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The Heart of the Salamander (Salamandra salamandra, L.), with Special Reference to the Conducting (Connecting) System and Its Bearing on the Phylogeny of the Conducting Systems of Mammalian and Avian Hearts

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Phil. Trans. R. Soc. Lond. B 1941 **231**, 99-130
doi: 10.1098/rstb.1941.0005

THE HEART OF THE SALAMANDER (*SALAMANDRA SALAMANDRA*, L.), WITH SPECIAL REFERENCE TO THE CONDUCTING (CONNECTING) SYSTEM AND ITS BEARING ON THE PHYLOGENY OF THE CONDUCTING SYSTEMS OF MAMMALIAN AND AVIAN HEARTS

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(*Communicated by W. E. Le Gros Clark, F.R.S.—Received 8 November* 1940)

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Nine salamander hearts have been studied histologically by means of serial sections, cut in each of three planes (transverse, frontal and sagittal), and stained with haemalum and eosin, van Gieson's acid fuchsin and iron-haematoxylin, and by the protargol method of Bodian. This study has demonstrated muscular continuity between the several cardiac chambers, and the entire absence of any specialized muscle or 'nodal tissue' at the junctional sites or in any other part of the heart. The heart muscle forms a continuum. The cardiac muscle fibres are characterized by their large size (i.e. breadth); they have the same general histological characters in all parts of the heart. Measurements are given for the fibres from various parts of the hearts of the salamander and frog.

The muscular connexions between the various cardiac chambers have been studied in detail. In each of the chambers the musculature is arranged in a basket-work fashion, but at each of the junctional sites the muscle suddenly changes to a regular circular arrangement. The sinus, at its junction with the right atrium, contains muscle only in its ventral wall, and it is this wall only of the sinus which thus establishes muscular continuity with the ring of

muscle (S-A ring) around the sinu-atrial opening. The musculature of both atria is continuous with that of the ventricle in two ways. From the ring of muscle (A-V ring) surrounding the common opening of the atria into the ventricle, the atrio-ventricular funnel dips down into the ventricle, and the caudal border of this funnel is continuous (*a*) with an invaginated part of the base of the ventricle, and (*b*) more extensively with ventricular papillary muscles, which, in their turn, are continued into the inner ventricular trabeculae about the middle level of the ventricle. The A-V funnel is homogeneous in structure; no one part of its circumference differs from another. The ventricular muscle is directly continued into that of the bulbus cordis, in which latter chamber the muscle is entirely circular.

The course which the wave of contraction takes during its transmission from the sinus throughout the heart has been deduced from the study of the details of the continuity of the musculature of the various cardiac chambers. To a large extent this deduction has been confirmed by superimposing tracings of the outline of the pulsating heart, made from the slow-motion cinephotographic records. This latter study has revealed many of the details of the phases of the cardiac cycle.

The delay in the transmission of the wave of contraction from one cardiac chamber to the next is accounted for by the relatively long path which the impulse has to traverse at the junctional sites, where the muscle is arranged in a circular fashion, without postulating the existence of specialized 'block fibres' at these sites. The branching of the muscle fibres has the effect of converting the morphological circular arrangement of the fibres at these junctions into a physiological spiral.

The glycogen content of the various parts of the frog's heart, as revealed by staining with carmine, is found to increase in the order sinus, atria, ventricle and bulbus cordis. This is correlated with a similar increasing order of density of musculature and work done, the glycogen being a reserve potency for the energy of muscular contraction. The fact that the intrinsic rhythmic rates of the several chambers decrease in the same order as the glycogen content increases may or may not be coincidental.

Cutting and ligature experiments, with cinephotographic and kymographic records, reveal the intrinsic rhythmic rates of the various cardiac chambers of the salamander heart.

No satisfactory reason has yet been adduced to account for the different intrinsic rhythmic rates of the several parts of the heart when these are isolated from each other.

The dorsal mesocardium has been traced in its entirety. The sinu-ventricular fold is a part of the continuous dorsal mesocardium and does not constitute a direct muscular sinu-ventricular connexion.

The distribution of the intracardiac nerve cells has been noted and the probable pathway of migration of these nerve cells in the embryo has been suggested.

The significance of the results of this investigation in relation to the phylogeny of the specialized conducting system of the hearts of homoiothermal vertebrates (mammals and birds) is discussed. The view is expressed that the cardiac conducting systems of homoiothermal vertebrates constitute a neomorphic development, correlated with functional requirements, and are not remnants of more extensive tissues of similar structure in the lower vertebrate heart. Variations in this newly evolved formation probably account for the different descriptions of such elements in various mammalian and avian hearts.

I. Introduction

The heart of the salamander has been chosen to form the basis of a comparative study of the conducting (connecting) systems of the hearts of vertebrates because a study of the gross anatomy of the entire animal (Francis 1934) revealed its very generalized nature, and the anatomical features of its heart appeared to us to be

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favourable for comparison with the hearts of fish, on the one hand, and those of reptiles, birds and mammals on the other. The main objects of the present study are to determine whether the regions of the heart possessing greatest rhythmicity are characterized by any specialized histological structure, and whether there are any special histological elements at the junctions of the cardiac chambers which may be responsible for the conduction of the impulse for co-ordinated contraction from one chamber to the next, and for the delay in the passage of the impulse across these junctional sites.

II. Historical

Very little study has previously been made of the cardiac conducting system of *Salamandra*, or for that matter of any caudate amphibian. The following review is a summary of existing work on lower vertebrates (fish, Amphibia, reptiles) which is relevant to the present study. Only those studies of higher vertebrates (birds and mammals) which are of immediate relevance are quoted; more extensive bibliographies concerning these have been published by one of the present authors (Davies 1930*a*, 1930*b*, 1931; Blair and Davies 1935).

The balance of evidence so far presented appears to favour the myogenic origin and conduction of the cardiac impulse, but there are considerable discrepancies in the findings of different authors, and much divergence of opinion concerning the phylogenetic relationship between the nodal and Purkinje tissues of the mammalian heart and the tissues connecting the cardiac chambers in the lower vertebrates.

On the one hand, many investigators claim that the muscle connecting the several chambers of the vertebrate heart has special histological characters. After Gaskell (1882, 1883, 1900) had stated that, in the frog and tortoise, the muscle joining the sinus to the atria and the atria to the ventricle had embryonic characters (less distinct striation and richer in sarcoplasm than the ventricular muscle), to which he attributed a slower rate of conduction and contraction than the ordinary cardiac muscle, and demonstrated that these special muscle junctions conducted the impulse for cardiac contraction from one chamber to the next, other investigators, with minor differences of detail, described specialized muscular junctions in various vertebrate hearts. Notable amongst these are the following. MacWilliam (1885) studied the hearts of the eel, dogfish, salmon and cod both histologically and experimentally, and found that the fibres of the auricular canal and basal wall of the atrium resemble those of the sinus, the fibres of which are more feebly striated than those of the atrium and ventricle. W. His, Junr. (1893) agreed with Gaskell that the varying rhythmicities of the several parts of the frog's heart are due to the different structure of their musculature. Ewald (1902) claimed that in osmium preparations of the frog's heart the musculature of the A-V funnel is distinguishable from that of the ventricle by its more yellow appearance. Although Keith and Flack (1907) observed a specialization of the muscle fibres of the A-V junction in the eel, they found no differentiation in the A-V ring of the frog.

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These authors trace the evolution of the conducting system of the mammalian heart from a simpler and more definite form seen in the fish, and they consider that the A-V node represents the only part of the A-V ring that has remained primitive and that the A-V bundle and its two limbs are remnants of the invaginated portion of the auricular canal (A-V funnel). They discovered the S-A node of the mammalian heart, and described it as consisting of tissue intermediate between nerve and muscle fibres to which they gave the non-committal name of 'nodal tissue'. They noted its close similarity in structure to that of the mammalian A-V node described by Tawara (1906). Keith and Mackenzie (1910) maintained that 'as one ascends the scale of animals the concentration (of nodal tissue) becomes more marked'. Thus they find the sinus venosus of the eel to be composed of nodal tissue which is especially accumulated at the bases of the venous valves; in the frog, lizard and tortoise, nodal tissue is massed mostly in the vestibular part of the left atrium at the end of the pulmonary vein, but extends also to the junction of the sinus with the right atrium, where a concentration occurs in intimate connexion with Remak's ganglion. Similarly, at the A-V junction the nodal tissue, which is described as forming a complete ring in the eel, shows evidence of concentration at certain points in the frog, which tendency becomes more marked still in the reptile. It should be noted that while Keith and Flack (1907) found no histological differentiation in the A-V ring of the frog, Keith and Mackenzie (1910) described in the same animal a complete ring of nodal tissue at this site, with evidence of concentration. Further, Mackenzie (1913) correlates the reduction of the S-A and A-V rings of nodal tissue of the fish heart to the S-A and A-V nodes of the mammal, with the concentration of nodal tissue in certain places and the development of fibrous rings separating the atria from the ventricles. Külbs (1912) also affirmed that there is a specialized muscular A-V connexion in lower vertebrate hearts (frog, tortoise, lizard), and that the specialized A-V connexion of mammals is evolved from this. However, he failed to find in these lower vertebrates any specialization of muscle either in the sinus or at the S-A junction, and maintained that Keith, Flack and Mackenzie confused nodal tissue with ganglion cells at the end of the pulmonary veins. Lange (1914), and Ohmori (1927), in a number of fish, Amphibia and reptiles, and Laurens (1913*a*, 1913*b*, 1915), in reptiles, found that the muscular A-V connexions show only slight histological differences from the rest of the heart muscle. Laurens examined also the S-A junction in numerous reptiles and failed to find any specialized muscle. Benninghoff (1920, 1922), in a number of Amphibia, found that the muscle fibres of the A-V ring and funnel stain paler and have more scanty fibrillation than the fibres of the ventricle, but he maintained that this type of fibre is also found elsewhere in the heart, and that the only peculiarity lies in the accumulation of such fibres at this site. He also strongly criticized the description by Keith, Flack and Mackenzie of nodal tissue in lower vertebrate hearts. Yokochi (1931) claims to have found specific muscle in a number of reptiles, either in the sinus or in the region of the S-A junction.

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It will thus be seen that there is no unanimity even amongst those workers who find evidence of histological specialization in the musculature in various parts of the hearts of lower vertebrates. The issue is still further confused by the results of other investigators who have failed to find any evidence of specialized muscle. Thus Braeunig (1904) in the frog and grass snake found that the musculature of the A-V junction has the same histological characters as the general myocardium. Carlson (1905), in the salamander (*Necturus maculatus*), could find no histological or microchemical difference in the muscle at the ventriculo-bulbar junction, although he noted that the pause in the wave of contraction was here greater than at the A-V junction. Haberlandt (1913, 1917) found no specialized muscle in the A-V funnel of the frog and determined experimentally that all parts of the circumference of the funnel have the same capacity to initiate automatic contractions of the ventricle. Mangold alone (1914*a*, 1914*b*) and with Kato (1914*a*, 1914*b*) concluded that no differentiation, either structural or functional, between purely contractile and purely conducting muscular tissue, occurs in lower vertebrate hearts, or in those of birds. Skramlik (1921, 1932) found that in the frog and toad the muscle fibres, apart from slight variation in size, have the same histological characters in all parts of the heart, and attributed the delay in the passage of the impulse across the junctional sites to the arrangement, rather than any specialization, of the junctional muscle.

In marked contrast with all of the investigators so far quoted, Dogiel and Archangelsky (1906), Dogiel (1907, 1910) and Imchanitzky (1908, 1909) failed to find in the frog, lizard and tortoise any muscular connexion whatever between the atria and ventricle, and maintain that the connexion between these chambers is purely a nervous one. In fact, Dogiel (1907) attributed the results of other workers, who found such muscular continuity, to the artificial dragging of muscle fibres across the A-V junction during the actual process of cutting the sections.

The foregoing survey has been mainly confined to the morphological aspect of the question, but there still remain a number of purely experimental investigations to be discussed. Omitting from the present discussion the numerous experiments designed purely to determine whether the origin and conduction of the cardiac impulse are neurogenic or myogenic, there are others which suggest the existence of specialized muscle in various parts of the heart. Thus Engelmann (1895, 1897) found that the speed of conduction of the impulse across the A-V junction of the frog's heart is much slower than that in the general atrial wall and attributed this to the muscle of embryonal type described by Gaskell and by His at the junctional sites of the heart. Garrey (1911) noted in the turtle, that by clamping the S-A junction, S-A block could be produced without interfering with the transmission of vagal impulses, and since Meek and Leaper (1911) had shown that the compression necessary to block the passage of impulses in nerve fibres and in skeletal muscle is not markedly different, he concluded that cardiac conduction must involve a tissue much more sensitive than ordinary motor nerves or skeletal muscle, or some sensitive mechanism of which we are not yet

cognisant. Nakano (1913), by cutting various parts of the A-V funnel of the frog and salamander (*Salamandra salamandra*) and recording the effects on A-V co-ordination with the kymograph, deduced that there were differences in the conducting powers of different parts of the funnel. Eckstein (1914), by electrical stimulation, showed that the A-V junction of the frog has a lower conducting power than either atria or ventricle. Scholomovitz and Chase (1916), as a result of localized warming, cooling, or electrical stimulation, claimed that the primary pacemaker of the turtle's heart is a definitely localized portion of the sinus wall, on the right side of the S-A junction. Haberlandt (1916, 1917) found that in the tortoise the lateral segments of the A-V funnel are more sensitive to electrical stimulation than the dorsal and ventral segments, maximum sensitivity occurring on the left. Veil (1917–18) described a light band on the dorsal surface of the frog's heart, extending from the sinus to the base of the ventricle, and she determined that this band has a chronaxie at least three times that of the general heart muscle. She called it a physiological dorsal A-V bundle and homologized it with the mammalian A-V bundle. Amsler and Pick (1920), by the application of strophanthin to the right and left halves of the frog's ventricle, both when separated from and attached to the rest of the heart, deduced that the conducting pathway is physiologically differentiated and shows a bifurcation into right and left limbs, the left limb being the stronger. Clark and Kingisepp (1935), studying the effect of low oxygen pressures on frog's cardiac tissue, liken the low oxygen requirements of the sinus and A-V conduction tissue (compared with the rest of the heart) to the low oxygen consumption of mammalian Purkinje fibres. Mitolo (1938), by the localized application of temperature changes, cauterization, faradaic stimulation and drugs, identified a point in the right half of the sinus, near the S-A junction, as the pacemaker of the toad's heart, and postulated the existence of specific nodal tissue as the anatomical basis of this pacemaker.

