J. Anat. (1980), **130**, 4, pp. 821–831
With 11 *figures*
Printed in Great Britain

Observations on the sinuatrial nodal artery of the rat

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(*Accepted* 11 *September* 1979)

INTRODUCTION

In 1906, Keith & Flack described the human sinuatrial node and drew attention to the presence of a special vasculature in it. Subsequently other authors, including James (1962, 1965) and James & Nadeau (1963, 1964), confirmed the richness of the arterial supply of the node in man and other species. It is clear that in many mammals, the node is disposed around a central nodal artery although in some the nodal artery breaks up as it enters the specialized tissue and forms several smaller arteries which then ramify through it. This arrangement is found in the bovine heart (James, 1965) and is occasionally seen in the rat also.

However, in 1974 Merrillees reported that the adventitial coat of the rodent sinuatrial artery did not always conform to the common description of an arteriolar adventitia. In a central part of the node, he described a discrete portion of the adventitia which contained nodal muscle cells. These cells were small and peculiar in shape and appeared to form a thin, incomplete labyrinthine cuff around the media. On either side of this central zone, the smooth muscle of the media was surrounded by more regularly shaped intermediate cell profiles to form a cuff one or two cells thick continuous with that of the central zone.

Although he found the central zone in only one group of thin sections taken from one of a total of 80 blocks derived from 20 rats, he suggested that the cuff of bizarrely shaped cells might be the site of pacing activity in this species. In view of the importance of this suggestion and the difficulty in locating this area of special interest, it was decided to look at a larger number of rats at various stages of maturation in an effort to confirm his observations. Young animals were studied in the hope that examination of smaller hearts would reveal the central zone more easily. In addition, electron histochemical techniques to disclose the presence of noradrenergic neurons and cholinesterases were applied to the nodal artery and surrounding tissues.

MATERIALS AND METHODS

Series 1. *The ultrastructure of the nodal artery*

A total of 80 rats comprising 30 adults and 10 each of newborn, 1, 2, 3 and 4 weeks old animals were examined. After decapitation, the heart of each rat was exposed and perfused via the left ventricle with a cold 2½ % paraformaldehyde and 2 % glutaraldehyde mixture in 0·1 M phosphate buffer at pH 7·4. The nodal artery was identified as it descended on the right superior vena cava and a block of tissue containing the artery dissected out. Each block was subdivided by transverse cuts across the artery to give between 4 and 8 slices which were kept in ice cold fixative for a further hour. After a brief wash in buffer, the specimens were fixed in 1 % osmium tetroxide for a further hour, routinely dehydrated in alcohol

0021-8782-80-2828-7690 $02.00

and embedded in Epon 812. The blocks were oriented so that transverse sections of the sinus artery could be prepared.

After areas of interest were identified on thick sections, thin sections were stained with lead citrate and uranyl acetate prior to examination in a Philips 300 electron microscope.

Series 2. *Histochemistry of the nodal artery*

(*a*) Nodal arteries from a further 10 adult rat hearts were examined for cholinesterase activity using the method of Mednick *et al.* (1971).

The hearts were fixed by perfusion with a freshly prepared solution of 1 % purified glutaraldehyde and 2 % formaldehyde in a 0·1 M phosphate buffer at pH 7·2. The nodal artery was dissected out as before and cut into 1 mm slices. After placing in cold fixative for a total of one hour, the tissue was washed for 16 hours at 4 °C in 0·05 M phosphate buffer containing 7·5 % sucrose.

Preparation of all reagents and substrates was carried out at 0 °C. Acetylthiolanilinium chloride (10 mg) was dissolved in 0·2 ml distilled water. Diazotization was carried out by the successive addition of 0·3 ml of 0·33 M hydrochloric acid and 0·3 ml of 1·14 % sodium nitrite. The solution was swirled for 5 minutes and, after the addition of 0·05 ml of 0·4 % urea, swirled for a further 2 minutes. This solution was then diluted with 19 ml of 0·1 M phosphate buffer pH 7·2 at room temperature and this incubation mixture poured onto the tissue slices. Incubation was carried out at 4 °C for 2 hours in the dark. The tissue was then washed twice in distilled water and placed in 1 % osmium tetroxide for 45 minutes at 55 °C. After standard dehydration in ethanol, the specimens were embedded in Epon and examined as in the first series.

To determine the specific activity of the cholinesterase demonstrated, some specimens were pre-incubated for 30 minutes with either 2×10^{-4} M tetraisopropylpyrophosphoramide (TIPA) or 2×10^{-4} M 1–5 bis 4 allyl dimethylammonium phenylpenta 3 one dibromide (BW284C51) inhibitors of non-specific and acetylcholinesterases respectively. As BW284C51 is a reversible inhibitor it was also added to the incubation mixture at a final concentration of 2×10^{-4} M. Pre-incubation with eserine at a molar concentration of 1×10^{-5} served to inhibit cholinesterases totally and no reaction product was ever seen after its use.

(*b*) A further group of 8 adult rats was given a single intraperitoneal injection of 25 mg of 5-hydroxydopamine (5-OHDA) dissolved in water. Four control animals were given a similar injection of water. Twenty four hours later, all the animals were killed and processed in an identical fashion to the first series of rats.

