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Morphology of Cardiac Nerves in Experimental Infarction of Rat Hearts

II. Electron Microscopical Findings *

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Summary. Alterations of cardiac nerves in myocardial infarction were investigated by electron microscopy after differing intervals in 28 rats. During the first 4 h there are, in non-myelinated nerves within the myocardium, a swelling of the axoplasm with the occurrence of 'pale' axons and swelling of axonal mitochondria and neurosecretory granules. After bursting of the axolemma, these are spilled into the adjacent interstitial space. After 4 h first myelin figures are observed, and in some axons an accumulation of neurofilaments takes place. During the second to seventh day an extensive vesicular disintegration of axonal structures develops. Because of regressive changes, axons cannot be identified with certainty within the necrosis. After two or three weeks nerves with lamellar enfoldings of cytoplasmic processes corresponding to Büngner bands can be seen at the infarction border. These nerves may contain only a few residual axons. Myelinated nerves show a mainly vesicular disintegration. The results are discussed with regard to their functional significance and the special conditions of the animal model, in which ligature of the coronary artery may not only produce ischemia, but may also, by simultaneous ligature of the adjacent cardiac nerves, induce Wallerian degeneration.

Key words: Myocardial infarction – Autonomic nerves – Electron microscopy – Rat experiments.

Introduction

Following ultrastructural and functional studies of experimental infarction, surviving Purkinje fibres have been thought to be of significance in postinfarction

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arrhythmogenesis (Friedman et al., 1975). By histochemical techniques, however, it has been shown that regressive changes take place in the intramyocardial adrenergic cardiac nerves, with consequent liberation of neurotransmitters (Borchard and Paessens, 1977; Paessens and Borchard, in press). These neural catecholamines may reach the adjacent myocardium by diffusion and may act locally on the contractility, fibrillation threshold and microcirculation (Paessens and Borchard, in press). To our knowledge, there is no information on ultrastructural changes of nerves in the necrosis of cardiac infarction. Degenerating nerves have usually been studied after trans-section of isolated motor nerves and the resultant changes are now quite well known. Ultrastructural alterations of autonomic nerves have been reported only after the neural trans-section or excision of ganglia in several peripheral organs in different species (van Orden et al., 1967; Pitha, 1969; Roth and Richardson, 1969; Iwayama, 1970; Csillik and Knyihár, 1970; Knoche and Terwort, 1973). In the present study the ultrastructural degenerative changes of nerves of the rat heart after coronary ligature are described.

Materials and Methods

In 131 rats a standardized myocardial infarction of the anterior wall of the heart was produced by ligation of the left coronary artery according to Bajusz (1967). Details of the experimental procedure are given in the preceeding paper (Paessens and Borchard, in press). Twenty-eight out of 65 surviving rats were investigated by electron microscopy. After survival periods between 1 h and 8 weeks the rats were killed by cervical dislocation. Since the area of the infarction would not have been infiltrated by perfusion fixation, the tissue of the infarction and adjacent parts were immediately fixed by immersion in glutaraldehyde phosphate buffer. This was followed by postfixation with 1% buffered osmiumtetroxide. After dehydration and embedding in metracrylate the blocks were cut and contrasted with lead hydroxide and uranyl acetate. Sections were examined with the Elmiscope 101 (Siemens). During the investigation of the infarcted area nerves were only studied when typical regressive changes in the adjacent myocardium were present.

Results

During the first 4 h after coronary ligation regressive changes of the autonomic cardiac nerves in the centre and at the border of the ischemic zone usually appear later than those in the infarcted myocardium (Fig. 1). After 3 h definite changes of Schwann cells can be detected in form of a hydropic swelling of the cytoplasm (Fig. 2a). After 4 h, there are also alterations of the axoplasm (Fig. 2b, c): The structure of the axonal vesicles may show a loss of granular cores and there are indistinct vesicular membranes which flow together. The mitochondria sometimes show myelin figures (Fig. 3) and swelling of the matrix. Up to the sixth hour after ligation there is an irregular ballooning of some neurosecretory vesicles. The axolemma may burst and some neurosecretory vesicles may be spilled into the adjacent interstitial space (Fig. 2c). In some nerves there is an axonal swelling with an accumulation of neurofilamentous structures in the axoplasm (Fig. 4). After 6 h the hydropic swelling of the axons

