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Rabbit heart nodal tissue, sinuatrial ring bundle and atrioventricular connexions identified as a neuromuscular system

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INTRODUCTION

The normal cardiac impulse is generated in pacemaker cells at the centre of the sinuatrial (S-A) node. It spreads radially within the node, excites the crista terminalis over a broad front and thereafter accelerates in two directions along this structure. In one direction, it passes down along the crista, round the openings of the inferior vena cava and coronary sinus to the atrioventricular (A-V) ring and the A-V node. In the other direction, it spreads to the left in front of the superior vena cava to where the crista terminalis meets the interatrial septum and interatrial (Bachmann's) bundle. From this site, the impulse continues into the interatrial septum and to the left atrium (Bachmann, 1916; Paes de Carvalho, De Mello & Hoffman, 1959; Horibe, 1961; Sano & Yamagishi, 1965; Wagner, Lazzara, Weiss & Hoffman, 1966; Spach *et al.* 1971). Although these pathways seem well established functionally it has been difficult to demonstrate them morphologically, and there is no identity between the descriptions either of the course of the pathways or of the cytological characteristics of the conducting cells (Paes de Carvalho *et al.* 1959; Robb & Petri, 1961; Truex & Smythe, 1965; James, 1967; Takayasu, 1967; Merideth & Titus, 1968; Emberson & Challice, 1970).

In the pig heart we have shown that the ChE-positive reaction of nerves in the nodal tissue and conducting system of the ventricles may be utilized to identify the specialized tissues (Bojsen-Møller & Tranum-Jensen, 1971*a*, *b*). In the present work the method was used to study the topography of the atrial tracts and atrioventricular connexions in whole mounts of the rabbit heart. Information from this mapping was used to secure a precise excision and orientation of tissue blocks for histological examination of the contained cells.

MATERIAL AND METHODS

The material comprised the hearts of 20 rabbits of both sexes (3–12 months old, body weight 1·3–3·5 kg). Both whole mounts and sections 2 μm thick were prepared from the hearts.

1. Block-staining for cholinesterase (ChE) was performed on 10 of the hearts. The animals were anaesthetized with an intravenous injection of Nembutal (about 60 mg per kg body weight). After thoracotomy, the hearts were stopped in diastole

by injecting about 20 ml KCl solution (150 mmol per l) into the inferior vena cava. They were then fixed by perfusion through the aorta and the coronary arteries of 500–800 ml 4 % formaldehyde dissolved in 0·125 M acetic acid/acetate buffer (pH 5·2). The hearts were opened as described by Paes de Carvalho (1961) and fixed further by immersion in the same fixative for three hours at 4 °C. They were then treated by a whole mount modification of Koelle's ChE-staining method as described earlier (Lewis, 1961; Bojsen-Møller & Tranum-Jensen, 1971 *a*, *b*). The specimens were examined under a stereomicroscope and photographed both before and after sulphide treatment. Five of the specimens were thereafter cleared in anise oil and examined. With the information thus obtained, well defined parts of the conducting system could be excised for histological examination. The excised blocks were dehydrated in ethanol, embedded in Epon and cut in 2 μm sections with a glass knife. The sections were stained with toluidine blue.

2. Supravital staining with methylene blue was performed on whole mounts of four hearts, using the method of Richardson (1969). The rabbits were given an intravenous injection of 3000 i.u. heparin and killed with a bolt pistol. The hearts were removed, cut open and, still beating, incubated for 45 minutes at 32 °C in a 0·002 % solution of methylene blue in phosphate/citric acid buffer. The incubation fluid was adjusted to pH 5·2, at which level both cholinergic and adrenergic nerves are stained (Richardson, 1969). Air was bubbled through the solution to maintain its oxygenation. Finally, the whole mounts were briefly rinsed in distilled water and fixed in an ice-cold 3 % solution of ammonium molybdate in phosphate/citric acid buffer, pH 5·5.

3. Perfusion fixation with glutaraldehyde was performed on the remaining six hearts in order to obtain optimum histological preservation. Under Nembutal anaesthesia and artificial ventilation through a tracheal tube, the hearts were stopped in diastole and immediately fixed by perfusion through the aorta and the coronary arteries and through the inferior vena cava. Outlet was provided by puncturing the right auricle and the pulmonary trunk. Three per cent glutaraldehyde in phosphate buffer, pH 7·2, passed through a cannula placed in retrograde position in the descending aorta, was used for the arterial perfusion. This was maintained for about 30 minutes at a flow of 50–80 ml/min. The hearts were then removed, cut open and fixed for a further 18 hours by immersion in the same fixative at 4 °C. Blocks measuring less than 3 mm were excised from well defined sites in the atria and the ventricles, post-fixed in 1 % OsO_4 in phosphate buffer (pH 7·2) for two hours and embedded in Epon after dehydration with ethanol. Sections 2 μm thick were stained with toluidine blue or paraphenylene diamine.

RESULTS

In the whole mounts stained for ChE a strong positive reaction was obtained in nerves and nerve cell bodies, while a weaker reaction was elicited in the specialized muscle cells and hardly any reaction was visible in the ordinary myocardial cells. In cleared specimens, the course of the nerves and the location of the nerve plexuses could thus be determined, and the topography of the sinuatrial node, sinuatrial ring bundle (SARB), atrioventricular node, and bundle of His and its crura could be visualized (Figs. 1, 13).

