Virchows Arch. A Path. Anat. and Histol. 386, 265–278 (1980)

Virchows Archiv A

Morphology of Cardiac Nerves in Experimental Infarction of Rat Hearts

I. Fluorescence Microscopical Findings *

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Summary. The effect of ischemia on the catecholamine content and morphology of autonomic cardiac nerves was studied in experimental myocardial infarction in 65 rats with coronary ligation. The area of infarction, of ligation and other regions of the heart were investigated at hourly and daily intervals up to 9 weeks after ligation. During the first 4 h there is a slight reduction of fluorescence in the area of infarction and a focal, inconstant diffusion of adrenergic transmitters shown by Falck method. After 4 h an increasing loss of catecholamines is observed while the fluorescence of the adrenergic nerves in the adjacent myocardium is maintained or even increased. After 8 h the fluorescence of terminal nerve fibres has decreased; such changes also take place in bigger perivascular nerve trunks. After 16 h specifically fluorescent nerves can be identified only weakly. After two days catecholamine fluorescence has faded. The serotonin fluorescence of mast cells is constant except for focal degranulation. In later stages non-specific fluorescence is caused by fatty degeneration of myocardial cells and lipid-laden phagocytes. The results are discussed in respect to the local effects of catecholamines liberated during the early phase of infarction.

Key words: Myocardial infarction – Rat experiments – Transmitter fluorescence – Adrenergic nerves – Mast cells.

Introduction

Many investigators after Goodall (1951) and von Euler (1956) have shown that the heart has a high concentration of catecholamines (ref.: Holzbauer and Sharman, 1971; Borchard, 1978). The distribution of adrenergic nerves

* With support by the DFG, SFB 30 Cardiology, Project E 6/14

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0340-1227/80/0386/0265/$02.80

in the heart has repeatedly been investigated by the Falck method (Ehinger et al., 1966; Jakobowitz, 1967; Schiebler und Heene, 1968; Laties et al., 1967; ref.: Borchard, 1978).

By morphometric analysis a statistically significant correlation was shown between the density of the adrenergic ground plexus and the catecholamine concentration (Borchard, 1978). It was also shown by these investigations that the so-called specific atrial granules contain no catecholamines. In hypertrophic human hearts apart from a distension of the ground plexus, there is a reduction of specific fluorescence and of catecholamine concentration which cannot be satisfactorily explained (Borchard, 1975). As hypoxia was assumed to be a main pathogenetic factor, we investigated the result of local ischemia on the adrenergic nerves of the rat heart using the model of experimental myocardial infarction. In the course of the studies we realized that the catecholamines liberated during the early phase of the infarction seemed to be of functional significance e.g. for arrhythmogenesis. – In this study, the light microscopical and fluorescence microscopical alterations of the nerves and the serotonin containing mast cells in the area of experimental infarction are reported. The electron microscopical findings are reported in another paper (Borchard and Paessens, 1980).

Material and Methods

In 131 female Wistar-rats with a medium body weight of about 200 grammes, a standardized infarction of the anterior wall of the left ventricle was produced by the method of Bajusz (1967): under light ether narcosis a left paramedian thoracotomy was performed. The left (descending) coronary artery was ligated between the pulmonary cone and the left auricle by atraumatic silk size 07. The thorax was closed by sutures and the pneumothorax was removed by pressure on the thorax. The skin was then closed with Michaelis-clamps. Animals that died spontaneously during operation (e.g., from bleeding or shock) were excluded from the investigation. The surviving animals received water and standard food ad libitum. The animals were allowed to survive as listed below:

Interval	1/2	1	2	3	4	5	6	8	9	10	16	20
	Number of experimental animals:											
Hours	2	8	6	6	5	–	2	2	–	2	2	2
Days	–	4	2	2	2	2	1	–	–	–	–	–
Weeks	–	1	4	3	2	–	2	2	1	–	–	–

After these periods they were killed by cervical dislocation. After opening of the thorax, the vena cava inferior was pinched off in order to prevent terminal inflow of adrenal catecholamines. In preliminary experiments the extension of the infarction was checked by the acridine orange method (Schümmelfeder, 1950; Hecht et al., 1961). Because of interference of the acridine orange fluorescence with the catecholamine fluorescence, this method was changed in other preliminary experiments in favour of the Indian ink injection method (Brumschtein et al., 1966; Camilleri, 1976) which, however gave variable results. The establishment of the border of the infarcted area during the first hours after ligation proved to be very difficult. Later on, during this period the border of the tissue investigated for fluorescence microscopy was controlled by electron microscopy.

