

OBSERVATIONS ON THE CONDUCTING SYSTEM OF THE HEART¹

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INTRODUCTION

THE present work was begun with the object of investigating the nerve supply of the conducting system of the heart. At an early stage in the study it was found that the neurohistological technique which we employed for the purpose of displaying the nerve elements had the additional effect of revealing the detailed structure of the muscular elements of the system more clearly than the methods commonly used for that purpose. We therefore decided to attempt a complete survey of the structure and nervous connections of the cardiac conducting system, in the hope of amplifying previous work and clearing up existing discrepancies. To begin with, attention was specially directed to the human heart, because of its clinical importance, and to the bovine heart, as providing in some ways a useful comparison and because its conducting system, richly supplied with nerves, seemed particularly suitable as an initial subject for neurohistological study. The present paper records the observations so far made in these two forms on the sinu-atrial and atrio-ventricular nodes, the atrio-ventricular bundle and its two limbs, together with their immediate neural connections. In the following text the two types of heart are dealt with concurrently, to avoid repetition; but we are careful to indicate whether statements are applicable to both types or only to one of them.

MATERIAL

All the bovine material was obtained from adult animals immediately after death. The human material comprised three hearts removed at post-mortem examination, one from a subject aged 14 years, 6 hours after death, one from a child aged 9 months, 12 hours after death, and one from a child aged 10 months, 12 hours after death.

The sinu-atrial region of the human and bovine hearts was studied in blocks cut out of the whole thickness of the heart wall so as to include more than the upper half of the sulcus terminalis, and the adjacent parts of the right atrial and sinus walls and the entry of the superior vena cava. These were sectioned, some at right angles to the sulcus terminalis, others parallel to it.

The specialised atrio-ventricular connections in the human heart were examined in blocks which included the adjacent parts of the interatrial and

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interventricular septa, the entry of the coronary sinus, and the region beyond (in front of) the pars membranacea septi. These blocks were sectioned parallel to the coronary sulcus. In the bovine heart, the atrio-ventricular node and its prolongations, together with the atrio-ventricular bundle and its two limbs, with some of the adjacent myocardium, were dissected out carefully with the aid of binocular magnifiers in the fresh heart, transferred immediately to the fixing fluid, and ultimately cut longitudinally. Further, in the bovine heart, tangential sections (parallel to the endocardial surface) were cut of blocks which included the atrio-ventricular node and proximal part of the atrio-ventricular bundle.

The blocks from both the sinu-atrial and atrio-ventricular regions in both types of heart were deliberately cut out in such a manner that they extended on all aspects well beyond the sites customarily ascribed to the specialised conducting system in these regions in the hearts of placental Mammals. All preparations were cut into serial sections $10\ \mu$ thick.

TECHNIQUE

A histological method was required which would give reasonably consistent results and be applicable to the high-power study of serial sections of fairly large blocks. To begin with, preliminary trial was made on cardiac material of numerous neurohistological methods, using silver nitrate, gold chloride, osmic acid or methylene blue. The special merit of silver impregnation in differentiating the muscular elements of the conducting system became early apparent and led us to concentrate on such methods.

In view of the remarks of Woollard in favour of methylene blue rather than silver technique, in his paper on the innervation of the heart (1926), we may be permitted to point out some reasons for the contrary view. The intravital methylene-blue technique cannot be practised on the human heart, and neither that nor the supravital method can be made to produce complete nerve differentiation throughout a comparatively thick block of tissue such as must be used in studying the conducting system in a large heart. Even when small pieces of tissue are used, it is difficult to make sure of precise nerve-muscle relationships without high-power study of thin serial sections, and we are convinced that the preparation of paraffin sections of methylene-blue material causes appreciable loss of staining.

Of all the silver methods tried, that of Ranson (1914) seemed likely to give good results most readily in comparatively large blocks. The undoubted excellence of Bielschowsky preparations, when successful, is offset by the notorious difficulty of the method, and by its limitation to very small blocks. One great drawback of Ranson's method, at any rate when applied to muscular tissues, is the amount of shrinkage and friable hardening produced owing to fixation in what is almost absolute alcohol. We found, however, that fixation in 10 per cent. neutral formalin, followed by 10 per cent. formalin with 5 per cent. of ammonia added, instead of the ammonia-alcohol fixation of the

original technique, obviated these undesirable effects and greatly improved the differentiation of the nerve and muscle fibres. The former were revealed with a clarity and fineness equalled only in the best Bielschowsky results, but much more readily and if necessary in large blocks, while the muscle fibres were rendered more translucent and their striations became much more clearly visible than when using the unmodified Ranson technique. Variations were tried out in every stage of the technique; in this experimental work we were much helped by suggestions from Dr E. W. McClelland, Lecturer in Chemistry, and Dr W. Robson, Reader in Biochemistry, of King's College; and Dr E. E. Hewer, Reader in Histology at the London School of Medicine for Women, was good enough to discuss with us various modifications in the light of her own experience of the Ranson technique (1933). What we came to adopt as our standard method is indicated in the following statement, where the times are those actually employed in the preparation of one of our most successful specimens:

1. Formalin 10 per cent. (4 per cent. formaldehyde), neutralised with ammonia 10 days
2. Formalin 10 per cent., 95 c.c. } 48 hours
- Ammonia fortis, 5 c.c. }
3. Running tap water 48 hours
4. Distilled water (many changes) 3 hours
5. Pyridine 24 hours
6. Distilled water (many changes) 4 days
7. Silver nitrate 2 per cent. in incubator at 35° C. 4 days
8. Distilled water (many changes) 3 hours
9. Pyrogallic acid, 4 g. } 24 hours
- Formalin 5 per cent., 100 c.c. }
10. Distilled water (changed) 1 hour
11. Transfer to 50 per cent. alcohol, dehydrate, clear in benzol or chloroform, and embed in paraffin wax.

Stages 7-10 are carried out in the dark.

The following notes are numbered to correspond with the stages in the process given above:

1. Neutralisation (to litmus) with ammonia is distinctly beneficial. Longer fixation does no harm. To give the best results, material must be fixed immediately after death; hence none of our human preparations has been quite so successfully impregnated as those of animal hearts.

2. This improves the differentiation of both nerve and muscle fibres and renders nuclei more visible; less or more ammonia is disadvantageous. This solution is *not* a fixative (hence must be preceded by 1), because in this strength the formalin is completely converted into hexamine (hexamethylenetetramine) by the ammonia, and any formic acid in the formalin into ammonium formate. An aqueous solution of hexamine and ammonium formate chemically equivalent to the theoretical product of the formalin-ammonia combination gives results

nearly but not quite so good as the formalin-ammonia solution; a solution of hexamine alone was still less good.

3. To remove formalin completely and to save distilled water.

4. Tap water distilled once over potassium permanganate in an all-glass still which has been in use for several years, so that no soluble material remains in the glass.

5. Longer in pyridine is undesirable, as the tissue becomes brittle and the neural impregnation is not so good.

6. Pyridine must be thoroughly removed; continue for 24 hours after all odour of pyridine has disappeared.

7. Longer in silver nitrate results in poorer impregnation of nerve fibres.

8. Less washing causes deposit.

9. Longer reduction disadvantageous. Several other reducing agents were tried without advantage.

10. Prolonged washing with distilled water is unnecessary as pyrogallie acid comes out still more readily in the alcohols, but the 90 per cent. alcohol should be changed until it remains colourless.

SINU-ATRIAL NODE

The sinu-atrial node of the heart of Man and other placental Mammals was originally described by Keith and Flack in 1907 as a mass of peculiar muscle fibres, some nerve cells and nerve fibres, embedded in the densely packed connective tissue which surrounds the artery or arterial circle that lies at the junction of sinus and right atrium. The fibrous tissue in which the nodal fibres are embedded is considered by these authors to belong to the epicardium and to correspond to the infolded epicardial fibrous tissue in the right venous valve at the sinu-atrial junction of the lower vertebrate heart. Further, these authors note that in the human heart the nodal fibres are cross-striated, fusiform, arranged in a plexiform manner and related mainly to the sulcus terminalis in the region of the entry of the superior vena cava. Many subsequent investigators have verified the main features of this description, though Tandler (1913), Koch (1922), Zimmermann (1923) and Monckeberg (1924) could not establish with certainty the transverse striations of the nodal fibres. Bruni (1924) illustrates cross-striation of the nodal fibres in the calf, and Burian (1925) gives a convincing description and illustrations (drawings) of cross-striation in the nodal fibres of the human heart, in material stained for the purpose with iron haematoxylin after fixation in Heidenhain's "Susa" mixture. Walmsley in 1929, although he refers to Burian's work, summarises the position with the statement that transverse striation of the sinu-atrial nodal fibres (in the human heart) has not been certainly demonstrated. Wingate Todd and van der Stricht in 1919, and Wingate Todd in 1928, from their study of the adult human heart, maintain that both nodes (sinu-atrial and atrio-ventricular) are non-specialised parts of, and structurally identical with, the general cardiac Purkinje system.

In the present work we have observed that the sinu-atrial node is more extensive than existing descriptions imply. In both human and bovine hearts the sinu-atrial node consists of a main mass lying in relation to the upper part of the sulcus terminalis, and of tapering extensions, both above and below the main mass, which pass beyond the scope of our blocks. The upper extension passes in front of and above the entry of the superior vena cava into the right atrium, so that *in situ* the sinu-atrial node would have a horseshoe shape, the entry of the superior vena cava lying below and behind the concavity of the horseshoe. Bruni (1924) has described a similar arrangement of the node in Ruminants, and Pace (1924) in the sheep. Segre (1926) has also described this arrangement in Man and further notes that the intermediate portion of specialised fibres connecting the two limbs of the horseshoe does not develop until after birth. The lower tapering extension of the node, as stated above, passes beyond the scope of our blocks, and therefore must extend downwards beyond the middle of the sulcus terminalis.

The main nodal mass, moreover, extends through the entire thickness of the heart wall, spreading from the subepicardial to the subendocardial connective tissue (Plate I, figs. 1-4); as it approaches the endocardium it spreads out so that in sections at right angles to the sulcus terminalis it appears wedge-shaped, with the base of the wedge towards the endocardium (Plate I, fig. 1). This does not seem to have been appreciated previously. In this situation the nodal fibres, together with the enveloping connective tissue, intervene between the sinus musculature (covered with a thick endocardial layer) and the atrial musculature (covered with a thin endocardium). Elsewhere the sinus and atrial musculature are continuous. The main mass of the node thus lies immediately on the sinus side of the crista terminalis, and, except where it impinges on the subendocardial or subepicardial connective tissue, it is directly contiguous all round with the adjacent atrial or sinus muscle. In the human heart the main nodal mass extends from the subepicardial connective tissue to the endocardium both behind (i.e. on the sinus side) and in front (i.e. on the atrial side) of the base of the crista terminalis.

