THE DEVELOPMENT OF THE SPECIALIZED CONDUCTING TISSUE OF THE HUMAN HEART

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INTRODUCTION

Views which have long been held with regard to the morphological nature of the specialized conducting tissue of the mammalian heart have recently been strongly challenged by Davies (1942). He believes that the system in mammals and birds is a neomorphic development and '...is not a "remnant" of more extensive tissues of similar structure in lower vertebrate hearts'. In support of this thesis Davies cites as collateral evidence the findings of Shaner (1929) in the developing calf heart. According to that author the atrio-ventricular (A.V.) node appears in the calf embryo at the 9 mm. stage as an excrescence on the part of the A.V. ring that lies behind the dorsal endocardial cushion, at a time when the developing musculatures of the atria and ventricles are in unbroken continuity. As it develops the node grows forwards on to the free edge of the inter-ventricular septum, becoming flask-shaped as it does so, and by the 23 mm. stage the pointed end of the flask has lengthened out into the Y-shaped A.V. bundle and its two limbs. It therefore would seem that the A.V. node and bundle are derivatives but not remnants of the original A.V. ring. The sinu-atrial (S.A.) node is not recognizable in the calf embryo till much later, 100 mm., but it too possesses a true individuality, appearing at a definite time and growing and differentiating in a regular manner. It is first seen as a structurally specialized part of the antero-lateral surface of the superior vena cava just above the atrium, but its appearance has been heralded for some time by the gradual accumulation of nerve cells and fibres in the region. As will be seen later Shaner's description corresponds closely to what occurs in human embryos; it differs, however, in some particulars from the account given by Calcagno (1941 *a*, *b*), who states that the A.V. node and bundle arise separately in the bovine embryo and become joined secondarily.

Accounts of the development of the conducting tissue of the human heart are few, accompanying photographic illustrations are fewer still, and only one writer, Sanabria (1936), has described the development of the entire system. But even more than these considerations, what prompted the present study was the desire to establish if possible whether the nodes and bundle appear, grow and develop as do the other organs of the body, or whether they are simply remnants of the junctional tissues found at the S.A. and A.V. rings.

The first attempts to identify the A.V. bundle in the human embryo succeeded only in fairly advanced specimens. Thus Tawara (1906) identified it at 100 mm., Keith & Flack (1906) at 45 mm., Fahr (1907) at 160 mm. and

Monckeberg (1908) at 75 mm. The position of the bundle on the summit of the inter-ventricular septum was explained by Keith & Flack as due to the fact that the upper edge of the septum represents the least disturbed part of the interior of the embryonic heart and the part which during systole is least affected. Histological differentiation of the bundle was described at the 165 mm. stage by Monckeberg, who also noted that in the embryo the limbs of the bundle behave as in the adult, the left branching early and the right remaining unbranched, and that their Purkinje fibres do not appear until quite late.

Amongst the earlier workers in this field Tandler (1912) and Mall (1912) inclined to different opinions with regard to the origin of the human A.V. bundle. According to Mall the bundle is an embryological remnant of the atrial canal of which the posterior musculature, connecting the sinus and the ventricle, never breaks down although showing early in development changes in structure which differentiate it from the rest of the heart muscle. In an embryo of 11 mm. C.R. length Mall was able to identify the bundle which was becoming isolated by the breaking down of the rest of the A.V. ring. He also claimed that in suitable sagittal sections the bundle may be recognized as early as the 8 mm. stage and, indeed, figures it in a specimen measuring only 7 mm.

Tandler, on the other hand, seemed to favour the view that the bundle does not represent the persistence of an ancient A.V. connexion but is a new development, the conclusion which had been reached by Retzer (1908) from his studies of the developing pig. In 10 mm. human embryos in which the atria and ventricles are continuous at the A.V. canal the bundle, according to Tandler, can be recognized as a strip of cells of darker colour on the posterior wall of the canal, and by 19·75 mm. the bundle can be seen on the summit of the inter-ventricular septum and its limbs followed a short way. The problem of the conduction of the impulse from atria to ventricles engaged Tandler's attention, and he pointed out that conduction in hearts without a ventricular septum must be different from that in hearts with one, just as it must differ in the same heart before and after the completion of the septum during development.

Sanabria described histological differentiation in, and a typical reticular arrangement of, the cells of the posterior atrial wall in a 6 mm. human embryo. By the 11 mm. stage he found the muscle of the posterior atrial canal noticeably different from that near it, although the general A.V. muscular continuity was then still unbroken. From its lower end this differentiated muscle gave rise to a line of cells which sank into the dorsal endocardial cushion to reach as far as its ventral free border. So placed, this muscular band, which was clearly the A.V. bundle, capped the upper border of the inter-ventricular septum just where it joined the dorsal cushion. Sanabria did not discuss whether or not he regarded the bundle as a remnant of the original A.V. connexion, but from his description it would appear that while the A.V. node is a part of the original muscular connexion between atrium and ventricle which undergoes exceedingly early differentiation, the bundle, on the other hand, is a new formation derived from this tissue by active proliferation into the dorsal endocardial cushion.

PRESENT WORK

Material used in the present investigation was as follows:

A. Complete serial sections of the following embryos:

No. 162. Cardiff Collection, C.R. length 8 mm.
No. 161. Glasgow Collection, C.R. length 8 mm.
No. H. 33. London Hospital Collection, C.R. length 10 mm.
No. H. 23. London Hospital Collection, C.R. length 13 mm.
No. 129. Cardiff Collection, C.R. length 16·5 mm.
No. 44. Cardiff Collection, C.R. length 18·8 mm.
No. 110. Cardiff Collection, C.R. length 22 mm.
No. 114. Cardiff Collection, C.R. length 25 mm.
No. 7. Cardiff Collection, C.R. length 88 mm.

B. Complete serial sections of the hearts from embryos of 40, 60 and 78 mm. C.R. length.

C. Interrupted serial sections of the hearts from foetuses of 105, 123 and 150 mm. C.R. length.

D. Interrupted serial sections of blocks from still-birth hearts.

All the material was cut at 10μ except embryo H. 33 which was cut at 8μ and embryo no. 7 which was embedded in celloidin and sectioned at 100μ. Embryo 110, the hearts from the 105, 123 and 150 mm. foetuses, and some of the still-birth material were impregnated with silver before cutting. All other sections were stained with haematoxylin and eosin.

To facilitate precise orientation of the specialized conducting tissue wax-plate reconstructions were made of the hearts of the 16·5 and 60 mm. embryos.

The main planes of section of the different hearts examined will be indicated as they are described, but it must be stressed that complete series of true transverse, sagittal or coronal sections of developing hearts cannot be obtained as the curvature of the embryo and of the heart itself cause the plane of section to change as cutting proceeds.

For convenience the usual terms will be used in describing the endocardial cushions, but it is felt, nevertheless, that anterior and posterior as usually employed are unsatisfactory, and that there is some justification for Frazer (1931) reversing the customary usage.

OBSERVATIONS

8 *mm.*

Two embryos of this size, each cut transversely, were studied. In one no specialized tissue could be found in the heart but in the other, no. 161, sections 19.1.9 to 19.2.7, the tissue which will become the definitive A.V. node and bundle can be identified. At this stage there is complete continuity between the atrial and ventricular primitive musculatures, and at one point, on the right side of the posterior wall of the common atrium at its junction with the ventricle, a localized proliferation of tissue can be seen budding off. As it grows forward it passes under the dorsal endocardial cushion, still not fused with the ventral, to reach the summit of the inter-ventricular septum which has by now succeeded in reaching the dorsal cushion in this position. The appearance presented is unquestionably that of an actively growing structure (Pl. 2, figs. 1, 2). Even at this early stage it is possible to recognize differences between the node and the bundle. The node is less compact than the bundle, which, in addition, stands out by reason of the very great density with which the nuclei of its cells stain. In cross-section the bundle presents a somewhat oval appearance and, as observed by Mall in sagittal sections of an embryo of similar size, is already becoming separated off although the spaces which surround it are as yet small. The bundle can be followed for a short distance down the left side of the septum.

