J. Anat. (1972), **111**, 3, pp. 453–468
With 23 figures
Printed in Great Britain

The disposition, morphology and innervation of cardiac specialized tissue in the guinea-pig

R. H. ANDERSON

Department of Anatomy, University of Manchester, Manchester, M13 9PL

(*Accepted 9 February* 1972)

INTRODUCTION

The morphology of the atrioventricular nodal area was originally described by Tawara (1906) and subsequently supported by the reports of many workers. Recently it has been observed that the cellular arrangement in the rabbit and human (De Felice & Challice, 1969; Anderson, 1971; Anderson & Latham, 1971) may differ from the original description. Since much of the evidence to support this differentiation in the rabbit was adduced from the use of a combined neurohistochemical–histological technique, it was decided to apply this technique to another of the species studied by Tawara (1906), namely the guinea-pig. In addition to the atrioventricular area, the entire heart was studied in an attempt to elucidate the full extent of cardiac specialized tissue in this animal.

MATERIALS AND METHODS

Twenty-five adult guinea-pigs were used. Following death, produced by intraperitoneal injection of Nembutal (pentobarbitone sodium – Abbott), the hearts were rapidly removed. Different hearts were orientated on cryostat chucks to allow sectioning in one of three planes relative to the atrioventricular ring of the heart. With the ring in the horizontal position sections were cut parallel to it and in sagittal and coronal planes. In additional hearts the cardiac septum was removed from the heart and prepared for sectioning in similar planes. Following orientation, the blocks were plunged into isopentane previously cooled in liquid nitrogen, and sectioned in a cryostat at 10–20 μm thickness.

From some hearts alternate sections were mounted on separate glass slides, and from others two or three sections out of ten were collected. Adjacent sections were processed to demonstrate histology, cholinesterase activity and catecholamine distribution.

Histology

The sections were fixed for five minutes in 4 % formaldehyde in sucrose–phosphate buffer (Karnovsky, 1964) at room temperature. They were then stained using Masson's modification of the trichrome technique.

Cholinesterase activity

The sections were fixed in buffered 4 % formalin–sucrose (Karnovsky, 1964) for five minutes at 4 °C. Following fixation the sections were incubated in Gomori's (1952) medium at pH 6·0 using acetyl and butyryl thiocholine iodide as substrates.

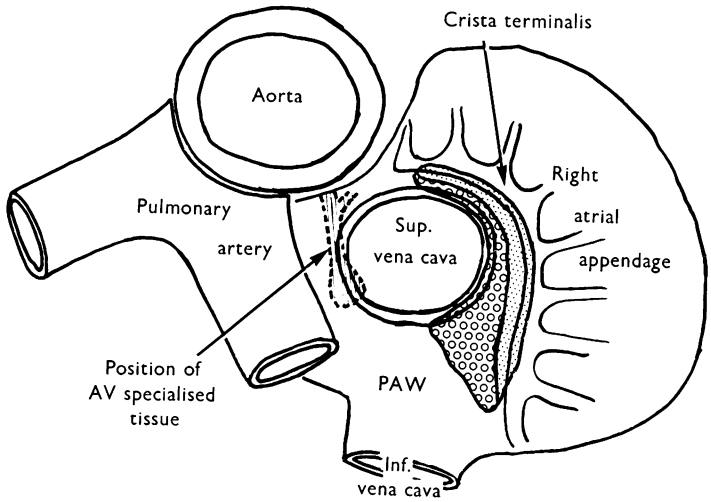


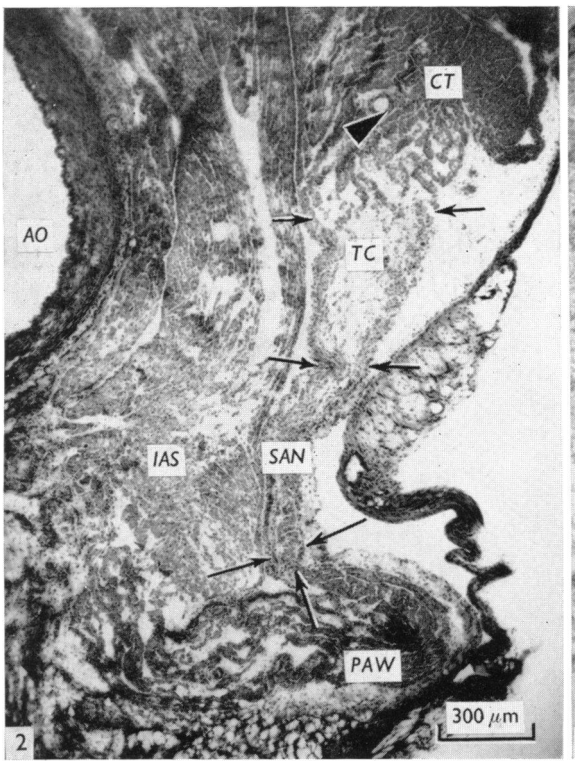
Fig. 1. Diagrammatic representation of the position of the sinuatrial node. The relative position of the atrioventricular specialized tissue is projected on to the diagram.

Nodal cells Transitional cells

Abbreviations given at this stage are used throughout subsequent figures.

A	Artery	*AM*	Atrial myocardium
AO	Aorta	*AV*	Aortic values
AVB	Atrioventricular bundle	*AVN*	Atrioventricular node
AVR	Atrioventricular ring	*CS*	Coronary sinus
CT	Crista terminalis	*FT*	Fibrous tissue
IAS	Interatrial septum	*IVS*	Interventricular septum
LAW	Left atrial wall	*LAOC*	Left atrial overlay cells
LBB	Left bundle branch	*LE*	Left extension of RAT
LV	Left ventricle	*MV*	Mitral valve
MVR	Mitral valve ring	*PAW*	Posterior atrial wall
RAOC	Right atrial overlay cells	*RAT*	Retro-aortic tissue
RAW	Right atrial wall	*RBB*	Right bundle branch
RE	Right extension of RAT	*RV*	Right ventricle
RVW	Right ventricular wall	*SAN*	Sinuatrial node
TC	Transitional cells	*TV*	Tricuspid valve
TVR	Tricuspid valve ring	*VM*	Ventricular myocardium

Incubation was conducted at 38 °C and proceeded for between two and eight hours. As controls some sections were incubated in the absence of substrate. Other sections were subjected to ChE inhibitors prior to incubation. The following inhibitors were employed, pre-incubation proceeding for 30 minutes: eserine sulphate (10^{-4} M) as an inhibitor of ChE activity; tetraisopropylpyrophosphoramide (TIPA, 10^{-6} M) as an inhibitor of non-specific ChE; and 1–5 bistrimethylammonium pentan 3–1 diiodide (BW chemical no. 62C47 – 2×10^{-5} M) as an inhibitor of acetylcholinesterase (Bayliss & Todrick, 1956). When eserine and 62C47 were used these compounds were also incorporated in the incubating media at the same concentrations as those used during pre-incubation. Following incubation the sections were washed twice in distilled water, developed for 30 seconds in freshly prepared 1% ammonium sulphide at



