DEVELOPMENT OF THE NERVE SUPPLY TO THE HUMAN HEART*

BY

V. NAVARATNAM†

From the Anatomy School, University of Cambridge

Received August 7, 1964

The nerve supply to the adult heart is complex and for the most part is devoid of any readily comprehensible pattern. The various individual cardiac nerve trunks lose their identities by repeated interconnexion with their fellows and it becomes impossible to trace their final distributions by simple dissection. His (1893), however, by investigating the embryos of several vertebrate species including man, indicated that the nerves to the embryonic heart initially exhibited an orderly pattern which became obscured during the subsequent alterations in cardiac position and form. He particularly drew attention to the influence of the pericardial attachments round the principal arterial and venous channels in guiding nerves towards the heart.

In more recent times, though cardiac innervation has been studied in embryos of several species including the chick (Abel, 1913; Kramer, 1950), rat (Hall, 1951), pig (Kuntz, 1910), and man (Perman, 1924; Fukutake, 1925; Ihdima, 1929; Wahlin, 1935), the subject of inquiry has usually been the time at which the embryonic heart is innervated. Shaner (1930), who investigated calf embryos, and Licata (1954), who studied human embryos, are among the few authors who have followed up attempts of His to elucidate the embryological pattern of cardiac innervation. Licata, in particular, emphasized the importance of appreciating the state of cardiac development as a whole, including the distribution of the specialized musculature, before one can comprehend the disposition of the cardiac nerves. Unfortunately he limited himself to human embryos in the ninth week of gestation and was therefore unable to demonstrate the fate of the cardiac nerves at later stages of development.

The purpose of this investigation is to present briefly the observations on cardiac innervation in human embryos and foetuses from the fifth week of gestation till full term.

MATERIAL AND METHODS

Serial sections through the heart in 91 human embryos and foetuses, ranging from 4 mm. to 310 mm. crown-rump (C.R.) length were examined; these specimens are included in the collection at the Anatomy School, Cambridge.

Eight of the specimens (H 214—15·5 mm. C.R. length; H 191—17·5 mm.; H 180—30 mm.; H 556—40 mm.; H 218—48 mm.; H 125—92 mm.; H 177—150 mm.) were impregnated with silver by the De Castro method while sections through 26 other specimens were treated by the Bodian technique using Roques’s silver proteinate. To follow the development of the specialized musculature in the heart, the remaining material was treated by a variety of procedures including a modified Masson’s trichrome method, Goldner’s trichrome method, the P.A.S. stain, and haematoxylin and eosin.

* Based on part of a thesis presented for the Ph.D. degree of the University of Cambridge in May 1964.
† Present address: Faculty of Medicine, University of Ceylon, Kynsey Road, Colombo 8, Ceylon.
DEVELOPMENT OF THE NERVE SUPPLY OF HEART

Fig. 1.—7-5-mm. embryo. Near its junction with the ventricular myocardium, the musculature of the dorsal wall of the atrio-ventricular canal is arranged to form a compact fascicle (b), which is the primordium of the atrio-ventricular bundle. (H. and E. \( \times 51. \))

Fig. 2.—11-mm. embryo. Nerve fibres at the base of the septum spurium. R.S. = right sinus horn. (Bodian. \( \times 400. \))

OBSERVATIONS

The 11-mm. stage (Streeter’s Horizon XVII) is the earliest one in which nerve fibres were identified in the heart. This is preceded at the 7-5-mm. stage (Horizon XV) by the differentiation of the atrio-ventricular bundle. The muscle cells of the atrio-ventricular canal are arranged circumferentially except where the dorsal wall of the canal merges with the ventricular wall; at the latter site the cells are arranged parallel to the longitudinal axis of the canal and they are more tightly packed than the surrounding myocardial fibres (Fig. 1). The differentiation gradually extends to the crest of the interventricular septum. Although the bundle primordium is evident by the 7-5-mm. stage, neither the sinus node nor the atrio-ventricular node can be identified.

11-mm. Stage. The heart lies between the levels of the dorsal root ganglia of the fifth cervical and second thoracic spinal nerves. On each side, vagal and sympathetic nerve fibres run towards the truncus arteriosus along the pulmonary arch artery, forming a plexus at the dorsal extremity of this vessel (see Navaratnam, 1963). No nerves, however, enter the arterial mesocardium at this stage. Many of them pass caudally towards the venous mesocardium by running along the ipsilateral branch to the lung bud, i.e. along the pulmonary artery, behind the transverse pericardial sinus.