In Sanabria's description of the posterior musculature of the A.V. canal in the 6 mm. human embryo it is stated that the fibres there show less advanced fibrillary differentiation and are paler than their neighbours. These features were not noted by Mall in 8 mm. embryos, nor were they observed in embryos of that size in the present study.

It should be clearly understood that in order to be able to identify the A.V. bundle at such an early stage as 8 mm. experience of its appearance in older embryos is essential, and although the present description necessarily proceeds from the younger to the older stages the investigation was carried out in the reverse way.

10 *mm.*

The plane of section of this embryo is transverse, but although the upper parts of the atria are cut in this plane the greater part of the heart has been cut in vertical section. By now, although the developing musculatures of the atria and ventricles are still largely in unbroken continuity, in places signs of commencing breakdown are evident. Pl. 2, figs. 3 and 4, show one such place, where on the left side only a slender strand of muscle connects the common atrium with the ventricle. These sections illustrate also that, as pointed out by Mall, the breakdown in muscular continuity begins about the time of appearance of the lateral endocardial cushions. On the posterior wall of the A.V. canal but towards its right side, there is a clearly defined, almost circumscribed, body of cells, which when traced serially can be recognized as the A.V. node from which the bundle is now very obviously arising (Pl. 2, fig. 3), and passing upwards into the base of the dorsal endocardial cushion. The node is in cellular continuity with the wall of the atrium but fibrous tissue, the future trigonum fibrosum dextrum, is filling in the A.V. sulcus and separating it from the ventricle. Tissue spaces around the developing bundle are now conspicuous, and these, together with its large deeply staining nuclei, make identification certain. There can be no question that whereas the A.V. node represents a part of the original A.V. canal destined to become structurally specialized *the bundle* *is a new formation which arises from that primitive nodal tissue by a process of active growth.*

The point where the nodal tissue joins the musculature of the right atrium is exactly the place at which the lower end of the right venous valve is attached (Pl. 2, fig. 3), so that in effect the A.V. node and right venous valve are at this stage continuous with one another. This brings to mind the connexion described by Tudor Jones (1932) between the S.A. and A.V. nodes in a 24 mm. human embryo by a tissue strand within the right venous valve.

When the bundle is traced farther it is found to run down the left side of the inter-ventricular septum for a short distance, but as yet no right limb is recognizable (Pl. 2, fig. 4). This agrees with Mall's statement that in the early stages of its development the bundle is related more to the left ventricle than to the right. Neither in the ordinary myocardial cells nor in those of the differentiating conducting tissue are cell outlines easily discernible. Some cells in the left limb of the bundle show vacuole formation (Pl. 3, fig. 5), a most interesting feature suggesting as it does extreme precocity in the histogenesis of Purkinje fibres. Incidentally, when Purkinje fibres do appear they do so first in the upper part of this limb.

The sinu-atrial node. At the S.A. junction at the 10 mm. stage there is a round clump of cells more closely packed together than those of the neighbouring tissue (Pl. 3, fig. 6). The compactness of the cell group and its position rather than any specific histological character of its individual cells suggest it as the anlage of the future pacemaker, although their nuclei do show a slightly stronger chromatic reaction than those of adjacent cells. Traced caudally this tissue can be found in the upper parts of the bases of both venous valves.

In older embryos there develop in close association with the S.A. node numerous ganglion cells from which nerve fibres pass into it, and in addition the artery which traverses the node, and which forms such a conspicuous feature in the adult, serves as a means of identification. But the special histological characters of the nodal fibres are not recognizable till birth. It is, maybe, as well to emphasize that these do not at any time constitute anything very striking, and in fact several investigators deny any structural specificity even of the adult node, but they are, nevertheless, real enough, e.g. smaller breadth compared with that of ordinary atrial fibres and, in the writer's opinion, less marked cross-striation.

Sanabria (1936) also described a localized thickening of the heart wall at the junction of the superior vena cava and the right atrium in an embryo of 11 mm., composed of fibres noticeably more compressed and paler than those of the contiguous muscle.

Examination of older embryos confirms that these aggregations of cells noticed at 10 and 11 mm. do in fact represent the S.A. node. It might be thought that, while the specialized conducting tissue can be recognized as early as 8 mm., its development is then in such a primitive state as to preclude any possibility of functional activity; however, there are solid grounds for the belief that thus early (8 mm. = approx. 38 days) the system is functioning as in the adult. This point will be discussed later under 'Foetal electrocardiography'.

13 *mm.*

Main plane of section transverse. The A.V. bundle is now very easily identified. Not only has it developed into a denser and more 'rounded-off' structure, but the tissue spaces around it have enlarged so that in places it seems quite isolated (Pl. 4, figs. 10, 12). If these figures are compared with figs. 24–28 of Mall's paper it will be appreciated that in the embryo here described, H. 23, the bundle has by 13 mm. reached a degree of differentiation equal to if not in advance of that reached by Mall's 21 mm. specimen. The bundle now bears its definitive relationship to the septal cusp of the tricuspid valve. The left limb of the bundle is now of considerable size, and fibres from it can be followed into the developing trabecular tendons, sometimes called false tendons, which bridge the ventricular cavity (Pl. 4, fig. 10). For the first time it is now possible to identify the right limb of the bundle; it is represented by one or two strands of cells which descend from the main bundle along the right side of the upper part of the inter-ventricular septum (Pl. 4, fig. 11).

The sinu-atrial node. Within the basal attachment of the fused venous valves below the opening of the sinus venosus there is a kidney-shaped mass of cells which forms the entire thickness of the heart wall between epicardium and endocardium. This is the future posterior horn of the S.A. node (Pl. 4, fig. 9). Traced upwards it just fails to become continuous with a similar cell clump situated within the upper part of the base of the right venous valve. In this specimen, therefore, the S.A. node is developing in two parts, an upper and a lower, which represent respectively the anterior and posterior horns of the adult node. There is still no artery traversing the node, but its cells stand out by reason of their greater affinity for eosin. The caudal end of the node extends along the right venous valve for a short distance as the valve passes to be attached to the dorsal endocardial cushion; farther down the heart the A.V. node is continuous with the attachment of the right valve to the dorsal cushion, but no direct connexion between the two nodes at this or any other stage has been found.

16·5 *mm.*

Main plane of section transverse. The A.V. node and bundle have now reached a considerable size and in many respects possess the topographical relationships of the fully developed structures. The bundle is very sharply defined (Pl. 1). It describes an arch upwards and slightly forwards from the node to the upper part of the inter-ventricular septum at its junction with the dorsal endocardial cushion. The point of junction is over towards the right side of the cushion, now really the fused endocardial cushions, an important point in the development of the pars membranacea septi and one which is well shown in the figure. The bundle then passes along the upper margin of the septum below the still largely patent inter-ventricular foramen, a position in which it has been successfully dissected in congenitally malformed hearts by Keith (1909). Differences in the conformation of the two limbs of the bundle are now apparent. In the 16·5 mm. embryo examined the left limb arises as a series of strands which begin to come off the main bundle some time before the right limb which is now more clearly defined and in effect the direct continuation of the bundle. This manner of division of the bundle corresponds to type C of the adult (Walls, 1945) and was the type most frequently met with in the series of embryos studied. Another point of difference between the limbs now is their relation to the endocardium. From its commencement the left limb is immediately subendocardial, whereas the right limb passes downwards for a short distance through the substance of the inter-ventricular septum before reaching its inner surface. According to Matsuda (1936) the course of the right limb in the foetus varies greatly, and he figures three possible courses which it may take, all of which are in part at least within the muscle of the septum. His findings, together with those of Sanabria (1936) and those of the present investigation, explain why it is that, in the adult, dissection of the right limb of the A.V. bundle varies so greatly in its ease of accomplishment. Clearly if the bundle maintains a position largely within the muscular septum then its exposure must necessarily be exceedingly difficult if not impossible.

In the bundle cell outlines are becoming clearer, and some of the fibres of the lower part of the right limb are noticeably larger than those of the adjacent myocardium and contain large sausage-shaped nuclei. Rondolini (1937) stated that the cells of the system can now be recognized by their larger volume and more swollen appearance, but these characters were only noted at 16·5 mm. by the present writer in some of the cells of the right limb of the bundle as mentioned above. There is still no evidence of a connective tissue sheath around the conducting tissue.