For fluorescence microscopical vizualization of the adrenergic nerves the method of Falck (1962) was used. Tissue blocks of 2 mm were placed in open copper containers precooled in liquid nitrogen and transferred to the cooling device of a freeze dryer (Leybold-Heraeus GT 1)

in combination with a gas ballast vacuum pump (V 3900, Leybold-Hereaus). On the first day the cooling temperature remained at 40° C, on the following at −30° C. The samples were freeze dried for 3–5 days and incubated in hot paraformaldehyde vapours of 80° C at 65–70% relative humidity and finally embedded in degassed paraffin. 5 µm serial sections were mounted in a xylene-Entellan mixture and immediately examined under a Orthoplan-microscope (Leitz) converted for transmitted and reflected light. The standardized filter combination was 2 × BG3 and S 405 (Leitz) as exciter and one K 470 or 490 as secondary filters. Photographies were taken on Highspeed or Ektachrom 135-20 (160 Tungsten) 23 DIN (For further details see Borchard, 1978).

Fluorescence intensity was judged in comparison with hearts from control rats semiquantitively (+to+++).

Results

A. Postoperative Mortality and Behaviour

The intra- and immediately post-operative mortality was 51% on the average, being higher in the beginning and lower at the end of the experiment. In the post-operative period the surviving animals sat crooled and showed signs of increased sympathetic activity with bristled neck hair and slight exophthalmus. These signs decreased within several hours up to two days in the longest.

B. Light Microscopic Investigations

After one hour there is patchy perivascular oedema and little diapedesis of leucocytes. Some myocardial fibres show slight acidophilic changes and vacuolations. The intramyocardial nerves have not altered. After 2 h there is a slight separation of myocardial cells by increased interstitial oedema. The myocardial cells show focal acidophilic changes, extensions of sarcomes and a formation of hyaline bands. Small, partly dilated vessels at the marginal zone of the infarcted area show regressive changes with swelling of the endothelial cells. There is an increase in perivascular oedema and a granulocyte emigration has begun. The epicardium shows fresh deposits of fibrin. Small nerves in this area are sometimes surrounded by inflammatory cells. – After 3 h regressive changes have increased considerably. There is a slight reduction of the diameter of muscle cells in the area of infarction. As a part of fresh fibrinous pericarditis, one can find an invasion of the oedematous epicardial nerves by granulocytes (Fig. 1). After 4 h degenerative changes of muscle cells may be fully developed, with partial sarcolysis, a narrowing of muscle fibres and a garland-like swelling of the myofibres in the sarcolemmal tubes may occur. Some small vessels on the border of the infarction and myocardium show thrombotic changes. Mast cells loose granules. There is an increasing oedema and little cellular infiltration of small nerve fibres.

After 6 h the changes described have progressed in the infarcted area. There, the nuclei of muscle fibres have pycnotic and sometimes lytic changes. Some nerves show increasing oedema, and a few pycnotic nuclei can be identified in Schwann cells. – After 8 h there is marked perivascular oedema (Fig. 2).

