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THE HEART AND ARTERIAL CIRCULATORY SYSTEM OF TICKS (ACARI: IXODOIDEA)[1]

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ABSTRACT

The heart and arterial (efferent) circulatory system in *Argas radiatus* and *Ornithodoros turicata* (Argasidae) and in *Ixodes scapularis*, *Dermacentor variabilis* and *Amblyomma tuberculatum* (Ixodidae) are consistent in form with the plan observed in other apulmonate Arachnida. Specialized sinuses or vessels for channelization of venous (afferent) hemolymph are absent, but heart, arterial vessels and sinuses are well-developed. The heart lies in a sinus formed by the pericardial septum which is continuous with connective tissue processes of dorso-lateral and ventro-lateral suspensory muscles of the heart. Hemolymph flows from the pericardial sinus into two segmental cardiac cavities via two pairs of ostia. Walls of this pulsatile portion of the heart are formed from radiating muscle bands. On contraction, hemolymph is pumped through an anterior thin-walled heart region (aortic-myocardial cone), past an aortic septal valve which prevents back flow, into the anterior aorta and on to the periganglionic sinus. Hemolymph reaches peripheral parts of the body via arterial vessels which surround the pedal nerve trunks. Hemolymph also flows anteriorly, through the periesophageal sinus which surrounds the esophagus and the pharyngeal musculature, and into vessels surrounding nerves to the capitular appendages.

An endosternum is present in argasid ticks. Its reported continuity with tissues forming the periganglionic sinus walls is confirmed in this group. No trace of an endosternum is observed in ixodid ticks. Loss of the endosternum appears to facilitate the extensive engorgement behavior observed in ixodid females. Extrinsic muscles present in the periganglionic sinus of all investigated tick species may be derived from dorso-ventral suspensory muscles of a prototypic endosternum. These muscles, together with the intrinsic muscles in the ventral wall of the aorta and the action of the aortic septal valve, may function in the maintenance of elevated arterial pressures. The specialized structure of the ventro-lateral suspensory muscles of the heart suggests that they may play an important role as proprioceptors. The presence of a highly condensed and regionally specialized heart, the existence of a pericardial sinus, and specializations of arterial vessels and sinuses, attest to the complexity and evolutionary advancement of the circulatory system in Ixodoidea.

INTRODUCTION

Classical data on the form of the heart and arterial circulatory system in ticks come from general anatomical studies by Christophers (1906) on species of *Ornithodoros* and *Hyalomma*, Nordenskiöld (1909) on *Ixodes*, Robinson and Davidson (1913-1914) on *Argas*, and Douglas (1943) on *Dermacentor*. Although there are many contradictory

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details in these reports, they indicate the presence of characteristic features from the basic plan of apulmonate arachnid-type circulatory systems in representative Argasidae and Ixodidae.

In Arthropoda the open-type circulatory system is considered an evolutionary consequence of the disintegration of coelomic walls which were present in their ancestral annelid-like prototypes (Beklemishev, 1968). Most arthropods retain only the dorsal pulsatile vessel (heart) and a few associated lateral arches of the annelid closed-type system. Certain higher Crustacea, some Myriapoda, larval Xiphosura and most pulmonate Arachnida (those taxa with book lungs) also retain ventral vessels (the paired thoracic arterial sinuses of Firstman, 1973) in association with the ganglionic chain of the central nervous system. Together, these vessels function as an arterial system; hemolymph is pumped through their branches into lacunar spaces in the body and appendages.

In those Xiphosura and pulmonate Arachnida (including the Scorpiones, Uropygi, Amblypygi and Araneae) which have been investigated (Kaestner, 1968; Firstman, 1973) hemolymph passes ventrally from lacunae of the appendages and body into one or more venous sinuses. Within connective tissue-lined extensions of these cavities it is channeled through the gills or leaves of the book lungs where dissolved respiratory pigments (hemocyanins) are oxygenated. Several pairs of lateral dorso-ventral sinuses, incorrectly called veins, transport oxygenated hemolymph to the pericardial cavity, a specialized sinus surrounding the multi-segmented heart and other portions of the dorsal vessel. The heart is suspended within the pericardial sinus by a series of dorso-lateral and ventro-lateral muscles or elastic ligaments (Stewart and Martin, 1974). During diastole the recoil of these tissues expands the previously contracted heart and hemolymph is pumped forward through the anterior aorta. Simultaneously, hemolymph may be pumped laterally through segmentally arranged lateral arteries or to the rear through a posterior aorta.

Anatomical data on the circulatory system in apulmonate Arachnida (including the Palpigradi, Opiliones, Acari, Pseudoscorpiones, Ricinulei and Solifugae) are limited (Firstman, 1973). Nevertheless, consistent differences between the pulmonate and apulmonate-type plans are known. In apulmonate arachnids, particularly those which show reductions in number of body tagmata, the heart is more condensed and the anterior (dorsal) aorta more highly differentiated, but unbranched (Beklemishev, 1969). In place of ventral vessels the thoracic arterial sinuses are expanded as a perineural sinus enclosing the entire central nervous system (Kaestner, 1968; Firstman, 1973). This perineural sinus receives the aorta and gives rise to an anterior (periesophageal) sinus and lateral arterial vessels which surround nerve trunks to the appendages. Hemolymph passes from these vessels into lacunae within each appendage, then flows into the general body lacunae and, finally, back to the heart.

Although preliminary anatomical studies have been made on the heart and arterial circulatory systems of ticks and other apulmonate Arachnida, there is no firm anatomical basis for the investigation of physiological and pharmacological processes. Such studies have not been initiated despite the relative ecological and economic importance of these taxa. The small size of most parasitic Acari makes them unsuitable for many basic anatomical and physiological investigations, but the larger size of ticks (Ixodoidea) and their importance as vectors of disease (Balashov, 1972) make them prime subjects for such studies. Furthermore, the possibly critical role of the circulatory system during feeding in Ixodidae makes an understanding of this system particularly important. Our previous examination of the central nervous system (Obenchain, 1974a) and neurosecretory system of *Dermacentor variabilis* (Obenchain, 1974b; Obenchain and Oliver,

1975) revealed the probable involvement of tissues forming the wall of the aorta and periganglionic arterial sinus in neuroendocrine mechanisms. The present study was undertaken as the basis for further studies on the roles of tick cardioglial tissues in such mechanisms and to provide the anatomical data prerequisite to a fuller understanding of the functional role(s) of the circulatory system in engorged and unengorged ticks.

MATERIALS AND METHODS

Anatomical and histological observations on tick hearts and arterial circulatory systems were made on laboratory-reared and wild-caught specimens representing four sub-families from the two major tick families. Species examined include *Argas radiatus* Railliet (Argasidae: Argasinae), *Ornithodoros turicata* (Duges) (Argasidae: Ornithodorinae), *Ixodes scapularis* Say (Ixodidae: Ixodinae), and *Dermacentor variabilis* (Say) and *Amblyomma tuberculatum* Marx (Ixodidae: Amblyomminae). Laboratory rearing conditions, tick-hosts and collection data were reported previously (Obenchain and Oliver, 1973). Anatomical observations on the heart of intact nymphal and adult ticks were facilitated by application of paraffin oil to the tick's dorsum. Under these conditions the cuticle became semi-transparent. Although the transparent heart musculature and associated tissues could not be observed directly, heart position and its rate of contraction were determined from the movements of tracheae and tracheoles. Detailed structural observations were made on dorsal and ventral dissections of the circulatory system, performed under Shen's physiological saline (9.0 g NaCl, 0.42 g KCl, 0.25 g $CaCl_4$/liter of distilled water).