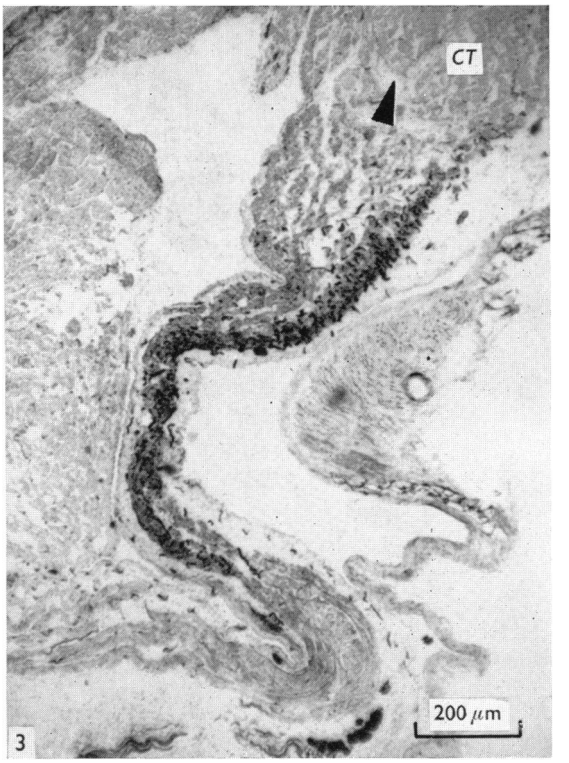


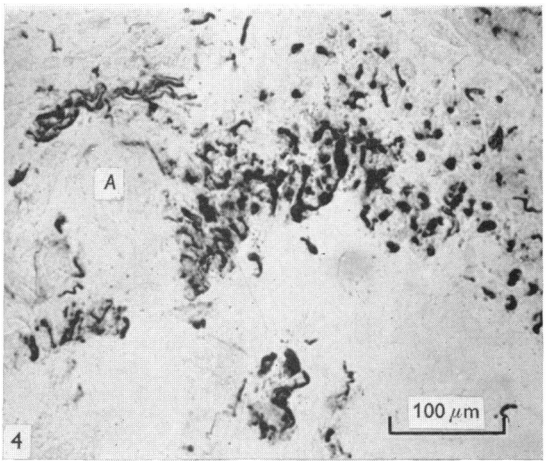
Fig. 2. Transverse section through right atrium showing the position of the SA node. Note the cells occupy the full thickness of the atrial wall (arrowed). Transitional cells are present connecting the node with the crista terminalis and passing around a large artery (large arrow). Trichrome stain.

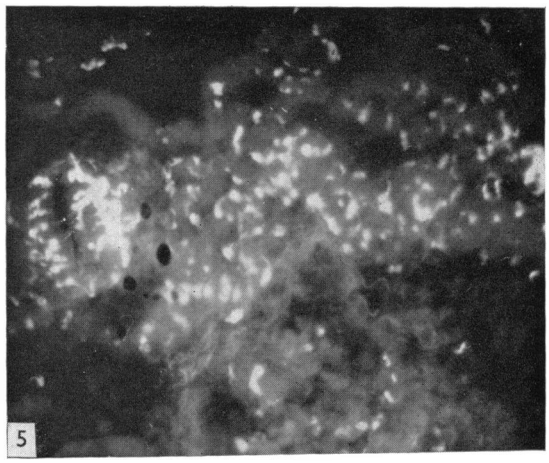
Fig. 3. Section close to that illustrated in Fig. 2. The nodal margins are indicated by the AChE content of nodal cells and their associated AChE-positive innervation. For all sections incubated for AChE the conditions were identical as follows: Gomori's medium was used at pH 6·0 with acetyl thiocholine iodide as substrate. Sections were pre-incubated for 30 minutes in 10^{-6} M TIPA, and then incubated for 4 hours in the medium. Fig. 3 was counterstained for 30 seconds with haematoxylin.

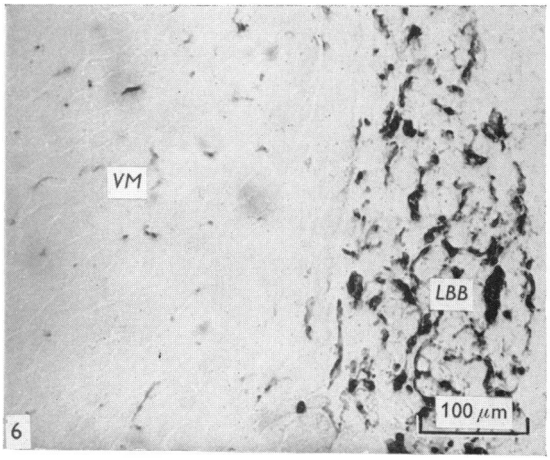
20 °C, and counterstained (if necessary) in haematoxylin for 30 seconds. The sections were dehydrated, cleared and mounted in Canada Balsam dissolved in tetrachlorethylene.

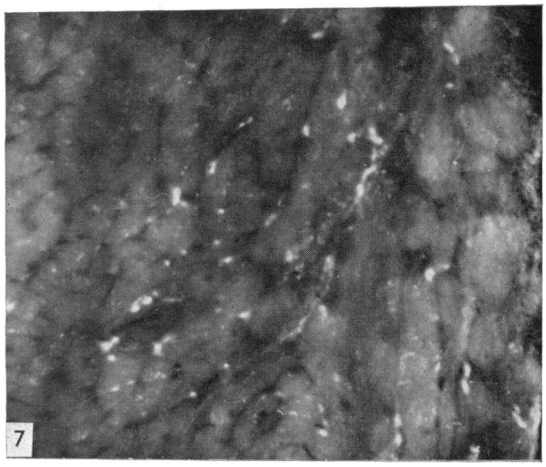
Catecholamine (CA) distribution

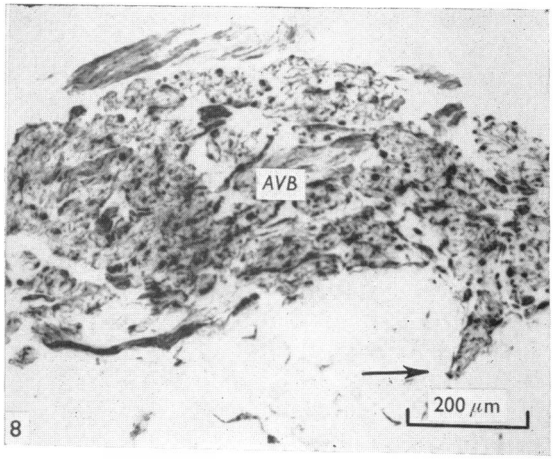
The formol fluorescence method of Spriggs, Lever, Rees & Graham (1966) was employed. The paraformaldehyde used for condensation was stored at a relative humidity of 70·4 %. Heating in paraformaldehyde at 80 °C proceeded for 1¼ hours. After reaction the sections were mounted in liquid paraffin and examined in a Zeiss photomicroscope using an ultraviolet light source, barrier filter GG 9/1 mm and excitor filter BG 12/4 mm.

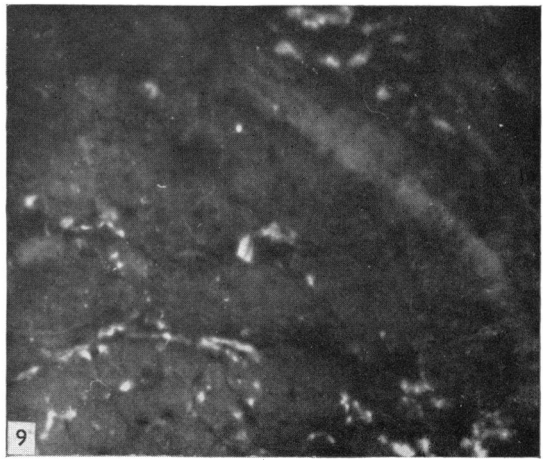

A
100 μm
4


5


VM
LBB
100 μm
6


7


AVB
200 μm
8


9

RESULTS

Sinuatrial node

This structure is identified in the lateral wall of the superior vena cava at its junction with the right atrial appendage and the posterior atrial wall (Fig. 1).

The nodal cells occupy the full thickness of the atrial wall. They are smaller than atrial cells and are packed tightly together by much connective tissue. In the epicardial tissues adjacent to the node are situated several large ganglia and nerve bundles. Although the nodal cells receive a rich vascular supply, a circumarterial arrangement is not identified. A large artery is constantly seen running along the crista terminalis, but attenuated cells encircle this artery, connecting nodal cells with the myocardium of the crista terminalis. These cells are considered to be transitional cells (Figs. 1–3).