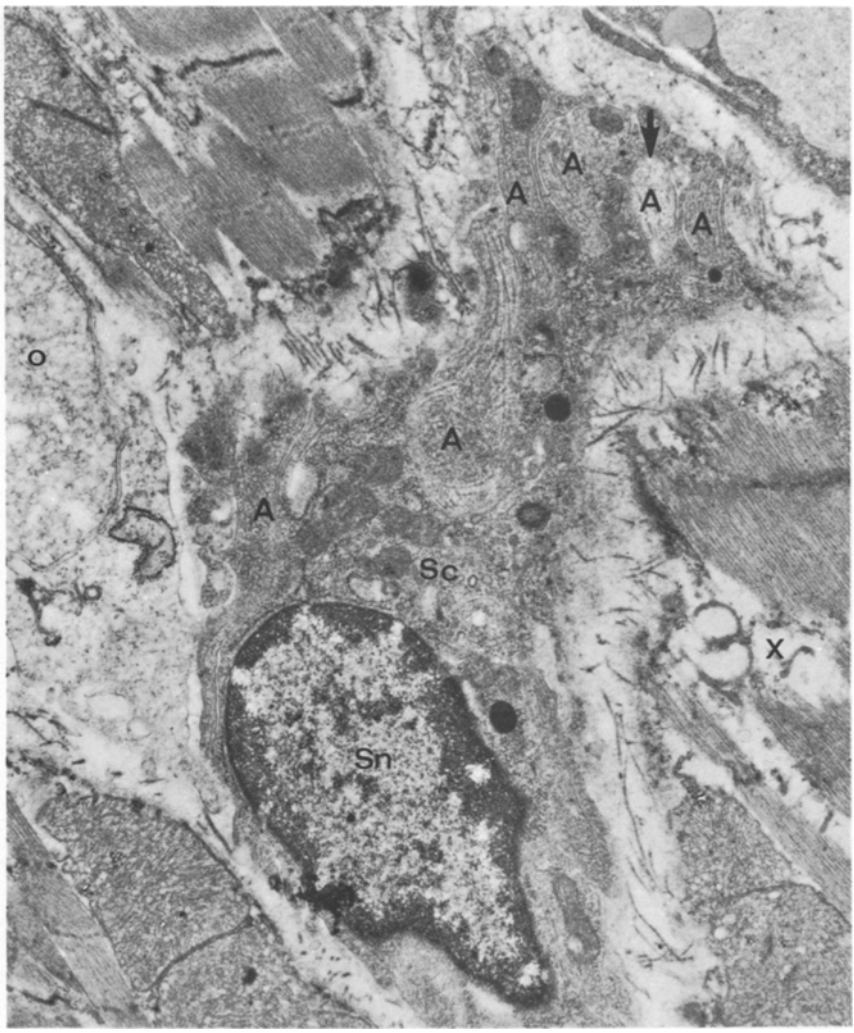
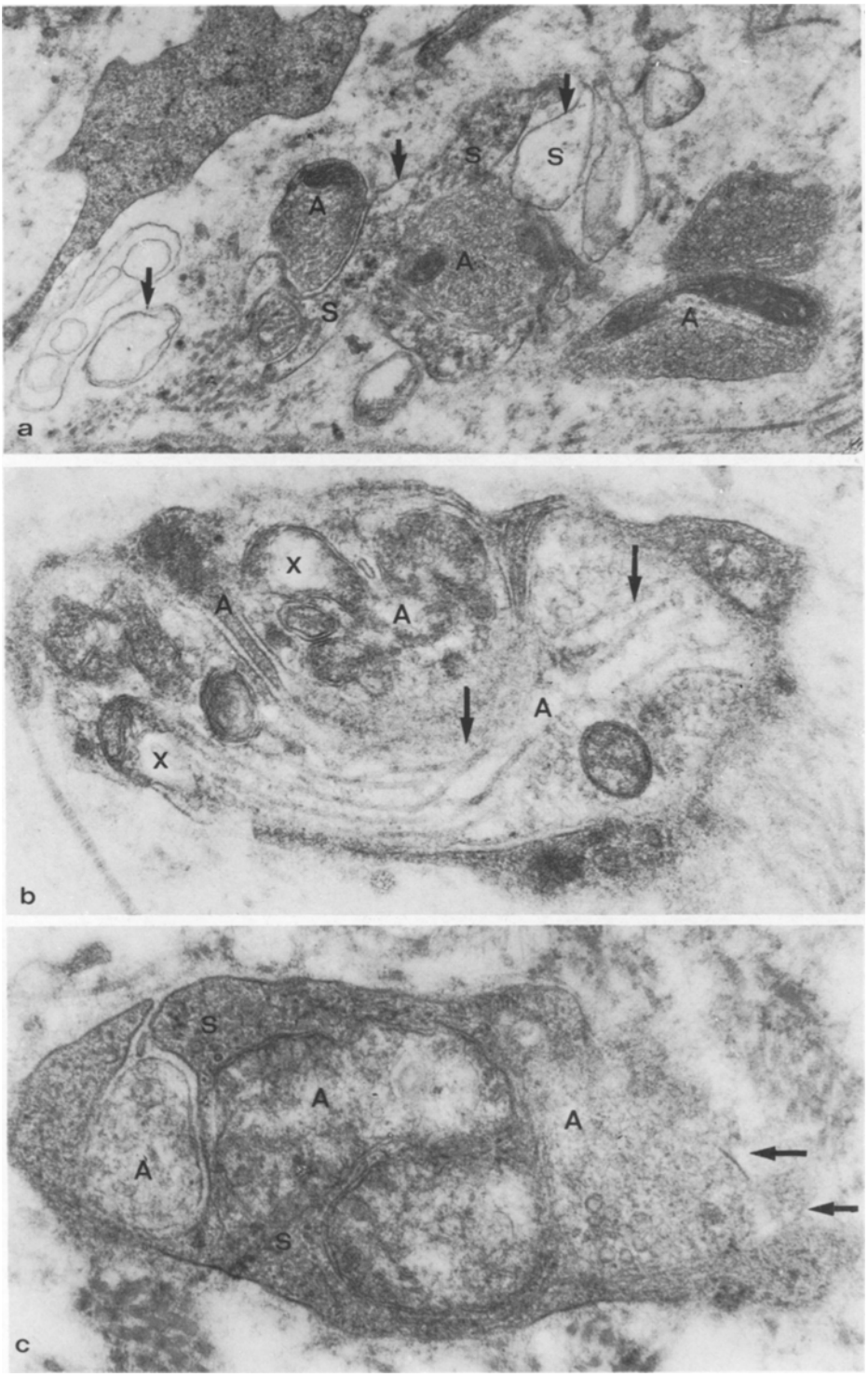