On the other hand, Skramlik (1921), by combining histological with experimental study in the hearts of the frog and toad, found that the delay observed in the passage of the impulse from one chamber to the next could be explained without postulating the presence of specialized fibres. He observed that at each junctional site the muscle fibres are arranged in a spiral manner, and, by measuring the time taken for an electrical stimulus to pass across the junction and a calculation based on the angles of the spirals, he estimated that the speed of conduction through the junctional muscle fibres is the same as that through the general heart muscle. His histological study, as explained above, also failed to reveal any specialized characters in the junctional muscle.

The markedly conflicting results of former workers, as revealed by this historical survey, render a further investigation of the hearts of lower vertebrates desirable.

III. Methods

Histological study has been made of serial sections of the hearts of nine adult salamanders, cut in the three conventional planes—transverse, frontal and sagittal—and stained with haemalum and eosin, van Gieson's Picro-Säurefuchsin and iron-haematoxylin, and with silver by the protargol method of Bodian (1937). The hearts were carefully excised from pithed animals under a binocular dissecting microscope and fixed while still beating, without artificial distension of the chambers. Experimental work comprised the careful cutting apart from each other of the several segments of the beating heart, and recording the contractions of each simultaneously on a slowly revolving kymograph. In this way the independent intrinsic rhythmicity of each chamber was observed. The course of the wave of contraction throughout the heart was studied by means of superimposing tracings made from the slow-motion cinephotographic records.

IV. Observations

(1) *Histological*

The parts of the heart are described in the order followed by the wave of contraction, viz. sinus venosus, sinu-atrial junction, atria, atrio-ventricular junction, ventricle, ventriculo-bulbar junction and bulbus cordis.

(*a*) *Sinus venosus.*

The sinus venosus (figure 1, *S.*) is a large triangular sac lying dorsal and to the left of the atria and ventricle. Its caudal apex receives the post-caval vein (*P.C.V.*), and its lateral basal angles receive the corresponding right and left ducts of Cuvier (figure 8, *R.D.C.*, *L.D.C.*). While the right duct of Cuvier is very long, the left duct is extremely short and sometimes does not exist as a discrete vessel, its tributary veins entering the sinus separately. The common pulmonary vein has an intimate relation to the sinus and to the S-A opening (figure 1, *C.P.V.*, *S-A.O.*). On its way to the left atrium, the pulmonary vein courses in a cranial direction across the right half of the dorsal surface of the sinus, and fuses with the dorsal sinus wall (figure 12, plate 7), so that here the ventral wall of the vein and the dorsal wall of the sinus form a common structure. After passing through the cranial part of the cavities of the sinus and right atrium, either freely, or attached to the internal surface of the dorsal sinus wall and to the cranial border of the S-A orifice, the vein finally pierces the atrial septum and opens into the left atrium. The wall of the sinus is very thin and its muscle content varies in different regions. Caudally, near the entrance of the post-caval vein, the muscle is sparse and is confined to the ventral wall of the chamber, the dorsal and lateral walls here being formed only of connective tissue lined with endocardium. Proceeding cranially the musculature gradually spreads round until it completely surrounds the sinus. It does not, however, form a continuous sheet, but a basket-work,

the meshes of which are occupied by connective tissue. The dorsal wall of the sinus, above the level of the caudal boundary of the S-A opening, contains very little muscle, and even this entirely disappears before the dorsal sinus wall finally fuses with the wall of the right atrium, the muscular union between the sinus and the right atrium being effected therefore only by the ventral wall of the sinus. The right lateral wall of the sinus is joined to the dorsal wall of the ventricle by a fold of the dorsal mesocardium, the sinu-ventricular fold, which is described in detail below (figure 8; figure 13, plate 7, *S-V.F.*). The attachment of the common pulmonary vein to the

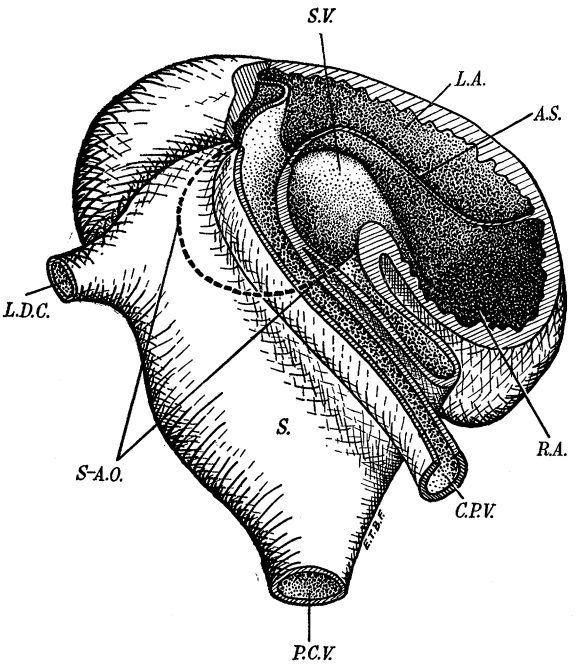

FIGURE 1. Dorsal view of dissection of salamander heart (ventricle and bulbus omitted) showing the relations of the common pulmonary vein to the sinus and atria; diagrammatically represented. *A.S.* atrial septum; *C.P.V.* common pulmonary vein; *L.A.* left atrium; *L.D.C.* left duct of Cuvier; *P.C.V.* post-caval vein; *R.A.* right atrium; *S.* sinus; *S-A.O.* sinu-atrial opening; *S.V.* sinus valve.

dorsal wall of the sinus lies slightly to the left of this fold. Immediately caudal to the entry of the right duct of Cuvier into the sinus, between these sinus attachments of the pulmonary vein and sinu-ventricular fold, a large nerve ganglion (the sinus ganglion) is embedded in the sinus wall (figure 8; figures 11 and 13, plate 7, *S.G.*). This ganglion passes obliquely through the sinus wall, its caudal end being sub-epicardial and its cranial end sub-endocardial. Where the sinus musculature is interrupted by the ganglion, the muscle of the ventral wall of the sinus is diverted and extends for a short distance into the dorsal mesocardium but ends far short of the ventricle. Apart from this slight extension of sinus muscle, the sinu-ventricular fold consists only of connective tissue containing some nerve cells and nerve fibres and in no way constitutes a direct muscular connexion between sinus and ventricle.

The muscle fibres of the sinus are approximately cylindrical in form and are transversely striated throughout, except for an extensive spindle-shaped perinuclear clear zone (figure 14, plate 8). In the nuclear region the fibres are slightly swollen and have here their maximum transverse diameter. The breadth of the fibres ranges from 12·3 to 20·5μ, the majority being about 16·4μ. The smallest fibres are found mainly in the caudal part of the sinus, very few small fibres being present in the cranial portion. These general histological characters, apart from size, are common to the musculature of all the regions of the heart. The muscle fibres of the sinus, as elsewhere throughout the heart, form a continuum, so that it is not possible to speak of a measurement for the length of individual fibres. There are no striated muscle fibres in the terminal parts of the post-caval vein and left duct of Cuvier. In the right duct of Cuvier striated muscle, with the same histological characters as those of the sinus muscle, extends from the sinus to about half-way across the base of the ventricle. This muscle is entirely longitudinal or slightly oblique in its disposition, and is continuous with the sinus musculature.

(*b*) *Sinu-atrial junction.*

As stated above, the musculature of the sinus immediately proximal to the S-A junction is arranged as a basket-work and is here limited to the ventral wall of the sinus, where it is continued into the musculature around the opening of the sinus into the right atrium. At this site, however, the fibres are arranged in a regular circular fashion, completely surrounding the S-A opening, this sudden change being a striking feature of this junctional region. The probable significance of this arrangement of the muscle fibres at the S-A junction is discussed below. This muscle ring is thickest at the caudal border of the orifice, but it is everywhere thicker and denser than the adjoining walls of both sinus and atrium (figure 13, plate 7, *S-A.R.*). Distally, the circular arrangement once more suddenly gives place to the basket-work arrangement of the right atrium, the fibres of which are directly continuous with those of the muscular ring. The epicardium over this thickened ring of muscle is itself thicker than that over the neighbouring regions of sinus and atrium. The present writers, after repeated observations, have failed to identify any nodal tissue—in the sense of Keith, Flack and Mackenzie—at the junction of sinus and atrium in the heart of the salamander. Where the common pulmonary vein fuses with the dorsal wall of the sinus, the sinus muscle extends for a very short distance into the ventral wall of the vein. Apart from this, the terminal part of the common pulmonary vein is devoid of striated muscle, both before its entry into and during its passage through the sinus and right atrium. No nodal tissue is present in relation to the terminal part of the pulmonary vein. The circular muscle at the S-A junction (figure 15, plate 8) consists of approximately cylindrical fibres with a maximum breadth ranging from 16·4 to 24·6μ, the majority being about 20·5μ. Their histological characters are identical, apart from their slightly greater breadth, with those of the sinus and atrial musculature. Arising from the atrial

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septum, on the left side of the S-A orifice, is the large flap-like sinu-atrial valve (figure 1; figures 12, 13, plate 7, *S.V.*), which consists of a muscular basket-work extending from the muscle of the atrial septum and covered on both sides with endocardium. There is no trace of a valve on the right side of the S-A opening.

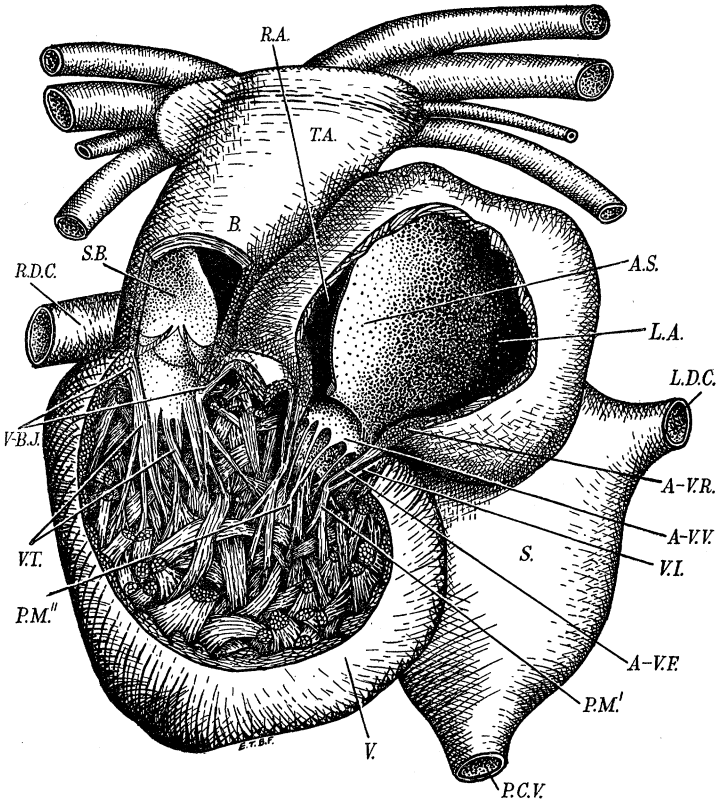

FIGURE 2. Ventral view of dissection of salamander heart showing details of atrio-ventricular and ventriculo-bulbar junctions; diagrammatically represented. *A.S.* atrial septum; *A-V.F.* atrio-ventricular funnel; *A-V.R.* atrio-ventricular ring; *A-V.V.* atrio-ventricular valve; *B.* bulbus; *L.A.* left atrium; *L.D.C.* left duct of Cuvier; *P.C.V.* post-caval vein; *P.M'.* papillary muscles attached to A-V funnel; *P.M''.* papillary muscles attached to chordae tendineae of A-V valve; *R.A.* right atrium; *R.D.C.* right duct of Cuvier; *S.* sinus; *S.B.* septum bulbi; *T.A.* truncus arteriosus; *V.* ventricle; *V-B.J.* ventriculo-bulbar junction; *V.I.* ventricular invagination; *V.T.* ventricular trabeculae.

