopsis

The larval heart of *Calliphora* consists of a cylinder of muscle cells. The cells are situated in pairs each member of a pair being placed laterally in the heart wall. The external and internal (luminal) surfaces of the myocardium are covered by acellular connective tissue membranes of the basement-membrane type. The outer connective tissue layer contains elastic fibres. The fibres of the outer connective tissue membrane connect with fibres from the alary muscles, the surface of which show a similar arrangement: a layer of acellular connective tissue with elastic fibres. Such fibres are never seen in the much thinner basement membranes of the skeletal muscles. At any stage the larval *Calliphora* heart consists of a fixed number of cells arranged according to a very constant pattern. The differentiation of particular cells can be followed: some of the cells become transformed into vacuolized intersegmental cells, other myocardial cells seem to divide in the larval heart, and the imaginal ostiae are formed by perforation of the heart wall between the daughter cells. The course of differentiation of each particular cell can be predicted by determining its exact position (number).

II. Materials and Methods

Calliphora larvae reared on pork-heart at 25° were used. The description is based on larvae at the time of crop-emptying.
The techniques used were as follows:

1) fixation in Carnoy's fixative, sections stained with hematoxylin-eosin.
2) dissection in saline, fixation in cacodylate buffered 2.5 % glutaraldehyde, stained with hematoxylin-eosin: stained whole mounts.
3) dissection in saline, fixation in cacodylate buffered 2.5 % glutaraldehyde, postfixation in 1 % osmium tetroxide and embedding in epon according to Luft (Luft, 1961). Thin (1–1.5 μ) sections were stained by 1 % toluidine blue in 1 % borate.

III. Structure of the Larval Heart (text. fig. 1)

The posterior part of the larval heart, the ventricle, is situated immediately beneath the dorsal cuticle. The hindmost part of the ventricle (text. fig. 2) is located in the 8. abdominal segment. Anteriorly the ventricle reaches the border between segments 5 and 6, the border being marked by two highly vacuolized cells (text. fig. 3). The ventricle is the only part of the larval heart supplied with ostiae. The portion of the heart in front of the ventricle is usually termed the aorta but is actually part of the heart which may be referred to as the "front-heart". At the level of the most anterior pair of alary muscles (on the border between abdominal segments 2 and 3) the front-heart passes into the aorta. Aorta passes through the ring gland and attaches anteriorly between the protractor muscles of the mandibular hooks. The anterior part of the aorta opens ventrally allowing the hemolymph to leave the dorsal vessel.

(1) *The ventricle* (text. fig. 2).

The ventricle is the most active pumping part of the larval heart. It is suspended by elastic connective tissue fibres. The posterior part of the ventricle is closed and attached to the transverse trachea connecting the two lateral trunks. Fibres from the dorsal part of the ventricle go to the integument, fibres from the dorsolateral parts to the skeletal muscles on either side of the heart. The fibres from the ventrolateral and ventral parts of the heart are connected to the alary muscles.

The distension of the ventricle is effected by three pairs of alary muscles inserting dorsolaterally on the hypodermis between the abdominal segments $^{5}/_{6}$, $^{6}/_{7}$, and $^{7}/_{8}$. From the insertions the muscles pass beneath the lateral tracheal trunks and split up near the heart forming a fan of muscle fibrils. The fibrils are connected to the heart wall by elastic fibres. The elastic fibres are profusely anastomosing, and they even form arched connections between two adjacent muscles. Along the posterior part of the ventricle the fibres from both sides branch and anastomose forming a dense meshwork. The fibres of this meshwork support a very delicate connective tissue membrane forming a sort of pericardial septum. However, the pericardial septum of the *Calliphora* larva is entirely acellular, the connective tissue membrane being similar to the membrane covering the muscle layer of the heart (adventitia).

Ten to fourteen pairs of large pericardial cells are situated on the ventrolateral surfaces of the heart. Each cell is placed like a ball in a net formed by the bundle of fibres connecting the alary muscles and the heart wall. Schematic drawings of the fibres attached to the heart wall have been given by WHITTEN (1962). The tension of the fibres cause the pericardial cells to assume pear-shape, the tip pointing away from the heart wall. It should be mentioned that the posterior large pericardial cells have nuclei containing polytene chromosomes quite similar to those found in the salivary glands.

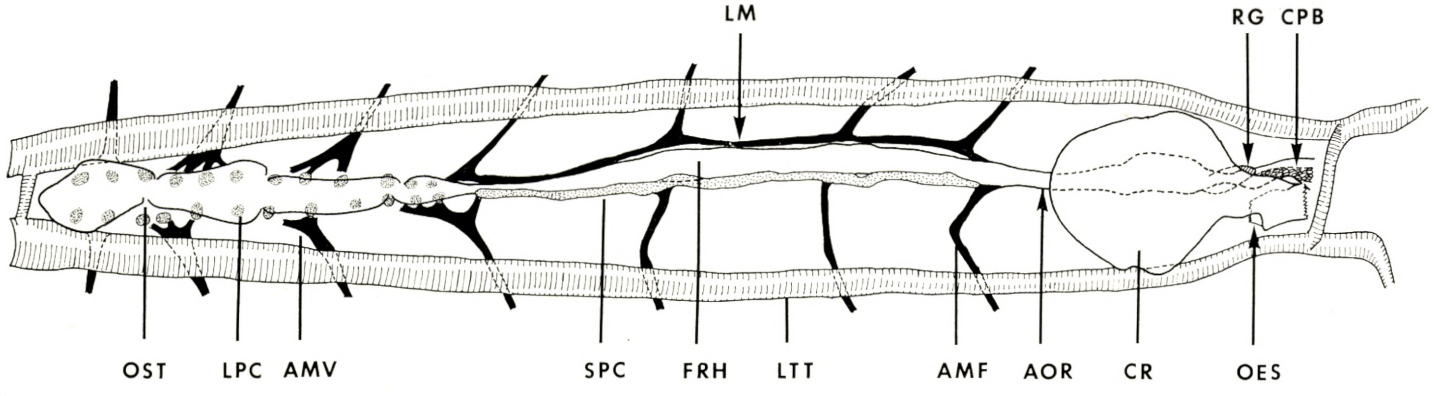


Fig. 1. Graphic reconstruction of the larval *Calliphora* heart. AMF = alary muscles to the front-heart, AMV = alary muscles to the ventricle, AOR = aorta, CPB = cephalo-pharyngeal band, CR = crop, FRH = front-heart, LM = longitudinal muscle, LPC = large pericardial cell, LTT = longitudinal tracheal trunk, OES = oesophagus, OST = ostia, RG = ring gland, SPC = small pericardial cells. The strand of small pericardial cells is only shown at the right side of the heart. The scale represents 0.2 mm.

(2) *The ostiae* (plate I, fig. 1).

The ventricle is provided with three pairs of ostiae. This number is in accordance with other observations: *Musca domestica* (HEWITT, 1910 and RANADE, 1967) and *Thrixion halidayanum* (PANTEL, 1914). The ostiae of the *Calliphora* larvae are vertical slits with two flaps protruding into the lumen. As the flaps are fused in the ventral and dorsal parts, the ostial openings are limited to the middle part of the ostial slit. Each flap contains a nucleus situated approximately in the middle of the flap providing the ostial flap to be a specialized muscle cell.

(3) *The ventricle wall* (plate I, fig. 2).

The wall of the ventricle consists of muscle cells arranged in a single layer. The muscle fibres in the cells always run circularly. The muscle layer is covered by two basement membranes the outer one called adventitia the inner one intima. The adventitia contains fibres similar to the suspending fibres from the alary muscles. The fibres are embedded in a basement membrane which appears homogeneous when examined with the light microscope. The connective tissue membranes of the *Calliphora* heart are acellular whereas in other insects the adventitia contains cells (*Aeschna*: ZAWARZIN, 1911, *Dytiscus*: KUHL, 1924, *Locusta*: HOFFMANN and LÉVI, 1965 and *Aiolopus*: BACETTI and BIGLIARDI, 1969). The elastic fibres from the alary muscles connect with fibres in the adventitia. In the adventitia the fibres form a dense meshwork covering the entire surface except at the inflected parts of the ostial flaps. Here they cross the slits connecting the heart wall on both sides forming a grate in which many haemocytes may be captured.

No fibres have been observed in the intima. In the contracted heart the adventitia and intima fold longitudinally; these folds are very regular and correspond to to the striation of the myocardium.

(4) *The nuclei of the myocardium* (plate I, fig. 2).

The nuclei are situated in pairs protruding into the lumen. This arrangement seems to reflect the ontogenetic formation of the heart from a right and left semicylindrical row of cells which later fuse dorsally and ventrally. According to POULSON (in DEMEREC, 1950) also the heart of *Drosophila* is formed in this way.

(5) *The tracheal supply to the ventricle.*

Stout tracheae leave the lateral trunks and run directly to the heart. Moreover more delicate tracheae penetrate the alary muscles and follow them towards the heart. They pass over to the adventitia along with the elastic fibres. In the ventricle wall the tracheae are mainly localized in the adventitia, however they are often seen crossing the muscle layer forming loops. The tracheal supply to the ventricle far exceeds that of the anterior parts of the heart.

