J. Anat. (1971), **108**, 3, pp. 375–386
With 11 *figures*
Printed in Great Britain

Whole-mount demonstration of cholinesterase-containing nerves in the right atrial wall, nodal tissue, and atrioventricular bundle of the pig heart

F. BOJSEN-MØLLER AND J. TRANUM-JENSEN
Anatomy Department C, University of Copenhagen, Universitetsparken 1, 2100, *Copenhagen Ø, Denmark*

(*Received* 24 *April* 1970)

INTRODUCTION

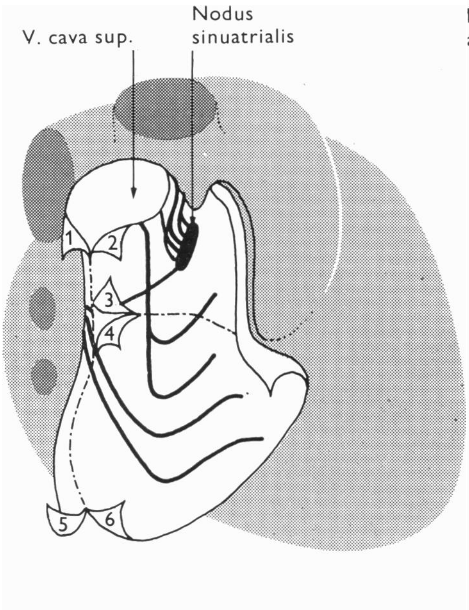
The nerves in the heart are distributed subepicardially, subendocardially, along the coronary arteries and along the conducting system. The innervation is ample, and complicated networks of autonomic nerves and nerve cells are formed. The topography of the nerves and ganglia and their relation to the conducting system have been investigated by dissection and by means of conventional histological sections or thick, cleared sections (Wilson, 1909; Aschoff, 1911; Wolhynsky, 1928; Licata, 1954; Davies, Francis & King, 1952; King & Coakley, 1958; Wensing, 1964). By these methods it is, however, difficult to demonstrate the exact course and arrangement of the nerves, and our knowledge of the innervation of the sinuatrial (*SA*) and atrioventricular (*AV*) nodes, of the course of the nerves between the two nodes, and of the position of ganglion cells in the atrium is still deficient.

The purpose of this study is to present a method for staining cholinesterase (ChE)-containing nerves in whole mounts and to attempt, by this method, to obtain a more complete mapping of the ChE-containing nerves and ganglion cells of the right atrium.

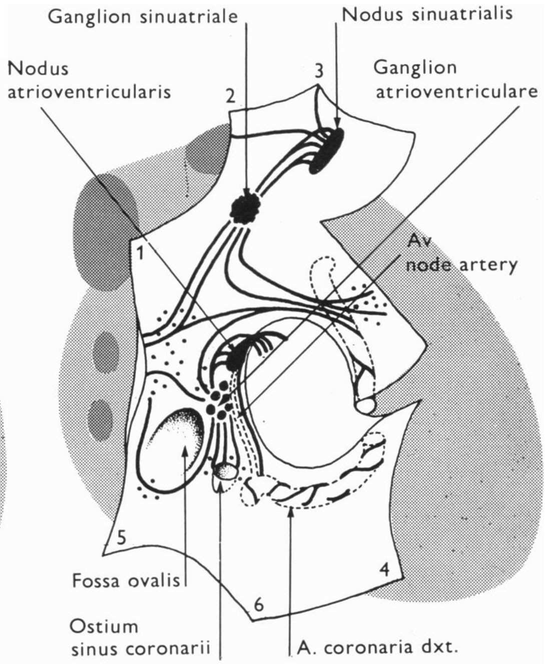
MATERIALS AND METHODS

ChE-containing nerves were stained and demonstrated in whole mounts of considerable thickness. All fixing and staining procedures were therefore performed by combined perfusion and immersion, the heart being kept immersed in a vessel into which the fluid was collected after having perfused the preparation. During prolonged perfusions, dissolved gases may be liberated into the perfusion equipment and into the blood vessels, making the perfusion heterogeneous and the result erratic. In order to avoid this, the water for the solutions was de-aerated before use (Thompsett, 1956).

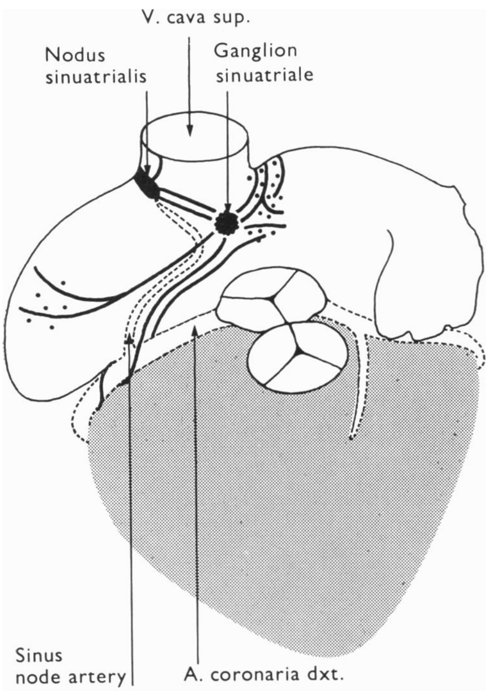
The study used nine hearts from pigs aged 3–17 days and weighing from 1·65 to 3·00 kg. The pigs were anaesthetized with Nembutal (100 mg i.p./kg body weight), and the chest was opened. Out of regard to the perfusion, it was important to avoid cardiac arrest in systole with a consequent closure of the intramural vessels, and a solution of KCl containing 100 mmol/l was therefore injected through the inferior vena cava until the heart stopped in diastole. The aorta was clamped, and the heart was removed and immersed in a 0·9 % solution of NaCl. A cannula was tied in the ascending aorta for the purpose of perfusion through the coronary arteries.