(*c*) *Atria.*

These chambers occupy an unusual topographical position in that both right and left atria are situated entirely to the left of the bulbus cordis (figure 2). Relative to each other the left atrium occupies a left ventral position, and the right atrium a right dorsal position, the atrial septum being thus very oblique (*A.S.*). The walls of both atria, like those of the sinus, consist of a basket-work of muscle fibres, but are considerably thicker and present prominent muscular trabeculae which form a loose spongework extending for a short distance into the atrial cavities. The atrial septum

is exceedingly thin and consists of a delicate, loose, muscular network, the fibres of which are continuous with those of both atria. Its musculature is sparse throughout, especially where the pulmonary vein enters the left atrium. There is no large collection of nerve cells in the atrial septum of the salamander heart comparable with Ludwig's ganglion (Ludwig 1848) in the frog's heart. The lower border of the obliquely placed atrial septum is attached on the left side to the dorsal membranous cusp of the atrio-ventricular valve (*A-V.V.*), and on the right to the ventral membranous cusp of this valve, while between these two attachments it is free, non-muscular, and thickened, and projects into the common atrio-ventricular orifice. The histological characters of the atrial muscle fibres (figure 16, plate 8) are the same as those of the sinus; they range in breadth from 14·4 to 18·5μ, the majority being about 16·4μ. No nodal tissue, or muscle having a structure different from that described, was found in either atrium.

(*d*) *Atrio-ventricular junction.*

The common opening of the atria into the ventricle is guarded by the atrio-ventricular valve (figure 2; figure 10, plate 7, *A-V.V.*), which consists of four non-muscular, membranous cusps. The principal pair are dorsal and ventral, the right and left being very small. The free borders of the cusps are attached by chordae tendineae to ventricular papillary muscles (*P.M''.*). The muscular union between the atria and the ventricle may be considered under two heads, (1) the atrio-ventricular ring, and (2) the atrio-ventricular funnel.

(1) The atrio-ventricular ring (*A-V.R.*). At the level of the attached bases of the membranous cusps of the A-V valve, the musculature of the atria changes, without losing continuity, from its irregular meshwork to a regular circular arrangement (figure 22, plate 8) and at the same time it becomes thickened, thus forming a sphincter-like ring around the opening which is common to both atria. The musculature of this A-V ring is continued caudally into that of the A-V funnel, and its muscle fibres (figure 17, plate 8) have the same histological characters as those of the atria and sinus; their breadth ranges from 16·4 to 20·5μ, the majority being about 19·7μ.

(2) The atrio-ventricular funnel (figure 2; figure 10, plate 7, *A-V.F.*) takes the form of an inverted truncated cone of muscle which extends downwards into the ventricle. As the base of the ventricle is itself invaginated at this site (*V.I.*), so that the muscle of the invaginated part is continuous with the caudal border of the funnel, the arrangement of the atria and the funnel in relation to the ventricle may be likened to an intussusception of the gut, in which the funnel corresponds to the entering tube, the ventricular invagination to the returning tube, and the base of the ventricle to the receiving tube or sheath. The epicardial connective tissue (figure 10, plate 7) extends between and separates the muscle of the funnel from that of the ventricular invagination, except at the caudal border of the funnel where the musculature of the two becomes continuous. In addition to this connection, the musculature of the funnel is continuous with papillary muscles in the ventricle (*P.M'.*), which join the funnel at

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its caudal border. It should be noted therefore that the musculature of the funnel has two connexions with that of the ventricle, firstly with the invaginated wall of the ventricular base, and secondly, a more extensive one, with the innermost ventricular fibres by means of papillary muscles. Both these junctions occur at the same level, which is approximately one-fifth of the way down from base to apex of the ventricle. The wall of the funnel is thin and its musculature has, in the main, a basket-work arrangement, although the majority of its fibres are approximately circular. The wall of the invaginated part of the base of the ventricle is even thinner, and here the majority of the muscle fibres are longitudinal in direction. The muscle fibres of both the funnel (figure 18, plate 8) and the ventricular invagination have the same histological characters as those of the previously described parts of the heart, and their breadth ranges from 14·3 to 16·3μ, the majority being about 15·4μ. Bearing in mind the contradictory results of experimentally interfering with the passage of the excitatory wave from the atria to the ventricle by damage to various parts of the A-V junction, the present workers have repeatedly and carefully examined all parts of the A-V muscular junction—A-V ring and A-V funnel—and have failed to find any differences between the various sectors of these junctional structures. We are convinced of the homogeneity of the muscular structure of these parts. No nodal tissue was found in any of the parts of the A-V junction. The ventricular papillary muscles (figure 2, and figure 10, plate 7, *P.M''.*) to which the membranous cusps of the A-V valve are attached by chordae tendineae are situated a little caudal to those (*P.M'.*) which are continuous with the lower border of the A-V funnel.

(*e*) *Ventricle.*

The single ventricular chamber (figure 10, plate 7, *V.*) consists, as is usual in Amphibia, of a coarse muscular spongework, but the peripheral muscle does not form a compact myocardial layer as in the frog, so that the endocardium lining the ventricular cavity extends throughout the meshes of the spongework, and in places reaches as far as the epicardium. The ventricular muscle is thus nourished by the blood in the chamber and is devoid of a coronary supply. The main cavity of the ventricle is somewhat L-shaped, extending from the A-V opening towards the apex and then returning cranially and to the right to the ventriculo-bulbar orifice. While most of the ventricular muscle bundles are arranged in a complicated irregular fashion, the innermost bundles are mainly longitudinal, and it is these latter which effect the principal connexions of the ventricular muscle with that of the A-V funnel and bulbus cordis. As explained above, the caudal border of the A-V funnel is continuous with the 'apices' of ventricular papillary muscles about one-fifth of the distance down the ventricle; the 'bases' of the papillary muscles subdivide and merge with the innermost ventricular muscle about half way down the ventricle, which is clearly shown in figure 10, plate 7 (*P.M'.*). The significance of these connexions is discussed below. The ventricular muscle (figure 19, plate 8), again, has the same histological characters

as those described for the sinus and atria. The fibres range in breadth from 12·3 to 16·4μ, the majority being about 15·4μ.

(*f*) *Ventriculo-bulbar junction* (figure 2, *V-B.J.*).

The junction of the ventricle with the bulbus cordis is not uniform, the right dorsal portion of the union (figure 23, plate 8, *R.V-B.J.*) being quite different from the left ventral part (*L.V-B.J.*). In the former part the muscular union is very close and intimate, the circular musculature of the bulbus wall (*B.W.*) being continuous with the superficial bundles of the ventricle (*V.W.*), which in this region are mainly circular, and, in addition, a very strong band of longitudinal muscle fibres (*V.T.*, see also figure 2), formed from the innermost ventricular trabeculae, originates about the centre of the ventricle and passes cranially to join the circular muscle just caudal to the ventriculo-bulbar semilunar valves (*V-B.V.*). In the left ventral part of the ventriculo-bulbar junction (*L.V-B.J.*), on the other hand, the peripheral circular muscle of the ventricle is not directly continuous with the circular muscle of the bulbus, but is separated from it by a short thin invagination (*V.I.*) of longitudinal muscle from the ventricular base, similar to that described at the junction of the A-V funnel with the ventricle. In addition the bulbar muscle is here connected with the central ventricular trabeculae by pillars of longitudinal muscle (*V.T.*), which, however, consist of isolated columns unlike the continuous broad sheet of longitudinal muscle connecting the ventricle to the right dorsal part of the bulbus. There is no direct connexion between the musculature of the A-V funnel and that of the bulbus as occurs in the frog. The change from the longitudinal trabecular muscle of the ventricle to the circular muscle of the bulbus is even more sudden and pronounced than the similar directional change of the fibres at the S-A and A-V junctions. The muscle fibres (figure 20, plate 8) at this junctional region have the same general histological characters as described above for other regions of the salamander heart, and no nodal tissue is present. Their breadth ranges from 12·3 to 20·5μ, the majority being about 15·4μ.

(*g*) *Bulbus cordis* (*conus arteriosus*) (figure 2, *B.*).

The bulbus is cylindrical at its junction with the ventricle but expands transversely as it proceeds cranially. Proximally, at its junction with the ventricle, is the ventriculo-bulbar valve consisting of three non-muscular semilunar cusps, while distally, at its junction with the truncus arteriosus (*T.A.*), is a similar valve with four cusps, of which one (the right dorsal) is prolonged caudally as the spiral valve—septum bulbi (*S.B.*)—as far as the ventriculo-bulbar valve. Distally, at its junction with the truncus arteriosus, the circular, striated, bulbar muscle completely ensheathes the unstriped, arterial muscle of the truncus, while proximally its junction with the ventricular muscle is as described in the preceding paragraph. The wall of the bulbus is uniformly thick, its muscle being compactly arranged in a circular manner throughout (figure 23, plate 8,

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B.W.), lacking the fenestration so characteristic of the other cardiac chambers, and is nourished by the coronary arteries which arise from the truncus arteriosus. The striated muscle fibres of the bulbus (figure 21, plate 8) do not differ in their histological characters from those described for the other parts of the heart. They range in breadth from 16·4 to 20·5μ, the majority being about 18·5μ.

(2) *The size of the cardiac muscle fibres* (figure 3)

The present authors regard the heart muscle of the salamander as a continuum, and accordingly are unable to give a measurement for the length of individual muscle fibres. Skramlik (1921) claimed that, by macerating the hearts of the frog and toad in 40% potassium hydroxide, he was able to isolate complete individual muscle fibres from the various parts of the heart, and he gives measurements for both their length and breadth. He finds very little difference between the two animals, and gives the following average measurements for the size of the fibres from the frog's heart: sinus—length 73μ, breadth 5·4μ; atrium—length 193μ, breadth 5·68μ; A-V funnel—length 116μ, breadth 9·1μ; ventricle—length 131μ, breadth 9·2μ; bulbus—length 136μ, breadth 5·9μ. This maceration with a strong solution of potassium hydroxide appears to us to be a drastic treatment, and we doubt whether the fibres measured by Skramlik were *complete* muscle fibres, and hesitate to place any reliance on figures pertaining to the length of fibres obtained by such means. Our own measurements of the breadth of the fibres in the hearts of the salamander and frog were made directly with micrometers on paraffin sections, prepared from hearts which had been fixed in 10% formalin and stained with haemalum and eosin. Owing to shrinkage of the material, which is unavoidable during histological preparation, these figures manifestly represent relative, not absolute values. The fibres have been measured in both transverse and longitudinal section, and the results are set out in the accompanying table (figure 3).

	sinus	S-A ring	atria	A-V ring	A-V funnel	ventricle	vent.-bulbar junct.	bulbus
				A. Salamander (*Salamandra salamandra*)				
majority of fibres	16·4	20·5	16·4	19·7	15·4	15·4	15·4	18·5
range	12·3–20·5	16·4–24·6	14·4–18·5	16·4–20·5	14·3–16·3	12·3–16·4	12·3–20·5	16·4–20·5
				B. Frog (*Rana temporaria*)				
majority of fibres	5·5	6·2	7·5	8·2	5·1	11·3	8·2	7·2
range	4·1–8·2	5·2–8·2	6·2–8·2	6·2–10·2	4·1–6·2	8·2–12·3	7·2–10·3	6·2–10·2

FIGURE 3. Size of cardiac muscle fibres (breadth) from various regions of the hearts of the salamander and frog, measured in microns (0·001 mm.).

Note. Hearts fixed in 10% formalin and stained with haemalum and eosin.

(3) *General remarks on the histology of the cardiac muscle fibres*

The muscle fibres in all parts of the heart have the same general histological characters (see figures 14–21 inclusive, plate 8). One striking feature of the myocardial fibres of the heart of the salamander is their very large size (breadth). They are two to three times as large as those of the frog, for example. Their nuclei also are very large; these vary in shape from ovoid to long sausage-shaped bodies, and, as observed when stained with haemalum, they are packed with chromatin material. The transverse striation, rendered very distinct by silver impregnation, extends through the substance of the fibres, and is not coarser in any one part of the heart than another. Nowhere in the heart are any fibres present which resemble the Purkinje fibres of mammalian and avian hearts, or the nodal tissue described by Keith, Flack and Mackenzie: neither are there any fibres which might be considered to be more 'embryonic' in character. When traced in longitudinal section the fibres are seen to branch at a very acute angle. After staining by the Bodian silver technique, a peculiar feature of the nuclear impregnation was observed. The nuclei of the muscle fibres of all the cardiac chambers —sinus, atria, ventricle (superficial layers only), bulbus—are in general darkened on the side nearest the heart cavity, and remain pale in their outermost parts. This darkening, due to reduction of the silver salt, shows a granular character, and has not a sharply defined border but shades gradually towards the lighter part. The different regions of the nuclei of the majority of the fibres of the S-A ring also reduce the silver salt unequally, but here it is the part of the nucleus towards the epicardium which is dark, while that towards the endocardium remains pale. The nuclei of the fibres in the remaining parts of the heart show a more or less uniform reduction of the salt throughout their substance, but, while those of the atrial septum and of the deep ventricular trabeculae show granular deposit, those of the A-V ring, A-V funnel and ventricular invagination are so uniformly black that the granular nature can only be discerned with difficulty. That this uneven reduction of the silver salt by the nuclei is not purely fortuitous, is rendered likely by the fact that three hearts, separately treated at different times by the Bodian technique, all revealed the same phenomenon. For this reason we record the observation. It is interesting to note that, if sections previously treated by the Bodian method be decolorized (by treating with iodine, potassium iodide and potassium cyanide) and subsequently stained with haemalum, the nuclei show the even distribution of the blue-stained chromatin material, similar in appearance to that observed in sections stained directly with haemalum. This would seem to indicate that the nuclear material responsible for reducing the silver salt is not identical with that stained by the haemalum.