The A.V. node has its fibres arranged in a loose open meshwork which is in continuity with the right atrium and with the bundle, the latter providing its only connexion with the ventricle.

In places the muscular continuity between the atria and ventricles has not yet been cut through by the invading fibrous tissue of the A.V. sulcus, and in Pl. 1 the left atrium and left ventricle can be seen still joined by a persisting part of the old A.V. canal. Muscular slips of this kind may well have been what Kent (1893) described as the first and third of his three connexions between the atria and ventricles of the mammalian heart. In most cases, of course, these muscle slips disappear, leaving the A.V. bundle as the sole connexion between atria and ventricles, but there is reason to believe that occasionally they do persist and give rise to phenomena which can be recognized clinically (Wolff, Parkinson & White, 1930; Ohnell, 1946).

The sinu-atrial node. The further development of the S.A. node is witnessed by the appearance of a small nodal artery within the tissue of the base of the right venous valve. Moreover, there can now be seen for the first time in any part of the heart primitive nerve cells. These occur on the epicardial aspect of the posterior surface of the common atrium, partly about halfway down this surface from where later on they can migrate into the inter-atrial septum and so supply the A.V. node, but mainly in relation to the S.A. junction. These cells do not as yet have clear-cut cell borders, possibly due to delay in fixing the embryo, their cytoplasm forming a bright pink syncytium in which are set nuclei possessing that perfect roundness so characteristic of nerve cells. The source of the intrinsic (vagal, parasympathetic) cardiac nerve cells is still in doubt, opinion being divided between a sympathetic and a vagal origin. For this reason the closest attention was given to this question, but unfortunately without shedding further light upon it. It would seem that for problems of this kind only the experimental method can provide the answer, and such work carried out by Jones (1942) indicates strongly a vagal origin for these cells.

18·8 *mm.*

This embryo was the only member of the series examined which was cut sagittally, and although in the writer's opinion this plane of section is not so generally useful for a study of the cardiac conducting tissue as transverse or coronal, some valuable information was obtained.

In Pl. 3, fig. 7, the S.A. node can be identified within the substance of the crista terminalis at the bottom of the sulcus separating the superior vena cava from the right atrium. It is placed towards the sinus side of the crista, the position which it occupies in the adult (Blair & Davies, 1935). The appearance presented by the sinus, atrium and ventricle lying in series in sagittal section in an embryo of this size is very reminiscent of an adult lowly vertebrate heart, and makes it easy to understand why the S.A. and A.V. nodes should have been accepted by earlier workers as the remnants of primitive junctional tissue. The first to object to this conception was Stiénon (1926), who described the S.A. node as a new growth that is properly located at the junction of the common cardinal vein and the sinus venosus and not as remnant tissue left over from a mass of similar character and more widespread distribution. This description has been confirmed. The position of the A.V. node close to the opening of the coronary sinus is well shown in this embryo, but the bundle is very difficult to find and follow as in no part of its course is it cut through its full thickness. In the sections in which it can be seen it is surrounded by a delicate connective tissue sheath. This investment never really increases during subsequent development, the explanation no doubt of the universal failure to reveal the conducting system by injection in the human heart as in the ox. The cells of the bundle as it passes between the fibrous tissue of the A.V. sulcus and the dorsal cushion are closely packed together and appear somewhat narrower than those of the unspecialized myocardium. On reaching the muscular septum the bundle swells out a little and its cells become broader, but none show any of the characters of Purkinje fibres.

22 and 25 mm.

New features noted in these transversely cut embryos are these: The right limb of the bundle can now be traced into the moderator band, and nerve cells are definitely present on the posterior surface of the atria at 25 mm. It is interesting to note that the silver impregnation method by which the 22 mm. specimen was prepared failed completely to show any nerve elements in the heart. His, Jr. (1893), stated that nerves arrived at the sinus region about the 12·5 mm. stage, but Mall (1912) was unable to find them in that situation till 21 mm. No doubt there is a fair range of variation in the development of embryos, although possibly the variation exists more in the measurement of their specimens by different observers. Stiénon (1926) reported a 22·4 mm. embryo as the youngest showing ganglion cells in the region of the sinus or atria and asserted that their appearance preceded that of the S.A. node. The same author did describe, however, in embryos of 6·4–15 mm. C.R. length, differences in the character of the muscle of the superior vena cava...greater density, increase in numbers of nuclei, etc....similar to those accepted by later writers as indicating the presence of the nodal anlage.

The A.V. node at 25 mm. (Pl. 3, fig. 8) forms a large mass of loosely arranged muscle fibres in the interstices of which capillary blood vessels are becoming numerous. The nodal fibres are thinner than those of the adjacent myocardium and are without a demonstrable nerve supply.

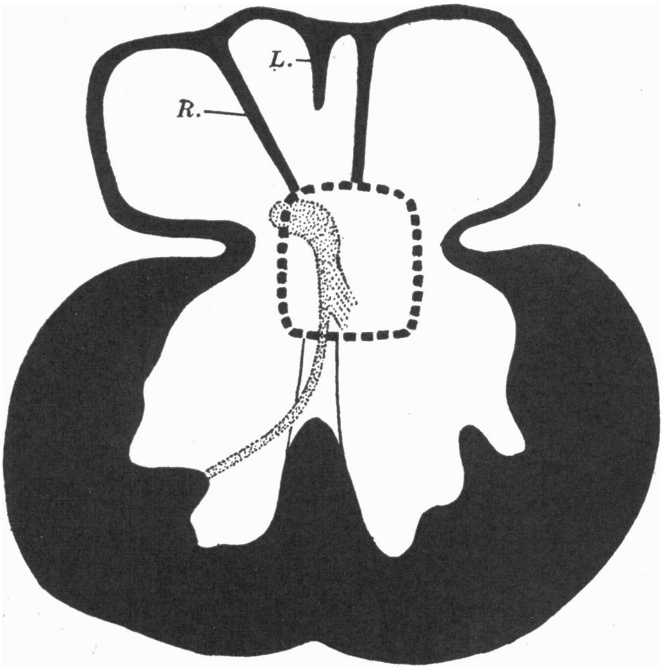
40 *mm.*

The plane of section is transverse, but much of the ventricles appears to have been cut coronally.

The S.A. node has now established itself in its adult form, i.e. an open horseshoe in front of the opening of the superior vena cava into the right atrium. Although in the 13 mm. embryo examined there was some evidence that the node consisted of two separate parts, it is not possible to agree with Segre (1926) who stated that only after birth do the anterior and posterior horns of the node become joined together. At 40 mm. the part of the node which lies in the crista terminalis and which connects its two horns is definitely present. It is shown in Pl. 5, figs. 13 and 14, together with the nodal artery here cut along its length.

Nor is it possible on physiological grounds to accept Segre's findings. The circumstances of his investigation were these: A patient with mitral stenosis gave an electrocardiogram in which a double auricular wave existed. This suggested the presence of two S.A. nodes, one for the right and one for the left atrium. From his examination of the hearts of two full-time foetuses, one newborn child and one young male adult, all cut at 30 μ, Segre decided that the node did in fact develop in two parts and that the case in question was an instance of the persistence of the node in two parts. Now if he were correct then electrocardiograms of foetal hearts should exhibit the phenomenon displayed by the case of presumed double node. But they do not: human foetal heart tracings are just like those of the adult which, even allowing for the small size of the heart, would be hardly likely if the S.A. node were in two parts.

Numerous nerve fibres derived from groups of cells in the sinus walls are now entering the node, but at no time are nerve cells to be found within the node. Tudor Jones (1932) described a communication between the caudal end of the S.A. node and the right phrenic nerve in a 24 mm. human embryo: in a few sections of the 40 mm. embryo here described the phrenic nerve is adherent to the surface of the lower part of the node, but no fibres from it can be seen entering the node either in this specimen or in any of the others examined. For the most part, as shown in Pl. 5, fig. 13, the nerve lies well clear of the heart wall.