Some dissections were made on specimens previously injected with supravital dyes, including 0.5% ammoniacal carmine, 0.02-0.5% trypan blue in Shen's saline, or 1.0% methylene blue in 1.3% NaCl. Other dissections were stained *in toto* with leucomethylene blue (about 0.1% after reduction with 0.01M ascorbic acid, Larimer and Ashby, 1964) for demonstration of neural elements. Whole mount preparations of the circulatory system were fixed in a calcium formal or 5% ammonium molybdate for direct microscopic examination. Other dissections were fixed in Heidenhain's SUSA or Carnoy's fixatives (Humason, 1967) and routinely dehydrated in Cellosolve, embedded in Tissuemat-Paraplast by the dioxane method, and sectioned at 7 μm. Staining techniques included Hubschman's (1962) simplified azan and Rosa's aldehyde fuchsin with Halmi's counterstain (Meola, 1970). Fresh or vitally stained tissues were stretched on slides in Shen's saline or 50% glycerol in distilled water for examination by bright field, dark field/fluorescence, phase contrast, or polarized light microscopy. Histological sections were dehydrated through xylene, mounted in Harleco synthetic resin and examined with the above mentioned optics. Photomicrographs were taken on a Wild M20KGS photomicroscope. Dimensions of tissues were determined with an ocular micrometer and from calibrated photomicrographs.

OBSERVATIONS

In all examined tick species the heart (Ht) is suspended by a series of dorso-lateral and ventro-lateral suspensory muscles (dlSM, vlSM) within a pericardial sinus (pcS) located medially below the dorsal cuticle. The heart and tissues forming the boundries of the pericardial sinus are bordered anteriorly by insertions of the cheliceral retractor muscles (Figs. 1, 2). Laterally, they lie between mid-dorsal loops of the malpighian tubules and

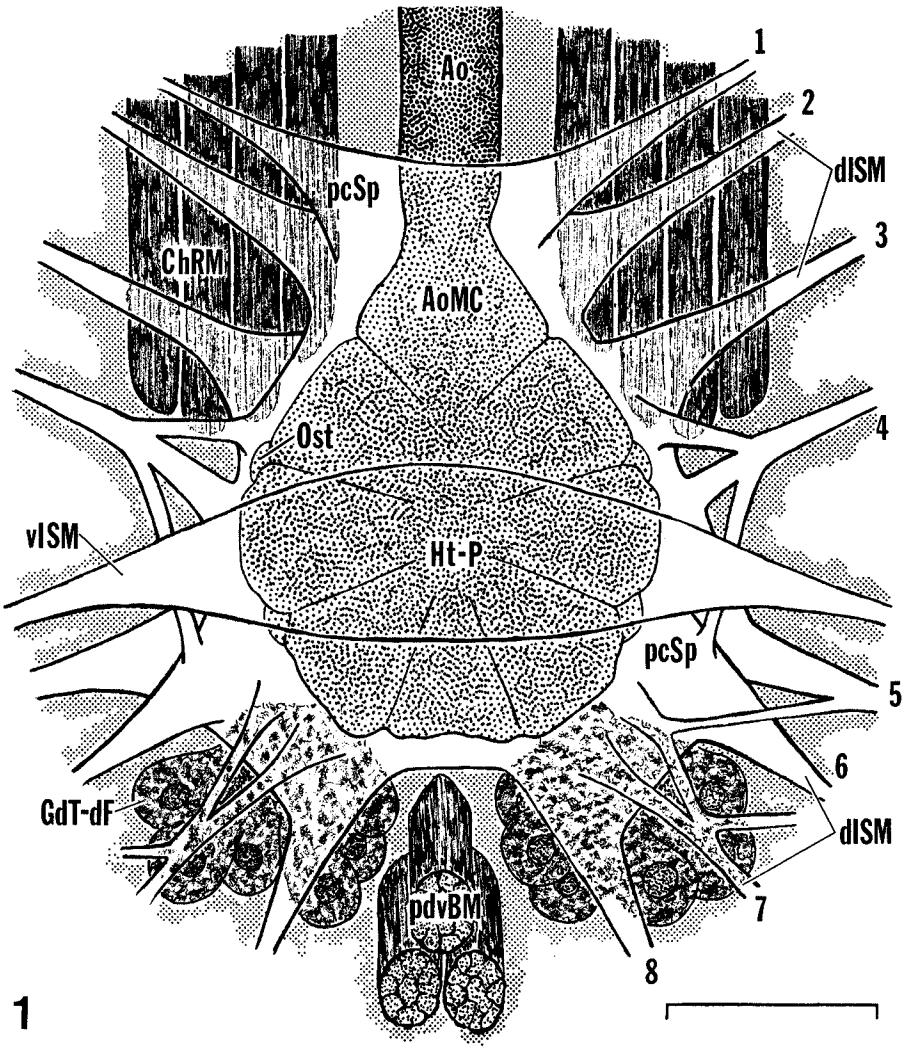
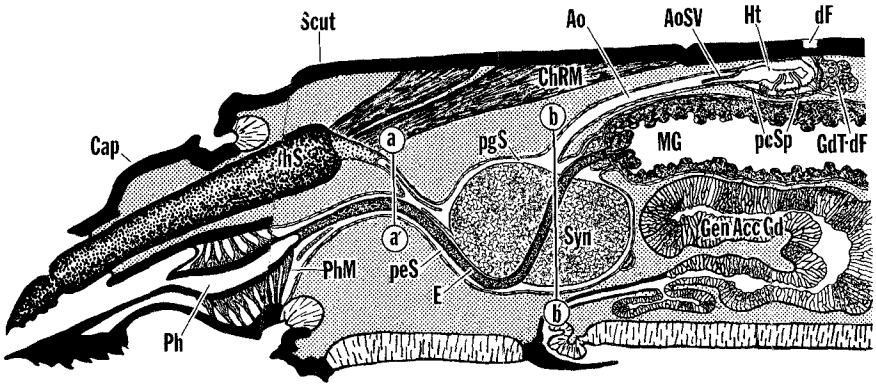


Fig. 1.–Diagrammatic representation of the heart, pericardial septum, suspensory muscles and associated tissues of *Amblyomma tuberculatum*, in ventral view. Scale equals 0.1 mm. Ao, anterior aorta; AoMC, aortic-myocardial cone; ChRM, cheliceral retractor muscles; dlSM, dorso-lateral suspensory muscles; GdT-dF, glandular tissues of the dorsal foveae; Ht-P, pulsatile portion of the heart; Ost, ostia; pdvBM, posteromedian dorso-ventral body muscles; pcSp, pericardial septum; vlSM, ventro-lateral suspensory muscles.

insertions of the dorso-genital muscles (and by insertions of the coxal adductor muscles III and IV in Argasidae). Posteriorly, they are bordered by insertions of the postero-median dorso-ventral body muscles (and by tissues associated with the dorsal foveae in Ixodidae-Amblyomminae). The tick heart is in the form of a dorso-ventrally flattened sack. During diastole the heart outline is sub-triangular (Argasidae) to pentagonal (Ixodidae, Fig. 1), but generally spherical in the contracted state (Figs. 4, 5). The thin-walled aorta (Ao) emerges from the anterior apex of the heart and runs forward below the cheliceral retractor muscles and above the central portion of the midgut (Fig. 2). At the median notch between the anterior ramifications (caeca) of the midgut, the aorta



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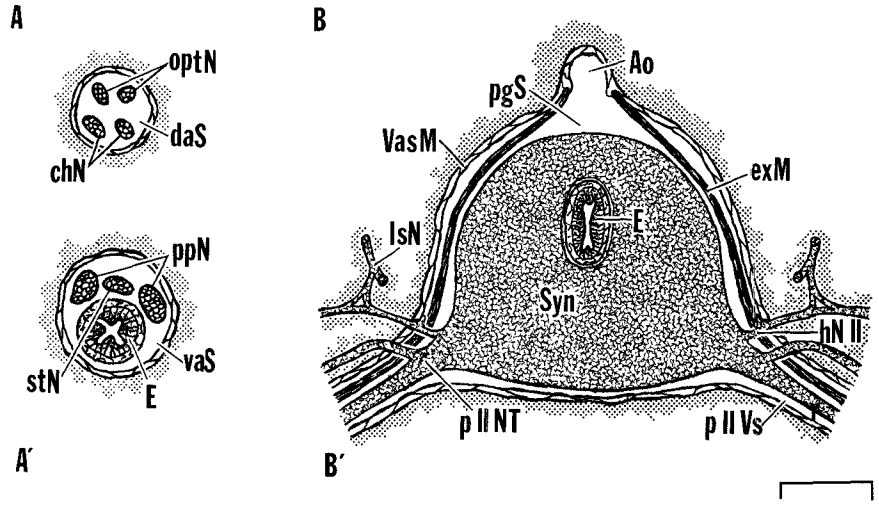