(4) *Glycogen content of the cardiac muscle fibres (of the frog)*

The present authors had intended to investigate the glycogen content of the muscle fibres in the several parts of the salamander heart, when present hostilities prevented their obtaining a further supply of animals. We decided therefore at present to use

the heart of the frog (*Rana temporaria*) as material for this part of our study, particularly as we had already examined the heart of this animal by the histological techniques employed for the salamander, and failed to find any specialized muscle or nodal tissue in any part of the frog's heart.

Buadze and Wertheimer (1928), as a result of their quantitative chemical estimations, claimed that the ordinary myocardium of the hearts of the dog, she-goat and sheep is richer in glycogen than the specialized fibres of the A-V bundle, and thus concluded that the microscopical tests for glycogen, which indicate that the mammalian conducting system is richer in glycogen than the myocardium, are unreliable. The later careful quantitative estimations of glycogen by Yamazaki (1929) in the hearts of the horse and ox, by Yater, Osterberg and Hefke (1930) in the horse, and by Noll and Becker (1936) in the horse and calf, however, all agree that the A-V bundle of these mammals is richer in glycogen than the myocardium. Noll and Becker employed the iodine and the carmine (Best) histological tests alongside their quantitative estimations, and found that the results agreed. Yater and his co-workers noted that, while the A-V bundle of the horse is much richer in glycogen than the myocardium, in the human the glycogen contents of the A-V bundle and the myocardium are approximately the same. This may be correlated with the observation by Blair and Davies (1935) that, while the fibres comprising the A-V bundle of the ox are large typical Purkinje fibres, those of the human bundle are identical in histological structure with the myocardial fibres.

These later studies therefore indicate that the microscopical tests (Best carmine or iodine) are reliable indicators of the relative glycogen content of the cardiac muscle fibres. Using the Best carmine technique, controlled both by the saliva test and by simultaneously staining a piece of liver, the following observations were made on the hearts of two frogs, removed very rapidly from pithed animals, thus avoiding the anoxaemia which has been shown by Evans (1934) to result in rapid disappearance of mammalian cardiac glycogen. The muscle fibres of the sinus and the S-A junction contain very little glycogen; those of the atria and A-V funnel contain slightly more; those of the ventricle, while not rich in glycogen, contain rather more than the atrial fibres. The fibres of the bulbus cordis are very rich in glycogen, the red-stained granules being so numerous that even under the low powers of the microscope the red colour of the bulbus stands out in marked contrast with the rest of the heart. The possible significance of this gradation of glycogen content in the several segments of the heart is discussed below. Clark *et al.* (1938) found by quantitative estimation that the total carbohydrate content of the ventricular muscle of the heart of the tortoise was slightly greater than that of the auricular muscle, the proportion being 1·67 to 1·5. As stated above, the glycogen content of the ventricular muscle of the frog would also appear to be slightly greater than that of the atria. It is probable that our findings in the frog's heart, so far as glycogen content is concerned, are applicable to the salamander, but this must be determined with certainty by later study.

(5) *The dorsal mesocardium* (figure 8)

The parietal layer of serous pericardium is reflected from the dorsal wall of the fibrous pericardium on to the back of the heart in the following manner. A fold (*L.F.*) passes to the left part of the dorsal wall of the sinus, where it extends from the post-caval vein to the left duct of Cuvier. The two layers of this fold then diverge, the right layer covering the back of the sinus as far as its right border, where it is met by the left layer which has passed round the left border and ventral surface of the sinus. From the right border of the sinus these two serous layers are reflected as a fold, the sinu-ventricular fold (*S-V.F.*; see also figure 13, plate 7), which passes to the back of the ventricle, reaching it along a line extending from just above the apex to the junction of the right duct of Cuvier with the sinus. On reaching the ventricle the two layers of this fold again part company, the right layer passing across the dorsal surface of the ventricle, while the left layer passes round the left border, ventral surface and right border of the ventricle to meet the right layer on the dorsal surface of the ventricle near its right border. Here both layers are reflected as a fold (*R.F.*) from the ventricle to the dorsal wall of the fibrous pericardium. This reflection becomes confluent with the sinu-ventricular fold a short distance above the apex of the ventricle. The pericardium covering the back of the ventricle is reflected, at the level of the atrio-ventricular and bulbo-ventricular junctions, around the right duct of Cuvier, forming a short Cuvierian fold (*C.F.*; see also figure 10, plate 7) which, on the left, becomes continuous with the sinu-ventricular fold. The atria and bulbus cordis are entirely covered with serous pericardium.

The part of the dorsal mesocardium of the salamander heart described above as the sinu-ventricular fold is homologous with the following structures: the fibrous band carrying the coronary nerve (and vein) described by Gaskell (1883) in the tortoise heart and shown by him to be of no importance for atrio-ventricular co-ordination; the dorsal ligament (ligamentum atrioventriculare) described by Dogiel and Archangelsky (1906) in the tortoise and containing the nerve connexion between atria and ventricle (these authors deny any muscular A-V connexion); the ligamentum interauriculo-ventriculare described by Roskam (1913) in the eel, which when cut does not produce A-V dissociation; the sinu-ventricular ligament described by Laurens (1913 *b*, 1915) in the lizard as containing nerve cells but not forming a muscular connexion between sinus and ventricle; the dorsal ligament described by Nakano (1913) in the salamander as passing from sinus to ventricle but as being of no importance for A-V conduction; the sino-auricular bundle described by Mackenzie (1913) in the British Guiana salempenter (*Tejus, vel Monitor, tejuixin*) as carrying a bundle of specialized muscle, lying free for a short distance behind the atrium isolated from the atrial muscle, and becoming continuous with the specialized muscle in the floor of the atrium, the latter being in turn continuous with the auricular canal and thence with the ventricular muscle, the whole thus forming a sinu-ventricular connexion consisting of specialized muscle. The

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sinu-ventricular fold of the dorsal mesocardium of the salamander heart was found in the present work, as stated above, to contain nerve cells and nerve fibres and a slight extension of muscle from the sinus in the region of the sinus ganglion, but it in no way constitutes a direct muscular connexion between the sinus and the ventricle.

(6) *The intrinsic rhythm of the several cardiac chambers*

The sinus, atria, ventricle and bulbus cordis were carefully cut apart from each other with a fine pair of scissors. A binocular dissecting microscope was used to ensure that the cuts were made as precisely as possible along the lines of junction of these parts of the heart. Each part was then pinned to a cork base and a lead taken from each to four levers which recorded on a slowly revolving kymograph. The tracings thus obtained (figure 4) show that, when completely isolated from each other, the various segments of the heart contract at the following rates (expressed in beats per minute): sinus 50, atria 14, ventricle 7, bulbus cordis 4.

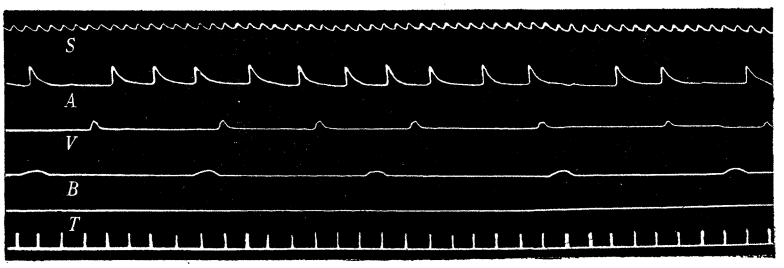

FIGURE 4. Kymograph tracings of the several chambers of the salamander heart which have been completely separated from each other by cuts through the junctional sites (room temp. 15° C). *A*. atria; *B*. bulbus; *S*. sinus; *T*. time signal (2 sec.); *V*. ventricle.

Cinephotographic records (Davies and Francis 1939), after ligaturing the S-A and A-V junctions, show similar results as regards the intrinsic rhythm of the sinus, atria and ventricle. A ligature tied at the junction of the ventricle and the bulbus shows that the rhythm of the bulbus is slower than that of the ventricle, but this part of the experiment has not yet been incorporated in the cine-film.

(7) *The course of the wave of contraction; cinephotographic study* (figures 5, 6 and 7)

The slow motion cinephotographic record (sixty-four exposures a second) of the normally beating salamander heart *in situ*, shows a ventral view of the left part of the sinus, the terminal parts of the post-caval vein and left duct of Cuvier, the right and left atria, the ventricle, the bulbus cordis and the truncus arteriosus. Part of the 16 mm. film thus prepared was passed, frame by frame, across the stage of a Leitz projector, and the images projected at a linear magnification of $\times 33$ on to sheets of paper. In this way the outlines of the cardiac chambers were traced on to separate sheets of paper. When successive tracings were superimposed on each other on a glass sheet illuminated from below, it was found that the seventy-seventh tracing coincided

almost precisely with the first. This indicated that a complete cardiac cycle occupied 77/64 sec., or 1·2 sec., the equivalent of a heart rate of 50 beats a minute. This was actually the rate of the heart measured by stop-watch at a room temperature of 15° C, and the correspondence indicated that the claimed rate of sixty-four exposures a second could be taken to be accurate. Two sides of each frame were also traced in order to act as guide lines when the tracings were superimposed.

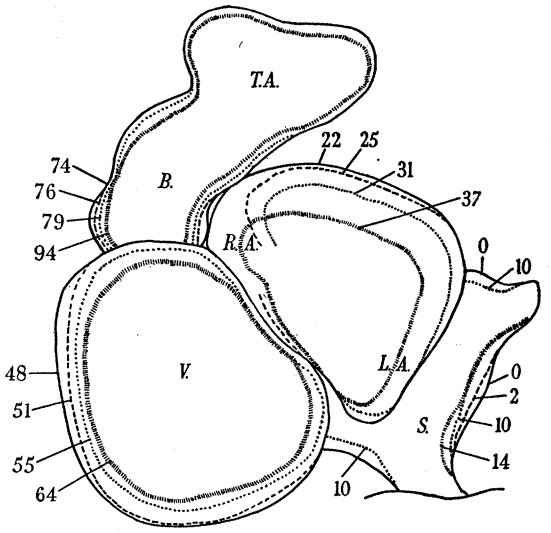

FIGURE 5. Superimposed tracings of cine-photographs showing phases of systole of the salamander heart (room temp. 15° C).

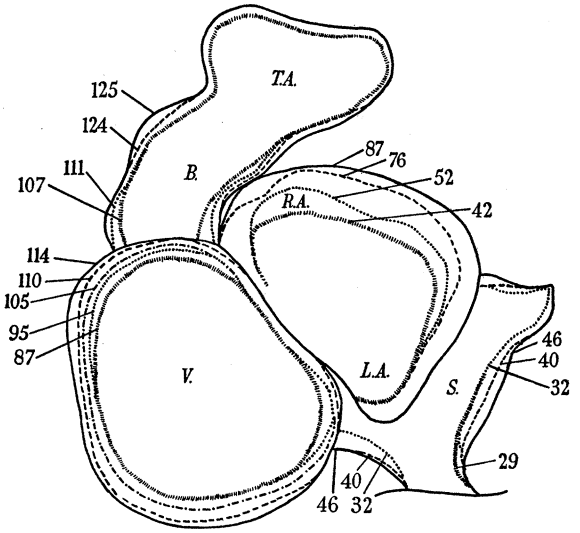

FIGURE 6. Similar tracings showing phases of diastole (room temp. 15° C).

Abbreviations in figures 5 and 6. *B.* bulbus; *L.A.* left atrium; *R.A.* right atrium; *S.* sinus; *T.A.* truncus arteriosus; *V.* ventricle.

Figures represent time, in 64ths sec., commencing with the end of the diastolic phase of the sinus as zero.

By such means the course of the wave of contraction and diastole could be followed (figures 5 and 6). The method is admittedly not so delicate as the electrocardiographic or oscillographic method, but it does give a rough estimate both of the order of events and the time intervals occupied by the various phases of the cardiac cycle.