RESULTS

The position of the sinuatrial node in the rat heart conforms with earlier descriptions (e.g. Halpern, 1955) and the node itself takes the form of a horseshoe shaped collar around the junction of the superior vena cava with the atrium. The nodal artery, a branch of the right internal thoracic artery, runs down the lateral side of the right superior vena cava, passes into the node and traverses it. *En route*, however, several branch arteries pass into the node and these may be the only arterial structures within the node when, as seen in occasional hearts, the nodal artery proper breaks up as it enters the specialized tissue.

The ultrastructural appearance of the nodal artery and its related nodal cells

is similar in both young and adult animals, although it is rather easier to identify in younger rats. No major differences are seen save for the innervation. This is sparse in newborn animals but shows considerable increase by the end of the first postnatal month. In view of these similarities, only features of the nodal arteries of adult animals will be illustrated.

The artery and its branches consist of an endothelial cell layer separated from the media by an irregular internal elastic lamina. The overlying media is usually 1 or 2 cells thick but on rare occasions as many as three layers of smooth muscle cells may be observed in adult animals.

Within the sinuatrial node, the media of the greater part of the nodal artery and of its major branches is surrounded by a cuff of nodal cells lying within the adventitial coat of fibroblasts and collagen. The cuff seen on the main nodal artery is continuous with that of the branch arteries and throughout its length is made up of specialized cells smaller than those seen elsewhere in the node.

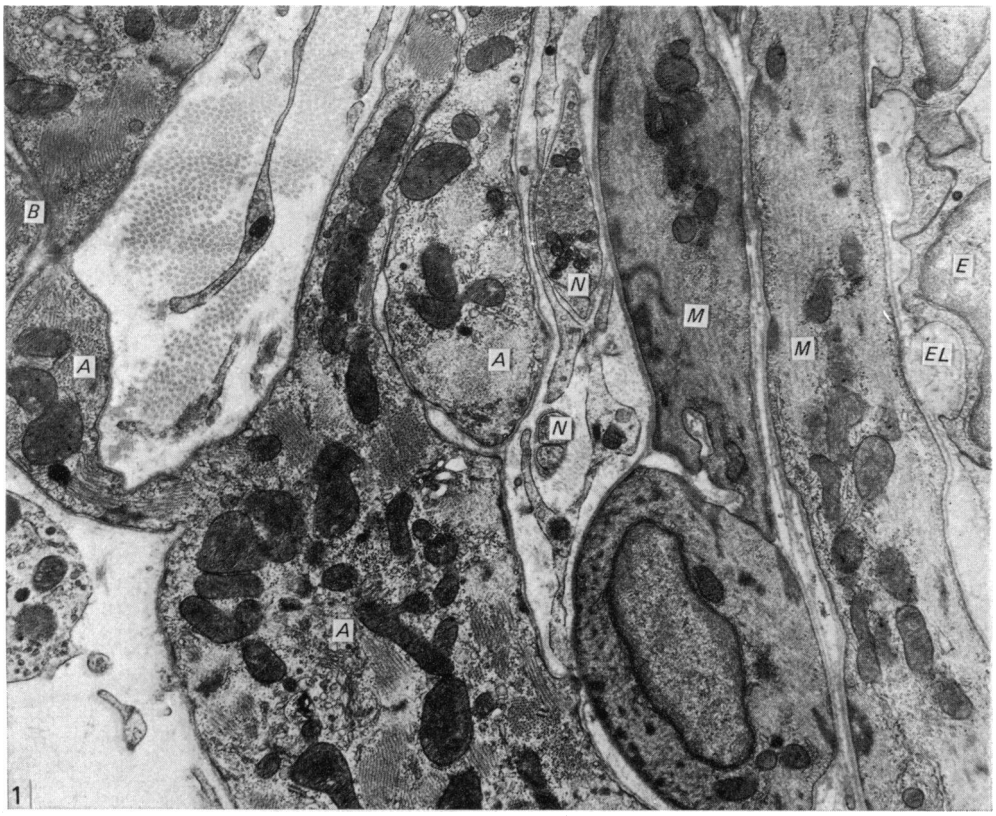
One small central part of the cuff on the sinuatrial nodal artery itself is made up of nodal cells with peculiar irregular profiles, possessing elongated processes which are often folded upon themselves (Figs. 1, 2). Adjacent processes of neighbouring cells form desmosomes and close contacts and thus the cells are linked together to form a cuff. The extracellular spaces contain fibroblasts and collagen and many unmyelinated nerves.

Nodal cell profiles vary considerably in their content of myofibrils. Some are filled with regularly arranged bundles of fibrils with Z bands. Others possess only a few disorganized fibrils or none at all, and when such cells and their processes are attenuated (Figs. 7, 8), they are often difficult to identify. However, the cholinesterase technique has proved helpful in this respect, although it is difficult to determine the precise ultrastructural localization of enzymic activity in cuff cells. On the basis of selective inhibition it can, however, be stated that adult cuff nodal cells contain acetylcholinesterase (Figs. 3, 4).

This has allowed the easy recognition of elongated, isolated profiles of nodal cells because other cell types, such as smooth muscle and fibroblasts, consistently exhibit very little or no reaction product in the presence of TIPA, an inhibitor of pseudocholinesterases.