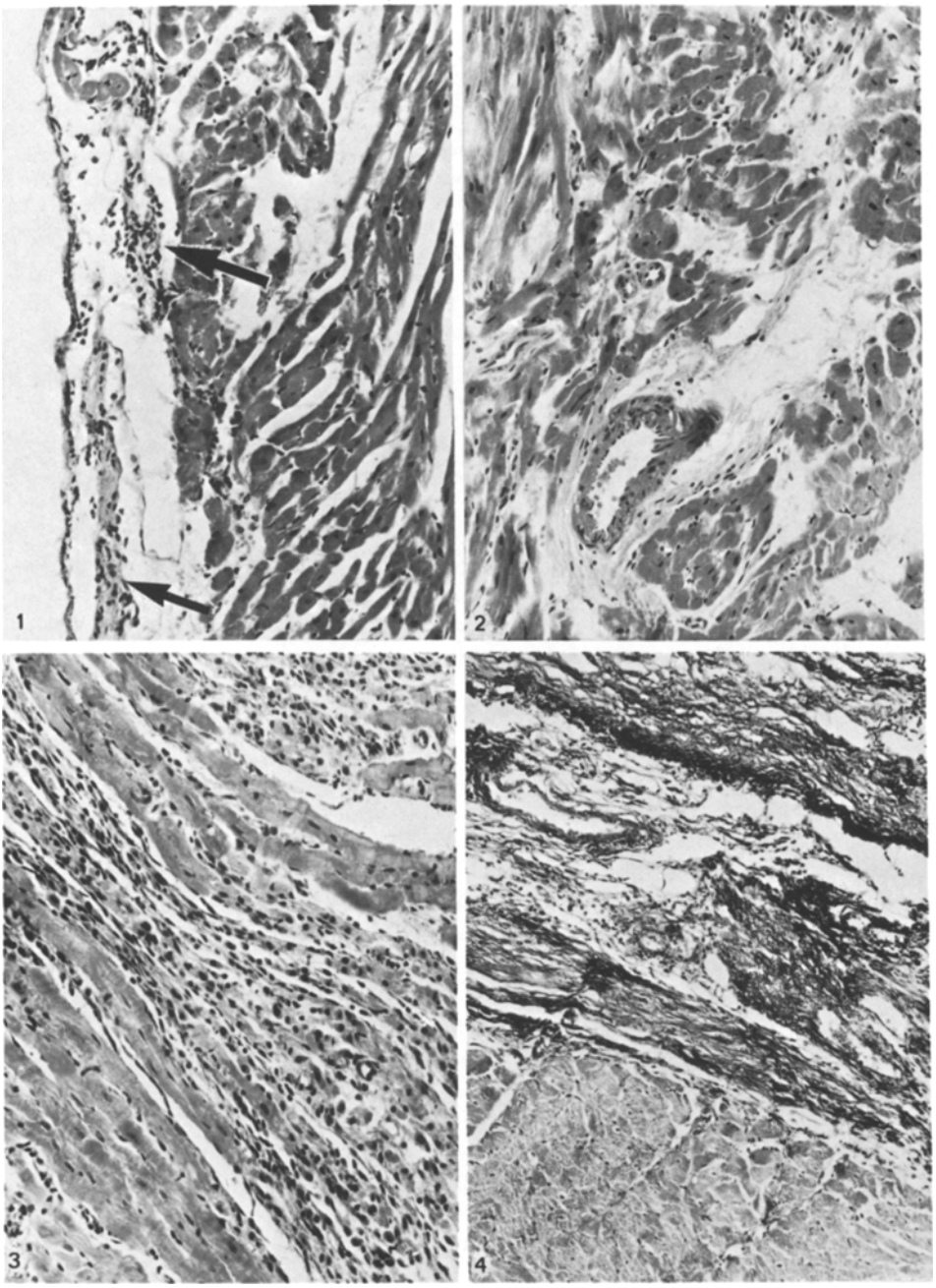


Fig. 1. Rat heart three hours coronary ligature: Infiltration of subepicardial nerves by polymorphonuclear leucocytes (↑↑). (HE 160×)

Fig. 2. Eight hours after coronary ligature: Perivascular edema at the border of the infarction. (HE 160×)

Fig. 3. Three days after coronary ligature: Border of the infarcted area. Dense inflammatory cell infiltration (HE 160×)

Fig. 4. Five weeks after coronary ligature: Scar tissue at the border of the infarction (Elastica-van Gieson 96×)