Text-fig. 1: Diagram to show the position of the A.V. node and bundle in the developing heart. The interrupted line represents the dorsal endocardial cushion. *R* and *L*, right and left venous valves.

The atrio-ventricular node and bundle. Pl. 5, figs. 15 and 16, show the bundle before and at division as well as many features of general interest in the development of the heart. As the bundle runs forward on the summit of the fleshy inter-ventricular septum it now bears its close and important relation to the mitral and tricuspid valves. It is relatively huge at this stage, being in section fully a third of the thickness of the septum. Its fibres and those of the limbs show well-developed myofibrillae (Pl. 5, fig. 17) but no cross-striations;

as yet they are unaccompanied by nerve fibres. The arrival of nerve fibres in the sinus wall was suspected by Mall to hasten the histological differentiation of the conducting fibres, and the present study confirms this suspicion. The appearance of Purkinje fibres in the limbs of the bundle occurs simultaneously with that of nerve fibres at about 100 mm. The A.V. node has quite a rich nerve supply by the 40 mm. stage, thick leashes of unmyelinated nerve fibres running forwards into it from the atrial septum (Pl. 5, fig. 18).

Since subsequent to 40 mm. the disposition of the specialized conducting tissue remains unaltered a brief recapitulation at this point may prove of value: The A.V. node represents part of the original A.V. canal musculature which becomes structurally differentiated early, and from it the A.V. bundle sprouts forwards into the base of the dorsal endocardial cushion. By continued new growth the bundle reaches the attachment of the inter-ventricular septum to the fused cushions and there divides, the type of division varying, but in most cases being that in which the left limb arises as a series of strands.

105 *mm.*

In vertical section the bundle is now seen to occupy a groove in the upper border of the fleshy septum, just below the pars membranacea septi, immediately beneath the septal attachment of the tricuspid valve (Pl. 6, fig. 19). Both the bundle fibres and those of the myocardium now show cross-striation, but with silver impregnation the bundle fibres are much more lightly coloured and distinguishable with ease. Nerve fibres can be seen running within the bundle and the upper parts of both limbs; they are few in number and seem non-myelinated, although with silver it is always difficult to be dogmatic on this point. In any event they are very fine fibres which show slight beading along their length and end as delicate threads on the surface of the conducting muscle fibres. In the upper part of the left limb, but nowhere else, a few Purkinje fibres have appeared, this being the youngest embryo in which these fibres were observed. Sanabria (1936) described Purkinje fibres in the bundle and upper part of the left limb in an embryo of 60 mm.

123 *mm.*

A notable feature of this embryo is the marked advance in the development of the cardiac ganglia in relation particularly to the sinus part of the atria. At no time before birth are there two cell types in these ganglia as in the adult human heart (Blair & Davies, 1935). The single type which occurs in the foetus corresponds generally with type 1 of these authors, and although the cells are rather darker this feature may well be due to slight differences in preparation.

150 *mm.*

The A.V. node has now a rich blood and nerve supply. Connective tissue fills the spaces between its freely branching striated fibres, the whole structure being somewhat more closely knit than hitherto. Purkinje fibres are now plentiful in both limbs of the bundle (Pl. 6, figs. 22, 23). Many of these fibres possess all the characters of the large bloated 'typical' Purkinje fibres of the ox or sheep, i.e. they are subendocardial in position, they are on the average about twice the breadth of adjacent ventricular fibres, 11μ compared with 5μ; they exhibit cross-striation only at their periphery, the myofibrillae being limited to the cortex of each fibre; some of the fibres show vacuolation and, while the majority have a single large round or oval centrally placed and (with silver) pale nucleus, a number have two nuclei. In none of the Purkinje fibres could peripheral multiple nucleation as described in the ox by Blair & Davies (1935) be observed. In general the fibres of the right limb seem smaller than those of the left, a feature which has been remarked upon by others also, but this may be due to the difference in direction of the two limbs with the consequent effect upon the planes of section of their constituent fibres. It must be pointed out that although figures have been quoted above of fibre diameters the precautions stated by Ashley (1945) to be necessary for complete accuracy in measurement could not in the nature of the investigation be taken. No Purkinje fibres occur elsewhere than in the subendocardium. Many of the fibres in the limbs of the bundle are similar in appearance to ordinary unspecialized myocardial fibres: in places these small limb fibres swell out into large Purkinje fibres (Pl. 6, fig. 22), and it would seem reasonable to conclude that Purkinje fibres are simply bundle fibres which have proceeded a step further in structural specialization. Nothing whatever has been observed in the present study to suggest that Purkinje fibres are embryonic fibres at an arrested stage of development as has been stated by Retzer (1920). At no time during their differentiation do myocardial fibres not belonging to the conducting system even remotely resemble Purkinje fibres. It is interesting to note that Erlanger (1909) found that the A.V. bundle possessed no powers of regeneration after severance, a finding which lends support to the conception of these fibres as highly specialized elements. Cady (1921), Tufts (1922) and Nonidez (1943) all agree with the present observations.

Still-born hearts

Only now do the fibres of the S.A. node possess the full characters by which they are usually identified, i.e. they are, with silver impregnation particularly, definitely cross-striated although not so strongly as the neighbouring atrial fibres; they are spindle shaped and of smaller breadth than atrial fibres and are arranged circumferentially around the nodal artery (Pl. 6, fig. 24). Groups of ganglion cells are numerous on the surface of the node and its nerve supply at birth is rich. The A.V. bundle is still relatively very large and the A.V. node has increased in vascularity, but no other features call for comment.

Foetal electrocardiography

Bell (1938) in this country and Ward & Kennedy (1942) in America succeeded in obtaining electrocardiograph tracings of the foetal heart using leads from the maternal abdominal wall. With great kindness Drs Ward & Kennedy sent the writer a large series of their original recordings covering the last 24 weeks of term, from which it was hoped correlation might be made between the anatomical development of the conducting system of the heart and its degree of physiological activity. This, however, is not yet possible owing to technical difficulties such as the smallness of the deflexion caused by the foetal heart recorded in this way. Tracings which can be analysed, however, have been taken from foetuses removed by hysterotomy by Heard, Burkley & Schaefer (1936) and by Marcel & Exchaquet (1938). It is difficult to regard records so obtained as physiological, particularly as the heart rates are definitely less than those of foetuses *in utero*, but until improved technique allows of earlier and better intra-uterine recordings these must suffice. The authors mentioned above used direct chest leads and obtained success from very young specimens indeed. Heard *et al.* (who unfortunately did not reproduce their earliest tracings) reported their youngest as being of 9½ weeks' gestation, but the ages of Marcel & Exchaquet's specimens appear to have been assessed by a method other than that commonly used. However, from the data given it seems that an adult-type electrocardiograph tracing was obtained from an embryo of between 6 and 7 weeks' gestation and of about 16 mm. C.R. length. This is a most remarkable thing, for although the bundle and both nodes are then recognizable their differentiation is far from complete and they are quite without a nerve supply.

The conduction time throughout intra-uterine life is approximately the same as in the adult: allowing for the difference in heart size it follows that the excitation wave must at first be very much slower than in the adult, but that with increase in heart size its speed will gradually approach the adult value. Consequently Purkinje fibres begin to appear in the limbs of the bundle in order that its passage may be facilitated. The extent to which the specialized conducting tissue is a neuromuscular mechanism is hard to say, but it is probable that, although functioning early on independent of nervous control, for the proper fulfilment of its purpose an adequate nerve supply is required.

An adult-type tracing from an embryo of 16 mm. is interesting for another reason; at that stage of development, as has been noted, the A.V. bundle is not the only connexion between the atria and ventricles, and it might be expected that a tracing from such a heart would show a double ventricular wave or similar abnormality. That it does not do so is due to the very small size of the heart in which the time difference in the arrival of separate impulses to the ventricle is infinitesimal and not recordable.

DISCUSSION

It seems clear from the evidence available that structural differentiation of the specialized conducting tissue of the human heart commences about the 8 mm. stage, the A.V. node and bundle being formed in advance of the S.A. node, and that the time of appearance of nerve elements in relation to this tissue, although variously reported on, may be said to be approximately 20 mm. Now how does this fit in with our present knowledge of the early functional development of the vertebrate heart?