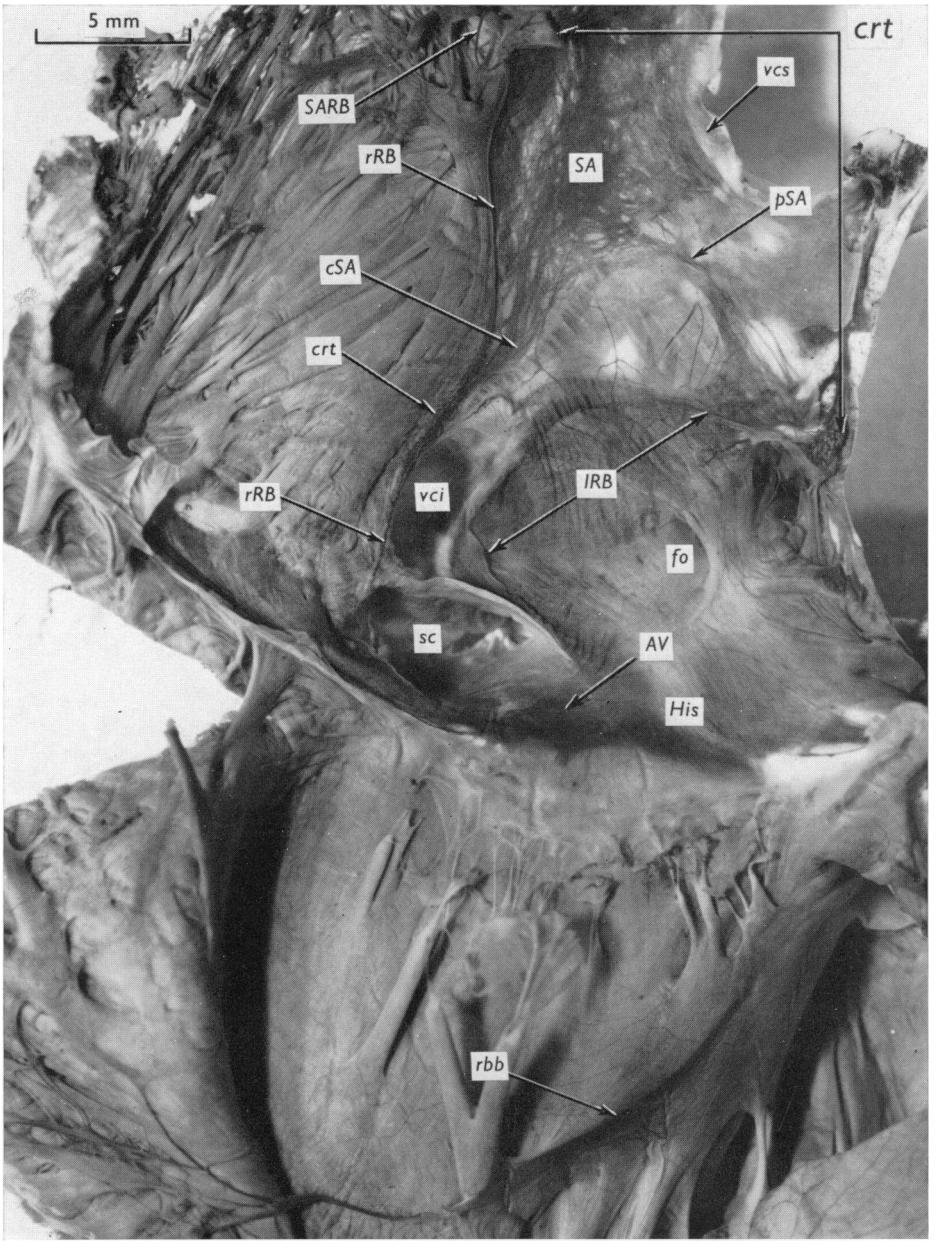


Fig. 1. ChE-stained preparation of the rabbit heart showing the localization of the specialized tissues. The right atrium and right ventricle have been cut open to expose the interatrial and the interventricular septa. The left branch of the crista terminalis is thereby divided at its junction with the interatrial septum and interatrial (Bachmann's) bundle. The S-A node, the specialized tissues along the crista terminalis, the A-V node, the bundle of His and the right bundle branch are arranged in the form of an S. In this heart the SARB runs as a free strand from the auricle to the crista terminalis. For list of abbreviations see p. 370.

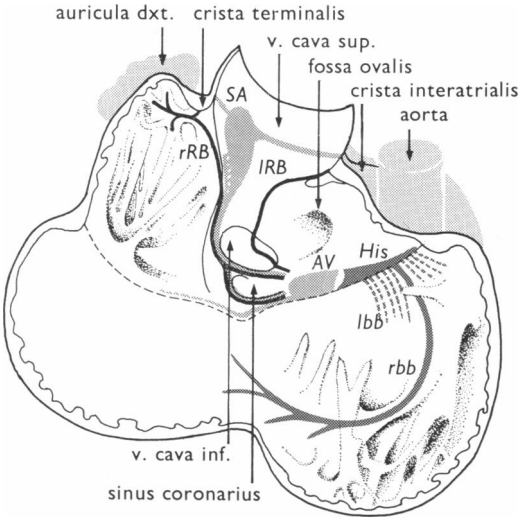


Fig. 2. Drawing of the specialized tissues in the right atrium and right ventricle.

LIST OF ABBREVIATIONS

SA sinuatrial node.
pSA the encircling fibres of the S-A node in the posterior wall of the superior vena cava.
cSA the cauda of the S-A node.
rRB right branch of the sinuatrial ring bundle.
lRB left branch of the sinuatrial ring bundle.
AV A-V node.
His bundle of His.
rbb right branch of the bundle of His.
lbb left branches of bundle of His.
vcs superior vena cava.
vci inferior vena cava.
sc coronary sinus.
crt crista terminalis.
ss sinus septum.
fo fossa ovalis.

After supravital staining with methylene blue, the nodes, SARB, and bundle of His with its crura were found to be stained, the most superficial structures most distinctly, i.e. the SARB and the distal part of the crura. The nerves were more intensely stained than the specialized muscle cells, so that the two tissues could be differentiated under the stereomicroscope.

The S-A node (Figs. 1, 2, 5, 6) is situated in the wall of the superior vena cava, the bulk of the node being found in the anterior wall at the junction with the auricle. It forms an oval, poorly demarcated body, the long axis of which is parallel to the sulcus terminalis. This part of the node measures 2–3 × 6–7 mm and has a thickness of about 200 μm. From the body of the node a process encircles almost completely the superior vena cava. The upper border of the node is situated subepicardially in the vein, whilst the lower border is related to the endocardium. At this site the node is in broad contact with the cells of the crista terminalis. From the lower border another process of nodal tissue passes down alongside the crista terminalis. This process, or