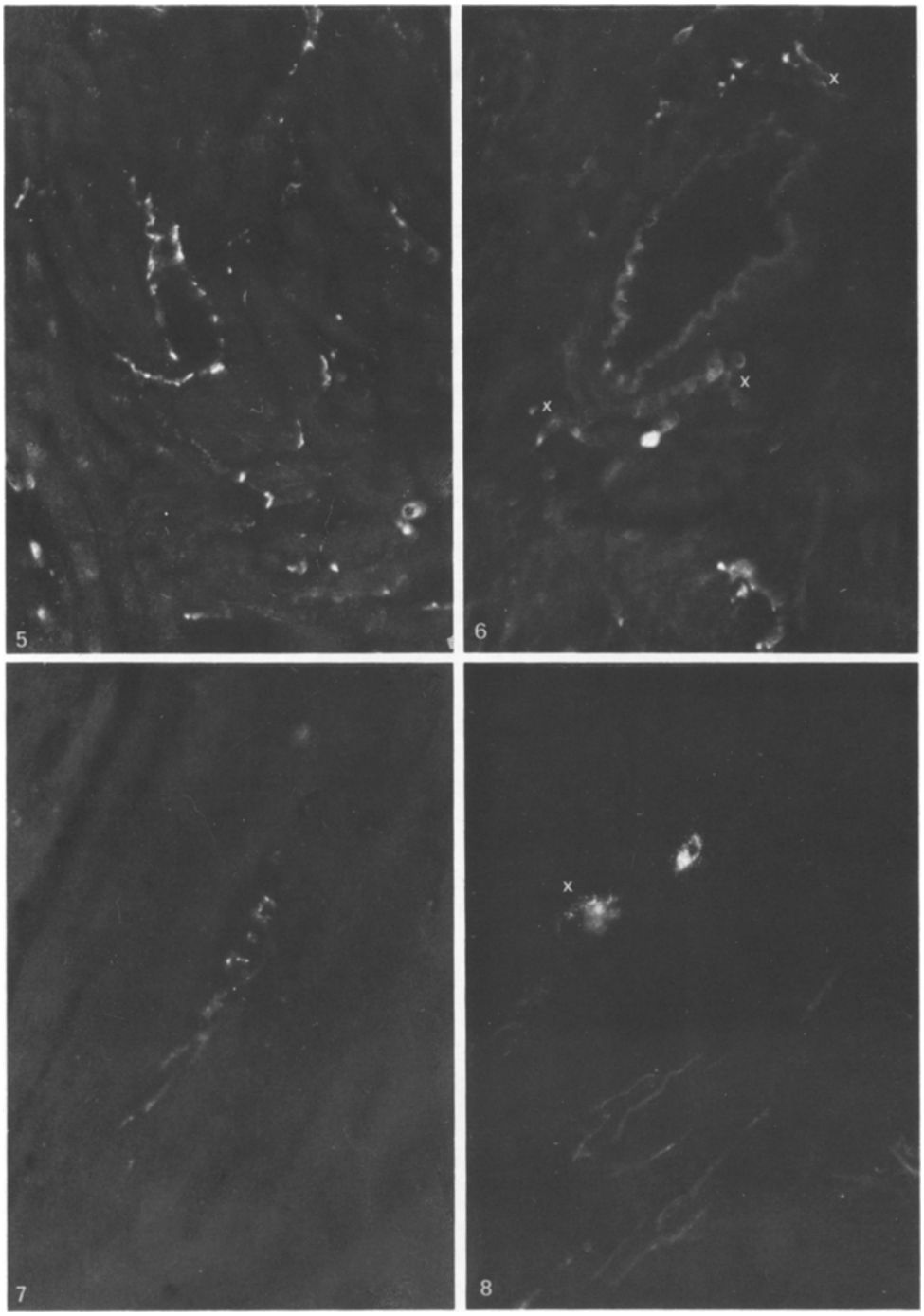


Fig. 5. Normal catecholamine fluorescence in the left ventricle myocardium of a control rat with perivascular fibres from which intramural fibres emerge (centre). Lighter spots correspond to socalled varicosities with a higher content of transmitters. (Falck method: 250×)

Fig. 6. Three hours after coronary ligature. The start of transmitter diffusion (×) from the fluorescing perivascular nerves and a diminution of catecholamine fluorescence (Falck method: 250×)

Fig. 7. Six hours after coronary ligature: Only sparse perivascular and intramural fluorescence of the adrenergic nerve fibres (Falck method: 160×)

Fig. 8. Sixteen hours after coronary ligature: Diffusion of serotonin from mast cells (×) in the border zone of infarction. In the lower part adrenergic nerves can be recognized only as shadows. (Falck method: 250×)

Sometimes one can find the beginning of loss of muscle cells in the infarcted area with patchy granulocytic infiltration. The fibrinous pericarditis has increased. After 16 h a dense granulocytic infiltration was observed in some animals.

By the second day, there is further thinning of the muscle fibres in the infarcted area, and in some animals many macrophages may be seen. Some nerves are split by oedema and infiltrated by inflammatory cells. After three days the infiltration of macrophages has increased considerably (Fig. 3). The empty sarcolemmal tubes collapse and have partly disappeared. Arteries in the area of infarction are thrombosed. In myocardial cells on the border of infarction some large vacuoles can be seen. Nerves can no longer be identified in the infarcted area, even not by silver techniques. Also after the fourth and fifth day, the fatty changes on the border of the infarction are prominent. Macrophages and inflammatory cells are forming a granulation tissue together with capillary sprouts. Reticulin fibres are formed within these areas and there is also a collaps of local reticulin fibres and empty sarcolemmal tubes.