The nodal fibres in both human and bovine hearts are spindle-shaped and branched, and are set rather loosely in the surrounding connective tissue (Plate III, fig. 9, and Plate V, fig. 17). For the most part they are arranged with their long axes parallel with the long axis of the sulcus terminalis, so that in sections at right angles to the sulcus they are cut almost transversely. In such sections, however, many of the fibres, particularly those near the epicardium and those near the atrial myocardium, are cut very obliquely or even longitudinally. The nodal fibres immediately adjacent to the main artery and its branches which, as all previous workers have noted, run along in the axis of the sulcus terminalis and embedded in the node, are arranged in a circular or spiral fashion around the artery (Plate I, fig. 2, and Plate II, fig. 5). In the bovine heart the majority of the fibres of the sinu-atrial node (with the technique employed) are about $9\ \mu$ in their widest diameter, with a common range

of from 3 to 17 μ . In the human heart (14 years old) the majority of the nodal fibres are about 5 μ wide, with a common range of from 2 to 7 μ . With the silver technique employed in the present work the nodal fibres are seen to be distinctly striated transversely (Plate III, fig. 9, and Plate V, fig. 17), the smaller fibres, which comprise the greater proportion, being striated throughout their entire thickness, while the larger fibres have an appreciable perinuclear unstriated zone, and thus resemble in structure (though not in size) some of the "typical" Purkinje fibres found in the bovine heart beneath the ventricular endocardium, or in the atrio-ventricular bundle and its two limbs. Each nodal fibre has a nucleus which is round or oval in shape and centrally situated; in some fibres two nuclei are present, close to one another in the centre of the fibre. The structural features of the nodal fibres revealed by silver impregnation leave no doubt as to the essential muscular nature of the sinu-atrial node.

With regard to the relation of the nodal fibres to the neighbouring myocardial fibres, Walmsley (1929) states that direct continuity of nodal and atrial muscle fibres (in the human heart) has not been established, but that there is no definite boundary of the node; its fibro-muscular tissue gradually fades into the surrounding myocardium. Wakefield and Chandler (1924) could not find any evidence of direct continuity of nodal and myocardial fibres in the adult human heart; they state that a delicate connective tissue sheath intervenes between the nodal and atrial muscle fibres; their sections, incidentally, were cut at a thickness of 25 μ . Shaner (1929), on the other hand, found evidence of direct continuity of the sinu-atrial nodal fibres with both sinus and atrial muscle in the heart of the foetal calf.

In the present work the peripheral nodal fibres of both human and bovine hearts were frequently seen to become directly continuous with the atrial or sinus fibres, either by end-to-end union, or by union through one or more side processes of the nodal fibres (Plate IV, fig. 14). One nodal fibre may thus become directly continuous with two or more atrial or sinus myocardial fibres. With a Zeiss binocular microscope and the highest power apochromatic lenses and aplanatic condenser, no interruption of continuity by any sheath formation or otherwise could be made out at the junctional zone; the nodal fibre passes directly over into the myocardial fibre in both bovine and human hearts. In places, at the periphery of the sinu-atrial node, it was difficult to say where nodal fibres ended and myocardial fibres began. This was particularly the case where the peripheral nodal fibres were of medium size and completely cross-striated so that they closely resembled the neighbouring myocardium in appearance. The precise site of continuity was most readily observed between the larger nodal fibres in the ox heart and the neighbouring myocardial fibres.

Another type of continuity was observed in the bovine heart. Subepicardial nodal fibres extend caudally in a subepicardial plane and become directly continuous with much larger fibres which have exactly the same

appearance as the large subendocardial ventricular Purkinje fibres in the same heart. These large atrial Purkinje fibres, in their turn, were traced caudally for a short distance and passed into direct continuity with the subepicardial atrial muscle fibres, joining these on their epicardial aspect (Plate IV, figs. 15 and 16). This observation is of interest in relation to the much discussed question of the presence or absence of "typical" large Purkinje fibres in the atria of placental Mammals. One of the present writers (Davies, 1931) has reviewed the literature on this question in a previous paper in this *Journal*. Various authors have described collections of Purkinje fibres in the right atrium of Man and other placental Mammals, but it is noteworthy that the situations in which they have been found differ with the investigators, and moreover the atrial Purkinje fibres appear, from the descriptions of these workers, to form isolated collections, unconnected with either the sinu-atrial or the atrio-ventricular node. In the avian heart one of us (Davies, 1930) observed that the rich abundance of atrial Purkinje fibres was continuous on the one hand with the sinu-atrial node, and on the other with the ordinary atrial muscle, but no specialised connection between the sinu-atrial and atrio-ventricular conducting systems could be made out; the atrio-ventricular nodal fibres became directly continuous only with the atrial myocardium. So far as the present work goes the bovine heart offers a parallel picture, namely, some of the sinu-atrial nodal fibres are continuous with large "typical" Purkinje fibres, which, in their turn, are continuous with the atrial myocardium, as described above, while, as mentioned later in this paper, the atrio-ventricular nodal fibres become directly continuous with the atrial myocardium without the intervention of large "typical" Purkinje fibres.

In the human heart the difficulty arises of recognition of the various types of fibres in the conducting system. With the silver technique used in the present study, the sinu-atrial nodal fibres are plainly seen to exhibit transverse striation (Plate III, fig. 9). Moreover, the breadth of the nodal fibres (human, aged 14 years) varies from 2 to 7 μ , while that of the atrial muscle in the same heart ranges from 5 to 11 μ (Plate IV, fig. 13). Hence many of the nodal fibres are as large as the atrial muscle fibres, and while the small nodal fibres or even the larger ones which exhibit only peripheral transverse striation are easily recognisable, the nodal fibres of intermediate size that show marked and almost complete transverse striation are only with difficulty distinguished from myocardial fibres. This difficulty must be borne in mind in assessing references in the literature to scattered collections of Purkinje fibres in the atria. After the examination of much material and bearing in mind our findings in the bovine and other hearts, we have come to the conclusion that continuity with undoubted specialised muscle is the criterion which best determines the nature of a doubtful muscle fibre. In our blocks from the right atrium of the human heart we have found no large "typical" Purkinje fibres such as are seen in the bovine heart; neither are such found in the human atrio-ventricular bundle, as will be shown later.

A further relation of the sinu-atrial nodal fibres, not hitherto described, was observed in the present investigation. As mentioned above, the nodal fibres in the vicinity of the nodal artery and its branches are arranged in a circular manner around these vessels (Plate I, fig. 2, and Plate II, fig. 5). In numerous places some of these peri-arterial nodal fibres turn into the arterial wall and become contiguous with the unstriated muscle fibres of the media. In the bovine heart some of these long, slender, cross-striated nodal fibres are seen actually to pass into the arterial tunica media and become directly continuous with the plain muscle fibres (Plate II, fig. 6). The presence of a distinctly cross-striated muscle fibre in the middle of the tunica media of an artery provided a very striking and unusual picture. In the human heart, while numerous peri-arterial nodal fibres pass into immediate contiguity with the outer surface of the arterial tunica media (Plate II, fig. 7), we could not with absolute certainty make out any penetration into the media, although the nodal fibres appeared to blend with the outermost plain muscle fibres of the arterial coat.

In both types of heart there is no nodal vein in the sense of a *vena comitans* of the nodal artery, but a number of small veins (*venae cordis minimae*) drain from the nodal tissue into the cavity of the right atrium. This agrees with the description of Géraudel (1928). We have seen no special relation between nodal fibres and the nodal venules.

In the endocardial connective tissue in the immediate neighbourhood of the sinu-atrial node in both types of heart, we have observed unstriated muscle fibres of ordinary visceral type. Such have been described in various situations in the endocardium, although no complete account of their arrangement and topographical distribution in any species has been given. They form a thick layer on the sinus side of the *crista terminalis*, but only a very thin layer on the atrial side of the *crista*. Near the sinu-atrial node we observed long tapering tongues of ordinary atrial muscle fibres which run into the endocardium and appear to become continuous with these endocardial unstriated muscle fibres (Plate II, fig. 8). We could, however, trace no direct connection between the sinu-atrial nodal fibres and these visceral muscle fibres, a thick layer of endocardial connective tissue intervening between the two. We hope to investigate further the topography of this endocardial plain muscle with particular reference to its possible relationship to the conducting system.

The question of direct connection between the sinu-atrial and atrio-ventricular nodes by means of specialised muscle is discussed at the end of the section dealing with the atrio-ventricular node.

The penetration of sinu-atrial nodal tissue from epicardium to endocardium, and its extent beyond the customarily recognised limits of this specialised structure, as above described, are of interest in relation to the experimental work that has been done on this region of the heart. For example, Hering (quoted by Lewis, 1925) claimed that, after isolation of the sinu-atrial and atrio-ventricular nodes from the mass of the right atrial tissue, the right atrium

continued to beat, and concluded that there are other atrial centres capable of rhythmic action. These hearts, however, were subsequently examined by Koch, who found nodal tissue attached to the remaining mass of atrial tissue, and, as Lewis asserts, this emphasises the importance of strict histological controls in experiments of this type. Furthermore, Lewis has shown that to cool the "head" of the sinu-atrial node in dogs is insufficient to induce atrio-ventricular rhythm. The present work further emphasises the difficulty of experimental removal of the entire sinu-atrial node, and as any nodal tissue remaining will be capable of initiating atrial contraction, throws open to still more serious question the validity of previous experimental work as a basis for the supposition of other (ectopic) centres with the same rhythmic powers as the node.