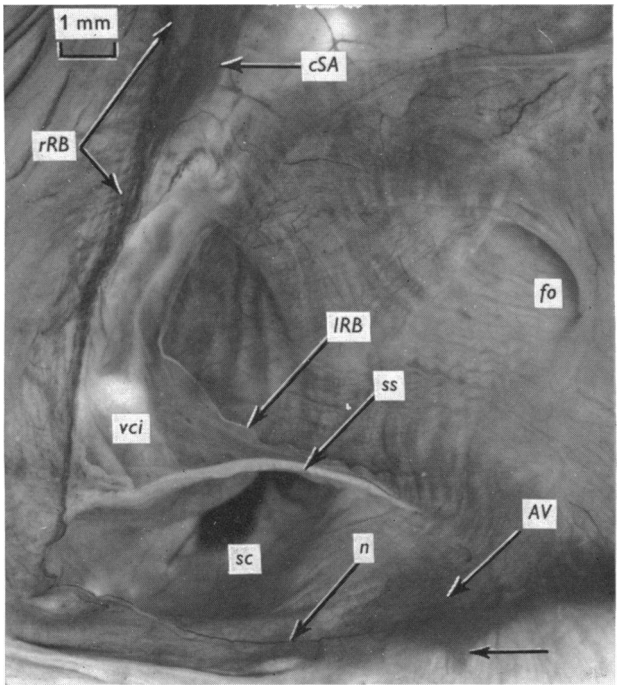


Fig. 3

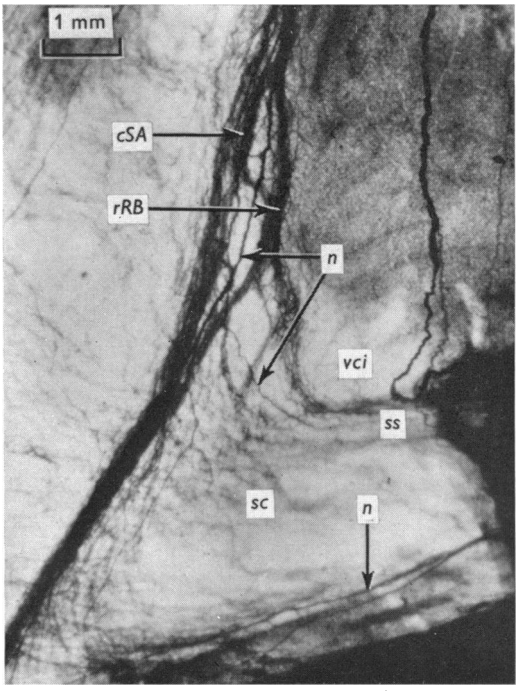


Fig. 4

Fig. 3. ChE-stained preparation showing the course of the distal parts of the right and left branch of the SARB and of the cauda of the S-A node. Detail from Fig. 1. Nerve fascicle (*n*) from the right branch of the SARB can be followed around the ostium of the coronary sinus and into the A-V node. From the lower border of the A-V node strands of specialized tissue (arrow) extend in the direction of the interventricular septum, cf. Figs. 14, 15.

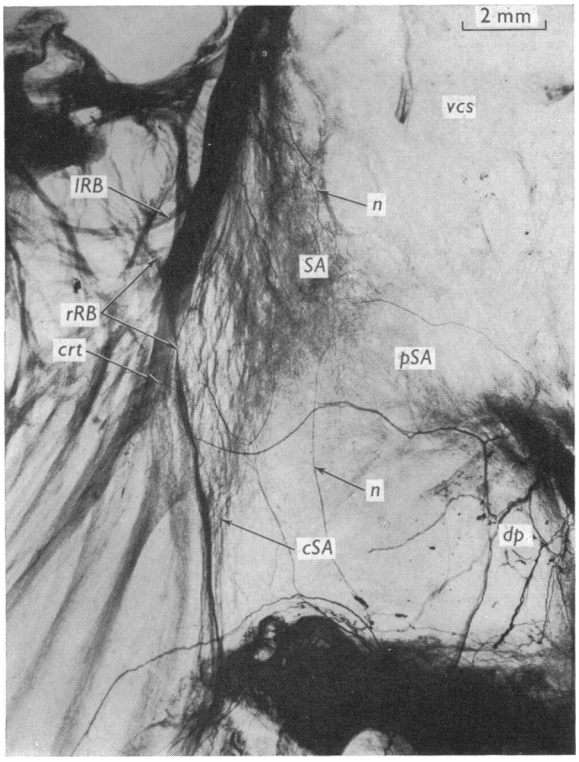
Fig. 4. ChE-stained and cleared whole mount of distal part of the right branch of the SARB and the cauda of the S-A node, cf. Fig. 3. Branches from both structures and their nerves (*n*) can be followed into the sinus septum and around the coronary sinus.

cauda, continues into the valve of the inferior vena cava and the valve of the coronary sinus and merges into the A-V ring just behind the A-V node. Just proximal to the ostium of the coronary sinus, a small branch is given off, which runs for a varying distance out into the so-called sinus septum separating the inferior vena cava and the coronary sinus (Fig. 4). The diameter of the cauda is initially about 500 μm, decreasing before the bifurcation to about 200 μm.

In the sections the S-A node and its processes can be seen to consist of a network of muscle fibres. These are pale cells with boundaries which are difficult to resolve. In longitudinal sections, only a weak, irregular cross-striation is seen. The diameter of the cells is 1–4 μm. A network of ChE-positive nerves and fine nerve branches invests the muscle cells.

The node receives ChE-positive nerves from along the superior vena cava and from a ganglion-containing plexus on the base of the heart. From this plexus the nerves pass both anteriorly and posteriorly around the superior vena cava to the node.

A sinus node artery was found in a number of hearts (Fig. 6).



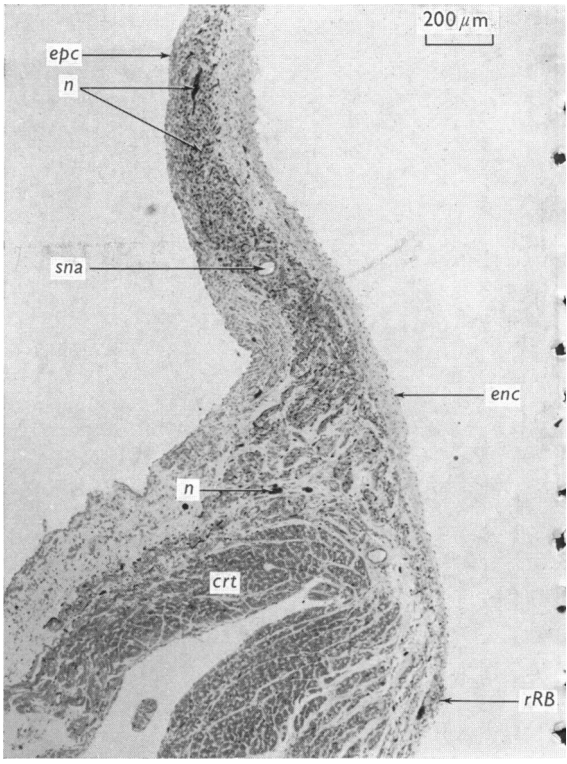


Fig. 5

Fig. 6

Fig. 5. ChE-stained and cleared whole mount of the S-A node and surrounding tissues. ChE-positive nerves (*n*) are seen passing from the ganglion-containing dorsal plexus (*dp*) to the S-A node. The right and left branches of the SARB each pass in a different direction from the auricle on to the crista terminalis. The right branch crosses the crista terminalis and joins the cauda of the S-A node.

Fig. 6. Section through the S-A node. 2 μm section stained with ChE and toluidine blue. The cranial margin of the sinus node lies under the epicardium (*epc*) in the superior vena cava. The opposite border lies under the endocardium (*enc*) and in broad contact with the cells of the crista terminalis. ChE-positive nerves (*n*) are seen everywhere between the muscle cells of the sinus node. In the centre of the node is the sinus node artery (*sna*).

