Linear densities in mitochondria of human myocardial cells¹

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Ultrastructural study of myocardial biopsies from 5 patients who underwent myocardial revascularization procedures because of impending infarction showed mitochondria with linear densities. These densities, which occupied the intracristal space, appeared to have a high protein but low mineral content. It is suggested that linear densities may be an expression of severe, acute myocardial cell injury.

Linear densities in mitochondria have been described by Denker, Bergman, and Nachlas (1969) and Dusek, Rona, and Kahn (1971) in association with ischaemic injury to the heart of experimental animals. In addition, a number of other pathological conditions of muscle cells in man have been shown to be associated with such densities (Fisher and Danowski, 1969; Sluga and Monneron, 1970; Tandler *et al.*, 1970; Woodhouse and Burston, 1969).

This paper deals with observations in five patients who were found to have linear densities in mitochondria of cardiac muscle cells obtained from the left ventricle at the time of cardiac surgery. The conclusion is drawn that these findings are likely to represent a cumulative effect of repetitive periods of ischaemic injury to the myocardium.

Patients and methods

The five patients reported in this paper were admitted to the Cardiovascular Surgery Service at the University of Wisconsin Hospitals for coronary revascularization procedures. The relevant clinical data of these patients are summarized in Table 1.

Full thickness biopsies were taken in all patients from the anterior free wall of the left ventricle, near the apex using a nitrogen gas driven drill (Brambridge and Niles, 1964). In this group of patients, no attempt was made to remove material from the ischaemic zone of the left ventricle for

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biopsy but it is likely that in Case 2 the control biopsy was taken from an injured zone as judged by the standard 12 lead scalar electrocardiogram, arteriogram, and operative findings. In 3 of the patients, biopsies were obtained at the beginning (control) and at the end (experimental) of cardiopulmonary bypass. In Case 2 only a pre-bypass specimen was obtained, while in another (Case 4) a single post-bypass biopsy was obtained. Care was exercised to assure that both biopsies were taken from adjoining areas of the left ventricular free wall near the apex, whose blood supply was the left anterior descending artery.

The biopsies were fixed within 30 seconds of removal from the heart by immersion into cold 4 per cent formaldehyde, 2 per cent glutaraldehyde, 0.5 mmol CaCl₂ in 0.1 mol Na cacodylate at pH 7.2. The formaldehyde was diluted from 10 per cent formaldehyde prepared by alkaline depolymerization of paraformaldehyde. After primary fixation, the tissue was diced and washed overnight at 4°C in three changes of 0.1 mol Na cacodylate buffer at pH 7.2. The tissue was then post-fixed in 2 per cent osmium tetroxide for two hours at room temperature, dehydrated in ethanol, contrasted in block with ethanolic uranyl acetate, and embedded in an Epon-Araldite mixture. Two to five blocks were obtained from each biopsy, and all were examined. Only central portions of the biopsy material were studied in order to avoid the possibility of examining tissue that was damaged as a result of the biopsy procedure. One hundred to three hundred sections were studied from each block obtained at two different levels within the

TABLE 1 Summary of clinical data

Case no.	Age (y)	Sex	Pre-infarction angina	Preop ECG	Coronary arteriographic findings	Surgical procedure and findings	Total pump time (min)	Aortic cross-clamp time	Post-operative course
1	51	M	Yes	Inferoseptal infarction	99% stenosis RCA 90% stenosis LAD 50% stenosis circ	3 vessels bypassed	171	4 times, 33 min total	Uneventful
2	57	M	Yes	T waves negative in I, aVL, V2-V5	95% stenosis LAD	LAD bypassed; anterior wall motion poor with dusky appearance	127	5 times, 42 min total	Uneventful
3	42	M	Yes	Normal	95% stenosis LAD	LAD bypassed; good left ventricular motion	104	3 times, 28 min total	Developed antero-septal wall infarction, with Q waves in V1-V3 and negative T waves in I, aVL, V2-V5
4	53	M	Yes	ST depressed 0.5 mm in V3-V5, T inversion in I and aVL	95% stenosis LAD 75% stenosis RCA 100% stenosis circ	3 vessels bypassed; cardiac action poor, patient expired in OR	533	8 times, 64 min total	—
5	47	M	Yes	Normal	99% stenosis RCA 95% stenosis LAD 75% stenosis circ	3 vessels bypassed	331	11 times, 93 min total	Uneventful