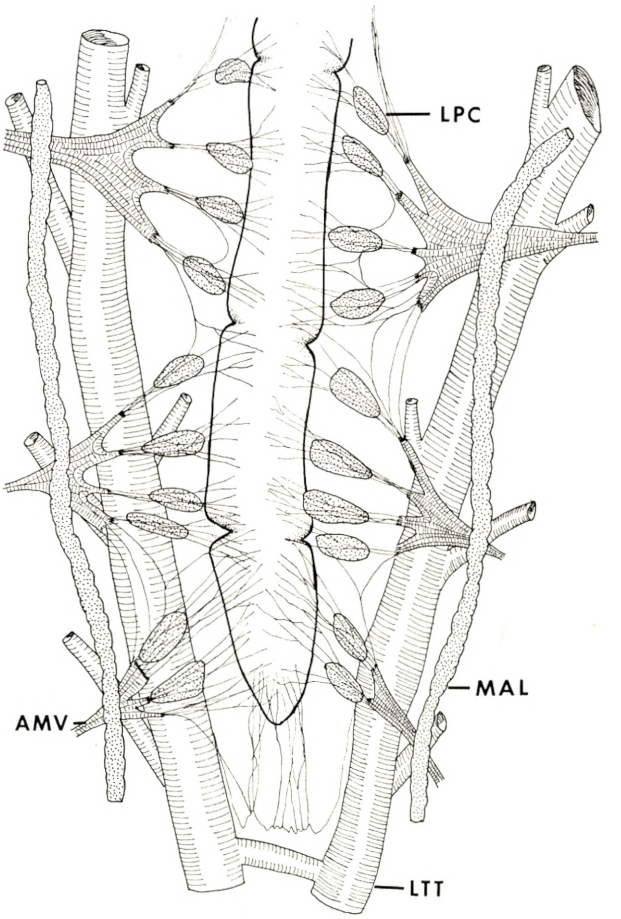


Fig. 2. Semi-schematic drawing of the ventricle (after a photograph) — AMV = alary muscle to the ventricle, LTT = longitudinal tracheal trunk, LPC = large pericardial cell, MAL = malpighian tubule. The scale represents 0.5 mm.

(6) *Ventricular valve* (text. fig. 3).

Between the ventricle and the front-heart two vacuolized cells form a "valve" allowing the hemolymph to pass only in a cephallic direction. Similar structures are described by PANTEL in *Compsilura concinnata* and *Rhacodineura antiqua* (1914), by JAWOROWSKY (1880) in *Tanypus varius* and by LEBRUN (1926) in the lumen of the *Corethra* heart.

(7) *The front-heart* (text, fig. 1, plate II, fig. 1).

The term front-heart refers to the portion of the heart from the ventricular valve to the foremost pair of alary muscles. This part of the heart is usually referred to as the aorta but there are several reasons for considering it part of the heart:

1) In other dipterous larvae (*Tipula*: WETTINGER, 1927, *Corethra*: LEBRUN, 1926, and TZONIS, 1936) the homologous part of the dorsal vessel is supplied with ostiae and therefore considered part of the heart.
2) In this portion of the vessel PANTEL (1914) found four pairs of rudimental ostiae in addition to the three posterior functional pairs.
3) During metamorphosis almost the whole imaginal heart is formed by transformation of this part of the larval heart.

The front-heart is suspended by four pairs of alary muscles attaching dorsolaterally between the segments. Like the three posterior pairs the alary muscles to

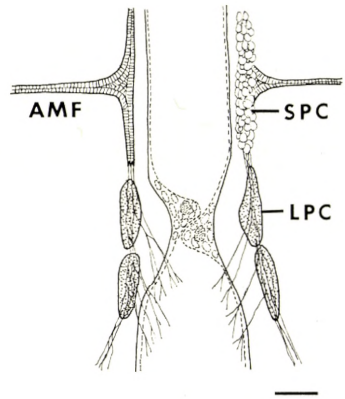


Fig. 3. Schematic drawing of the ventricle/front-heart transition. — AMF = alary muscles to the front-heart, LPC = large pericardial cell, SPC = small pericardial cells. The small pericardial cells are only shown at the right side of the hearts. The scale represents 0.1 mm.

the front-heart pass under the longitudinal tracheal trunks towards the heart. The muscles to the front-heart are joined by longitudinal muscles on either side of the heart as already described by WEISMANN (1864). The longitudinal muscles are formed by branching of each alary muscle into two bundles, one turning forward and one to the rear. At the level of the posterior pair of alary muscles to the front-heart, the longitudinal muscles pass into elastic fibres connected to elastic fibres from the anteriormost pair of alary muscles of the ventricle. The foremost pair of large pericardial cells is suspended in this pair of fibres (text. fig. 3). From the most anterior pair of alary muscles muscle fibres follow and eventually enwrap the aorta (plate II, fig. 2).

(8) *Pericardial cells in connection with the front-heart.*

Small pericardial cells (diam. 25–30 μ) accompany the longitudinal bands (plate II, fig. 1). They form two dense bands along the dorsal side of the muscles. The nucleus of these cells do not contain polytene chromosomes as found in the posterior pericardial cells.

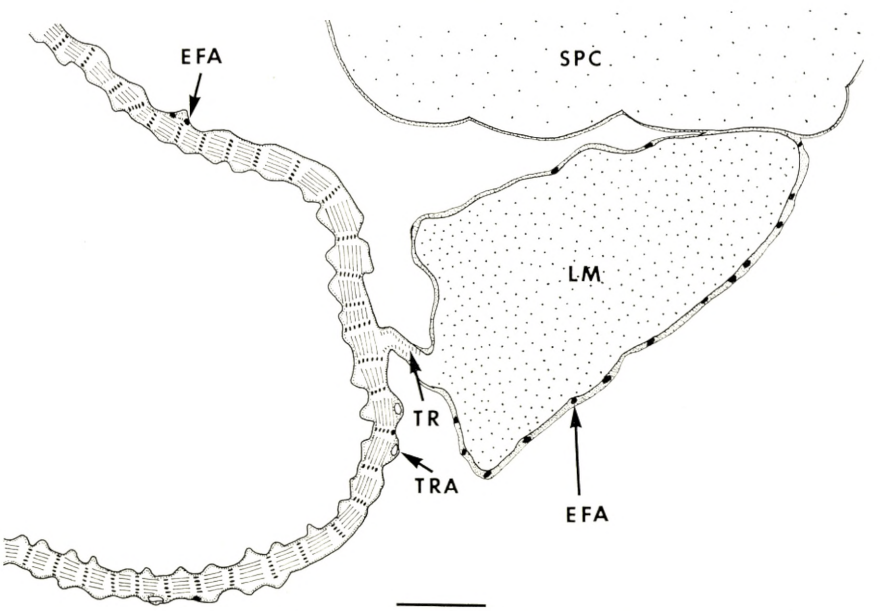


Fig. 4. Diagrammatic, transverse section of the front-heart (after a photograph). — EFA = elastic fibres of the adventitia, LM = longitudinal muscle, SPC = small pericardial cells, TR = trachea supplying the heart. TRA = tracheole in the adventitia of the heart. The scale represents 10 μ.

(9) *Histology of the front-heart* (text. fig. 4).

The front-heart contains a muscle layer one cell in thickness. At the outside the muscle layer is covered by an adventitia containing fibres; towards the lumen an intima devoid of fibres is found. The adventitial fibres are thinner and more sparse than those of the ventricle. The "adventitia structure" (a fibrous basement membrane) also covers the surfaces of the longitudinal muscles, the alary muscles, and the surface of the pericardial cells. However, at the surfaces of the pericardial cells the fibres are sparse. Fibres from the adventitia of the heart cross over and anastomose with fibres of the longitudinal muscles. In addition fibres from the longitudinal muscles go to the pericardial cells, and finally, fibres run directly from the pericardial cells to the adventitia of the heart. At a short distance from the heart the fibres from the longitudinal muscles ramify into an anterior and a posterior branch which join the adventitia of the heart. The longitudinal fibres in the heart wall are anastomosing by more or less oblique cross connections the whole fibre system forming oblong polygonal figures. The wavy appearance of the contracted front-heart is similar to that of the ventricle.

(10) *The tracheal supply* (text. fig. 4).

All tracheae to the front-heart pass through the alary muscles to the longitudinal muscle bands. From these they pass to the adventitia of the heart. The tracheae in the front-heart are seemingly confined to the adventitia. The tracheal supply to the front-heart is sparse compared to that of the ventricle.

(11) *The aorta* (plate II, fig. 2).

The aorta begins just anterior to the foremost pair of alary muscles and it is attached to the posterior edge of the inflection between the foremost segment and the prothorax. The aorta opens ventrally just in front of the central nervous system. The posterior part of the aorta is close to the ventral surface of the crop with which however it has no connection. The very thin-walled aorta has a transverse striation indicating longitudinal muscle fibres whereas the front-heart only contains circular fibres. The more distinct striation of the aorta compared to the front-heart is similar to the striation of the alary muscles and suggest that the muscle layer of the aorta is actually formed by the forward directed fibres of the foremost pair of alary muscles. The aorta wall varies in thickness with the presence and absence of muscle fibres. Thus when muscle fibres are absent the two basement membranes are close together forming a very delicate membrane approximately 0.5 μ thick. The adventitia contains fibres of the same type as in the front-heart. The nuclei of the aorta are arranged in pairs.

(12) *The ring gland* (plate III, fig. 1).