Fig. 1. Cardiac nerves 3 h after coronary ligature. Regressive changes of the cardiocates with oedema of the sarcoplasm (*O*), swelling of mitochondria and rupture of myofibrils (*X*). Fairly intact structure of the axons (*A*) with focal oedema (/). Nucleus (*SN*) and cytoplasm of the Schwann cell (*Sc*) without major alterations. (5,000:1)

increases while the membranes of the axolemmas are still intact. One day after the ligature there may be an accumulation of myelin figures marking the place of the axon (Fig. 3b). These processes finally result in total axolysis after some time.

In the debris of the infarction during the first to the seventh day, several vesicular structures resembling nerves were seen that could no longer be identified as nerves with certainty. During the formation of the scar there are only a few surviving nerves with swollen axons. The cytoplasm of the Schwann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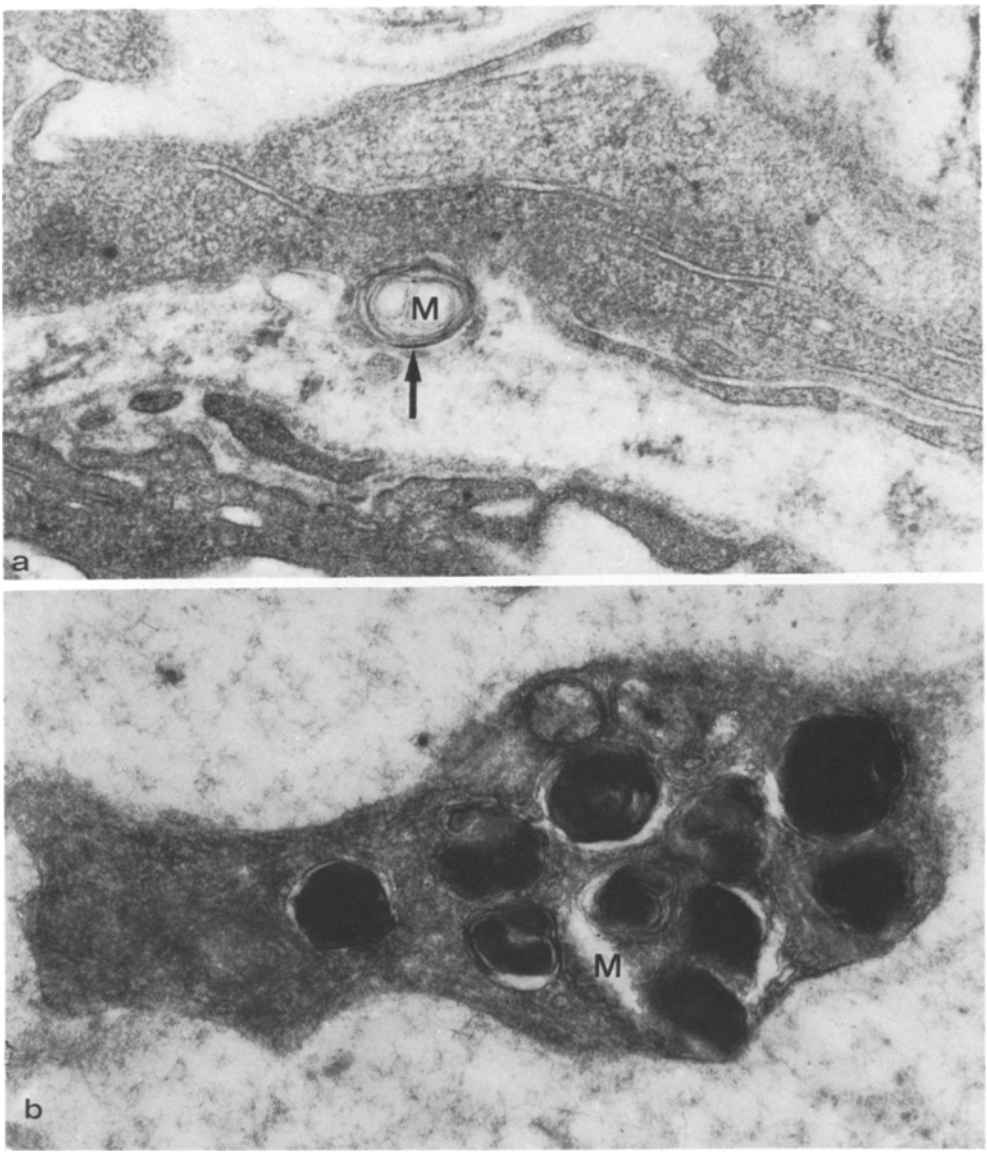
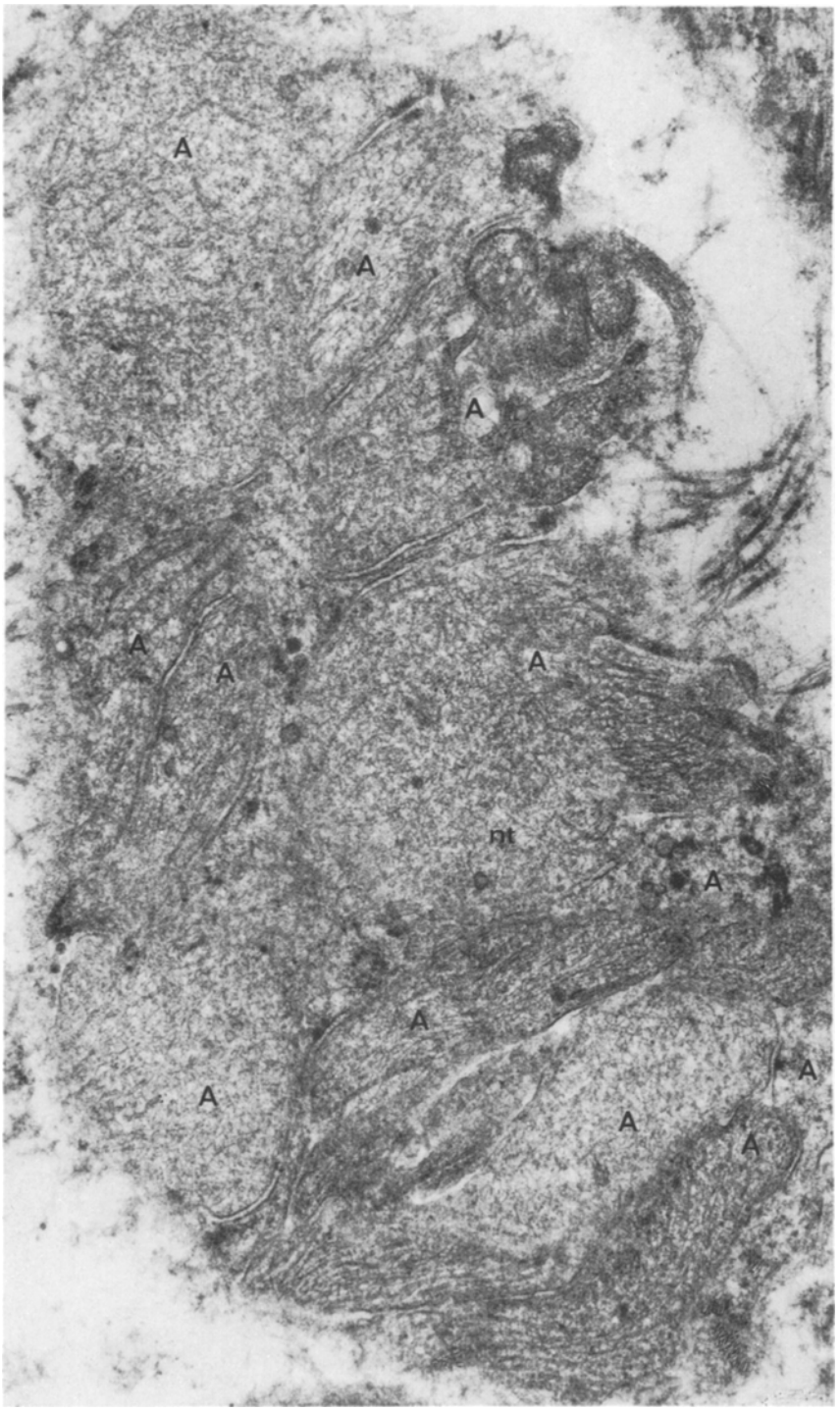