V. cava sup.
Nodus sinuatrialis
1
2
3
4
5
6

1


Ganglion sinuatriale
Nodus sinuatrialis
Nodus atrioventricularis
Ganglion atrioventriculare
1
2
3
4
5
6
Av node artery
Fossa ovalis
Ostium sinus coronarii
A. coronaria dxt.

2


V. cava sup.
Nodus sinuatrialis
Ganglion sinuatriale
Sinus node artery
A. coronaria dxt.

3

For demonstrating ChE, Koelle's method, as modified by Lewis (1961) and Tsuji (1968), was used. The procedure was as follows:

(1) Fixation by perfusion of 500 ml 10 % formol saline, followed by immersion in the same fixative for 3 h at approx. 10 °C.

(2) Perfusion by 300 ml water.

(3) Incubation at room temperature in a medium containing acetylthiocholine-iodide, 8 mmol/l, and including copper (pH 4·9). 200–300 ml of the incubation medium was perfused, the medium being collected after each perfusion and re-perfused after filtration. In the course of 20–25 h a total of 1200 ml was perfused in this manner, while the heart was kept immersed in the medium all the time. The pH, checked during the incubation, ranged from 4·9 to 5·2.

(4) Perfusion of 300–400 ml water followed by immersion in water for 16 h at 4 °C, and further perfusion of 300 ml water.

(5) Intermittent perfusion, over a period of about 4 h, of 300 ml of a Na_2S solution in 0·2 M-HCl containing 3 g Na_2S/100 ml, pH around 7.

(6) Perfusion of 200 ml water, followed by immersion for 24 h.

In all the perfusions the pressure was 50–60 cm of water except during the fixation, where it was 3–4 times higher.

The right atrium was removed and opened as shown in Figs. 1 and 2. The auricle was cut off and an incision made between the two venae cavae. From the intervenous tubercle another incision was made at right angles to the first one through the right wall of the atrium to the atrioventricular ostium. Thereafter, the atria were stretched out on a frame and postfixed in 10 % formalin for 12 h at 4 °C, dehydrated through ethanol and xylene, and cleared in anise oil.

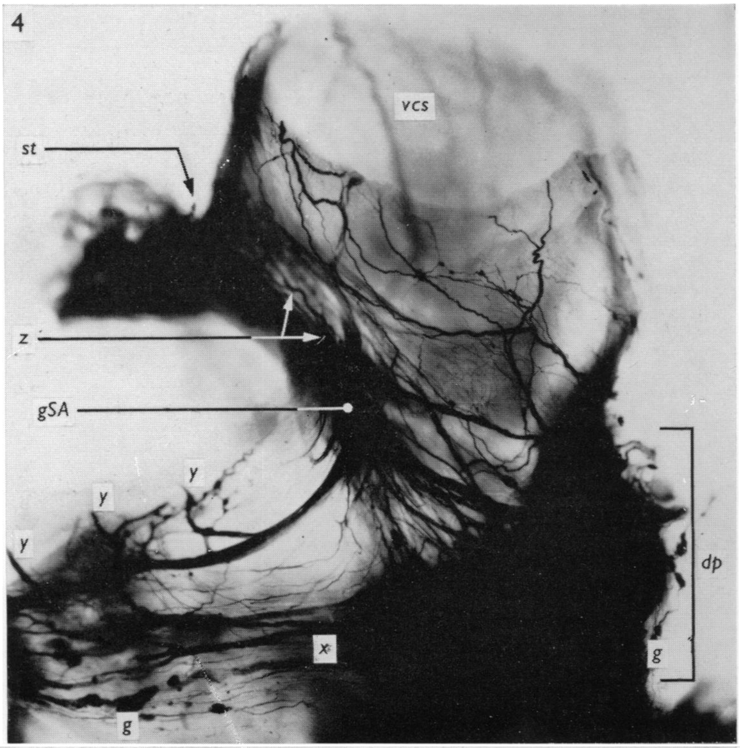
After examination in a stereomicroscope and photographing, specimens were taken from selected areas for histological study.