During its passage through the ring gland (the postcerebral endocrine complex) the aorta makes connection with this organ dorsally and ventrally whereas the lateral parts of the aorta are free. At the dorsal connection the vacuolized cells of the cephalopharyngeal band (Lowne, 1893–95) separate the aorta and the ring gland proper. Corpus allatum of the gland is located beneath the vacuolized cells. The lateral parts of the gland contain cells belonging to the prothoracic gland system. The nervus recurrens from the frontal ganglion passes below the aorta to reach the hypocerebral ganglion behind the ring gland. This ganglion emits two nerves: the nervus proventricularis running backwards to the proventriculus and the cardio-hypocerebral nerve which bifurcate and then terminate in the ventral part of the ring gland. Two additional nerves run from the cerebral ganglia into the ring gland some distance from the sagittal plane. These are the two nerves to the corpus cardiacum. The picture is further complicated by the presence of delicate muscular bands in this region. Two muscular bands leave the ventrolateral aorta wall and join the nervus cardio-hypocerebrale which at this level is fused to a single nerve. The distal attachment points of these muscles are not known but the nervus recurrens contains muscle fibres of a similar type far anterior to the ring suggesting that the fibres form part of the nerve throughout its full length. In addition to these muscles which are not mentioned by Frazer (1959) in his description of this region in *Lucilia* two muscle bands each consisting of two parts leave the ventral wall of the aorta just anterior to the ring. These muscles appear similar to the aorta wall at this point: a stout outer connective tissue membrane containing fibres. In this they differ from the muscles connecting with the cardio-hypocerebral nerve which has only a delicate outer connective tissue membrane. The latter two bands, also mentioned by Frazer (1959) in *Lucilia*, fuse at a distance from the

aorta forming a single band which is still separated into four sectors by massive connective tissue. I have not been able to locate their attachments, Frazer suggests the cerebroid commissure which seems likely.

(13) *The anterior part of the aorta* (plate III, fig. 2).

The part of the aorta anterior to the ring is connected to the following structures: the frontal sacks forming the imaginal head early in metamorphosis and the cephalo-pharyngeal band. The frontal sacks originate from the ring fold between the foremost segment and the prothorax. Posteriorly they fasten via the optic nerve rudiments ventrolaterally on the cerebral ganglia. The anterior parts of the segmental nerves also makes connections with the aorta. The cephalo-pharyngeal band follows the dorsal wall of the aorta from the ring gland to the anterior insertion. Figures showing the anatomy of the anterior parts of the larvae are given by Ludwig (1949), by Possompès (1953), and by Frazer (1959).

Transverse sections of the region anterior to the ring gland show a ventral tubular membrane surrounding the oesophagus. This membrane emerges from the neutral lamella of the oesophageal canal. The membrane is very delicate and contains only scattered muscle fibres between the two connective tissue membranes. Anterior to the ventral ganglion mass this membrane is open ventrally, the free edges containing the two mandibular-maxillar-labial nerves. The frontal sacks make contact by a transverse commissure and just anterior to this point the ventral membrane fuses with the aorta. In the angle formed between the aorta and the ventral membrane the frontal sacks penetrate. Here the two ventral fringes approach the frontal sacks. Weismann (1864) described the anterior part of the aorta as being distended within a frameword corresponding to the position between the frontal sacks. Over the aorta a strand of vacuolized cells is present from the ring gland to the anterior attachment point of the aorta: the cephalo-pharyngeal band. Posteriorly the band is formed by a single row of cells which appear highly vacuolized. The main part of the cytoplasm is confined to the central part of the cells. From here fine cytoplasmic projections radiate into the thin peripheral cytoplasmic layer. Anteriorly a transverse section will contain more than one cell and the vacuolization is less pronounced. The foremost part of the band consists of cells showing little vacuolization but very deep invaginations of the cell membrane (plate IV). Often the invaginations almost traverse the cells and make the organ appear lamellar. A stout basement membrane containing powerful elastic fibres covers the cells. At the anterior part the thick basement membrane bridges the gaps formed by the invaginations, and the fibres attain their maximum thickness. The nucleoli of the cells are well developed. Electron micrographs show the cells to be filled with microtubules parallel to the invaginations. Points of contact between two cells show well developed desmosomes. Wahl (1915) considered this cell band to be an anterior extension of the ring. However the cells are very different, and the function is not known. It may be added that two muscle strands are found in sections of the anterior aorta. They are similar in structure to the alary muscles. Posteriorly they

fasten on the inside of the cerebroid commissure, anteriorly they are still found in the region where the aorta fastens. FRAZER (1959) reports that in *Lucilia* they are inserted near the openings of the frontal sacks.

Histology of the anterior membranes.

The anterior membranes (the aorta anterior to the ring gland and the membrane surrounding the oesophagus) contain sparse longitudinal muscle fibres between two basement membranes. It should be mentioned that the segmental nerves are covered by fibrous membranes.

IV. Observations on the Living Heart

The ostiae are usually considered to be thin membraneous flaps opening and closing passively according to the pressure on the in- and outside. However the individual ostial flaps of *Calliphora* consist of single muscle cells with maximum thickness along the edges.(Plate I, fig. 1). The contraction of the muscle bands along the edges during systole is obvious whereas the compression of the thin parts of the ostiae might result from the increased pressure in the ventricle. The connective tissue fibres bridging the ostial clefts probably serve to keep the cleft sufficiently narrow to be closed by the flaps.

(1) *The ventricle* (text, fig. 2).

The ventricle is suspended by elastic fibres from the integument and from the alary muscles. At the systole the ostiae are closed and the hemolymph pressed forward through the narrow opening between the ventricular valve (cf. p. 4). The systole causes considerable elongation of the elastic fibres from the alary muscles but only little elongation of the muscles themselves. Immediately following the systole the valve closes, probably through action of a powerful ring muscle layer contained in these cells. In the larvae of *Chironomus* JAWOROWSKY (1880) described a ring muscle in this region. When systole is over the diastolic expansion is due to the elastic fibres. Thus the ventricle is filled by hemolymph only through the ostiae. It should be noted that the two cells of the ventricular valve apparently contract during diastole and are therefore out of phase with the other muscle cells of the heart.

(2) *The front-heart.*

The front-heart appears much less active in the contractions; it is difficult to determine whether the pulsations of this part of the heart is mainly a function of its own contractions or of the pulsations of hemolymph propagated by the ventricle. Anteriorly the hemolymph leaves the aorta ventrally just anterior to the transverse connections between the frontal sacks.

V. The Metamorphosis

The most important events during larval-adult metamorphosis are described by PEREZ (1910), ROBERTSON (1936), and by SNODGRASS (1955).

(1) *The muscid heart during metamorphosis.*

The literature concerning the transformations of the heart during metamorphosis is sparse. WEISMANN (1864) saw the alary muscles undergo histolysis and the heart shorten in consequence of the shortening of the abdomen following the evagination of the head. Weismann believed that the heart as an organ remained intact during metamorphosis. KOWALEWSKY (1886–87) observed heart transformations after the third day of pupal life. The contractions became irregular and heart activity ceased. At the same time the striation of the myocardium disappeared. Kowalewsky considered the middle and anterior part of the heart to be transformed into the imaginal heart whereas the posterior part was engulfed by phagocytic cells. Furthermore he observed the middle part of the heart to approach the integument. A different opinion was held by LOWNE (1893–95) who proposed the imaginal heart to be formed by strethcing of the posterior part of the larval heart (here termed the ventricle) pointing out that the posterior part of the larval heart and the adult heart maintain the same close contact with the dorsal integument. Lowne regarded the imaginal heart to be formed by embryonic cells joining the larval heart laterally.

(2) *The Calliphora heart during metamorphosis.*

At formation of the puparium the heart pulsates regularly with a frequency of about 180/min. After four hours the heart pulsations are arrested during periods of steadily increasing duration until heart action ceases. The pulsations are not resumed until shortly before imaginal ecdysis.

(a) *The ventricle during metamorphosis.*

The ventricle undergoes partial histolysis. While evagination of the head occurs the abdomen shortens thus reducing the length of the ventricle from appr. 1.3 mm to 0.5–0.6 mm. Thirty hours after puparium formation haemocytes gather at the distal attachments of the alary muscles, and at 50 hours the alary muscles are free distally and contract the adventitia into transverse folds. The striations of the alary muscles rapidly become very indistinct. At the same time the large pericardial cells assume an irregular surface, they are then attacked by phagocytes commencing at the most posterior pair. The decomposition of the ventricle becomes visible after 70 h. starting at the hindmost tip and progressing in a forward direction. Phagocytic cells apply closely to the ventricle wall and begin to take up material from the wall. The adventitia material containing fibres can be recognized as inclusions in the phagocytes. At 50 h.

the ventricle loses its connection with the hypodermis, the larval cells being pushed into the hemocoele by advancing imaginal cells. At the time of eclosion the regression of the ventricle has proceeded until between the anteriormost and the second pairs of larval ostiae. The part of the ventricle situated in the 5. abdominal segment and the anterior pair of larval ostiae persist in the imago. The "surviving" larval ventricle is supplied with a pericardial septum of the imaginal type as described later. The three pairs of alary muscles to the larval ventricle are completely destroyed. The fourth pair of alary muscles from behind is reconstructed by imaginal myoblasts which fuse with remnants of the old muscle.

