J. Anat. (1970), **107**, 2, pp. 271–279
With 13 *figures*
Printed in Great Britain

The development of the intrinsic innervation of the human heart between the 10 and 70 mm stages

R. B. SMITH

Department of Anatomy, University of Manchester

(*Received* 25 *August* 1969)

Marshall (1878) provided evidence of the early origin of the vagal ganglia in the chick embryo from the part of the neural crest which is associated with the ninth and tenth cranial nerves. This was later confirmed by Jones (1942) who noted that, after destruction of the hind brain vagal 'apparatus' at a sufficiently early stage, the cardiac and pulmonary plexuses failed to develop. Kuntz (1953) agreed, stating that the primordia of the vagal ganglia develop early, probably from mantle layer cells which are displaced from the neural crests and which migrate from the hind brain down the vagi.

In the 10·2 mm human embryonic heart, Perman (1924) identified nerves passing between the aorta and pulmonary artery to end in nerve cells situated between these two vessels. A further group of cells became apparent in relation to the dorsal atrial walls at the 19·0 mm stage, together with a nerve plexus which extended caudally to the atrioventricular furrow. However, Fukutake (1925) could not fine neurons lying in the atrial subepicardial layer before the 17·0 mm stage, although he noted that the neurons had invaded the upper quarter of the heart by the 25 mm stage, and also that in the 40 mm human heart nerves had penetrated to the ventricular septum. Idhima (1929) described a small number of nerve cells appearing in the superior aspect of the 20 mm human enbryonic heart and Navaratnam (1965) saw ganglion cells arranged around the ductus arteriosus in embryos between 21 and 30 mm crown-rump (C–R) lengths. The cells also extended over the dorsal surface of the left atrium, while others were associated with both the aorta and pulmonary artery and with the coronary nerve plexuses. Other clumps were scattered over the right atrium and on the surfaces of the sinuatrial and atrioventricular nodes. The cells were either round or piriform in shape. Consequently this study was undertaken to determine the time of arrival of the nerve cells in the heart and their subsequent distribution. A morphological classification of the ganglion cells has also been attempted.

MATERIALS AND METHODS

Owing to the difficulty of obtaining satisfactory human material, embryos H. 10, H. 15, H. 18, H. 24 and H. 28 in the embryological collection in Manchester University were studied. These were already stained with either haematoxylin and eosin or by a modification of Masson's trichrome method.

In addition, 30 embryos and foetuses were collected from various hospitals in the Manchester region. After fixation in 20% formalin for periods ranging from 3 to 5 weeks the hearts were removed from the larger specimens (i.e. those of C–R length exceeding 50 mm) and embedded in paraffin wax. In smaller specimens the entire thorax was cut out and embedded in wax. Alternate hearts were sectioned longitudinally and transversely at 10 μm and every section was mounted. Alternate sections from both series were stained either with haematoxylin and eosin or by a modification of Masson's trichrome technique, but every fifth section was stained with silver employing a modification of Samuel's (1953*a*, *b*) method.

Observations

Although nerve fascicles containing small, darkly stained cells are seen between the aorta and pulmonary artery in the 10·0 mm human embryonic heart, no nerve fibres or cells are identified in the heart itself. However, small nerve bundles soon begin to penetrate the superior aspects of the atria (Fig. 1) and a clump of 20–25 darkly staining cells lying between the aorta and pulmonary artery are visible at the 15·0 mm stage. The cells have spherical, slightly eccentric and densely staining nuclei, surrounded by dark cytoplasm. Measuring between 10 and 15 μm, they are larger than any other cells found in the heart at this stage and, as they closely resemble the mantle layer cells of the developing spinal cord, they are presumably neuroblasts.

Between the 15 and 22 mm stages of development this 'aortico-pulmonary' group of neuroblasts (Fig. 2) is the only one clearly definable in the heart. The cells gradually begin to exhibit granular cytoplasm and the primitive ganglion remains constant in position while slowly increasing in size. Slightly later (25 mm) neuroblasts can also be detected scattered in small groups over the posterosuperior aspect of the atria close to the mid-line (Fig. 5). Some of the cells approach 20 μm in size and possess large eccentric reticular nuclei, while others have small darkly staining nuclei and resemble neuroblasts more closely (Fig. 3).

