

# Development of innervation of coronary arteries in human foetus up until 230 mm. stage (mid-term)

R. B. Smith

*From the Anatomy Department, The University, Manchester*

*Innervation of the coronary vessels is initiated before the 30 mm. stage of development. All the main branches of the coronary arteries are formed and in their definitive positions by the 40 mm. stage. Two plexuses have been shown for all the larger vessels after the 120 mm. stage. There are coarse-fibre and fine-fibre plexuses, situated at different levels in the tunica adventitia. Ganglion cells have been found in relation to the coronary arteries over the ventricles. This confirms the part played by the vagal system in the innervation of the ventricle. No nerve endings were seen in the tunica media.*

Since Willis (1684) described the contributions of the vagus and recurrent laryngeal nerves to the coronary vessels, many reports have been published on their innervation. Behrends (1792) confirmed the distribution of nerves along the coronary arteries, and Scarpa (1794) noted that right and left coronary plexuses followed the coronary arteries to the cardiac apex, nerves leaving the vessels at intervals to supply the myocardium.

Speculating upon the functional significance of the nerve plexuses, Blackhall-Morison (1926) used the presence or absence of ganglion cells to determine their sympathetic or parasympathetic origins. As the coronary plexuses contained ganglion cells in their meshes he concluded that they contained vagal elements. Woollard (1926) considered that the coronary arterial nerve plexus consisted of two distinct parts. The coarse plexus of large nerve bundles lying in the adventitial coat of the vessels was considered to contain vagal fibres. A finer plexus of nerves close to the tunica media consisted of sympathetic fibres and supplied nerve terminals which penetrated the tunica media to supply the muscle cells. Woollard thought that the nerves followed the vessels down to the arteriolar level but they could not be traced beyond this. He suggested that the smaller vessels (arterioles) possessed only a vagal nerve supply.

Ihdima (1929) claimed that, in the 7-month human foetus, all the coronary arteries were supplied with nerves situated in both the adventitial and medial coats. He could not identify them in the tunica intima. Stöhr (1932) described an adventitial nerve plexus running along the longitudinal axis of large medium arteries, while a subsidiary, deeper, plexus consisting of fine varicose fibres lay on the surface of the tunica media. However, he asserted that, contrary to Woollard's (1926) and Ihdima's (1929) views, no nerves penetrated the medial coat. Capillaries were associated with a scanty plexus composed of varicose fibrils. Though Jones (1927) was uncertain whether the vascular nerve plexus aided the myocardial nerve supply, Stöhr confirmed Scarpa's (1794) opinion that the coronary arterial nerve plexuses made a significant contribution. Nettleship (1936) described large nerve bundles accompanying the coronary arteries which he thought subserved sensory functions, and Hirsch and Orme (1947) suggested that the cardiac sensory fibres were all closely associated with the coronary arteries. The fibres left the vessels to branch in the myocardium, terminating as unmyelinated neurofibrils. Mitchell (1956) stated that the nerves of the coronary plexuses (which are a continuation of the cardiac plexus) contained both motor and sensory elements destined for the supply of the coronary vessels themselves and the myocardium, with afferent fibres from the endocardium and valves of the heart.

Contrary to Woollard's (1926) opinion that the capillary nerve supply was absent, King (1939), Lavrentiev (1946), and Hirsch and Borghard-Erdle (1961) all stated that the coronary arterial nerve plexuses followed the vessels down to their terminal ramifications.

### Materials and methods

Twenty-six foetuses ranging in size from 30 mm. to 230 mm. crown-rump length were collected from hospitals in the Manchester Region. After fixation for periods of up to six weeks, all hearts from foetuses of 90 mm. crown-rump length or less were serially sectioned at  $10\ \mu$  and every section was mounted. Alternate hearts were sectioned both longitudinally and transversely at  $10\ \mu$ , and the resulting sections were stained with either a modification of Masson's trichrome method or a silver staining procedure (Samuel, 1953). A number of hearts were serially sectioned after staining with an osmic acid-zinc iodide mixture. This was composed of one part 2 per cent osmic acid which was mixed with three parts of 3 per cent zinc iodide after the latter had been filtered. Both solutions were made up from deionized water, and the specimens were placed in the mixture, in the dark, for periods varying between 12 and 18 hours.