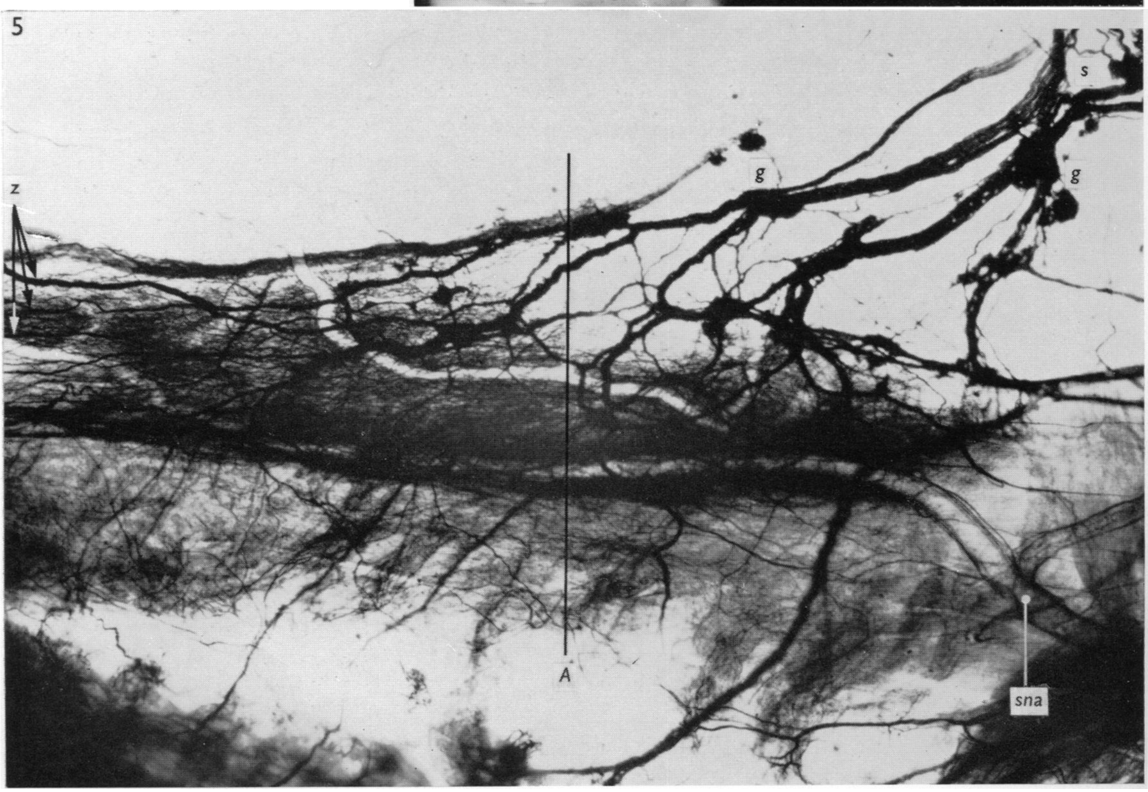
Since the reaction product, copper sulphide, is soluble in xylene and anise oil, these fluids were saturated with copper sulphide before use. This appreciably reduces decoloration of the preparations which proved stable for several months. In the histological sections the reaction product was stable after being embedded in Canada balsam.

Fig. 1. Diagram of right atrium. A few nerves from the dorsal plexus and from the superior vena cava to the *SA* node and to the lateral wall of the atrium are traced. The lines of cutting are dotted.

Fig. 2. Diagram of cut and unfolded atrium showing the nodal tissues, the largest ganglionic areas (dots), and nerve paths (solid lines). The majority of the nerves enter the dorsal wall of the atrium where they form the dorsal plexus. From this, nerves proceed by way of the sinuatrial ganglion to the sinuatrial node, to the coronary sulcus and, by way of the anterior and posterior limbus of the fossa ovalis, to the atrioventricular ganglion. The atrioventricular ganglion also receives nerves from the coronary sinus and from the plexus in the coronary sulcus. The *AV* node and the *AV* bundle receive nerves along the posterior and superior border.

Fig. 3. Diagram of the atria from the anterior aspect. The nerves from the dorsal plexus proceed to the left of the superior vena cava, distributing to the sinuatrial ganglion, to the plexus in the coronary sulcus, and to the left atrium. The sinuatrial ganglion is connected with the medial wall of the right auricle and with the *SA* node.


4
vcs
st
z
gSA
y
y
y
y
dp
x
g
g


5
z
s
g
g
A
sna

RESULTS

When the heart is cut open, cholinesterase reaction is found in all parts, including the thickest, the left ventricular wall. In the cleared preparations of the atrial wall the reaction product is visible in ganglion cells and in unmyelinated nerves which stands out distinctly from the surrounding tissue. In myelinated nerves there is a weaker reaction, and in addition a diffuse reaction is observed in the nodal tissue.

The right atrium is amply supplied with ChE-containing nerves which in several areas form complicated networks. Ganglia lie, in major and minor groups, along the nerves. The greatest accumulations of ganglia are found in the plexuses formed where the nerves enter the heart and around the *SA* and *AV* nodes.

The nerves enter the atrium at the cardiac base, along the wall of the superior vena cava, along the coronary sinus, and along the right coronary artery (cf. Figs. 1–3).

The nerves entering the cardiac base represent the most important supply. They enter the heart superiorly at the junction of the right and left atrium, forming a network with numerous ganglia. From this dorsal plexus the nerves are distributed to both atria, to the right by six routes:

(1) Many of the nerves proceed towards the *SA* node, passing on the left of the superior vena cava at its entrance into the atrium. *En route*, they run into the sinuatrial ganglion whence they continue to the *SA* node in the sulcus terminalis (Fig. 4, *z*).

(2) Other nerves cross the superior vena cava in its anterior wall above the *SA* node to curve down the right lateral wall of the atrium.

(3) A dense strand of nerves proceeds forward beneath the medial surface of the auricle and along the sinus-node artery to its origin in the coronary sulcus (Fig. 4, *x*). At this site they form a ganglion-containing plexus which surrounds the right atrioventricular ostium together with the right coronary artery.

From the nerve plexus at the cardiac base further nerves proceed, in the interatrial septum by way of the anterior (4) and posterior (5) limbus fossae ovalis, to the atrioventricular ganglion, which is situated in the septum immediately behind the *AV* node (Fig. 8).

(6) Finally, nerves with many ganglia proceed laterally in the posterior and right atrial wall in order to supply these parts and the *SA* node (Fig. 5, *s*).

A few nerves are embedded longitudinally in the wall of the superior vena cava. They occur especially in the anterior and right walls of the vein. A number of them end in the plexus proximal to the *SA* node, while others proceed down along the sulcus terminalis, where they deflect forward and branch into the auricle.