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Figs. 2, 3.—Diagrammatic representation of the form and anatomical associations of heart, arterial sinuses and vessels in a representative male ixodid tick (about the size of *Dermacentor variabilis*): 2, Near-sagittal reconstruction, with addition of a cheliceral shaft, associated muscles, and dorsal foveae (non-mid-line structures); 3, Cross-sections of tick arterial sinuses at levels indicated in Fig. 2. Scale on figures equals 0.1 mm. Ao, anterior aorta; AoSV, aortic septal valve; Cap, capitulum; ChRM, cheliceral retractor muscles; ChS, cheliceral shaft; chN, cheliceral nerves; daS, dorsal anterior sinus; dF, dorsal foveae; E, esophagus; exM, extrinsic muscles of the periganglionic sinus; GdT-dF, glandular tissues of the dorsal foveae; Gen Acc Gd, male genital accessory gland; Ht, heart; hN II, second hemal nerve; lsN, lateral "sympathetic" nerve; MG, midgut; optN, optic nerves; Ph, pharynx; PhM, pharyngeal musculature; pcSp, pericardial septum; peS, periesophageal sinus; pgS, periganglionic sinus; p II Vs, second pedal arterial vessels; Scut, scutum; Syn, synganglion; stN, stomodeal nerve; VasM, vascular membrane; vaS, ventral anterior sinus.

descends into the periganglionic arterial sinus (pgS). No traces of a posterior aorta are observed in any of the examined tick species.

Anatomically, the periganglionic sinus and associated arterial vessels of *Dermacentor variabilis* are representative of similar circulatory structures in other tick species. Sinus walls completely envelop the condensed mass of the central nervous system (synganglion)

and the esophagus which passes through it. These walls are closely applied to ventral, lateral and posterior surfaces of the synganglion, but are more removed dorsally (Figs. 2, 3). Here, the aorta enters the sinus just above the point where the esophagus passes out of the synganglion. The periganglionic sinus communicates anteriorly with the periesophageal arterial sinus (peS) and laterally with four pairs of pedal vessels (p I-IV Vs) which surround the major pedal nerve trunks (Fig. 3BB′) Postero-dorsally it terminates around the neuroendocrine retrocerebral organ complex at the level of the proventriculus. The four pairs of pedal vessels extend to the coxal-trochanter junction in the walking appendages. A short distance in front of the synganglion (Figs. 2, 3AA′) the periesophageal sinus divides into a dorsal anterior sinus (daS) surrounding paired optic and cheliceral nerves and a ventral anterior sinus (vaS) surrounding paired pedipalpal nerves, the unpaired stomodeal nerve and the esophagus. Extensions of the dorsal anterior sinus surround the peripheral nerves which enter cheliceral shafts, while similar vessels arise from the ventral anterior sinus and surround nerves which enter the pedipalps. The median extension of the ventral anterior sinus expands broadly and irrigates the pharyngeal musculature (Fig. 2).

Differences in the proportions of the arterial circulatory system among species of Ixodidae parallel changes in overall body dimensions, as in the series of increasing body size from *Ixodes scapularis*, through *D. variabilis* and to *Amblyomma tuberculatum* respectively. Similar proportional differences are observed between *Ornithodoros turicata* and *Argas radiatus* in Argasidae. The form of the periganglionic sinus is modified in argasid ticks by its continuity with tissues of the well-developed endosternum. The periesophageal sinus of argasid ticks is also slightly different in form from that observed in ixodids. Paired arterial vessels to the cheliceral shafts arise separately at the anterior boundary between periganglionic and periesophageal sinuses; consequently, the dorsal anterior sinus, as observed in representative Ixodidae, is poorly developed in Argasidae. This anatomical difference appears to be related to the different orientation of the capitulum and its associated musculature in Argasidae as compared to Ixodidae. In both groups, however, the appendages of the capitulum are supplied by arterial vessels and the pharyngeal musculature is contained within an extension of the ventral anterior (periesophageal) sinus. Sexual differences in the form of the heart and arterial circulatory system are minor in the species studied. Differences in the degree of tracheation are pronounced, particularly in Ixodidae, where the more extensive tracheal supply to tissue and organ systems in females allows for considerable expansion and differentiation of the idiosoma during engorgement.

The heart rate appears extremely variable in all tick species. In observations on restrained specimens of newly ecdysed *D. variabilis*, the rate varies between 18 and 120 beats per minute, with periods of inactivity from less than one to several minutes in duration. These observations were made at room temperature with low intensity fluorescent illumination. Effects of changing levels of incandescent light intensity (and radiant heat) on ticks treated with paraffin oil are pronounced. Increasing or decreasing the intensity randomly accelerates or depresses the heart rate in *Amb. tuberculatum*, *D. variabilis* and *A. radiatus*.

Tick hemolymph contains a variety of hemocyte cell types (Dolp, 1970; Brinton and Burgdorfer, 1971). In specially dissected preparations, numerous cells are observed in circulating hemolymph within the dorsal aorta. Prohemocytes, plasmatocytes and various spherule cells are observed within the arterial vessels and sinuses in histological sections. In all observed ticks, with the exception of *Amb. tuberculatum*, the hemolymph is

generally colorless. In this single species, however, the distinct blue-green color of oxygenated hemolymph seems to indicate the presence of hemocyanin pigments.