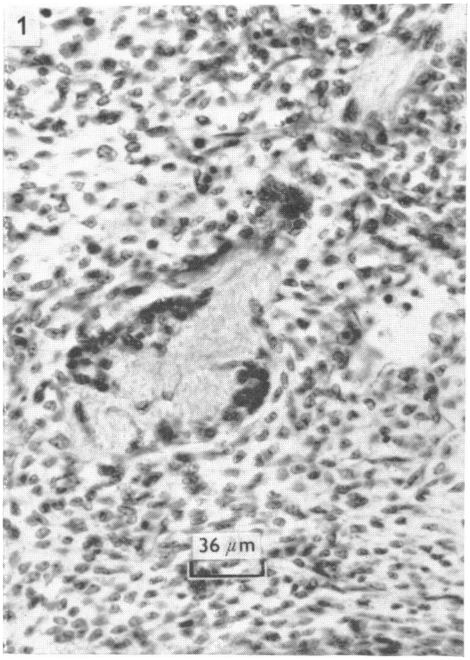
The human foetal heart at the 35 mm stage has more ganglia situated posterosuperiorly lying near both the superior walls of the atria and the posterior interatrial groove (Fig. 6). Occasionally, isolated groups of cells are located in the atrioventricular groove. The aorticopulmonary ganglion is much better developed at this stage and the two types of cell described above (Fig. 3) are now positively distinguishable with a silver staining technique. No cell processes are visible. Nerve fibres which, at the 22 mm stage, are present only in the delicate epicardial tissue of the superior atrial walls and aortic adventitia advance into the heart so that, at the 40 mm stage, they

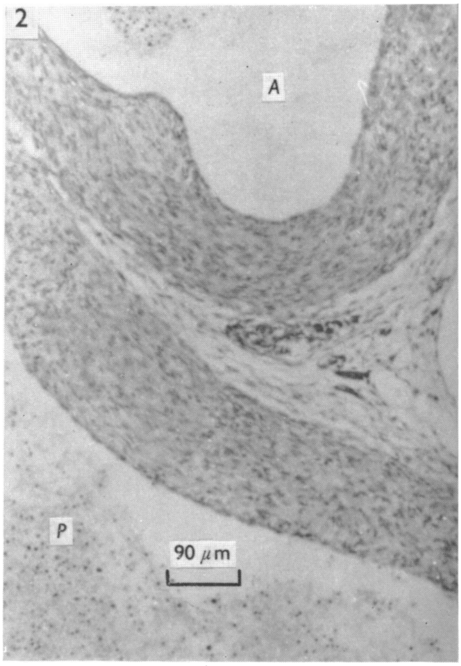
Fig. 1. A small nerve bundle, containing neuroblasts, in the superior aspect of the atria of a 15 mm human embryo (H and E).

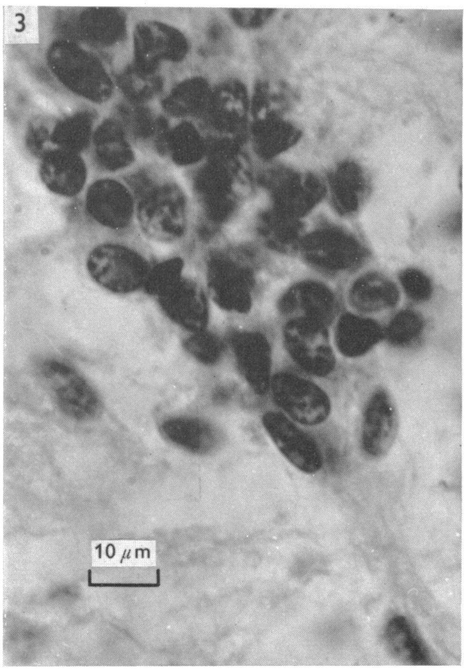
Fig. 2. The aorticopulmonary ganglion of a 22 mm human embryo lying between the aorta (*A*) and the pulmonary artery (*P*) (H and E).

