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The disposition and innervation of atrioventricular ring specialized tissue in rats and rabbits

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INTRODUCTION

Although Davies & Francis (1946) expressed the belief that the specialized tissue was a neomorphic development in homoiothermic animals, others have described such tissue in poikilothermic hearts (Gaskell, 1883, 1900; Keith & Mackenzie, 1910; Robb & Petri, 1961; Robb, 1964). Keith & Mackenzie (1910) thought that the mammalian AV bundle was a remnant of the more widespread specialized atrioventricular junctional musculature of such lower animals, whilst Lewis (1920) suggested that the additional specialized tissue described by Kent (1893, 1913, 1914) might also be a remnant of this musculature. A similar explanation would account for the extranodal specialized tissue adjacent to the AV junction described by Shaner (1929), Truex, Bishof & Hoffman (1958), Lev, Fielding & Zaeske (1963), Gossrau (1971), and others. However, Robb & Petri (1961) indicated briefly that in human and monkey fetal hearts, and in adult guinea-pig hearts, specialized tissue was related to the entirety of the primitive atrioventricular junctions. Subsequent investigation has confirmed the presence of rings of specialized tissue related to the AV orifices in guinea-pig and human fetal hearts (Anderson, 1972*a*; Anderson & Taylor, 1972). The present investigation was performed in order to establish the presence of such tissue in other mammalian hearts. The species selected were the rabbit, in which specialized AV rings have been reported in an electrophysiological investigation (Paes de Carvalho, 1961) and the rat, in which junctional tissue was mentioned by Kent (1893).

MATERIALS AND METHODS

Fifteen adult animals of each species were studied. Rats were killed by chloroform inhalation, rabbits by intravenous injection of pentobarbitone sodium. The hearts were rapidly removed and orientated on cryostat tissue holders to allow subsequent sectioning in one of three planes relative to the cardiac septum. With the atrioventricular junction horizontal these planes were transverse, coronal and sagittal in relation to the septum. The blocks were frozen in isopentane previously cooled in liquid nitrogen and sectioned in a cryostat at 10–20 μm thickness. Two series of sections were collected on glass slides, taking either adjacent sections, two sections in five or two sections in ten. The slides were then processed to demonstrate either histology or cholinesterase (ChE) activity.

Routine histology

The sections were allowed to dry in air at room temperature for 5–10 minutes. They were then fixed in formol–sucrose–ammonium mixture (Pearson, 1964) for 5–120 minutes at room temperature and stained using Masson's trichrome technique.

Cholinesterase activity

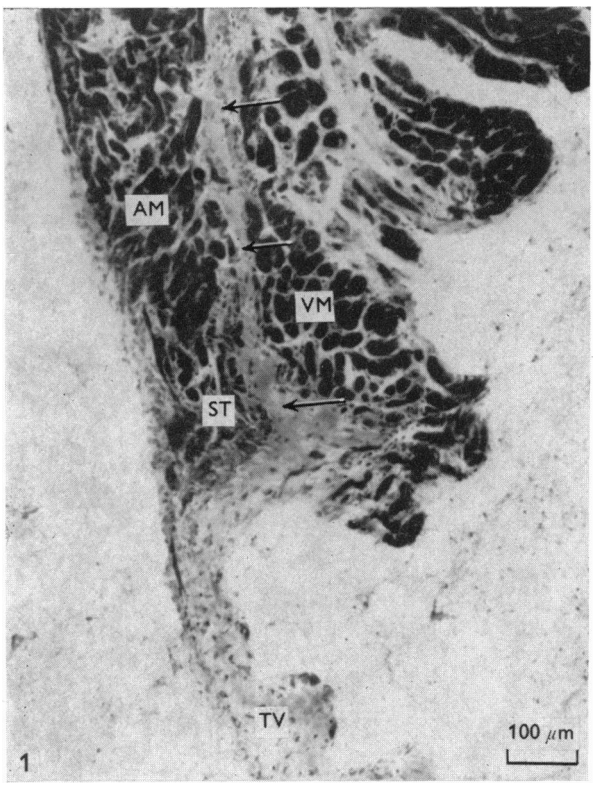
The method of Gomori (1952) was employed. Acetylthiocholine iodide was employed as substrate, and tetraisopropylpyrophosphoramide (TIPA) as an inhibitor of non-specific cholinesterase (Bayliss & Todrick, 1956). The sections were dried on slides for 5–10 minutes and fixed at 4 °C in formal–sucrose–ammonium mixture (Pearson, 1964) for 5–60 minutes. Sections to demonstrate acetylcholinesterase (AChE) were pre-incubated for 30 minutes in 10^{-5} molar TIPA. These and uninhibited specimens were then incubated in Gomori's medium at pH 6·0. Incubation proceeded at 38 °C from 1 to 4 hours for rat tissues, and from 2 to 16 hours for rabbit tissues. Control sections were incubated in the absence of substrate. Following incubation, sections were washed twice in distilled water, developed in fresh 1 % ammonium sulphide and counterstained for 30 seconds in haematoxylin. They were then dehydrated, cleared, and mounted in Canada Balsam dissolved in tetrachlorethylene.

Demonstration of adrenergic innervation

Three rats hearts and one rabbit heart were taken to demonstrate catecholamines by a formol-fluorescence technique. The hearts were rapidly removed after death, and blocks of the atrioventricular septum containing the AV specialized tissue and the lateral AV junctions were removed. These blocks were frozen in isopentane cooled in liquid nitrogen, and freeze dried in an Edwards–Pearse freeze drier for 24 hours. They were then heated at 80 °C for 1¼ hours with 5G paraformaldehyde previously stored at a relative humidity of 70·4 % (Spriggs, Lever, Rees & Graham, 1966). The blocks were then vacuum embedded in paraffin wax and serially sectioned at 8 μm thickness. One section in 25 was dewaxed and mounted in liquid paraffin and examined in a Vickers photomicroscope, using an ultraviolet light source, barrier filter GG 1/9 mm and excitor filter BG4/12 mm. When necessary intermediate sections were subsequently mounted and examined.

Electron microscopic examination

As a preliminary stage of a more extensive investigation of the atrioventricular ring tissue (Humpherson & Anderson, unpublished observations), a block was taken from the posterior region of the right AV ring of one rat heart. The heart was perfused with glutaraldehyde immediately following death, and the selected block was subsequently fixed in osmium tetroxide for 30 minutes. The block was then embedded in epoxy resin and sectioned on a Huxley ultramicrotome. Thin sections were double stained with lead citrate and uranyl acetate, and examined in a Philips EM 300 electron microscope.