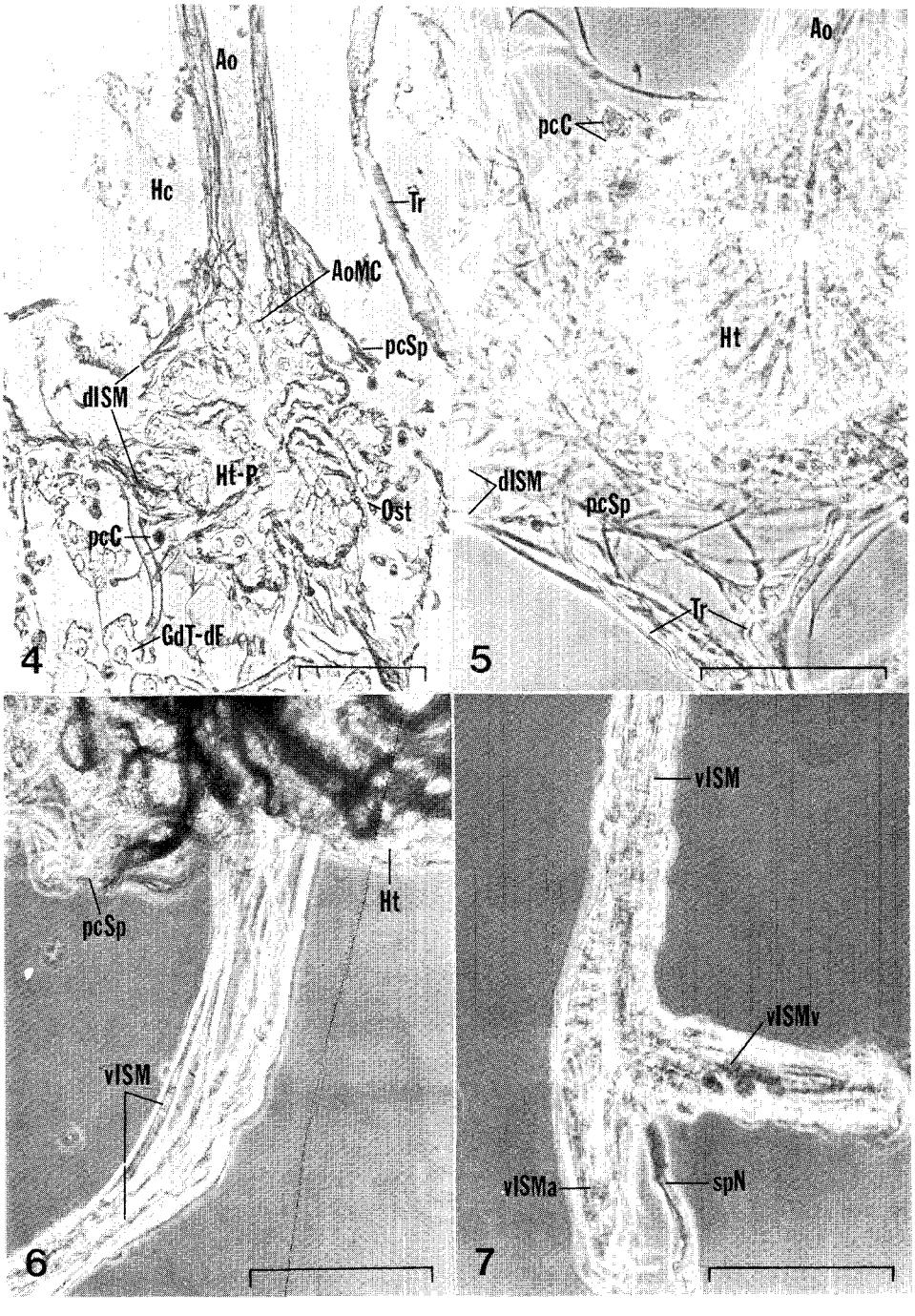
Structure of the Myocardium

The anatomical form and histological structure of the heart is similar in all investigated tick species. The heart wall (myocardium) is formed from striated muscle. Cellular margins are indistinct and there is no detectable internal intima. Myofibrils take up acidophilic stains, but the myoplasm is generally chromophobic. The basophilic nuclei of these cells seem to be distributed randomly throughout the myocardium. Histological sections of contracted heart muscle demonstrate a complicated network of connective tissue fibers over the external surface of the heart muscle. In preparations of relaxed heart muscle, however, these tissues are recognized as the terminal processes of dorso-lateral and ventro-lateral suspensory muscles.

The tick heart is divided into two anatomical regions on the basis of constituent muscle fiber orientation. Each region seems to have a distinctive function. In the larger (posterior) region (Figs. 1, 5, 8) the myocardium is formed from seven semi-circular bands of muscle which radiate from slightly thickened central areas on the dorsal and ventral surfaces of the heart. The dorsal thickened area is loosely anchored to the overlying cuticle by scattered connective tissues resembling the tonofibrillae of major body muscles. The semi-circular muscle bands receive connective tissue processes from dorso-lateral suspensory muscles along the lateral margins of the heart (Figs. 1, 4). Ventro-laterally, two pairs of ostia open between radiating muscle bands. The muscular lips of these ostia (Figs. 2, 4) extend internally into the segmental cardiac cavities of the heart. When small amounts of 0.5% trypan blue solution are applied laterally to physiological preparations of the heart of *Amb. tuberculatum* or *A. radiatus*, anterior and posterior cardiac chambers are readily observed. These two bilaterally symmetrical chambers receive afferent hemolymph via the first and second pairs of ostia, respectively. This is the major pulsatile portion of the heart (HtP).

The anterior region of the myocardium (aortic-myocardial cone) links the pulsatile portion of the heart with the aorta (Figs. 1, 4, 8; AoMC). Muscle fibers in this portion of the heart are not organized into radiating bands. Instead, they have a primarily longitudinal orientation. Some muscle fibers appear to be extensions of the longitudinal striated fibers which lie in the ventral wall of the anterior aorta.

The myocardium is tracheated by way of an anastomosing plexus of the postero-dorsal tracheal trunks (Figs. 4, 5). Branches of these tracheae generally follow the paths of suspensory muscles on their way to the myocardium. Both regions of the myocardium are tracheated, but the posterior pulsatile portion receives the more extensive supply. After treatment with leucomethylene blue the presence of an intrinsic cardiac ganglion associated with the heart of *Amb. tuberculatum* is supported by the staining response of eight or more small cells. These are presumably neurons, and they are grouped mid-dorsally above the thickened central area of the myocardium. Nuclei of these cells stain more intensely than the axoplasm. Some neurons appear to be bipolar, others appear to be unipolar. Their processes penetrate the myocardium and can be traced into the cardiac cavity along the internal projections of radiating muscle bands. Similarly staining tissues are observed in other investigated tick species, but in the latter the presence of a discrete cardiac ganglion was not confirmed with vital staining methods.



Other axon-like fibers were observed after application of leucomethylene blue in ventral and posterior heart walls in *Amb. tuberculatum* and *A. radiatus*. The paths of these fibers seem to indicate an extrinsic myocardial innervation. In *Amb. tuberculatum* some of these putative axons were traced posteriorly into adjacent gland-like tissues of the dorsal foveae or ventrally into the ventro-lateral suspensory muscles. In *A. radiatus*

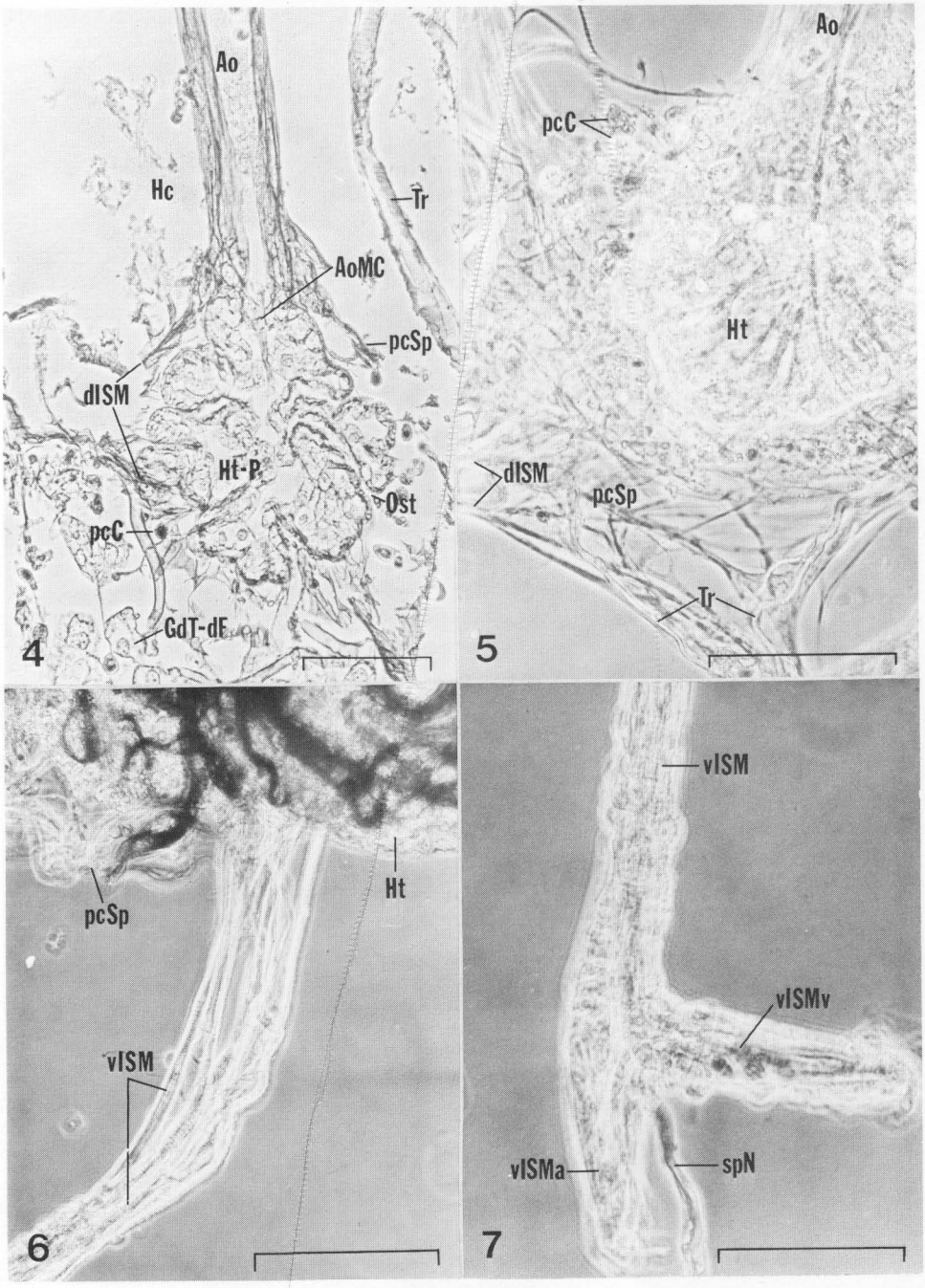


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Fig. 4.–Frontal section through heart and anterior aorta of an adult male *Amb. tuberculatum*, aldehyde fuchsin stain, phase contrast optics. Anterior orientation to the top of figure, scale equals 0.1 mm.