An analysis of the tracings (figure 7) enables the following conclusions to be drawn. Each chamber takes 77/64 sec. (1·2 sec.) to complete its cycle. The time interval from the beginning of systole of the sinus to the end of systole of the bulbus (i.e. the time taken for the wave of contraction to pass from the sinus to the end of the bulbus) is 94/64 sec. (1·47 sec.). The time taken for the wave of contraction, followed by the wave of relaxation, to pass from its origin in the sinus to its termination in the bulbus is 151/64 sec. (2·36 sec.). Although the passage of the contraction wave in a heart lacking specialized muscle has very probably a uniform rate throughout the muscular continuum (see also Skramlik p. 104), the apparent pause between two consecutive chambers, due to the time taken for the impulse to traverse the 'spiral' pathway of the

junctional muscle, is roughly indicated by the interval between the end of the *visible* contraction of one chamber and the beginning of the *visible* contraction of the succeeding one. These pauses are: sinu-atrial 8/64 sec. (0·125 sec.), atrio-ventricular 11/64 sec. (0·172 sec.), ventriculo-bulbar 10/64 sec. (0·156 sec.). It is evident that these time intervals are longer than actual, because the deeper parts of the cardiac musculature will probably commence contracting before any change in the surface outlines of a given chamber will be effected. Of the parts of the sinus which are visible, the central part

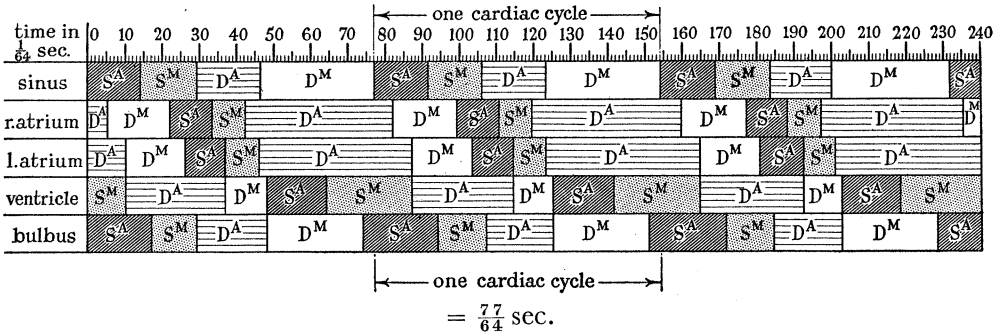

FIGURE 7. Graphical analysis of the cardiac cycle of the salamander, based on cinephotographic records. D^A, active phase of diastole; D^M, maintenance phase of diastole; S^A, active phase of systole; S^M, maintenance phase of systole.

contracts first, the wave of contraction extending thence towards the left duct of Cuvier and the post-caval vein. The right duct of Cuvier and the S-A junction are not visible, so that no information can be deduced concerning the time relation of the onset of contraction in these parts to that in the centre of the sinus. The right atrium undergoes systole and diastole slightly (4/64 sec.) before the left atrium. The first part of the ventricle to contract is the part approximately midway between base and apex. The contraction spreads thence to the apex and base. The wave of contraction spreads progressively from the caudal to the cephalic part of the bulbus. The wave of diastole follows the same order as systole in each of the chambers. Systole of each chamber occurs during the terminal part of the diastolic phase of the succeeding chamber. Both systole and diastole of each chamber consists of two phases—an *active* phase of contraction (S^A) or relaxation (D^A), and a *maintenance* phase of maximum contraction (S^M) or relaxation (D^M).

By means of more precise electrocardiographic studies, Lewis (1915, 1916) in the toad, and Holzlöhner (1929, 1930) in a number of fish, Amphibia and reptiles, found that the middle level of the ventricle is the first part to receive the stimulus from the atria and that the wave of contraction proceeds thence towards the apex and base. By similar methods Meek and Eyster (1912) found the wave passed over the heart of the tortoise in the following sequence—sinus, right duct of Cuvier, right atrium, left atrium, A-V ring, base of ventricle, apex of ventricle. In 1916 they showed in the

turtle that the part of the sinus in which the wave is initiated is in the region of the S-A junction. Goldberg and Eyster (1940), on the other hand, demonstrated electrically in the snapping turtle that the wave spreads over the ventral surface of the ventricle from the left part of the base to the apex and thence to the right part of the base. Roskam (1919), purely by visual inspection, claimed that in the eel the cardiac contraction can start in either of the ducts of Cuvier, depending on the relative pressures in them (as induced by clamping or by withdrawing blood).

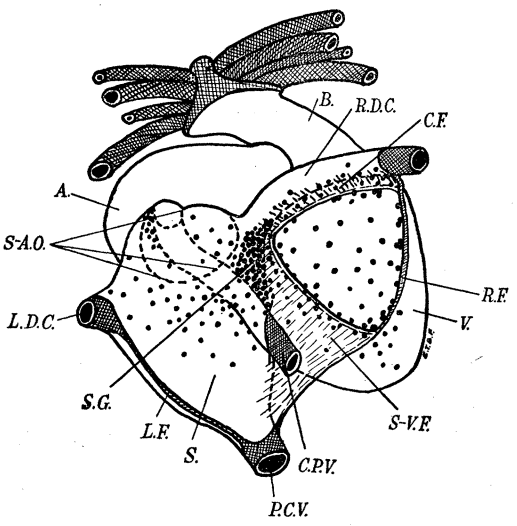

FIGURE 8. Diagram of dorsal view of the salamander heart showing the dorsal mesocardium and the distribution of the *subepicardial* nerve cells (solid dots).

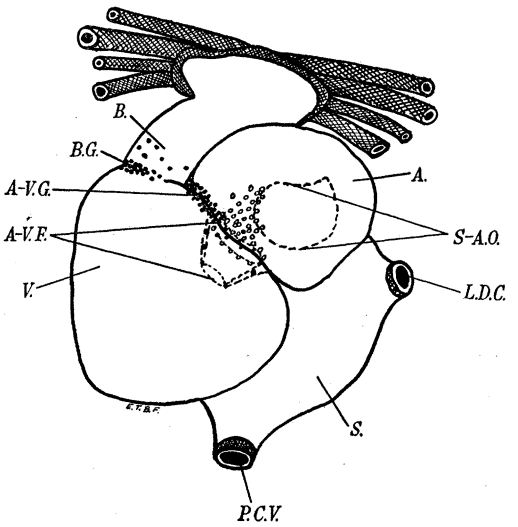

FIGURE 9. Diagram of ventral view of the salamander heart showing the distribution of the *subendocardial* (open circles) and *subepicardial* (solid dots) nerve cells.

(Abbreviations in figures 8 and 9.) *A.* atria; *A-V.F.* atrio-ventricular funnel; *A-V.G.* atrio-ventricular ganglion; *B.* bulbus; *B.G.* bulbus ganglion; *C.F.* Cuvierian fold; *L.F.* left fold of mesocardium; *P.C.V.* post-caval vein; *R.D.C.* right duct of Cuvier; *R.F.* right fold of mesocardium; *S.* sinus; *S-A.O.* sinu-atrial opening; *S.G.* sinus ganglion; *S-V.F.* sinu-ventricular fold; *V.* ventricle.

(8) *Intracardiac nerve cells and nerve fibres* (figures 8, 9, and figure 11, plate 7)

The largest collection of nerve cells in the salamander heart is that related to the right wall of the sinus and S-A junction, corresponding to Remak's ganglion (Remak, 1844) in the frog (figure 8, *S.G.*, and figure 11, plate 7), and situated in the region where the sinu-ventricular fold of the dorsal mesocardium leaves the right border of the sinus. Caudally the nerve cells are subepicardial, becoming subendocardial cranially and interrupting the myocardium of the sinus during this transition. From this ganglion nerve cells spread subepicardially and are scattered over the cranial two-thirds of the dorsal wall of the sinus; they become more sparse to the left of the pulmonary vein. They accordingly become intimately related to the terminal part of the common pulmonary vein which fuses with the sinus wall in this region. The cranial

part of the sinu-ventricular fold is continuous on the right with the mesocardial fold which attaches the right duct of Cuvier to the back of the ventricle—Cuvierian fold (*C.F.*)—along which nerve cells extend beneath the epicardium, on the caudal and dorsal walls of the right duct of Cuvier. They also spread from the sinus ganglion through the S-V fold to the dorsal wall of the ventricle. Cells are found scattered about in the S-V fold itself, being most numerous in its cranial part. No nerve cells are found in the caudal third of the dorsal wall, or in any part of the ventral wall, of the sinus, or in relation to the entry of the left duct of Cuvier.

From the cranial part of the sinus ganglion, which lies subendocardially just caudal to the right part of the S-A junction, nerve cells spread both subepicardially and subendocardially to the ventral part of the S-A opening (figure 9, *S-A.O.*), the subendocardial cells being limited to its right sector. From here subendocardial cells extend along the adjacent part of the wall of the right atrium to the A-V junction where they spread around the A-V ring beneath the endocardium. Apart from the cells above described, there are no nerve cells, either subendocardial or subepicardial, in the general walls of the right and left atria. In the atrial septum, however, while there is no collection of cells like Ludwig's ganglion in the frog, a few isolated scattered nerve cells are found.

At the A-V junction, in addition to these subendocardial cells related to the A-V ring, there is a small ganglion of cells (*A-V.G.*) lying subepicardially in the right and ventral sector of the A-V sulcus, which corresponds with Bidder's ganglion in the frog (Bidder 1852). A few outlying cells of this ganglion extend, in some specimens, into the connective tissue separating the two laminae of the A-V funnel (*A-V.F.*); apart from these, no nerve cells have been found in the funnel itself.

Only subepicardial nerve cells have been observed in the ventricle, and these are practically confined to the dorsal surface (figure 8), most of them being found between the S-V, right and Cuvierian mesocardial folds. They are fairly evenly distributed over this area, occurring singly or in pairs rather than in clusters, except at the edges (i.e. at the sites of pericardial reflexion), where they are more numerous and aggregated into small groups. Isolated nerve cells occur on the dorsal surface even as far as the apex of the ventricle.

Both the ventriculo-bulbar junction and the bulbus are likewise devoid of subendocardial nerve cells. Subepicardial cells occur chiefly as a small group in the right ventral quadrant of the V-B junction (figure 9, *B.G.*); a few cells extend on to the proximal part only of the bulbus itself.

Whereas detailed distribution of nerve fibres has not been traced, coarse leashes have been found to extend from the sinus ganglion into the wall of the right atrium directly, into the atrial septum and wall of the left atrium by way of the pulmonary vein, and to the back of the ventricle by way of the S-V fold. From the A-V ganglion fibres extend into the A-V funnel and thence by way of the papillary muscles to the ventricular wall itself. From the ventricular subepicardial nerve cells,

nerve fibres pass into the ventricular muscle, and appear to be confined in their distribution to the superficial musculature, the deeper ventricular muscle being supplied by the fibres passing down the funnel from the A-V ganglion. Throughout the heart fine nerve fibres permeate the muscle bundles and run on, and between individual myocardial fibres. Nothing resembling motor end plates or other specialized endings has been observed.

The disposition of the intracardiac nerve cells just described, appears to indicate that, during development, migration of nerve cells has occurred from a locus, represented by the site of the sinus ganglion, to the other parts of the heart, (*a*) by means of the mesocardial reflexions to give rise to the subepicardial groups, and (*b*) through the sinus wall to become subendocardial and thence migrating caudally beneath the endocardium through the S-A orifice and along the right atrial wall to and around the A-V ring. If this postulation is correct, and it can only be substantiated by embryological study, it suggests that there is only one site of migration of the subendocardial cells from without, and that is by way of the sinus wall.

V. Discussion

The present investigation has shown that the muscle fibres in all parts of the heart of the spotted salamander have the same general histological characters (cf. figures 14–21 inc., plate 8), as revealed by various staining methods. Neither in the sinus, nor at the sites of junction of sinus and atria, atria and ventricle, or ventricle and bulbus cordis, is there any muscle which can be considered to be embryonic in type, or to conform to the description of nodal tissue.

In the absence of invocation of any muscle with special histological structure, it becomes necessary to seek other causative factors to account for the delay in the passage of the impulse for cardiac contraction across the sites of junction of the various heart chambers, and for the different intrinsic rhythmic rates of the several parts of the heart. As direct muscular continuity between all segments of the heart has been clearly observed in the present work, and as most of the experimental investigations of other authors indicate that it is the muscular and not the nervous elements which conduct the impulse, we are convinced that it is in the muscle itself that these factors must be sought. It has been noted above that at each of the junctional sites the musculature of the chambers, both proximal and distal to each junction (except the bulbus), is arranged in a basket-work manner, and quite suddenly changes to a circular arrangement at the actual site of junction. The heart muscle forms a continuum, the muscle fibres branching at an acute angle. It thus appears that the wave of contraction, arriving for instance at the S-A junction from the basket-work musculature of the sinus, will have to traverse the circular pathway of the S-A ring on its way to the atrial muscle. Skramlik (1921) maintained that the S-A ring (in the frog and toad) is really in the form of a spiral, with connective tissue between the

muscular whorls. We have observed that there is exceedingly little delicate connective tissue between the muscle fibres of the S-A ring (or the A-V ring) in the salamander heart; no more, in fact, than that between the muscle fibres in any other part of the heart. It is extremely difficult, if not actually impossible, to decide by an examination of serial sections that the fibres of the S-A ring are in the form of a spiral, rather than arranged in a regular circular fashion. Assuming that the impulse passes along the length of the muscle fibres and not across from one muscle bundle to a neighbouring one through the connective tissue separating them, the acute branching of the fibres will have the effect of converting the 'morphological ring' into a 'physiological spiral', so that the wave of contraction will have to traverse a relatively long spiral pathway from the sinus to the atrium. As similar arrangements of the musculature exist at the A-V and V-B junctions, the delay in the passage of the impulse at each junctional site is readily explained in the absence of specialized tissue.

Present hostilities prevented us from obtaining further material for a detailed electrocardiographic study of the origin and course of the wave of cardiac contraction, but by tracing muscular continuity in serial sections, the pathway which the impulse would appear to take is indicated, and an analysis of the cinephotographic records confirms many of these deductions.