ATRIO-VENTRICULAR NODE

In both bovine and human hearts the arrangement of the atrio-ventricular nodal tissue has a characteristic appearance quite distinctive from that of the ordinary atrial myocardium or of the sinu-atrial node. In the bovine heart the atrio-ventricular nodal fibres form a loose spongework of muscular trabeculae, with the meshes occupied by loose connective tissue and abundant capillary blood vessels (Plate V, fig. 18). The spongework is much more compact in the human atrio-ventricular node, with less connective tissue between the nodal fibres (Plate III, fig. 10). In both types the trabeculae branch and anastomose with each other, and are not orientated in any particular plane. The individual nodal fibres are cylindrical in shape and branch like ordinary cardiac muscle. Walmsley (1929) states that, at the best, transverse striation of the fibres of the atrio-ventricular node (in the human heart) is necessarily insignificant. With the silver technique employed in the present investigation the nodal fibres in both human and bovine hearts are seen to be distinctly cross-striated (Plate III, fig. 10, and Plate V, fig. 18). For the most part the striation extends throughout the fibres, except for a narrow perinuclear clear zone. Each nodal fibre contains one or sometimes two centrally situated round or oval nuclei. As stated above for the sinu-atrial node, the appearance revealed by the silver method leaves no doubt as to the muscular nature of the atrio-ventricular node.

In the human heart (aged 14 years) the nodal fibres vary commonly from 3 to 11 μ in breadth, with the majority about 7 μ . The bovine nodal fibres are rather larger, mostly about 10 μ in breadth, with a common variation from 3 to 14 μ . In both human and bovine hearts, the atrio-ventricular nodal fibres differ from those of the sinu-atrial node in that they are shorter, rather thicker, more cylindrical and more branched than the sinu-atrial nodal fibres, which are commonly of elongated fusiform type. We do not agree with the observations of Shaner (1929), who, while noting that the sinu-atrial and atrio-ventricular nodes in the adult bovine heart are not especially alike in structure, states that the clear-cut cell walls or membranes of the atrio-ventricular node are absent in the sinu-atrial node, and that the sinu-atrial node forms a diffuse branching

meshwork that does not resemble anything found in the atrio-ventricular node. The differences between the sinu-atrial and atrio-ventricular nodes that we have observed comprise those of the arrangement, shape and size of the constituent muscle fibres as stated above. Our observation that the fibres of the sinu-atrial node are in general rather smaller than those of the atrio-ventricular node may be of importance in relation to the correlation, expressed by Lewis as the law of cardiac muscle, between the size (thickness) of a cardiac muscle fibre and its rate of conduction, rhythmicity, length of systole and glycogen content; the fibre size, glycogen content and rate of conduction increase in the following order: nodal, ventricular, atrial, Purkinje; while the length of systole and rhythmicity diminish in the same order.¹ The smaller size of the sinu-atrial nodal fibre noted in the present work might have a bearing on any difference that may be found to exist in the rhythmicity of the sinu-atrial and atrio-ventricular nodes, which difference might be correlated with the onset of the wave of excitation for cardiac contraction at the sinu-atrial node (the pacemaker, Lewis).

Dissections of the atrio-ventricular node in the fresh bovine heart were made with the aid of binocular magnifiers. Atrial prolongations of the main nodal mass as described and figured by Curran (1903) were made out. The entire dissected mass, including the atrio-ventricular node and its prolongations, was subjected to histological section (after treatment with the silver technique), which revealed that the prolongations consisted of nodal fibres which after a very short course became directly continuous with the ordinary atrial myocardium. One of these prolongations extended towards the opening of the coronary sinus, others upwards in the atrial septum to become continuous with the right and left atrial muscle components of the septum. These atrial septal prolongations did not extend, as Curran states, almost as far as the superior vena cava, but after a very short course the nodal fibres comprising them became directly continuous with the atrial myocardium. In the human heart a large prolongation from the atrio-ventricular node to the left atrial muscle component of the atrial septum was seen piercing the upper part of the trigonum fibrosum dextrum in its upward and backward course. Further, in the human heart (14 years old) an extension of the nodal tissue was traced for a short distance into the anterior wall of the left atrium, where it became continuous with the atrial myocardium. In its course it passed upwards and to the left, immediately behind the basal attachment of the right posterior (non-coronary) cusp of the aortic valve. This nodal prolongation appears to us to correspond in position to at least a part of the interatrial node described by Shaner (1929) in the early foetal calf heart. Shaner, however, observed that

¹ Our preparations of the adult bovine heart show that the order of fibre size in the ox is the same as that given by Lewis in the dog, which is here referred to. But in our preparations of human heart (aged 14 years) the order is different; the thickness of fibre increases in the order: nodal, atrial, atrio-ventricular bundle, ventricular. (Compare figs. 9, 10, 13, 11 and 12, which are all from the same human heart, aged 14 years, and of the same magnification.)

the interatrial node had no connection with the atrio-ventricular node, and, in the calf at least, disappeared in the later stages of foetal life, gradually fading into ordinary atrial muscle.

No "typical" Purkinje fibres were seen in the immediate vicinity of the atrio-ventricular node in either atrial wall in either the human or the bovine heart; the nodal fibres become directly continuous with the atrial myocardium. In both the human and the bovine heart the change from nodal to atrial myocardial fibres is a very gradual one, in contrast with the sudden change in character from atrio-ventricular nodal fibres to large "typical" Purkinje fibres at the beginning of the atrio-ventricular bundle in the bovine heart, as described below. It is difficult, if not impossible, to say exactly where nodal fibres end and atrial muscle begins. Branching of both nodal and atrial muscle fibres occurs in the territory of junction, and the fibres, both atrial and nodal, vary so much in diameter that it is often difficult to assign any one fibre to node or atrial muscle with certainty. The network formation of the nodal fibres is continued up to the junctional territory; in the junctional zone the network formation is rather less marked, but the parallel arrangement of very slender fibres at the upper part of the atrio-ventricular node, described by Cohn (1909) as the atrio-nodal junction, was not observed by us in either the bovine or human heart.

The prolongation from the atrio-ventricular node towards the ventricular septum described by Curran (1903) in the calf heart was also noted by us in the dissected heart of the ox. In the human heart we traced this prolongation in the serial sections to the base of the septal cusp of the tricuspid valve, but no direct continuity of these nodal fibres with the myocardium of the ventricular septum could be made out, connective tissue, continuous with the trigonum fibrosum dextrum, intervening in all sections between nodal and ventricular muscle. Holmes (1921) came to the same conclusion from his study of the hearts of the sheep and ox. In this region, however, we observed continuity between these nodal fibres and the myocardium of the right atrium which descends for a short distance into the base of the septal cusp of the tricuspid valve. Further, we noted in this region of the human heart no direct continuity between the myocardial fibres of the right atrium and those of the ventricular septum, although in a few sections the terminal fibres of the ventricular septum and right atrium approached very close to each other. This was one of the regions of the heart where Kent (1893) noted direct continuity between the myocardial fibres of the right atrium and those of the ventricular septum in the hearts of the rat and young rabbit and to a lesser extent in the guinea-pig and hedgehog. In the monkey, only very few fibres were found by Kent to establish atrio-ventricular continuity, and these were described as transversely striated, branched, spindle-shaped cells, intermediate in character between ordinary heart muscle and unstriated muscle, with long tapering ends extending almost completely through the atrio-ventricular fibrous ring from atrium to ventricle and establishing muscular continuity between atrium and ventricle.

Similar fibres were seen in the present work in the human heart in the fibrous tissue between the right atrium and the ventricular septum, beneath the base of the septal cusp of the tricuspid valve, but they appear to us to be of the nature of the "insertions" of the cardiac muscle fibres (atrial and ventricular) into the right fibrous ring. Pace (1924), who studied these fibres carefully, believed likewise and referred to them as "fibres terminales spéciales". We certainly could not find in the human heart evidence of direct continuity by means of these fibres between the right atrium and the ventricular septum. In this connection it may be remarked that Lewis states that the physiological evidence is strongly opposed to the idea of the fibres described by Kent taking part in conducting impulses from atrium to ventricle.

The sinu-atrial node, as stated above, extended beyond the scope of our blocks, but the atrio-ventricular node appeared to be entirely contained within the blocks of tissue removed in both human and bovine hearts. The atrial extensions of the atrio-ventricular node were all traceable into continuity with atrial myocardium. Recently Tudor Jones (1932) has described direct continuity between the sinu-atrial node and the atrio-ventricular node in the 24 mm. human embryo, the connection lying in the base of the right venous valve. Previously Thorel (1906), in his study of adult human hearts (over 40 years of age), found a direct connection between the sinu-atrial and atrio-ventricular nodes consisting of fibres of Purkinje type, but subsequent investigators have failed to corroborate this. While we would defer final opinion on this question until we have studied sections of entire hearts, our present study reveals no indication of such a connection in the post-natal heart. It may be that the connection described by Tudor Jones in the embryonic heart is of a temporary nature, and, if confirmed, would obviously be important from the point of view of the development of the nodes and their embryological and phylogenetic relation to one another. On the other hand, Shaner (1929) found that, while the atrio-ventricular node appeared in the 9 mm. calf embryo, the sinu-atrial node did not appear until the 100 mm. stage, and he does not describe any direct connection between the two.

ATRIO-VENTRICULAR BUNDLE

In the bovine heart the atrio-ventricular bundle arises from the centre of the main mass of the atrio-ventricular node, as the shaft fits into the knob of a walking stick (Plate VI, fig. 20). In the proximal part of the atrio-ventricular bundle, as it leaves the atrio-ventricular node, the specialised muscle fibres are arranged in plexiform strands with a general direction in the long axis of the bundle (Plate V, fig. 19, and Plate VI, fig. 22); subsequently the bundle fibres form parallel longitudinal fasciculi. From the very commencement of the bundle the fibres stand out clearly from either the nodal or ordinary myocardial fibres because of their relatively enormous size, about $30\ \mu$ in average diameter. Commonly two, three or more fibres of the atrio-ventricular node become uninterruptedly continued into one Purkinje fibre, joining the latter end on or

on its side; the transversely striated myofibrillae of the nodal fibres can be clearly seen to pass into the Purkinje fibre to become continuous with the transversely striated portions of the Purkinje fibre (Plate VI, fig. 23). There is no sign of any sort of septum across the junction of nodal and Purkinje fibres, and frequently the continuity provides the striking appearance of a thin nodal fibre suddenly swelling out into a broad bundle fibre. It is not easy to determine the exact configuration of these bundle fibres. But it seems to us that descriptions, such as those of Wingate Todd (1928), are mistakenly based on deceptive appearances seen in oblique sections. The fluted column fibre form suggested by these descriptions, we consider to be an erroneous impression of a fasciculus of several individual fibres lying very close together, or of sections at the side-to-side junctions of individual Purkinje fibres. The fibres in a fasciculus are continuous with one another by branches split off longitudinally in regions where a slight interval occurs between the fibres, or by localised areas of side-to-side union where they are more compactly arranged. The fasciculi themselves fork and join with one another; this is more obvious in the proximal, plexiform part of the bundle. In the distal part of the bundle and in the limbs, long lengths of cylindrical fibres without any side connections can be traced in longitudinal sections of the dissected specimen. In these elongated cylindrical fibres numerous small rounded nuclei are seen, mostly placed immediately below the surface of the fibre, exactly as in skeletal muscle (Plate VII, fig. 26). Multiple central nuclei, however, are commoner, particularly in the shorter fibres. The fibres are definitely cross-striated; this striation may be indistinct or absent in localised areas within the fibre, but the cross-striation is not confined to the periphery, and the central part of the fibre is not entirely unstriated. In other words, in these large "typical" Purkinje fibres the transversely striated longitudinal myofibrillae are irregularly distributed in the fibre and do not completely fill it as they do in ordinary cardiac muscle (Plate VI, fig. 23, and Plate VII, fig. 26).