After two weeks more collagenous and elastic fibres have been produced. Nerves cannot be identified by light microscopical methods. After four weeks there is a scar of different cell contents. It consists of collagenous and partly of elastic fibres (Fig. 4). There may be calcifications and cartilagenous changes. The endocardium and a subendocardial muscle layer survive. These findings remain unchanged until the ninth week.

C. Fluorescence Microscopical Findings

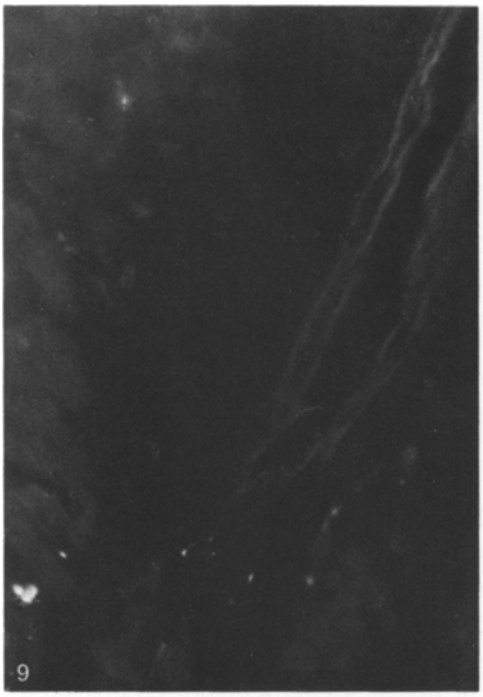
In the control animals, there is a typical distribution of adrenergic cardiac nerves forming a subendocardial, subepicardial and an intramural plexus. In the myocardium the nerves surround great vessels and branch into smaller fibres accompanying the muscle fibres (Fig. 5). These show bright fluorescent swellings, so-called varicosities.

Fig. 9. One and a half day after coronary ligature: Nearly complete loss of catecholamine fluorescence in the area of infarction; in the upper part of the illustration slightly enhanced autofluorescence of the lamina elastica of a larger artery (Falck method: 250×)

Fig. 10. Four days after coronary ligature: Total loss of catecholamine fluorescence with preserved serotonin fluorescence of some mast cells. An ochre to yellow background fluorescence by the onset of fatty degeneration of the myocardial fibres in the border zone of infarction (Falck method: 250×)

Fig. 11. Four weeks after coronary ligature: numerous mast cells with specific serotonin fluorescence and many phagocytes with non-specific yellow fluorescence in the area of infarction (Falck method: 250×)