The sinuatrial ring bundle (SARB) is a bundle of muscle cells and ChE-positive nerves situated in a rudiment of the two venous valves which in the embryo separate the sinus venosus from the atrium proprium. These valves fuse in front of the superior vena cava to form the septum spurium. In the adult heart, a pectinate muscle is found to connect the roof of the auricle with the crista terminalis at the site of the septum spurium. The SARB passes alongside this pectinate muscle either embedded in its surface or as a free strand (Figs. 1, 2, 5). It was not possible with the techniques applied to point out any site of origin in the auricle. At the crista terminalis the SARB divides into a right and a left branch.

The right branch (Figs. 3, 4, 5, 7, 8) passes from the auricle out on to the crista terminalis, which it crosses to take up a position over the cauda of the S-A node

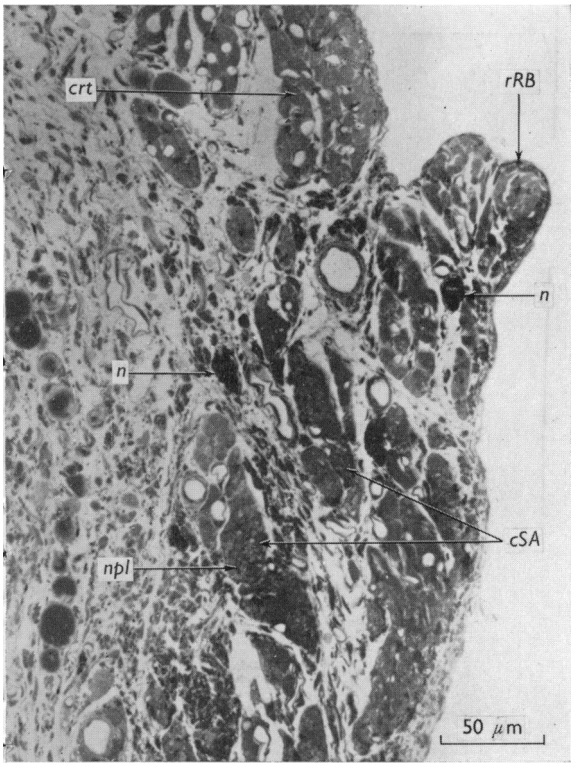


Fig. 7

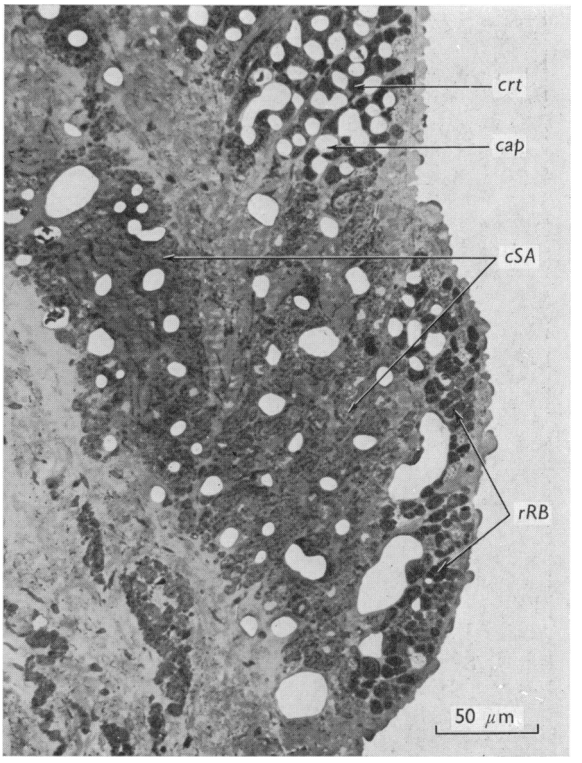


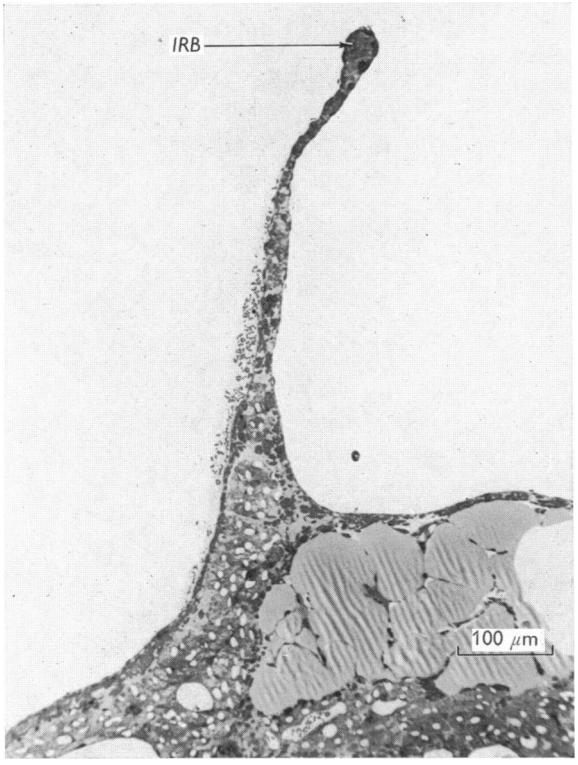
Fig. 8

Fig. 7. Section through the upper part of the crista terminalis. 2 μm section stained with ChE and toluidine blue. ChE-positive nerve plexuses (*npl*) and nerve fascicles (*n*) are seen in connexion with the SARB and the cauda of the S-A node.

Fig. 8. Section through the crista terminalis just above the ostium of the inferior vena cava. Paraphenylene diamine stained 2 μm section of specimen fixed by glutaraldehyde perfusion. Three kinds of muscle cells are seen: (1) ordinary atrial muscle cells of the crista terminalis, (2) small compact muscle cells forming a bundle 4–5 cells thick under the endocardium, constituting the right branch of the SARB, and (3) small muscle cells with ill-defined cell borders comprising the cauda of the S-A node. The latter two form a small elevation running parallel to the crista terminalis. *cap*: capillary, open because of the fixation by perfusion.

as a thin layer, 4–5 cells thick, between the cauda and the endocardium. With the cauda of the S-A node, it passes into the valve of the inferior vena cava and the valve of the coronary sinus, giving off a fascicle to the sinus septum. Muscle fibres in both terminal branches can be traced until they disperse at the A-V node, while the nerves can often be followed into the node itself (Fig. 3).

