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Fibrillolysis and Electrophoretic Determination of Actin and Myosin in Hypertrophied Human Myocardium *

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Summary. Destructive myofibrillar lesions, particularly degenerative fibrillolysis in hypertrophied hearts, were studied with the electron microscope in left ventricular myocardium samples obtained from autopsies with post mortem periods not exceeding 195 min. The contractile protein composition of these samples was determined electrophoretically. 18 cases were studied, grouped as follows: Group I, composed of 5 cases showing normal hearts which were employed in the determination of normal electrophoretic values. Group II: 10 cases with hypertrophic hearts, 1 of them showing contraction bands and decreased myosin, 6 others with fibrillolytic lesions of which 3 presented a decrease in myosin and 3 possesed normal myosin values. None of the cases showed a decrease in actin values. Group III was composed of 3 cases of hearts with normal weights that showed ultrastructural alterations of the myofibrils: 1 with myofibrillar disarray and normal electrophoretic values, 1 with incomplete contraction bands and normal protein values and 1 with focal myofibrillar disgregation and myosin decrease.

It is concluded that in some cases of degenerative fibrillolytic lessions a decrease of myosin occurs. The method employed does not rule out that apparently normal actin and myosin values might be due to the pressence of contractile proteins in a denatured state that does not affect their electrophoretic mobility.

Key words: Myocardial fibrillolysis – Contractile protein electrophoresis – Heart hypertrophy – Myocardial ultrastructure

Degenerative fibrillolytic alterations have been clearly demonstrated in cardiac hypertrophy due to a variety of causes (Ferrans 1977 and 1978; Maron

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and Ferrans 1978; Maron et al. 1975a and b; Schaper et al. 1978; Themann et al. 1980). Information currently available does not allow one to decide if a decrease in actin and myosin content occurs in these alterations, or whether these contractile proteins may be preserved in a submicroscopic state. The purpose of this comparative preliminary study carried out by electron microscopy and electrophoresis was to answer this question. Since we have not encountered references on similar studies on human myocardium we have included some cases of hearts which did not present myocardial hypertrophy but did show other abnormalities of the cell contractile apparatus. The study was carried out on necropsy material. A complete autopsy was performed on all cases studied.

Material and Methods

Left ventricular myocardium was examined in 18 cases, whose principal data are listed in Table 1. In the last column, the principal clinical and pathoanatomic diagnoses are shown (excepting the diagnosis of cardiac hypertrophy). The age of the subjects varied between 32 and 80 years, 9 were male and 9 were female. The post mortem period (at initiation of autopsy) was less than 3 h except in 2 cases (No 9: 3 h 15′ and No 11: 3 h).

The cases were divided in 3 groups:

Group I. In order to establish a normal reference electrophoretic pattern, several cases presenting no evidence of cardiovascular disease were studied. They were selected with special attention to normal heart weight (Zeek 1942) and satisfactory ultrastructural preservation of the cell contractile apparatus. 5 cases composed this group (1–5, Table 1).

Group II. Cases presenting signs of left ventricular hypertrophy (increase in thickness or size of the left ventricle or both, and heart weight above the maximum as established by Zeek (1942)). In 2 of the cases in this group heart weight was less than 400 g (No 6 and No 13). In both of them the left ventricle showed signs of concentric hypertrophy, with a thickness of 18 mm and 16 mm respectively. 10 cases composed this group (6–15, Table 1).

Group III. Composed of cases which complied with the conditions set for group I but presented ultrastructural alterations in the cell contractile appratus (16–18, Table 1).

The heart was opened as usual. Samples for electron microscopy and electrophoresis were taken from the lateral wall (obtuse angle) of the left ventricle, 2–3 cm below the mitral annulus. An area of approximately 1 cm^2 was exposed with a razor blade, discarding a layer 2–3 mm thick parallel to the scissors' plane of section. The sample for electron microscopy was taken from this area, as a thin block running from epicardium to endocardium. Both ends of the block were discarded. The sample for electrophoresis was taken from the same zone, a tissue block 0.5–1 ml in volume being obtained after discarding the epicardial and endocardial ends. It was immediatly frozen in liquid nitrogen and kept at −90° C until examination.

