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The Relaxation of Sarcomeres in Ischemic Injury of Myocardium

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Summary. Polarization-microscopic and micrometric investigations of the myocardium during infarction at autopsy and in experimental ischemic conditions, has shown that one of the earliest and most typical morphological signs of ischemic myocardium is sarcomere relaxation. The latter is expressed by the loss of contractility of muscle cells in life time and in strongly frozen corpses, and due to the effects of fixatives. Increase of the percentage of relaxed sarcomeres parallels the duration of experimental ischemia and the time after myocardial infarction. This allows the indirect calculation of irreversible cell change.

Key words: Myocardium – Ischemia – Infarction – Sarcomere relaxation.

Introduction

Local ischemia of the functioning heart, exceeding the critical duration for myocardial cell survival (Jennings and Ganote, 1975), leads to coagulative necrosis, the main form of cell injury in myocardial infarction (Baroldi, 1973).

The morphological signs of coagulative necrosis can only be detected some time after cell death, as some time is required for their manifestation following the activity of autolytic enzymes. The morphological diagnosis of pre-necrotic stages of myocardial infarction thus remains an unsolved problem (Report of the Scientific Group of W.H.O., 1971). In this respect the data of some authors on sarcomere relaxation in ischemically injured myocardial cells are of interest (Caulfield, 1963; Ferrans et al., 1973; Hack and Helmy, 1967; Hort, 1968; Jennings et al., 1965; Page and Polimeni, 1977; Socolov et al., 1972). There is even a study on sarcomere measurement during myocardial infarction (Hort, 1965), but this interesting investigation has not been further developed.

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Material and Methods

We have continued Hort's (1965) investigation into the measurement of sarcomere size in myocardial ischemia and infarction. For this purpose a polarization-micrometric measurement method was worked out, which allowed us to determine the sarcomere sizes in histological preparations accurately and without complicated technical apparatus (Shperling, 1977). The method consisted of counting the anisotropic or isotropic discs in pieces of longitudinal by sectioned muscle fibres and measuring them with an ocular micrometer. Measurement and counting were carried out in polarized light. The mean value of the sarcomeres in each section is calculated by dividing the length of the section by the number of the discs. The sarcomeres are grouped according to their sizes, and their relative percentage is determined.

The measurements were carried out on patients dying from myocardial infarction at periods from 4.5 h to 6 days following the onset of pain, as well as in patients dying from other causes. The diagnosis of myocardial, infarction was based on the clinical picture of the disease and on the typical E.C.G. changes in standard and thoracic leads.

The autopsies were carried within 24 h of death. Pieces of the myocardium were excised from the affected zone or from any region of evident infarction. The affected zone in infarctions not older than two days was determined on the basis of electrocardiographic findings and on the results of dissection of coronary arteries.

In order to examine the early development of sarcomere relaxation, measurements of sarcomere lengths were carried out following experimental occlusion of the left coronary artery of adult white rats. The thorax and the pericardium were dissected under ether anaesthesia without injuring the pleural cavities. The left cardiac coronary artery was tied (after Selye). After 6–60 min under the same anaesthetic, the heart was removed, put on ice, dissected and placed in fixative (10% neutral formalin). Pieces were excised from the left and right ventricles, dehydrated in alcohol and embedded in paraffin. Sections 7–10 mcm thick were stained by conventional methods. The preparations were studied in normal and polarized light.