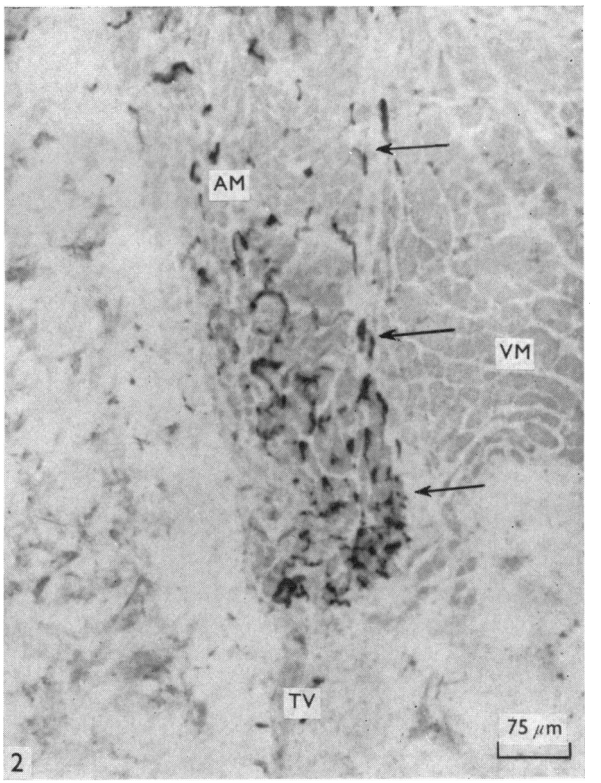


Fig. 1. Rat heart. Section from the lateral portion of the tricuspid ring showing the relationship of atrial (AM) and ventricular (VM) myocardium. The tricuspid valve (TV) is seen originating from the fibrous ring (arrows) which separates the two segments of myocardium. The portion of atrial myocardium adjacent to the valve-ring junction (ST) shows specialized characteristics. Trichrome stain.

Fig. 2. Adjacent section to that illustrated in Fig. 1, processed to demonstrate acetylcholinesterase (AChE) activity. Note that the AChE-positive nerves are abundant in the specialized tissue, which can be demarcated by its innervation. Section counterstained with haematoxylin.

RESULTS

Rat heart

As the atrial musculature inserts into the atrioventricular valve rings, its distal portions are noted to possess different staining characteristics (Fig. 1). The cells in these regions are more attenuated and stain palely with the trichrome technique. When adjacent sections processed for cholinesterase are studied the differentiation is confirmed (Fig. 2), and the distal portions are noted to be weakly acetylcholinesterase (AChE)-positive and to be associated with numerous AChE-positive nerves. The arrangement of nerves and cells is similar to the arrangement of specialized conducting tissue elsewhere in the rat heart. It is of interest that the AChE-positive nerves are much more evident following pre-incubation in TIPA than in sections incubated in acetyl substrate alone.

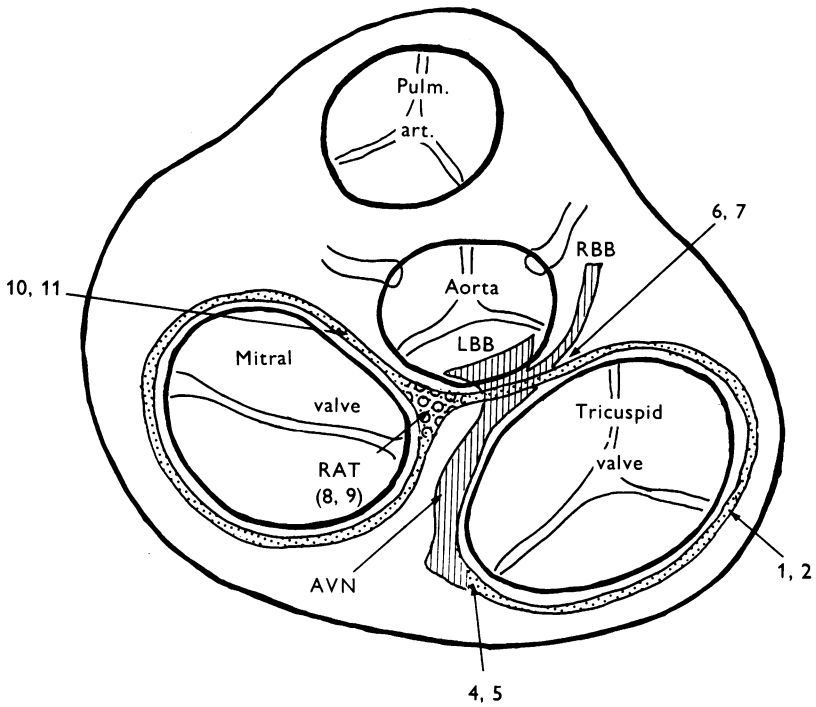


Fig. 3. Diagrammatic representation of the fibrous skeleton of the rat heart. The atrioventricular node (AVN), the bundle and its branches (LBB and RBB) are indicated by hatching. The disposition of the ring specialized tissue is indicated by stippling. Note the enlargement of the left ring in the retro-aortic region (RAT), and its connexion with the right ring which crosses the AV bundle. The figures refer to the figure numbers, and represent illustrations of the appropriate portions of the ring tissue.

When traced through serial sections, the specialized tissue is found to occupy the atrial margins of the entire circumference of the atrioventricular valve rings (Fig. 3). Throughout its extent the atrial myocardium communicates with the superior part of the tissue. Although in places the tissue comes very close to ventricular myocardium, fibrous tissue of the atrioventricular ring is always identifiable between the two. The