At the margins of the node apart from the crista terminalis the specialized cells merge directly with the myocardial cells of the atrial walls (Fig. 2). The extent of the node is clearly delineated by its acetylcholinesterase (AChE) content (Fig. 3). Both nodal and transitional cells are AChE-positive and are associated with a rich plexus of AChE-containing nerves. More nerves are found related to nodal cells than to transitional cells, but in contrast the atrial myocardium is poorly innervated, whilst no AChE-containing nerves are identified around the cardiac vasculature (Figs. 3–5). Sections of the node processed to reveal catecholamine (CA) distribution show a plexus of nerves similar to that demonstrated by AChE activity (Figs. 4, 5).

However, the myocardial cells and cardiac blood vessels are well supplied with plexuses of CA-containing nerves.

Atrioventricular specialized tissue

The specialized cells constituting the atrioventricular node are located within the right fibrous trigone of the heart, and are encased within connective tissue (Fig. 10). The input to the node is via the myocardium beneath the coronary sinus. Apart from this junctional region, no other tracts of atrial myocardial cells make contact with the nodal specialized tissue. As the myocardium of the posterior atrial wall sweeps beneath the coronary sinus the cells become separated by fibrous tissue, and show specialized characteristics. These cells are considered to be transitional, and as they pass forwards towards the fibrous trigone they interweave and form lattice patterns (Fig. 11). Numerous ganglion cells are identified in this position, and large nerve bundles pass from the posterior atrial wall towards the transitional cells (Fig. 12).

Figs. 4, 5. Adjacent transverse sections of the sinuatrial node, and artery demonstrating (4) AChE and (5) CA. Note that the nodal plexus is similar but that only CA-containing nerves are found in relation to the artery. AChE as for Fig. 3. CA demonstrated by the technique of Sprigg's *et al.* (1966).

Figs. 6, 7. Adjacent coronal sections of the left bundle branch and associated myocardium processed for (6) AChE and (7) CA. The CA-containing nerves are present mainly in the myocardium, whilst the AChE-containing nerves are related to the specialized tissue.

Figs. 8, 9. Similar coronal sections of the atrioventricular bundle, with a septal branch (arrowed) demonstrating (8) AChE and (9) CA. Note that the specialized tissue is profusely supplied with AChE-containing nerves, but devoid of CA-containing nerves. Fig. 8 should be compared with Fig. 14, an adjacent section stained with trichrome.

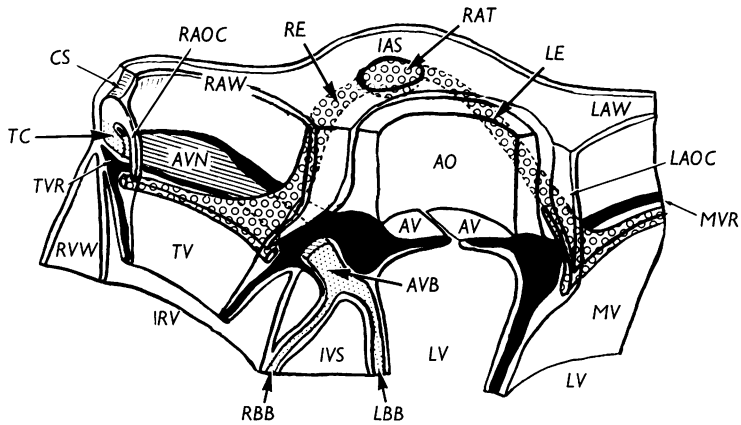


Fig. 10. Diagrammatic representation of the relationship between the fibrous ring, AV specialized tissue and retro-aortic specialized tissue.

AV node and bundle

Bifurcation (cut end on)

Retro-aortic specialized tissue and extensions

As the cells are traced forwards they merge together to form columns of small cells. These columns are separated by connective tissue, and are firmly embedded within the fibrous trigone. Multiple nerve bundles again interweave with the nodal columns, and further ganglion cells are identified in the surrounding connective tissue (Fig. 13). This collection of nerves and specialized cells constitutes the compact atrioventricular node. When traced anteriorly the nodal cells enlarge and become arranged individually. The fascicle of these larger single cells, widely spaced by connective tissue containing nerve bundles, pierces the atrioventricular fibrous ring to become the

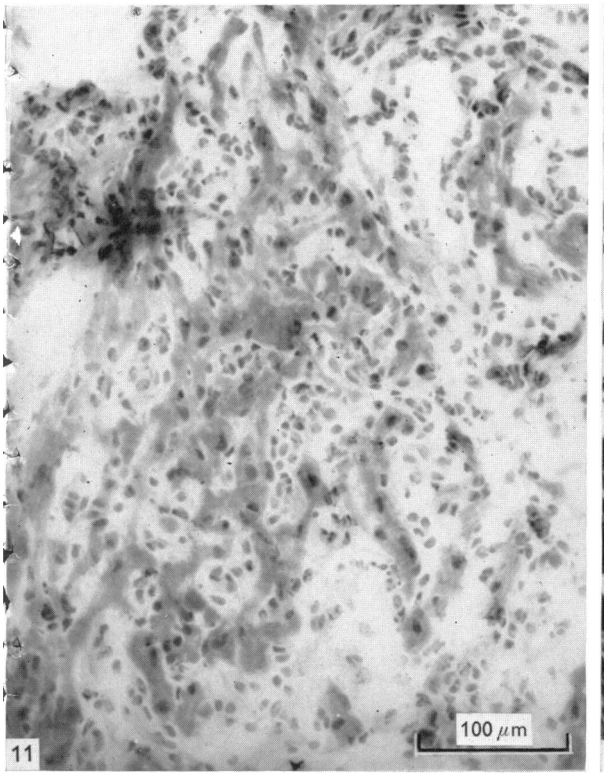
---

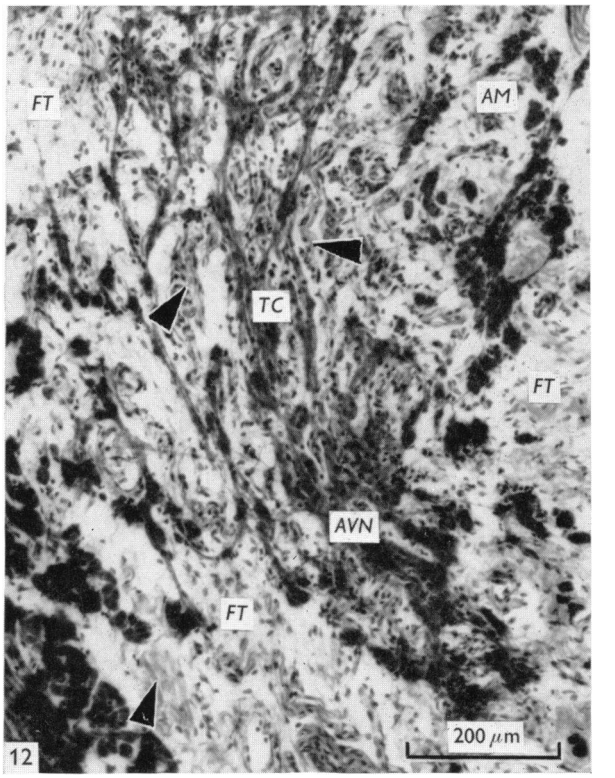
Fig. 11. Transitional cells from the area beneath the ostium of the coronary sinus. Note the cells are elongated and pale staining, and are separated by connective tissue septa in which ramify numerous small blood vessels. Trichrome stain.