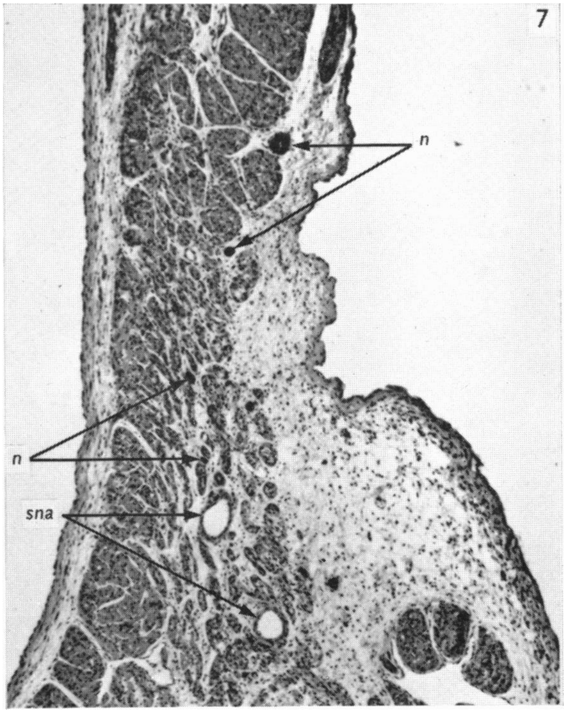
In the wall of the coronary sinus densely arranged nerves pass to the right atrium,

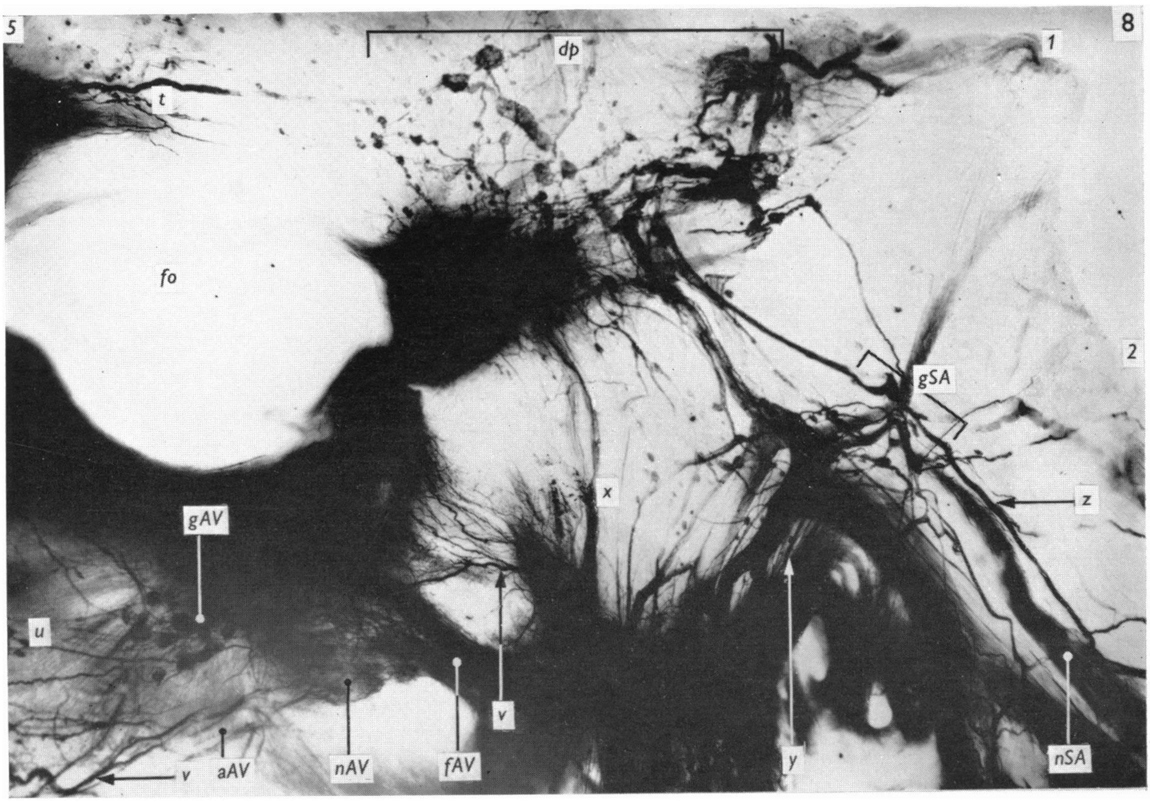
---

Fig. 4. Whole mount of antero-medial part of the right atrium with the entrance of the superior vena cava. Koelle staining (cf. Fig. 3), 8:1. *dp*, dorsal plexus; *vcs*, superior vena cava; *st*, sulcus terminalis; *gSA*, sinuatrial ganglion; *x*, nerves to the coronary sulcus; *y*, nerves to the medial wall of the right auricle; *z*, nerves from the *SA* ganglion to the *SA* node; *g*, ganglia.

Fig. 5. Whole mount of the *SA* node. Koelle staining. The node presents itself diffusely dark-stained and surrounded by a dense plexus of nerves (*z*) from the *SA* ganglion and (*s*) from the dorsal plexus. Within the node, the sinus node artery divides into two branches. 17:1. Section *A*, see Fig. 7. *sna*, sinus node artery; *g*, ganglia.