Passing along the artery away from the area of bizarrely shaped cells just described, the nodal cells of the adventitia become more fusiform (Figs. 5, 6). However, they still form a more or less complete sheath which may be as many as three cells thick (Fig. 6). A similar appearance is also seen on the proximal portions of the major branches of the nodal artery. Connective tissue is more prominent both outside the sheath and within it, and long thin fibroblast processes are seen lying between nodal cells. The specialized cells of the cuff do not exhibit a consistent degree of fibrillation in any one area. Sometimes cells with considerable and well-ordered myofibrillar development are seen adjacent to other nodal cells containing practically none of these structures (Fig. 7). As in the central area, sheath cells share the basal lamina of the smooth muscle cells of the media. Commonly the nodal cell just dips down at intervals by small projections to make contact, but occasionally a considerable length of a smooth muscle cell may be covered by nodal cells without interruption (Fig. 6).

Further along the main artery and its branches, the sheath changes its character again and is now seen to consist of layers of nodal cells alternating with layers of fibroblasts and collagen in which run small nerve bundles (Fig. 8).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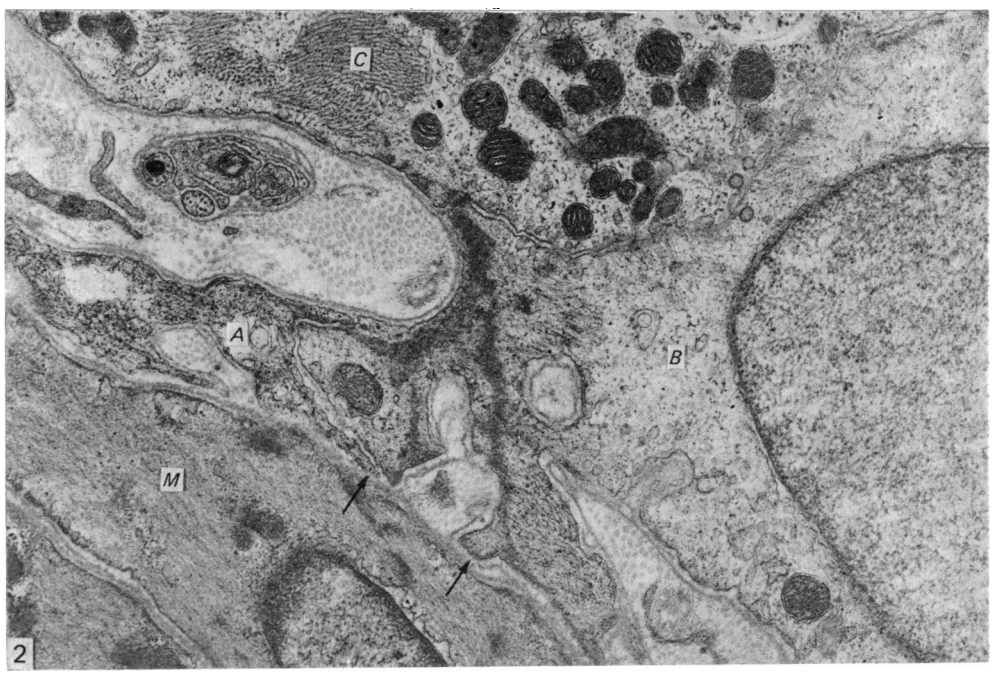
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The nodal cells are much more elongated and slender except in the nuclear region, and are increasingly separated from the media by connective tissue. The cuff is now discontinuous and larger nerve bundles are seen in and around the adventitia (Fig. 9).

Finally, a distinct cuff of nodal cells is no longer seen as the edge of the node is reached, typical atrial cells are observed, separated from the media in the usual way by a thin adventitia (Fig. 10).

Examination of the node as a whole reveals a profuse innervation but nowhere is this more true than around the nodal artery and its branches. Nerves are found in bundles of various sizes and although most are unmyelinated, the occasional myelinated axon is seen at the periphery of the node. Within the adventitia are many small bundles of axons completely surrounded by Schwann cells. However, many naked axon terminations are seen in relation to both smooth muscle cells of the media and nodal cells of the cuff. Close contacts between nerves and muscle cells of both types are frequently found and they are comparable with those seen elsewhere in the specialized tissues of the heart.

After treatment with 5-hydroxydopamine, two distinct types of nerve terminal can be recognized. One possesses 40–50 nm diameter vesicles each containing a small dense core (Fig. 11). The second type contains agranular vesicles which are of similar size and, in addition, the occasional larger 80–100 nm vesicle containing a granule of faint to medium density. Both types of vesicle are frequently encountered throughout the lengths of both the main and branch nodal arteries although profiles exhibiting small agranular vesicles predominate. Profiles of sensory endings have not been identified so far.