Fig. 12. Four weeks after coronary ligature: Beginning of non-specific autofluorescence of the collagenous and elastic fibres of the infarction scar in the upper part of the illustration (Falck method: 250×)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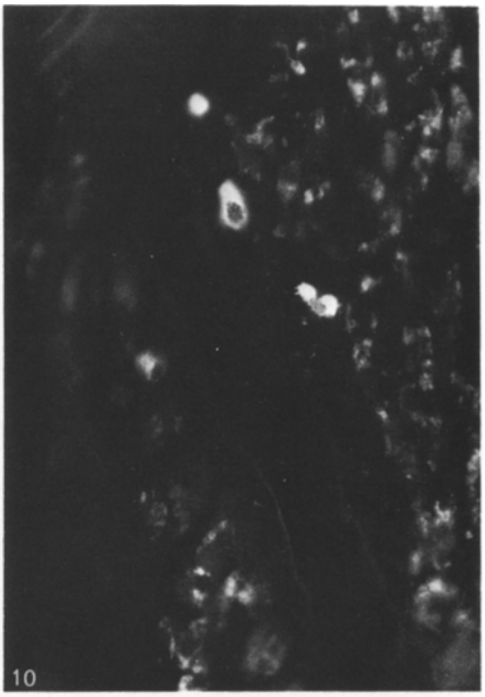
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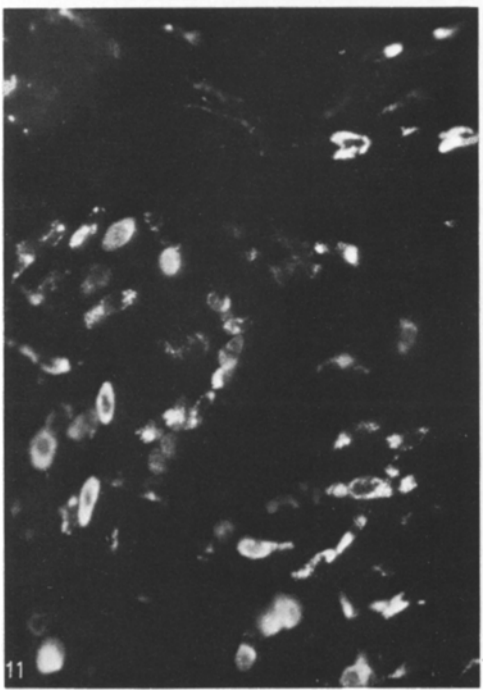
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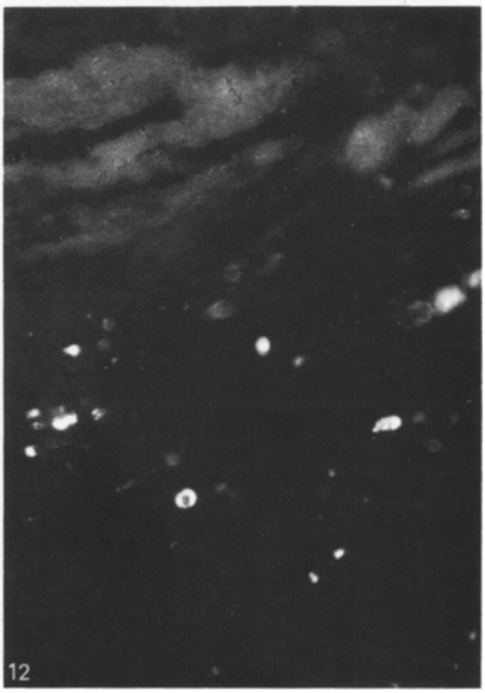
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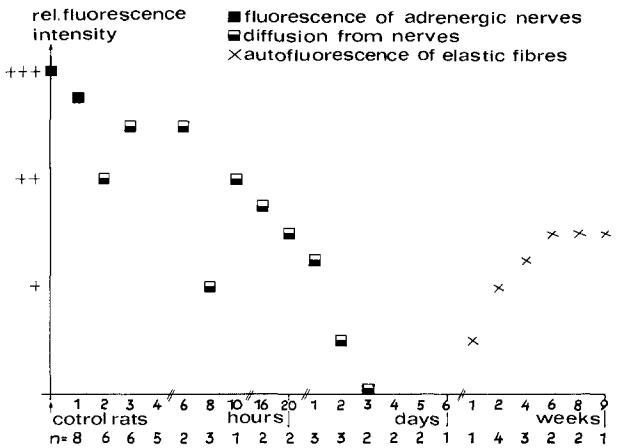

Fig. 13. Fluorescence intensity of adrenergic nerves in experimental myocardial infarction of rats

In the experimental animals there are inconstant changes of the catecholamine fluorescence in the infarcted area three to four hours after ligation. They consist of a slight reduction of the fluorescence and the beginning of diffusion of the transmitter substances (Fig. 6). The adjacent myocardium sometimes shows an increased specific background fluorescence by which non-specific fluorescent structures, e.g., the autofluorescent internal elastic lamina of great arteries may be accentuated (Fig. 6). The diffusion and the loss of catecholamine fluorescence during the first hours do not depend on the site of the tissue sample within the infarcted area, since there may be considerable variations in the loss of catecholamines, especially in the border zone of the infarction. Six hours after ligation the fluorescence of intramural nerves is partly faded, and there is only a little remaining fluorescence of the perivascular nerves (Fig. 7). Occasionally, degranulation of serotonin containing yellowish fluorescing mast cells can be found at the same time (Fig. 8). The serotonin fluorescence of the mast cells, however, is relatively stable compared with the nervous catecholamine fluorescence. Thus mast cells with intact fluorescence may be seen during all intervals in the infarcted area (Figs. 7–12). After one and a half day, but not later than two days, the specific fluorescence of the adrenergic nerves ceases. Only specifically fluorescent mast cells and autofluorescent fibres of the internal and external elastic laminae can be recognized (Fig. 9).