The muscular connexion of the ventral wall of the sinus with the right atrium through the S-A ring will conduct the impulse from sinus to right atrium. Thence it will spread to the atrial septum and left atrium. Both atria are in muscular continuity with the ventricle by means of the A-V ring and funnel, the latter being continuous with the ventricular muscle in two ways; firstly by means of the ventricular invagination, which will conduct the impulse to the base of the ventricle, and secondly, by the more extensive continuation into the papillary muscles which join the innermost ventricular trabeculae about midway between apex and base. By this latter connexion the impulse would reach the middle region of the ventricle and spread thence to apex and base. Finally, the muscular ventriculo-bulbar junction would conduct the wave from the central ventricular trabeculae and ventricular base to the bulbus cordis. The circular arrangement of the bulbus musculature probably accounts for the slower rate of spread of the wave along the bulbus. The shortest distance between the S-A and A-V muscular rings is manifestly that along the dorsal wall of the right atrium, from the caudal border of the S-A ring to the dorsal part of the A-V ring. This portion of the atrial wall is presumably the morphological equivalent of the basal wall of the auricle of the eel, which MacWilliam (1885) claimed to have a special histological structure and to form a direct connexion between the sinus and the ventricle, and of the 'band of lighter colour' described by Veil (1917–18) on the back of the frog's heart, stretching from the sinus to the middle of the base of the ventricle, and which she claimed to be the physiological homologue of the mammalian bundle of His. We have not found any histological difference between the musculature of this part of the atrial wall and the general myocardium of the heart of the salamander.

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The different intrinsic rhythmic rates of the cardiac chambers of the salamander, when separated from one another (figure 4), cannot be correlated with gross histological differences between the muscle fibres. The size, i.e. breadth (figure 3), of the fibres varies but slightly; those of the S-A and A-V rings are largest, those of the bulbus slightly smaller, and those of the sinus, atria, A-V funnel, ventricle and V-B junction, while of equal size amongst themselves, are still smaller. Now in the heart of the mammal (dog), Lewis (1925) has expressed as 'The Law of Cardiac Muscle' a correlation between the size (breadth) of a cardiac muscle fibre and its physiological properties; namely, that the fibre size, glycogen content, and rate of conduction increase in the order, nodal, ventricular, atrial, Purkinje, while the length of systole and property of rhythmic contraction (when nourished under natural conditions) diminish in the same order. Blair and Davies (1935), however, observed that while the order of size of the muscle fibres in the adult bovine heart is the same as that given by Lewis for the dog, that of the fibres of the heart of the human (aged 14 years) is different, the thickness of fibre increasing in the order, nodal, atrial, A-V bundle, ventricular. In the salamander the different intrinsic rhythmic rates cannot be correlated with differences of size or structure of the muscle fibres in the several parts of the heart.

With regard to possible differences of a chemical nature, while the present authors have not personally pursued this line of inquiry, the results of other workers on the mammalian heart and our observations on the glycogen content of the various parts of the frog's heart may be significant. The glycogen content has been shown above to increase in the following order in the frog's heart—sinus, atria, ventricle, bulbus; the muscle fibres at the junctional sites are not characterized by a rich glycogen content. The intrinsic rhythmic rates of the several segments of the frog's heart thus decrease in the same order as the glycogen content increases. But a further correlation can be made in that the density of the musculature and the work done increase in the same order as the glycogen content. While this latter correlation suggests that the order of increasing glycogen content is related to the necessity for an increasing potency of energy for the work of contraction in the successive cardiac chambers, whether the relation of the glycogen content to the intrinsic rhythmic rate of a chamber is merely coincidental, or is indicative of a causal relationship, remains to be determined.

In the mammalian heart Demoor and his colleagues (1921 *et seq.*) and Paes (1939) claimed to have extracted from the S-A node of the right atrium a substance which will cause rhythmical contractions in strips of the left atrium, devoid of nodal tissue, which previous to treatment were beating in an irregular, aperiodic manner. They separated this substance into thermostable (sensitizing) and thermolabile (rhythm-producing) components, the latter of which could be successfully replaced by a mixture of acetylcholine and adrenalin. This led Paes to postulate that the normal cardiac rhythm was due to the tonic action of the heart nerves by the constant liberation of acetylcholine (vagus) and adrenalin (sympathetic), together with a chemical substance

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specific to the nodal tissue. Rothberger and Sachs (1939) criticized the work of Demoor, and found that strips of the left atrium of the rabbit and guinea-pig which contain no nodal tissue (the specimens were examined histologically by Aschoff) exhibited spontaneous rhythmical contractions when immersed in nutrient fluid; the rhythm of these strips was slower than that of the S-A node. The absence of nodal tissue in the salamander heart, which exhibits the same phenomenon of intrinsic rhythmicity as the mammalian heart, appears to indicate that nodal tissue is not essential as a source of a substance responsible for rhythmicity alone. Mansfield and Szent-Györgyi (1920) observed that acapnia (deprivation of carbon dioxide by perfusion with alkali) affects first those parts of the heart with greatest automatism, namely the sinus in the frog and toad and the S-A and A-V nodes in the mammal, suggesting some chemical difference between these parts and the rest of the myocardium. While we cannot explain the uneven nuclear reduction of the silver salt in different parts of the salamander heart, it is of interest to note that Drury (1936) and others quoted by him found that nucleic acid derivatives have a depressor action on the cardiac conducting systems of both cold- and warm-blooded vertebrates.

Various factors, chemical, physical and physico-chemical, have from time to time been held by different workers to act as the origin of the heart's 'internal stimulus', and to account for the fact that the heart of either a cold- or warm-blooded animal is able to maintain rhythmical beats for a long time after all nervous, vascular and supporting connexions between the heart and the rest of the animal's body have been severed, provided that the heart is perfused with a liquid containing certain inorganic salts in proper proportions. This automaticity of the heart has been attributed at different times to the following: metabolic products of the activity of the intracardiac nerve cells; metabolic materials elaborated by the heart muscle during the resting period in the cardiac cycle and destroyed during systole; inorganic cations (sodium, potassium, calcium) and their interaction with certain cellular contents of the heart muscle; changes in the hydrogen-ion concentration at interfaces within the cardiac muscle structure; variations in the permeability of the lipoid-containing cell surfaces; rhythmic building up and discharging of a potential difference across semipermeable membranes depending on differences in hydrogen-ion concentration within the cardiac muscle cells and the fluid bathing them; carbon dioxide; oxydation-reduction reactions in the muscle cells; 'nodal extracts'; various heart 'hormones'; and electrical variations in the cardiac muscle fibres. These theories have been reviewed by McDonald and McDonald (1933) and none appears to be entirely satisfactory as the explanation of the automaticity of the heart. Moreover, no satisfactory explanation has been advanced to account for the different intrinsic rhythmic rates of the several cardiac chambers when these are isolated from each other.

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VI. Phylogeny of the conducting systems of the mammal and bird

It is evident from the present investigation that the S-A node, A-V node and A-V bundle of the hearts of mammals cannot be considered as remnants of more extensive tissues of similar structure in a lowly, generalized vertebrate heart like that of the spotted salamander. Further, whilst detailed study of sections of the hearts of the eel, gurnard, frog, tortoise, Mississippi alligator and Sphenodon still await completion, preliminary inspection of such sections has failed to reveal the presence of any muscle with specialized structure in the hearts of these animals. One of the present authors (Davies 1930*a*, 1930*b*) showed that the bird's heart possessed S-A node, A-V node, A-V bundle and terminal ventricular Purkinje ramifications, similar in structure and general topographical disposition to those in the mammalian heart. Presumably these avian structures subserve the same functions as those which have been demonstrated for the similar structures in the mammal, namely the initiation and conduction of the impulse for cardiac contraction. It appears, therefore, that in the hearts of homoiothermal vertebrates (mammals and birds), a new system, composed of muscle fibres of specialized structure, has been evolved, and we would postulate that this neomorphic structural development is to be correlated with the functional requirements of these hearts, namely their more rapid rate of contraction (in proportion to their size) than that of the hearts of poikilothermal vertebrates (fish, Amphibia, reptiles).

Braeunig (1904) observed that the auricular canal, joining the atria to the ventricle, is simple in the fish and, by invagination, becomes more complicated in Amphibia and reptiles, although still without histological specialization, whereas in mammals a new apparatus is developed in the newly formed ventricular septum to effect this connexion—the A-V bundle. Tandler (1912) inclines to the view that the mammalian A-V bundle does not represent the persistence of an ancient A-V connexion, but that it is a new development arising only after a complete ventricular septum has been evolved. As he points out, this interpretation would involve the conclusion that the conducting apparatus of hearts without a ventricular septum would be quite different from that of hearts which possess one, and that the conduction of stimuli in hearts with a ventricular septum would be different in the embryo before the development of the septum (i.e. before the development of the A-V bundle) from what it is later on. Shaner (1930) investigated the ontogenetic development of the conducting system in the heart of the fœtal calf and found that the A-V node, which is the first part of the conducting system to appear, arises behind the dorsal endocardial cushion as an excrescence of the inner layer of the, at this time, complete and unbroken A-V muscular ring which is connecting the atrial and ventricular musculature. From its first appearance the node is continuous with the inner layer of ventricular muscle, to which it takes the impulse in the adult; from the node the A-V bundle grows along the free edge of the ventricular septum. Thus both node and bundle are not 'remnants' of the A-V muscular ring but embryonic specializations. The S-A node develops

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later as a definite structure, appearing at a definite time; it originates and grows like any other embryonic structure and is not a 'remnant' of a more widespread mass of the same tissue in the early embryo.

Such a newly evolved system might be expected to be prone to minor variation, and it is probable that the descriptions of isolated collections of subendocardial Purkinje fibres in the atria of mammals (the site varying with the observer), and the occasional discoveries of direct connexions (of specialized structure) between the S-A and A-V nodes in the mammalian right atrium may be explained in this way. In the avian heart also, variation in this system has been noted. For instance, Adams (1937) found that the right A-V ring of Purkinje fibres, which Davies (1930*a*) described in the hearts of a number of birds as establishing connexions between the atrial and ventricular myocardial components of the muscular right atrio-ventricular valve, was not present in the heart of a yellow-crested penguin (*Megadyptes antipodum*) examined by him; instead, direct myocardial connexions passed across the invaginated epicardial connective tissue between these two muscular components of the valve.

Finally, as between mammal and bird, it may be observed that, size for size, the heart-rate of the bird is faster than that of the mammal, and we may correlate with this the fact that the Purkinje ramifications are more extensive in the avian heart, permeating the thickness of the wall of the atria as well as those of the ventricles, in order that the impulse for cardiac contraction may be propagated rapidly to all parts of the more quickly beating bird's heart. In the mammal, Purkinje fibres have been described penetrating into the walls of the ventricles only (Cardwell and Abrahamson 1931; Abrahamson and Margolin 1936). It is in the avian heart, therefore, that the Purkinje system, although having the same fundamental ground plan as in the mammal, reaches its maximum extent of distribution, and this development is governed by functional requirements as indicated above.

Part of the expenses involved in this work was defrayed by a grant from the Medical Research Council to one of us (F. D.).

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Abbreviations used in the plates and text-figures

A. Atria.
A.S Atrial septum.
A-V.F. Atrio-ventricular funnel.
A-V.G. Atrio-ventricular ganglion.
A-V.R. Atrio-ventricular ring.
A-V.V. Atrio-ventricular valve.
B. Bulbus.
B.G. Bulbus ganglion.
B.W. Bulbus wall.
C.F. Cuvierian fold.
C.P.V. Common pulmonary vein.
D^{A}. Diastole (active phase).
D^{M}. Diastole (maintenance phase).
D.S.W. Dorsal sinus wall.
L.A. Left atrium.
L.D.C. Left duct of Cuvier.
L.F. Left fold of mesocardium.
L.V-B.J. Left ventriculo-bulbar junction.
P.C.V. Post-caval vein.
P.M'. Papillary muscles attached to A-V funnel.
P.M''. Papillary muscles attached to chordae tendineae of A-V valve.
R.A. Right atrium.
R.D.C. Right duct of Cuvier.
R.F. Right fold of mesocardium.
R.V-B.J. Right ventriculo-bulbar junction.
S. Sinus venosus.
S^{A}. Systole (active phase).
S^{M}. Systole (maintenance phase).
S-A.O. Sinu-atrial opening.
S-A.R. Sinu-atrial ring.
S.B. Septum bulbi.
S.G. Sinus ganglion.
S.V. Sinus valve.
S-V.F. Sinu-ventricular fold.
T. Time signal.
T.A. Truncus arteriosus.
V. Ventricle.
V-B.J. Ventriculo-bulbar junction.
V-B.V. Ventriculo-bulbar valve.
V.I. Ventricular invagination.
V.S.W. Ventral sinus wall.
V.T. Ventricular trabeculae.
V.W. Ventricular wall.

Description of plates

(All the figures are untouched photomicrographs)

Plate 7

Figure 10. Sagittal section of salamander heart × 24, showing details of atrio-ventricular junction.

Figure 11. Sagittal section of salamander heart through sinus ganglion × 160.

Figure 12. Sagittal section of salamander heart × 18, showing sinu-atrial junction and relation of common pulmonary vein to sinus.