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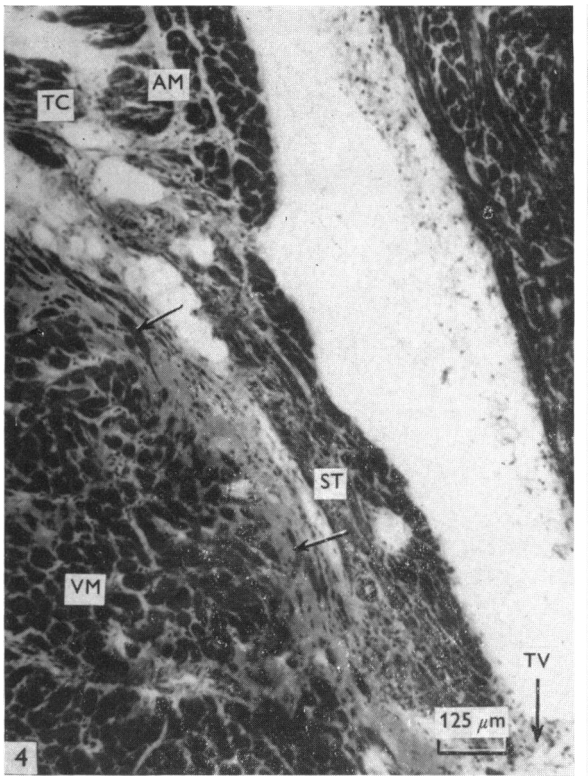
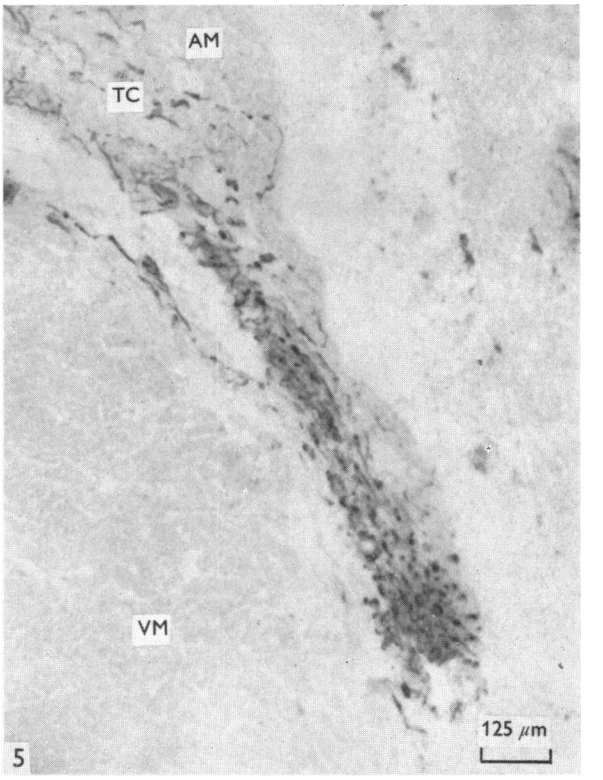
Fig. 4. Rat heart. Section taken posteriorly to the junction of the ring tissue (ST) with the posterior extent of the AV node (see Fig. 3). Note how the atrial myocardium (AM) joins the superior aspect of the ring tissue via transitional cells (TC). The fibrous ring (arrowed) separates the tissue from ventricular myocardium (VM) and is continued into the tricuspid valve (TV). Trichrome stain.

Fig. 5. Adjacent to Fig. 4 processed for AChE. Abbreviations as for Fig. 4. Note that the specialized tissue is distinguished by its AChE content and AChE-positive innervation. Section counterstained with trichrome.