There are additional non-specific fluorescence phenomena in the area of infarction: Three days after ligation at the latest, there occurs an increasing granular fatty degeneration of the myocardial yellowish green serotonin fluorescence of mast cells. The fats are absorbed by phagocytes. After two to three weeks, there are numerous specific fluorescing mast cells between non-specific fluorescing phagocytes and granular deposits of small autofluorescent fat droplets in the interstitial space (Fig. 11). After three to four weeks, during the development of the infarction scar, there is a partly coarse-reticular, partly delicate fibrillar autofluorescence of collagenous and elastic fibres (Fig. 12). In this scar, nerves can no longer be identified by catecholamine fluorescence.

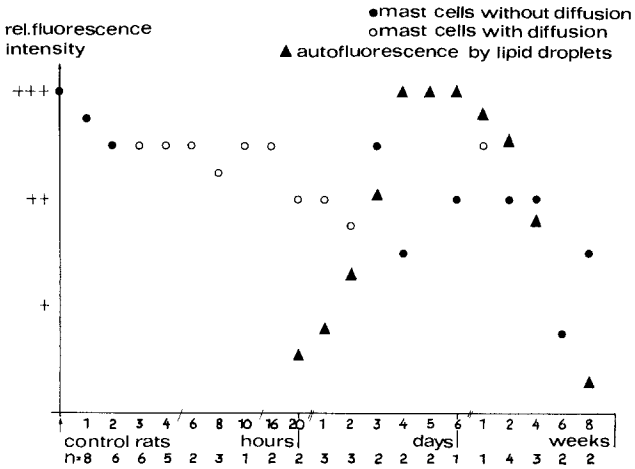

Fig. 14. Fluorescence intensity of mast cells in experimental myocardial infarction of rats

The specific fluorescence microscopical findings in adrenergic nerves and mast cells within the myocardial infarction and the non-specific fluorescence phenomena are summarized in Figs. 13 and 14.

The investigation of the border of the infarction shows a retained specific fluorescence during the first hours after ligation, which may occasionally be increased. Sometimes there are accumulations of mast cells in the outer infarction zone.

The investigation of the ligation area showed a focal loss of catecholamine fluorescence and an increase of specific transmitter fluorescence, above and below the point of ligation. The other cardiac nerves of the non-infarcted areas did not show any constant and distinct alteration at various intervals after the operation.

Discussion

Our light microscopic observations of myocytes in the infarcted myocardium largely correspond to those published earlier by various authors (Hort and Da Canalis, 1965; Korb and Totovic, 1969; ref.: Hecht, 1970). Changes of the heart nerves were only investigated by Hirsch (1970) in human infarction by means of an non-specific silver method; he found a complete disintegration of neural structures within the area of infarction. However, histochemical studies on transmitter fluorescence of cardiac nerves in infarction have not been reported. Our findings show that during the first four hours after ligature an unstable reduction of catecholamine fluorescence occurs which constantly progresses during the following hours. Thus initially the intramural terminal fibres

are affected and later the perivascular nerve fibres. As a consequence of transmitter flow into the interstitium a light diffuse specific fluorescence of the adjacent infarcted myocardium develops. This however is reduced after 10 h like the remaining catecholamine fluorescence remaining in the infarcted area. After two days no more fluorescing nerves can be found the infarcted myocardial tissues. This investigation shows that local ischemia does not lead to an immediate discharge or loss of intramural catecholamine stores (Borchard and Paessens, 1977). A considerable loss of adrenergic transmitters occurs only after several hours by diffusion of the catecholamines into the adjacent tissue. The increase of fluorescence intensity of adrenergic nerves at the border zone of the infarction in some animals probably indicates a diffusion into the non-infarcted myocardium and a specific uptake$_1$ (Iversen, 1973) by intact nerves.

In our experiment preventing blood outflow by simultaneous ligature of venous and lymphatic vessels the metabolic degradation of liberated catecholamines mainly takes place in the area of the infarction and its border zone. Within the infarction the enzymatic degradation of noradrenalin may be performed by mitochondrial monoaminooxidase (Kopin, 1972) of myocardial cells. These, however except from ischemia may be additionally damaged by the local action of the liberated catecholamines. The contraction bands observed during the early necrobiotic changes in the infarcted area may be interpreted as an indication for the action of a sympathomimetic stimulus (Rona et al., 1977).

The time course of reduction of the specific fluorescence is in agreement with biochemical findings in experimental infarction in the dog, where the catecholamine stores were gradually depleted within two days (Russel et al., 1961; Shabab et al., 1969; Mathes and Gudbjarnason, 1971). – The regressive changes in nerves in the infarcted area are presumably related to the reduction of specific catecholamine fluorescence in postmortem myocardium observed by Elbadawi et al. (1970), Pentillä (1971) and Borchard (1975/8) and which has also been demonstrated biochemically in the latter studies.