Results

Investigation of the affected myocardium of patients dying 1–6 days after clinically documented myocardial infarction have all shown the picture of sarcomere relaxation, with some addition necrosis. Marked sarcomere relaxation was detected during investigation of the histological preparations of longitudinal

Table 1. The characteristics of sarcomeres in patients dying at various intervals after infarction

Number	Time following the fit	Number of sarcomeres measured	Size of sarcomeres in mcm				
			minimum–-maximum	less than 1.5	1.5–1.69	1.7–2.19	2.2 and more
				Percentage			
1	4.5 h	449	2.0 –2.5	–	–	44	56
2	5 h	327	1.42–2.45	6	–	29	65
3	2 days	426	2.12–2.5	–	–	8	92
4	5 days	454	2.0 –3.37	–	–	18	82
5	6 days	421	2.25–2.65	–	–	–	100
6	–	623	1.16–3.0	45	21	25	9
7	–	239	1.04–2.1	51	–	49	–

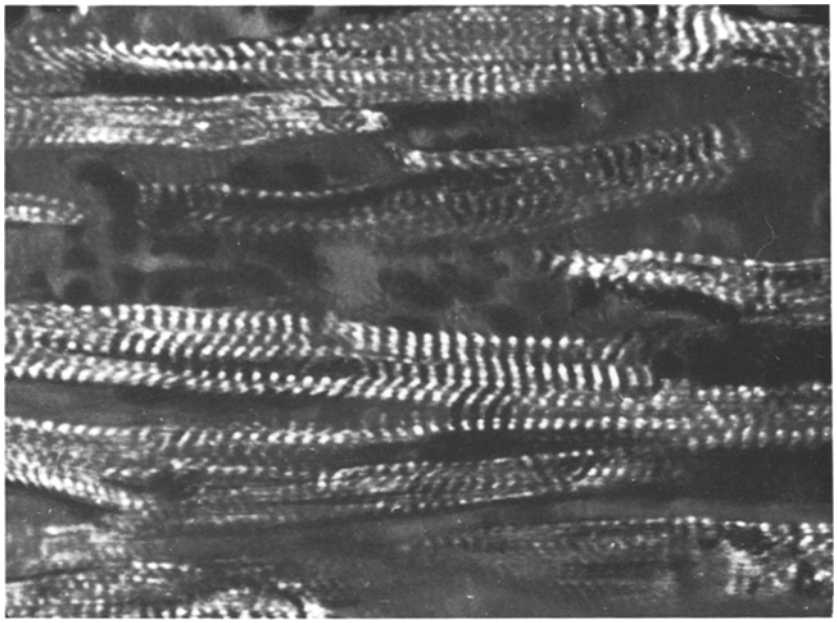

Fig. 1. Relaxation and extension of sarcomeres in necrotic muscular fibres

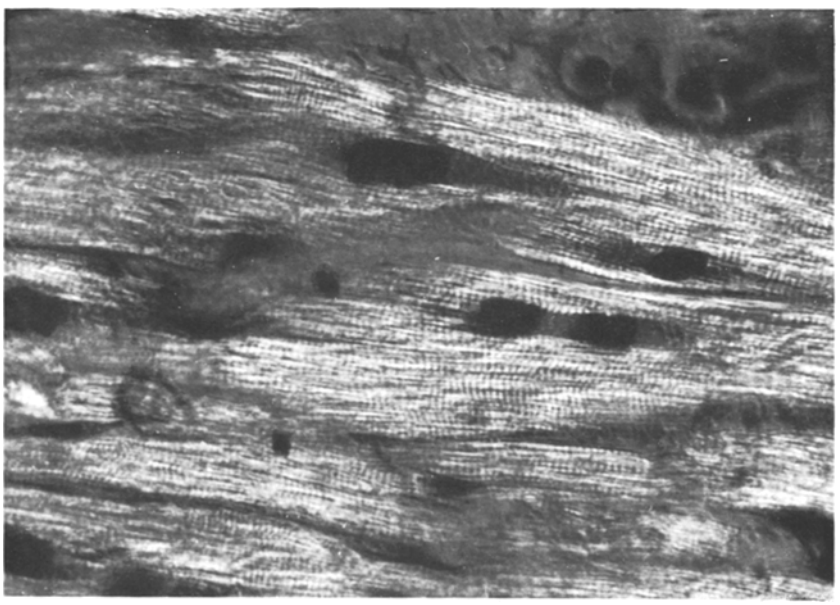

Fig. 2. Contracted sarcomeres in normal muscular fibres. Pictures in polarized light, stained in hematoxylineosyne, ocular 10, objective 40