RCA=right coronary artery; LAD=left anterior descending coronary artery; circ=circumflex coronary artery; OR=operating room.

TABLE 2 Summary of ultrastructural findings

Case no.	Pre-bypass biopsy	Post-bypass biopsy
1	Normal structure	Shown considerable intracellular swelling, loss of glycogen particles, and moderate mitochondrial swelling; linear densities were present in about 20% of mitochondrial profiles
2	Abnormal myocardial cell morphology, including some intracellular swelling, some loss of glycogen particles, and moderate mitochondrial swelling; linear densities were present in about 10% of mitochondrial profiles	No ne obtained
3	Normal structure	Moderate intracellular swelling, considerable loss of glycogen particles, severe mitochondrial swelling with clearing of matrix and loss of cristae; linear densities were present in about 50% of mitochondrial profiles
4	None obtained	Severe intracellular swelling, total loss of glycogen particles, badly swollen and disrupted mitochondria, with clearing of matrix and loss of cristae; linear densities were present in about 60% of mitochondrial profiles
5	Normal structure	Intracellular swelling, almost total loss of glycogen particles, and severe mitochondrial swelling with loss of cristae and matrix density; linear densities were present in nearly 80% of mitochondrial profiles

same block. The frequency of the appearance of linear densities within mitochondrial profiles was estimated from 18 micrographs taken at random from these sections (Table 2) which were cut on a Porter-Blum MT-2 ultramicrotome, further contrasted with lead citrate, and studied in a Hitachi HU-11B electron microscope.

Micro-incineration, similar to that described by

Shen and Jennings (1972), was employed in an attempt to gain information on the mineral composition of the linear densities and amorphous granules observed. Sections of tissue were mounted on grids coated with silicon monoxide, routinely stained, and examined and photographed in the electron microscope. Diagrams were made of the grids to permit location of the area photographed after

FIG. 1 (A) An electron micrograph of left ventricular muscle obtained from Case 5 before initiation of cardiopulmonary bypass, showing abundant glycogen particles and lack of intracellular swelling. A portion of a nucleus is visible (N), and lipid droplets, seen frequently in human myocardial cells, are apparent (L). The mitochondria (M) appear dense and well organized. ($\times 31\,900$.) (B) A high magnification view of a mitochondrion from cardiac muscle obtained from Case 5 before initiation of cardiopulmonary bypass. Numerous, well-organized cristae are apparent, demarcating a dense appearing matrix. The area of the mitochondrial profile occupied by the transparent intracristal space appears to be approximately equal to area occupied by the dense matrix. ($\times 60\,000$.)

incineration. After photography, the grids were subjected to periods of heat in air sufficient to bring the grids to a dull red glow. The grids were re-examined at three-minute intervals, and the degree of destruction observed. This procedure was continued until complete destruction of the sections occurred, and tissue detail was totally lost.