Some of the nerves coursing along the pulmonary arteries accompany these vessels into the lung buds but many others pass off the vessels to enter the venous mesocardium, where they are joined by further branches from both vagal and both sympathetic trunks. One branch, derived from the right vagus nerve, courses near the right pulmonary artery and enters the venous mesocardium where the right pleuro-pericardial fold blends with the mediastinum; this nerve, which can be termed the right sinus nerve, arborizes in the wall of the right sinus horn, particularly near the mural attachments of the septum spurium and upper part of the right venous valve (Fig. 2). The corresponding branch on the left side, i.e. the left sinus nerve, reaches the heart by traversing the mediastinal attachment of the left pleuro-pericardial fold and terminates on the left sinus horn. It does not pass near the left pulmonary artery which, unlike the right pulmonary artery, lies entirely behind the hilum of the corresponding lung bud; the right pulmonary artery, on the other hand, runs caudally in front of the right upper lobe bronchus and then turns backwards between the upper lobe bronchus and the stem bronchus.
FIG. 3.—17.5-mm. embryo. Reconstruction of the antero-superior view of the heart after removal of the atria and a portion of the pulmonary trunk. Of the nerves related to the arterial end of the heart, the right cardiac nerves (r) mainly course towards the left coronary artery but a few of them (r') wind round the right side of the ventral aortic sac (v.s.) to pass towards the right coronary artery. The left cardiac nerves (l) end mainly near the right coronary artery. (×33.)

Most of the nerves entering the venous mesocardium accompany the single pulmonary vein. They ramify mainly in the wall of the vein itself but a few nerve fibres extend through the sinus septum to the dorsal wall of the atrio-ventricular canal to terminate in the atrial extremity of the primordium of the atrio-ventricular bundle. During the 11–13-mm. stage, the musculature of the atrial extremity of the bundle undergoes further differentiation; it becomes loosely and irregularly arranged and can be identified as the atrio-ventricular node. There are no nerve fibres in the interventricular septum or in the walls of either ventricle.

The nerve fibres ramifying in the heart are accompanied by oval cells containing little cytoplasm; these cells for the most part are presumably developing sheath cells (lemnoblasts).

13–20-mm. Stage. At the 13.5-mm. stage many of the nerve fibres accompanying the pulmonary arch artery on each side enter the arterial mesocardium by piercing the corresponding flank of the aortico-pulmonary spur of extracardiac mesoderm. The nerves from the right side and the nerves from the left side remain in separate groups within the arterial mesocardium (Fig. 3), though both sets give off twigs that wind circumferentially round the aorta and pulmonary trunk. The nerves from the right side mainly course along the corresponding border of the spiral septum and

FIG. 4.—17.5-mm. embryo. Reconstruction of the dorsal view of the heart showing the nerves related to the pulmonary arteries (P.A.) and to the principal venous channels. The right sinus horn (R.S.) is supplied by the right sinus nerve (r.s.n.), and the left sinus horn (L.S.) is supplied by the left sinus nerve (l.s.n.). Several nerves accompany the pulmonary venous channel (P.V.). (×33.)
come into relation with the primordium of the left coronary artery (a few right cardiac nerves wind round the right side of the ventral aortic sac towards the right coronary artery). The nerves from the left side run along the opposite border of the spiral septum and reach the developing right coronary artery. Although the nerves in the arterial mesocardium pass towards the coronary arteries, they do not give off branches to these vessels at this stage.

The nerves traversing the venous mesocardium (Fig. 4), which are more numerous than the nerves in the arterial mesocardium, maintain a distribution similar to that seen at the 11-mm. stage. The right and left sinus nerves, each accompanied by sympathetic twigs, reach the corresponding sinus horns by passing through the mesocardial plicae suspending these vessels (Fig. 5). The plicae are fashioned from the original pleuro-pericardial folds, which in most cases have completely occluded the corresponding pleuro-pericardial openings by the 13-mm. stage; the plica suspending the left sinus horn is particularly prominent because of the expansion of the pericardial cavity behind the horn. At about the 20-mm. stage, the sinus node differentiates at the base of the septum spurium and upper part of the right venous valve, i.e. precisely at the site of maximum innervation of the right sinus horn. The sinus node like the atrio-ventricular node consists of loosely and irregularly arranged muscle cells, which are stained lighter than the surrounding myocardium.

Although the sinus node is innervated by nerves of the right side, the atrio-ventricular node is supplied by nerves derived from both sides of the body accompanying the pulmonary venous channel (Fig. 6). These latter nerves insinuate themselves into a little fossa between the coronary sinus and the inferior vena cava and then pierce the sinus septum to reach the atrio-ventricular node; they cannot, however, be traced into the atrio-ventricular bundle.