In the human heart the fibres at the beginning of the atrio-ventricular bundle differ very little in histological structure from those of the atrio-ventricular node. Holmes (1921) has likewise commented on the absence of clear distinction between the node and the beginning of the bundle. The fibres in the proximal part of the bundle are slightly larger than the atrio-ventricular nodal fibres, averaging about 9μ in diameter as against 7μ for the nodal fibres, and have a less ragged appearance because they branch less markedly. When running parallel in the more distal part of the bundle and in the proximal parts of its right and left limbs, the fibres closely resemble the ordinary ventricular muscle fibres; the latter are slightly larger with an average diameter of about 12μ (Plate III, figs. 11 and 12). Only a small proportion of the bundle fibres exceed this size. Most of the fibres in the bundle and in the proximal parts of its right and left limbs present transverse striation throughout their thickness (Plate III, fig. 11, and Plate VII, fig. 25), with the exception of a narrow, clear, central, perinuclear zone, and thus resemble the

neighbouring ventricular myocardial fibres. The few larger fibres bear some resemblance to "typical" Purkinje fibres in respect of irregular and incomplete transverse striation, but even these only slightly exceed the ventricular muscle fibres in size. In some of the bundle fibres two centrally situated rounded or oval nuclei are found. The elongated peripherally nucleated Purkinje fibres observed in the bovine heart are not seen in the human bundle; indeed in the bundle fibres in the human heart there is no room for nuclei elsewhere than in the centre of the small fibres. Holmes (1921) has previously noted these relative sizes of the bundle fibres and ventricular muscle fibres in the human heart, but gives no actual measurements. The bundle and its limbs are surrounded with a delicate connective tissue sheath, which in places is so thin as hardly to be recognised at all. In these places, particularly in the proximal part of the right limb, where the limb fibres closely resemble the neighbouring myocardial fibres, the limb itself is recognised histologically in isolated transverse sections only with some difficulty. The thinness and delicate nature of the sheath, as Holmes has also observed, explain the difficulty or impossibility of injection (e.g. with Indian ink) in the human heart, rupture and extravasation through the sheath taking place immediately it is exposed to even a slight pressure. Such was the experience, also, of Aagaard and Hall (1915).

NERVE CELLS IN RELATION TO THE CONDUCTING SYSTEM

We hope later to make a complete topographical survey of the intracardiac ganglia by means of serial sections of entire hearts, but the following observations on the present material may be recorded. Groups of nerve cells are placed in the immediate neighbourhood of the sinu-atrial node in both ox and man; they are more numerous in the ox. For the most part they are situated on the epicardial aspect of the node, where in both types of heart some of the groups of nerve cells lie in juxtaposition with the superficial nodal fibres. We found no nerve cells in the interior of the sinu-atrial node in either type of heart; neither did Meiklejohn (1913) in the heart of the monkey (*Callitrix*) or of Man. Fahr (1910), on the other hand, occasionally found nerve cells in the sinu-atrial node of the human heart. Groups of nerve cells were also found close to the atrio-ventricular node in both forms, in the lower and back part of the atrial septum, mostly placed in the connective tissue between the right and left atrial muscle components of the septum. Here again nerve cells are more numerous in the bovine heart, where a few small groups of cells were found buried within the atrio-ventricular node, an observation previously made by Fahr (1910). In the bovine heart, small groups of nerve cells were found in relation to the atrio-ventricular bundle and the proximal parts of its right and left limbs, some immediately adjacent to the surface of the bundle and its limbs, some amidst the Purkinje fibres (Plate VI, fig. 21, and Plate VIII, figs. 28 and 29). In the human heart, however, we observed no nerve cells within the atrio-ventricular node, or in the course of the atrio-ventricular bundle or

its right or left limb. Fahr (1910) and Holmes (1921) also failed to find nerve cells in these situations in the human heart. Wilson (1909) has described nerve cells in the atrio-ventricular bundle and its two limbs in the calf, sheep and pig, and Meiklejohn (1913) in the left limb of the atrio-ventricular bundle in the guinea-pig. Woollard (1926) states that intracardiac ganglia in the dog, cat, rabbit and guinea-pig (but compare findings of Meiklejohn mentioned above) are limited caudally by the coronary sulcus. Perman (1924) and Francillon (1928) failed to find subepicardial ganglia caudal to the coronary sulcus in the human heart. But, in the bovine heart, as stated above, nerve cells are present in both limbs of the atrio-ventricular bundle. Further, sections of the entire calf heart impregnated with silver are in the process of preparation, and a preliminary survey of those already cut has revealed numerous ganglia in the epicardium more than half way down the ventricles.

We noticed that the silver technique we employed tended to differentiate the nerve cells found in relation to the conducting system into two types: a generally larger type of rounded form and very finely stippled texture, which assumed a pale yellow colour, and a smaller type of somewhat angular form, which stained more densely with a deep brown or almost black coloration which prevented any study of the internal texture of the cell body. Often the small, darkly staining cells showed branching dendrites. Both types of cell were to be seen in most collections of nerve cells in the heart. This recalled the histological differentiation of ganglion cell types described by Kiss (1932) on the basis of his prolonged osmic acid method of staining. Prof. Kiss himself was good enough to examine some of our specimens and agreed that the cell types we observed corresponded to those described by him. Further, Bacsich (1933), a colleague of Kiss, using the prolonged osmic acid technique has found these types of nerve cells in the coronary plexus and in the posterior wall of the right atrium of the pig. Although we draw attention to the similarity of the two types of nerve cells noted by us to those described by Kiss in ganglia elsewhere, we are at present unable to offer any views on any possible functional significance which this morphological difference may imply. We tried the experiment of photographing these nerve cells with infra-red sensitive plates (1933*a*) and found that the pale cells appeared as seen by the eye under the microscope, but the dark cells revealed a coarsely granular texture not otherwise visible (Plate VIII, figs. 30 and 31). Careful study of the infra-red photomicrographs and of enlargements of them showed that this "granularity" was of a reticular nature. Bacsich (1932) has analysed the coloration of the dark nerve cells by the osmic acid method of Kiss as being due to the presence of coarse lipoidal granules within the cells. It may be that the darkly staining reticulum observed in the dark cells with the silver technique and infra-red photography corresponds to a neurofibrillary network in the meshes of which lie the lipoidal granules stained by the osmic acid method.

Intricate terminal arborisations of nerve fibres could be made out in connection with some of the cells in the cardiac ganglia. But to trace precise

relationship, or lack of relationship between the nerve cells and the nerve fibres is very difficult if not impossible.

NERVE FIBRES IN RELATION TO THE CONDUCTING SYSTEM

All parts of the bovine conducting system were found to be profusely supplied with nerve fibres; in the human heart the nerves are much fewer but are not inconsiderable. The contrast was least in the sinu-atrial node and greatest in the atrio-ventricular bundle and its limbs. In both sinu-atrial and atrio-ventricular nodes in the bovine heart there is an abundance of fine nerve bundles or isolated nerve fibres twining amidst the nodal fibres; in the sinu-atrial node the nerves appear in part at any rate to be related to the ganglia in the vicinity, while in the case of the atrio-ventricular node large nerve trunks, connected with the adjacent ganglia, sweep forward in the lower part of the atrial septum to the node. The human sinu-atrial node is well supplied with nerve fibres, the atrio-ventricular node less so. The atrio-ventricular bundle and its right and left limbs in the bovine heart are so richly supplied with nerves—large fasciculi, finer pencils and isolated fibres—that they might truly be called neuromuscular bundles (Plate VI, fig. 21). On the other hand, the nerves in the human atrio-ventricular bundle and its limbs are much fewer and do not form large fasciculi, but run in very small bundles, or, more commonly, as individual fibres between the muscle fibres. Cohn (1909), using iron haematoxylin and van Gieson's stain, failed to find any nerves in the region of the atrio-ventricular node or bundle in monkeys. Meiklejohn (1913), however, noted a nerve supply to the atrio-ventricular bundle in the monkey (*Callitrix*) much finer than in Ungulates, but found only a few small nerve fibres in the human atrio-ventricular bundle. Wilson (1909) found abundant nerves in the atrio-ventricular bundle and its limbs in the calf, sheep and pig.

All the nerve fibres seen in the conducting system in both types of heart appear to be unmyelinated; in the large nerve bundles associated with the ganglia close to the sinu-atrial node, however, some myelinated fibres were seen. It must be remembered, however, that with silver impregnation it is difficult to distinguish between unmyelinated and finely myelinated fibres, particularly in longitudinal section.

A detailed search was made for possible nerve endings in the conducting system muscle, in particular for intra-protoplasmic endings such as described, for example, by Boeke (1932), Woollard (1926) and Tudor Jones (1927) in the ordinary myocardium, and by Boeke (1932) in the muscle fibres of the atrio-ventricular bundle. But the most careful study has failed to reveal in our specimens any such ending within a muscle fibre of the conducting system. This is, of course, no positive proof that such do not exist. But our technique, in our best preparations, did reveal intra-protoplasmic nerve endings in the plain muscle fibres of the tunica media of branches of the coronary arteries in the bovine heart (1933*b*), so that we feel justified in placing some weight on

our negative findings in respect of the conducting system. In every case where at first sight a nerve fibre seemed as if it might be penetrating a muscle fibre, we each of us made careful independent examination with a binocular microscope and in every case agreed that the nerve fibre did not penetrate into the striated portion of the muscle fibre. It is of interest to note that Woollard, in his remarks made when our present work was communicated to the Anatomical Society of Great Britain and Ireland, then stated that his general impression was that the nerve endings were invariably pericellular in position (*J. Anat.*, Lond., 1933, vol. LXVIII, *Proc. Anat. Soc.*, p. 151).