Results

Examination of myocardial cells obtained from the pre-bypass biopsy material showed normal human myocardial ultrastructure (except from Case 2), with abundant glycogen particles, little or no intracellular swelling, and dense, well-organized mitochondria (Fig. 1A). The mitochondrial matrix

FIG. 2 An electron micrograph of left ventricular muscle obtained after cardiopulmonary bypass in Case 1, showing the morphological changes associated with ischaemic injury, and, in addition, linear densities in mitochondria. Considerable intracellular swelling is obvious, along with vesiculation of the sarcoplasmic reticulum (SR) and extreme elongation of the sarcomeres (MF). Glycogen particles are scarce. The mitochondria appear swollen, with reduction of matrix density. Linear densities and amorphous dense granules can be seen in several of the mitochondria (arrows). ($\times 26\,600$.)

material appeared dense and the intracristal space transparent, with the two compartments occupying approximately equal volumes (Fig. 1B). Varying degrees of cellular change were seen in the biopsy specimens obtained after bypass. These are summarized in Table 2. Intracellular swelling, loss of glycogen particles, and mitochondrial swelling were commonly noted in the post-bypass samples, though in case 2 considerable cellular swelling and linear densities were present in the pre-bypass sample of myocardium. In three cases (Cases 1, 4, and 5), more severe damage was apparent, with appearance of vesiculation of the sarcoplasmic reticulum, extreme cellular swelling, and margination of nuclear chromatin.

In all instances, linear densities and amorphous granules were noted in mitochondria of myocardial cells from post-bypass biopsies. The frequency of this finding varied from patient to patient (Table 2) and appeared to correlate best with the degree of overall cellular damage. Thus, in the more severely damaged myocardial cells, a larger number of mitochondria were found to contain linear densities. However, this was not true of the number of linear densities in each mitochondrial profile. In general, one or two linear densities were found in any particular mitochondrial profile, regardless of the degree of cellular damage. Frequently, amorphous granules were also observed, often in the same mitochondria containing linear densities. They were more common in severely damaged tissue (Fig. 2). The distribution and frequency of these alterations were uniform throughout the samples studied.

When studied at higher magnification, the linear densities appeared to consist of electron opaque material deposited between a pair of cristal membranes, in the intracristal spaces (Fig. 3A, B, C). The contrast of this material was enhanced by staining with lead citrate (Fig. 3B, and C). In

favourable sections, an apparent core was observed, appearing as a fifth electron dense line midway between the four electron dense lines representing the cristal membranes (Fig. 3B). Resolution of this core into individual subunits, such as the intracristal rods reported by Hall and Crane (1971), was suggested in some instances (Fig. 3C), but could not be consistently obtained. Measurements of the intracristal space indicated that the spaces occupied by linear densities were slightly narrower (approximately 140Å) than those without such densities (about 200 to 400Å). The linear nature of these structures suggests that the dense material imparts a rigidity to the occupied cristae.

Microincineration studies of sections showed an even loss of structure, with increasing duration of incineration. Membranes disappeared rapidly, leaving rows of electron dense dots which delineated the former location of these structures. Amorphous granules and mitochondrial cristae could not be recognized after 6 minutes of incineration while outer mitochondrial membranes and linear densities required 9 minutes of incineration before they too disappeared. Once all recognizable morphology was destroyed, no deposits that corresponded to the location of the linear densities or amorphous granules could be seen.

Discussion

The ultrastructural findings reported in this paper indicate that human myocardial cells are altered to varying degrees when subjected to ischaemia. In the post-bypass sample of heart muscle, structural damage was severe, suggesting that the added periods of ischaemia and reperfusion, a necessary part of the surgical procedure, contributed significantly to the production of these abnormalities. That cardiopulmonary bypass with aortic cross-clamping results in intracellular swelling, loss of

FIG. 3 (A) An electron micrograph of left ventricular muscle obtained after cardiopulmonary bypass in Case 5, showing two typical mitochondria that are swollen with loss of matrix density and cristae. Several linear densities are apparent, and a core or central filament can be observed (arrows) in favourable orientations of the densities. ($\times 175\ 000$.) (B) Linear densities in mitochondria from post-cardiopulmonary bypass muscle samples of Case 3 are shown at high magnification. The dense material appears to be deposited in the intracristal space, and to have a core or central filament (single arrows). The upper density appears to be interrupted at one point, and then to continue without the core or dense material (double arrow). This section did not receive additional contrasting with lead citrate. ($\times 243\ 000$.) (C) Further examination at high magnification (Case 5) shows, in this instance, the core of the density to be resolvable into dense dots or rods (arrows) similar in appearance to the structures reported by Hall and Crane (1971) in isolated beef heart mitochondria. This section did receive additional contrasting with lead citrate. ($\times 180\ 000$.)