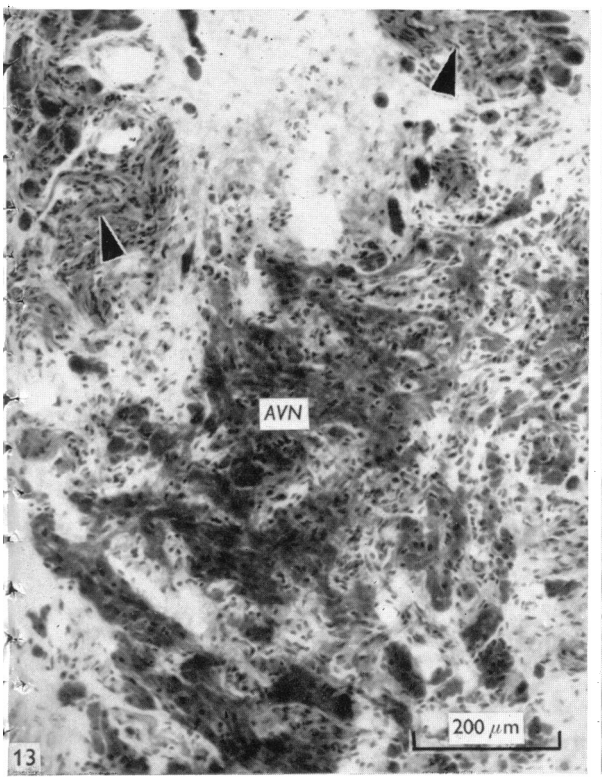
Fig. 12. This transverse section from the septum beneath the ostium of the coronary sinus shows the interweaving transitional cells joining anteriorly to form the compact atrioventricular node. At their other extremity the transitional cells are in contact with atrial myocardium. Note how the atrioventricular node is surrounded by fibrous tissue except on its posterior aspect. Numerous nerve bundles (arrowed) ramify amongst the connective tissue and between the specialized cells. The node is separated by the atrioventricular fibrous ring from ventricular myocardium. Trichrome stain. This section is adjacent to the section processed for AChE illustrated in Fig. 16.

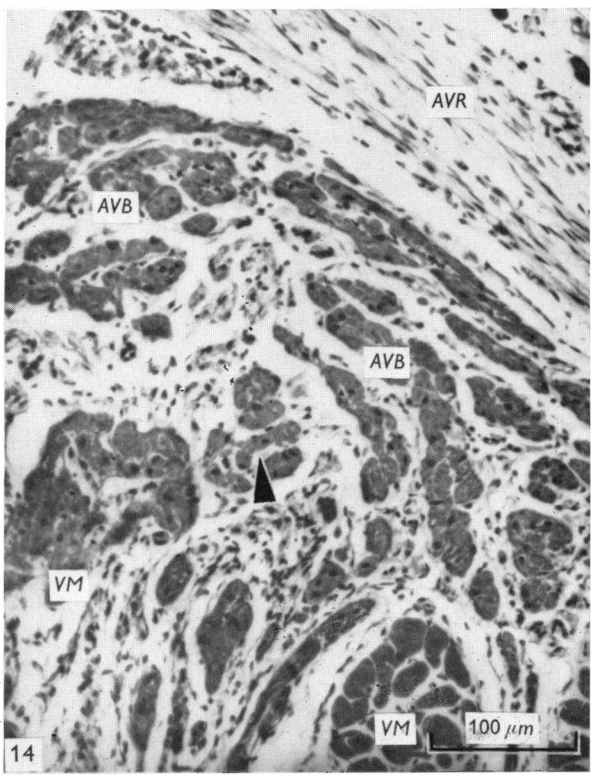
Fig. 13. Transverse section through the compact atrioventricular node. The node is made up of interweaving bundles of small cells, and is surrounded by fibrous tissue. Large nerve bundles and ganglia (arrowed) are seen adjacent to the node, whilst nervous and vascular plexuses ramify amongst the nodal bundles. Trichrome stain.

Fig. 14. Coronal section of the atrioventricular bundle lying astride the interventricular septum beneath the atrioventricular fibrous ring. Note that the bundle cells are individually arranged, surrounded by connective tissue, and are no larger than myocardial cells. Note also the direct communications with the ventricular myocardium (arrowed). Trichrome stain. This section is an adjacent section to that illustrated in Fig. 8.


100 μm
11


FT
AM
TC
FT
AVN
FT
200 μm
12


AVN
200 μm
13


AVR
AVB
AVB
VM
VM
100 μm
14

atrioventricular bundle (Fig. 14). This structure forms a loose crescent of cells astride the muscular interventricular septum in relation to the right border of the aortic root and outflow tract of the left ventricle. As the bundle sits astride the septum some specialized cells pass directly into the underlying ventricular myocardium (Figs. 8, 14).

The left bundle branch is given off along the length of the main bundle. It is a thin extensive sheet of subendocardial cells which passes down the septal aspect of the

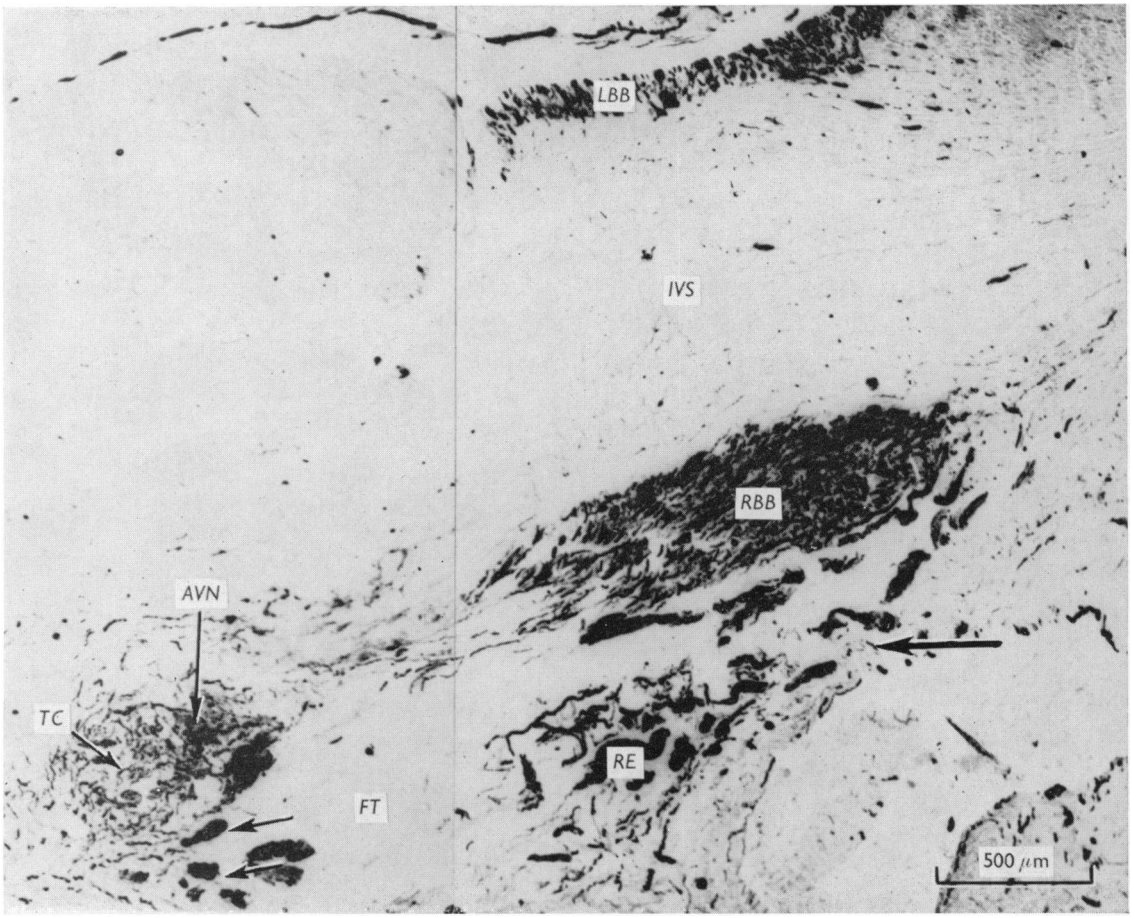


Fig. 15. Composite photograph of the atrioventricular specialized tissues demonstrated by AChE content (method as indicated for Fig. 3). The well innervated transitional cells are in contrast to the poorly innervated interventricular septum. The node is strongly reactive and enclosed in fibrous tissue. Ganglion cells are arrowed. The AV bundle is out of the plane of section but both bundle branches are clearly evident as is the right extension of the retro-aortic tissue separated from the right bundle branch by fibrous tissue (large arrow).

left ventricular outflow tract (Figs. 6, 15). The right bundle branch is a continuation of the main bundle, and is a thicker fascicle of cells which passes intramyocardially to reach the subendocardium of the right ventricle (Fig. 10). The constituent cells of the bundle and proximal bundle branches are no larger than ventricular myocardial cells, but are paler staining and are separated from the myocardium by connective

tissue sheaths in which ramify large nerve bundles (Fig. 18). In the distal parts of the ventricles it becomes increasingly difficult to differentiate the specialized cells from the myocardial cells, since the dimensions of the two are still similar. However, tracing of serial sections facilitates their recognition, and the ramifications are clearly recognizable by their AChE content (see below).