It may be noted in this place that P. Stöhr, Jr. (1932) has been unable, after studying hundreds of specimens, to demonstrate any sign of nerve fibres within the muscular coat of arteries, and asserts that this agrees with the results of most authors. So far as the branches of the coronary arteries of the bovine heart are concerned, the present technique certainly demonstrates a rich muscular innervation of the media. We have also seen clearly the endings of these nerves in the form of a paranuclear loop (Plate VII, fig. 27) similar to that described in relation to the plain muscle of the ciliary body by Boeke (1932), and that of the gastro-intestinal tract by Lawrentjew (1926), Stöhr, Jr. (1932) and Hill (1927).

One complicating factor in studying the ultimate nerve-muscle relationships in the conducting system is that the capillary blood vessels in the system, themselves very plentiful and closely applied to the muscle fibres, are accompanied by fine nerve fibres. As the use of a pyridene bath in a silver technique tends to diminish the impregnation of capillary endothelium, great care must be taken to determine that a nerve fibre, suspected of ending in connection with a muscle fibre, is not really a capillary nerve. Our observations in respect of nerve endings have invariably been checked by examination with a Zeiss binocular microscope and one-twelfth inch apochromatic objective.

We find that ultimate nerve-muscle relationships in the conducting system of the bovine heart which can be considered as true nerve endings are of two quite different types. One of these has been described by several previous authors, and is readily seen in the atrio-ventricular bundle of the ox, where with our technique the large clear Purkinje fibres form a pale yellowish background against which it is easily seen. It takes the form of a network of extremely delicate nerve fibres wrapped round the Purkinje fibre and in immediate contact with it. The nerve fibres show no varicosities, nor are any nuclei seen in special relation to the network. Although clearly seen, this network may lie in relation to a small part only of a Purkinje fibre; in fact, long lengths of Purkinje fibres are devoid of any perifibrillar nerve network. A similar nerve network was observed in relation to the nodal fibres, both sinu-atrial and atrio-ventricular. We are satisfied that such a perifibrillar network is entirely on the surface of the muscle fibre (i.e. epilemmal) and sends no processes into the interior of the muscle fibre. Its arrangement suggests the possibility of afferent function.

The second type of nerve ending which we have made out, we suggest may be the true motor nerve ending of the bundle or nodal fibre (Plate VII, fig. 24). It is formed by a single unmyelinated nerve fibre, usually slightly beaded and finely varicose, which runs close alongside and parallel with the muscle fibre for some distance, and then ends in the centre of a group of rounded or oval nuclei. These nuclei are set in a yellow-coloured protoplasm which seems to be directly continuous with the cytoplasm of the muscle fibre, but the cross-striations of the muscle fibre do not extend into this nucleated zone. When such an ending is seen in profile in a longitudinal section, the nucleated zone appears as an elongated slight bulging on the side of the muscle fibre. Beyond this localised region, the straight edge of the muscle fibre has a definite limiting "line" of slightly darker tint, which is not an optical effect only, but suggests the presence of a definite sarcolemma. In the region of the bulging, this sarcolemma cannot be identified, so that it is difficult to trace the exact boundary of the pale-stained nucleated mass on its free surface; but since it seems to be directly continuous deeply with the cytoplasm of the muscle fibre, it is presumably hypolemmal in nature. We could not observe, however, any evidence of penetration of branches of the nerve into the muscle fibre to become continuous with the cross-striations as Tudor Jones (1927) has described in the case of the ordinary cardiac muscle. Within the clear cytoplasm of the bulging zone, the nerve takes a slightly angulated course amidst the nuclei and shows slight branching by means of very short side processes at the angulations. No trace was seen of a periterminal network as described by Boeke. The whole structure recalls the motor end-plate of skeletal muscle; we would characterise it as a greatly elongated nerve ending of simple filiform type in a richly nucleated sole-plate. Our observation that some of the long Purkinje fibres have multiple nuclei disposed like those of skeletal muscle strengthens the possibility that the motor nerve endings in the two types of muscle may approximate in structural arrangement. Meiklejohn (1913) has described nerve fibres ending in relation to groups of nuclei, some of which she thinks may be muscle nuclei of the nodes or bundle. Undoubtedly some of these endings described and figured by her are endings in the connective tissue of the conducting system, such as we have ourselves noticed. But we think that others may be of the nature of sole-plates similar to those we have just described, although she does not clearly indicate the relationship to the muscle fibre cytoplasm.

In the human heart we could not find the fine perifibrillar network we observed in the bovine heart. This may be due to failure of impregnation of these very fine nerve fibres, as the freshest human material we used was obtained 6 hours post-mortem. But we have observed numerous extremely fine isolated nerve fibres twining amidst the muscle fibres of the conducting system and in intimate relation to their surface. Fresher material has recently been obtained for further study of this question. We did, however, observe in relation to the muscle fibres of the human atrio-ventricular bundle, single nerve

fibres running on the surface of a muscle fibre and, after becoming extremely fine, ending in a terminal knob apparently in a slight unstriated bulging of the surface of the muscle fibre (Plate VII, fig. 25). It resembled a diminutive arrangement of what we had observed in the bovine heart, but without the nest of nuclei, and may possibly be an even simpler type of motor nerve ending in the human heart. In relation to the coronary arteries in the human heart, we observed many nerve fibres penetrating the media, but, probably for the reason given above, we could not make out with certainty penetration of the nerve endings into the plain muscle fibres.

In both bovine and human hearts the capillary blood vessels were observed to be accompanied by nerve fibres, often in the form of a plexus. From these pericapillary plexuses nerve fibres were often seen to pass to the muscle fibres of the conducting system.

CONCLUDING REMARKS

Before concluding this paper we would like to refer to two points in connection with the use of the term Purkinje fibres with which we have been impressed by our study of the literature in conjunction with our own observations.

In the first place, the term Purkinje fibre has been used in different connections by different workers. On the one hand, it has been used to cover all the muscle fibres in the conducting system which itself is referred to as the Purkinje system. On the other hand, the term has been restricted to large muscle fibres such as are seen, for example, in the atrio-ventricular bundle of the ox, and which are specially characterised by the irregular and incomplete distribution within the fibre of cross-striated myofibrillae; in the present paper we have used in this sense the expression "typical" Purkinje fibres, and we think the use of the term Purkinje fibre should always be thus limited.

In the second place, in comparing our observations on the human and bovine conducting systems we have been struck by the marked difference in appearance of the muscle fibres in the atrio-ventricular bundles of these two types; the term "typical" Purkinje fibres would be applicable to the bovine bundle but not to the human, yet the two must be regarded as homologous. Therefore the term in its restricted use is not always applicable to corresponding parts of the conducting system in different types.

We think it important to call attention to these two points because if they are not kept in mind, reports of the presence or absence of Purkinje fibres in different parts of the heart may be misinterpreted. For example, a statement that no Purkinje fibres are present in the atria of a particular type of heart does not necessarily imply that no ramifications of the conducting system are present in those atria. We have already stressed our view that continuity with a known part of the conducting system, one or other of the nodes or the atrio-ventricular bundle or its limbs, is the real criterion of conducting system fibres, and not peculiarities of histological appearance.

In conclusion, we would like to say that we are fully conscious of the incomplete nature of our present work due to the limitations imposed by the study of blocks and dissections. On this account we have not attempted to give in this paper complete topographical descriptions of the nodes, the atrio-ventricular bundle or its limbs; we have not described the distribution of the limbs of the bundle; and we have not fully surveyed the extent of the sinu-atrial node or the possibility of internodal connections in the atria. We hope to undertake further study of these matters in sections of entire hearts which are now being prepared.

SUMMARY

1. The conducting system of the human and bovine hearts has been studied with the aid of a pyridine silver histological technique evolved by experimental modification so as to secure good impregnation of nerve fibres and at the same time clear differentiation of striated muscle fibres with the minimal amount of shrinkage, even in large blocks.

2. The technique employed emphasises the undoubted muscular nature of both sinu-atrial and atrio-ventricular nodes.

3. The extent of the sinu-atrial node beyond the customarily recognised limits is noted, and its bearing on experimental work in this region of the heart is discussed.

4. The connections of the sinu-atrial nodal fibres with the sinus and atrial musculature and with the musculature of the nodal artery and its branches are described.

5. In the bovine heart, continuity of the sinu-atrial nodal fibres with large "typical" Purkinje fibres, and of these in turn with ordinary atrial muscle fibres, is described, and a parallel drawn with the condition previously observed in the avian heart.

6. A description is given of the structure of the atrio-ventricular node, of the atrial prolongations of the node, and of the continuity of the nodal fibres with those of the atrial myocardium and of the atrio-ventricular bundle.

7. The structure of the fibres of the atrio-ventricular bundle and the proximal parts of its limbs is described.

8. The relative sizes of the muscle fibres of the sinu-atrial node, atrio-ventricular node, atrio-ventricular bundle, atrial myocardium and ventricular myocardium are noted in the human and bovine heart.

9. The general arrangement of nerve cells in relation to the conducting system is noted, and the occurrence of pale and dark staining types (with the silver technique employed) is observed.

10. Two types of nerve ending are noted in relation to the conducting system muscle fibres:

(a) A fine perifibrillar network, epilemmal in position, and possibly of afferent function.

(b) A simple filiform ending, hypolemmal in position, somewhat similar to the motor nerve ending of skeletal muscle, which may be the motor nerve ending of the conducting system fibres.

11. The presence of nerve fibres with intra-protoplasmic endings has been demonstrated in the tunica media of branches of the coronary arteries.

12. The use of the term Purkinje fibres is discussed.

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EXPLANATION OF PLATES

(All figures are untouched photomicrographs)

PLATE I

- Fig. 1. Sinu-atrial node. Ox. $\times 15$. Shows extent of node from epicardium (above) to endocardium (below), with sinus muscle on right and atrial muscle on left.
 Fig. 2. Sinu-atrial node. Human. $\times 46$. Shows extent of node from epicardium on right to endocardium on left below, with sinus muscle at lower edge and atrial muscle above and on left.
 Figs. 3 and 4. Sinu-atrial node. Ox. $\times 100$. Fig. 3 shows subepicardial nodal fibres and Fig. 4 subendocardial nodal fibres from same section.