The left branch (Figs. 3, 9, 10) of the SARB is somewhat thinner than the right branch. On the crista terminalis it turns to the left in front of the superior vena cava to continue on the interatrial septum, in the rudiment of the venous valve which is seen here as a little endocardial fold. It courses in a posteriorly convex curve behind the fossa ovalis to the medial wall of the inferior vena cava. It may pass for a varying distance down into this vein, but thereafter it runs forward to the medial aspect of the



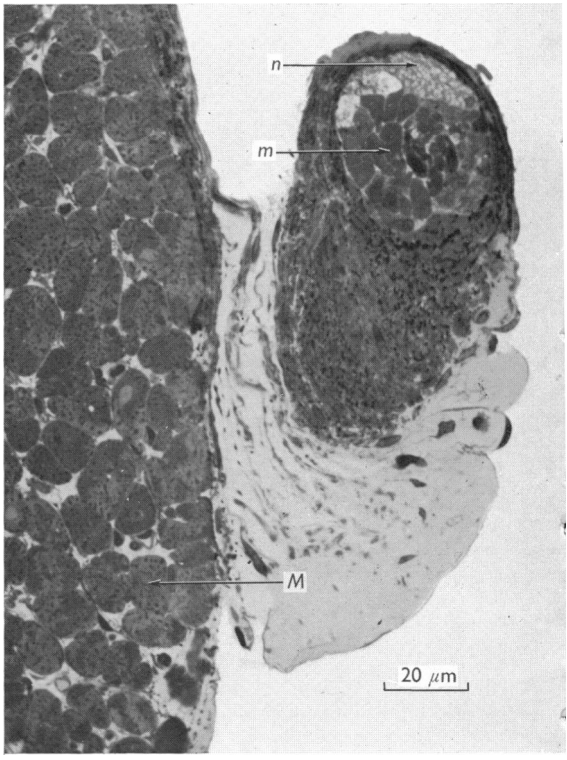


Fig. 9 Fig. 10

Fig. 9. Section through the proximal part of the left branch of the SARB. 2 μm section stained with toluidine blue. The cells constituting the left branch of the SARB lie at the tip of an endocardial fold representing the vestigial left venous valve.

Fig. 10. Section through the distal part of the left branch of the SARB. Toluidine blue 2 μm section of a specimen fixed by glutaraldehyde perfusion. In the vestigial valve small compact muscle cells (*m*) and a nerve fascicle (*n*) are seen invested with a sheath of dense connective tissue. *M*: ordinary myocardial cells.

sinus septum. Here it continues in company with the fascicle from the right branch of the SARB in the direction of the A-V node.

The SARB can be discerned in the opened, unstained heart as a little elevation on the crista terminalis, and on the interatrial septum as an endocardial fold or a free strand.

Histologically, the SARB consists of muscle fibres and nerves (Figs. 8, 10). The muscle cells present a rather uniform morphology, only 3–5 μm in diameter, often closely packed and, if so, of polygonal cross-section. The cells contain abundant myofibrils in regular array, and appear therefore very compact when compared with the nodal cells. They usually have one nucleus, but occasionally up to three in a row. Intercalated discs connect the cells. A weak ChE reaction was found in these muscle cells.

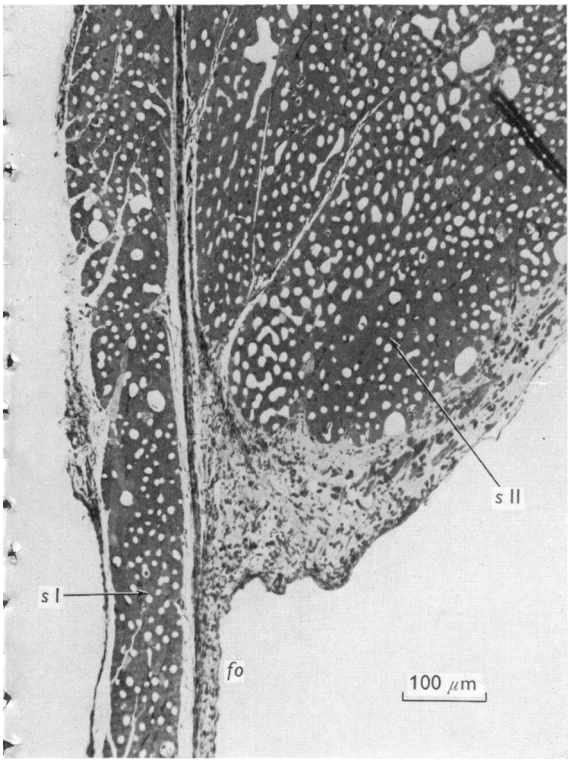


Fig. 11

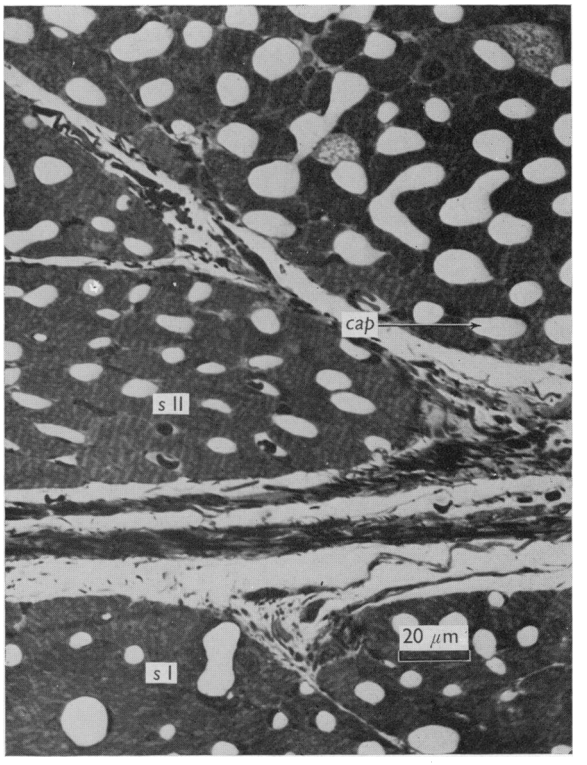


Fig. 12

Fig. 11. Section through the upper anterior part of the limbus of the fossa ovalis. Toluidine blue stained 2 μm section of specimen fixed by glutaraldehyde perfusion. The septum primum (*s* I) and the septum secundum (*s* II) are separated by a layer of dense connective tissue. Due to the parallel arrangement of the muscle fibres, regular cross-sections are seen.

Fig. 12. Detail of Fig. 11. The septum primum and the septum secundum consist of ordinary myocardial cells. In the upper right corner two light cells are seen. No Purkinje-like cells are present.

Numerous ChE-positive nerve fibres are found in the SARB between and in close relation to the muscle cells. In addition, both the right and the left branches are accompanied by one or several small nerve fascicles (Figs. 7, 10).

Dense connective tissue invest the SARB except at the crista terminalis, where the ring bundle cells are in contact with the underlying cauda of the S-A node (Figs. 8, 10).

The A-V node (Figs. 1, 13, 14) is situated just in front of the orifice of the coronary sinus between the anterior end of the sinus septum and the insertion of the septal cusp. The oblong node measures about 800 μm in diameter and about 1·5 mm in length. Anteriorly it merges smoothly into the bundle of His, and posteriorly it continues in a thin strand of nodal tissue coursing for a varying distance along the A-V ring.