Fig. 6. Rat heart. Anterior region of the tricuspid ring (see Fig. 3 for location) showing the specialized tissue (AVRT) passing to the left through the interatrial septum (IAS) above the atrioventricular bundle (AVB) to reach the retro-aortic specialized tissue (RAT). IVS, Interventricular septum. Section processed to demonstrate AChE.

Fig. 7. Detail of Fig. 6 (in box) from adjacent section stained by the trichrome technique. Note that the specialized cells (AVRT) are minimally differentiated from the atrial myocardium.


TC
AM
ST
VM
TV
125 μm
4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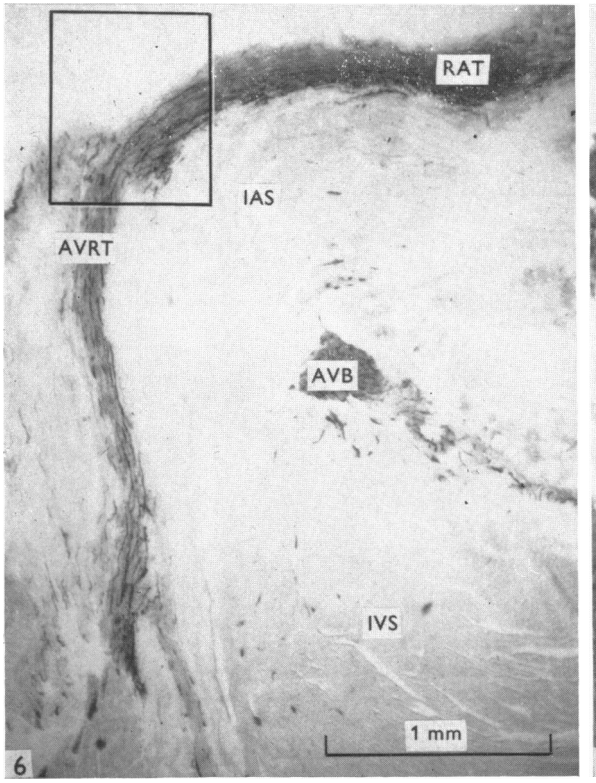
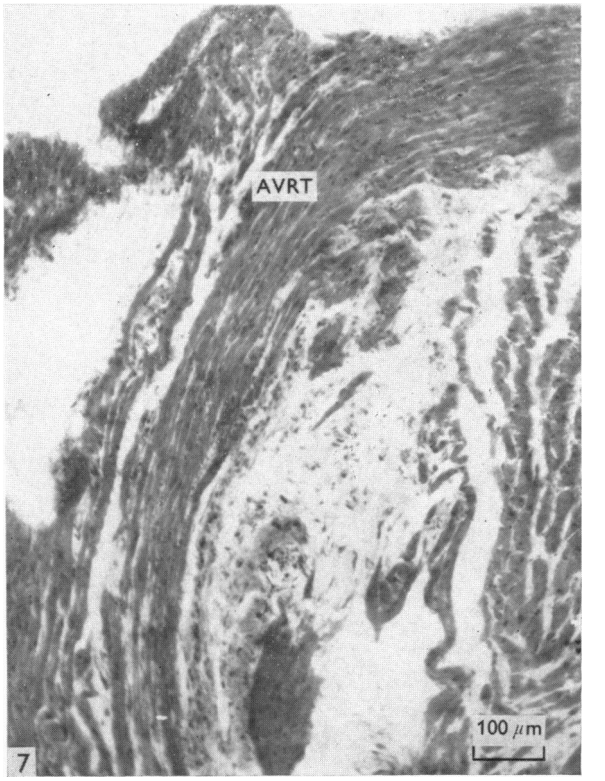
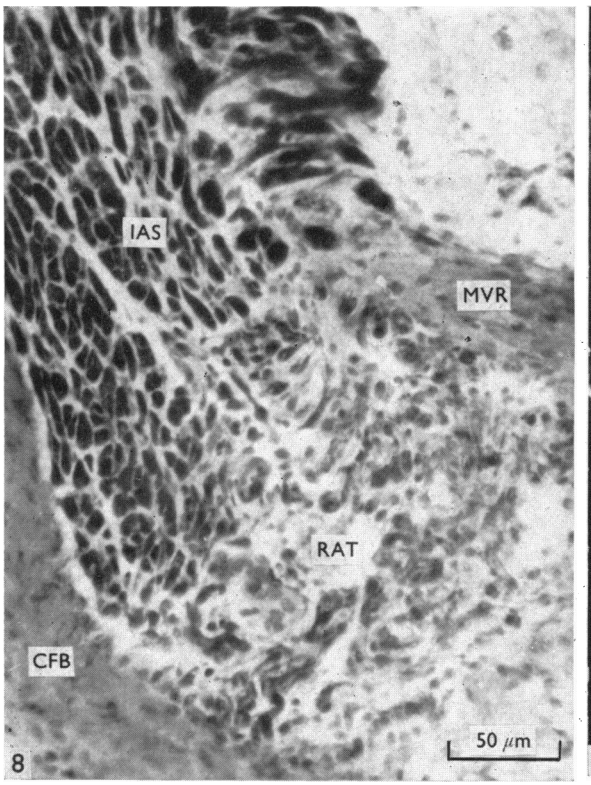
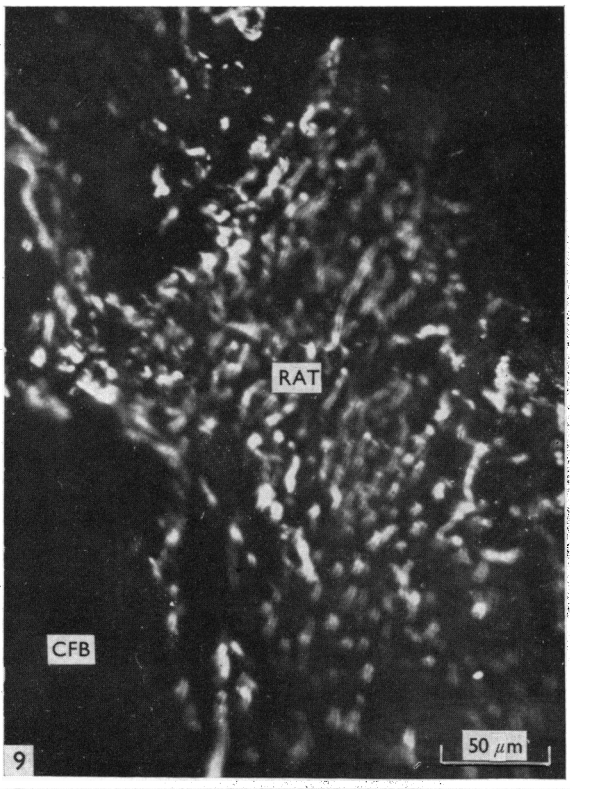
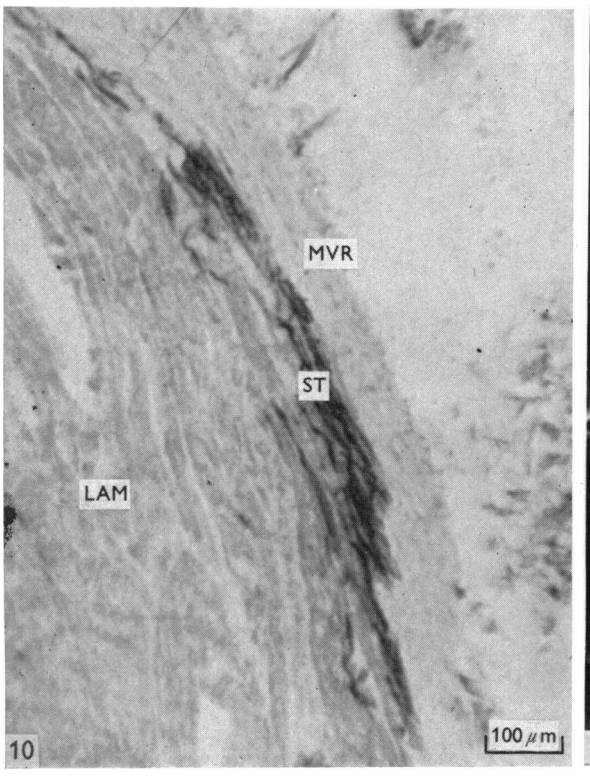
TC
VM
125 μm
5