Fig. 5.–Dissected heart preparation of an adult female *Ornithodoros turicata* after formol calcium fixation, in dorsal view with phase contrast optics. Scale equals 0.1 mm.

Fig. 6.–Dissected preparation of ventro-lateral suspensory muscle from the heart of an adult female *Amb. tuberculatum* in the proximity of the pericardial septum, leucomethylene blue technique, after ammonium molybdate fixation with phase contrast optics. Scale equals 25 μm.

Fig. 7.–Ventro-lateral suspensory muscle of *Amb. tuberculatum*, as in Fig. 6, at junction of atrial and ventral contributing muscle fibers. Scale equals 25 μm. Ao, anterior aorta; AoMC, aortic-myocardial cone; dlSM, dorso-lateral suspensory muscles; GdT-dF, glandular tissues of the dorsal foveae; HC, hemocoel; Ht, heart; Ht-P, pulsatile portion of the heart; Ost, ostia; pcC, pericardial cells; pcSp, pericardial septum; spN, branch of the spiracular nerve; Tr, trachea; vlSM, ventro-lateral suspensory muscle; vlSMa, atrial contributing branch of vlSM; vlSMv, ventral contributing branch of vlSM.

similar fibers were traced posteriorly along branches of the postero-dorsal tracheal trunks. These fibers were followed to the bilateral ganglionic masses of dorsal photoreceptors (Binnington, 1972) which lie below the first pair of cuticular disks associated with the second rows of postero-accessory dorso-ventral body muscles.

Structure of the Pericardium and Suspensory Muscles

The pericardial sinus surrounds the heart on its ventral, lateral and posterior surfaces. It is bound by the perforated pericardial septum (Figs. 1, 5; pcSp). This septum is formed by overlapping membranous sheets and strands of loose connective tissue, bound together and supported by fibrous terminal processes of the suspensory muscles of the heart. Along its lateral and posterior margins the septum is loosely attached to the dorsal body wall. Anteriorly, it connects to the walls of the aortic-myocardial cone (Fig. 4). Connective tissues of the pericardial septum serve as a support for pericardial cells (Figs. 4, 5; pcC). The distribution of these pinocytotic cells is best demonstrated in dissections treated with 0.02% trypan blue or other particulate vital dyes. In *Amb. tuberculatum* and *D. variabilis* pericardial cells are distributed over the surface of the septum with only a slight peripheral concentration. In *A. radiatus* and *O. turicata*, however, lateral concentrations of these cells are pronounced.

The dorso-lateral suspensory muscles of the heart have their origins on specialized cuticular structures associated with origins of principal body muscles. In Ixodidae they arise from integumental grooves and/or unsilvered areas of the dorsal cuticle. These areas are also associated with the origins of the anterior and posterior genital muscles and the marginal dorso-ventral and posteroaccessory dorso-ventral body muscles. In Argasidae the dorso-lateral suspensory muscles of the heart have their origins on dorsal cuticular disks associated with adductor and abductor muscles of the coxae and with postero-accessory dorso-ventral body muscles.

The dorso-lateral suspensory muscles (dlSM) of the tick heart are long and filamentous with a markedly striated appearance under phase-contrast, dark field, or polarizing optics (Figs. 4, 5). Histological properties of suspensory muscle tissues are similar to those of myocardial muscles but their nuclei have a more consistent peripheral distribution. Near the pericardial septum, these muscles become highly branched. In *Amb. tuberculatum* we identify eight bilaterally symmetrical groups of dorso-lateral muscles (Fig. 1) which terminate as strands of connective tissue (Figs. 4, 5) contributing to the fibrous matrix of

the pericardial septum. The first and second groups also send some terminal processes into the wall of the aortic-myocardial cone. Terminals of the third group of muscles insert on lateral margins of the anterior pair of muscle bands (pulsatile portion of the myocardium). Fourth and fifth dorso-lateral suspensory muscle groups insert on the second pair of muscle bands between the two pairs of ostia (Figs. 1, 4). Sixth and seventh groups insert on muscle bands behind the second pair of ostia, while terminals of the eighth group insert on either side of the complex (unpaired) posterior band. Similar patterns are observed in *A. radiatus*, but dorso-lateral suspensors are grouped in three major sets. The first set seems to include suspensory groups one to four from *Amb. tuberculatum*, the second set includes suspensory groups five and six, and the posterior set includes groups seven and eight. Different grouping patterns of dorso-lateral suspensors between ixodid and argasid ticks seem to determine the shape of the uncontracted heart (pentagonal in Ixodidae and sub-triangular in Argasidae). In ticks from both families the dorso-lateral suspensory muscles are so highly branched that the degree of overlapping myocardiac insertions is not readily determined. A few scattered fibers and presumptive axon-terminals stain with leucomethylene blue in all four tick species. Although the complex patterns of peripheral innervation remain obscure, some dorso-lateral suspensory muscles appear to be innervated by the same nerves which supply body muscles with adjacent origins.

Dorso-lateral suspensory muscles hold the pericardium in place with respect to the heart, but the volumetric integrity of the pericardial sinus (especially in fully engorged adult ixodid ticks) is maintained by the action of right and left ventro-lateral suspensory muscles (vlSM). Branches of these muscles are joined medially as a flattened belt of fibrous connective tissue and slips of striated muscle, all embedded in the pericardial septum (Fig. 1). Other processes of the ventro-lateral suspensory muscles are larger in diameter and longer than dorso-laterals. In *A. radiatus* they have a flattened strap-like appearance, but in *Amb. tuberculatum* (Fig. 6) these muscles branch near the heart and have a gradually tapered outline. In ticks from both families the ventro-lateral muscles of the heart have dual origins (Fig. 7). Separate contributing muscles arise (1) from the atrial wall of the spiracular plate and (2) from the ventral body wall. In some species (particularly *O. turicata*) several muscle slips may arise from the general sites of origin, but these slips soon fuse within the common sheath of the atrial or ventral contribution. Subsequently, the sheaths of the two muscles fuse and their fibers coalesce as the common ventro-lateral muscle to the heart.