(b) *The front-heart.*

The front-heart develops to form the main part of the imaginal heart. While the larval front-heart is a tube of uniform thickness the corresponding part of the imaginal heart is divided into chambers separated by narrow intersegmental parts. Four pairs of ostiae are formed in this part of the heart which in the larvae shows no traces of ostiae. As a consequence the imaginal heart possesses 5 pairs of ostiae: the anteriormost larval pair and four pairs formed during metamorphosis. The alary muscles and the longitudinal muscles are histolysed early in pupal life in the same way as the posterior pairs of alary muscles. The distal attachments lose the contact with the hypodermis during formation of the imaginal hypoderm. The wall of the front-heart is not histolysed. Only in the zones of ostial formation contact between phagocytes and the heart wall occurs. The small pericardial cells which have multiplied vividly in the beginning of the third larval stage stop the divisions at the time of puparium formation.

(c) *The number of cells in the front-heart.*

The larval front-heart corresponds to the abdominal segments 1–4. Each "segment" of the front heart contains 6 pairs of nuclei in the third larval stage. Consequently the whole front-heart contains 24 pairs of muscle cells. The cells may be numbered from the vacuolized pair of cells between the larval ventricle and the front-heart and may then be called no. 1. Cell pair no. 1 retains its structure in the imago where it is situated between abdominal segments 4 and 5. The cell pairs 2, 7, and 12 are not differentiated visibly but continue to form "ordinary" myocardial cells in the imago. The cell pairs 3a/3b, 8a/8b, 13a/13b, and 18a/18b presumed to be the result of divisions of the cells 3, 8, 13, and 18 early in larval life are mush smaller than the other nuclei of the heart. The "a" and "b" nuclei are always lying close together in thin-walled areas of the front-heart thus suggesting their formation being the mitosis of a single cell. The thin areas are the future ostial flaps, and the nuclei are the small nuclei found in the flaps, The cells 4 and 5 in the 4th segment, 9 and 10 in the third segment and 14 and 15 in the second are not transformed visibly, and they remain "ordinary" myocardial cells. In addition to the differentiation into imaginal ostiae

intersegmental vacuolized cells are formed by the cell pairs 6 and 11 at the segment borders $^{3}/_{4}$ and $^{2}/_{3}$. These cells develop the same structure as the cells in pair no. 1. In the abdominal segments 1 and 2 the strictly segmental pattern of the heart is lost, and a single great chamber is formed. Thus instead of differentiating into vacuolized cells like pairs no. 6 and 11 cell pair no. 16 form ordinary myocardial cells. Nevertheless the two ostiae of this chamber are formed from the cells 13a/13b and 18a/18b as expected. At the anterior end of the chamber the cells of no. 20 together with no. 21 differentiate into four vacuolized vells.

PANTEL (1914) described the heart of *Thrixion halidayanum*. In the portion of the heart referred to as the "troncon intermédiaire" and which corresponds to the front-heart he found four ostioles i.e. rudimentary ostiae. Each ostiole was seen to contain two nuclei referred to as "noyaux géminés". Like in *Calliphora* four pairs of cells were found between two ostioles. Probably these ostioles form the imaginal ostiae by perforation of the wall between the nuclei. Pantel points out that the number of nuclei in the heart of *Thrixion* is constant (though in other species the total cell number may differ from the number found in *Thrixion*) and this applies also to *Calliphora* where deviations from the schedule described are never seen.

(d) *The pericardial septum.*

At 70 h. imaginal myoblasts of the pupa make close contact with the front-heart and penetrate between the pericardial cells. These myoblasts form the pericardial septum of the imago which consequently is devoid of larval parts. The myoblasts also make contact with the imaginal hypodermis cells all the myoblasts together forming a half-cylinder surrounding the heart. In this period the front-heart moves from the larval position to a position just beneath the integument. This movement may be due to the activity of the myoblasts. At first the myoblasts form intimate contacts with the ventral and lateral parts of the front-heart wall forming longitudinal rows. Later they retire from the heart wall forming longitudinal strands running parallel to the heart. The strands are fixed laterally by transverse connective tissue strands (plate V, fig. 1). The connections between the myoblasts and the heart wall are preserved when the myoblasts move away from the heart. The myoblasts also make contact with the pericardial cells; these connections later stretch to form connective tissue strand. The early myoblasts contain little cytoplasm and are strongly stained by hematoxylin. Later they acquire more cytoplasm, and they show striation in late pupal life. The pericardial cells laterally to the larval heart accompany the heart during its movement towards the hypoderm. Later they spread laterally under the integument making contact with the hypodermic cells. The connections between pericardial cells and other tissues always consist of connective tissue strands.

(e) *The ostiae.*

About 100 h. after puparium formation phagocytes gather outside the thin areas of the front-heart wall. The perforation of the wall occurs between the neighbour

nuclei 3a/3b, 8a/8b, 13a/13b, and 18a/18b. The thin-walled areas form the imaginal ostial flaps. Four pairs of ostiae are formed almost simultaneously.

Details of ostial formation in insects are hardly known. In small larvae of *Chironomus* the ostiae appear to be formed in the same way as in *Calliphora* during metamorphosis, by division of laterally situated cells (POPOVICI-BAZNOSANU, 1905). The vacuolized intersegmental cells in the imaginal *Calliphora* heart are also prominent in the heart of *Drosophila* (MILLER, in Demerec, 1950).

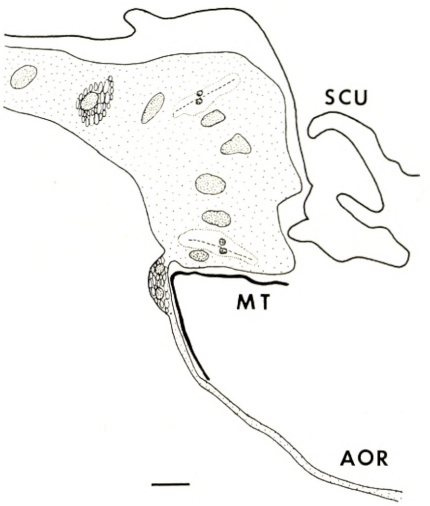


Fig. 5. Graphic reconstruction showing the anterior part of the imaginal heart. — AOR = aorta, MT = metathorax, SCU = scutellum. The scale represents 0.1 mm.

(f) *The anterior chamber* (text fig. 5).

One big chamber possessing two pairs of ostiae occupies segments 1 and 2 in the adult. In the cryptocephalic stage the longitudinal axis of this chamber is horizontal but the formation of the constriction between thorax and abdomen causes a rotation of the axis to an almost vertical position. The connection to the aorta is consequently found at the "bottom" of the chamber. The posterior part of the metathorax forms two projections penetrating on each side of the heart/aorta transition. On these projections the alary muscles of the imago fasten as already described by WEISMANN. The alary muscles of the imago appear more diffuse than in the larvae. They consist of individual fibres running parallel rather than of compact muscles. It is surprising that the ventral part of the pericardial septum is attached to the midgut which has a prominent multicellular layer at the dorsal side. In the space between the attachment of the alary muscles at the metathorax and the attachment of the pericardial septum at the midgut numerous connective tissue fibres cross directly from the adventitia of the heart to the protrusions of the metathorax. The transition to the aorta is formed by four strongly vacuolized cells differentiated from the larval cell pairs no. 20 and 21.

(g) *The tracheal supply.*

The larval heart is supplied with tracheae via the alary muscles, consequently the supply to the front-heart is interrupted by their histolysis. This condition persists until 170 h. after pupation when tracheoblasts enter the wall. Later on the tracheal supply increases considerably especially in the anterior chamber.

(h) *The aorta.*

The hindmost part of the larval aorta is accompanied by branches of the first pair of larval alary muscles. At about 50 h. a vigorous infiltration of phagocytes occurs between the anterior branches and the aorta wall which are thus disconnected. The aorta is considerably elongated through the evagination of the head. The ring gland situated above the cerebral ganglia in the larva moves backwards to become located in the prothorax at hatching. At 70 h. phagocytes are seen on both sides of the aorta wall apparently concerned with uptake of its material (plate VI, fig. 1). The following period (72–143 h.) shows no changes in the aorta which only consists of two connective tissue membranes (adventitia and intima) lying very close together except where nuclei are found. At 143 h. a third layer can be distingushed between the two membranes and tracheoblasts make connections with the outer membrane ventrally and dorsally. The tracheoblasts penetrate between the layers and can be followed over long distances. At 170 h. the lateral nuclei seem to resume their activity, and new contractile substance is synthesised (plate VI, fig. 2). Formation of the muscle layer of the aorta occurs shortly before eclosion. Simultaneously with the formation of new contractile substance the surface of the adventitia and intima changes now acquiring a wavy appearance similar to the surface of the larval ventricle and front-heart. The muscle fibrils tend to form spirals rather than a regular circular array.

(i) *The anterior part of the larval aorta.*

The frontal sacks fuse very early in metamorphosis. Their expansion towards the midline follows the aorta. The cephalo-pharyngeal band lying "outside" the aorta is displaced to be found above the fused sacks. Later it is histolyzed. The degradation of the pharyngeal muscles causes the aorta to lose its anterior attachment. It is withdrawn and contracts much like the alary muscles while they lose their distal attachments. Having passed through the ring gland the aorta now opens just behind the brain. Because of the contraction a thick layer of muscles and connective tissue is found in the ring gland of the early pupae. The histolysis of the muscle layer in the anterior part of the aorta continues until eclosion. At this time the aorta opens between two muscles fastening ventrally at the trachea supplying the head and dorsally on the head capsule. In *Drosophila* the aorta opens behind the brain (Miller in Demerec, 1950).