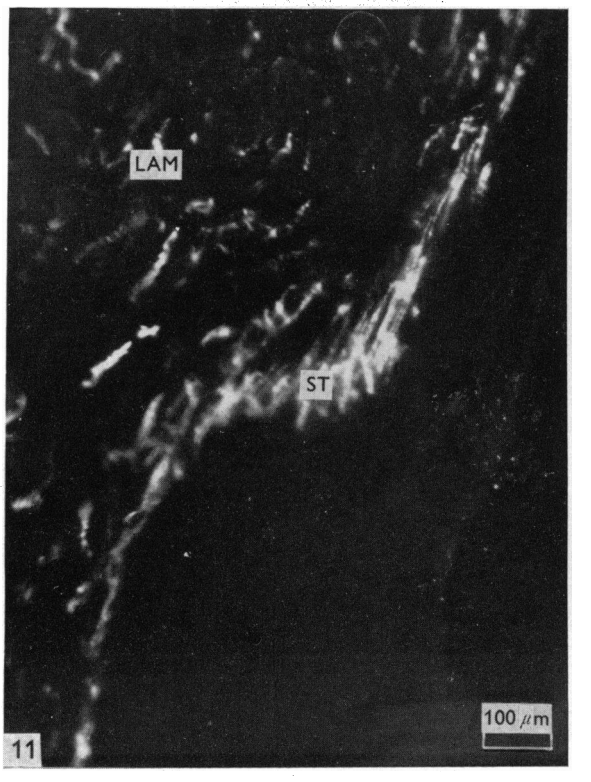

RAT
IAS
AVRT
AVB
IVS
1 mm
6


AVRT
100 μm
7


IAS
MVR
RAT
CFB
50 μm
8


RAT
CFB
50 μm
9


MVR
ST
LAM
100 μm
10


LAM
ST
100 μm
11

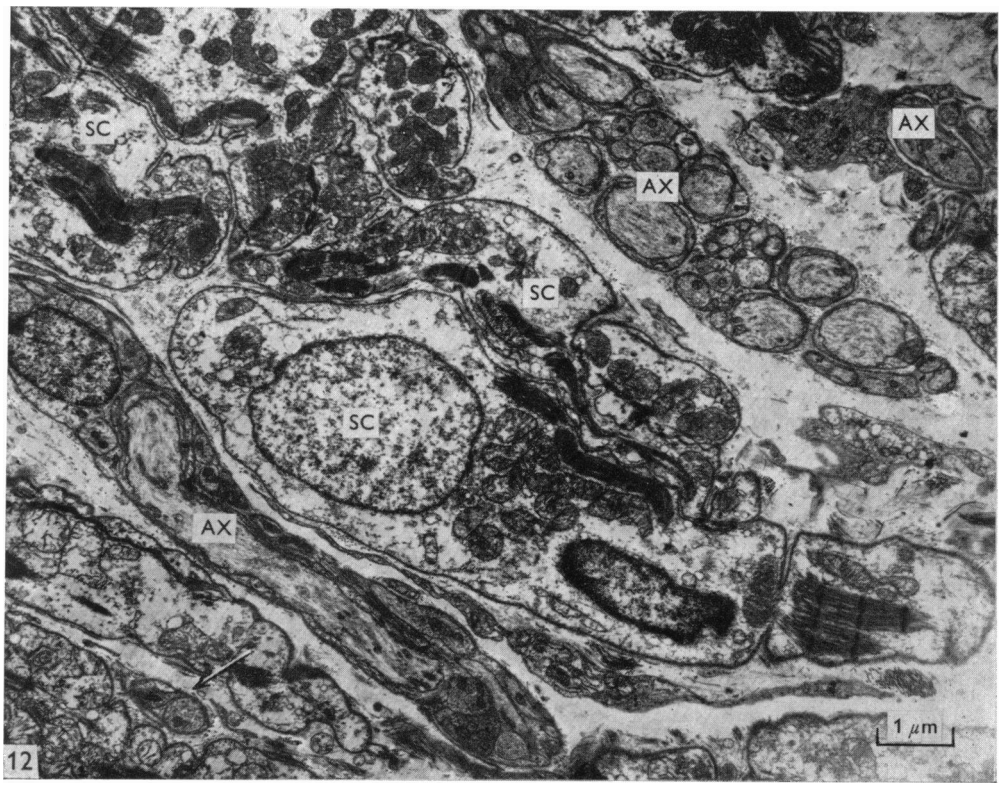


Fig. 12. Rat heart. Electron micrograph of posterior region of tricuspid ring tissue. Note the typical features of specialized tissue in the cells of the ring tissue (SC). The cells contain large areas of clear cytoplasm, with peripherally arranged myofibrils. Note also the numerous axon bundles (AX) running between the cells, and individual axons (arrowed) passing into crevices in the specialized cells. Section stained with uranyl acetate and lead citrate. (Photomicrograph prepared in collaboration with Dr J. R. Humpherson.)

ring specialized tissue is a direct posterior continuation of the atrioventricular specialized tissue, which occupies the distal segment of the interatrial septum anterior to the coronary sinus and adjacent to the right fibrous trigone. The cells of the atrioventricular node are continued posteriorly into the tricuspid valve base as the ring

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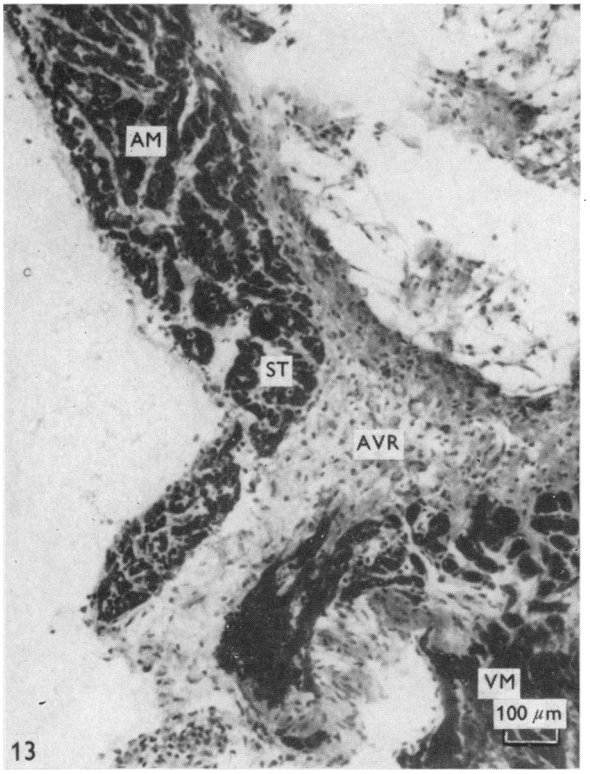
Fig. 8. Rat heart. Section showing the histological differentiation of the retro-aortic tissue (RAT) from the myocardium of the interatrial septum (IAS). CFB, central fibrous body; MRV, mitral valve ring. Trichrome stain.

Fig. 9. Section taken in a similar plane to Fig. 8, processed to demonstrate catecholamines. Note the rich nerve supply to the retro-aortic tissue.

Fig. 10. Rat heart. Section of the mitral valve ring (MVR) (see Fig. 3 for location) showing the well-innervated specialized tissue (ST) adjacent to the ring, and differentiated from the left atrial myocardium (LAM) by its innervation. Section processed for AChE and counterstained with haematoxylin.

Fig. 11. Section in similar plane to Fig. 10 processed to demonstrate catecholamines. Note the similarity in nerve plexuses demonstrated by the two techniques.



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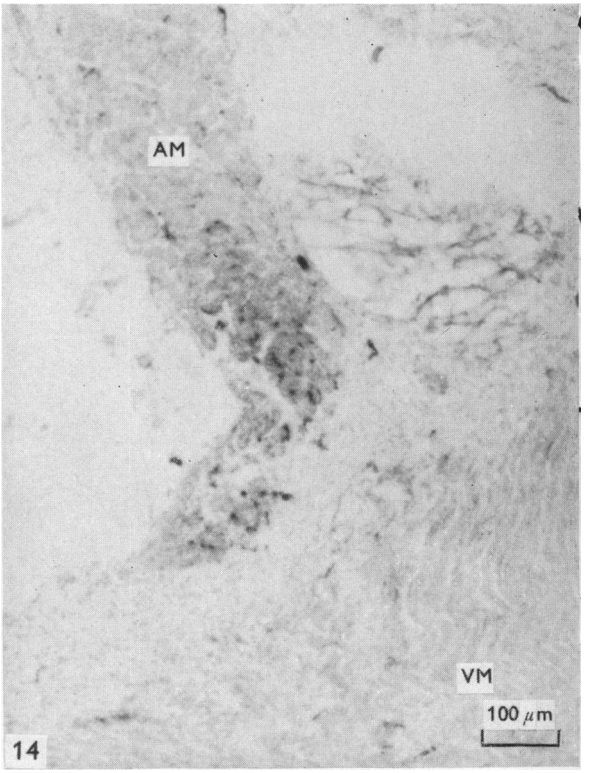