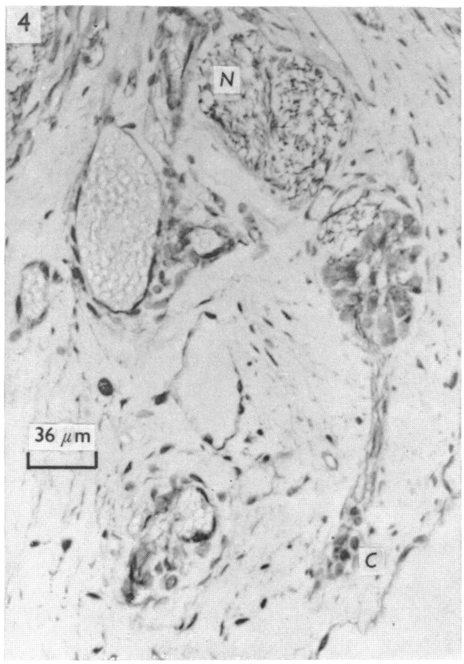
Fig. 3. A ganglion from a 26 mm embryo heart containing two types of cell. A ganglionic capsule is not present (H and E).

Fig. 4. Ganglion with capsule in the 65 mm foetal heart, associated with a nerve bundle (*N*) and many small vessels. Small darkly staining cells (*C*) can be seen lying along the nerve bundle entering the ganglion. (Modification of Samuel's Silver method.)


1
36 μm


2
A
P
90 μm


3
10 μm


4
N
36 μm
C

have colonized the upper two-thirds of the atria forming a wide-meshed tenuous plexus.

The intrinsic cardiac ganglia of the 50 mm human foetal heart can be classified into the following groups: (1) an aorticopulmonary group in the adventitial tissues around the roots of the aorta and pulmonary artery; (2) a left superior atrial group; (3) a right superior atrial group; (4) a group lodged in the posterosuperior aspect of the interatrial groove.

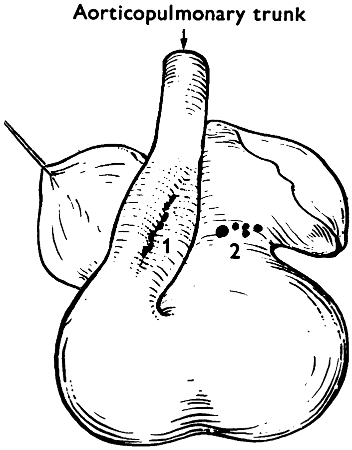


Fig. 5

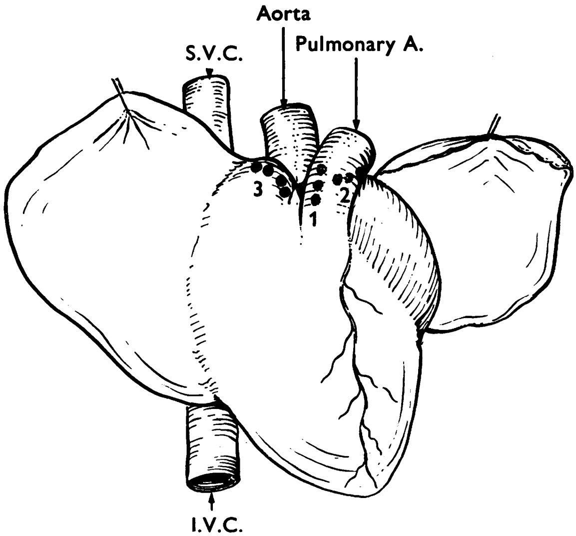


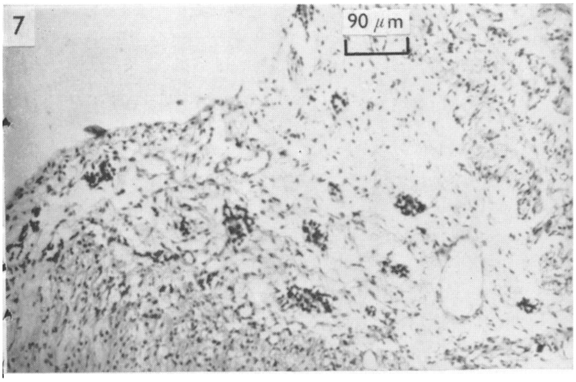
Fig. 6

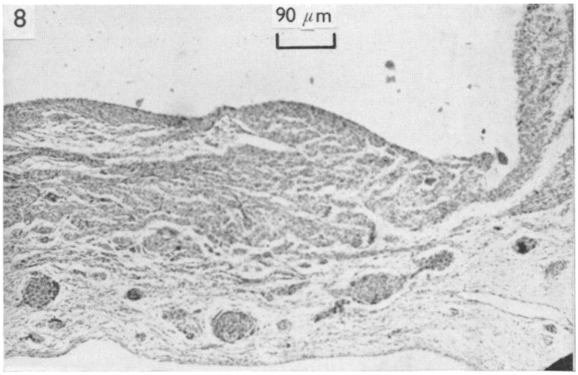
Fig. 5. Diagram of a 25 mm embryo heart to show the position of the aorticopulmonary (1) and left ati ial (2) groups of ganglia.