DISCUSSION

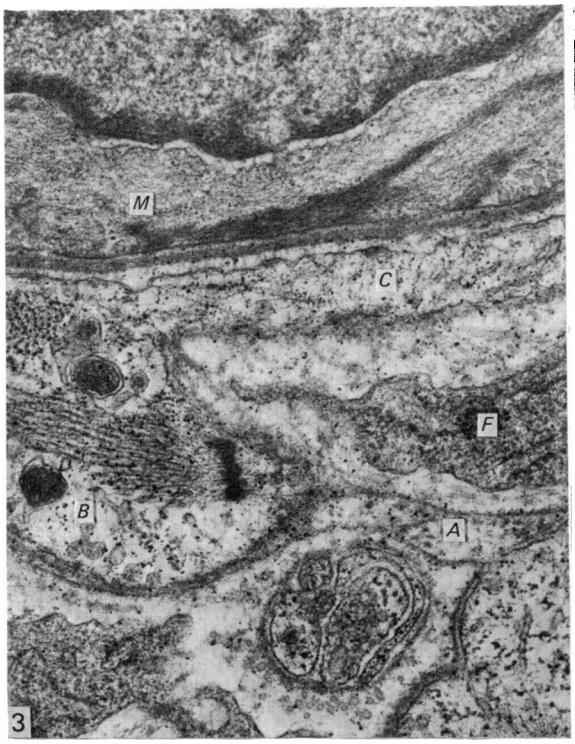
A prominent feature of the nodes of man, rat and certain other mammals is a central nodal artery. Söderström (1958) described the node as resembling an enormous adventitia of the artery in these species and several authors such as James & Nadeau (1963) have suggested that it might be of unusual physiological significance.

In a light microscope study of the human heart, Ryback & Mizeres (1965) found that the outer longitudinal coat of muscle of the sinuatrial nodal artery was lost just before the artery entered the node and in its place was found a thick adventitia consisting of elastic and collagenous tissues. This was also lost when the node was entered and replaced in turn by fibres of the sinuatrial node. Because a similar histological appearance is found in the wall of the carotid sinus they suggested that this area of the nodal artery might also be related to a pressor receptor function.

However, Brooks & Lu (1972), in their review of the sinuatrial node, concluded that there appeared to be nothing peculiar about the vasculature.

Fig. 1. Arterial cuff in the mid-zone. Nodal cells are found within the adventitial layer which is limited internally by a media 2 cells thick (*M*). Nodal cell A is folded back on itself and sends a process out to contact cell B. Separating these cells are thin fibroblastic processes and collagen and these structures are also seen around the nerves (*N*). The endothelium (*E*) is separated from the media (*M*) by a thin elastic lamina (*EL*). × 10344.

Fig. 2. Arterial cuff in the mid-zone. Three nodal cell profiles, (*A*), (*B*) and (*C*) are seen. Profiles (*A*) and (*B*) are irregular, especially on the side nearest to the smooth muscle cell of the media (*M*). The areas where nodal cells and smooth muscle cells share basal lamina are arrowed. × 19000.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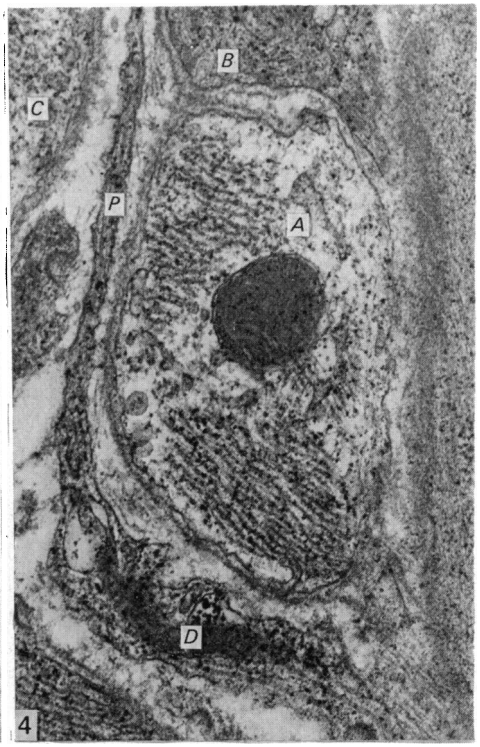
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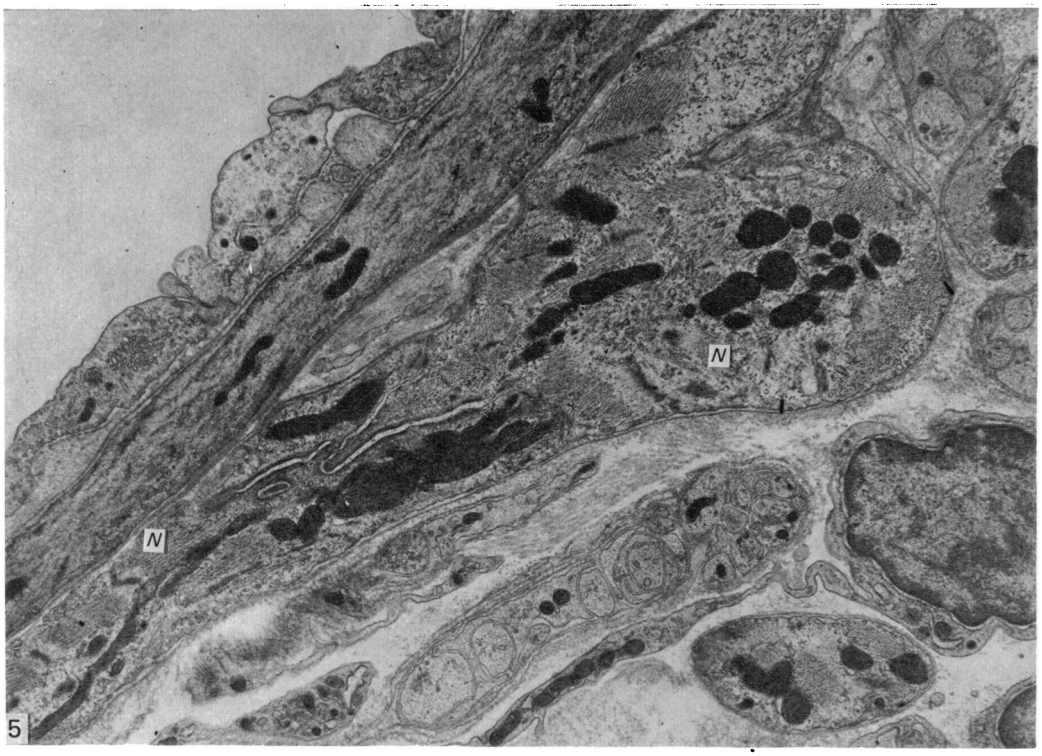
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Nevertheless, it is surprising that so little ultrastructural study of the nodal artery and its environs was made prior to Merrillees' study in 1974. While it is clear that his findings and those outlined above do not substantiate Söderström's concept, they do suggest that an unusual anatomical relationship does exist between the artery and certain nodal fibres found within its adventitia rather than outside it.