6


7
n
n
sna


8
5
dp
1
t
fo
2
gSA
x
z
gAV
u
v
y
v
aAV
nAV
fAV
nSA

forming around the ostium of the coronary sinus a ganglion-containing plexus. Branches from this plexus proceed, in the interatrial septum, to the atrioventricular ganglion (Fig. 9, *u*).

From the nerve plexus between the bulb of the aorta and the pulmonary trunk the right coronary plexus is formed. Beneath the right auricle part of this plexus meets the nerves which continue from the dorsal plexus along the sinus node artery. Together they form a plexus which surrounds the right atrioventricular ostium and gives off branches to the auricle. It could not be decided whether the branches to the auricle originate exclusively from the dorsal plexus or whether they too are connected with the plexus on the aorta. The plexus around the atrioventricular ostium also gives off branches to the atrioventricular ganglion. Some of these branches come from the anterior part and course dorsally across the membranous part of the interventricular septum. The remainder come from the dorsal part of the coronary sulcus and course forward to the ganglion along the *AV* node artery (Fig. 8, *vv*).

The sinuatrial ganglion (Remak's ganglion) is situated in the antero-medial wall of the superior vena cava immediately before it debouches into the atrium. It may present itself as a large, collected ganglion, as shown in Figs. 4 and 6, or as a group of smaller ganglia, each containing a considerable number of nerve cells (Fig. 8). ChE-containing nerves to this ganglion arise partly in the dorsal plexus and partly in the medial wall of the right auricle (Fig. 4). From the ganglion a dense plexus of nerves proceeds laterally in the sulcus terminalis to the *SA* node.

The *SA* node lies in the sulcus terminalis anterior to the entrance of the superior vena cava. Its medial end is situated where the crest of the right auricle meets the superior vena cava, and its lateral end extends a variable length laterally and caudally along the sulcus terminalis. It is surrounded by a dense plexus of ChE-containing nerves, including ganglion cells and a few myelinated nerves (Figs. 5, 7). The plexus receives branches from the sinuatrial ganglion, from the nerves in the anterior wall of the superior vena cava and from the dorsal plexus. From the plexus delicate branches enter the node where they lie on the surface of the nodal cells. In the middle of the *SA* node is the sinus node artery. It has no or only very few ChE-containing nerves in its adventitia.

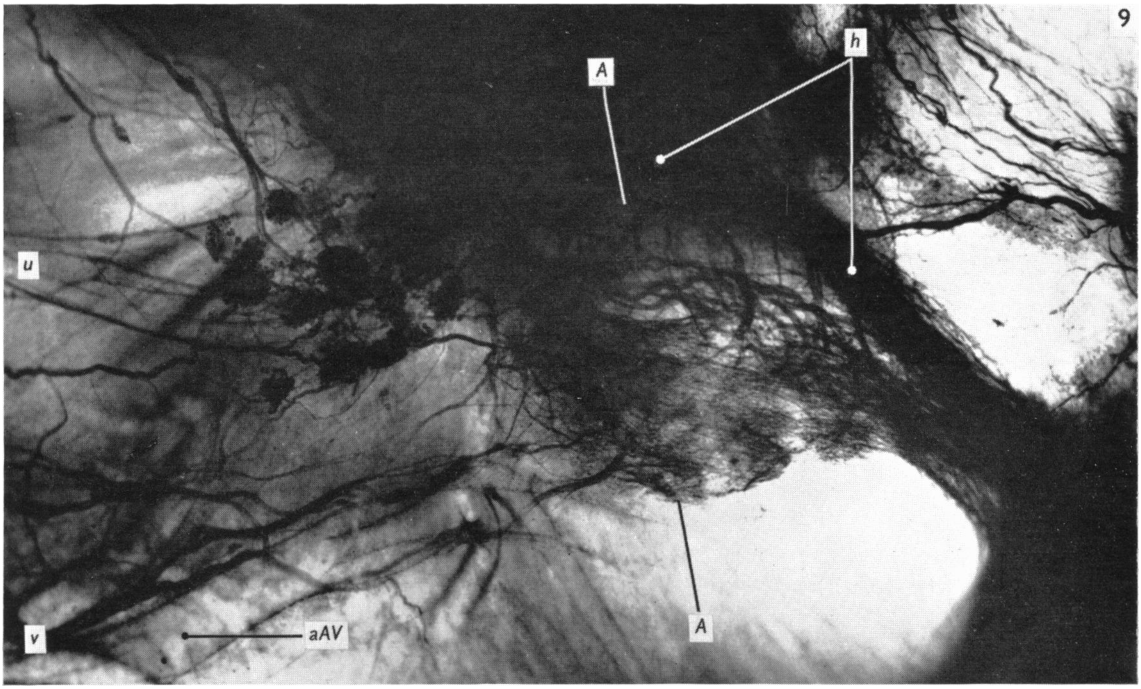
Histological cross sections of the crista terminalis have shown, flattened in the