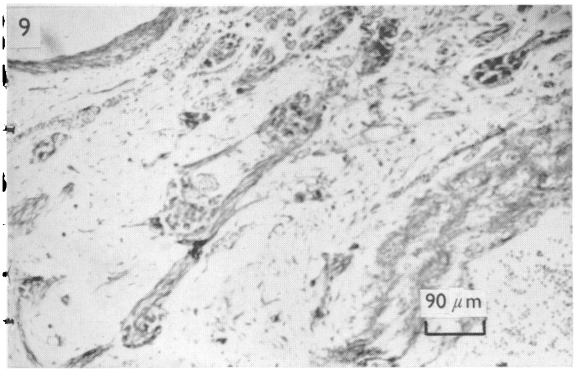
Fig. 6. Diagram of a 35 mm foetal heart showing the positions of the aorticopulmonary (1), the left atrial (2) and the right atrial (3), groups of ganglia.

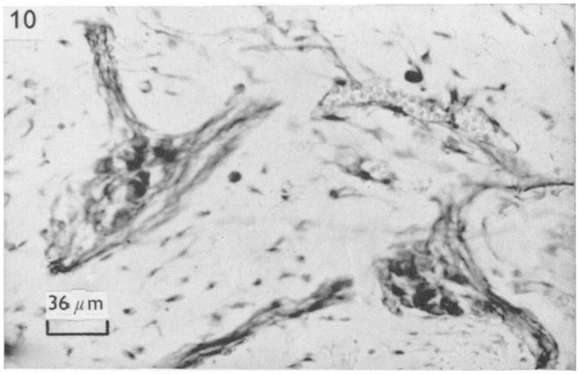
All the groups are interconnected through nerve fibres of the atrial epicardial plexus. While most of the intrinsic ganglia measure 180 μm $\times$ 60 μm or less, some of the aorticopulmonary ganglia are larger and a few, fusiform in shape, measure about 350 μm $\times$ 80 μm.

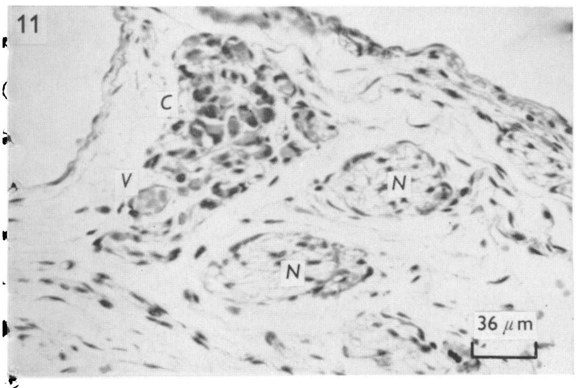
At the 55 mm stage, most hearts examined exhibited only sporadic ganglia lying in the atrioventricular groove (Fig. 7) which contain only small densely stained cells. As the interatrial group is limited to the upper part of the sulcus posteriorly and does not extend caudally as far as the atrioventricular groove, the ganglion cells found in this latter situation may have migrated along the coronary nerve plexuses to gain this site. Furthermore, the cardiac plexus contains numerous neuroblasts at this time and the cells in the atrioventricular groove are similar, while those of the interatrial groove present a more mature appearance. Everywhere over the atria the ganglia are arranged in chains (Fig. 8). Ganglionic capsules are absent at this stage. Apart from the two types of cell referred to previously, occasional binucleated cells make their appearance at this stage in development. The reticular nucleated variety (*vide supra*) is now very irregular in shape and may develop into a future multipolar cell while the smaller, darkly stained cell may be either a neuroblast or a future supporting