Merrillees described a cuff of nodal cells of varying specifications extending around the media of the nodal artery. This is confirmed, although it appears to be more extensive than he suggested, being found along the major branches of the nodal artery also. In those hearts where the central artery breaks up as it enters the node the cuff may indeed only be seen on these branch arteries.

The cuff was identified in all the animals examined and careful semi-serial thick sectioning showed that the central zone is a small but constant feature.

Merrillees commented that cells of the central part of the cuff were small and irregular in shape and contained few or no filaments. This has often been found in the present study although cells with many myofibrils may be equally peculiar in shape and may also be found in the central area. Indeed, neighbouring cells in the same section through the cuff frequently displayed quite disparate degrees of fibrillar development. There did not appear to be any absolute correlation between the type of specialized cell seen outside the adventitia and those seen within it which formed the cuff.

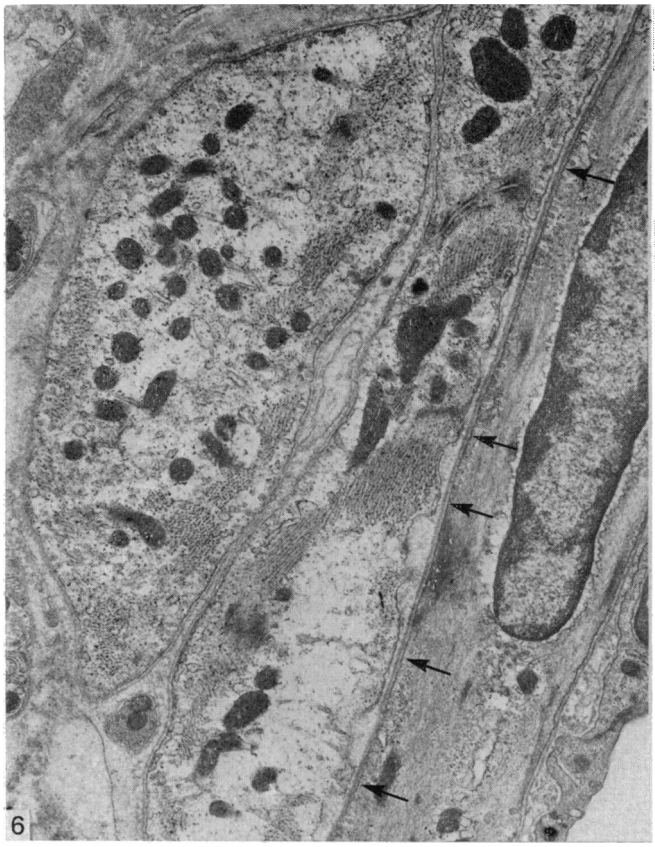
The cholinesterase technique has proved useful in the identification of small portions of elongated cells without obvious distinguishing features, for it clearly differentiates both nodal cells and certain nerves from other components of the vessel wall.

Cholinesterase activity has been observed in the media of many peripheral blood vessels in several species. For example, Navaratnam & Palkama (1966) found butyrylcholinesterase activity in the media of the aorta and pulmonary arteries but were unable to demonstrate this in the coronary arteries. Carbonell (1956), however, observed a strikingly high concentration of enzymatic activity in relation to the nodal artery of man and this has also been observed previously in the rat. While some of this activity may be due to accompanying nerves, its appearance is rather more diffuse than might be expected if they were the only reason for its presence. Rat and human specialized tissues of both the sinuatrial and atrioventricular nodes characteristically contain cholinesterase whose specific activity, in the rat at least, depends on the degree of maturity of the animal (Taylor, 1977). The ultrastructural observation of acetylcholinesterase activity in the nodal cells of the cuff of the adult rat is in keeping with these light microscope histochemical

Fig. 3. Arterial cuff in the mid-zone. Tissue examined for cholinesterase activity without inhibition. Reaction product in the form of small osmiophilic drops is seen in relation to nodal cells (*A*), (*B*) and process (*C*). No reaction product is seen on the fibroblast (*F*) and only a very little on the basal lamina of smooth muscle cell (*M*) which is otherwise devoid of activity. × 25650.