glycogen particles, and mitochondrial alteration is well documented (Bellotti *et al.*, 1974; Bittar *et al.*, 1974; De Gasperis, Miani, and Donatelli, 1970). However, linear densities within myocardial mitochondria after cardiopulmonary bypass have not been previously described.

In Case 2 these changes were observed in the pre-bypass biopsy. In this case, the preoperative electrocardiogram, and observation of the anterior wall of the left ventricle at operation, suggested that this portion of the left ventricle was indeed ischaemic. This finding indicates that myocardial

ischaemia in man results in similar cellular alterations as seen in experimental animals. In three other cases where pre-bypass biopsies were obtained, these were not taken from ischaemic muscle. Ultrastructural examination confirmed that they were essentially normal. Since the post-bypass biopsies of all patients showed significant morphological alterations, which included linear densities in mitochondria, it appears that the bulk of cellular damage seen was induced by the cardiopulmonary bypass procedure. This is not surprising since the heart was subjected to periods of ischaemia and reperfusion, conditions known to result in pronounced cellular damage (Herdson, Sommers, and Jennings, 1965; Kloner *et al.*, 1974; Whalen *et al.*, 1974). These findings appeared identical to those induced by myocardial ischaemia (Case 2) alone. Though linear densities in mitochondria have not been described as a feature of the morphological alterations induced by either cardiopulmonary bypass (Bellotti *et al.*, 1974; Bittar *et al.*, 1974; De Gasperis *et al.*, 1970) in man, or by ischaemia followed by reperfusion in animals (Herdson *et al.*, 1965; Kloner *et al.*, 1974; De la Iglesia and Lumb, 1972), they have been associated with acute infarction in experimental animals (Denker *et al.*, 1969; Dusek *et al.*, 1971). It is thus possible that linear densities in human mitochondria may be a feature of acutely ischaemic myocardial cells, whether induced by chronic degenerative processes or surgical interventions, or a combination of both.

Since linear densities have been shown to occur under conditions other than ischaemia, such as malignant hypertension (Huttner, Rona, and More, 1971), metastatic calcification in a patient with renal failure (Woodhouse and Burston, 1969) and in several myopathies (Fisher and Danowski, 1969; Sluga and Monneron, 1970), it appears that other causes of cell injury can result in similar mitochondrial alterations. However, it is interesting to note that these findings have all arisen from pathological conditions of muscle cells, whether cardiac or skeletal. Structures of similar appearance have also been found in apparently normal mitochondria obtained from several non-mammalian sources (Munn, 1974).

It is possible that linear densities result from calcium deposition as suggested by Woodhouse and Burston (1969). However, the microincineration of grids with sections containing linear densities did not support this idea. It may be that the amount of calcium deposited was insufficient to leave detectable ash. Another explanation is that these densities may represent protein deposition or accumulation in the intracristal space (Dusek

et al., 1971; Fisher and Danowski, 1969; Sluga and Monneron, 1970). The observation that exposure to lead citrate results in increasing electron opacity supports this idea. Whether or not the 'intracristal rods' reported by Hall and Crane (1971) in isolated beef heart mitochondria are structures similar to or identical to the linear densities reported here is uncertain. Hall and Crane (1971) showed that aldehyde fixation was necessary to visualize 'intracristal rods' and, therefore, concluded that they were protein in nature.

Regardless of the nature of these densities, their appearance in mitochondria in a variety of pathological conditions suggests that they may reflect a common mitochondrial lesion induced by various conditions. Insight into the nature of linear densities may contribute to a better understanding of the pathogenesis of cell injury.

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