The hearts from foetuses measuring more than 90 mm. crown-rump length were studied following a different method. After fixation, careful dissection was employed to remove the epicardium from both the atria and the ventricles and also the aortic adventitia. It was found that, following this procedure, the coronary vessels could be detached with the epicardium, and that the resulting strip of tissue was thin enough to allow transmission of sufficient light for adequate photography. Staining of the strips was by an osmic acid-zinc iodide mixture (*vide supra*) either before or after detachment of the epicardium from the hearts.

### Observations

In foetuses of 30 mm. crown-rump length, both the aorta and pulmonary artery are surrounded by five or six small nerve fascicles, branches of which are continued into the stems of the coronary arteries. At the 55 mm. stage an adventitial plexus of small nerve bundles is associated with both the main coronary arteries and their medium-sized branches (Fig. 1). At this stage no nerves can be observed near the tunica media of the vessels. In later foetuses (75 mm. C-R length) the innervation of the coronary vessels is well established and is beginning to fall into the pattern observed for older specimens. Each main branch of the coronary arteries is accompanied by an attendant plexus of 2-4 small nerve bundles which travel parallel to the vessels communicating with each other infrequently (Fig. 2). The smaller vessels possess only a sparse plexus of tenuous beaded fibrils (Fig. 3). By the 90 mm. stage the nerve

bundles travelling longitudinally with the main coronary vessels have become thicker, with many more constituent fibres. There are still very few communications between the nerve bundles. The nerve fascicles associated with the branches of the coronary arteries tend to travel in the epicardium parallel to, and some distance from, their respective vessels. Gradually communications develop between the attendant nerve bundles so that at the 110 mm. stage the larger vessels are surrounded by between two and five medium-sized bundles arranged in a long, loose spiral. The very small coronary branches are still only accompanied by a single bundle which splits up into finer branches as it progresses along the vascular tree.

Development now proceeds rapidly so that in the foetal heart at the 120 mm. stage the main coronary arteries are associated with two or more nerve bundles of  $25\ \mu$  diameter spiralling round the vessels, together with 2-4 smaller bundles of approximately  $7\ \mu$  diameter. The corresponding veins, however, possess only two or three of the smaller bundles arranged in a loose spiral along their length. There are frequent communications between the general epicardial plexus and the coronary arteries (Fig. 4 and 5), and the nerve bundles rapidly break up and become lost in the coronary plexuses. A finer nerve network surrounding the larger vessels and lying in the deeper layers of the tunica adventitia is now clearly visible (Fig. 5). The fine nerves are arranged in a more closely woven spiral than the coarse plexus previously described. Smaller vessels have only two or three small nerve bundles arranged in a loose spiral formation along their length while their attendant veins are accompanied by only a single small nerve bundle which is sometimes absent (Fig. 6). At infrequent intervals reinforcing fibres are fed into the nerve plexuses of these small arteries. Occasionally small groups of ganglion cells are found along the course of the large nerve bundles of the adventitial plexus (Fig. 7), and these rapidly increase in size as development proceeds. The contrast between the innervation of the anterior interventricular branch of the left coronary artery and the great cardiac vein of the 150 mm. foetus can be seen in Fig. 8. While the artery shows both coarse and fine plexuses, the innervation of the vein is limited to two or three small bundles. These follow the vessels only loosely, lying some distance from it, with infrequent communications between the bundles winding round the vessels.