Fig. 4. Arterial cuff away from the mid-zone. Tissue examined for cholinesterase activity in the presence of TIPA. Reaction product is seen on cells (*A*), (*B*) and (*C*) which are clearly nodal cells. Cell (*D*) is also nodal and exhibits acetyl cholinesterase activity and so does its long process (*P*) which, if seen in isolation, would be difficult to identify positively. × 17100.

Fig. 5. Arterial cuff away from the mid-zone. The nodal cells (*N*) are more fusiform in shape and those seen here are intermediate in shape between those seen centrally and those seen in Fig. 6. × 8444.



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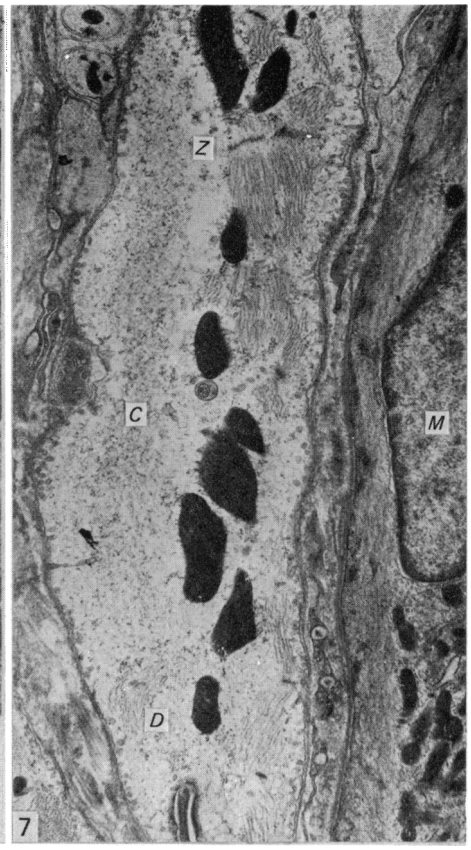

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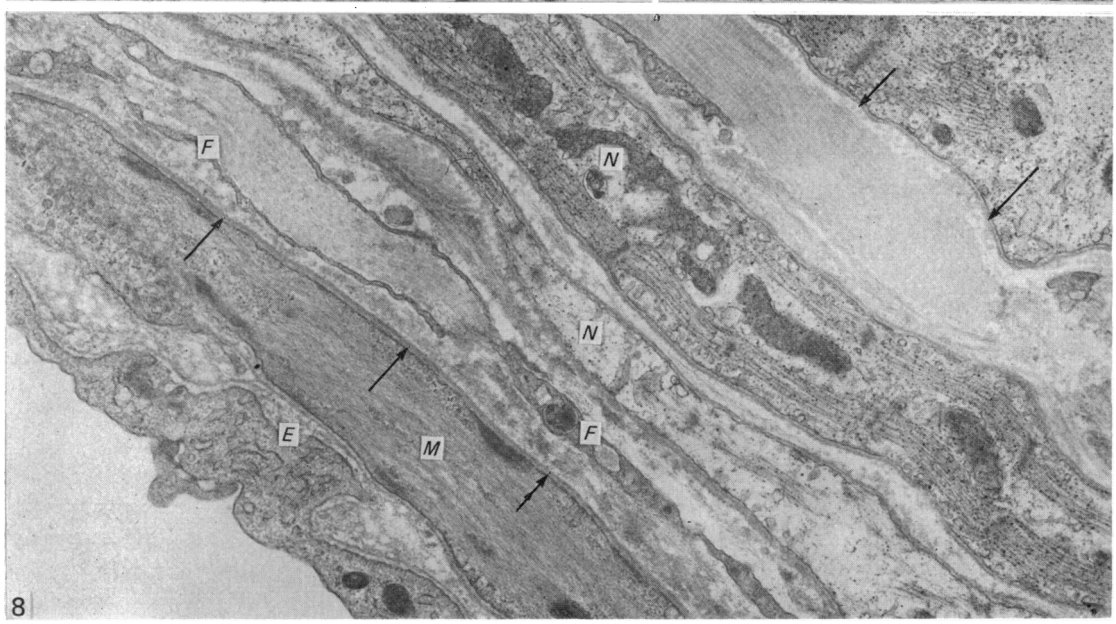

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observations. The absence of a cuff in relation to the main coronary arteries might explain the absence of cholinesterase activity in this site, as described by Navaratnam & Palkama (1966), although their observations in other vessels cannot be understood on this basis. However, Carbonnell's (1956) findings of enzyme around the human nodal artery suggests that a nodal cell cuff might also be present in man.

The rich innervation of the cuff around the central artery is a major feature of the sinuatrial node and is particularly striking because the whole node is well innervated by comparison with the rest of the atrial myocardium. Because of the unique morphology of the arterial cuff, it is tempting to speculate that in this area, nodal cells, nerves, and the various components of the vascular wall such as collagen and smooth muscle cells may interact with each other both biochemically and mechanically. Merrillees (1974) thought it reasonable to suggest that the cuff is the site of pacemaking activity in the node and Ryback & Mizeres (1965) proposed that the artery might be specialized to form a pressoreceptor mechanism. Some evidence of this was found earlier by James & Nadeau (1963) who demonstrated changes in the rate of sinus firing in response to pressure changes in the lumen of the nodal artery.