The A-V node consists of muscle cells surrounded by a fine plexus of nerve fibres. In both of these tissues a ChE reaction is found. Several large nerves with ganglia

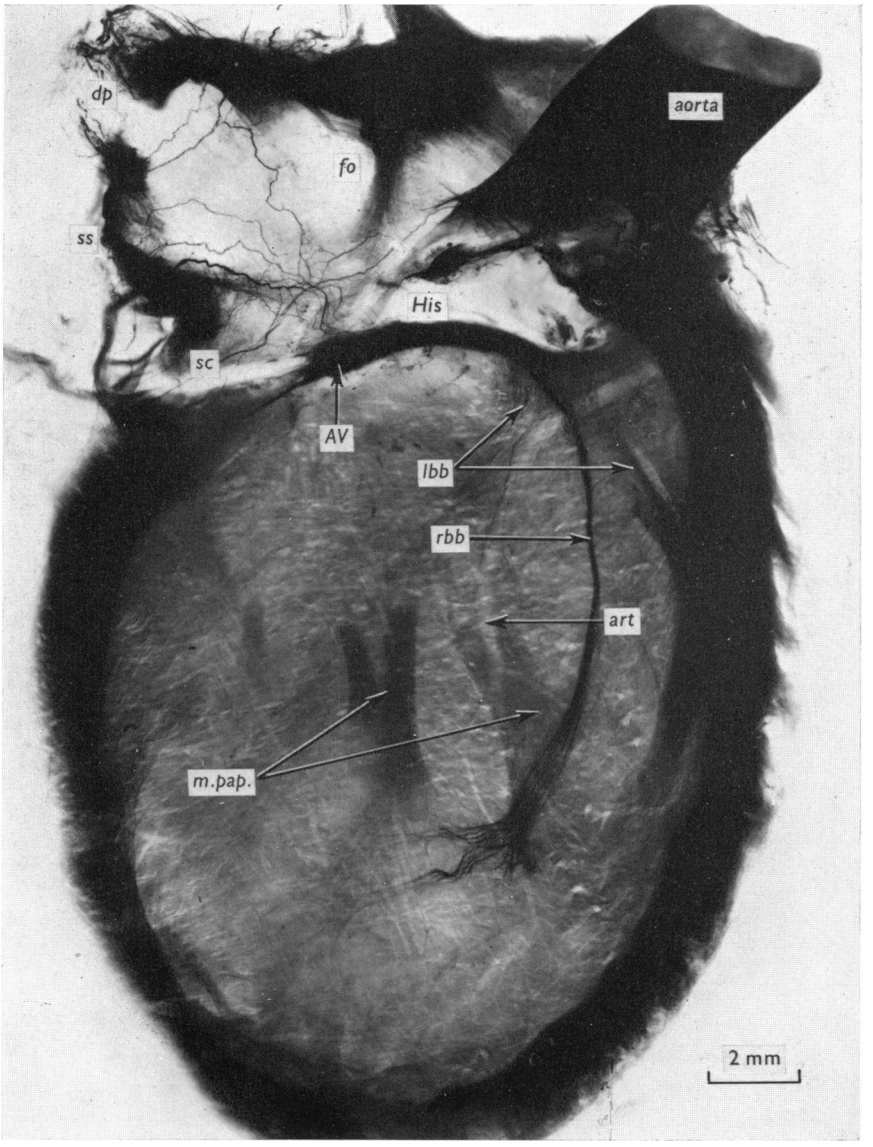
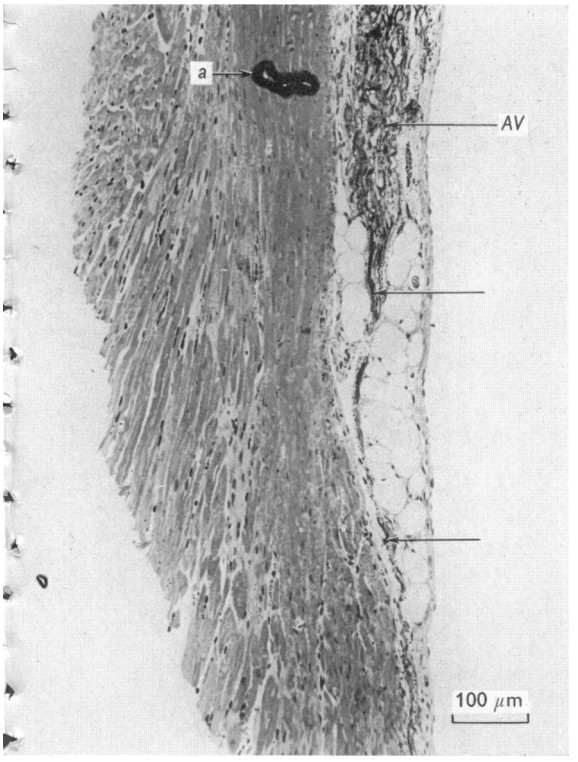


Fig. 13. ChE-stained and cleared whole mount of the interventricular and part of the interatrial septum. The aorta leaving the left ventricle is seen through the cleared tissue. The A-V node passes anteriorly into the bundle of His whilst it continues posteriorly in a thin process along the atrioventricular ring. The right bundle branch curves down over the right side of the septum to the base of the anterior papillary muscle. At this site it ramifies to the subendocardial plexus. The left bundle branches, given off in two diverging groups, can be seen through the septum. A perforating branch (*art*) from the anterior interventricular artery proceeds in the substance of the interventricular septum between the right and left bundle branches. Nerves pass from the dorsal plexus (*dp*) on the base of the atria along the sinus septum to the atrial margin of the A-V node and bundle of His (see also Fig. 16). The node receives further nerves from the plexus surrounding the aorta. *m.pap.*: papillary muscles of the right ventricle.



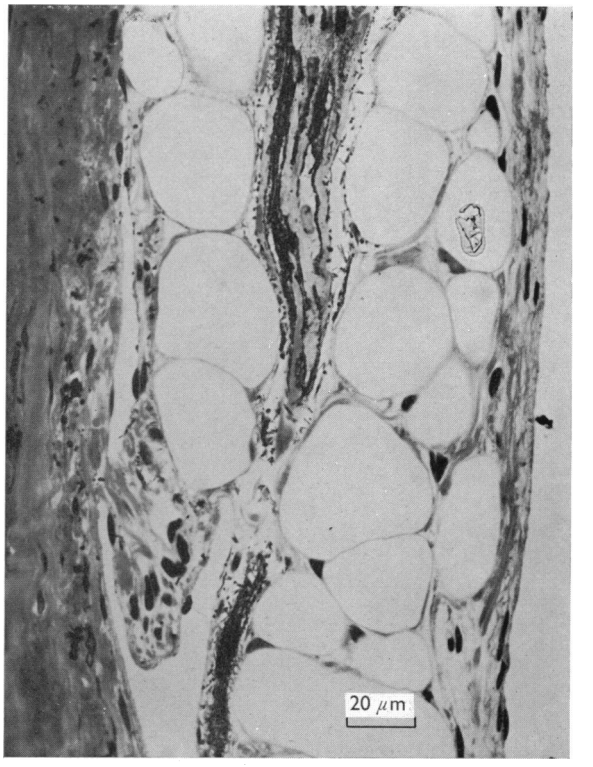


Fig. 14 Fig. 15

Fig. 14. Section through the A-V node and the specialized tissue marked in Fig. 3 with an arrow. ChE and toluidine blue stained 2 μm section. Specialized tissue (arrows) can be followed from the lower border of the A-V node through adipose tissue to the right aspect of the interventricular septum. *a*: fold of Epon.