The EM sample was covered with fixative and sectioned into cubes of approximately 1 mm. It was fixed in 3% glutaraldehyde in 0.1 M Veronal buffer, pH 7.4 for at least 24 h. After post fixation in OsO_4 1% in Veronal buffer for 1 h the blocks were stained in 2% aqueous uranyl acetate for 2 h. They were then dehydrated in ethanol and embedded in Epon. The sections were obtained with a Reichert OMU3 Microtome; 180 to 360 ultrathin sections from each case were examined after lead citrate staining with a Siemens Elmiskop 102.

For electrophoresis, tissue blocks approximately 0.2 ml in volume were cut from the frozen samples, thawed at 4° C and homogenized (Potter Elvehjem homogenizer, 800 rpm, 25–30 strokes) in an equal volume of phosphate-buffered saline solution. A 50 µl aliquot of this homogenate was employed in total protein determination according to Lowry et al. (1951).

Table 1. Data of the 18 cases examined

No	PMP[a]	Age	Sex	Heart w.	Main diagnoses
1	95	65	m	345	Prostatic carcinoma. Multiple metastases. Bronchopneumonia
2	70	34	m	346	Common bile duct carcinoma. Acute pancreatitis. Shock
3	60	40	f	236	Common hepatic duct carcinoma. Acute pyelonephritis
4	55	32	f	250	Cerebral artery aneurysm. Cerebral hemorrhage
5	120	47	f	260	Laennec's cirrhosis. Acute pyelonephritis
6	90	42	m	390	Laennec's cirrhosis. Purulent meningitis
7	60	49	m	464	Rheumatic mitro-aortic valvulopathy. Heart failure
8	50	50	m	497	Cholelithiasis. Suppurative cholangitis. Arterial hypertension
9	195	60	m	590	Gastric ulcer. Gastrointestinal hemorrhage. Arterial hypertension
10	60	34	m	595	Rheumatic mitral valvulopathy. Valvular replacement. Pleural empyema
11	180	62	m	640	Chronic glomerulonephritis. Renal insufficiency
12	30	50	m	747	Coronary atherosclerosis. Myocardial infarction scar. Heart failure. Arterial hypertension
13	90	54	f	350	Erdheim's disease. Dissecting aortic aneurysm. Arterial hypertension
14	120	60	f	486	Arterial hypertension. Chronic myeloid leukemia. Chronic pyelonephritis
15	50	50	f	489	Rheumatic mitral valvulopathy. Heart failure. Thrombotic pulmonary embolism
16	60	60	f	251	Cerebral artery aneurysm; operated. Purulent meningitis
17	60	80	f	267	Cecal carcinoma. Multiple metastases. Portal vein thrombosis. Thrombotic pulmonary embolism
18	60	50	f	289	Cerebral artery aneurysm. Cerebral infarction

[a] PMP: post mortem period (in minutes). m, male; f, female; heart weight in grams

The remainder was completely solubilized in sample buffer (Laemmli 1970) by thorough mixing and heating at 100° C for 4 min. Adequate volumes were electrophoresed in 11% polyacrylamide gels in the presence of sodium dodecyl sulfate (Laemmli 1970). The standards employed were the purified proteins actin and myosin as well as rabbit psoas myofibril preparation. The quantities of actin and myosin were determined by densitometry with an integrating gel scanning device. Electrophoretic runs of independently prepared samples of two of the cases (N° 7 and No 14) presenting myosin decrease were carried out in order to verify the data originally obtained. The values determined were 3.5 and 5.2 μg/100 μg total protein, respectively, in excellent agreement with previous results (Table 2).