Goss (1942), in rat embryos, observed the initiation of contraction occurring in embryos of three somites in which the heart consisted of two lateral rudiments. The first activity was the feeble twitching of a few cells of the left heart just to the ventricular side of the A.V. junction. The contractions had a regular rhythm of 34–42/min. and were followed in 2 hr. time by similar activity of the same part of the right heart, the 'beats' of which were independent of and slower than those of the left. By five somites the ventricle was single and contracting as a unit. Towards the end of the six-somite stage a few cells on the atrial side began to contract 0·1–0·2 sec. before the ventricle and the atrium became the pacemaker with a consequent increase in rate. At eight somites the circulation began, the co-ordination of the whole heart being made possible by the establishment of the A.V. interval which appeared along with atrial contraction and inhibited the spontaneous ventricular rhythm.

These results agree with Copenhaver's (1939) in *Amblystoma* and with those of Patten & Kramer (1933) in the chick. The latter authors especially emphasize that the change of pacemaker occurs before any neuroblasts approach the heart and before even the 'suggestion' of a specialized conducting system.

The work of Davis (1927) on the development of the human heart up to the twenty-somite stage has shown that the heart tube is formed by a progressive fusion of paired primordia as in other forms, so that in all probability development of contractility is as just described. Since in the amphibian, avian and mammalian hearts, at a time when no specialized tissue has yet developed, contraction proceeds from the venous to the arterial end with, as in the adult, an A.V. interval to ensure the co-ordination of the heart, why does the specialized tissue develop at all in birds and mammals? The reason appears to be this: Whereas the slow rate of contraction (30–40/min.) of the very young heart will persist throughout life in the Amphibia, in birds and mammals very rapid rates may develop (for example, when first detected clinically the human foetal heart rate is about 160/min.), necessitating a mechanism capable of immediately propagating the impulse throughout the heart. The cardiac conducting tissue provides this mechanism, and *pari passu* with its appearance the atria and ventricles become separated by fibrous tissue until the A.V. bundle is left as their sole connexion, thus ensuring a single rapid impulse being carried to the ventricles which therefore contract together. The speed of conduction is important, and Lewis (1925) has stated that in Purkinje tissue the excitation wave travels ten times as fast as in ordinary cardiac muscle. On the other hand, in the slowly beating amphibian heart there is no specialized tissue (Davies 1942); instead there is present at the junctional zones a peculiar arrangement of the ordinary muscle fibres (Skramlik, 1921; Davies & Francis, 1941) sufficient to cause the delay in transmission which occurs there and which corresponds to that caused by the A.V. node in higher forms.

All heart muscle possesses rhythmic activity independent of any nervous connexions whatever, and as this is of greatest frequency at the venous end of the heart transmission of the beat normally starts from there. In order that the needs of the body as a whole may be served the automatic activity of the heart must be subject to the central nervous system. For this reason the S.A. node from a very early stage is in the closest relation with the nervous system, and by discharging its duties under nervous control ensures for mammals and birds a degree of circulatory efficiency not enjoyed by lower animals.

SUMMARY

1. The atrio-ventricular bundle can be recognized in a human embryo of 8 mm. C.R. length.
2. It arises from the A.V. node by a process of rapid growth which carries it from a position behind the dorsal endocardial cushion to the summit of the inter-ventricular septum where it divides.
3. The A.V. node represents a part of the original A.V. canal which undergoes early structural specialization.
4. Purkinje fibres of typical appearance can be recognized at the 105 mm. stage. They are myocardial fibres which have become further specialized. There is no evidence that they are fibres which have remained in an embryonic condition.
5. The sinu-atrial node can be identified at 10 mm., but only at birth does it show the histological features which characterize it in the adult.
6. The facts of foetal electrocardiography are discussed in the light of new information regarding the development of the conducting tissue.
7. The close relation borne by nerve elements to the developing S.A. and A.V. nodes is described.
8. The significance of the development of specialized conducting tissue in the mammalian heart is discussed.

I wish to express my thanks to Prof. C. M. West for helpful advice throughout the course of this work and to Mr A. Welch for his technical assistance. Profs. J. D. Boyd and W. J. Hamilton kindly gave me on loan valuable human embryos and Drs Ward and Kennedy of Vanderbilt University, Minnesota, U.S.A., with characteristic generosity, sent me a large number of their original and highly prized foetal electrocardiograph tracings; through the good offices of Prof. G. I. Strachan the resources of the Department of Obstetrics of the Welsh National School of Medicine were made readily available. To all I express my deep gratitude.

The drawing from which Pl. 1 was prepared was made by Miss M. White of the Histology Department, University College, Cardiff.

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EXPLANATION OF PLATES

Abbreviations used in the figures

Ao.	Aortic valve cusp.	*P.*	Phrenic nerve.
A.V.B.	Atrio-ventricular bundle.	*P.F.*	Purkinje fibre.
A.V.N.	Atrio-ventricular node.	*R.*	Right venous valve.
C.S.	Coronary sinus.	*R.D.C.*	Right duct of Cuvier.
D.	Dorsal endocardial cushion.	*S.A.N.*	Sinu-atrial node.
G.	Nerve ganglia.	*S.Pr.*	Septum primum.
I.V.C.	Inferior vena cava.	*S.V.*	Sinus venosus.
L.	Left venous valve.	*S.V.C.*	Superior vena cava.
L.L.	Left limb of A.V. bundle.	*T.*	Tricuspid valve cusp.
M.	Mitral valve cusp.		

Plate 1

Transverse section of 16·5 mm. human embryo to show the A.V. bundle on the summit of the inter-ventricular septum at its junction with the dorsal endocardial cushion. ×60.

Plate 2

Fig. 1. Transverse section embryo 161, 8 mm., section 19.2.2. The A.V. bundle, stained very darkly, is situated on the summit of the inter-ventricular septum which at this point has succeeded in joining the dorsal endocardial cushion. A prolongation of the bundle down the left side of the septum can also be seen. ×18.

Fig. 2. Part of the preceding section at a higher magnification. The appearance of the bundle is that of an actively growing structure. ×280.

Fig. 3. Transverse section embryo H. 33, 10 mm., section 19.1.2. The bundle can be seen arising from the A.V. node and passing into the base of the dorsal endocardial cushion. In this section as in the next a slender strand of muscle still connects the left atrium and ventricle. × 30.

Fig. 4. Transverse section embryo H. 33, 10 mm., section 18.2.6. To show the continuation of the bundle down the left side of the inter-ventricular septum. × 30.

PLATE 3

Fig. 5. Part of section 18.2.6. of embryo H. 33. To show vacuole formation in some of the cells of the primitive left limb of the A.V. bundle—the forerunners of Purkinje fibres proper. × 1000.

Fig. 6. Transverse section embryo H. 33, 10 mm., section 17.1.4. A cell cluster in the base of the right sinus valve represents the future S.A. node. × 30.

Fig. 7. Sagittal section embryo 44, 18·8 mm., section 23.2.5. To show the S.A. node within the substance of the crista terminalis. × 15.

Fig. 8. Transverse section embryo 114, 25 mm., section 38.2.2. The A.V. node is here shown close to where it will give origin to the bundle. × 40.

PLATE 4

Fig. 9. Transverse section embryo H. 23, 13 mm., section 25.3.5. To show the kidney-shaped mass forming the S.A. node within the base of the venous valves. × 150.

Fig. 10. Transverse section embryo H. 23, 13 mm., section 26.2.3. To show the left limb of the A.V. bundle passing into trabeculae (false tendons) of the left ventricle. The relation of the bundle to the septal cusp of the tricuspid valve is well seen. × 30.

Fig. 11. Transverse section embryo H. 23, 13 mm., section 26.1.8. To show the first identifiable strands of the right limb of the bundle. × 30.

Fig. 12. Transverse section embryo H. 23, 13 mm., section 26.2.7. To show the space which has now developed around the bundle. × 150.