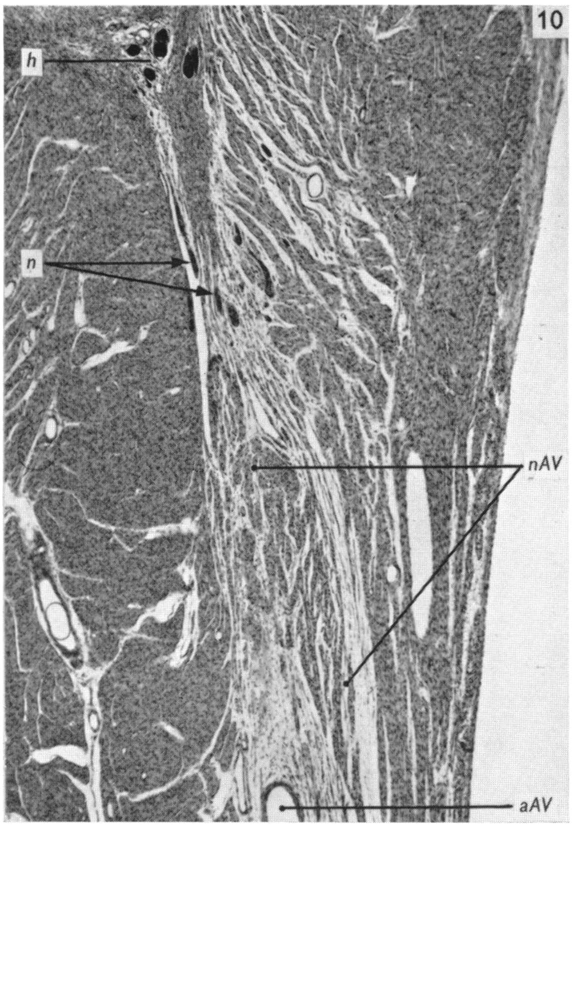
---

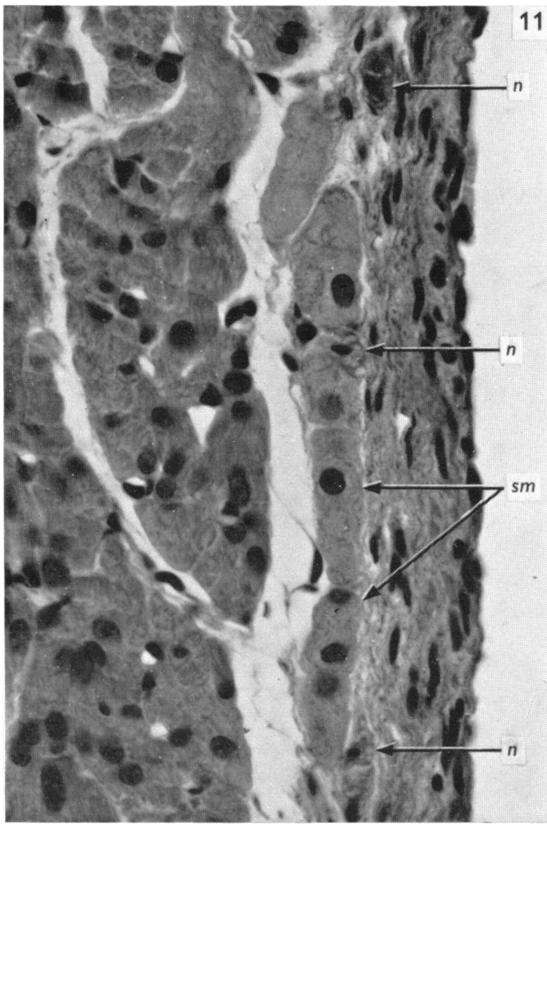
Fig. 6. Section through the *SA* ganglion depicted in Fig. 4. The reaction product in the ganglion cells has been almost completely eluted in the course of the histological procedure. H. and E. The ganglion is situated in the subepicardial connective tissue, and is composed of nerve cells surrounded by capsule cells. Most of the nerve cells have an eccentric nucleus. A major nerve trunk enters at the upper end of the ganglion. 85:1.

Fig. 7. Section through the *SA* node at *A* in Fig. 5. H. and E. The nodal tissue surrounds the two branches of the sinus node artery (sna). Several ChE-containing nerves (*n*) are seen in and around the node. 60:1.

Fig. 8. Whole mount of the cut and unfolded atrium showing the nodal tissues, ganglia, and nerves. Koelle staining. This preparation corresponds to Fig. 2, but is rotated through 90°. Points 1, 2 and 5 correspond to the numbering in Figs. 1 and 2. 9:1. *dp*, dorsal plexus; *fo*, fossa ovalis; *nSA*, sinuatrial node; *gSA*, sinuatrial ganglion (dispersed type); *nAV*, atrioventricular node; *gAV*, atrioventricular ganglion; *fAV*, atrioventricular fascicle; *aAV*, atrioventricular node artery; *t*, nerves and ganglia in the posterior limbus of the fossa ovalis; *u*, nerves from the ostium of the coronary sinus to *gAV*; *vv*, nerves from the plexus in the coronary sulcus to *nAV*; *x*, *y* and *z* correspond to the symbols in Fig. 4.


9
h
A
u
v
aAV
A


10
h
n
nAV
aAV


11
n
n
sm
n

subendocardial connective tissue, a fine strand of tissue of the same architecture as the conducting system of the ventricles. It is composed of 20–30 sarcoplasm-rich muscle fibres of a greater diameter than the fibres in the ordinary atrial muscle. The muscle fibres are surrounded by connective tissue and are accompanied by a few ChE-containing nerves (Fig. 11). This strand of specialized tissue is too delicate to be demonstrable in the whole mounts, and as we have not studied the atria in serial sections we have not been able to ascertain whether it forms a connexion between the *SA* and the *AV* nodes.

The atrioventricular ganglion is situated immediately behind the *AV* node, in the area between the fossa ovalis, the ostium of the coronary sinus, and the insertion of the septal cusp. It is composed of a collection of ganglia, 200–400 μm in diameter (Figs. 8, 9). This ganglion is connected with the plexus around the ostium of the coronary sinus. In addition, it receives nerves from the dorsal plexus by way of the anterior and posterior limbus of the fossa ovalis and from the plexus in the coronary sulcus by way of anterior and posterior branches. From the ganglion nerves curve forward to the *AV* node and the *AV* bundle. The former grasp the node along its posterior and superior border, while the remainder continue above the node to the superior border of the *AV* bundle (Figs. 9, 10). A few of these enter the bundle, while the rest continue together until they divide to follow its right and left branches. Thus, there appears to exist a direct connexion between the atrioventricular ganglion and the *AV* bundle, by-passing the *AV* node.