Fig. 3. a Autonomic cardiac nerve in the area of infarction with initial formation of myelin figures (M) 4 h after coronary ligature. (3,600:1). **b** Dense accumulation of myelin figures 12 h after coronary ligature. (3,600:1)

Fig. 2. a Autonomic cardiac nerve 3 h after coronary ligature. Oedematous swelling (/) of the cytoplasm of the Schwann cell (*S*). Axons (*A*) still normal. (24,000:1). **b** Axonal oedema and swelling of the matrix of axonal mitochondria. (40,800:1). **c** Rupture of the axolemma with discharge of neurosecretory vesicles into the adjacent interstitial space (/). (40,800:1)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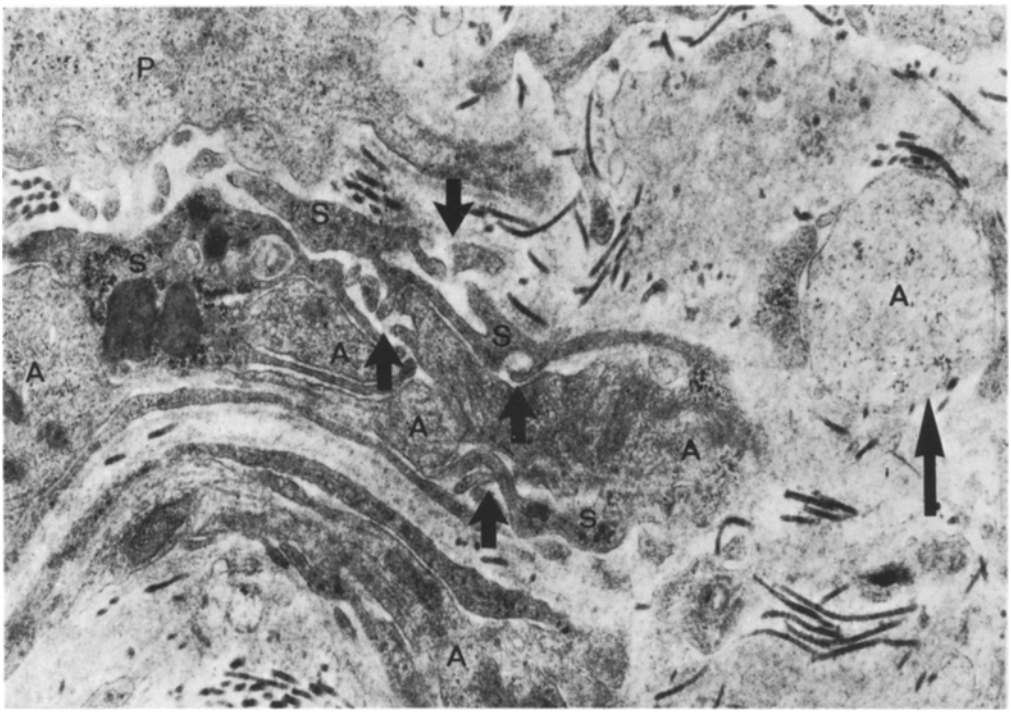

Fig. 5. Autonomic cardiac nerve 2 weeks after coronary ligature. The cytoplasm of Schwann cell show two lipid droplets (*S*) and a beginning enfolding of cytoplasm process (*I*). One swollen axon seems to be surrounded by cytoplasm of a macrophage (*P*). (7,800:1)

cells shows an increasing enfolding of plate-like cytoplasmic processes (Fig. 5). Some degenerating nerve fibres are removed by macrophagocytes.