PLATE II

- Fig. 5. Artery of sinu-atrial node. Ox. $\times 115$. Shows nodal fibres encircling artery.
 Fig. 6. Sector of artery of sinu-atrial node. Ox. $\times 730$. Shows striated nodal fibres invading media (on right).
 Fig. 7. Sector of artery of sinu-atrial node. Human. $\times 400$. Shows striated nodal fibres approaching media (lumen below on left).
 Fig. 8. Right atrium in vicinity of sinu-atrial node. Ox. $\times 100$. Shows tongues of atrial muscle extending amongst plain muscle of endocardium (on right).

PLATE III

- Fig. 9. Sinu-atrial node. Human (14 years). $\times 400$.
 Fig. 10. Atrio-ventricular node. Human (14 years). $\times 400$.
 Fig. 11. Atrio-ventricular bundle. Human (14 years). $\times 400$.
 Fig. 12. Ventricular muscle. Human (14 years). $\times 400$.

PLATE IV

- Fig. 13. Atrial muscle. Human (14 years). $\times 400$.
 Fig. 14. Edge of sinu-atrial node. Ox. $\times 460$. Shows a nodal fibre lying horizontally in centre of figure establishing continuity by side processes with atrial myocardial fibres above it.
 Fig. 15. Right atrium near sinu-atrial node. Ox. $\times 100$. Shows Purkinje fibres in epicardium (below and to right) and extending (above) between bundles of atrial muscle.
 Fig. 16. Similar region to fig. 15. Ox. $\times 260$. Shows epicardial Purkinje fibres becoming continuous above with atrial muscle fibres.

PLATE V

- Fig. 17. Sinu-atrial node. Ox. $\times 400$.
 Fig. 18. Atrio-ventricular node. Ox. $\times 400$.
 Fig. 19. Junction of atrio-ventricular node (above) with atrio-ventricular bundle (below). Ox. $\times 144$.

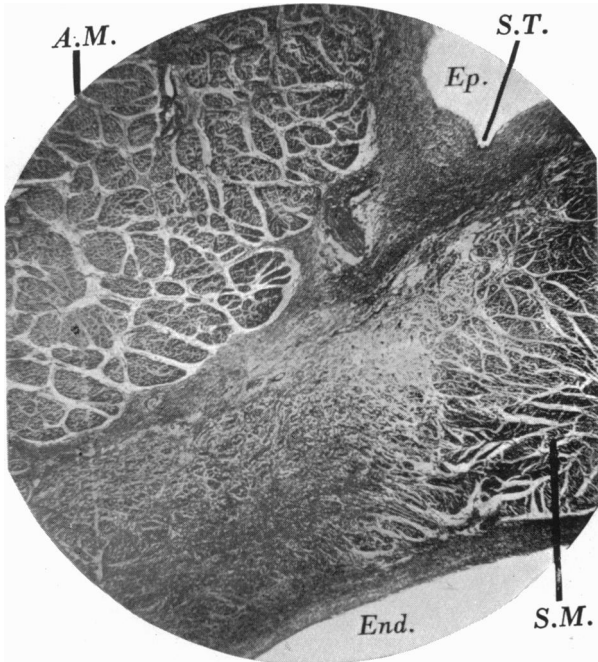


Fig. 1.

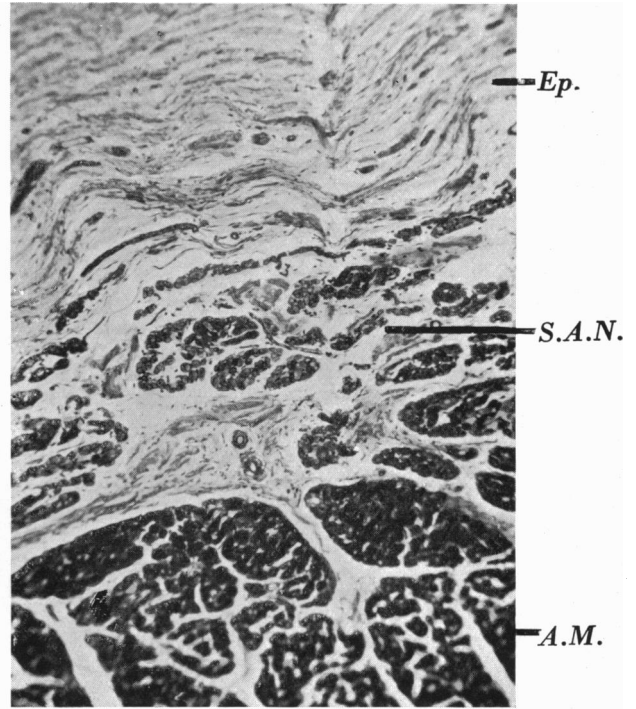


Fig. 3.

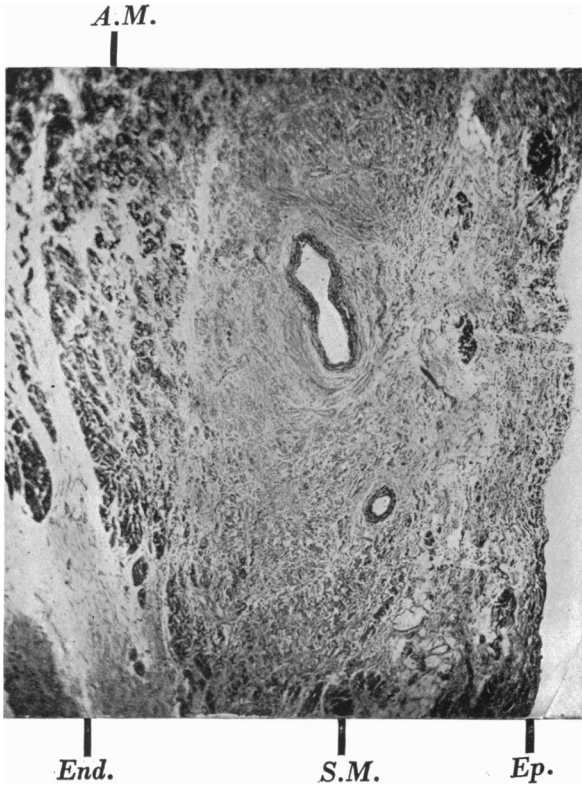


Fig. 2.

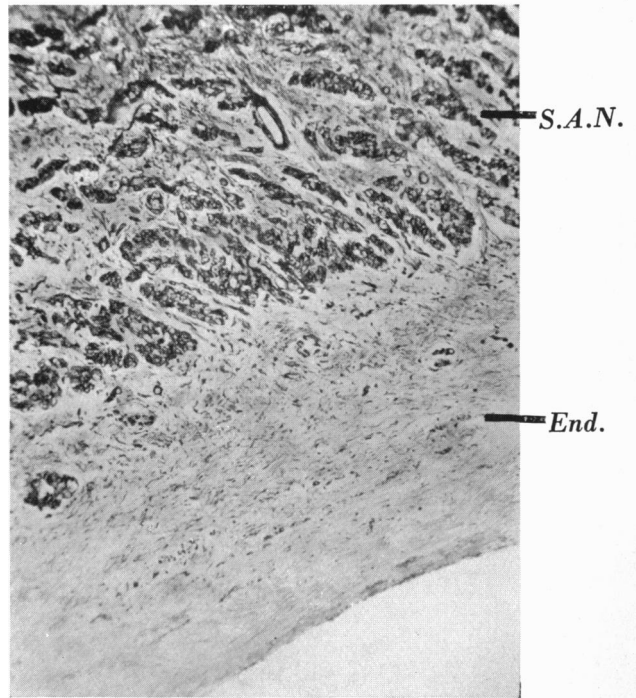


Fig. 4.

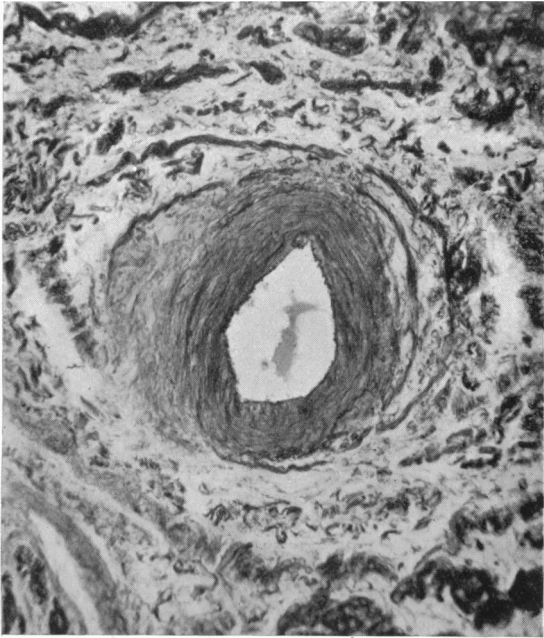


Fig. 5.

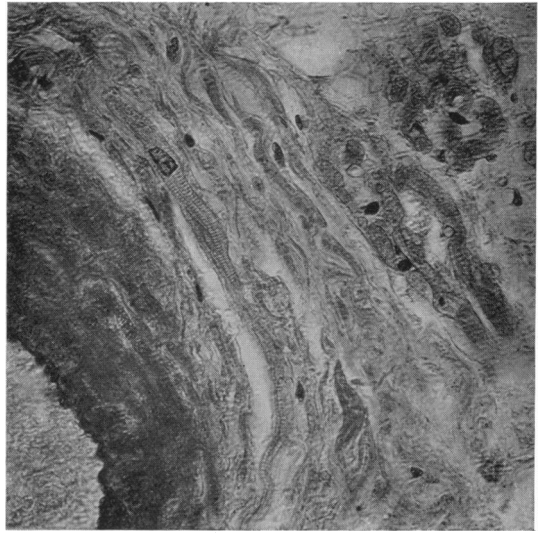


Fig. 7.



Fig. 6.