By the 170 mm. stage of development the finer network, which borders on the tunica

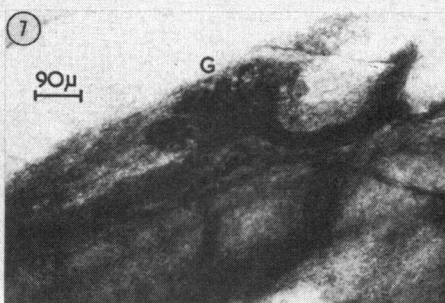
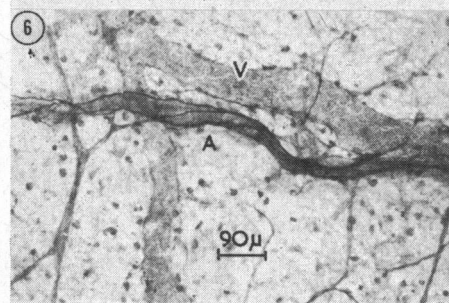
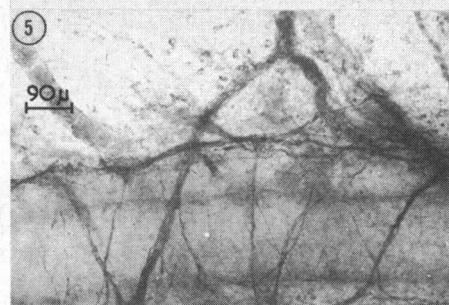
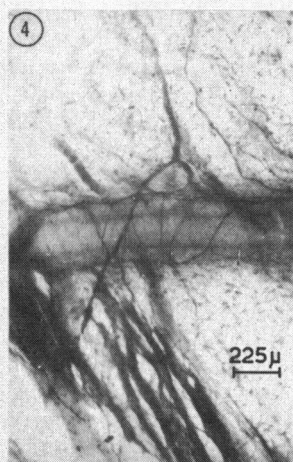
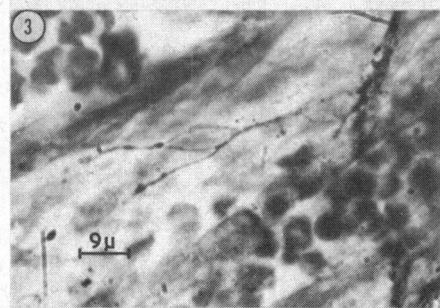
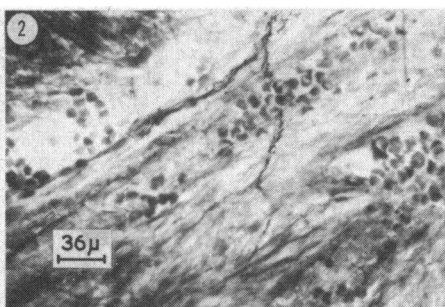
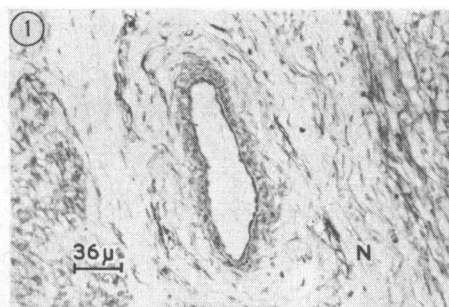


FIG. 1 55 mm. foetus. Transverse section of a coronary artery to show small nerves (N) in the tunica adventitia. (Samuels.)

FIG. 3 75 mm. foetus. Beaded nerve fibrils lying in the sparse tunica adventitia of an arteriole. (Osmic acid-zinc iodide.)

FIG. 5 120 mm. foetus. A higher magnification of Fig. 4 to show the presence of a fine adventitial nerve plexus. (Osmic acid-zinc iodide.)

FIG. 6 120 mm. foetus. An arteriole (A) accompanied by a venule (V) showing the contrast between the degree of innervation of the two vessels. (Osmic acid-zinc iodide.)

FIG. 2 75 mm. foetus. Longitudinal section of a coronary artery showing a nerve fascicle crossing it. (Osmic acid-zinc iodide.)

FIG. 4 120 mm. foetus. A nerve bundle from the epicardial plexus joining a coronary artery. (Osmic acid-zinc iodide.)

FIG. 7 150 mm. foetus. A ganglion (G) lying in the meshes of the coarse adventitial plexus of a branch of the left coronary artery. (Osmic acid-zinc iodide.)