Fig. 15. Detail of Fig. 14. The specialized tissue connecting the A-V node with the interventricular septum consists of muscle cells and ChE-positive nerves.

supply the node. Most of the nerves pass through the sinus septum from plexuses in the wall of the coronary sinus and at the base of the heart. At the node they meet nerves coming from the plexus surrounding the aorta. The nerves ramify along the atrial borders of the node and the bundle of His. Some of the branches supply these structures while others continue to the right and left bundle branches.

The bundle of His forms a smooth curve in continuation of the A-V node (Fig. 13). It is about 6 mm long and 600–700 μm in diameter. From the anterior half of the bundle the branches to the left ventricle are given off in a posterior and anterior group which as fine sheets pass downwards over the left side of the interventricular septum. The right branch leaves the bundle as a single unit about 4 mm from the A-V node, after which the bundle tapers off. The right branch continues without major ramifications through the interventricular septum and out on to the moderator band. At the base of the anterior papillary muscle, it ramifies to the walls of the right ventricle.

In histological sections of ChE-stained specimens (Figs. 16, 17) numerous nerve fascicles of varying size are found between the bundles of Purkinje fibres in the bundle

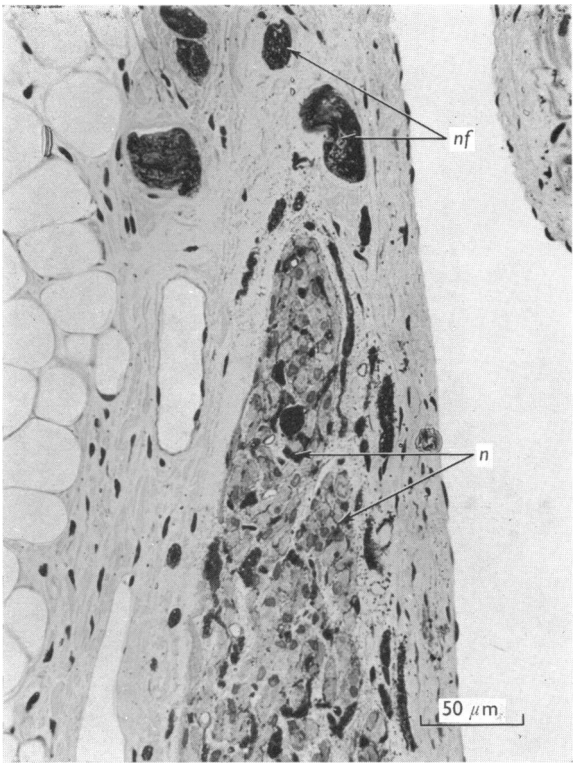


Fig. 16. Section through the bundle of His. ChE and toluidine blue stained 2 μm section. ChE-positive nerves (*n*) are seen as plexuses between the Purkinje cells. Large nerve fascicles (*nf*) are localized along the atrial border of the bundle.

of His and in particular at its atrial margin. In addition, each Purkinje cell is invested by a plexus of fine ChE-positive nerves.

In whole mounts from several hearts tissue with ChE reaction could be observed projecting from the A-V node and the posterior portion of the bundle of His down on the right aspect of the interventricular septum (Fig. 3). From histological sections made at right angles to the bundle of His it appeared that the tissue consists of muscle cells and ChE-positive nerves and that these cells terminated in connexion with the muscle cells of the septum (Figs. 14, 15).

In *ordinary myocardium*, ChE-positive nerve fibres and nerve fascicles are few as compared with the numbers in the nodes and the bundles. This also applies to the tissue forming the crista terminalis itself, the interatrial (Bachmann's) bundle, and to that part of the interatrial septum which lies over and in front of the fossa ovalis and through which pass the anterior and middle internodal pathways described by James (1967). In both whole mounts and sections, the only characteristic of these structures seems to be a parallel orientation of the muscle fibres (Figs. 11, 12). In particular, no large, pale, Purkinje-like cells were found in any of these atrial sites or among the atrial muscle fibres which come in contact with the surface of the A-V node.

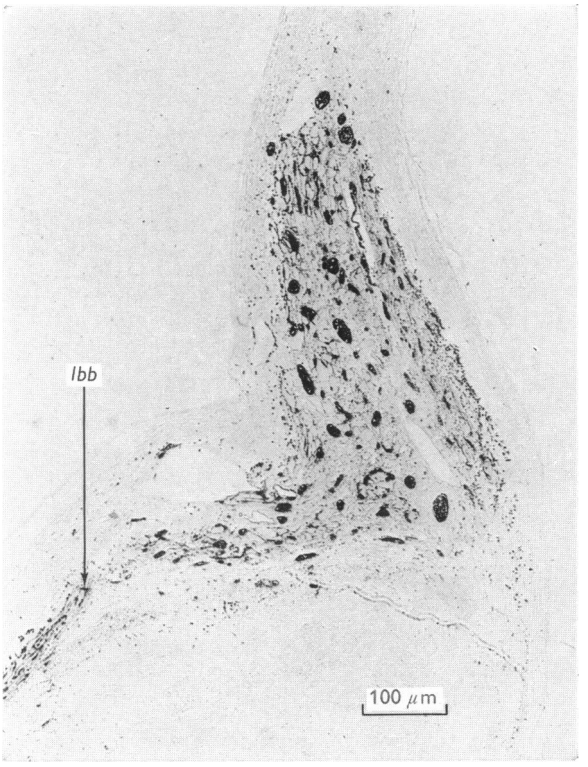


Fig. 17. Section through the bundle of His at the level of the departure of the left bundle branches. ChE-stained 2 μm section. Background unstained. The section shows that ChE-positive nerves and nerve fascicles are a prominent part of the bundle.

DISCUSSION

The muscle cells in the S-A node, SARB, A-V node, and the bundle of His and its two branches are specialized, among other things, in exhibiting a greater ChE activity and in being accompanied by more ChE-positive nerves than the muscle cells of the ordinary myocardium. In the present investigation this difference proved to be sufficient to permit cleared whole mounts to be made, in which the above structures were clearly visible against a lightly stained background. The specialized tissue in the heart could thereby be located and photographed, and tissue blocks containing well defined parts of the system could be accurately removed and oriented for sectioning.