Like the sinuatrial node, the atrioventricular specialized tissue is demarcated by its AChE content (Fig. 15). The transitional cells are weakly AChE-positive; the compact node is intensely reactive and the bundle and ramifications are less intensely stained but clearly definable from ventricular myocardium. In addition the tissue is profusely innervated by AChE-containing nerves. A plexus of small nerves is identified around the specialized cells, being most profuse in relation to transitional and nodal cells (Figs. 15–17). In addition, multiple AChE-positive nerve bundles stream into the node from the posterior wall and adjacent ganglia, and are continued into the bundle, branches and ramifications (Figs. 6, 8, 15 and 19).

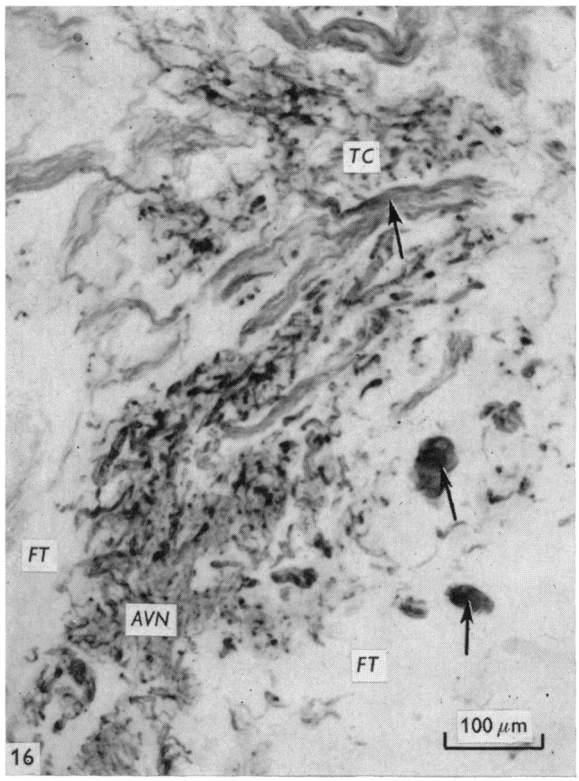
In contrast to the rich AChE-positive innervation, sections processed to reveal catecholamines show few CA-containing nerves. The density of innervation in the transitional cells and node is similar to that in atrial myocardium, but the bundle (Fig. 9) and bundle branches (Fig. 7) are very poorly innervated. The ventricular and coronary vessels are well supplied with CA-containing terminals, but these structures receive very little AChE-positive innervation.

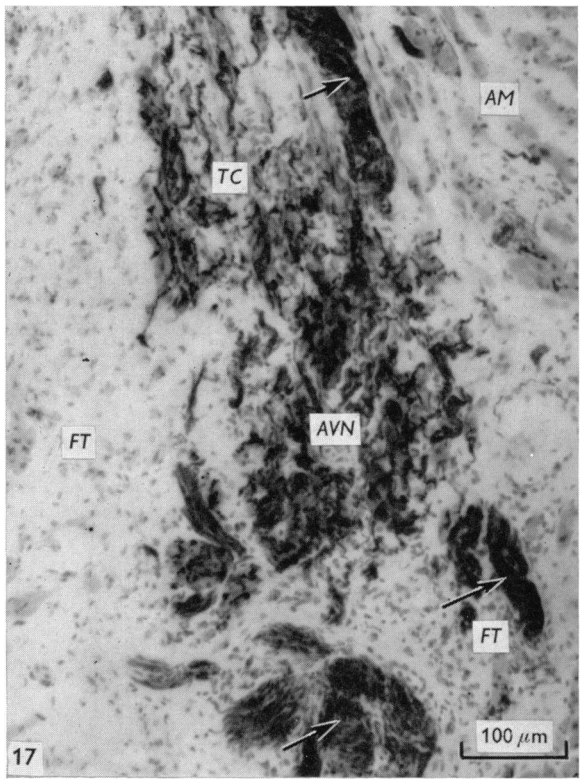
Internodal myocardium

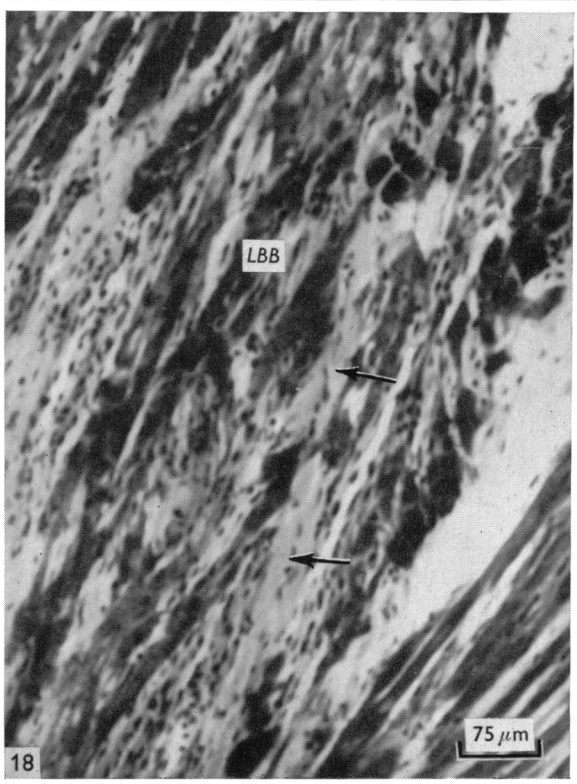
The myocardium of the posterior atrial wall and interatrial septum has been extensively examined in this investigation. It has not been possible to define histologically distinct tracts between the nodes. As pointed out above, the only nodal input is via the posterior atrial wall. The innervation of the myocardium is less profuse than the nodal areas, and again distinct internodal tracts are not identifiable.

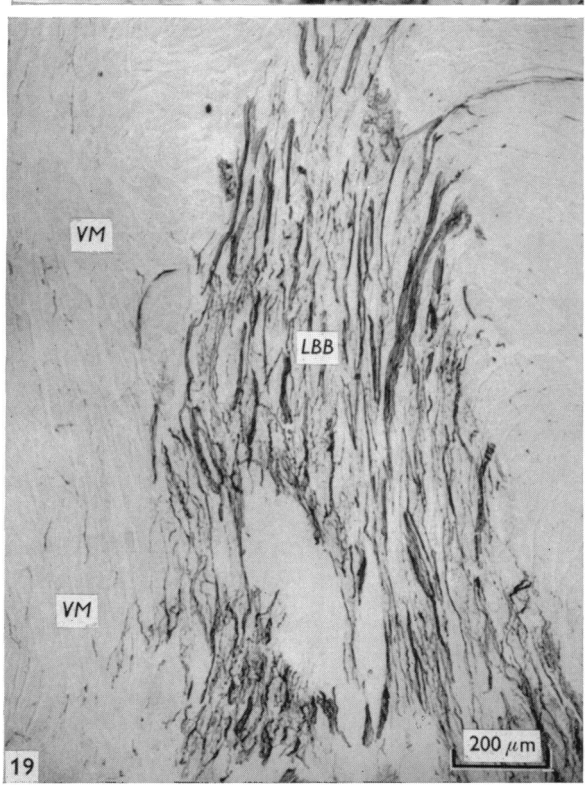
Extranodal atrial specialized tissue

A collection of cells, smaller, elongated and pale-staining, is identified in the atrial septum behind the aortic root (Fig. 10). Atrial myocardial cells contribute to this retro-aortic knot, which is set in fibrous tissue. The knot is well vascularized and nerve bundles pass through the septum to impinge upon it; collections of individual ganglion cells are identified in its vicinity (Fig. 20). Extensions of this specialized tissue pass to right and left of the aortic root (Fig. 10), and the right extension crosses the AV specialized tissue but makes no contact with it. Both extensions pass into the atrial margins of the atrioventricular valves, where they form a circumferential ring of specialized tissue (Fig. 22), which is definable from the atrial myocardium in all cusps of the AV valves. The retro-aortic knot and its extensions are well innervated with AChE-containing nerves, which confirms their distinction from atrial myocardium (Figs. 21, 23). It must be emphasized that no connexions are identified between retro-aortic tissue and AV specialized tissue or ventricular myocardium.