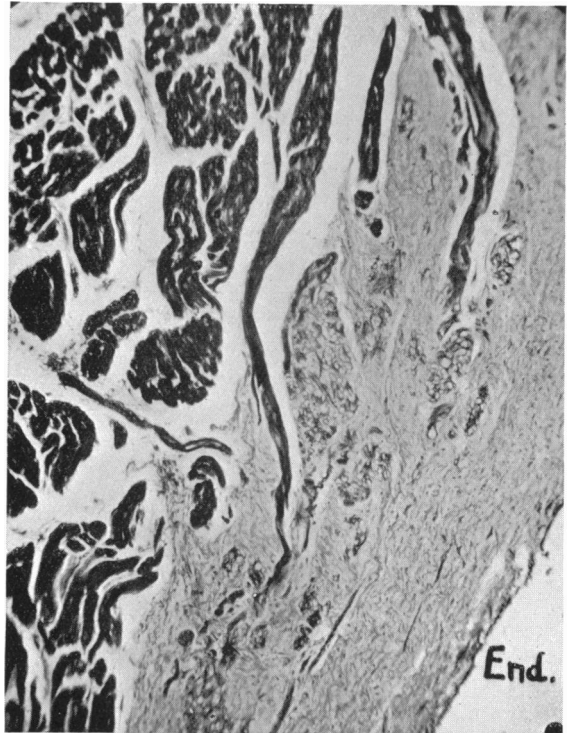


Fig. 8.

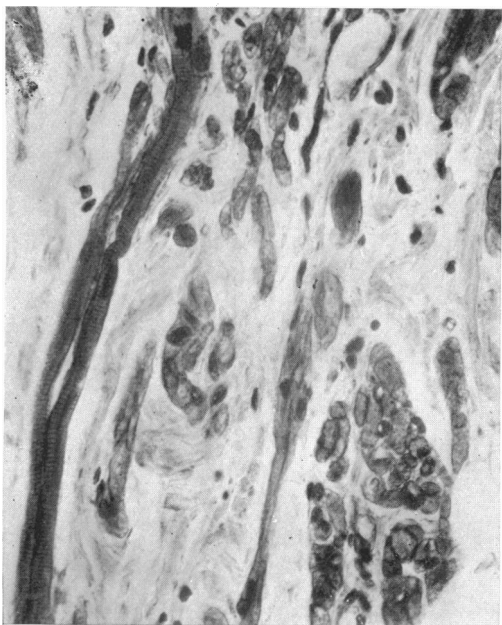


Fig. 9.



Fig. 10.



Fig. 11.



Fig. 12.



Fig. 13.

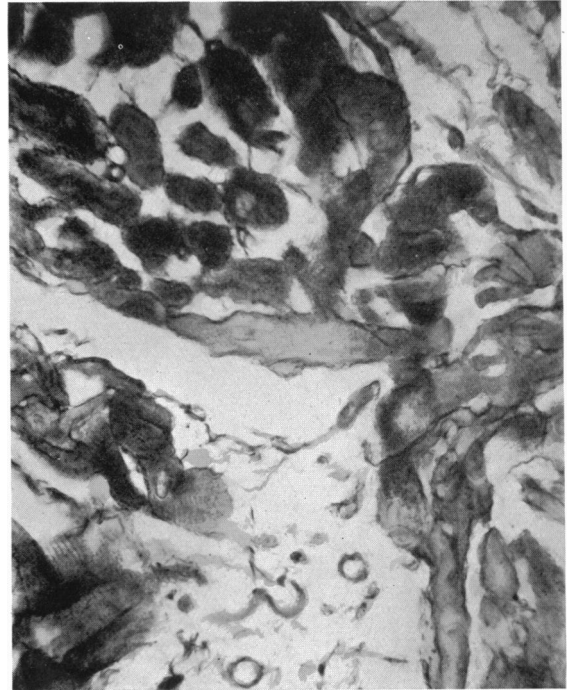


Fig. 14.

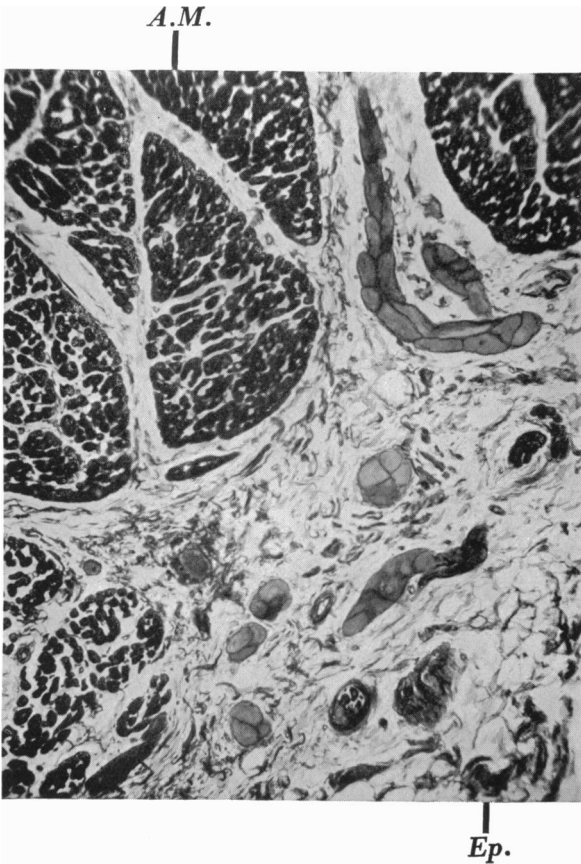


Fig. 15.



Fig. 16.

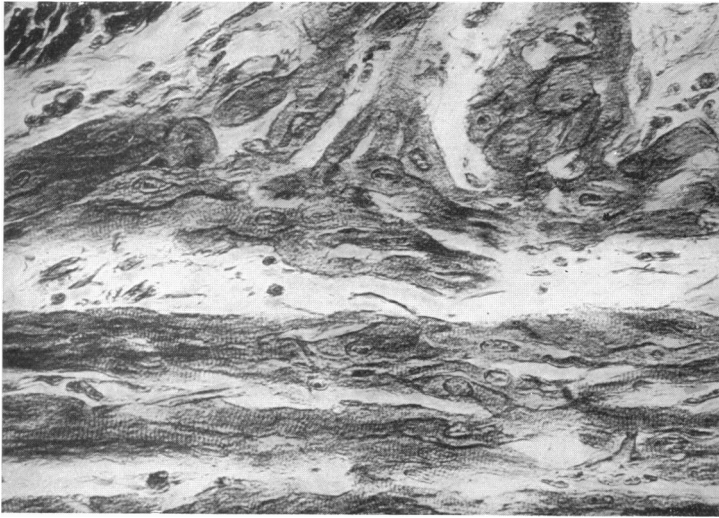


Fig. 17.



Fig. 18.

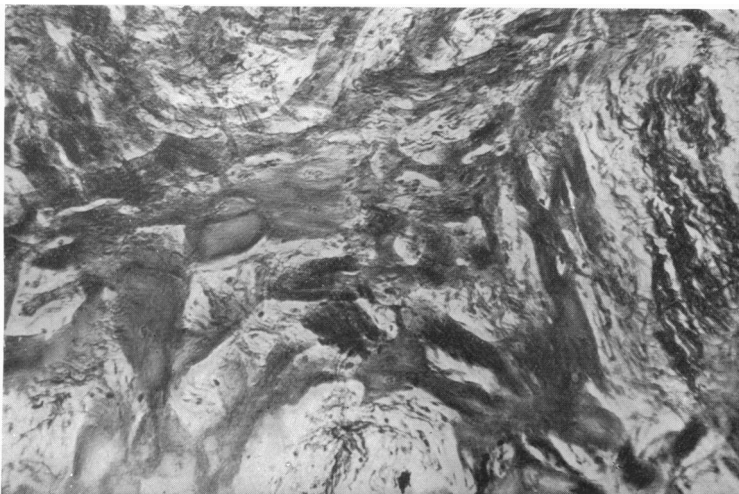


Fig. 19.

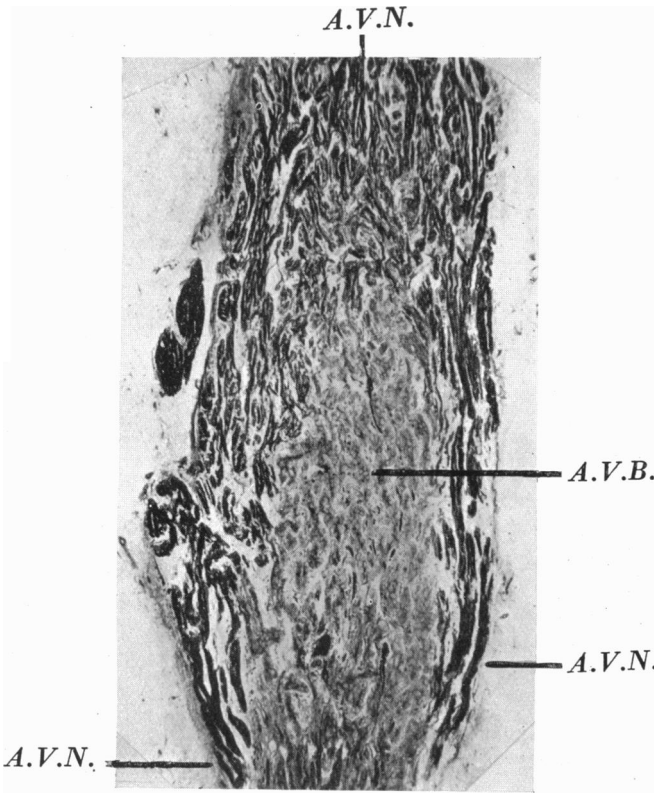


Fig. 20.



Fig. 21.