In the early phase after ligature, local hypoxia is likely to be the major factor in catecholamine decrease. In an electron microscopic investigation, however, a relative resistance of axonal ultrastructure in comparison with the infarcted myocardium could be shown (Borchard and Paessens, 1980). The hypoxic and hyperosmoloric environment of the infarcted area seems to influence nutrition and function of the peripheral axons by damage to surrounding Schwann cells. In the axons, degenerative lesions gradually occur but with a certain delay compared to the adjacent myocardium. These processes have already been described and discussed in detail for the ischemic myocardium (Trump et al., 1976).

For the later periods after coronary ligature, the particular conditions of the experimental model seem to be important, since during ligature of the coronary artery adrenergic nerves were also ligated. Two processes have to be discussed, disturbance of the proximodistal transport and Wallerian degeneration. With some animals, we could demonstrate interruption of proximodistal transport in the form of a dislocation of adrenergic transmitter substances

to proximal and distal axonal areas in the region of the ligature, a change also described by Mayor and Kapeller (1967). – Concerning the Wallerian degeneration there is no information about the time course and possible differences between nerves in the normal and infarcted myocardium. Wallerian degeneration in intact myocardium, after experimental neural ablation of cardiac nerves biochemically leads to a disappearance of cardiac noradrenaline content, when examined after one week (Cooper et al., 1962; Jakobowitz, 1967). Histochemical investigations, however, have shown that the specific fluorescence in various peripheral organs usually fades within two days after neural ablation (Falck, 1962; Malmfors and Sachs, 1966; van Orden et al., 1967; Blümcke, 1968; Olson, 1969). Since there are no such studies in the heart it is not clear whether there are differences in the axonal degeneration in the intact or infarcted myocardium respectively.

Infarction of man is mainly due to coronary occlusion (Hort et al., 1977). If this is compared to experimental infarction, which is usually induced by ligature there are considerable differences. These depend on trauma to the cardiac nerves and simultaneous disturbance of the lymphatic and venous outflow which is obstructed togehter with the arteries in the experimental model. However, in man catecholamines partly may reach the periphery by open cardiac venous and lymphatic vessels.

The fluorescent microscopical findings obviously bear a functional meaning, since it is known that catecholamines either administered exogenously or liberated endogenously may exert profound disturbances in the metabolism and function, above all contractility and excitability of the muscle cells. So apart from electrolyte imbalances and injury currents the catecholamines believed to play an important role in arrhythmogenesis (Harris and Bisteni, 1955; Harris, 1966; Gillis, 1971). This process may be due to the membrane labilisating effects of membrane potentials by catecholamines (Kent and Epstein, 1976) in which shifts in the electrolyte concentrations may be involved (Haeusler et al., 1968). Some authors have attributed only certain phases of arrhythmia to the action of catecholamines (Antoni, 1978). This author has claimed that also subendocardial Purkinje fibres might be of an importance for the genesis of ventricular arrhythmias. Although we could not trace the conduction system for reasons of methodology in this experiment, it is conceivable that the Purkinje fibres with their rich adrenergic supply (Borchard, 1978) are influenced by local diffusion of catecholamines out of the infarcted area.

The liberation of endogenous noradrenaline may also influence the vascular perfusion by inducing contraction of vascular smooth muscles or by alteration of the local microcirculation (Rona et al., 1977), possibly thus contributing to the extension of the infarction size, which has been found to be variable during the first hours after coronary ligation (Frenzel et al., 1977).

In respect to the various functional disturbances that may induced by systemic or endogenous catecholamines during heart infarction the recent clinical beneficial application of β-blocking substances seems noteworthy (Multicentre international study, 1975).

Since the behaviour of the adrenergic nerves in the infarcted human heart

is still obscure, at least in our rat experiments changes of the transmitter content are feasable: In the initial phase after complete ischemia only a few inconstant alterations of the adrenergic nerves can be found. After a few hours however, these nerves liberate appreciable amounts of endogenous catecholamines which is in good agreement with previous biochemical investigations. The noradrenergic transmitters may be of great importance for the functional state of the adjacent myocardium, for the size of the infarction and for the survival because of arrhythmogenesis in animals and man.

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Accepted January 2, 1980