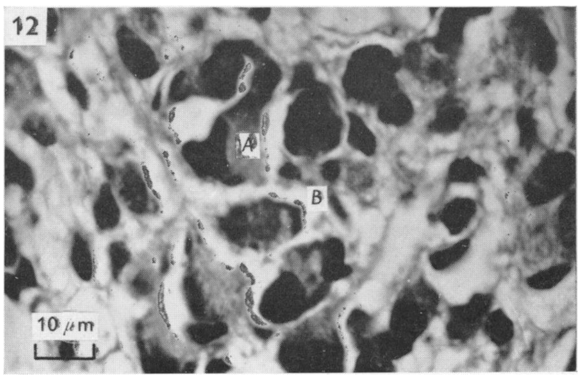


Fig. 7. A group of small ganglia in the atrioventricular groove of a 35 mm foetal heart. (Modification of Masson's Trichrome.)

Fig. 8. A chain of ganglia situated over the superior aspect of the left atrium of a 35 mm foetal heart (H and E).

Fig. 9. A right superior atrial ganglionic chain in the 65 mm foetal heart. (Modification of Samuel's Silver method.)

Fig. 10. Two ganglia lying in the superior part of the interatrial groove in the 65 mm foetal heart. The nerve bundles connect the ganglia with the atrial epicardial plexus. (Modification of Samuel's Silver method.)

Fig. 11. A ganglion in the interatrial septum of the 65 mm foetal heart associated with the two nerve trunks (*N*). The capsule (*C*) is tenuous. A small vessel (*V*) lies within the ganglion (H and E).

Fig. 12. A higher power view of Fig. 11 showing large cells (*A*) and small dark cells (*B*) (H and E).

cell. The latter are often seen lying along nerve fibres passing into the ganglia (Fig. 4).

The ganglia occupy similar positions in foetuses of 65–70 mm C–R length to those described above (Fig. 13). However, the total number of ganglia has increased, they are arranged in chains (Fig. 9) and are integrated into the general atrial epicardial nerve plexus (Fig. 10). The superior atrial groups, formerly lying close to the midline, are now seen to be extending laterally. The group lying in the interatrial sulcus has only spread caudally as far as the middle of the sulcus. Only a few ganglia are located in the atrioventricular groove and these are arranged in sporadic groups situated around the circumference of the groove. Distinct from the small dark cells,

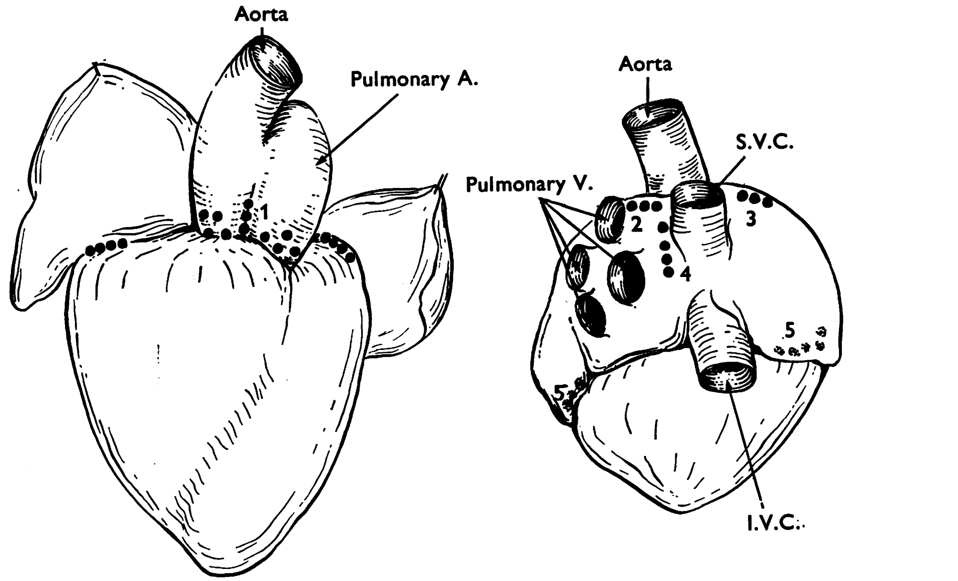


Fig. 13. Diagram to show the positions of the aorticopulmonary (1), left atrial (2), right atrial (3), interatrial septal (4) and atrioventricular groove (5) groups of ganglia in the 70 mm foetal heart.