PLATE 5

Fig. 13. Heart of 40 mm. embryo, section 24.1.5. To show the position of the S.A. node between the sinus and atrium. × 20.

Fig. 14. The previous section at a higher magnification. Nerve elements can be seen entering the anterior part of the node. × 75.

Fig. 15. Heart of 40 mm. embryo, section 27.2.4. To show the A.V. bundle cut in coronal section on the summit of the inter-ventricular septum between the mitral and tricuspid valves. × 20.

Fig. 16. Heart of 40 mm. embryo, section 26.2.5. To show the bundle dividing. × 20.

Fig. 17. Part of the previous section to show the finer structure of the right limb of the bundle. × 650.

Fig. 18. Heart of 40 mm. embryo, section 29.2.4. To show a leash of unmyelinated nerve fibres which runs into the A.V. node. × 650.

PLATE 6

Fig. 19. Heart of 105 mm. embryo, section 42.3. The inter-ventricular septum is here shown in vertical section. × 50.

Fig. 20. Heart of 123 mm. embryo, section 7.2.2. Nerve ganglion of the posterior atrial wall. × 350.

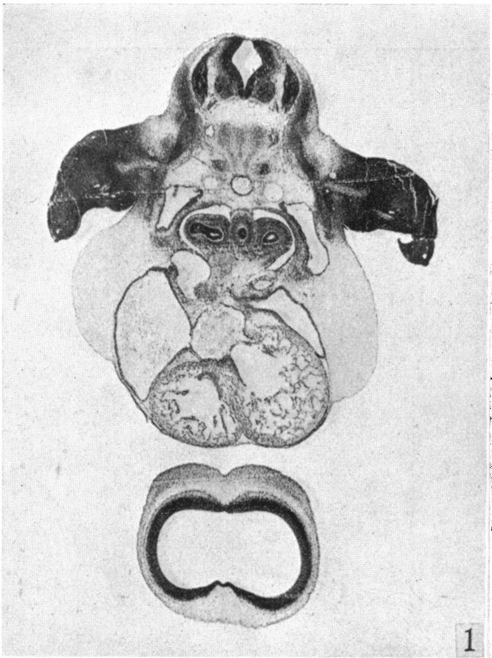
Fig. 21. Heart of 150 mm. embryo, section 14.4. Vertical section of the inter-ventricular septum to show the left limb arising from the main bundle with no evidence of the right limb. This is the commonest type of division, the right limb seeming really the continuation of the bundle. × 100.

Fig. 22. Heart of 150 mm. embryo, section 14.4. Part of the left limb of the A.V. bundle is here shown cut longitudinally. It contains a few Purkinje fibres and in the top right corner of the photomicrograph an apparently unspecialized myocardial fibre can be seen swelling out into a large Purkinje fibre containing two nuclei. Endocardial surface above and to the left. × 300.

Fig. 23. Part of the same left limb shown in the previous figure. Many large, typical Purkinje fibres are shown in which cross-striation is marked. Endocardial surface to the right. × 450.

Fig. 24. Transverse section of sinu-atrial region of a still-born heart, section 9.1.2. Endocardial surface above and to the right. × 60.

WALLS—SPECIALIZED CONDUCTING TISSUE OF THE HUMAN HEART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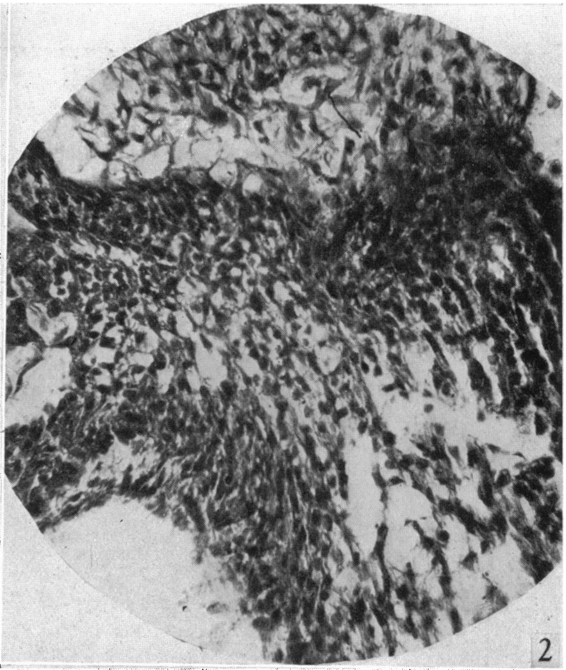
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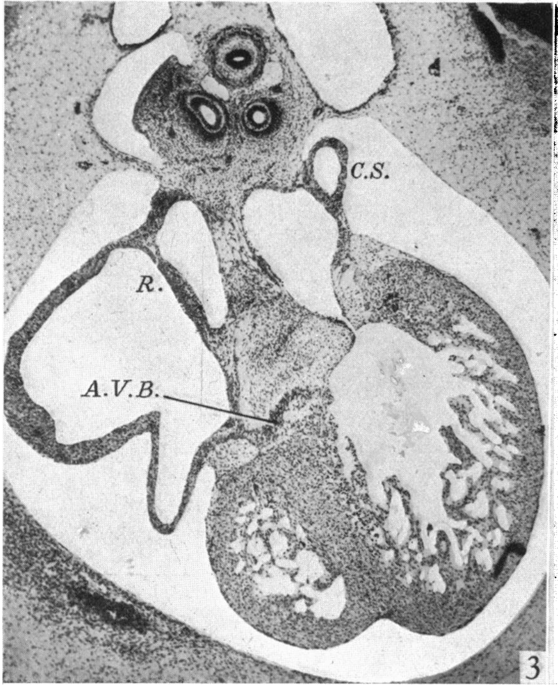

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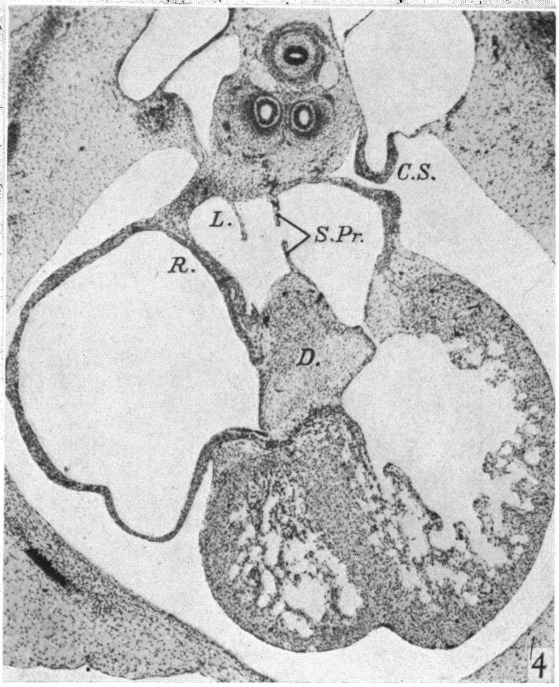

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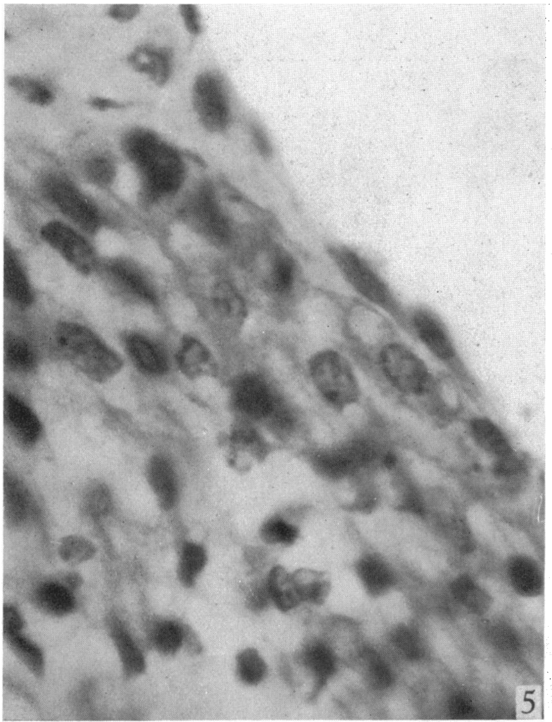