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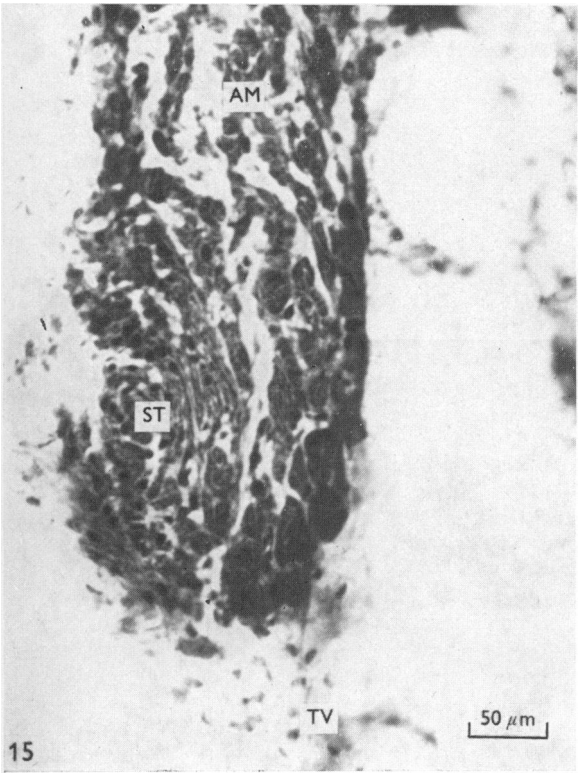

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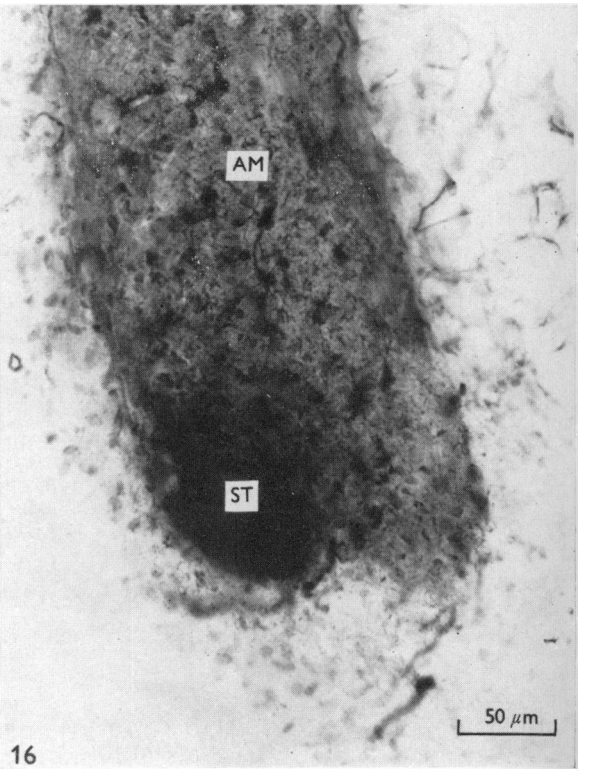

16

tissue, and in this situation transitional cells intervene between atrial myocardium and both nodal and ring tissue specialized cells (Figs. 4, 5). Having passed round the tricuspid ring to reach the region of the crista supraventricularis, the ring tissue inclines upwards into the interatrial septum and passes superior to the penetrating AV bundle and its bifurcation (Figs. 6, 7). The tissue then passes through the anterior connecting interatrial myocardium and expands into a node-like structure related to the aortic root and the mitral valve ring. This tissue is clearly distinguishable histologically as specialized tissue (Fig. 8). Its constituent cells are small, well vascularized, and interspersed with numerous nervous elements. The tissue is also AChE-positive and profusely supplied with AChE-positive nerves (Fig. 6). From this knot the specialized tissue is continued into the mitral ring, where it forms a further annulus in the atrial margin (Fig. 10).

Throughout its extent the ring specialized tissue is also associated with large numbers of catecholamine-containing nerves. The disposition of these nerves is similar to the arrangement of the AChE-positive plexuses (compare Figs. 10, 11) and the retro-aortic knot is similarly profusely innervated (Fig. 9).

Electron microscopy shows that the area of atrial myocardium adjacent to the fibrous ring possesses the ultrastructural features of specialized tissue (Fig. 12). The cells are smaller than atrial myocardial cells, and contain few myofibrils, which are randomly arranged in the cytoplasm. Large numbers of axon bundles run between the specialized cells, and axon varicosities are seen, containing both agranular and granular vesicles. In contrast, few axon bundles are identified in the working atrial myocardium, and specialized features are not seen in the ventricular myocardium adjacent to the fibrous ring.

Rabbit heart

The distribution of AV ring specialized tissue in this animal is basically similar to that observed in the rat heart, but the specialized tissue is not nearly so well demarcated histologically (Fig. 13). However, the distal edge of the atrial myocardium is found to be associated with many more AChE-containing nerves than adjacent atrial myocardium (Fig. 14). Furthermore, the atrial cells themselves are weakly AChE-positive, and after extended incubation periods this reaction is greatly intensified (Figs. 15, 16).

As in the rat, the ring tissue is a continuation of the tract of lower atrioventricular nodal cells (Figs. 17, 18). Anteriorly the tract does not make contact with the retro-aortic knot of cells, which is again less well formed than in the rat. However, this

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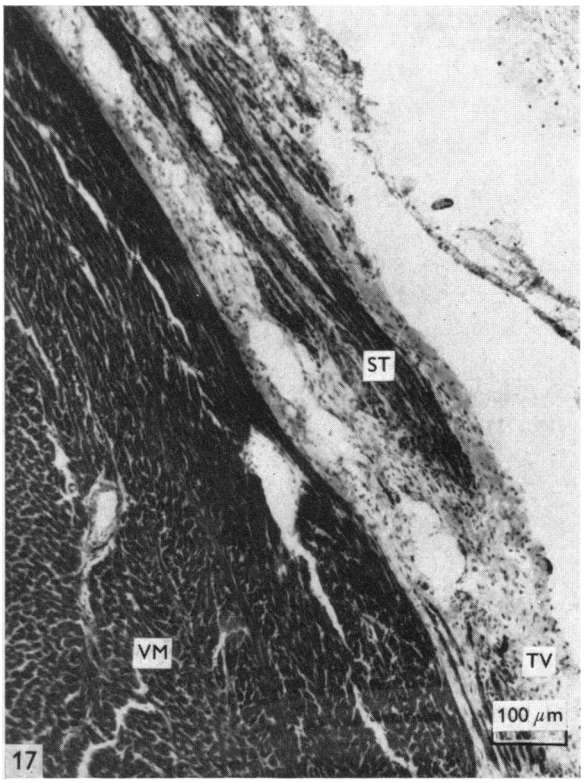
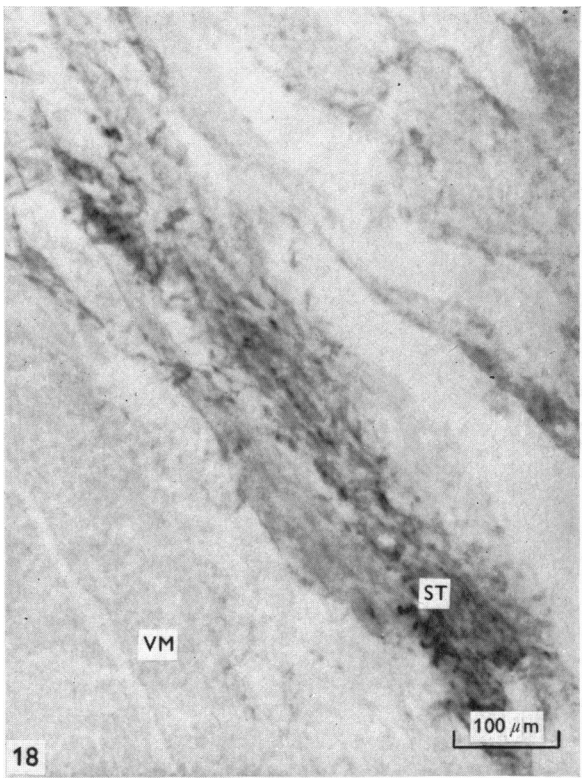
Fig. 13. Rabbit heart. Section taken from segment of the tricuspid ring corresponding to Figs. 1 and 2. Note the specialized nature of the ring tissue (ST) adjacent to the fibrous ring (AVR). AM, atrial myocardium; VM, ventricular myocardium. Trichrome stain.