In the rabbit and a number of other mammals, three morphological pathways, mainly consisting of large, pale, Purkinje-like cells, between the S-A and the A-V node have been described. A particularly large number of these cells has been found in the internodal pathways in the rabbit. One of the three pathways passes along the crista terminalis and the two others through the interatrial septum to the A-V node with the surface of which they come into contact (James, 1967). Preliminary studies showed us that the number of large, pale cells in the atria decreased as fixation of the heart improved. We therefore made a series of preparations where we sought to attain

optimal fixation of heart tissue. This was achieved by keeping the heart supplied with oxygenated blood until the moment of fixation, by stopping the heart in diastole, thus avoiding a systolic pinching of the intramural vessels, and by using these vessels to carry the primary fixative to the cells. Excised blocks were post-fixed in OsO_4. Both fixatives were dissolved in an isotonic vehicle with a neutral pH. Examination of thin sections of blocks removed from these hearts revealed no large, pale, Purkinje-like cells. This agrees with the findings of Truex & Smythe (1965) who, in a number of mammals including the rabbit, could not distinguish a pathway of differentiated muscle fibres between the nodes.

In the pig heart, we have demonstrated large, pale, Purkinje-like cells in the upper part of the crista terminalis (Bojsen-Møller & Tranum-Jensen, 1971*a*). These hearts were, however, not finally fixed until, after a short preliminary fixation, they had been treated for about 40 hours in different solutions to demonstrate a ChE activity. Although this finding is compatible with the presence of Purkinje cells in the atria, we prefer at this stage, like Emberson & Challice (1970), to show reservation in accepting such cells as a normal and widely distributed component of atrial myocardium.

Bachmann's bundle, the interatrial septum (both septum primum and septum secundum) and the crista terminalis itself seem to consist of ordinary atrial muscle tissue with relatively few nerves. With the technique employed, the only visible special characteristic of the muscle bundles was the parallel orientation of the fibres. Goodman, van der Steen & van Dam (1971) found that the impulse spreads more rapidly along the crista terminalis and the pectinate muscles than across them, and suggested that this could be due to the parallel orientation of the muscle cells in these structures. The question is, therefore, whether the rapid propagation of the impulse which is found in the interatrial septum (Sano & Yamagishi, 1965; Yamada *et al.* 1965) can likewise be due to a particular orientation of the muscle fibres in this structure.

The question of the presence of specialized tracts is complicated by the fact that in a zone along the crista terminalis cells have been found exhibiting different specialized electrophysiological properties such as spontaneous depolarization, low amplitude, prominent plateaus and ability to conduct at high K^+ concentrations (Paes de Carvalho *et al.* 1959; Paes de Carvalho, 1961; De Mello & Hoffman, 1960; Horibe, 1961; Hogan & Davis, 1968; Takayasu *et al.* 1969). Paes de Carvalho *et al.* (1959) found that these properties were associated with a superficial bundle-like structure which they called the sino-atrial ring bundle (SARB). It does not follow, however, that all the above-mentioned properties, in addition to that of rapid impulse propagation, are possessed by a single cell type, and it may therefore be significant that three kinds of muscle cells were found at this site in the present investigation:

(1) Ordinary atrial muscle cells arranged in parallel and constituting the bulk of the crista terminalis.

(2) Small compact muscle cells accompanied by ChE-positive nerves and forming a bundle four to five cells thick below the endocardial surface. The bundle which constitutes the right branch of the SARB contrasts with the septal branch in not being invested by a connective tissue sheath. It is therefore in immediate contact with the adjacent tissue, which consists of

(3) small muscle cells with abundant sarcoplasm and boundaries which are difficult to resolve with the light microscope. The cells, which are accompanied by ChE-

positive nerves, form a process of nodal tissue coursing from the S-A node towards the crista terminalis to lie deep to the right branch of the SARB.

Electron microscopic investigations have confirmed that areas with nodal cells are present along the crista terminalis in the heart of the monkey (Takayasu, 1967) and that cells containing few myofibrils, together with smaller ones full of myofibrils, are located in this area in the rabbit heart (Emberson & Challice, 1970).

Combined electrophysiological and morphological investigations could decide how the specialized functions and the different cell types described are correlated.

Besides through the bundle of His and its branches, it is possible that in the rabbit there may be another direct connexion from the A-V node and the posterior part of the bundle of His to the right aspect of the interventricular septum. The connexion consists of muscle cells and ChE-positive nerves. Direct branches of this kind are of great interest for the understanding of impulse propagation under normal and pathological conditions. Connexions consisting of muscle cells have been observed in a number of animals including rabbit and man (Cohn & Trendelenburg, 1910; Curran, 1910; MacKenzie, 1910; Lloyd, 1930; Mahaim & Winston, 1941; Mahaim, 1947). Their nerve content is, however, as elsewhere in the conducting system, difficult to ascertain when the preparations are not specially stained. Well marked nerves, intimately associated with 'early down-going branches', were occasionally discerned by Lloyd (1930) in the rabbit.

These early descending branches are different from the A-V connexions described by Kent (1914) in the right free wall of the ventricle. This area was not included in our investigation.

The present study of the rabbit heart, like earlier studies in that of the pig (Bojsen-Møller & Tranum-Jensen, 1971*a*, *b*), suggests that ChE-positive nerves are an obligatory and prominent component of the specialized tissue of the atria and ventricles.

SUMMARY

The muscle cells in the S-A node, the sinuatrial ring bundle (SARB), the A-V node and the atrioventricular connexions of the rabbit heart are specialized in exhibiting a greater ChE activity and in being accompanied by more ChE-positive nerves than the muscle cells of the ordinary myocardium. This feature has been used to identify the tissues in whole mounts and in 2 μm sections of 20 rabbit hearts. The sinuatrial node continues in fibres encircling the superior vena cava and in a cauda which extends alongside the crista terminalis to the A-V ring. The SARB, which is composed of small, compact muscle cells and nerves, extends from the roof of the auricle to the region around the atrioventricular node. At the crista terminalis the right branch of the SARB is situated between the cauda of the S-A node and the endocardium. The A-V node continues posteriorly in a thin strand of nodal tissue along the A-V ring and anteriorly into the bundle of His. The right bundle branch leaves the His bundle as a single unit while the left bundle branches are given off in two groups, which descend as fine sheets in the subendocardial tissue of the left side of the interventricular septum. Nerves from the plexus around the A-V node continue alongside the bundle of His to the bundle branches. From the A-V node and the posterior part of the bundle of His several fine bundles of thin muscle fibres and ChE-positive nerves pass to the right aspect of the interventricular septum. No Purkinje-like cells were found in the internodal or interatrial tracts.

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