two main types of ganglion cell now exist. While both have reticular nuclei, one variety often contains two nucleoli and is more irregularly shaped than the second type which is often piriform. The former variety exceeds the latter in the ratio 4:1. Binucleate cells are again present in slightly greater numbers than before, but they are confined mainly to the ganglia of the superior atrial and aorticopulmonary groups. Most ganglia are now surrounded by a tenuous capsule and small capillaries are often intimately related to them (Figs. 4, 11). At the 70 mm stage the arrangement of the intrinsic cardiac ganglia and nerves is found to be integrated into a composite whole.

DISCUSSION

There is general agreement (Marshall, 1878; Jones, 1942; Kuntz, 1953) that, after the vagi have sprouted from the hind brain, neuroblasts migrate down the nerves into the heart. Whether the nerves reach the heart first and the cells migrate along this pathway or the cells reach the heart at the head of the developing nerves is more uncertain. The majority opinion seems to indicate that the nerves grow into the heart in advance of the nerve cells (Navaratnam, 1965). Estimates of the time at which the invasion of the heart by the nerves is initiated vary considerably. Kuntz (1953) described nerves at the base of the heart in embryos of 7–9 mm C–R length although Fukutake (1925) failed to find them at this early stage. Perman (1924) found them in a 10·2 mm embryo and Navaratnam (1965) noted nerves close to the pulmonary artery in 11·0 mm embryos, although Idhima (1929) could not stain nerves until the 16·0 mm stage. This investigation supports the theory that nerves are present in the superior aspect of the heart in advance of the ganglion cells; the time of their arrival is slightly later (15 mm) than Kuntz's estimate but accords with Idhima's findings. Although Perman (1924) stated that nerves reach the atrioventricular groove by the 19·0 mm stage and Idhima (1929) found them in the interventricular septum in 20·0 mm embryos, Fukutake (1925) only noted nerves extending throughout the upper one-quarter of the heart by the 25 mm stage. Licata (1954) agreed with Fukutake and described nerves entering the interatrial sulcus near the bases of the atrioventricular valves at the 40 mm stage. The author's results support Fukutake's and Licata's views that the progressive invasion of the heart by the cardiac nerves is a relatively slow process which is not complete until the 55 mm stage for the atria and the 70 mm stage for the ventricles.

Perman (1924) described a small knot of ganglion cells first appearing between the aorta and the pulmonary artery in a 10·2 mm embryo. Although this group of cells was the first of the intrinsic cardiac ganglia to become apparent, it was not found until a slightly later stage (15 mm). Perman's second group, lying in the dorsal atrial walls at the 19·0 mm stage, was not seen in the present series until the 25 mm stage when it was found to be situated superiorly. Navaratnam (1965) stated that cardiac nerve cells appeared at about the 20 mm stage and he described their distribution both over the dorsal atrial walls and in relation to the sinuatrial and atrioventricular nodes at the 30 mm stage; this observation could not be confirmed in this investigation as ganglion cells were detected only in relation to the superior part of the interatrial septum posteriorly and the atria posterosuperiorly. There was no extension of the ganglia to surround the pulmonary vein entrances.

Navaratnam's (1965) concept of nerves passing from the aorticopulmonary arterial plexus into the coronary plexus is correct as ganglion cells are found scattered throughout these plexuses, particularly after the 35 mm stage. Licata (1954) has described neuroblasts in the 40 mm human embryo heart in: (1) the interatrial sulcus; (2) the atrioventricular groove and atrioventricular node anlage; (3) the dorsal walls of both venae cavae; (4) and groups related to the coronary vessels.

In general his findings have been confirmed in this study, except that the groups related to the inferior vena cava have not been identified while those related by Licata to the superior vena cava really appear to belong to the right superior atrial aggregation. After the 40 mm stage the ganglia develop rapidly until five chief groups can be regularly identified in the 70 mm human foetal heart.