In the *AV* node the nerves branch, placing themselves in close relation to the surface of the nodal fibres. From the *AV* node ChE-containing nerves proceed, together with Purkinje fibres, into the *AV* bundle.

Ganglion cells have not been observed inside the node.

DISCUSSION

Lewis (1961) stated that to obtain staining of the interior of a tissue block it would be an advantage if the incubation medium could be introduced by perfusion, but that as yet a method suitable for general application had not been conceived. By the present perfusion technique blocking of the vascular bed by liberated air was reduced and the method proved well suited for topographical studies of the ChE-containing nerves and ganglia in the heart. For histological study its suitability is limited because of crystal formation and diffusion phenomena during the long incubations.

In a number of mammals, Ehinger, Falck, Persson & Sporrong (1968) found ChE-containing as well as catecholamine-containing nerves included in the dorsal plexus, in the plexuses at the nodal tissues, and in the *AV* bundle. It is known that non-cholinergic neurons contain small quantities of cholinesterase (Giacobini, 1967).

Fig. 9. Whole mount showing the *AV* ganglion, *AV* node and *AV* bundle. Segment of Fig. 8. Koelle staining, 20:1. *h*, nerves by-passing the *AV* node connecting the *AV* ganglion with the *AV* bundle. Line *A-A*, see Fig. 10. Other symbols as in Fig. 8.

Fig. 10. Section through the *AV* node at *AA* in Fig. 9. H. and E. 40:1. Symbols as in Figs. 8 and 9. *n*, nerves which connect *gAV* with *nAV*.

Fig. 11. Crista terminalis. H. and E. and Koelle, 570:1. Specialized muscle fibres (*sm*) accompanied by ChE-containing nerves (*n*) in the subendocardial connective tissue.

Considering the long incubation period used in the present study, therefore, non-cholinergic nerves may have been visualized together with the cholinergic ones. The subendocardial sensory end organs, demonstrated by Holmes (1957) and Miller & Kasahara (1964) by methylene-blue staining, were not visualized by the present method.

Embryologically and functionally there is a fundamental difference between the nerves proceeding to the heart behind the tranverse sinus, supplying the 'venous base', and those proceeding in front of it, supplying the 'arterial base' (His, 1891; Keith & MacKenzie, 1910; Perman, 1924). The former appear to exert inhibitory and accelerating actions upon the heart through the 'specialized nodal system'. In stimulation and cutting experiments it has been demonstrated that the right-sided nerves act especially upon the *SA* node just as the left-sided ones act upon the *AV* node (Rothberger & Winterberg, 1910).

The morphological explanation appears to be that the right-sided nerves form the dorsal plexus which supplies the *SA* node via the sinuatrial ganglion. The left-sided nerves reach the heart along the oblique vein of the left atrium and the coronary sinus (Perman, 1924; Wolhynsky, 1928; Licata, 1954; King & Coakley, 1958). From the ostium of the coronary sinus they proceed to the neighbouring atrioventricular ganglion and node. As nerves from the dorsal plexus proceed through the anterior and posterior limbus of the fossa ovalis to the atrioventricular ganglion, the possibility exists of the *AV* node being affected by right-sided nerves too. Thus, there seems to be a right–left difference in the nerve supply in those parts of the heart which develop from the sinus venosus, while a convergence takes place in the parts which develop from the atrium proprium.

In his study of the pig heart, Wensing (1964) found that nerves proceed from the *AV* node, by way of the sulcus terminalis and the interatrial septum, to the area around the *SA* node. He mentioned the possibility of a direct connexion between the two nodes. We did not find ChE-containing nerves proceeding direct from one node to the other. However, we are not able to exclude that a connexion may exist either by way of nerve cells in the dorsal plexus or by way of the crista terminalis. Paes de Carvalho (1961) has demonstrated that the impulse from the *SA* node in the rabbit heart spreads with particular speed along the crista terminalis *en route* to the *AV* node. It should therefore be noted that the specialized tissue we have found in the crista terminalis of the pig heart is built up like the conducting system in the ventricle, i.e. of muscle fibres and ChE-containing nerves, and that it is situated in the course of the bundle described by Thorel (1909) as connecting the *SA* and *AV* nodes in the human heart.

The bundle of His is considered to be the only connexion between the atria and ventricles of the heart in higher mammals. This bundle is made up of Purkinje fibres accompanied by cholinesterase- and catecholamine-containing nerves (Ehinger *et al.* 1968). The present study has shown that cholinesterase-containing nerves are particularly densely arranged in the superior border of the bundle and that proximally these nerves pass the *AV* node in order thereafter to become connected with the atrioventricular ganglion. Distally, they continue into the right and left bundle branches. Wolhynsky (1928) studied the situation of nerves and ganglion cells in calf hearts by dissection. He found three ganglionic areas situated close together behind the *AV* node.

The nerves from these ganglia proceeded partly to the *AV* node and partly past this node into the upper part of the bundle of His. Some of them ran parallel to the bundle as far as the site of bifurcation, whereupon they continued into the right and left branches; others penetrated into the crus commune to place themselves in relation to the Purkinje fibres. In this, as well as in a number of other respects, there is good agreement between the course of the nerves found in calf hearts by Wolhynsky and the course of the ChE-containing nerves found by us in the pig heart.