Figure 13. Frontal section of salamander heart × 18, passing through sinu-atrial and ventriculo-bulbar junctions.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

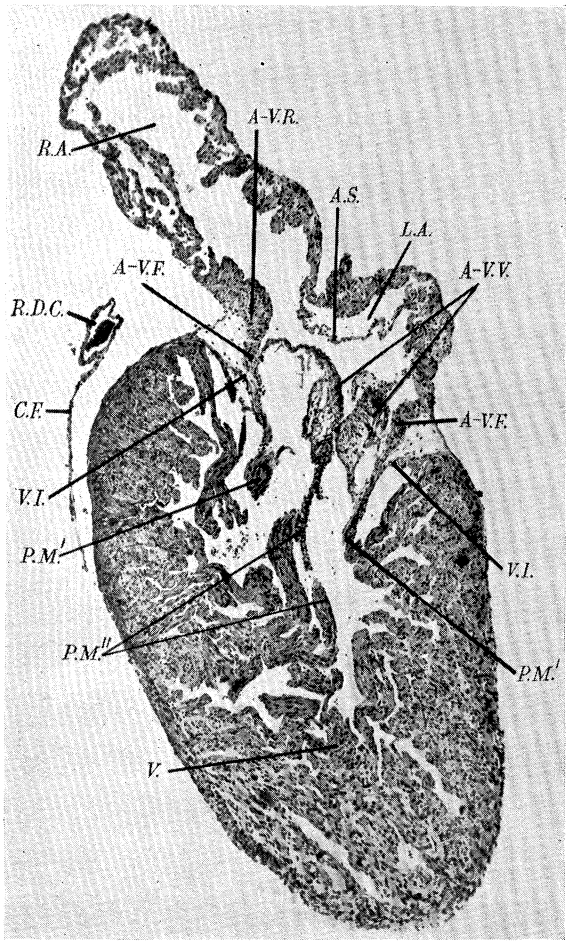

FIGURE 10

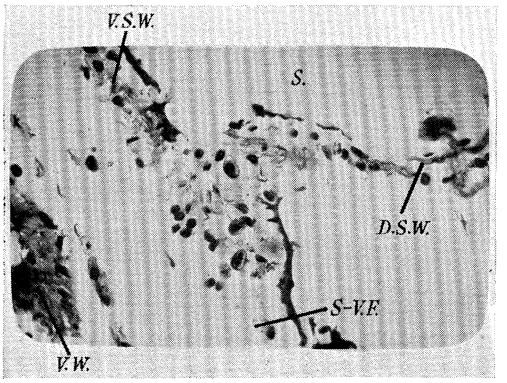

FIGURE 11

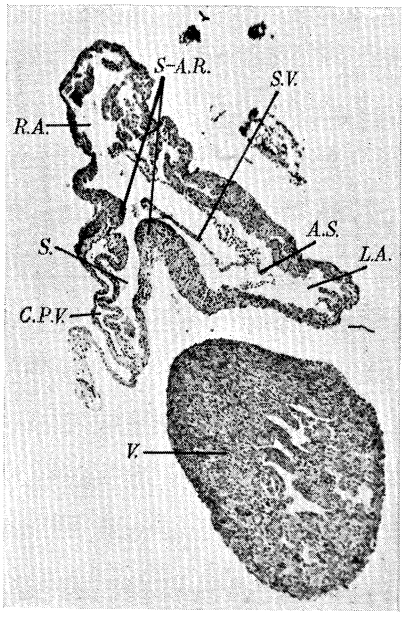

FIGURE 12

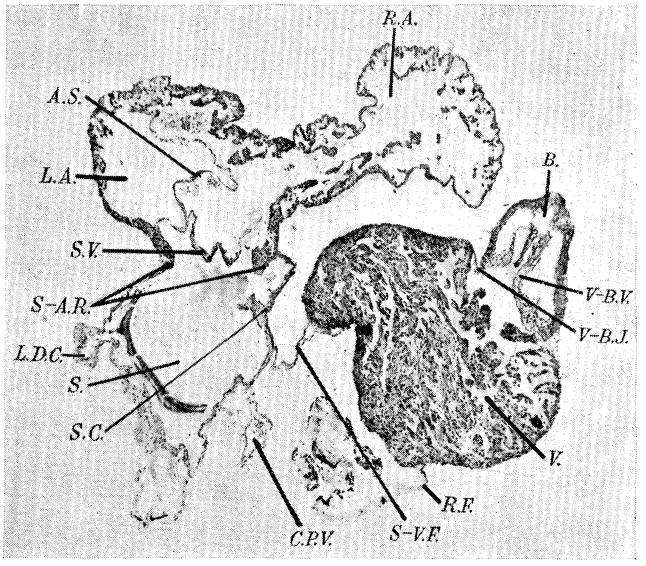

FIGURE 13

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Plate 8

Figure 14. Longitudinal section of muscle fibres of sinus × 800. Bodian protargol method.

Figure 15. Longitudinal section of muscle fibres at sinu-atrial junction × 800. Bodian protargol method.

Figure 16. Longitudinal section of muscle fibres of right atrium × 800. Bodian protargol method.

Figure 17. Longitudinal section of muscle fibres of atrio-ventricular ring × 800. Bodian protargol method.

Figure 18. Longitudinal section of muscle fibres of atrio-ventricular funnel × 800. Bodian protargol method.

Figure 19. Longitudinal section of muscle fibres of ventricle × 800. Bodian protargol method.

Figure 20. Longitudinal section of muscle fibres at ventriculo-bulbar junction × 800. Bodian protargol method.

Figure 21. Longitudinal section of muscle fibres of bulbus × 800. Bodian protargol method.

Figure 22. Sagittal section of salamander heart × 65, showing atrio-ventricular ring cut tangentially.

Figure 23. Frontal section of salamander heart × 65, showing details of ventriculo-bulbar junction.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 14

FIGURE 15

FIGURE 16

FIGURE 17

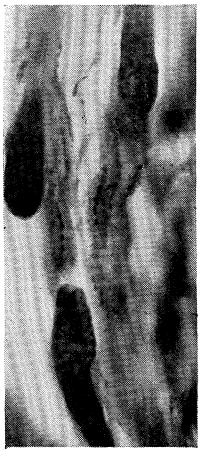

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FIGURE 20

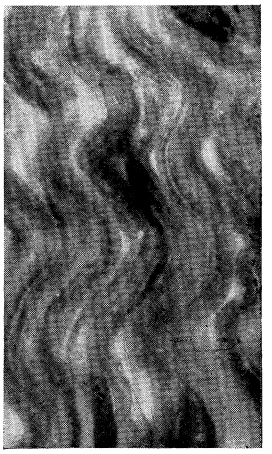
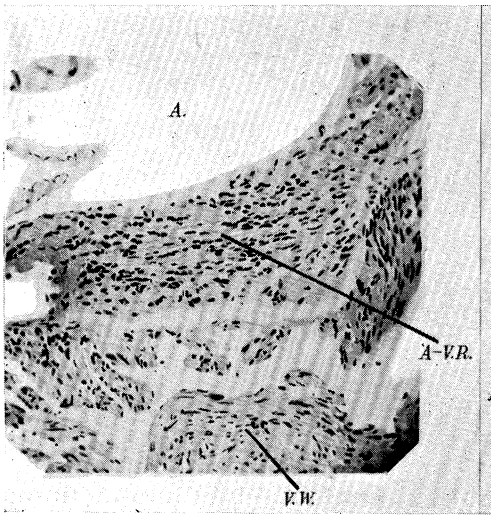


FIGURE 21



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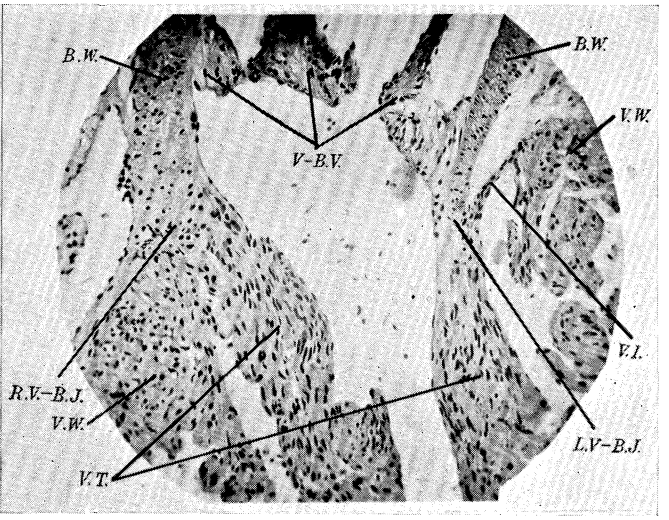