TC
FT
AVN
FT
100 μm
16


AM
TC
FT
AVN
FT
100 μm
17


LBB
75 μm
18


VM
LBB
VM
200 μm
19

DISCUSSION

The position of the sinuatrial node in relation to atrial structures is as documented by Keith & Flack (1907) and subsequently confirmed by many workers. However, unlike the situation in human and canine material (Hudson, 1960; James, Sherf, Fine & Morales 1966), the nodal cells in the guinea-pig are not arranged in circumarterial fashion, but occupy the full thickness of the atrial wall, as in the golden hamster (Walls, 1942) and the rabbit (James, 1967). The rich innervation of sinuatrial musculature is well documented (Keith & Mackenzie, 1910; and many subsequent workers). The present investigation shows that the nerves present contain both acetylcholinesterase and catecholamines, and the similarity between the demonstrated plexuses could indicate that AChE and CA are contained within the same nerves. The presence of CA alone in vascular nerves, whilst not proof in itself, mitigates against this possibility. It is more likely that separate nerves containing CA and AChE ramify in an arrangement resembling a ground plexus (Hillarp, 1959). Such a relationship would be in keeping with the ultrastructural findings of Nillson & Sporrong (1970) in the rabbit SA node, and is to be expected since it has been shown that the pacemaker is under both vagal and sympathetic influence (Hoffman, 1967; Vassalle, 1971).

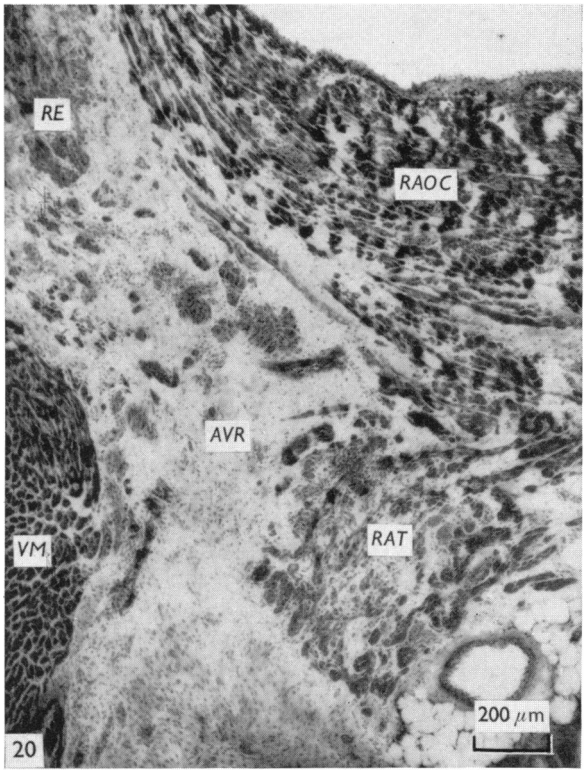
The findings concerning the atrioventricular node indicate that the architecture in the guinea-pig is as described by Tawara (1906). This is in contrast to the results of studies in the rabbit (Anderson, 1971) and in man (Anderson & Latham, 1971), both also studied by Tawara and reported to conform to a basic plan. These recent findings indicate that the nodal arrangement is similar to the plan constructed by electrophysiologists (Paes de Carvalho, 1961) and electron microscopists (De Felice & Challice, 1969). This arrangement produces a trilaminar node in the horizontal plane. In contrast, the findings reported by Tawara and most subsequent morphologists in many species and now also in the guinea-pig indicate that the node is trilaminar in a vertical plane, with a progression from transitional cells through the compact node to the atrioventricular bundle. However, in all nodes studied the morphological arrangement of interweaving transitional cells impinging on closely packed, small nodal cells, is present: this arrangement was implicated by Hoffman & Cranefield (1960) in the production of nodal delay. A further marked difference between the

Fig. 16. Transverse section of atrioventricular node showing AChE-positive nodal cells enclosed in a fibrous tissue collar and profusely innervated with AChE-positive nerves. Transitional cells are seen impinging on the posterior aspect of the node. AChE-positive nerve bundles and ganglion cells are evident (arrowed). Section processed for AChE as Fig. 3.

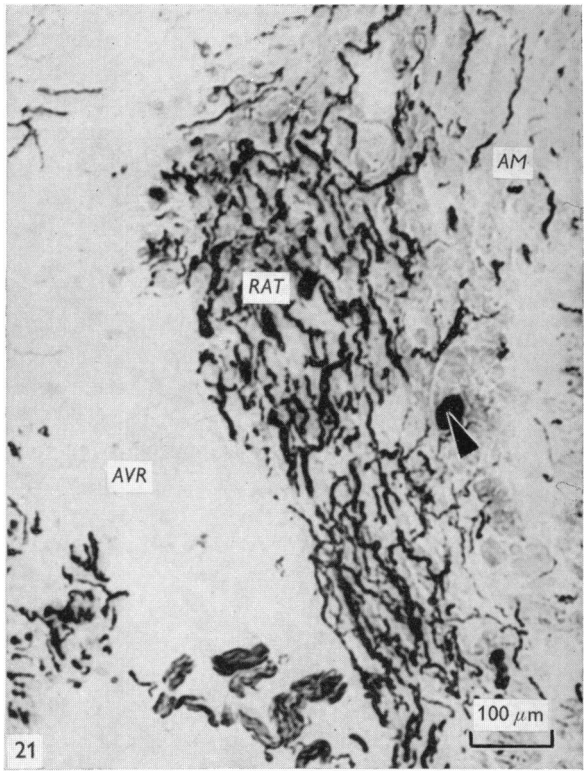
Fig. 17. Coronal section through posterior extent of atrioventricular node showing well innervated transitional cells contrasting with poorly innervated atrial myocardium. Note large AChE-positive nerve bundles approaching the node which is enclosed by fibrous tissue. Section processed for AChE as for Fig. 3.

Fig. 18. Section through subendocardial left bundle branch in the plane of the endocardium. Note that the specialized tissue is enclosed by connective tissue. The individual cells are no larger than ventricular myocardial cells. Nerve bundles are well demarcated (arrows). Trichrome stain.

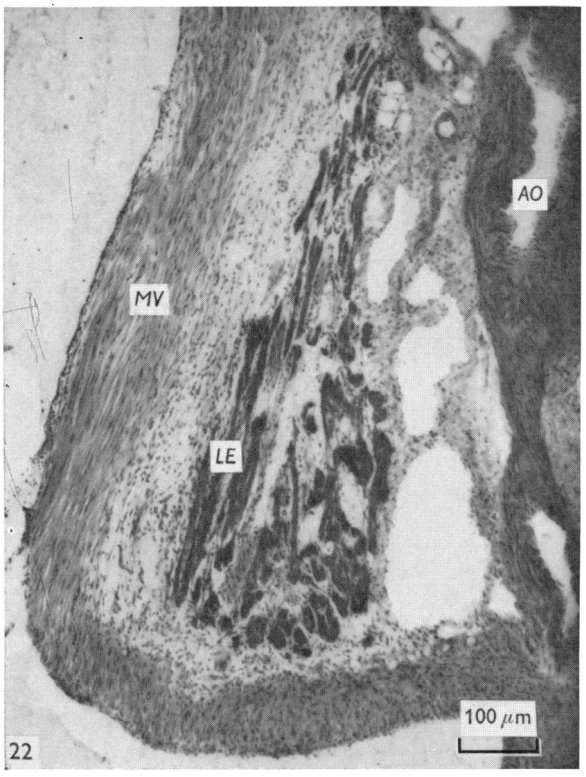
Fig. 19. Section a short distance from Fig. 9 processed to demonstrate AChE-activity (as for Fig. 3). Note that the specialized cells are AChE-positive, and the profuse innervation is confined to the specialized tissue. The ventricular myocardium is poorly innervated.