Both Idhima (1929) and Navaratnam (1965) have described two cell types in 30 mm human embryo hearts. Idhima noted both spherical and oval cells which he thought represented unipolar and bipolar cells respectively. Navaratnam regarded the smaller round cell as a sheath cell, and the larger cells, which were either spherical or piriform, as ganglion cell precursors. Although Blair & Davies (1935) stated that two distinct types of ganglion cell exist in the adult human heart, Walls (1947) considered that the two types do not appear before birth and that the only type present in the foetal heart corresponds to Blair & Davies's type I cell. This was a large cell which stained palely with silver methods. Kuntz (1953) on the other hand, saw unipolar, bipolar and multipolar cells in foetal hearts at the 7th month of development. Apart from the very early embryos in which only a single type of cell, small and darkly staining, is observed, one could always distinguish two distinct cell types in the cardiac ganglia. These findings correlate with those of Navaratnam (1965) as one type of cell is small and darkly staining and may be a future supporting cell while the other is large and spherical. However, at the 65–70 mm stage, the larger cells appear in two main varieties. One is irregular in shape while the other is piriform. They perhaps represent future multipolar and pseudo-unipolar cells respectively. In addition to the types noted above, binucleate cells occur sporadically in the cardiac ganglia.

Much of the controversy over times of appearance of ganglia, etc., may be due to difficulties in estimating accurately the age of the embryo or foetus. This is usually inferred from crown–rump measurements as the exact dates of conception are seldom known. Mensuration may be impaired by different degrees of spinal flexion of the foetus. Some of the cell types described could be fixation artefacts, but this is improbable as the cell types described exist in foetal hearts fixed by different methods.

SUMMARY

The intrinsic innervation of the human embryonic heart is comprised of both nerves and nerve cells from a very early stage. The first group of ganglion cells to appear, associated with a plexus of nerves and lying between the aorta and pulmonary artery, is visible in the 15 mm embryo.

During the next 7 weeks up to the 70 mm stage, the following ganglia appear: a group lying in the adventitia of both the aorta and pulmonary artery; a left atrial group; a right atrial group; an interatrial septal group; an atrioventricular groove group.

The ganglion cells and their processes are parts of an epicardial nerve plexus mainly located over the atria. Nerve fibres enter the atrial plexus from the cardiac plexus lying above the base of the heart.

The ganglia are usually small (180 μm × 60 μm average size) and unencapsulated until the 65 mm stage. Ganglia located between the aorta and pulmonary artery tend to be larger (350 μm × 80 μm) and fusiform, and lie in the long axis of the great vessels. Small capillaries are usually closely associated with the ganglia lying both inside and outside their capsules.

Four main cell types can be differentiated from the 65 mm stage onwards.

The author wishes to thank Professor G. A. G. Mitchell for his help and guidance during this study and also Dr J. B. Jones of Withington Hospital, Manchester, for supplying much of the human foetal material used in this investigation.

REFERENCES

BLAIR, D. M. & DAVIES, F. (1935). Observations of the conducting system of the heart. *J. Anat.* **69**, 303–325.

FUKUTAKE, K. (1925). Beiträge zur Histologie und Entwicklungsgeschichte Herzennervensystems. *Z. Anat. EntwGesch.* **76**, 593–639.

IDHIMA, K. (1929). Embryonal study of the nervous system of various organs of the human foetus. Part I. The cardiac nerves of the human foetus. *Jap. J. Obstet. Gynec.* **12**, 234–253.

JONES, D. S. (1942). The origin of the vagi and the parasympathetic ganglion cells of the viscera of the chick. *Anat. Rec.* **82**, 185–197.

KUNTZ, A. (1953). *The Autonomic Nervous System*, 4th ed., pp. 117–151. Philadelphia: Lea and Febiger.

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

MARSHALL, A. M. (1878). The development of the cranial nerves of the chick. *Q. Jl microsc. Sci.* **69**, N.S. 18, 10–40.

NAVARATNAM, V. (1965). Development of the nerve supply to the human heart. *Br. Heart J.* **27**, 640–650.

PERMAN, E. (1924). Anatomische Untersuchungen über die Herznerven bei den höheren Säugetieren und beim Menschen. *Z. Anat. EntwGesch.* **71**, 382–457.

SAMUELS, E. P. (1953*a*). Impregnation and development in silver staining. *J. Anat.* **87**, 268–277.

SAMUELS, E. P. (1953*b*). The mechanism of silver staining. *J. Anat.* **87**, 278–286.

WALLS, E. W. (1947). The development of the specialized conducting tissue of the human heart. *J. Anat.* **81**, 93–110.