FIGURE 23

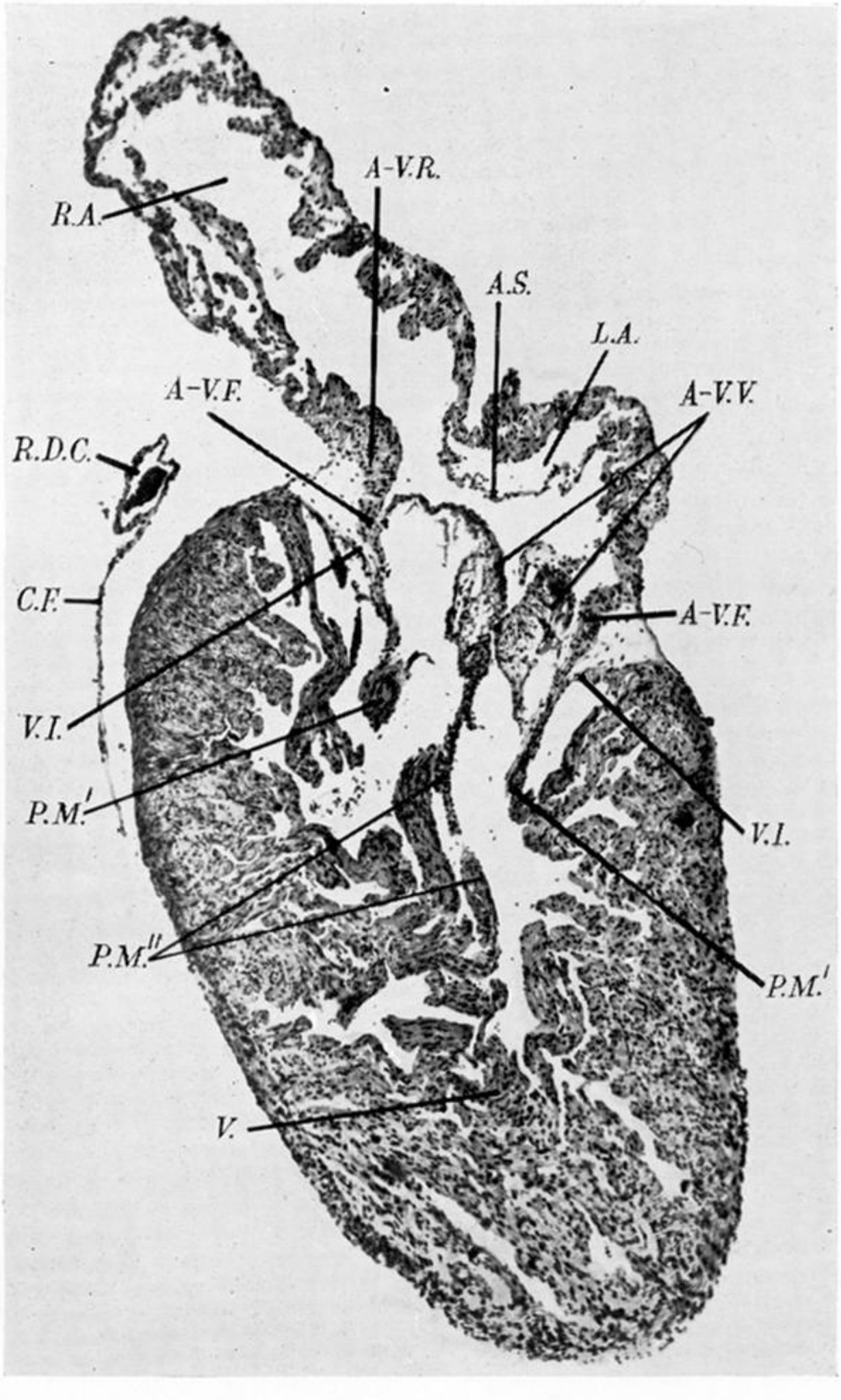


FIGURE 10

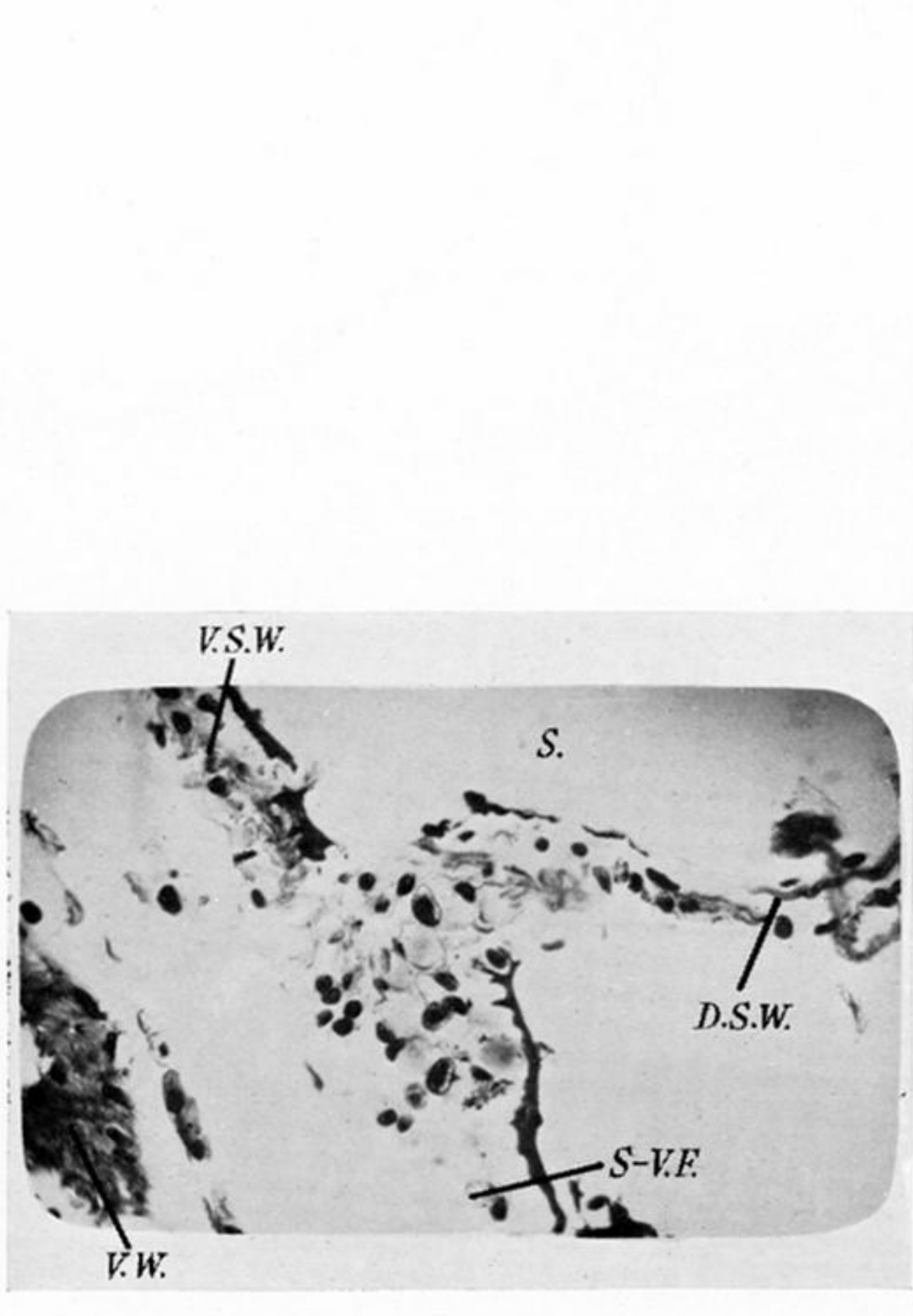
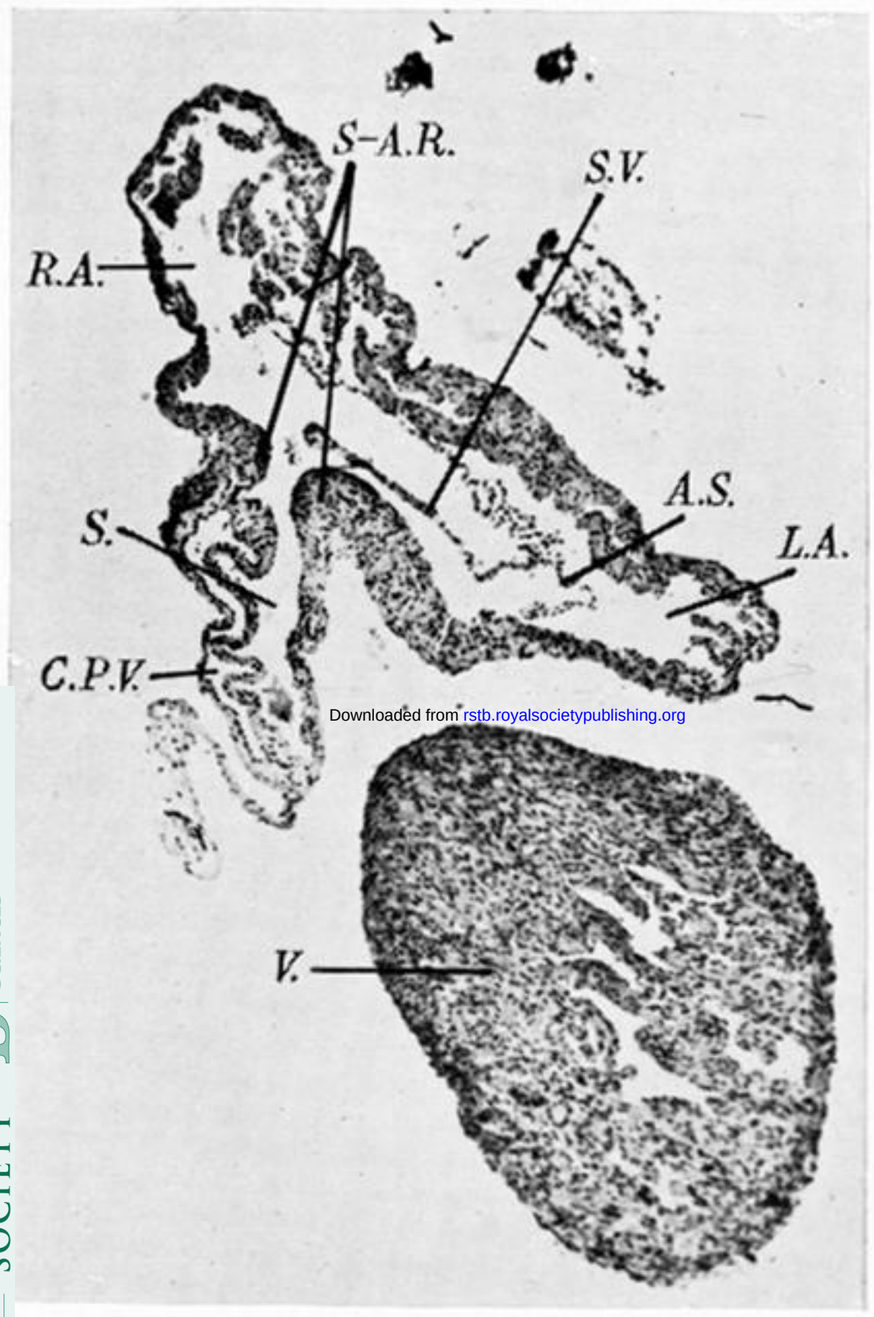


FIGURE 11



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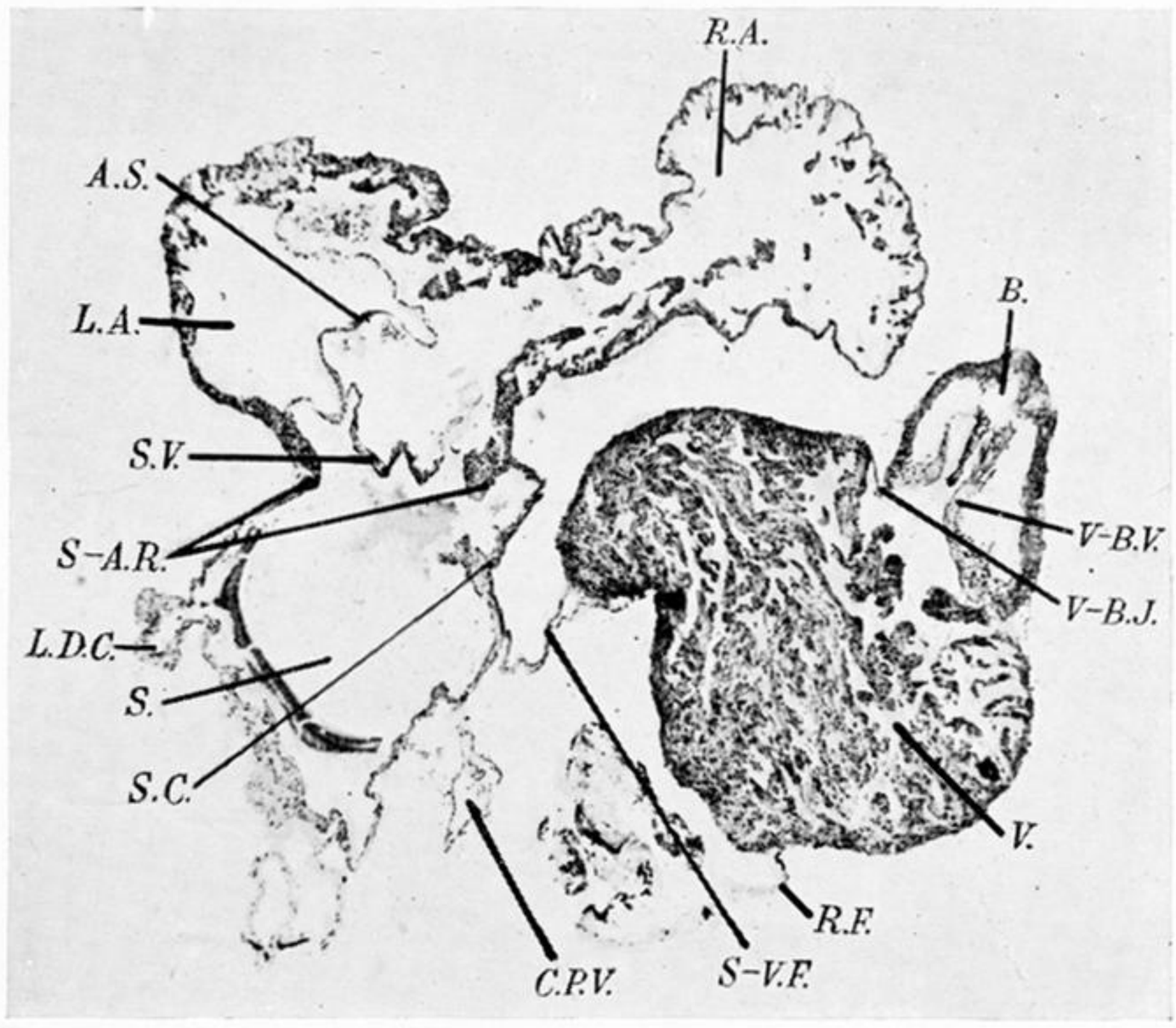

FIGURE 13

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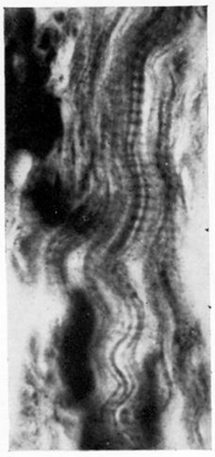

FIGURE 14

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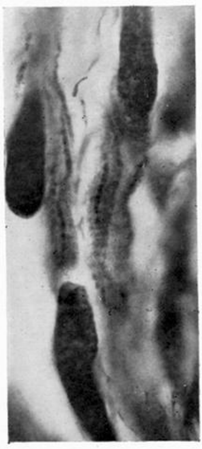
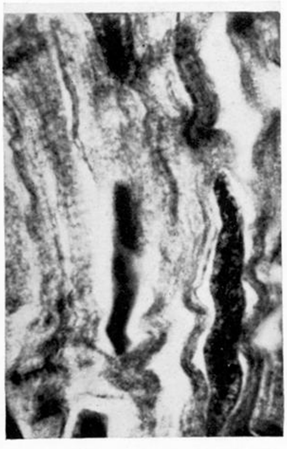


FIGURE 18

FIGURE 19



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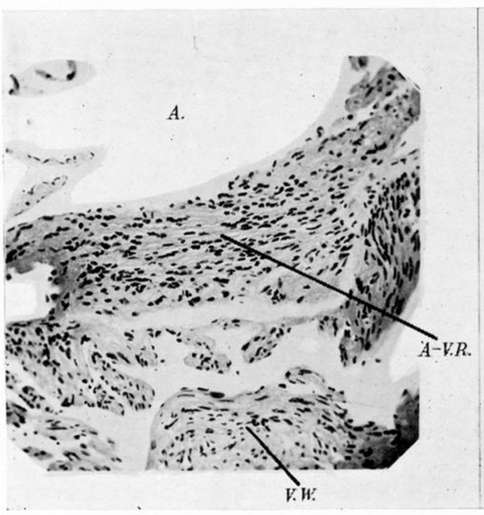

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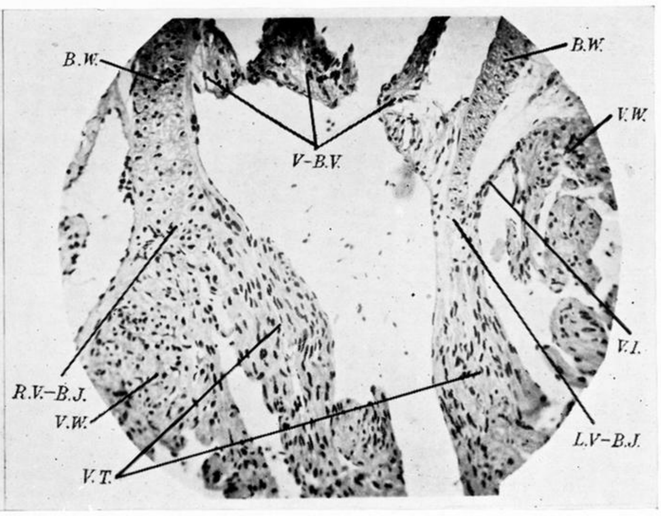

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