After 2 weeks only few nerves can be seen at the border of the scar tissue. They may be identified by their surrounding basal membranes (Fig. 6). These nerves contain sometimes more than twenty profiles of small, round or ovoid, sometimes elongated cytoplasmic processes which are usually of Schwann cell origin and sometimes appear to correspond to axonal processes (Fig. 6a). There were also nerves with few surviving axon profiles and multiple invaginations and enfoldings of the cytoplasm of the Schwann cells resulting in stacks of Schwann cell processes (Fig. 6b). Larger nerve trunks in the periphery of the infarction show an oedematous swelling of the axoplasm. There was sometimes an accumulation of vesicular myelin-like and tubular structures (Fig. 7).

Fig. 4. Autonomic cardiac nerve 4 h after ligature; accumulation of neurofilaments and neurotubular structures in enlarged axons (*A*). (28,800:1)

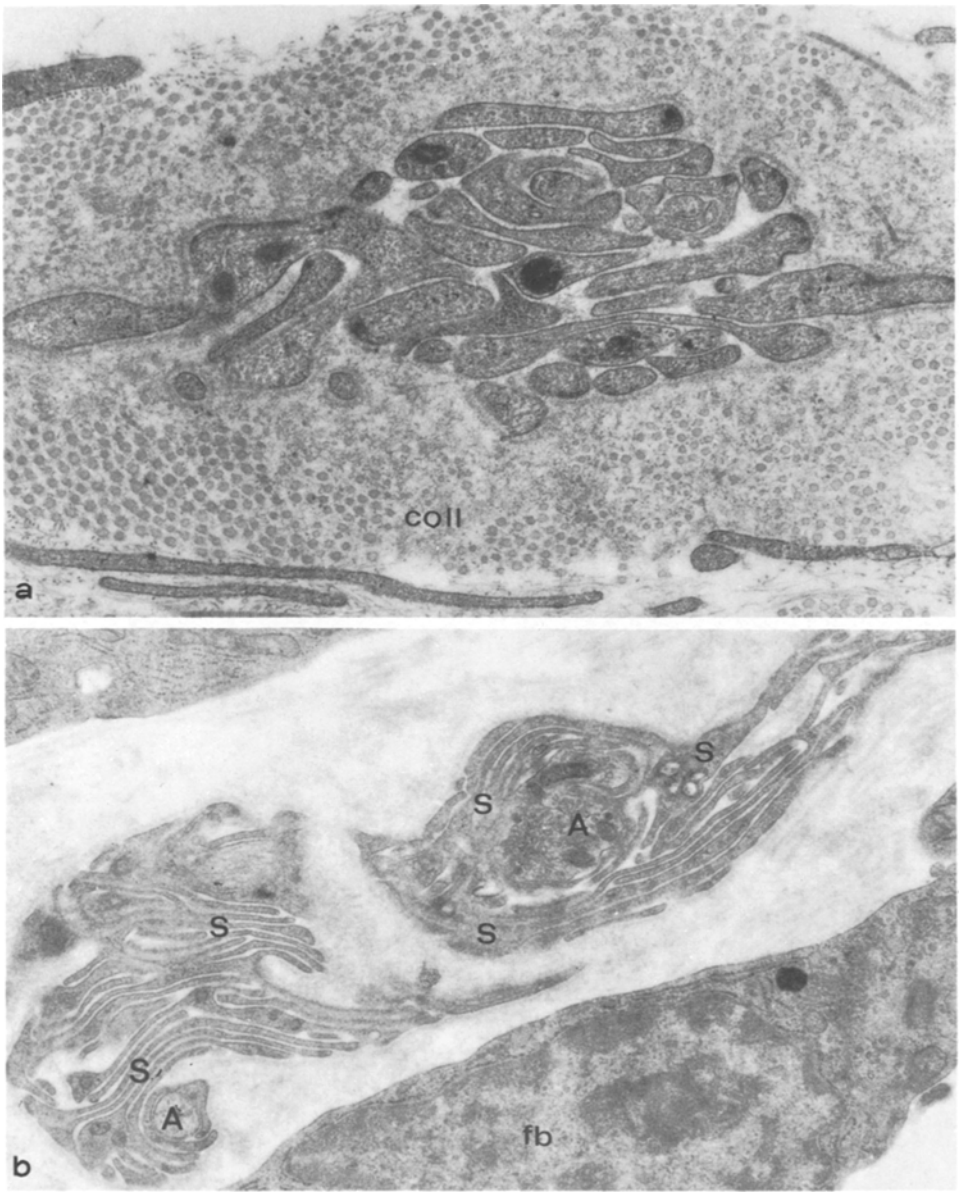

Fig. 6.a Cardiac nerves in the scar tissue of the infarction. Eight weeks after coronary ligature an atrophic axon (*A*) surrounded only by a basal membrane lying in a collagenous scar tissue (*coll*). (7,800:1). **b** Nerve profile with single axons (A) surrounded by multiple membraneous enfoldings. (5,500:1)