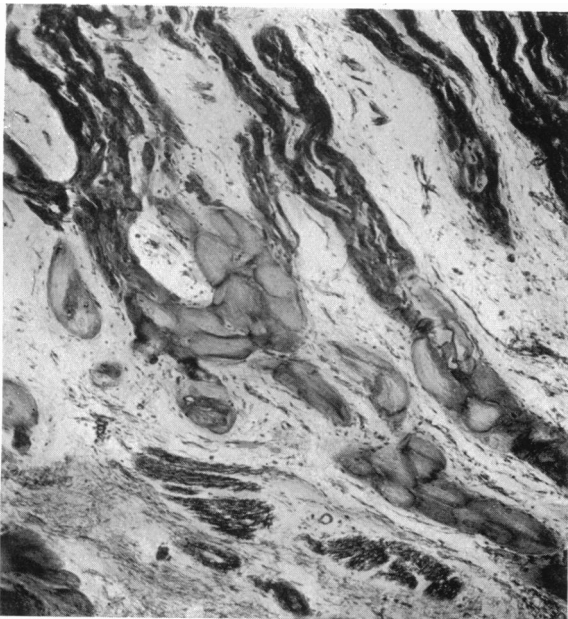


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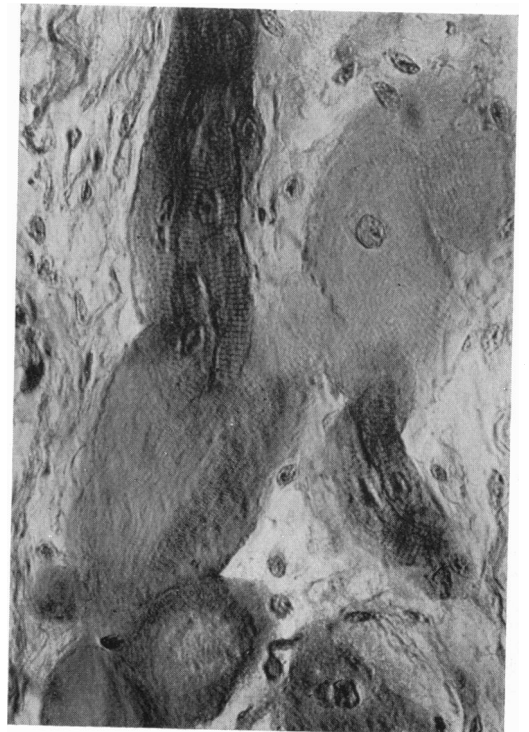


Fig. 23.

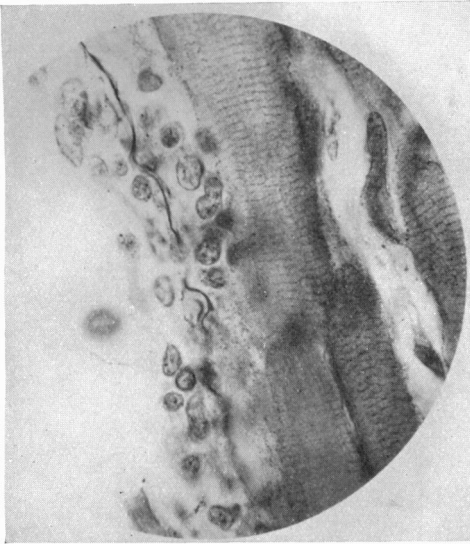


Fig. 24.



Fig. 25.



Fig. 26.

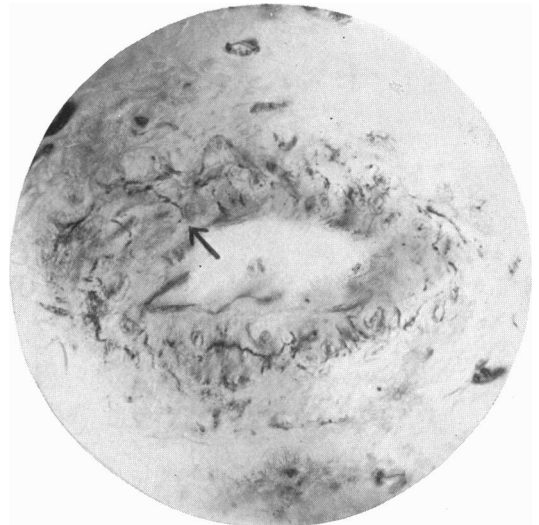


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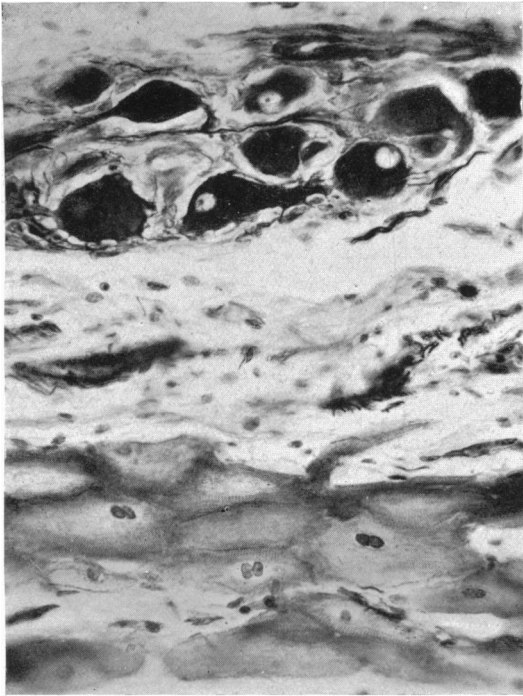


Fig. 28.

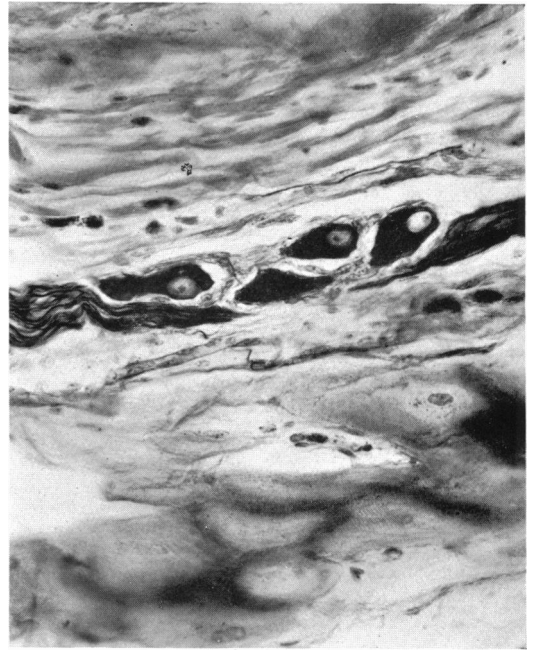


Fig. 29.

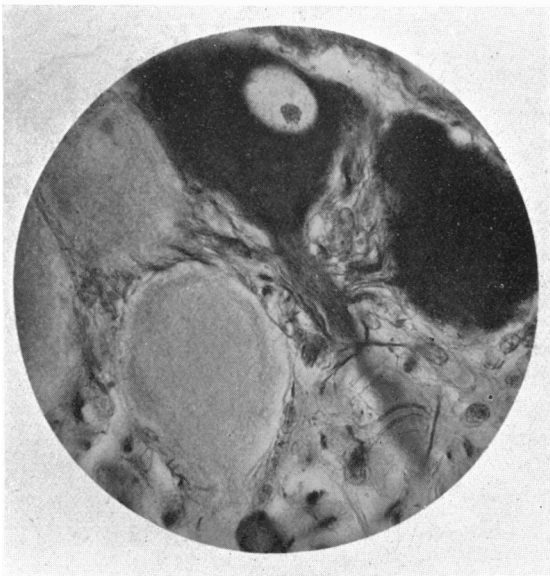


Fig. 30.

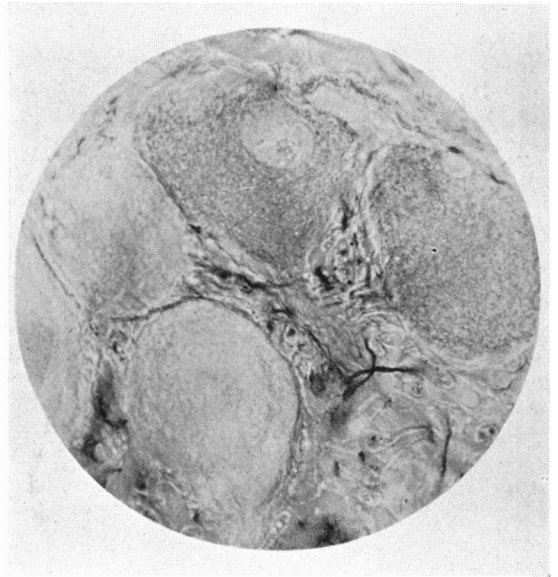


Fig. 31.

PLATE VI

- Fig. 20. Origin of atrio-ventricular bundle (pale fibres) from centre of atrio-ventricular node (dark fibres). Ox (dissected specimen). $\times 15$.
 Fig. 21. Atrio-ventricular bundle. Ox. $\times 40$. Shows Purkinje fibres, nerve fibres and nerve cells.
 Fig. 22. Junction of atrio-ventricular node and bundle. Ox. $\times 90$.
 Fig. 23. Junction of atrio-ventricular nodal fibres with a Purkinje fibre of bundle. Ox. $\times 500$.

PLATE VII

- Fig. 24. Atrio-ventricular bundle. Ox. $\times 900$. Shows nerve fibre ending amidst group of nuclei in relation to Purkinje fibre (see text).
 Fig. 25. Atrio-ventricular bundle. Human. $\times 1100$. Shows fine nerve fibre in upper half of figure running downwards with sinuous course between bundle fibres and ending in terminal enlargement on left side of a striated bundle fibre. Striations of muscle fibre can be seen, in a slightly different focal plane to that photographed, to approach still more closely to the nerve ending. (See text.)
 Fig. 26. Purkinje fibre from left limb of atrio-ventricular bundle. Ox. $\times 400$. Shows peripherally placed nuclei.
 Fig. 27. Branch of coronary artery. Ox. $\times 500$. Infra-red plate. Shows nerves in media with paranuclear terminal loop in upper left quadrant. (\uparrow .)

PLATE VIII

- Fig. 28. Left limb of atrio-ventricular bundle. Ox. $\times 260$.
 Fig. 29. Right limb of atrio-ventricular bundle. Ox. $\times 260$.
 Figs. 30 and 31. Nerve cells in wall of right atrium. Ox. $\times 700$. Fig. 30 panchromatic plate and fig. 31 infra-red plate. Shows dark and pale types of nerve cells.

ABBREVIATIONS USED IN FIGURES

<i>A.M.</i>	Atrial myocardium.	<i>Ep.</i>	Epicardium.
<i>A.V.B.</i>	Atrio-ventricular bundle.	<i>S.A.N.</i>	Sinu-atrial node.
<i>A.V.N.</i>	Atrio-ventricular node.	<i>S.M.</i>	Sinus myocardium.
<i>End.</i>	Endocardium.	<i>S.T.</i>	Sulcus terminalis.