Table 2. Summary of findings

No	Group	Ultrastructural findings	Electro-phoresis	Actin[a]	Myosin[a]
6	IIa	Ultrastructure preserved	Normal	24.1	16.3
7	IIa	Contraction bands. Anomalous Z material	Myosin decrease	20.6	3.2
12	IIa	Tubular and vesicular proliferation. Coated vesicles. Anomalous Z material	Normal actin and myosin. Clathrin-like band	19.8	17.1
13	IIa	Anomalous Z material	Normal	20.7	14.9
8	IIb	Fibrillolysis. Anomalous Z material. Myofibrillar disarray	Myosin decrease	23.4	4.7
9	IIb	Fibrillolysis. Isolated cytolysis. Anomalous Z material. Myofibrillar disarray	Myosin decrease	25.1	6.9
10	IIb	Fibrillolysis. Anomalous Z material	Normal	23.6	19.3
11	IIb	Fibrillolysis. Anomalous Z material	Normal	21.6	17.9
14	IIb	Fibrillolysis. Dehiscence of inter-calated discs. Anomalous Z material	Myosin decrease	18.8	5.0
15	IIb	Fibrillolysis. Anomalous Z material	Normal	18.5	14.7
16	III	Focal myofibrillar disgregation	Myosin decrease	24.5	2.8
17	III	Myofibrillar disarray	Normal	21.2	13.9
18	III	Incomplete contraction bands	Normal	17.9	12.8

[a] µg/100 µg total protein

Results

Reference values for content of actin and myosin were obtained from duplicate electrophoretic runs performed with samples obtained from the cases composing group I. The values we have considered *normal* are: myosin, 18.1 ± 4.2 (max.: 24.8, min: 13.5) µg/100 µg total protein; actin: 23.4 ± 4.0 (max.: 28.8, min.: 17.5) µg/100 µg total protein. The myosin content was considered to be *decreased* when the values obtained were 10 µg/100 µg total protein or less. Assuming a normal distribution of myosin content values in myocardial samples, the difference between *decreased* and *normal* values for myosin is significant, by Student's *t*-test with p values of the order of 0.002. We did not detect variations in the actin content of the material studied (Fig. 1).

In keeping with the purpose of this study only those findings pertinent to the filamentous ultrastructure of the myocardial fiber will be described in relation to the electrophoretic analysis.

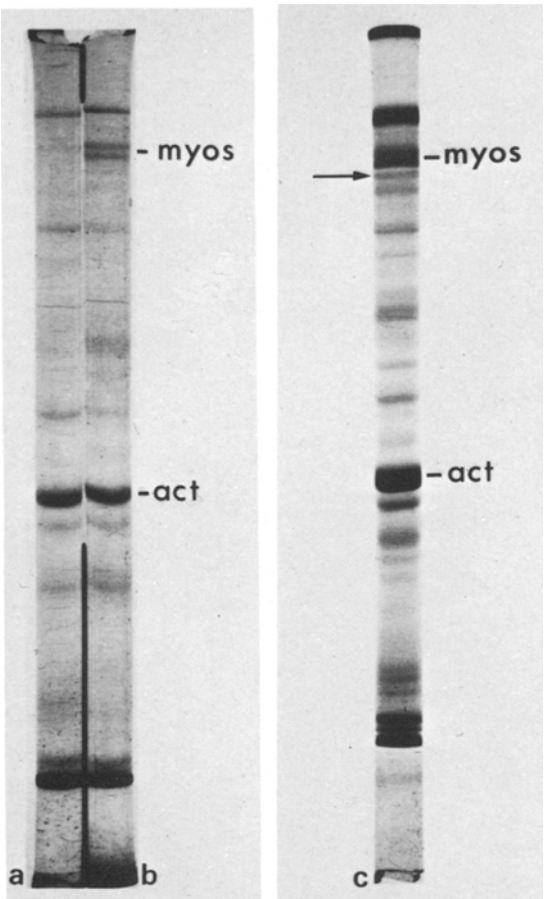

Fig. 1 a-c. Comparison between polyacrylamide gel electrophoreses of cases with decreased (**a**) and normal (**b**) myosin values. **c** Shows a gel from case No 12, which exhibits a small clathrin band (*arrow*). Clathrin is the protein which constitutes the coats of coated vesicles

Group II. In all cases, with the exception of No 6, anomalous Z-disc material was found either beneath the sarcolemma or among the myofibrils, and eventually in continuity with thickened Z discs. In cases 8 and 9 focal myofibrillar disarray was found. Myolytic lesions were identified in 6 out of 10 cases.