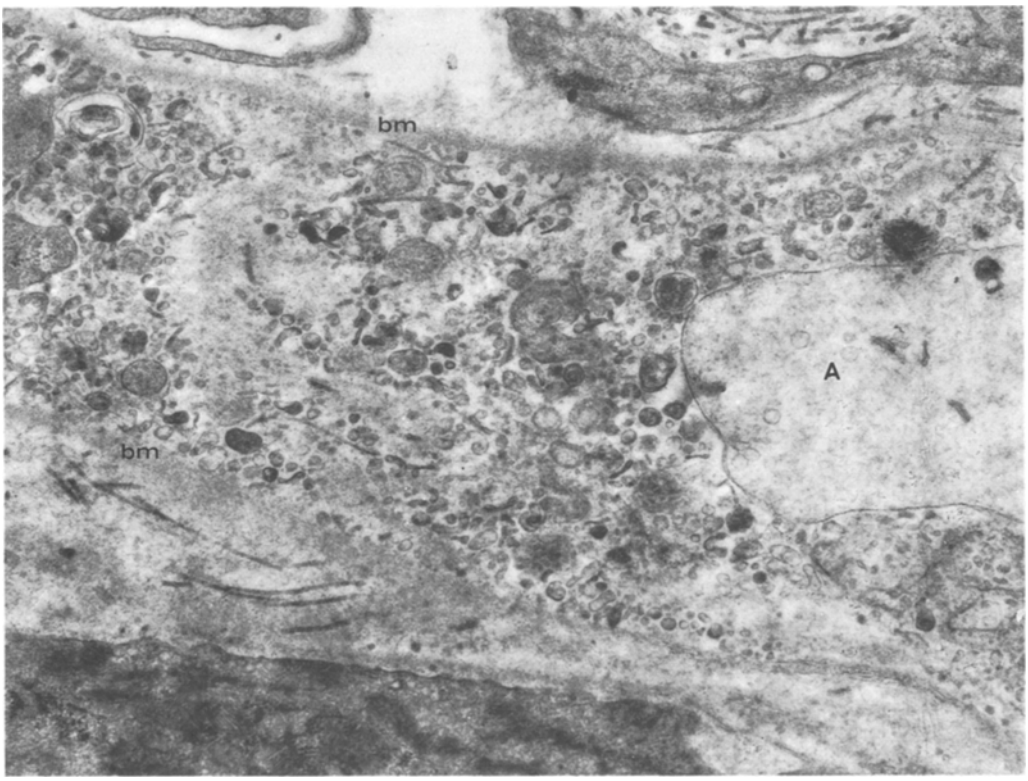

Fig. 7. Large nerve trunk at the border of the infarction, eight weeks after the cardiac ligature. A preserved basal membrane (*bm*) shows multiple lysosomal particles and one swollen axon. (10,000:1)

Discussion

There are numerous studies of the ultrastructure of the normal vertebrate cardiac nerves (Fawcett and Selby, 1968; Kisch, 1958; Viragh and Porte, 1961; Kawamura, 1961; Maekawa et al., 1967; Hadek and Talso, 1967; Yamauchi, 1969; Thaemert et al., 1969; Hayashi et al., 1970; Nilsson and Sporrong, 1970; Chiba and Yamachi, 1970; ref.: van der Zypen, 1974, and Borchard, 1978). Degenerative changes in cardiac nerves were described electron microscopically in few human hearts (Borchard, 1975; Kyösola, 1976; Borchard, 1978). To our knowledge, the process of axonal degeneration after denervation, infarction or transplantation in myocardium has not previously been investigated by electron microscopy, apart from Potter and co-workers (1965) who stated that after denervation and transplantation there were no axonal degenerative changes. However, degenerative changes in form of a transmitter loss have been noted after surgical cardiac neural ablation by fluorescence histochemical techniques (Falck-1962; Malmfors and Sachs, 1965). From these studies it was not evident

whether the observed loss of catecholamines was due to a destruction of the axons. From other studies such a degeneration would be expected after denervation of autonomic nerves in various organs (Pitha, 1969; van Orden et al., 1967; Blümcke et al., 1969; Roth and Richardson, 1969; Iwayama, 1970; Knoche and Terwort, 1973).