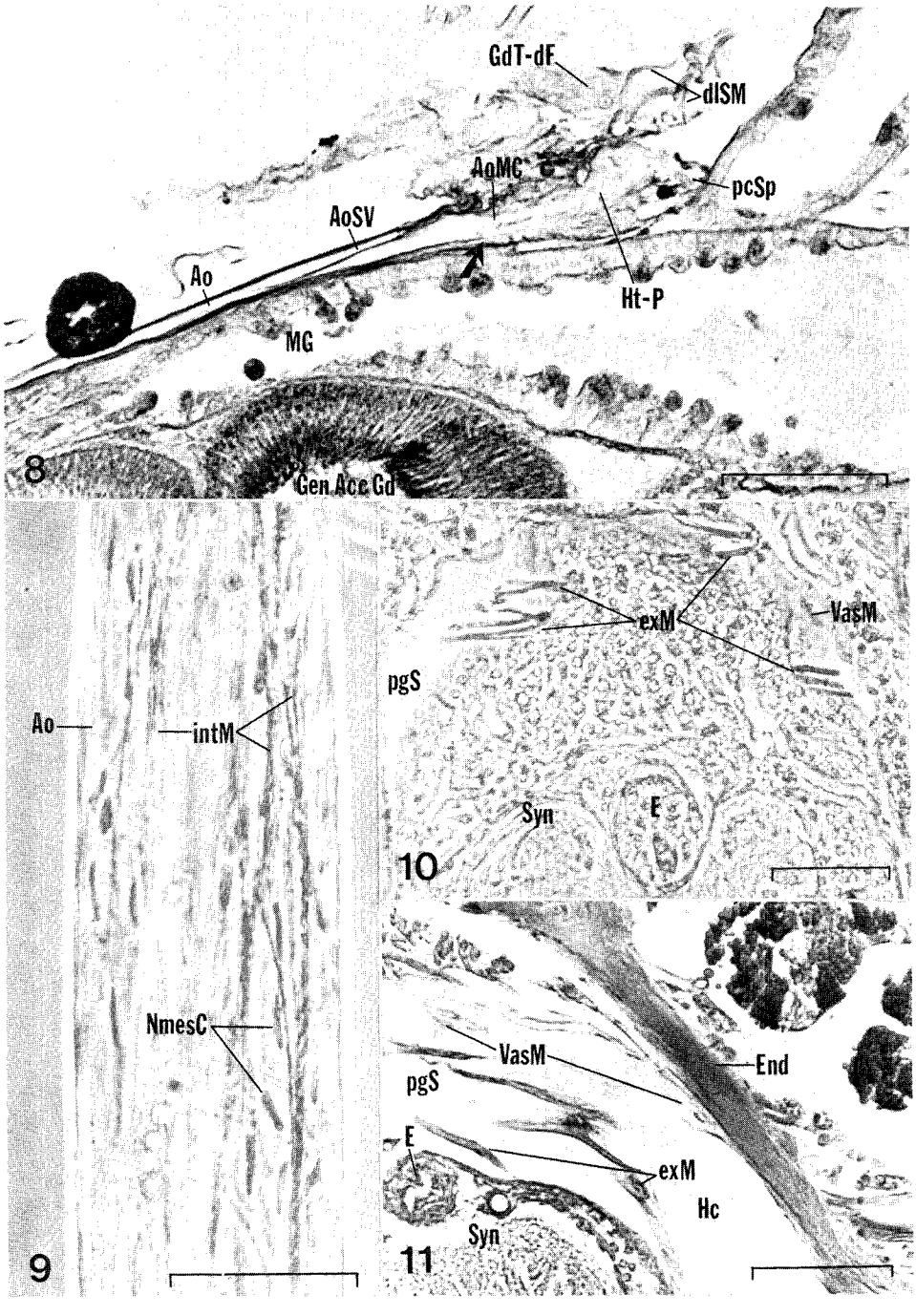
Certain cells associated with ventro-lateral suspensory muscles in *Amb. tuberculatum* and *A. radiatus* stain with leucomethylene blue. These are presumed to be the perikaryia of sensory neurons. Axons of these cells, together with other stained fibers which may represent a peripheral motor innervation, form a plexus over the surface of the ventro-lateral suspensors. This plexus is particularly dense in the region where atrial and ventral contributing muscles are joined. In both species some peripheral fibers can be traced into paired spiracular nerves, branches of which innervate the right or left ventro-lateral suspensor at that point. Spiracular nerves arise from the posterior margin of the synganglion in *A. radiatus* (Obenchain and Oliver, unpublished), but in *Amb. tuberculatum* and *D. variabilis* they have a dual origin from the posterior margin of the synganglion and from the lateral "sympathetic" nerves (Obenchain and Oliver, 1975).

Structure of Arterial Vessels and Sinuses

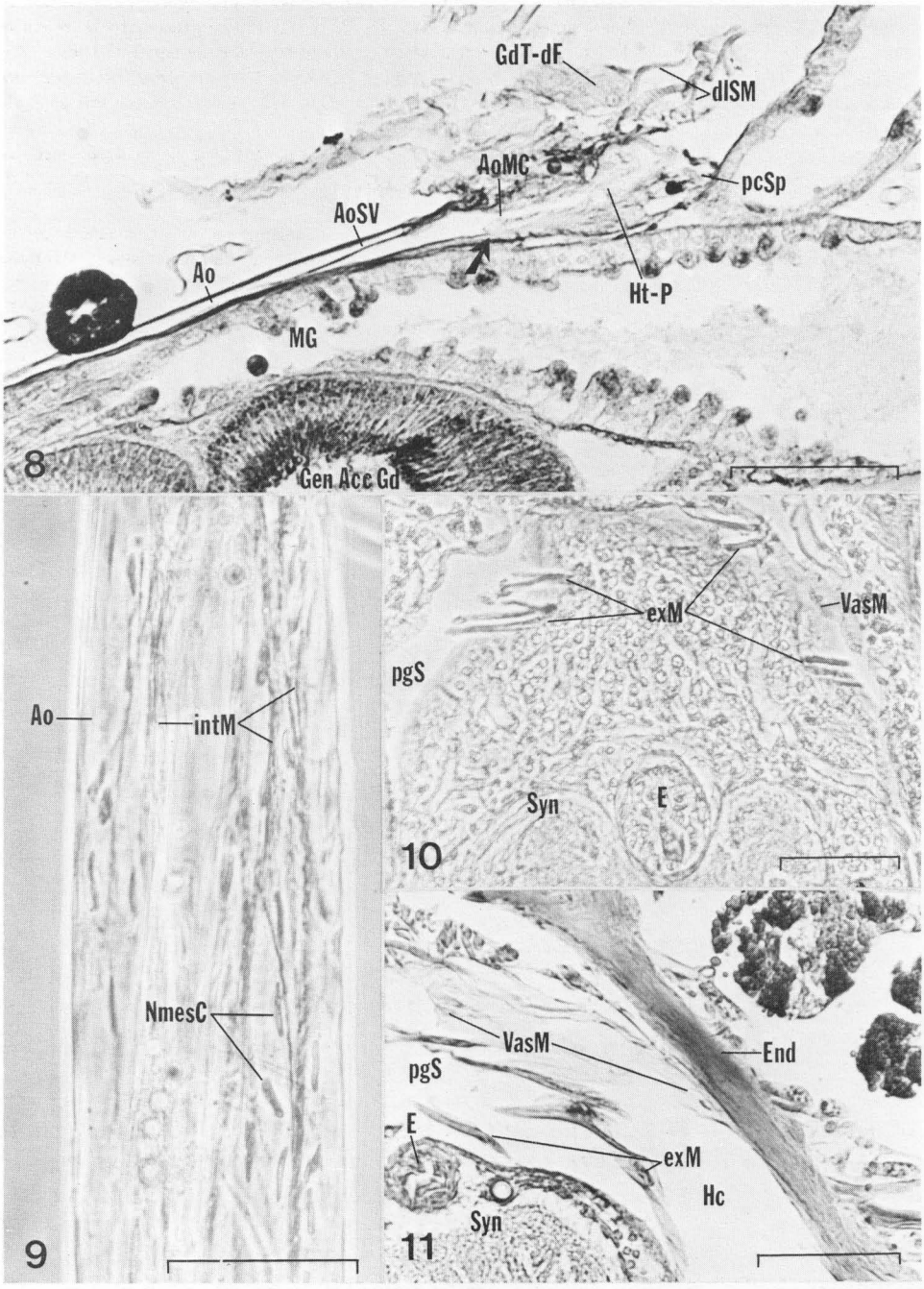
A complex layer (possibly stratified) of squamous mesenchyme forms the cellular matrix within walls of the tick arterial circulatory system. These vascular structures are bound on their outer and inner surfaces by distinct basement membranes (collagenous matrices approximately 1 μm thick) which stain intensely with basophilic stains. In argasid and unfed ixodid adults constituent cells of the vascular membranes (vascular sheaths of Obenchain, 1974a; Obenchain and Oliver, 1975) are extremely flattened (Fig. 8) and have indistinct margins. The expansion and apparent secretory activity of mesenchymal cells is pronounced, however, in engorged ixodid females. At this time, several tissue types can be identified within the vascular membranes. Margins of adjacent mesenchymal cells overlap extensively, presenting the appearance of two distinct layers. Nuclei are large (2.5 to 4.0 μm) and stain heavily with acidophilic components of the aldehyde fuchsin and azan staining techniques. Ground cytoplasm is chromophobic, extensively vacuolated and becomes progressively filled with basophilic granules following the blood meal. When intrinsic muscles (restricted to the ventral wall of the anterior aorta), tracheal or neural tissues are observed within the vascular membranes, they lie between the overlapping margins of mesenchymal cells. Some putative axons, stained with the leucomethylene blue technique, can be traced from the walls of the periganglionic sinus back to various pedal nerve trunks in *Amb. tuberculatum* and *A. radiatus*. These axonal pathways resemble the putative neurosecretory innervation to the periganglionic sinus walls described previously from *D. variabilis* (Obenchain and Oliver, 1975).