It is not known whether the nerves by-passing the *AV* node are afferent or efferent. If they are afferent, there appears to be morphological substance for an intracardial proprioceptive reflex arc comprising receptors in the periphery (e.g. stretch receptors), the *AV* ganglion – in which there are bipolar as well as multipolar nerve cells (King & Coakley, 1958) – and the *AV* node, in which the presence of neuromuscular junctions is likely (Thaemert & Emmett, 1968). A similar reflex arc may be imagined to exist at the *SA* ganglion and node if the very large nerves running in the medial wall of the right auricle are also afferent.

SUMMARY

Cholinesterase-containing nerves and ganglia were demonstrated in whole mounts of the right atrium by a Koelle technique in which the incubation medium was introduced by perfusion.

Nerves enter the atrium at the cardiac base, in the wall of the superior vena cava, along the coronary sinus, and along the right coronary artery, forming ganglion-containing plexuses. From the cardiac base nerves proceed on the left of the superior vena cava to the sinuatrial ganglion where they meet nerves from the right auricle. From the ganglion, nerves proceed to the sinuatrial node in the sulcus terminalis, forming a dense plexus of nerve fibres ending in relation to the nodal cells. From the cardiac base other nerves proceed along the sinus node artery to its origin to form, together with nerves along the right coronary artery, a plexus in the coronary sulcus.

The atrioventricular ganglion is situated in the interatrial septum behind the atrioventricular node. It receives nerves from the plexus around the coronary sinus, from the cardiac base by way of the limbus of the fossa ovalis, and from the plexus in the coronary sulcus. From the ganglion nerves proceed to the atrioventricular conducting system, some entering the atrioventricular node, others by-passing this node to join the bundle of His.

REFERENCES

ASCHOFF, L. (1911). *Pathologische Anatomie*. 2. Auflage, Bd. 2. Jena: G. Fischer.

DAVIES, F., FRANCIS, E. T. B. & KING, T. S. (1952). Neurological studies of the cardiac ventricles of mammals. *J. Anat.* **86**, 130–143.

EHINGER, B., FALCK, B., PERSSON, H. & SPORRONG, B. (1968). Adrenergic and cholinesterase-containing neurons of the heart. *Histochemie* **16**, 197–205.

GIACOBINI, E. (1967). Cholinergic and adrenergic cells in sympathetic ganglia. *Ann. N.Y. Acad. Sci.* **144**, 646–659.

HIS, W. (1891). Die Entwickelung des Herznervensystems bei Wirbelthieren. *Abh. Math. Phys. Kl. Sächs. Ges. Wiss.* **18**, 1–64.

HOLMES, R. L. (1957). Structures in the atrial endocardium of the dog which stain with methylene blue, and the effects of unilateral vagotomy. *J. Anat.* **91**, 259–266.

KEITH, A. & MACKENZIE, I. (1910). Recent researches on the anatomy of the heart. *Lancet* **1**, 101–103.

KING, T. S. & COAKLEY, J. B. (1958). The intrinsic nerve cells of the cardiac atria of mammals and man. *J. Anat.* **92**, 353–376.

LEWIS, P. R. (1961). The effect of varying the conditions in the Koelle technique. *Bibl. Anat.* **2**, 11–20.

LICATA, R. H. (1954). The human embryonic heart in the ninth week. *Am. J. Anat.* **94**, 73–125.

MILLER, M. R. & KASAHARA, M. (1964). Studies on the nerve endings in the heart. *Am. J. Anat.* **115**, 217–234.

PAES DE CARVALHO, A. (1961). Cellular electrophysiology of the atrial specialized tissue. *The Specialized Tissues of the Heart*, (eds. A. P. de Carvalho, W. C. de Mello, and B. F. Hoffman) pp. 115–133. Amsterdam: Elsevier Publishing Company.

PERMAN, E. (1924). Anatomische Untersuchungen über die Herznerven bei den höheren Säugetieren und beim Menschen. *Z. Anat. EntwGesch.* **71**, 383–457.

ROTHBERGER, J. & WINTERBERG, H. (1910). Über die Beziehungen der Herznerven zur Form des Elektrokardiogramms. *Pflügers Arch. ges. Physiol.* **135**, 506–558.

THAEMERT, J. C. & EMMETT, S. S. (1968). The ultrastructure of neuromuscular relationships within the atrioventricular node of the heart. *Anat. Rec.* **160**, 518.

TOMPSETT, B. H. (1956). *Anatomical Techniques*, pp. 1–240. Edinburgh: Livingstone.

THOREL, Ch. (1909). Vorläufige Mitteilung über eine besondere Muskelverbindung zwischen der Cava superior und dem Hisschen Bündel. *Münch. med. Wschr.* **56**, 2159.

TSUJI, M. S. (1968). Sur la localisation des cholinestérases á l'aide d'iodures d'esters de thiocholine. *C. r. hebd. Séanc. Acad. Sci., Paris* **267**, 801–802.

WENSING, C. J. G. (1964). The conductive system and its nervous component in the pig's heart. Thesis, pp. 9–78. G. Van Dijk, N. V., Breukelen.

WILSON, J. G. (1909). The nerves of the atrio-ventricular bundle. *Proc. Roy. Soc. Lond.* B **81**, 151–163.

WOLHYNSKY, Th. (1928). Innervation des Herzkammer- und Vorhofsseptums des Kalbes. *Z. Anat. Entw-Gesch.* **86**, 608–638.