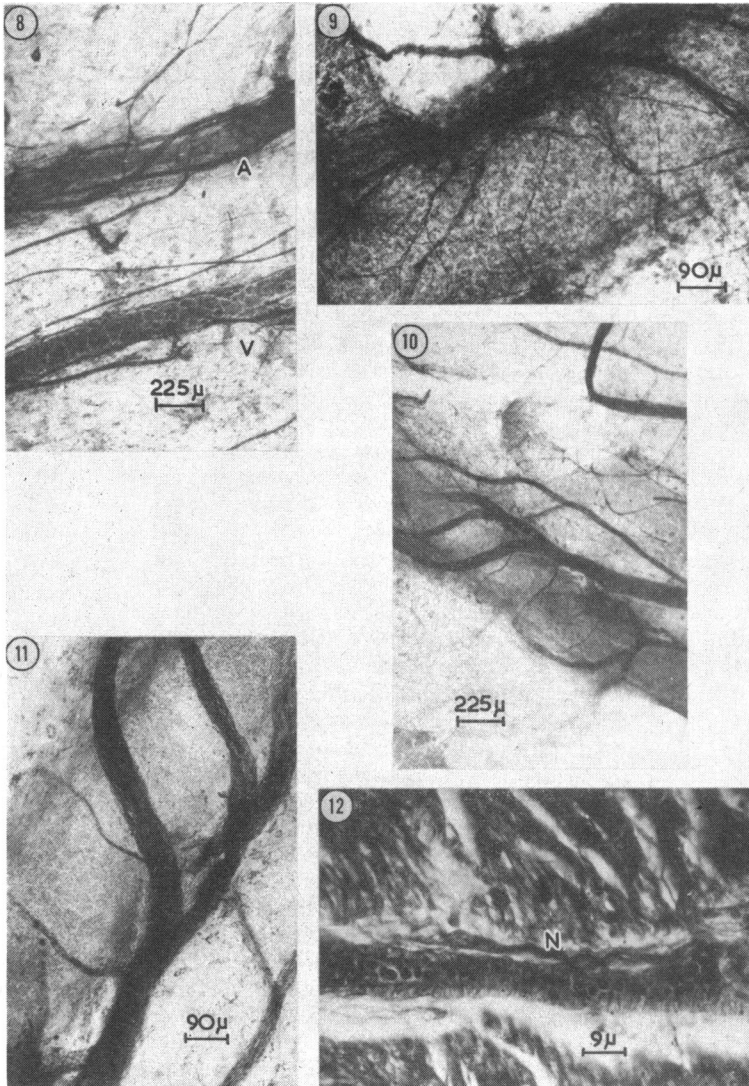


FIG. 8 150 mm. foetus. The anterior inter-ventricular branch of the left coronary artery (A) and great cardiac vein (V). Note the marked difference in the number of nerves surrounding the vessels. (Osmic acid-zinc iodide.)

FIG. 11 170 mm. foetus. A higher magnification of Fig. 10.

FIG. 9 170 mm. foetus. Note the deep adventitial plexus consisting of fine nerves. (Osmic acid-zinc iodide.)

FIG. 10 170 mm. foetus. A large nerve bundle joining the coarse adventitial nerve plexus of a coronary artery. (Osmic acid-zinc iodide.)

FIG. 12 230 mm. foetus. A nerve bundle (N) following an arteriole in the ventricular myocardium. (Osmic acid-zinc iodide.)

media but does not appear to enter it, is composed of a closely woven lattice of fine varicose fibres spiralling along the vessels (Fig. 9). It receives numerous communications from the coarse adventitial plexus. This latter is now composed of four or five medium-sized bundles, and receives communications from large epicardial nerve bundles (Fig. 10 and 11) which rapidly break up into reinforcements for the smaller fascicles. Arterioles receive a rich supply at this stage and are accompanied by 1-3 finer nerve bundles of approximately 6  $\mu$  diameter. Besides receiving nerve bundles from the general epicardial plexus, the arteriolar nerve plexus also appears to transmit branches back into the epicardial nerve plexus. By mid-term the coronary nerve plexuses have extended into even the terminal ramifications of the vascular tree. Even the smallest arterioles have an attendant nerve bundle (Fig. 12), and the capillaries are accompanied by one or more beaded nerve fibrils. The innervation of the capillaries by varicose fibrils is closely associated with similar nerve fibrils lying close to the individual myocardial muscle cells, so appearing as subdivisions of the same general plexus. The association between vessels and nerves is anatomically striking from an early stage in development and is maintained as development proceeds.