James (1967) has also shown that delivery of an artificial pulse into the sinus node artery altered the rate of sinus impulse formation. This is suggestive because the phasic radial distension of the nodal artery which normally occurs during cardiac contraction is of necessity interposed between two successive sinus impulses and this consistent interposition into the cardiac cycle recurs at exactly the same time. Whether it is this mechanical event or some more subtle physical process, associated with the flow of blood through the lumen, which triggers the response is unknown (James, 1977).

Hashimoto, Tanaka, Hitata & Chiba (1967) found an inverse relationship between sinus node arterial pressure and nodal rhythmicity over the pressure range 20–100 mm of mercury.

After an extensive review Pathak (1973) also concluded that mechanical stretch of sinuatrial nodal fibres can, within certain limits, influence pacemaker activity in a linear fashion. However, this response is not dependent on autonomic innervation. It appears that the intrinsic autoregulatory control is normally overshadowed by powerful neurohumeral influences which permit a smoother, more effective and quicker adjustment in cardiac performance then does the isolated node.

SUMMARY

The structure of the sinuatrial nodal artery has been investigated in young and old rats.

Fig. 6. Arterial cuff away from the mid-zone. The nodal cells are much more regular in shape in this area and contain scattered bundles of myofibrils. Note the areas (arrowed) where cuff cells and smooth muscle cells share basal lamina. × 12644.

Fig. 7. Arterial cuff away from the mid-zone on branch nodal artery. A cuff cell (*C*) containing very few myofibrils is seen adjacent to smooth muscle cell (*M*). Some fibrils (*Z*) are organized around a *Z* line, while others at (*D*) are poorly organized. × 8440.

Fig. 8. Arterial cuff further away from the mid-zone. The boundaries of the adventitial territory are indicated by the arrows. Endothelial cells (*E*) and smooth muscle cell (*M*) are seen on its luminal aspect. Nodal cells (*N*) are long slender cells containing varying amounts of myofibrils separated from each other and the media by elongated fibroblast processes (*F*) and collagen. × 14991.