When the musculature of the atrio-ventricular canal is disrupted owing to invasion by fibrous tissue during the 10–16-mm. stage, the bundle is invariably spared, so that it remains usually as the sole atrio-ventricular connexion. By the 17-mm. stage, when the partition between the two ventricles is completed, differentiation of the bundle has extended on either side of the muscular septum to form both bundle branches.

The nerves in the arterial and venous mesocardia are connected together by nerves passing behind the transverse pericardial sinus along the pulmonary arteries (Fig. 7).

---

**Fig. 5.**—20-mm. embryo. The right (r.s.n.) and left (l.s.n.) sinus nerves enter the mesocardial attachments of the right (R.S.) and left (L.S.) sinus horns respectively. The plica suspending the left horn is particularly prominent. Note the close relation between the right sinus nerve and the ipsilateral pulmonary artery. (Bodian. X40.)

**Fig. 6.**—17.5-mm. embryo. Several of the nerves accompanying the pulmonary veins (P.V.) pass towards the sinus septum (S.S.). L.S. = left sinus horn. (De Castro. X81.)
21-30-mm. Stage. During this stage the nerves in the arterial mesocardium form plexuses that accompany the coronary arteries for a short distance (Fig. 8) and supply the coats of these vessels; however, they do not as yet accompany the coronary arterial branches that penetrate the myocardium.

In the younger specimens in this group the nerves entering the arterial mesocardium from the
right side can be distinguished from the nerves from the left side. By the 28-mm. stage, however, the two sets of nerves coalesce, before entering the arterial mesocardium, to form a single plexus on the right side of the ductus arteriosus. The single juxta-ductal or "arterial" plexus contains many developing ganglion nerve cells as well as several oval and stellate cells which are heavily impregnated with silver in Bodian preparations; several of these stellate argyrophil cells accompany the nerves into the arterial mesocardium, where they are grouped to form the inferior aortico-pulmonary glomus, and some extend as far as the coronary nerve plexuses.

Owing to the relative cranial "migration" of the common cardinal veins and the widening of the area of pericardial reflection round the pulmonary venous openings, which now are usually four in number, the cranial limit of the venous mesocardium lies very near the arterial mesocardium. However, in spite of this proximity the "venous" plexus of nerves can still be distinguished from the "arterial" nerves.

The right sinus nerve passes through the pericardial fold on the medial side of the superior caval inlet and ramifies in the sinus node (Fig. 8). The developmental involution of the left common cardinal vein and left sinus horn (Fig. 9), which commences about the 25-mm. stage, has an effect on the course of the left sinus nerve. The involution usually begins as a constriction of the left sinus horn, near its junction with the common cardinal vein; subsequently, before the 40-mm. stage, a considerable length of the channel completely disappears. Nevertheless, the portion of the left sinus horn below its junction with the great cardiac vein actually enlarges during this period and becomes the coronary sinus; in addition an attenuated portion of the horn, just above the great cardiac vein, persists as a slender vein, namely the oblique atrial vein. The pericardial plica carrying the reduced left sinus horn is gradually obscured by progressive rearrangement of its layers within the general venous mesocardium; as a result, the cul-de-sac of the transverse pericardial sinus extending between the plica and the posterior atrial wall is obliterated, and the left sinus nerve together with the oblique atrial vein comes to lie beneath the epicardium on the back of the left atrium. The left sinus nerve thus becomes surrounded by the numerous nerves accompanying the pulmonary veins (Fig. 9). The latter nerves supply the walls of the pulmonary veins as well as the wall of the left atrium between the pulmonary venous openings; moreover, several nerve fibres pierce the sinus septum (Fig. 10) to reach the atrio-ventricular node.

A further development at about this stage is the differentiation of the cardiac ganglia. Many of the cells accompanying the cardiac nerves are oval and contain scantly cytoplasm; these cells are probably developing sheath cells. Other cells, however, are round or pear-shaped and contain plenty of cytoplasm; in the nucleus of each of the latter cells, which are presumably young nerve cells, one or two prominent nucleoli can be identified. The ganglia are predominantly distributed on the back of the left atrium (Fig. 11 and 12), but some clumps extend across the back of the right atrium while others lie on the surfaces of the sinus and atrio-ventricular (Fig. 10) nodes. In addition, many ganglionic clumps lie in relation with the aorta and pulmonary trunk within the arterial mesocardium, and several nerve cells lie enmeshed within the coronary nerve plexuses.