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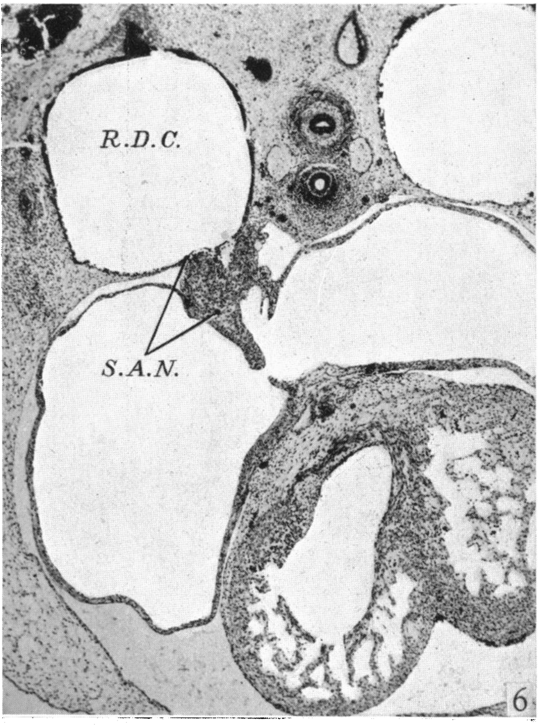

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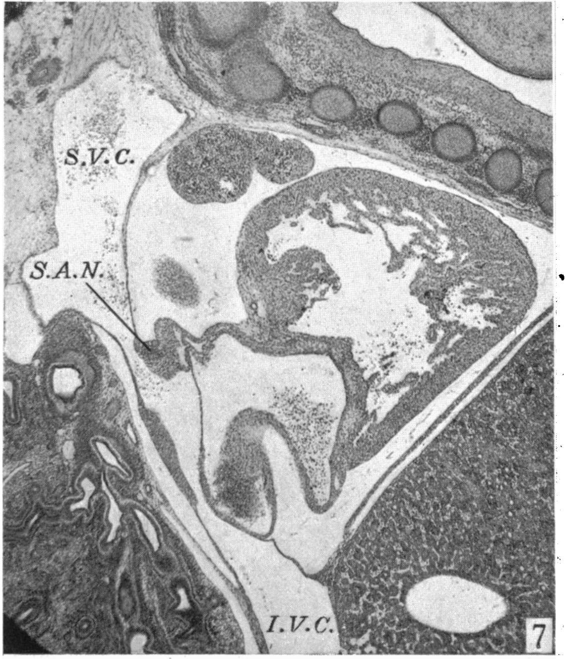

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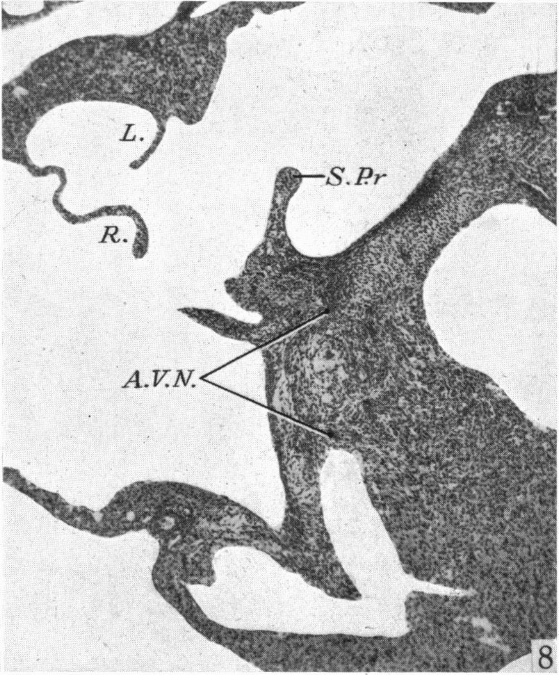

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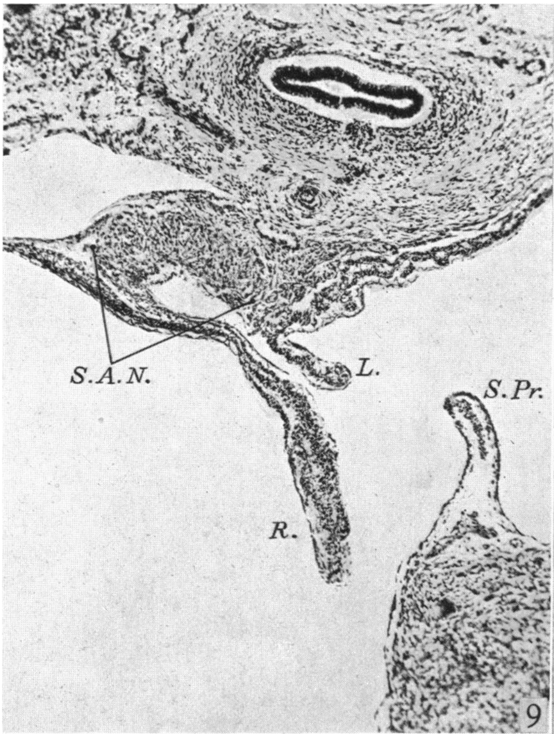
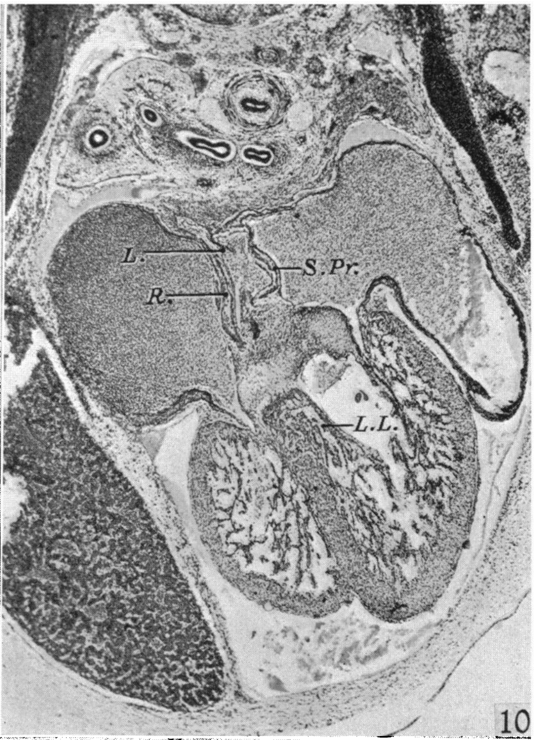
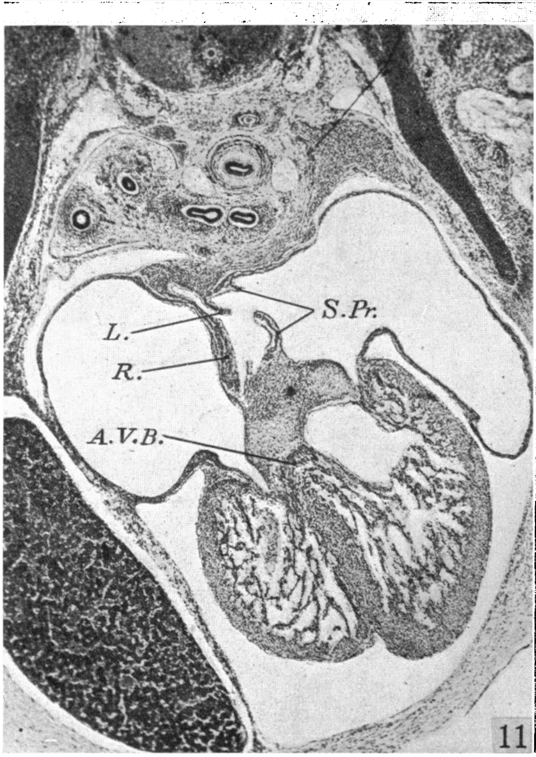
WALLS—Specialized conducting tissue of the human heart