### Discussion

The appearances of the coronary arterial plexuses up until the 50 mm. stage correlate well with the findings of Navaratnam (1965) who described nerve cells and nerves from the aortic plexus surrounding the coronary arteries. The initiation of coronary vessel innervation is by a caudal progression from the cardiac plexus and, as Navaratnam stated, the coronary plexuses increase in size and extent after the 40 mm. stage. Licata (1954), studying human foetuses of 9 weeks (40 mm. approx.), considered that all the branches of the coronary arteries were formed and in their definitive positions. This is confirmed by the present study. The nerves of the coronary plexuses, progressing caudally, are found to colonize this lattice.

The coarser adventitial plexus is associated with the major vessels before the 40 mm. stage is reached, and thereafter, as development proceeds, the number of its fibres gradually increases. Up to the 90 mm. stage, judging by the appearance of beaded filaments in the osmium preparations, the adventitial plexus consists of unmyelinated fibres. Myelination of this plexus appears to be well established by the 120 mm. stage. About this time also (110-120 mm.) the finer plexus of

nerves in the deeper layers of the adventitia makes its appearance. This consists of unmyelinated fibres until the 230 mm. stage.

Navaratnam (1965) described nerve cells at the 30 mm. stage in relation to the coronary arteries, and their presence has been noted in this investigation. They occur much more rarely in later foetuses and usually lie in relation to the vessels in the atrioventricular furrow. However, occasional ganglia have been identified in later foetal hearts (120-150 mm.), situated in the meshes of the coarse coronary arterial plexuses as they follow the vessels over the ventricles (Fig. 7). The finding of such ganglia lends weight to the opinions of such workers as Blackhall-Morison (1926) and Woollard (1926) who thought that the thicker adventitial plexus was composed of vagal fibres. As some fibres of this plexus may help to innervate the ventricle, then the presence of such ganglia strengthens the case for a ventricular parasympathetic supply (Mitchell, 1956).

The finer nerve plexus surrounding the vessels has been held to be sympathetic in nature (Woollard, 1926) consisting of non-medullated nerves which enter the tunica media to innervate the muscle cells. More recently, Norberg and Hamberger (1964) have described this plexus of varicose fibres, between the deepest layers of the adventitia and the media, and considered that it belonged to the sympathetic system. Berkley (1893), Davies, Francis, and King (1952), and Woollard (1926) have described the fibres of this fine plexus terminating in bulb-like and tapering endings in the tunica media. No such end-apparatus was seen in this investigation and, in agreement with Stöhr's (1932) findings, the nerve fibres were not seen to penetrate the medial coat. This finer nerve plexus was found to be absent from the smaller arterioles, and this accords with the findings of Woollard (1926). This worker considered that the single plexus associated with these vessels was composed only of vagal fibres.

Woollard also thought that the fine capillaries were devoid of a nerve supply and Stöhr (1932) stated that capillaries were instantly innervated. However, Lavrentiev (1946) disagreed, stating that the coronary arteries were richly innervated down to capillary level. This finding was confirmed by Sato (1954) and verified in the present investigation from the 110 mm. stage onwards. The capillaries and the muscle cells appear to be innervated through the same fine network of varicose nerve fibrils.

Generally, from the 120 mm. stage onwards there are more constituent nerve fibres in the

coronary plexuses than would be required for their innervation. The consensus appears to be that the vagal fibres comprise both motor and sensory elements while the sympathetic subserves motor functions (Hirsch and Borghard-Erdle, 1961). The fibres supply the coronary vessels: they are motor to the myocardium and sensory to both the myocardium and endocardium, particularly in the region of the valves (Mitchell, 1956).

I wish to thank Professor G. A. G. Mitchell for his help and advice and also Dr. J. B. Jones of Withington Hospital for supplying me with the human foetal material.

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