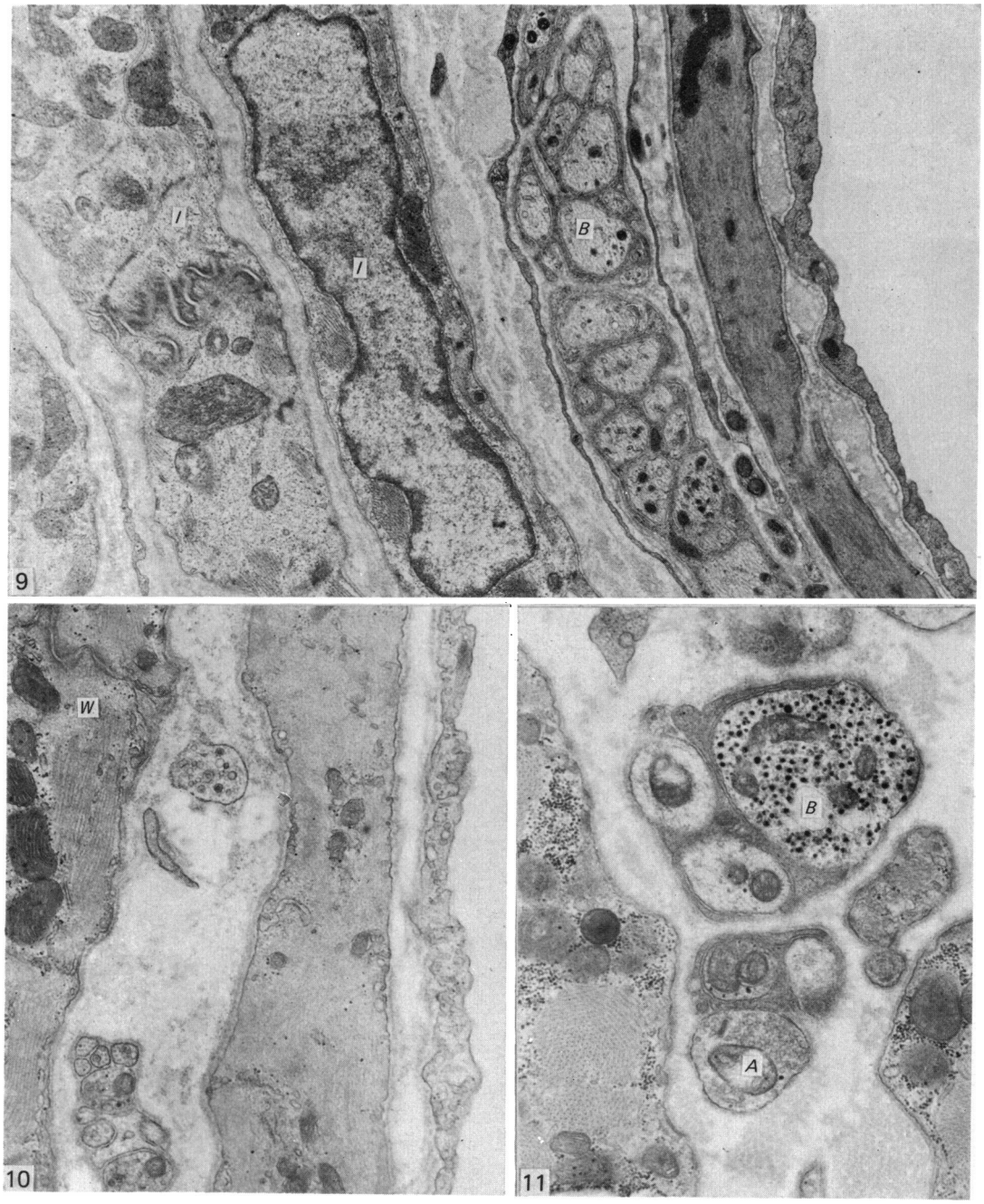

Fig. 9. A peripheral part of the nodal artery. The presence of intermediate type cells (*I*) indicates that this part of the artery is still within the node. However, the cuff is discontinuous in this area. Increasingly, large nerve bundles (*B*) area a feature as the edge of the node is approached. ×10344.

Fig. 10. A typical atrial muscle cell (*W*) is seen adjacent to the media of the nodal artery at the periphery of the node. No cuff is visible but a naked axon is seen in the thin adventitia. ×25650.

Fig. 11. Two adventitial nerves are seen in the central portion of the cuff after treatment with 5-OHDA. One (*A*) contains agranular vesicles and the second (*B*) contains vesicles with small very dense black cores. ×25650.

The nodal artery is derived from the internal thoracic artery, runs centrally through the node and supplies it by several branches. An extensive cuff of nodal cells is seen within the adventitia of the artery and its branches adjacent to the smooth muscle cells of the media. The cuff cells vary in both shape and myofibrillar content and contain acetylcholinesterase. The cuff is extensively innervated by both adrenergic and cholinergic axons.

The significance of these findings with regard to earlier morphological and physiological reports is discussed.

I am indebted to C. G. T. Watterson for his excellent technical assistance. This work was supported by the Atkinson Charitable Foundation and the Medical Research Council of Canada.

REFERENCES

BROOKS, C. McC. & LU, H. H. (1972). *The Sinoatrial Pacemaker of the Heart*. Springfield: Thomas.

CARBONELL, L. M. (1956). Esterases of the conductive system of the heart. *Journal of Histochemistry and Cytochemistry* **4**, 87–95.

HALPERN, M. H. (1955). The sinoatrial node of the rat heart. *Anatomical Record* **123**, 425–434.

HASHIMOTO, K., TANAKA, S., HIRATA, M. & CHIBA, S. (1967). Responses of the sinoatrial node to change in pressure in the sinus node artery. *Circulation Research* **21**, 297–304.

JAMES, T. N. (1962). Anatomy of the sinus node of the dog. *Anatomical Record* **143**, 251–266.

JAMES, T. N. (1965). Anatomy of the sinus node, AV node, and os cordis of the beef heart. *Anatomical Record* **153**, 361–372.

JAMES, T. N. (1967). Pulse and impulse in the sinus node. *Henry Ford Hospital Medical Bulletin* **15**, 275–299.

JAMES, T. N. (1977). The sinus node. *American Journal of Cardiology* **40**, 965–986.

JAMES, T. N. & NADEAU, R. A. (1963). Sinus bradycardia during injections directly into the sinus node artery. *American Journal of Physiology* **204**, 9–15.

JAMES, T. N. & NADEAU, R. A. (1964). The effects of vagal stimulation, eserine and atropine on retrograde pressure in the sinus node artery. *Henry Ford Hospital Medical Bulletin* **12**, 23–36.

KEITH, A. & FLACK, M. (1906). The form and nature of the muscular connections between the primary divisions of the vertebrate heart. *Journal of Anatomy and Physiology* **41**, 172–189.

MEDNICK, M. L., PETRALI, J. P., THOMAS, N. C., STERNBERGER, L. A., PLAPINGER, R. E., DAVIS, D. A., WASSERKRUG, H. L. & SELIGMAN, A. M. (1971). Localization of acetylcholinesterase via production of osmiophilic polymers. New benzenediazonium salts with thiolacetate functions. *Journal of Histochemistry and Cytochemistry* **19**, 155–160.

MERRILLEES, N. C. R. (1974). The fine structure of the sinus node in the rat. *Advances in Cardiology* **12**, 34–44.

NAVARATNAM, V. & PALKAMA, A. (1966). Cholinesterases in the walls of the great arterial trunks and coronary arteries. *Acta anatomica* **63**, 445–448.

PATHAK, C. L. (1973). Autoregulation of chronotropic response of the heart through pacemaker stretch. *Cardiology* **58**, 45–64.

RYBACK, R. & MIZERES, N. J. (1965). The sinus node artery in man. *Anatomical Record* **153**, 23–30.

SÖDERSTRÖM, N. (1958). Myocardial infarction and mural thrombosis in the atria of the heart. *Acta medica scandinavica Suppl.* **217**, 1–114.

TAYLOR, I. M. (1977). The relationship between innervation and the cholinesterases of the rat atrioventricular node. *Journal of Histochemistry and Cytochemistry* **25**, 21–26.