WALLS—SPECIALIZED CONDUCTING TISSUE OF THE HUMAN HEART

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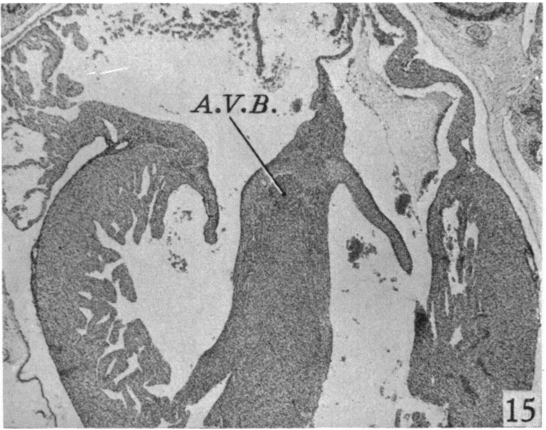

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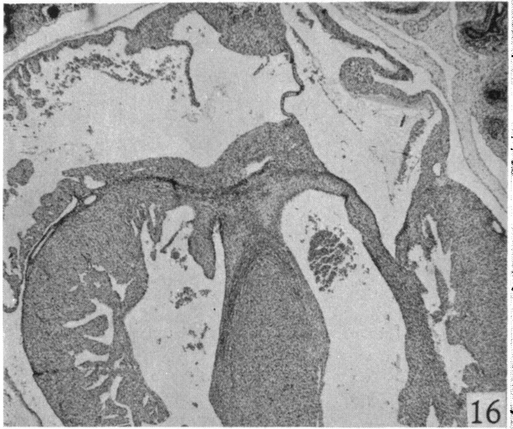

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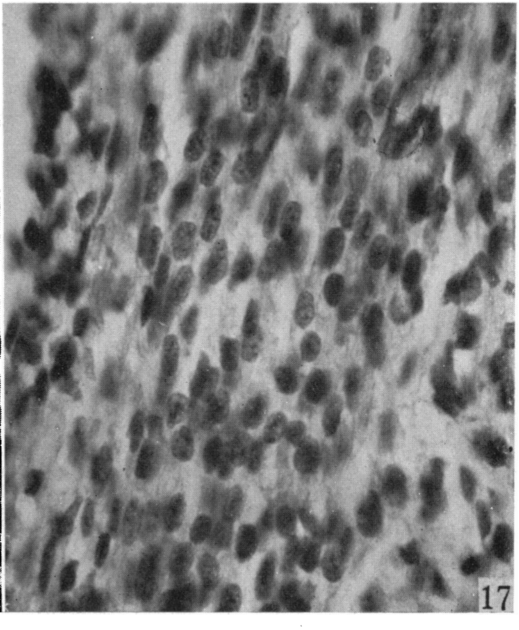

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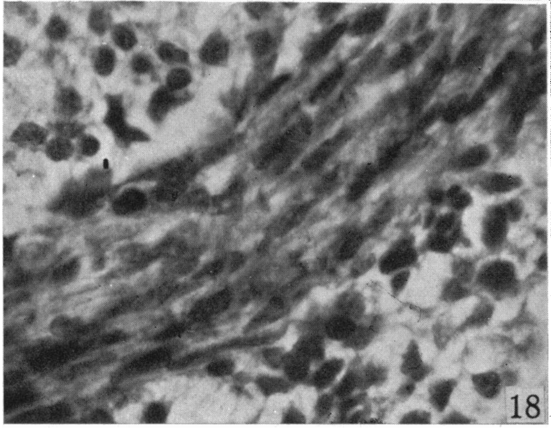

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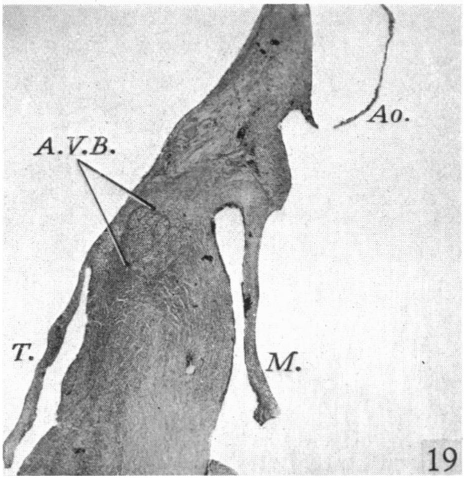
WALLS—SPECIALIZED CONDUCTING TISSUE OF THE HUMAN HEART



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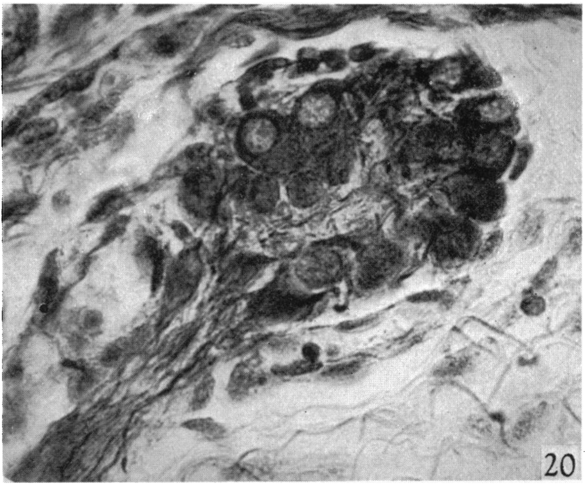

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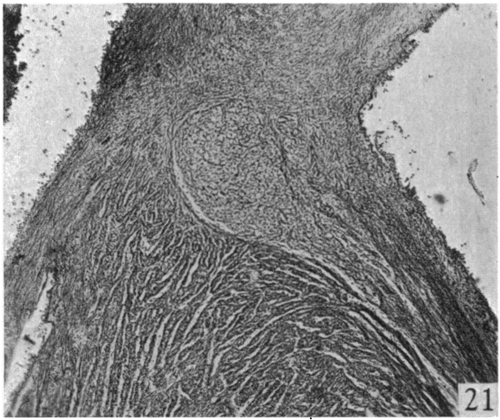

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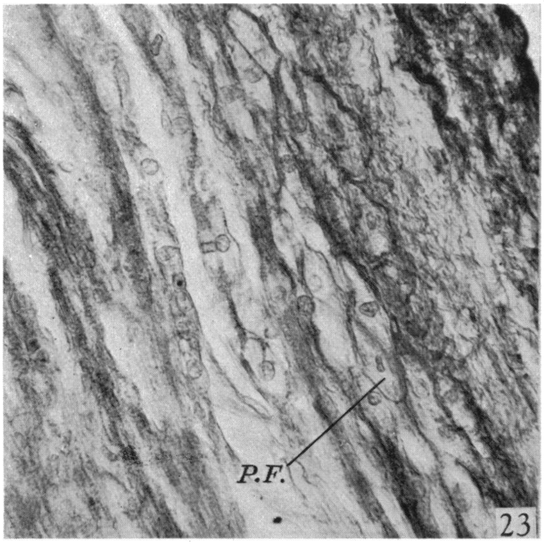

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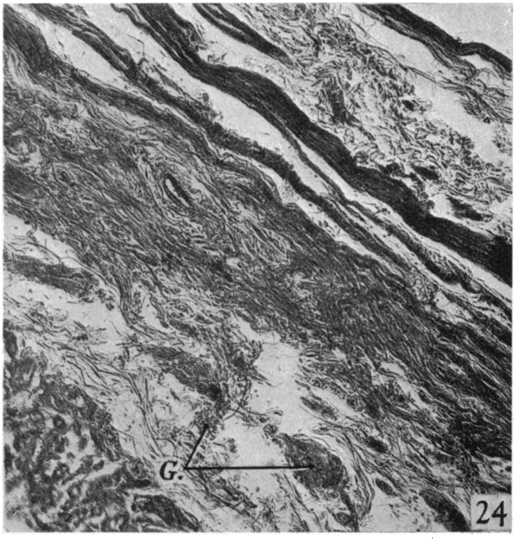

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WALLS—SPECIALIZED CONDUCTING TISSUE OF THE HUMAN HEART