(j) *Aorta between the prothorax and the head.*

The anterior segment of the *Calliphora* larvae is ventilated by four stout tracheae branching off from the longitudinal trunks just anterior to the transverse connection.

These tracheae are transformed to supply the head of the imago. When the constriction between head and prothorax is formed these tracheae approach one another to form two pairs along each side of the oesophagus. Each pair of tracheae is surrounded by a common matrix of tracheoblasts forming only one big trachea in the imago. Aorta is situated between the two dorsal tracheae ealy in pupal life. Later the imaginal tracheoblasts spread over and make intimate contact with the aorta wall.

(k) *The ring gland.*

The ring gland has been observed during pupation. The lateral parts (the thoracic glands) still exist at eclosion but are then histolyzed. However, an infiltration of the thoracic gland by haemocytes occurs early in pupal life even if it does not cause an immediate histolysis of the gland.

References

Bacetti, B. & Bigliardi, E., 1969: Studies on the fine structure of the dorsal vessels of arthropods. I. The "heart" of an orthopteran. Z. Zellforsch., *99*, 13–24.

Demerec, M. (ed.), 1950: Biology of *Drosophila*. John Wiley & Sons, Inc., London.

Frazer, A., 1959: The anatomy of the central nervous system and retrocerebral glands of the larvae of *Lucilia caesar* and certain other Diptera Cyclorrhapha. Proc. Roy. Ent. Soc. London, ser. A., 186–192.

Hewitt, C. G., 1910: The house fly. Manchester University Press, 1910. Cambridge: at the University Press, 1914.

Hoffmann, J. A. & Lévi, C., 1965: Étude au microscope électronique du vaisseau dorsal de *Locusta migratoria*. Comp. Rend. Acad. Sc. Paris, *260*, 6988–90.

Jaworowsky, A., 1880: Über die Entwicklung des Rückengefässes und speziell der Muskulatur bei *Chironomus* und einige anderen Insekten. S. B. Akad. Wiss. Wien, *80*, 238–258.

Kowalewsky, A., 1886–87: Zum Verhalten des Rückengefässes und des guirlanden förmigen Zellstranges der Musciden während der Metamorphose. Biol. Ztrbl., *6*, 74–79.

Kuhl, W., 1924: Der feinere Bau des Zirkulationssystems von *Dytiscus marginalis*. Zool. Jb. Abt. Anat., *46*, 75–198.

Lebrun, H., 1926: L'appareil circulatoire de *Corethra plumicornis*. La Cellule *37*, 183–200.

Lowne, B. T., 1983–96: The anatomy, physiology and development of *Calliphora erythrocephala*. R. H. Porter, London, vol. 1–2.

Ludwig, C. E., 1949: Embryology and morphology of the larval head of *Calliphora erythrocephala*. Microentomology *14*, 75–111.

Pantel, J., 1914: Région postérieure du vaisseau dorsal des larves des muscides et particularités remarquables de cette region chez la larve de *Ceromasia rufipes*, Mg. La Cellule *29*, 381–389.

Perez, C., 1910: Recherches histologiques sur la metamorphose des muscides, *Calliphora erythrocephala*. Arch. Zool. Exp. Gén., ser 5, *4*, 1–274.

Popovici-Baznozanu, A., 1905: Beiträge zur Kenntnis des Cirkulationssystem der Insekten. Jean Zeitsch. f. Nat., *40*, 667–696.

Possompés, B., 1953: Metamorphose de *Calliphora erythrocephala*. Arch. Zool. Exp. Gén., *89*, 204–345.

Ranade, D. R., 1967: The circulatory system of the third instar larvae of *Musca domestica*. Bull. Ent. Dep. Zool., *8*, 13–16.

Robertson, C. W., 1936: The metamorphosis of *Drosophila melanogaster* including an accurately timed account of the principal morphological changes. J. Morph., *59*, 351–398.

Snodgrass, R. E., 1955: The metamorphosis of a fly's head. Smiths. Misc. Coll., *122* no. 3, 1–25.

Tzonis, K., 1936: Beitrag zur Kenntnis des Herzens der *Corethra plumicornis*. Zool. Anz., *116*, 81–90.

Wahl, B., 1915: Über die Kopfbildung cychlorrhaphen Dipterlarven und die postembryonale Entwicklung des Fliegenkopfes. Arb. Zool. Inst. Wien, *20*, 35–114.

Weismann, A., 1864: Die Entwicklung der Dipteren. Leipzig, Wilh. Engelmann.

Wettinger, O., 1927: Das Cirkulationssystem der Tipulidenlarven mit besonderer Berücksichtigung von *Tipula selene*. Z. Wiss. Zool., *129*, 453–482.

Whitten, J. M., 1962: Breakdown and formation of connective tissue in the pupal stage of an insect. Quart. J. Micr. Sci., *103*, 359–367.

Zawarzin, A., 1911: Histologische Studien über Insekten. A: Das Herz der Aeschnalarven. Z. Wiss. Zool., *97*, 481–509.

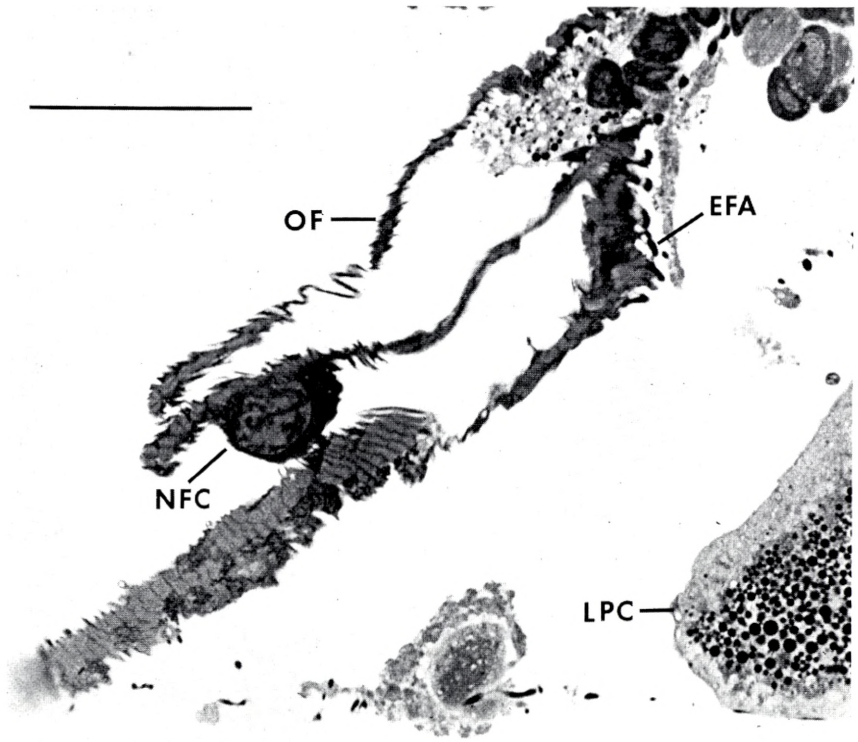
Indleveret til Selskabet den 23. oktober 1972.
Færdig fra trykkeriet den 8. august 1973.

PLATES

PLATE I

Fig. 1. Transverse section of a larval ostia. — EFA = elastic fibres of the adventitia, LPC = lagre pericardial cell, NFC = nucleus of flap cell, OF = ostial flap. The scale represents 50μ.

Fig. 2. Transverse section of the wall of the larval ventricle. — EFA = elastic fibres of the adventitia, NM = nucleus of the myocardium, TR = tracheole situated in the intima. The scale represents 10μ.