31–40-mm. Stage. By this stage the heart has descended to lie at the levels of the fifth to eighth
FIG. 10.—48-mm. fetus. Clumps of ganglion nerve cells lie in the sinus septum which separates the inferior vena cava (I.V.C.) from the mouth of the coronary sinus (C.S.). These nerve cells are closely related to the atrio-ventricular node. (Bodian. × 36.)

FIG. 11.—30-mm. embryo. Reconstruction of the dorsal view of the heart showing the distribution of the ganglia related to the atria. The stippled area denotes the position of the sinus node. (×23.)

FIG. 12.—28-mm. embryo. Many ganglionic clumps are related to the dorsal wall of the left atrium (L.A.) particularly near the pulmonary venous openings (P.V.). L.S. = reduced left sinus horn. (H. and E. ×100.)
DEVELOPMENT OF THE NERVE SUPPLY OF HEART

By the 40-mm. stage, however, the venous mesocardium is so close to the arterial mesocardium that plexuses 2 and 3 coalesce to form a single plexus which extends from the arterial mesocardium to the back of the atria; this plexus corresponds to the definitive deep cardiac plexus. The pulmonary arteries pass almost horizontally through this plexus and carry several nerves into the plexuses at the hila of the lungs. The asymmetry of the pulmonary arteries can still be recognized; the right pulmonary artery runs into the right lung between the superior lobe (eparterial) bronchus and the stem bronchus, whereas on the left side the pulmonary artery enters the lung above all the bronchi.

Fetuses Exceeding 40 mm. C.R. With further development, the coronary nerve plexuses increase in thickness and complexity. Several nerve fibres terminate in the medial and adventitial coats of the coronary arteries, but many others accompany the arterial branches that penetrate the ventricular myocardium. As the coronary arteries divide, each branch is accompanied by an extension of the nerve plexus; several small arterioles and even some capillaries are accompanied by nerve fibres as they meander in the myocardium. Although a nerve fibre may thus come in contact with several cardiac muscle fibres, no neuro-muscular junctions of the end-plate type can be identified. The appearance of a nerve ending is often simulated by the cut end of a nerve fibre being applied to the surface of a muscle fibre; when viewed “broadside on” such a nerve may even appear to penetrate the muscle fibre.

The regions where the nerve supply is richest are predictably at the venous end of the heart (Fig. 13). The spatium pulmonale, i.e. the left atrial wall between the pulmonary venous openings, the sinus node, and the atrio-ventricular node are particularly well innervated. On the other hand, it is noticeable that even at full term the innervation of the atrio-ventricular bundle is no richer than that of the general myocardium; apart from a few nerve fibres in the part of the bundle just next to the node, the only nerves in the bundle are related to the arteries.

Some nerves coursing over the left atrium transgress the superficial surface of the coronary sinus
FIG. 14.—93-mm. fetus. Reconstruction of the antero-superior view of the heart after the atria and part of the pulmonary trunk are cut away. The nerves passing through the arterial mesocardium pass into the coronary nerve plexuses. Scattered along the paths of these nerves are many clumps of ganglion nerve cells (stippled areas) and cells of the inferior aortico-pulmonary glomus (solid black). (x 7.75.)

to come in relation with the coronary arteries, the chief innervation of which, however, is derived from nerves in the arterial mesocardium (Fig. 14).

**DISCUSSION**

In the present study, the ingress of nerves into the heart was found to commence at about the 11-mm. stage, i.e. in the sixth week of intrauterine life, which accords well with the observations of His (1893), Perman (1924), Fukutake (1925), Wahlin (1935), and Volcher (1963). Since, by this stage, the dorsal mesocardium is already fenestrated by the formation of the transverse pericardial sinus, the nerves can reach the heart only by passing through the pericardial reflections round the principal arterial and venous channels.

The cardiac nerves reach the venous mesocardium before they reach the arterial mesocardium although the latter lies nearer the hindbrain, which is the source of the preganglionic vagal nerve fibres. The delay in the entry of nerves into the arterial mesocardium is perhaps due to the interposition of the bulky embryonic pharynx and pharyngeal pouches between the dorsal body wall and the mesocardium. Be that as it may, the innervation of the venous end of the heart remains, at all stages, much richer than that of the arterial end.