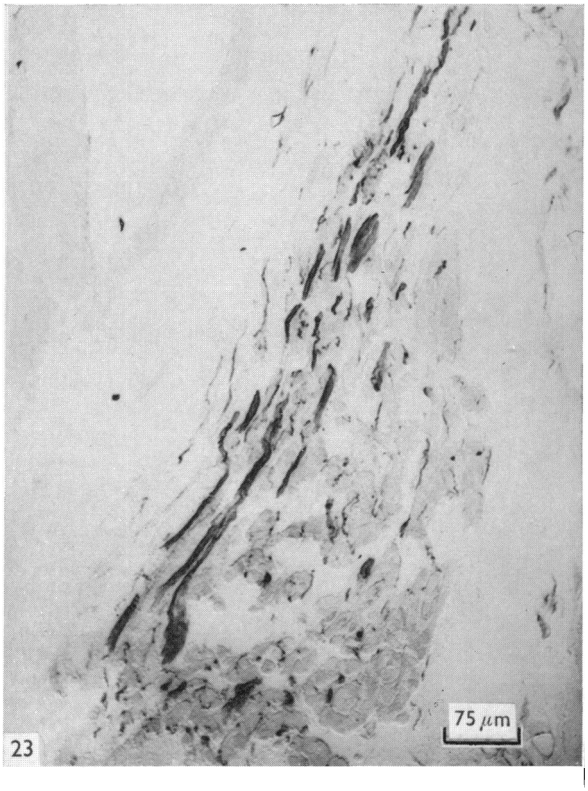
20



21



22



23

guinea-pig and the rabbit is the presence of AChE-containing nerves throughout the node (in the rabbit these nerves are confined to transitional cells). It is well known that vagal activity contributes to nodal delay (Hoffman & Cranefield, 1960; Hoffman, 1967), and it could be that the nervous component is of more import in producing delay in the guinea-pig heart. The significance of these reported differences in nodal architecture remains uncertain, and requires further investigation.

The profuse AChE-positive innervation in the guinea-pig node is continued into the AV bundle and terminal arborizations of the ventricular specialized tissue. Although Field (1951) reported a rich nervous component in the guinea-pig bundle, Lev (1960) summed up reports on innervation in this animal as follows: 'nerve fibres are present in the node, a lesser number are noted in the bundle, few in the bundle branches and none distally'. This statement is inconsistent with the present results. It is furthermore of note that, whilst rich in AChE, the plexus contains no catecholamines. It is therefore likely that the nerves are parasympathetic effector, the more so since cholinergic endings have been reported in guinea-pig ventricular specialized tissue (Hirano & Ogawa, 1967; Humpherson & Anderson, 1970). With regard to a parasympathetic ventricular supply Vassalle (1971) has stated that vagal stimulation produced ventricular standstill, but felt this was unlikely to be due to vagal inhibition, since: 'for such an inhibition to occur, two conditions should be met: (1) the vagus nerve should be shown to innervate the entire ventricular Purkinje network; and (2) acetylcholine should suppress spontaneous activity of ventricular Purkinje fibres'. The present results indicate that, certainly in the species studied, the first criterion of Vassalle is satisfied, and that vagal effects can be mediated to the entire ventricular specialized tissue.

The absence of catecholamine-containing nerves throughout the ventricular specialized tissue is interesting and surprising. An adrenergic effect on the ventricular network is well documented electrophysiologically (Hoffman, 1967; Vassalle, 1971), and adrenergic plexuses have been demonstrated in such situations in several species (Ehinger, Falck & Sporrong, 1966; Anderson, 1971). The situation in the pig, however, is identical to that presently described in the guinea-pig (Bojsen-Møller & Tranum-Jensen, 1971). Such marked species variation indicates that care must be taken when comparing experimental data produced with differing species.

Although the cells constituting the ventricular specialized tissue are histologically and neurohistochemically differentiated from ventricular myocardium, they are no

---

Fig. 20. Transverse section of interatrial septum behind the aortic root. The retro-aortic knot of specialized tissue is quite separate from the right atrial overlay cells. The right extension is seen passing towards the tricuspid valve margin. The atrioventricular fibrous ring separates the tissue from ventricular myocardium. The tissue is well supplied with nerves and blood vessels. Trichrome stain.

Fig. 21. Transverse section of retro-aortic tissue showing AChE-positive innervation (processed as for Fig. 3). Note AChE-positive ganglion cell (arrowed) and poorly innervated atrial myocardium.

Fig. 22. Musculature running in mitral valve base as it comes into relation with the aortic root. Note the left extension of the retro-aortic tissue with specialized characteristics. Trichrome stain.

Fig. 23. Adjacent section to that illustrated in Fig. 22 processed to demonstrate AChE-activity (as in Fig. 3). Note rich innervation of specialized tissue.

larger than the myocardial cells. They do not possess 'Purkinje' characteristics. This finding contrasts with the results of Truex (1961) who reported guinea-pig ventricular specialized cells to be larger than the myocardial cells.

The AV node in the guinea-pig is embedded in the fibrous tissue of the right trigone. This means that the only input to the AV specialized tissue is via the myocardium of the posterior atrial wall. A similar finding was reported in the rabbit (Anderson, 1971) and electrophysiological findings in this animal support such a morphological arrangement (Janse, 1969). If the node is enclosed in such a fashion, the reported middle and anterior internodal pathways (Robb & Petri, 1961; James, 1963, 1967) would, in these animals, impinge upon connective tissue, making no contact with nodal musculature. Although the internodal myocardium was extensively studied in this investigation, no evidence was produced to substantiate the claim that histologically distinct tracts exist between the nodes. James (1963) has remarked that controversy exists concerning such tracts because workers expect them to be composed exclusively of 'Purkinje' tissue. Truex (1961) doubted whether such tissue existed in the atria, and I would support this contention. Whilst Emberson & Challice (1970) were able to differentiate internodal tracts in rodents following vital staining, ultrastructural studies revealed that few of the fibres possessed specialized characteristics. Thus it is possible that characteristics which allow the phenomenon of preferential conduction (Eyster & Meek, 1916; Holsinger, Wallace & Sealy, 1968) by some atrial fibres do not allow their histological differentiation to be made. In this context the internodal regions are of sinus venosus origin, and James (1963) has indicated that this origin may contribute to preferential conduction through these regions.

Although internodal specialized cells are not seen in the guinea-pig, a retro-aortic collection of specialized cells has been identified. Extranodal specialized tissue has been previously reported in the guinea-pig by Robb & Petri (1961), particularly in the AV valve bases. However, they did not describe the exact nature and disposition of this tissue. The present results indicate that the valvular tissue originates in a well innervated retro-aortic node, unconnected with the AV specialized tissue. The first description of additional interatrial specialized tissue was made by Shaner (1929) in a calf embryo. Additional tissue has also been described in the golden hamster valve bases by Walls (1942). He considered these tracts to be extensions from the AV node, but in this animal Gossrau (1971) has reported a well innervated retro-aortic knot unconnected with the AV tissue. It is likely that this tissue is homologous with that presently reported. Its functional significance is not clear. The connexion with the valve bases suggests a possible function in controlling valvular activity, and Sarnoff, Gilmore & Mitchell (1962) have shown that valve closure is dependent on atrial activity and can be influenced by nerve stimulation. Alternatively it is possible that the tissue represents a remnant of the primitive atrioventricular canal musculature, which has specialized features in lower animals, and has been implicated in the embryologic development of the AV node (Keith & Flack, 1906; Lieberman, 1970). These suggestions remain conjecture, however, and much has still to be investigated concerning this tissue.