OF
EFA
NFC
LPC

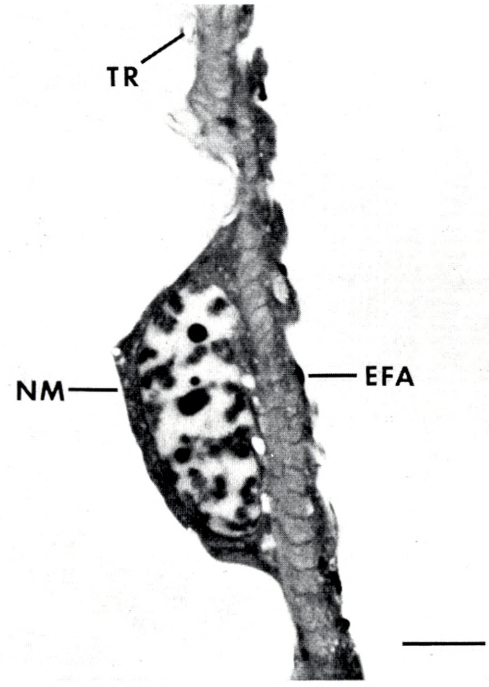
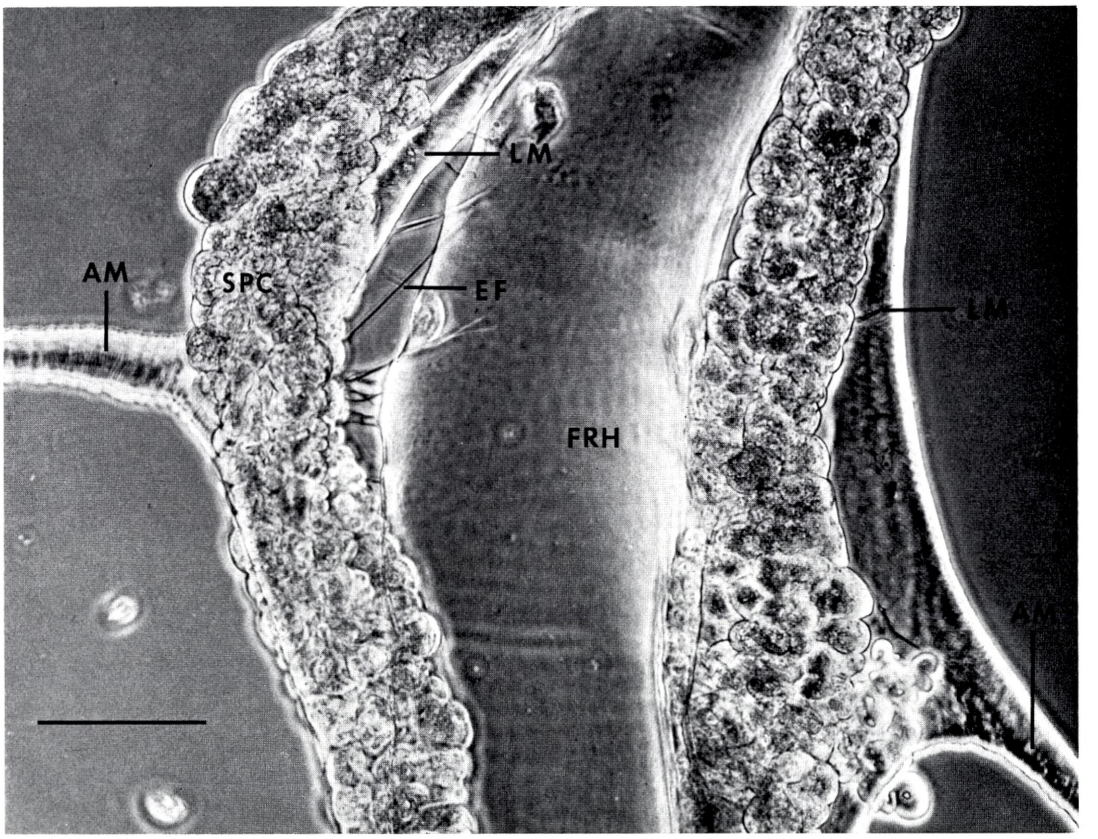

TR
NM
EFA

PLATE II

Fig. 1. Phase-contrast micrograph of an untreated larval front heart. — AM = alary muscle, EF = elastic fibre, FRH = front-heart, LM = longitudinal muscle, SPC = small pericardial cells. The scale represents 50 μ.

Fig. 2. Stained, whole mount showing the front-heart/aorta transition. — AOR = aorta, AM = alary muscle, FRH = front-heart, SPC = small pericardial cells. The scale represents 50 μ.


LM
AM
SPC
EF
FRH
LM
AM

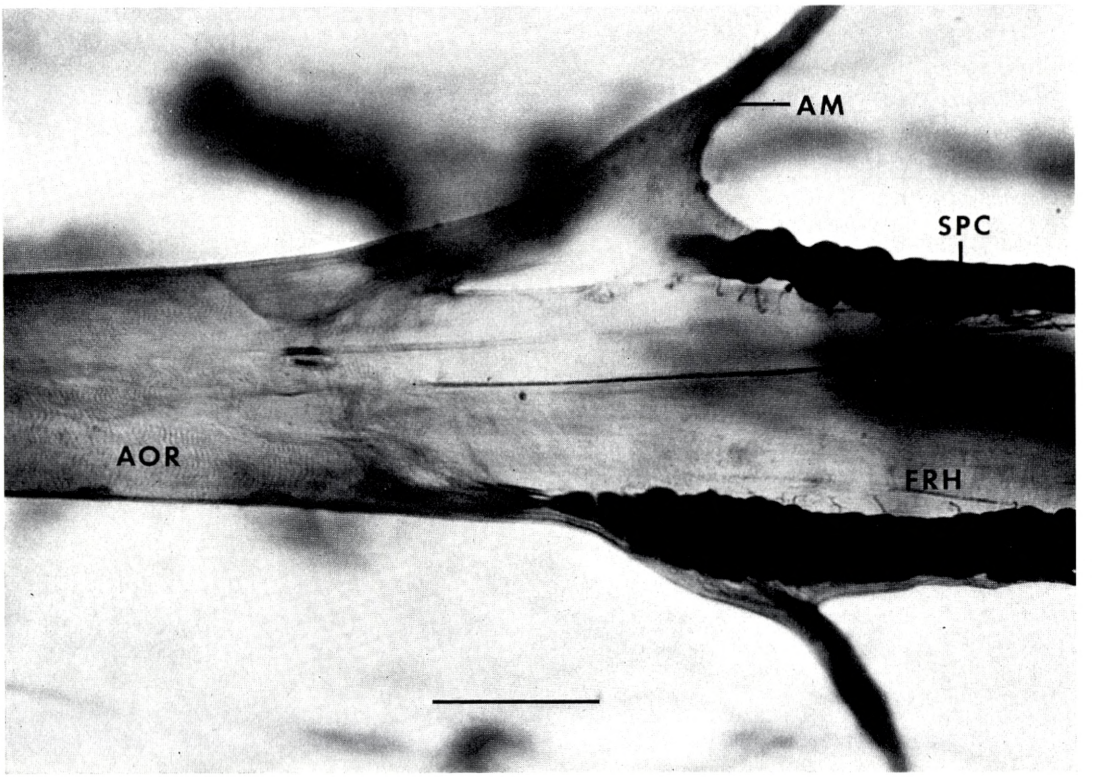
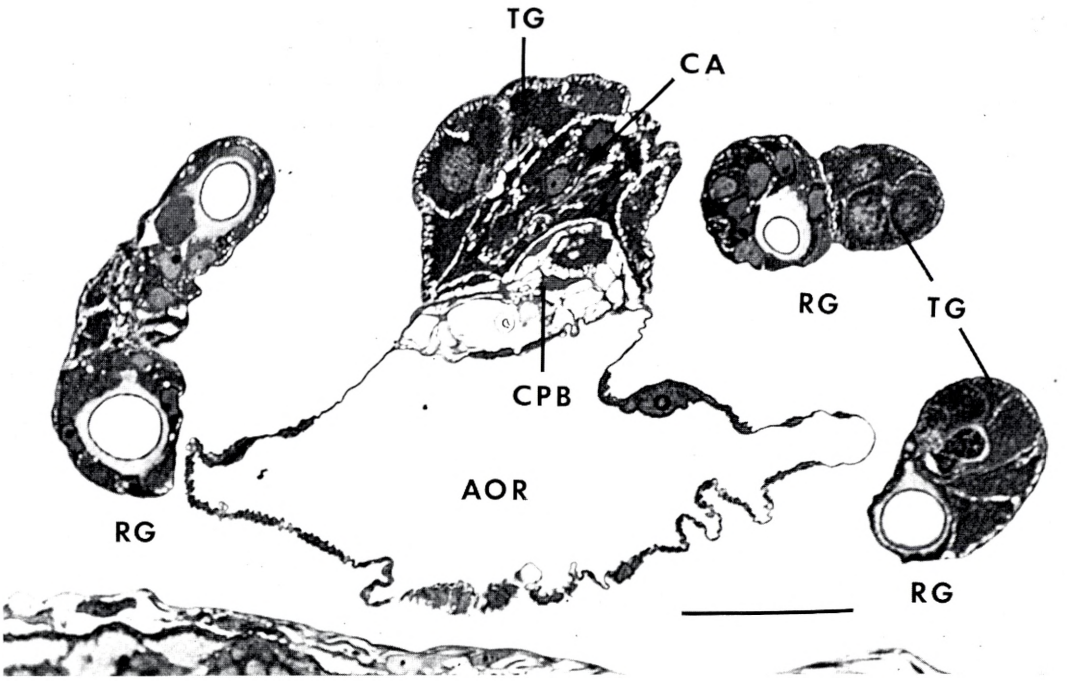

AM
SPC
AOR
FRH

PLATE III

Fig. 1. Transverse section of the aorta passing through the ring gland. — AOR = aorta, CA = corpus allatum part of the ring gland, CPB = cephalopharyngeal band, RG = ring gland, TG = thoracic gland part of the ring gland. The scale represents 50 μ.

Fig. 2. Transverse section of the aorta anterior to the ring gland. — AOR = aorta, CPB = cephalo-pharyngeal band, FS = frontal sack, MB = muscle bands, MML = mandibular-maxillar-labial nerve, NR = nervus recurrens, OES = oesophagus, VG = ventral ganglionic mass, VM = ventral membrane system. The scale represents 50 μ.