At the 11-mm. stage, the right sinus horn and the dorsal wall of the atrio-ventricular canal (the atrial end of the primordium of the atrio-ventricular bundle) are the sites of maximum innervation in the heart and it is precisely at these sites that the sinus node and the atrio-ventricular node, respectively, differentiate. One is thus reminded of the speculation by several investigators (Stiénon, 1926; Shaner, 1930) that nodal differentiation is induced by neural elements, where the latter can exert maximum influence on the initiation (right sinus horn) and conduction (atrio-ventricular canal) of the cardiac impulse.
On the other hand, the differentiation of the common trunk of the atrio-ventricular bundle commences before the arrival of neural elements; moreover, at no stage do numerous nerve fibres course through the bundle. Thus, it is unlikely that the cardiac impulse is transmitted from the atria to the ventricles by the nerves in the atrio-ventricular bundle as Wilson (1909), Morison (1912), Holmes (1921), and Field (1951) have suggested; these authors found many nerve trunks within the atrio-ventricular bundle in several species and considered that the musculature of the bundle was merely a highway for the nerves that passed into the ventricular myocardium. In contrast with the above-mentioned authors, Stotler and McMahon (1947), who investigated the heart in man and dog, indicated that the nerves entering the bundle from the adjacent atrio-ventricular node terminated within the bundle itself without passing to the general myocardium and they thus emphasized the exclusiveness of the bundle innervation. Such a distribution suggests that the nerves in the atrio-ventricular connecting system are concerned with regulating the speed and frequency of muscular conduction rather than with actually transmitting the cardiac impulse.

Cardiac Ganglia. His (1893) inclined to the view that the cardiac nerve cells do not migrate to the heart till a preliminary path of nerve fibres is laid down by the outgrowth of axons from cells in the neural tube. On the other hand, Kuntz (1910) and Streeter (1912) were of the opinion that the ganglion nerve cells migrate to the heart ahead of the growing cardiac nerves. In the present investigation cardiac nerve cells could not be identified with certainty till the 20-mm. stage whereas nerve fibres reach the heart by the 11-mm. stage. Similarly, Shaner (1930) in the calf, Kramer (1950) in the chick, and Hall (1951) in the rat have reported that the cardiac ganglia do not appear for some time after the cardiac nerves reach the heart. Nevertheless, it is possible that undifferentiated precursors of nerve cells are present near the heart at earlier stages but cannot be identified by standard techniques.

Summary and Conclusions

The development of the cardiac nerve supply has been studied in serial sections through the heart in 91 human embryos and fœtuses, ranging from 4 mm. to 310 mm. C.R. length; the 11-mm. stage is the earliest one in which nerve fibres were identified in the heart.

The cardiac nerves enter the venous mesocardium before the arterial mesocardium. At the 11-mm. stage three groups of nerves pass through the venous mesocardium; on each side, a sinus nerve supplies the corresponding sinus horn while the third set of nerves, derived from both sides of the body, accompanies the then single pulmonary vein. The right sinus nerve innervates the presumptive site of the future sinus node while the atrio-ventricular nodal anlage is supplied by offsets from the nerves accompanying the pulmonary vein. When the left sinus horn involutes at about the 25–40-mm. stage, the left sinus nerve maintains the relation with the persisting derivatives of the horn.

Nerves enter the arterial mesocardium at the 13·5-mm. stage. They course along the pulmonary arch artery on each side, forming a plexus near the dorsal extremity of this vessel, and pierce the corresponding flank of the aortico-pulmonary spur of extracardiac mesoderm. The nerves from each side follow the border of the spiral septum with which they come into relation; the right cardiac nerves pass towards the left coronary artery while the left cardiac nerves pass towards the right coronary artery. The coronary nerve plexuses develop as extensions of the nerves in the arterial mesocardium at about the 22-mm. stage.

The nerves in the arterial and venous mesocardia are connected together by nerves coursing along the pulmonary arteries. On the right side the pulmonary artery and sinus nerve are more closely related than their counterparts on the left side. This difference is correlated with the asymmetrical course of the two pulmonary arteries.

At the 40-mm. stage, when the heart reaches its definitive level, the arterial mesocardium lies very close to the venous mesocardium. The corresponding sets of nerves have coalesced to form a single plexus—the deep cardiac plexus; only the nerves at the dorsal end of the ductus arteriosus form a separate plexus—the superficial cardiac plexus.
Cardiac nerve cells can be identified at about the 20-mm. stage. They are usually related to the atria, including the nodal regions, the roots of the great arterial trunks, and the coronary arteries.

I wish to thank Professor J. D. Boyd for his valuable advice during this study and for permission to use his excellent collection of human embryos and fetuses. I am also grateful to Dr. G. H. Wright for helpful discussions, to Mr. A. K. Maxwell and Dr. Inderbir Singh for help with the illustrations, and to Mr. J. F. Crane and Mr. G. Oakes for the photographs.

REFERENCES