Walls of the periganglionic and periesophageal arterial sinuses and vessels to the appendages are formed from unspecialized vascular membrane. No intrinsic musculature is observed in the walls of these portions of the circulatory system, but the squamous layer of membrane-bound mesenchyme is frequently penetrated by tracheae which supply the central and peripheral nervous system. At the level of the retrocerebral neuroendocrine complex and the proventricular (esophageal) valve, the wall of the periganglionic sinus fuses with similar mesodermal layers which form the annular walls of the esophagus, the sheath of the retrocerebral organ complex and the outer covering of the midgut. Structural details of these associations and evidence for the endocrinological involvement of mesenchymal tissues within the aorta and sinus wall (as a type of cardioglial tissue) were reported previously for *D. variabilis* (Obenchain and Oliver, 1975). Progressive expansion of these tissues, with the formation of intracellular basophilic granules, is observed in all three species of ixodid ticks during and after the adult blood meal. A similar involvement in adult argasids is not indicated at the light microscope level, but expansion of these mesenchymal tissues (complete with development of basophilic granular inclusions) occurs during the molt from last stage nymph to adult in *A. radiatus*.

Extrinsic muscle fibers, observed within the periganglionic arterial sinus of all examined ticks, have their origins on internal antero-dorsal projections of coxae I, II and IV. No similar muscles were associated with coxae III in the species examined. From their origins, these transversely striated muscles penetrate adjacent walls of pedal arteries, pass up those vessels into the periganglionic sinus, continue over the surface of the synganglion and penetrate the ventral wall of the anterior aorta (Figs. 3, 10). In ixodid and argasid ticks the sheaths of extrinsic muscles are occasionally fused with the internal basement membrane of the sinus wall, but in both groups they remain free within the sinus for most of their length. In *Amb. tuberculatum* and *D. variabilis* there are two muscle slips



within each pedal vessel (I, II and IV). In *A. radiatus* and *O. turicata* there seem to be two muscle slips within the first pair of vessels, but pedal vessels II and IV contain single muscles. In both tick families there is a general agreement between the number of extrinsic muscle slips and the number of intrinsic muscle fibers in the ventral wall of the anterior aorta.



within each pedal vessel (I, II and IV). In *A. radiatus* and *O. turicata* there seem to be two muscle slips within the first pair of vessels, but pedal vessels II and IV contain single muscles. In both tick families there is a general agreement between the number of extrinsic muscle slips and the number of intrinsic muscle fibers in the ventral wall of the anterior aorta.

Fig. 8.–Sagittal section through heart, aorta and aortic septal valve in an engorged adult male *D. variabilis* (scutum removed), after aldehyde fuchsin stain with bright field optics. Arrow indicates demarcation between aorta and heart. Anterior orientation to left of figure, scale equals 0.1 mm.

Fig. 9.–Dissected preparation of anterior aorta from an unfed adult female *Amb. tuberculatum*, leucomethylene blue technique after ammonium molybdate fixation with phase contrast optics. Scale equals 25 μm.

Fig. 10.–Frontal section through extrinsic muscles in the periganglionic sinus of a partially engorged adult *D. variabilis*, azan stain, with phase contrast optics. Posterior orientation to the top of figure, scale equals 0.1 mm.

Fig. 11.–Frontal section through the synganglion and the endosternum of an adult female *Argas radiatus*, showing association of the periganglionic sinus wall with endosternal tissues, aldehyde fuchsin stain, bright field optics. Orientation as in Fig. 10, scale equals 0.1 mm. Ao, anterior aorta; AoMC, aortic-myocardial cone; AoSV, aortic septal valve; dlSM, dorso-lateral suspensory muscles; E, esophagus; End, endosternum; exM, extrinsic muscles of the periganglionic sinus; GdT-dF, glandular tissues of the dorsal foveae; Gen Acc Gd, male genital accessory gland; Hc, hemocoel; Ht-P, pulsatile portion of the heart; intM, intrinsic muscles of the aorta; MG, midgut; NmesC, nuclei of mesenchymal cells; pcSp, pericardial septum; pgS, periganglionic sinus; Syn, synganglion; VasM, vascular membrane.

No trace of an endosternum (End) is observed in the three species of ixodid ticks, but in *A. radiatus* and *O. turicata* paired collagenous masses lie alongside the dorso-lateral margins of the periganglionic sinus. These connective tissue formations bear the origins or insertions of a number of intercoxal, genital and dorso-ventral body muscles. Only enlarged posterior portions of the endosternites are continuous with posterior projections of the sinus walls in *A. radiatus* (Fig. 11), but in *O. turicata* the anterior arms of the endosternites are continuous with sinus walls. In these two argasids the right and left halves of the endosternum are joined by a series of transverse muscles. Preliminary observations on *Antricola mexicanus* Hoffman (Argasidae: Ornithodorinae) reveal that right and left halves of the endosternum are fused medially and that all internal margins of the horseshoe-shaped endosternum are continuous with the walls of the periganglionic sinus.

DISCUSSION

Most previous workers consider the circulatory system of ticks to be primitive or poorly developed (Robinson and Davidson, 1913-1914; Balashov, 1972). This judgement may rest in part, on the absence of a closed system of venous sinuses and segmental and posterior aortae, as well as the presence of a single periganglionic sinus in place of paired thoracic arterial sinuses. On the other hand, venous vessels found in Xiphosura and pulmonate Arachnida are believed to be secondary in origin, representing a "canalization of lacunar spaces" associated with the specialized respiratory structures of the gills or book lungs (Beklemishev, 1969). Absence of a channeled venous circulation, together with the general absence of dissolved respiratory pigments, may be correlated with the extensive tracheation of apulmonate Arachnida. Similarly, well-developed peripheral arterial systems, complete with metameric vessels arising from the dorsal vessel, are considered to be primitive in character (Beklemishev, 1969). Moreover, the normal developmental sequence in Xiphosura involves condensation of paired thoracic arterial sinuses, as found in larval stages, into the single perineural sinus of the adult (Firstman, 1973). In comparison to the periganglionic arterial sinus of apulmonate Arachnida, Firstman considers the persistence of paired arterial sinuses in pulmonate Arachnida as an example of "neotenous developmental retardation."