TG
CA
RG
TG
CPB
AOR
RG
RG

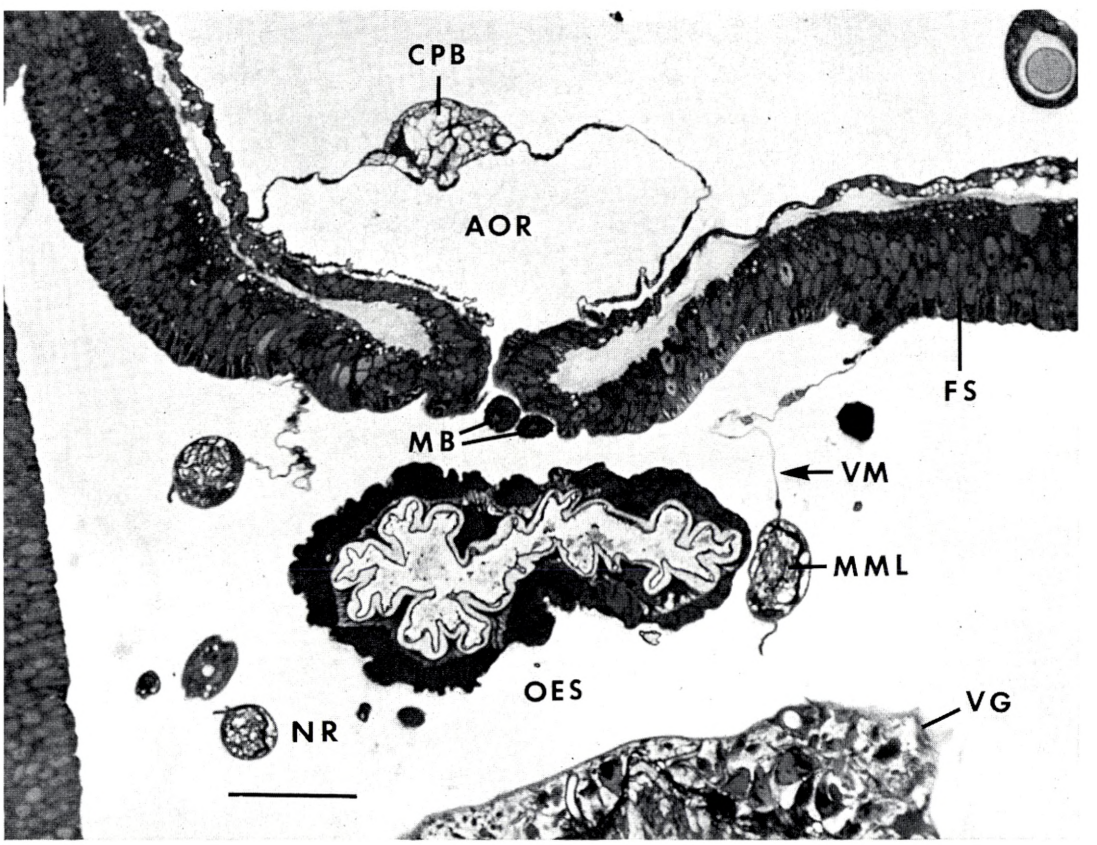

CPB
AOR
FS
MB
VM
MML
OES
VG
NR

Plate IV

Transverse section of the anterior part of the cephalo-pharyngeal band. — AOR = aorta, EFA = elastic fibres of the adventitia, FS = frontal sack, NCP = nucleus and well developed nucleolus of a cell in the cephalo-pharyngeal band. The scale represents 10 μ.

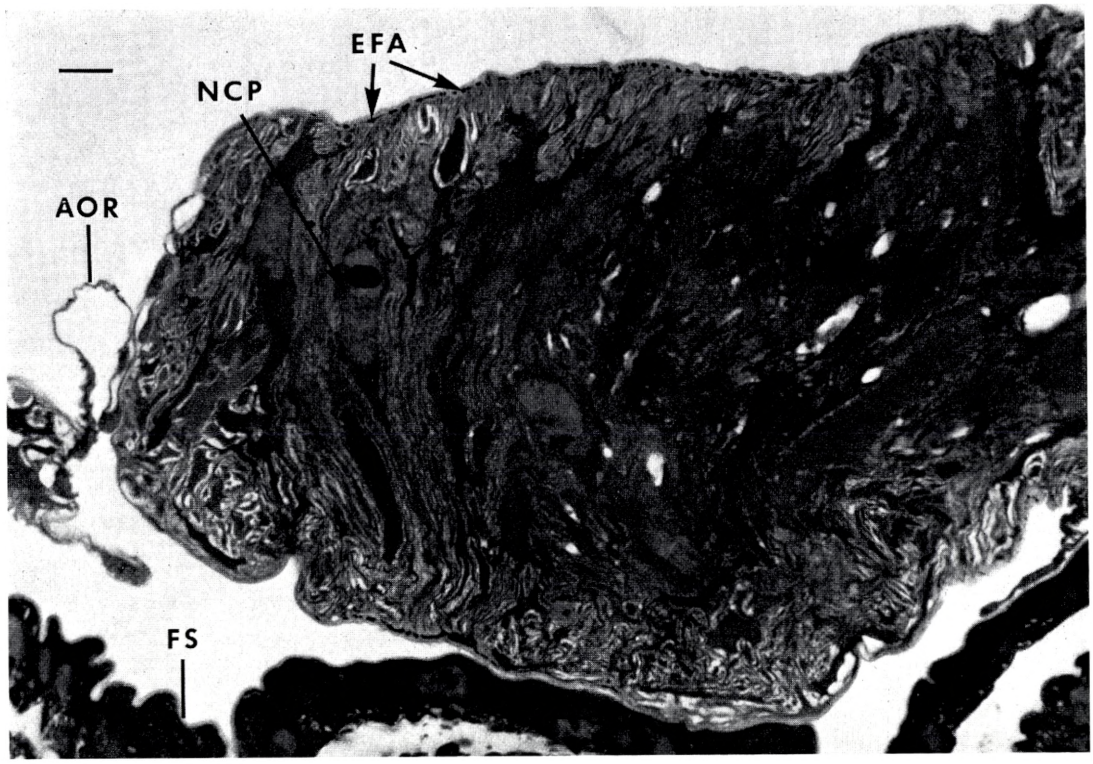
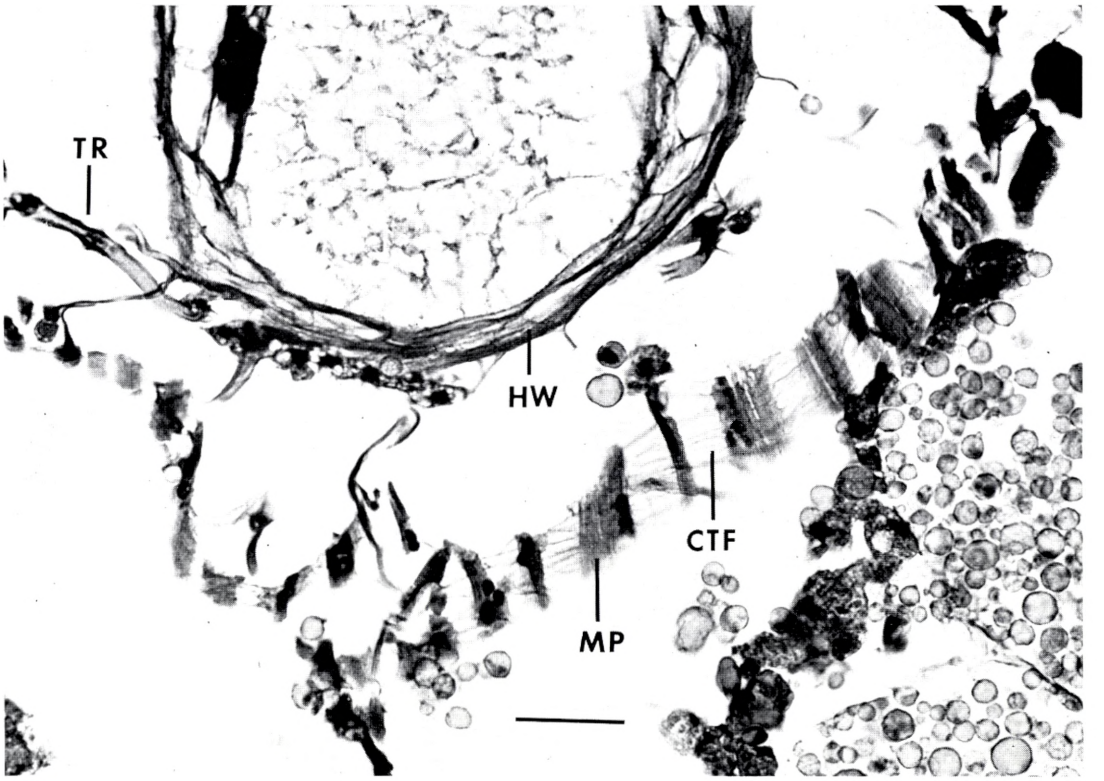

EFA
NCP
AOR
FS

PLATE V

Fig. 1. Transverse section of a pupal heart shortly before imaginal eclosion. — CTF = connective tissue fibres, HW = heart wall, MP = muscle strand of the pericardial septum, TR = trachea supplying the heart. The scale represents 25 μ.