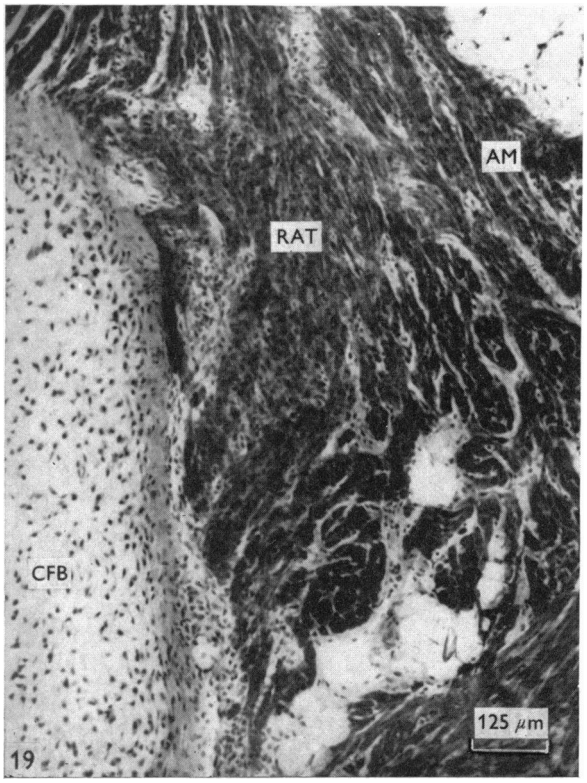
Fig. 14. This section, adjacent to that demonstrated in Fig. 13, has been processed for AChE, receiving 2 hours incubation. Note the weakly positive specialized tissue and the associated AChE-positive nerves. Section counterstained with haematoxylin.

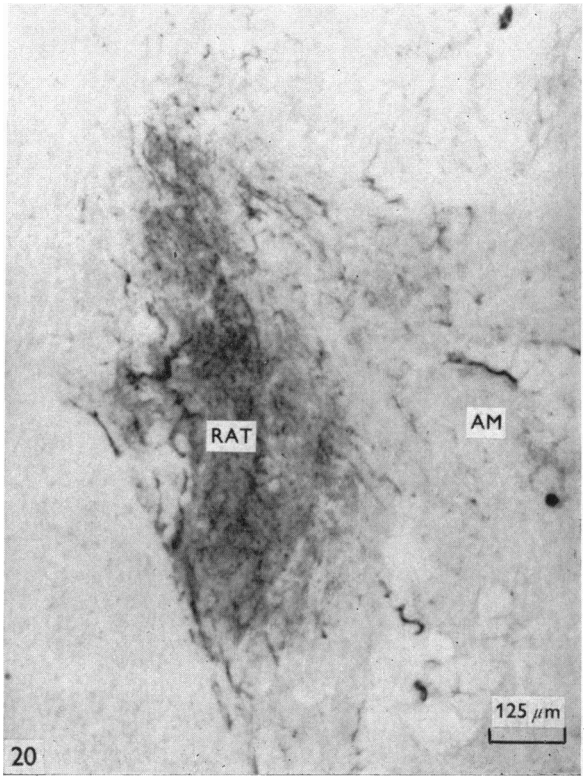
Fig. 15. Similar section to Fig. 14, again showing specialization of ring tissue. Abbreviations as for Fig. 13. Trichrome stain.

Fig. 16. This section, taken from a similar region to Fig. 15, has been incubated for 14 hours to demonstrate AChE activity. Note that the ring tissue is now intensely reactive, and the adjacent tissue is well supplied with AChE-positive nerves. Section counterstained with haematoxylin.


ST
VM
TV
100 μm
17


ST
VM
100 μm
18


AM
RAT
CFB
125 μm
19


RAT
AM
125 μm
20

retro-aortic knot is AChE-positive and is associated with AChE-positive nerves (Figs. 19, 20). The mitral ring is also not well formed, but is present and can be distinguished from atrial myocardium by both its histological and histochemical appearances. The ring tissue, particularly the retro-aortic knot, is well supplied with CA-containing nerves which form a plexus similar in disposition to the plexus of AChE-positive nerves.

DISCUSSION

The present investigation establishes the presence of specialized tissue in the atrial margins of the atrioventricular orifices of the rat and rabbit hearts. The arrangement demonstrated is very similar to that previously reported in the adult guinea-pig (Anderson, 1972*a*) and fetal human (Anderson & Taylor, 1972) hearts. Previous morphological documentation of complete annuli of specialized ring tissue is confined to the brief report of Robb & Petri (1961), who mentioned that they had discovered such tissue in human, monkey and guinea-pig hearts, but did not illustrate their findings. However, the presently reported distribution of specialized tissue bears close resemblance to that described by Davies (1930) in the bird heart. Particularly close similarities are noted in the disposition of the tricuspid ring tissue. Davies then believed that his results indicated that the extent of specialized tissue in the bird represented an intermediate stage between the arrangements reported in poikilothermic and mammalian hearts. However, in subsequent publications (Davies & Francis, 1946, 1952), the presence of specialized tissue in poikilothermic hearts was questioned, and he and his collaborator concluded that cardiac specialized tissue was a neomorphic development in mammalian and bird hearts. Subsequent researchers have questioned this concept, and specialized tissue has been recently described in poikilothermic hearts by Robb & Petri (1961) and Robb (1964). Our findings show that the specialized tissue in several mammals is almost identical to that in birds. Furthermore, it has recently been suggested (Anderson & Taylor, 1972) that the arrangement of specialized tissue in the early stages of human development is very close to that reported in poikilothermic animals. Thus the evidence available supports the earlier phylogenetic hypothesis of Davies (1930) regarding the origins of specialized tissue, rather than the later statement suggesting neomorphic differentiation.