SUMMARY

1. The cardiac specialized tissue and its associated nerves have been investigated in the guinea-pig.

2. The disposition of the sinuatrial node conforms with previous descriptions, but it is not arranged around a central artery. The nodal cells are supplied with plexuses of both acetylcholinesterase- and catecholamine-containing nerves.

3. The morphological arrangement of the atrioventricular node is similar to that reported by previous morphologists, but differs from the nodal architecture identified in rabbits and humans.

4. Histologically distinct tracts of specialized cells are not identified between the cardiac nodes.

5. The node, bundle and branches are well supplied with an acetylcholinesterase-positive nerve plexus, but are poorly supplied with catecholamine-containing nerves.

6. No cells with 'Purkinje' characteristics can be identified in the guinea-pig heart.

7. An additional collection of specialized cells is identified in the retro-aortic atrial septum. Extensions of this collection pass into both AV valve bases. The cells are well innervated.

8. The significance of these findings is discussed with regard to previous morphological and electrophysiological reports.

I wish to express my grateful thanks to Professor G. A. G. Mitchell, in whose department the work was performed; to Dr J. A. Gosling for his valuable advice and criticisms, and to Mr P. Howarth for his photographic skills.

REFERENCES

ANDERSON, R. H. (1971). Histologic and histochemical evidence concerning the presence of morphologically distinct cellular zones within the rabbit atrioventricular node. *Anatomical Record* (in the Press).

ANDERSON, R. H. & LATHAM, R. A. (1971). The cellular architecture of the human atrioventricular node, with a note on its morphology in the presence of a left superior vena cava. *Journal of Anatomy* **109**, 443–455.

BAYLISS, B. J. & TODRICK, A. (1956). The use of selective acetylcholinesterase inhibitors in the estimation of pseudo-cholinesterase activity in rat brain. *Biochemical Journal* **62**, 62–73.

BOJSEN-MØLLER, F. & TRANUM-JENSEN, J. (1971). On nerves and nerve endings in the conducting system of the moderator band (septomarginal trabecula). *Journal of Anatomy* **108**, 387–395.

DE FELICE, L. J. & CHALLICE, C. E. (1969). Anatomical and ultrastructural study of the electrophysiological atrioventricular node of the rabbit. *Circulation Research* **24**, 457–475.

EHINGER, B., FALCK, B. & SPORRONG, B. (1966). Adrenergic fibres to the heart and to peripheral vessels. *Bibliographica Anatomica* **8**, 35–45.

EMBERSON, J. W. & CHALLICE, C. E. (1970). Studies on the impulse conducting pathways in the atrium of the mammalian heart. *American Heart Journal* **79**, 653–667.

EYSTER, J. A. E. & MEEK, W. J. (1916). Experiments on the origin and conduction of the cardiac impulse. VI. Conduction of the excitation from the sino-auricular node to the right auricle and the atrioventricular node. *Archives of Internal Medicine* **18**, 775–799.

FIELD, E. J. (1951). The nervous component of the A.V. bundle. *Journal of Anatomy* **85**, 105–113.

GOMORI, G. (1952). *Microscopic Histochemistry – Principles and Practice*. Chicago: University of Chicago Press.

GOSSRAU, R. (1971). Über zusatzliches Reizleitungsgewebe im Goldhamsterherzen. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **115**, 587–592.

HILLARP, N. A. (1959). Constructional and junctional organisation of the autonomic innervation apparatus. *Acta physiologica scandinavia* **46**, Supplementum 157, 1–38.

HIRANO, H. & OGAWA, K. (1967). Ultrastructural localization of cholinesterase activity in nerve endings in guinea pig heart. *Journal of Electron Miscroscopy* **16**, 313–321.

HOFFMAN, B. F. (1967). Autonomic control of cardiac rhythm. *Bulletin of the New York Academy of Medicine* **43**, 1087–1096.

HOFFMAN, B. F. & CRANEFIELD, P. (1960). *Electrophysiology of the Heart*. New York: McGraw Hill.

HOLSINGER, J. W., SEALY, W. C. & WALLACE, A. G. (1968). The identification and surgical significance of the atrial internodal conduction tracts. *Annals of Surgery* **167**, 447–463.

HUDSON, R. E. B. (1960). The human pacemaker and its pathology. *British Heart Journal* **22**, 153–161.

HUMPHERSON, J. R. & ANDERSON, R. H. (1970). A preliminary investigation into the ultrastructure of the guinea pig atrioventricular node and bundle. *Journal of Anatomy* **107**, 376–377 (P).

JAMES, T. N. (1963). The connecting pathways between the sinus node and AV node and between the right and left atrium in the human heart. *American Heart Journal* **66**, 498–508.

JAMES, T. N. (1967). Anatomy of the cardiac conduction system in the rabbit. *Circulation Research* **20**, 638–648.

JAMES, T. N., SHERF, L., FINE, G. & MORALES, A. R. (1966). Comparative ultrastructure of the sinus node in man and dog. *Circulation* **34**, 139–163.

JANSE, M. J. (1969). Influence of the direction of the atrial wave front on A.V. nodal transmission in isolated hearts of rabbits. *Circulation Research* **25**, 439–449.

KARNOVSKY, M. J. (1964). The localization of cholinesterase activity in rat cardiac muscle by electron microscopy. *Journal of Cell Biology* **23**, 219–232.

KEITH, A. & FLACK, M. (1906). The auriculo-ventricular bundle of the human heart. *Lancet* ii, 359–364.

KEITH, A. & FLACK, M. (1907). The muscular connexions of the primary divisions of the heart. *Journal of Anatomy and Physiology* **41**, 172–189.

KEITH, A. & MACKENZIE, I. (1910). Recent researches on the anatomy of the heart. *Lancet* i, 101–103.

LEV, M. (1960). The conduction system: comparative anatomy in mammalian heart. In *Pathology of the Heart* (Ed. S. E. Gould). Springfield, Illinois: Thomas.

LIEBERMAN, M. (1970). Physiologic development of impulse conduction in embryonic cardiac tissue. *American Journal of Cardiology* **25**, 279–284.

NILLSON, E. & SPORRONG, B. (1970). Electron microscopic investigation of adrenergic and non-adrenergic axons in the rabbit S-A node. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **111**, 404–412.

PAES DE CARVALHO, A. (1961). Cellular electrophysiology of the atrial specialised tissues. In *The Specialised Tissues of the Heart* (Ed. A. Paes de Carvalho, W. C. de Mello and B. F. Hoffman). Amsterdam: Elsevier.

ROBB, J. S. & PETRI, R. (1961). Expansions of the A.V. system in the atria. In *Specialised Tissues of the Heart* (Ed. A. Paes de Carvalho, W. C. de Mello and B. F. Hoffman). Amsterdam: Elsevier.

SARNOFF, S. J., GILMORE, J. P. & MITCHELL, J. H. (1962). Influence of atrial contraction and relaxation on closure of mitral valve. *Circulation Research* **11**, 26–35.

SHANER, R. F. (1929). The development of the atrioventricular node, bundle of His and sinuatrial node in the calf, with a description of a third embryonic node-like structure. *Anatomical Record* **44**, 85–99.

SPRIGGS, T. B. L., LEVER, J. D., REES, P. M. & GRAHAM, J. P. D. (1966). Controlled formaldehyde-catecholamine condensation in cryostat sections to show adrenergic nerves by fluorescence. *Stain Technology* **41**, 323–327.

TAWARA, S. (1906). *Das Reizleitungssystem des Saugetierherzens*. Jena: Gustav Fischer.

TRUEX, R. C. (1961). Comparative anatomy and functional considerations of the cardiac conduction system. In *Specialised Tissues of the Heart* (Ed. A. Paes de Carvalho, W. C. de Mello and B. F. Hoffman). Amsterdam: Elsevier.

VASSALLE, B. (1971). Automaticity and automatic rhythms. *American Journal of Cardiology* **28**, 245–252.

WALLS, E. W. (1942). Specialized conducting tissue in the heart of the golden hamster (*Cricetus auratus*). *Journal of Anatomy* **76**, 359–369.