In the infarction zone there are other conditions for autonomic axonal degeneration than in Wallerian degeneration in so far as not only the nerve process is primarily subjected to degeneration, but also the whole environment including the Schwann cell envelope. According to our results it must be assumed that dissociated survival of either axonal processes or Schwann cells does occur in the infarcted area. In our experiment the small axons, after a short period, show alterations which are similar to 'light', 'pale' or 'watery' axons in experimental Wallerian degeneration (Bray et al., 1972; Dyck and Hopkins, 1972; Knoche and Terwort, 1973). We are aware that conventional fixation might contribute to such changes (Kinley and Usherwood, 1978). There is also a swelling of axonal organelles, especially of mitochondria, which was found by several authors. Although in the initial stage of electron microscopy these changes were interpreted as fixation artefacts (Wechsler and Hager, 1962; Webster, 1962; Lee, 1963), others regarded them as typical for the initial stage of degeneration (Knoche and Terwort, 1973). In degenerating axons a swelling of axonal vesicles was also found (Lloret and Saavedra, 1975; Borchard, 1978). These changes are due to a disturbance of the energy dependent Na^+-pump in the region of the axolemma which also affects the ischemic myocardial cell (Trump, 1976). For the neurons this means an increased water uptake following the inflow of Na^+-ions, finally resulting in the rupture of the axolemma. Similar extrusions of neurovesicles have been described by Grillo (1970) who considered them to be artifactual or to be a special mode of neurosecretion. In our experiment, where other cells also burst from osmotic pressure, the latter explanation can be excluded. It is reasonable to assume that neurosecretory granules thus reach the adjacent interstitial space and muscles. We observed early formation of vesicular structures corresponding to myelin figures also described by Webster (1962). In the first hours after infarction there was another peculiar change, a dilatation of axons by vesicular and neurofilamentous material which resembles the disintegrated neurofilamentous structures described by Causey and Hoffman (1955), Vial (1958), and Lee (1963). This phenomenon may be due to disturbed axonal cytoplasmic flow, which may follow proximal ligature.

The alterations in the bigger nerve trunks in the scar of the infarction are similar to those which occur in the degeneration of motor nerves. The myelin structures represent a mainly vesicular disintegration of myelin and axonal organelles (Blümcke et al., 1966; Kapeller and Mayor, 1966; Morris et al., 1972; Bray et al., 1972). In the cytoplasm of some Schwann cells there were several lipid droplets possibly resulting from catabolism of engulfed and destroyed axonal membranes (O'Daly and Imaeda, 1967). We finally found enfoldings of the cytoplasmic membranes of Schwann cells that corresponded to Büngner's bands. Similar findings have been described in the periphery of degenerate motor nerves (Nathaniel and Pease, 1963; Lee, 1963; Dyck and Hopkins, 1972; Weller and Cervós-Navarro, 1977).

Whether the lesions of bigger nerve trunks are due to ischemia is a problem which has to be judged with care, since the mechanical effects of the ligature (Kapeller and Mayor, 1966) and additional effects due to Wallerian degeneration have to be taken into account. Since degenerative changes due to Wallerian degeneration are not usually complete for 48 h, the early changes, especially in smaller nerve processes, are probably due to ischemia. However, the sequelae of ischemia on one hand and Wallerian degeneration on the other cannot be separated in an experiment with ligature of the cardiac arteries and simultaneous ligature of nerves with consequent Wallerian degeneration.

In conclusion our findings show that there is ultrastructurally observable damage to the cardiac nerves in experimental heart infarction, which correlated with the histochemical alterations described earlier (Borchard and Paessens, 1977; Paessens and Borchard, in press). These changes may induce arrhythmias in the early stage of infarction. In later stages, the alterations do not always result in disintegration of neuronal structures in the infarcted area, which has been postulated after light microscopic investigations (Hirsch, 1970),, but single axons or Schwann cells may survive, mainly at the border of the infarction but within the scar. These surviving structures show a reduced transmitter content as demonstrated in additional histochemical studies (Borchard and Paessens, 1977). Scarring of myocardial tissue has been related to functional impairment of transmitter release and diffusion to the myocardiocytes (Borchard, 1978). Moreover, the degeneration of nerves within the scar of infarction may also lead to a disturbance or loss of neural acitivty distal to the scar.

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