In other joint publications (Blair & Davies, 1935; Blair, Davies & Francis, 1942) Davies referred to the different histological characteristics of atrial and ventricular muscle fibres at their attachments to the fibrous rings. However, the conclusion was

Fig. 17. Rabbit heart showing the backward extension (ST) of nodal tissue into the tricuspid ring (TV). The section is from a position corresponding to Fig. 4. VM, ventricular myocardium. Trichrome stain.

Fig. 18. The adjacent section to that demonstrated in Fig. 17, processed for AChE, again shows the specialized tissue to be AChE-positive and to be well supplied with AChE-positive nerves. Section counterstained with haematoxylin.

Fig. 19. Rabbit heart. Section of the retro-aortic tissue (RAT) showing the staining differential from atrial myocardium (AM). CFB, central fibrous body. Trichrome stain.

Fig. 20. Adjacent section to that demonstrated in Fig. 19, processed for AChE. Note that the retro-aortic tissue is AChE-positive and well supplied with AChE-positive nerves. Section counterstained with haematoxylin.

reached that these differences represented 'insertions' of the myocardium, supporting the work of Pace (1924). Insertions of skeletal muscle have been shown to be rich in AChE activity (Couteaux, 1955; Beckett & Bourne, 1958). Thus the present finding of AChE-rich atrial muscle at the valve rings could be interpreted as supportive evidence for the 'insertion' theory of Pace (1924). However, the activity is noted only in atrial muscle, the ventricular insertions being AChE-negative. Furthermore, the atrial ring tissue is also associated with numerous AChE- and CA-containing nerves, a feature again lacking in relation to the ventricular myocardium. Thus one would conclude that the features observed in the atrial ring tissue represent specialization of the muscle and that the tissue is true cardiac specialized tissue. This contention is borne out by the preliminary ultrastructural studies, which show that the myocardial cells in the ring tissue possess electron microscopic appearances of specialized tissue, and also reveal the numerous axon bundles associated with them. The electron microscopic evidence also weighs against possible criticisms of the techniques employed. For instance, it could be claimed that the activity presently reported in atrial ring tissue cells represents diffusion from the associated nerves, even though diffusion is absent elsewhere in the sections. Similarly, it could be said that all fluorescing structures demonstrated are not nervous in origin. In both these cases the ultrastructural findings support the interpretation presented in this report.

Davies (1930) also supported the contention of Lewis (1920) that the lateral connections of Kent (1893, 1913) could represent segments of primitive ring tissue. In his first study Kent used the rat heart, and the present investigation shows that complete rings of tissue do exist in this animal. Kent was concerned to establish the presence of lateral atrioventricular connexions and did not comment on annular formations. In our experience fibrous tissue was always present between the two regions, although in some places this was very tenuous. If imperfect formation of the fibrous ring occurred during embryological development then an accessory connexion could easily be made via a segment of ring tissue. It may be speculatively argued that such a segment, if present, would be more distinct and easily recognizable. Such an explanation of well-developed segments of ring tissue would account for the reports of specialized tissue knots by Shaner (1929); Walls (1942); Truex *et al.* (1958); Lev *et al.* (1963), and others.

The portion of ring tissue which is best developed in the rat and rabbit hearts, and is also clearly seen in the guinea-pig heart (Anderson, 1972*a*), is the retro-aortic knot. This knot can be defined solely on its histological appearance, but its connexions with the rings do not become evident until cholinesterase-processed sections are studied. It is likely that this structure corresponds to the well-innervated mass of specialized tissue reported by Gossrau (1971) in the hamster heart and may be equivalent to the left-sided node reported by Nomura (1952) in the mouse heart. The connexion of the tricuspid ring with this structure, and thence with the mitral ring, is one discrepancy between this study and that of Davies (1930), for in the bird the tricuspid ring tissue was found to run posteriorly round the aorta and join the left bundle branch.

It seems unlikely that these rings of specialized tissue are merely atrophic remnants of more widespread primitive tissues, since in the rabbit heart the rings possess the electrophysiological characteristics of specialized tissue (Paes de Carvalho, 1961).

The disposition of the tricuspid ring demonstrated by Paes de Carvalho is very similar to the description given in the present paper. It differs to some extent in that he found the ring to join the 'AN' or transitional fibres of the atrioventricular node, whereas morphologically the tract is continuous with the lower nodal cells, which probably represent the 'NH' electrophysiological region (Anderson, 1972*b*). However, in the posterior regions of the node distinct transitional cells impinge on both the lower nodal cells and the ring tissue, and a recording electrode could easily monitor transitional type cells in this position.

Atrial myocardial cells impinge on the ring tissue throughout its circumference, and it may well be the tract 'collects' atrial impulses and forms an input route to the AV node. If this were so, then the ring tissue and the lower nodal cells would form a route to the ventricles which by-passed the nodal crest of transitional and upper nodal cells. The presence of other by-pass tracts such as those described by James (1961) has been used to explain various forms of the pre-excitation syndrome (Ferrer, 1970). The route presently described would represent another such pathway, and it is significant that the nodal architecture in human and rabbit hearts is very similar (Anderson & Latham, 1971; Anderson, 1972*b*). It is of further significance that the lower nodal cells are rich in acetylcholinesterase, and possess a rich cholinergic innervation. In normal hearts it is possible that this may serve to depress conduction through the by-pass, allowing pre-excitation to occur only in abnormal hearts. Lown, Ganong & Levine (1952) found the occurrence of ventricular pre-excitation to increase in the presence of increased adrenergic activity.

Since specialized tissue is related to both mitral and tricuspid rings, it is also conceivable that the tissue may be implicated in the regulation of valvular activity. Davies (1930) showed that in the bird the tricuspid valve was a muscular structure, and it was reasonable to suggest that the specialized tissue regulated its activity. In mammals the valves are fibrous structures and therefore relatively inert. However, Sarnoff, Gilmore & Mitchell (1962) have demonstrated that, in the dog, valve closure is under neural control, and thus the nerves associated with ring specialized tissue (if present in the dog) may be involved in producing alterations in timing of valvular closure. Clearly extensive physiological and electrophysiological studies are required before the functional significance of atrioventricular ring specialized tissue can be elucidated.

SUMMARY

1. Additional extranodal specialized tissue is described in the rat and rabbit hearts, related to the atrial margins of the atrioventricular orifices.

2. The tissue is differentiated by its histological appearance, acetylcholinesterase content and its pattern of AChE-positive and CA-containing innervation.

3. The tricuspid ring tissue is a posterior continuation of the atrioventricular node.

4. Anteriorly the tricuspid ring tissue crosses the atrioventricular bundle to form a second node-like structure related to the aortic root.

5. The mitral ring tissue is in continuity with this retro-aortic knot of tissue.

6. The results are compared with previous reports of specialized tissue in poikilothermic animals, and the functional significance of the findings is discussed.

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