Fig. 2. Transverse section of pupal heart 98 h. after the formation of the puparium. — DI = dorsal integument, HW = heart wall, OF = ostial flap, PHA = phagocytic cell, SPC = small pericardial cells. The scale represents 25 μ.


TR
HW
CTF
MP

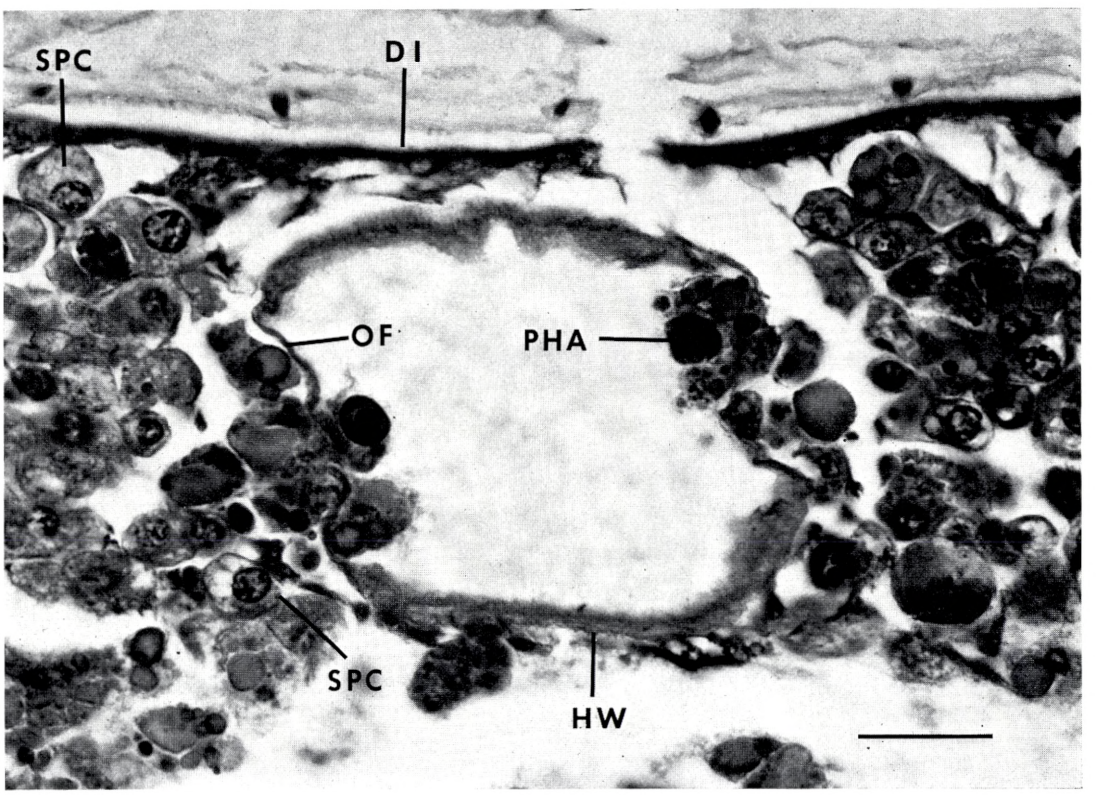
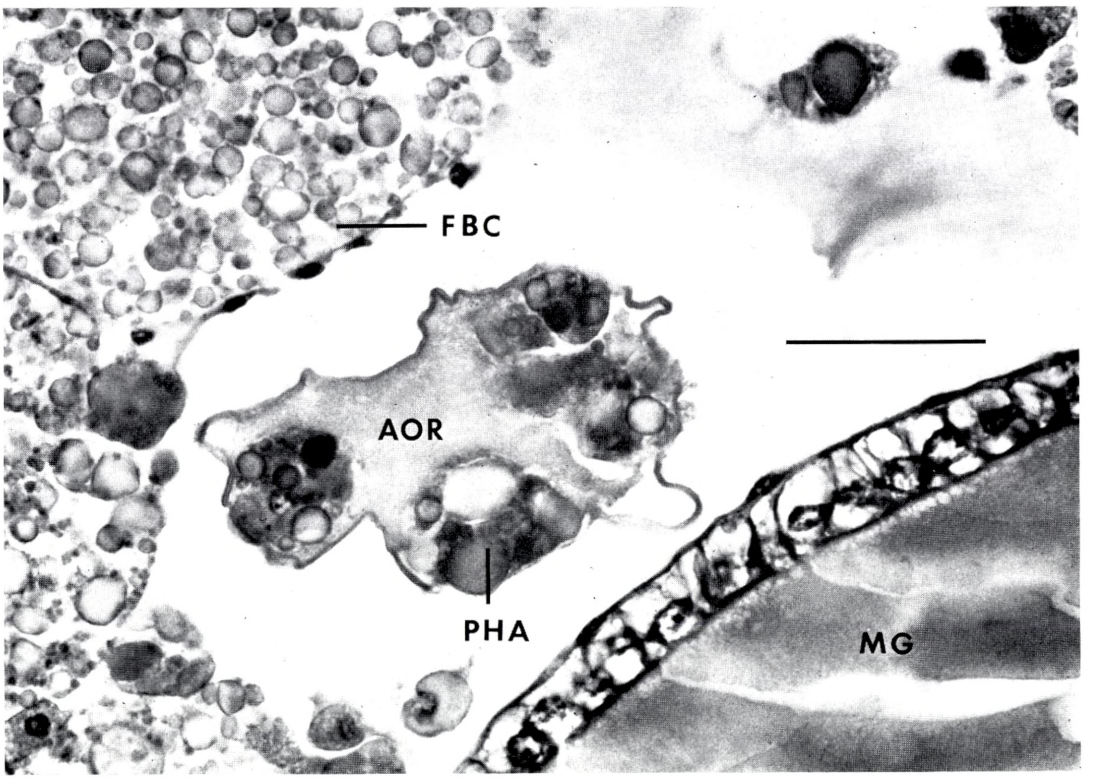
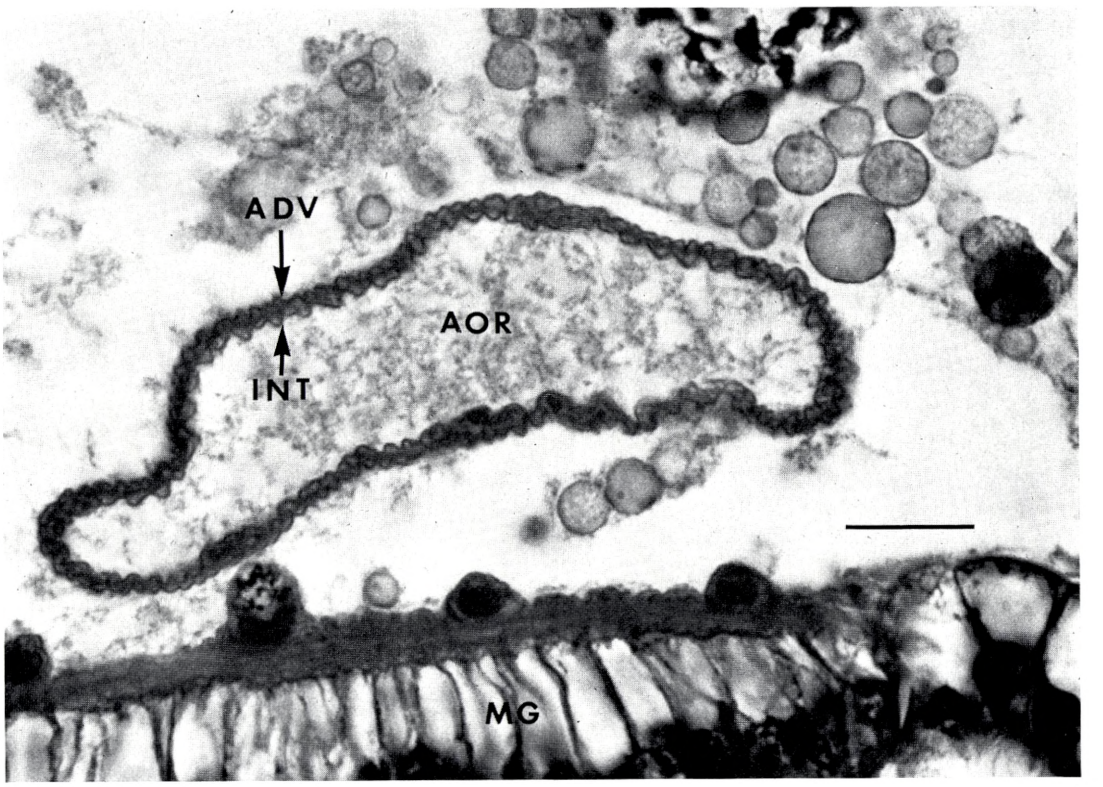

SPC
DI
OF
PHA
SPC
HW

PLATE VI

Fig. 1. Transverse section of the aorta, pupa, 98 hours old. — AOR = aorta, FBC = fat body cell, the cell indicated is being attacked by young phagocytic cells, MG = midgut, PHA = phagocytic cell in the aorta. The scale represents 25 μ.

Fig. 2. Transverse section of the aorta, pupa 196 hours old. — ADV = adventitia, AOR = aorta, INT = intima, MG = midgut. The scale represents 25 μ.


FBC
AOR
PHA
MG


ADV
AOR
INT
MG