In ticks representing the sub-families Argasinae and Ornithodorinae (Argasidae) and Ixodinae and Amblyomminae (Ixodidae) the heart is highly condensed and regionally specialized. Various authors have reported only a single cardiac chamber and a single pair of ostia in both argasid (Robinson and Davidson, 1913-1914) and ixodid ticks (Douglas, 1943). Our data show that the posterior (pulsatile) portion of the heart contains two cardiac compartments in both tick families, although irregular internal projections of the myocardium often obscure this segmental nature in histological preparations of contracted hearts. The arrangement of radiating muscle bands (three pairs) which surround the two pairs of ostia (Fig. 1) seems to reflect the primitive metamerism of this portion of the dorsal vessel. In that light, the complex postero-median (unpaired) muscle band may represent a condensed remnant from posterior portions of the ancestral vessel. The anterior portion of the tick heart (aortic-myocardial cone) appears to be a transition zone between the muscular posterior myocardium and the aortic wall with its discrete internal basement membrane (Fig. 8).

In those Xiphosura and pulmonate Arachnida which have been most studied, intrinsic elements of the cardiac ganglion serve as pacemakers for the heart. Recent data on the organization of the cardiac ganglion, the pattern of peripheral innervation and sensory modulation of heart rhythmicity come largely from Xiphosura (*Limulus polyphemus*) (Corning and VonBurg, 1968; Bursey and Pax, 1970a, 1970b; Sperelakis, 1971; Stephens and Greenburg, 1973) and from Araneae (Wilson, 1967; Sherman and Pax, 1968; Bursey and Sherman, 1970; Ude and Richter, 1974). Similar data from Scorpiones is provided by Zwicky (1968). In these taxa the heart rhythm is neurogenic, but subject to modulation by various sensory stimuli and by potential neuroendocrine regulators (Kadziela and Kokocinski, 1966; Sundara and Krishnan, 1968; Ude and Richter, 1974). Identification of a putative cardiac ganglion in *Amb. tuberculatum* and evidences of peripheral innervation from the central nervous system and from adjacent sensory complexes in other ticks (dorsal photoreceptors of Argasidae and dorsal foveae of Ixodidae-Amblyomminae), together with observations on the effects of various stimuli on heart rate, suggest that the tick heart also has a neurogenic rhythm which is subject to complex sensory modulation.

Although there is no closed system of venous vessels in ticks, there is a well-defined pericardial sinus. The highly perforated wall (pericardial septum) of the sinus is formed from connective tissues which are continuous with similar tissues associated with dorso-lateral and ventro-lateral suspensory muscles of the tick heart. No previous report of a pericardial sinus or septum in ticks is known to us. Although Douglas (1943) provides a brief description of the number and disposition of some suspensory muscles (dorso-laterals) from the heart of *Dermacentor andersoni* Stiles, the ventro-lateral suspensory muscles appear to be previously undescribed. One function of these suspensory muscles seems to be the maintenance of the space within the pericardial sinus.

The dorso-lateral suspensory muscles of tick hearts seem to resemble the alary muscles of insect hearts (McCann, 1970), both in terms of their general form and their structural coupling to the myocardium. Still, further studies at the ultrastructural level are needed in order to establish that these couplings constitute myo-myocardial junctions as reported from insect hearts (Sanger and McCann, 1968). Moreover, the functional importance of the dorso-lateral and ventro-lateral suspensory muscles, in relationship to the coordination of diastole and systole in the tick heart, remains to be determined.

The ventro-lateral suspensory muscles of the tick heart appear to be homologous to the dorso-ventral muscles of scorpion and spider hearts (Kaestner, 1968). In these pulmonate Arachnida the dorso-ventral muscles have their origins on cuticular invagina-

tions from the book lungs. The number of paired dorso-ventral muscles usually corresponds to the number of book lungs instead of the number of paired ostia in the heart. There is a similar correspondence in Ixodoidea; there are two pairs of ostia in tick hearts and the single pair of ventro-lateral suspensory muscles have some fibers which arise from the atrial wall of the spiracular plate. Furthermore, the specialized structure of the ventro-lateral suspensors may indicate their importance in processes not directly related to heart contraction or the maintenance of pericardial sinus integrity. The dual origins of these muscles and their complex association with putative neural elements may be indications that several of the muscle fibers are specialized as stretch receptors. In this respect they resemble stretch receptors of certain insects (Horridge, 1965; Wigglesworth, 1972). As proprioceptors they could function in the coordination of engorgement behavior or in the initiation of neuroendocrine mechanisms such as those demonstrated in the blood-sucking insect *Rhodnius prolixus* (Maddrell, 1963).

Certain specializations of the tick arterial circulatory system, as observed in this study, have not been previously reported. One such structure is the septal valve of the anterior aorta which effectively prevents the back-flow of hemolymph into the aortic-myocardial cone during diastolic expansion of the heart. In those arachnids which have distinct prosomatic and opisthosomatic body regions, the function of similar valves insures the maintenance of a higher internal pressure in the prosoma. This creates a pressure differential which assists venous circulation of unoxygenated hemolymph in pulmonate Arachnida and which is implicated as the principal mechanism for limb extension in Araneae (Wilson and Bullock, 1973; Stewart and Martin, 1974). Antagonistic extensor and flexor muscles are described from the appendages of ticks (Ruser, 1933), but pressure differentials may still be necessary for extension of the appendages. Specializations for maintenance of increased pressure within arterial vessels and sinuses may also be important in extension of the capitulum during attachment and engorgement. From fragmentary observations on *Rhipicephalus sanguineus* (Latreille) Chow, et al. (1972) postulated that muscles at the entrance of the aorta into the periganglionic sinus function as valves which might prevent the reversal of arterial flow. That function seems to be fulfilled by the septal valve at the posterior end of the aorta and the muscles observed by Chow, et al., seem to be extrinsic muscles of the periganglionic sinus. If hemolymph flow from lacunae in the appendages into the general body cavity is controlled in ticks by valve-like structures similar to those described from the leg bases of pulmonate Arachnida (Kaestner, 1968), then the contraction of extrinsic periganglionic muscles and intrinsic aortic muscles may elevate the intra-arterial pressure above that of the idiosoma.

Firstman (1973) postulates that dorso-ventral muscles attached to the endosternum in ancestral arachnids originally played a role in generation of arterial pressures. It seems likely that the reduction of the endosternum in argasid ticks and its loss in ixodids is an adaptation which facilitates the expansion of the body during engorgement. Extrinsic muscles of the periganglionic sinus may then be interpreted as derivatives of ventral suspensors which originally inserted on the prototypic endosternum. Since the pharyngeal musculature and the entire length of the esophagus are contained within arterial sinuses, any increase in arterial pressure over that of the general body cavity should favor passage of ingested fluids into the midgut. Such a selective advantage might account for the evolutionary retention of the aortic septal valve and the modification of endosternal musculature (as extrinsic periganglionic muscles) in representative Ixodoidea.

Previous workers have reported the reduction of the arterial circulatory system (Beklemishev, 1969) or even the absence of a functional heart (Mitchell, 1957, 1964;

Whitmoyer, et al., 1972) in many mite species. Their data raise a serious question: Within the Acari, how representative is the circulatory system of ticks? Reduction of the circulatory system in dwarf forms of most tracheate arthropods seems to parallel a similar reduction in the tracheal system. Reduction or loss of both of these systems is then seen as a secondary adaptation related to the lower oxygen transport requirements of smaller organisms. Anatomical comparisons of the heart and arterial circulatory system among Acari and other Arachnida should be more instructive when those larger Acari with well developed tracheal systems are considered. In this respect, the apparent presence of circulating hemocyanin pigments in the hemolymph of *Amb. tuberculatum* is of particular interest. This species is the largest ixodid tick found in the United States and parasitizes relic populations of the burrowing gopher tortoise, *Gopherus polyphemus*. In the absence of concrete data on the role of the pigment in oxygen transport, it is not known whether the presence of circulating hemocyanin should be considered as a retained primitive characteristic or as a secondary adaptation correlated with the large size of this tick or some other parameter of the tick-host relationship. In any case, our identification of a condensed and regionally specialized myocardium (probably with an intrinsic cardiac ganglion), a well-developed pericardial septum associated with the dorsolateral and ventro-lateral suspensory muscles of the heart, and arterial specializations, including the septal valve and the intrinsic and extrinsic musculature, attests to the complexity and evolutionary advancement of the circulatory system in Ixodoidea.

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