Table 2. Characteristics of sarcomeres in myocardium experimental ischemia

Number of animals	Time of occlusion	Left ventricle				
		Number of sarcomeres measured	Size of sarcomeres in mcm			
			minimum–maximum	less than 1.5	1.5–1.69	1.7–2.19
				Percentage		
1	6	295	1.2–1.9	52	39	9
2	38	231	1.3–1.8	9	60	31
3	40	314	1.5–1.8	–	49	51
4	52	315	1.6–1.8	–	37	63
5	60	309	1.3–2.0	5	20	75
6	60	316	1.5–1.9	–	41	59

myocardial sections in polarized light: in the affected muscle cells the thickness of the isotropic band was often equal to the value of A-band (Fig. 1). Unaffected zones of the myocardium showed a picture of contracted sarcomeres, with thin isotropic and thick A-band (Fig. 2).

The sarcomere lengths (Table 1) in the presumed infarcted zone of myocardium of the left ventricle, 4.5–5 h after the onset of ischemia, fluctuated between 1.42 and 2.45 mcm, and in this case 60% of the sarcomeres were relaxed and extended (2.2 mcm and more). In the case dying after two days the sarcomere lengths in the infarction zone were equal to 2.12–2.5 mcm, and the percentage of the relaxed and extended sarcomeres was 92. Death after 5 or 6 days showed sarcomere lengths between 2 and 3.37 mcm, the relaxed and extended sarcomeres were 82–100% of the total. In those dying from diseases other than myocardial infarction the sarcomere lengths in the myocardium of the left ventricle were 1.04–3 mcm; contracted sarcomeres (less than 2.2 mcm) were 91–100% of the total.

In experimental animals (Table 2) at intervals of 6–60 minutes after occlusion the number of the contracted sarcomeres in the left ventricular myocardium (i.e. those less than 1.7 mcm) decreased and the number of relaxed ones (1.7–2.19 mcm) increased, from 9 to 59–75%. Over the same period the percentage of contracted sarcomeres in the myocardium of the right ventricle was 85–100.

Discussion

The results of polarization-microscopic investigation and comparison of the sarcomere measurement with established data (Sonnenblick et al., 1964; Spiro, 1966) suggest that the early appearance and correlation with increase in length of the sarcomeres with the increased duration of ischemia in man and experimental animals, presents valuable information.

Right ventricle				
Number of sarcomeres measured	Size of sarcomeres in mcm			
	maximum–minimum	less than 1.5	1.5–1.69	1.7–2.19
		Percentage		
375	1.1–1.5	90	10	–
224	1.4–1.8	11	74	15
281	1.1–1.5	95	5	–
308	1 –1.8	65	30	5
244	1.2–1.5	97	3	–
194	1.3–1.7	52	40	8

The presence of relaxed sarcomeres in the ischaemic myocardium suggests a loss of contractility. In dead muscle the affected cells do not contract in rigor mortis, nor, in experimental animals, on fixation. It is well established that normal muscle cells are contractile in frozen corpses (Hort, 1965) and after some fixation (Hort, 1961; Schmalbruch, 1970). Increase in sarcomere tension in cells which have lost their contractility is explained by contraction of surrounding intact fibres and by the effects of intraventricular pressure (Baroldi, 1974; Bouchardy, Majno, 1974). The size increase of sarcomeres in the injured cells in prolonged infarction is conditioned by the time-dependance of these effects.

Total arrest of contractile function develops earlier than irreversibility of ischaemic injury. However, the increase in the percentage of relaxed sarcomeres when ischaemia is prolonged, is an indirect indication of the onset of a critical survival period for most of the muscular cells of the affected zone. From experimental investigations (Jennings, Ganote, 1975) an increase in relaxed sarcomeres to more than 50% of the total is an indication of irreversible change.

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