a) Cases Without Myolytic Lesions

Case No 6: good ultrastructural preservation of the cell contractile apparatus. Normal electrophoresis. *Case No 7:* contraction bands, some 4–12 sarcomeres in length were seen. The structure and limits of these bands were poorly defined. Z discs and myofilaments were partially disgregated. Electrophoretic analysis revealed an abnormally small amount of myosin. *Case No 12:* the interfibrillar and subsarcolemmal spaces appeared to be widened in certain locations. These spaces were occupied by microvesicular and microtubular formations among which abundant coated vesicles were especially evident (Fig. 2). Electrophoresis revealed normal actin and myosin

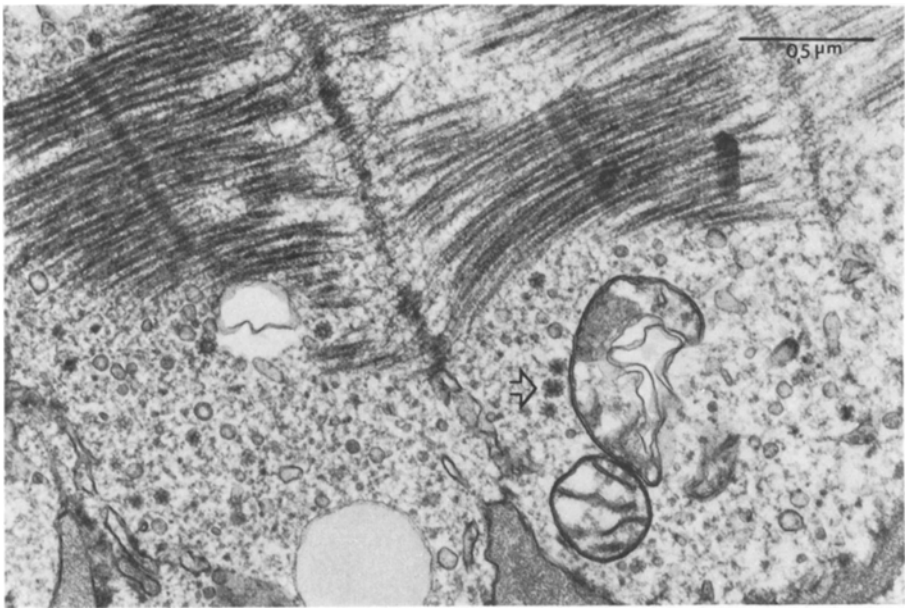

Fig. 2. Subsarcolemmal space occupied by granular material (glycogen?) and by tubular and vesicular structures; among them many coated vesicles (*arrow*). Case No 12, heart weight 747. ×25,000

quantities; a clathrin-like band was present (Pearse 1975) (Fig. 1). *Case No 13:* anomalous Z material was present as the sole cell contractile apparatus abnormality. Electrophoretic analysis was normal.

b) Cases with Myolytic Lesions

These lesions where similar in character to those described in detail by several authors (Ferrans 1977 and 1978; Maron and Ferrans 1978; Maron et al. 1975a and b; Schaper et al. 1978; Themann et al. 1980); in particular, lysis affected mainly the thick filaments. The foci were predominantly found in the neighborhood of intercalated discs and the sarcolemma (Figs. 3 and 4).

Fibrillolysis was found in cases No 8, 9, 10, 11, 14 and 15. In case 9 one cell was encountered which had undergone complete cytolysis, with preservation of the basal lamina (Fig. 5). In case 14 partial dehiscence of some intercalated discs was seen. Electrophoresis showed a reduction in myosin in cases 8, 9 and 14, and normal values for this protein in cases 10, 11 and 15.

Group III. Case No 16: multiple focal lesions were present. These consisted in disgregation of myofilaments and Z discs (Fig. 6). The alterations did not appear as myolytic lesions with its typical myofibrillar rarefaction nor as contraction bands with characteristic shortening of the sarcomeres. The

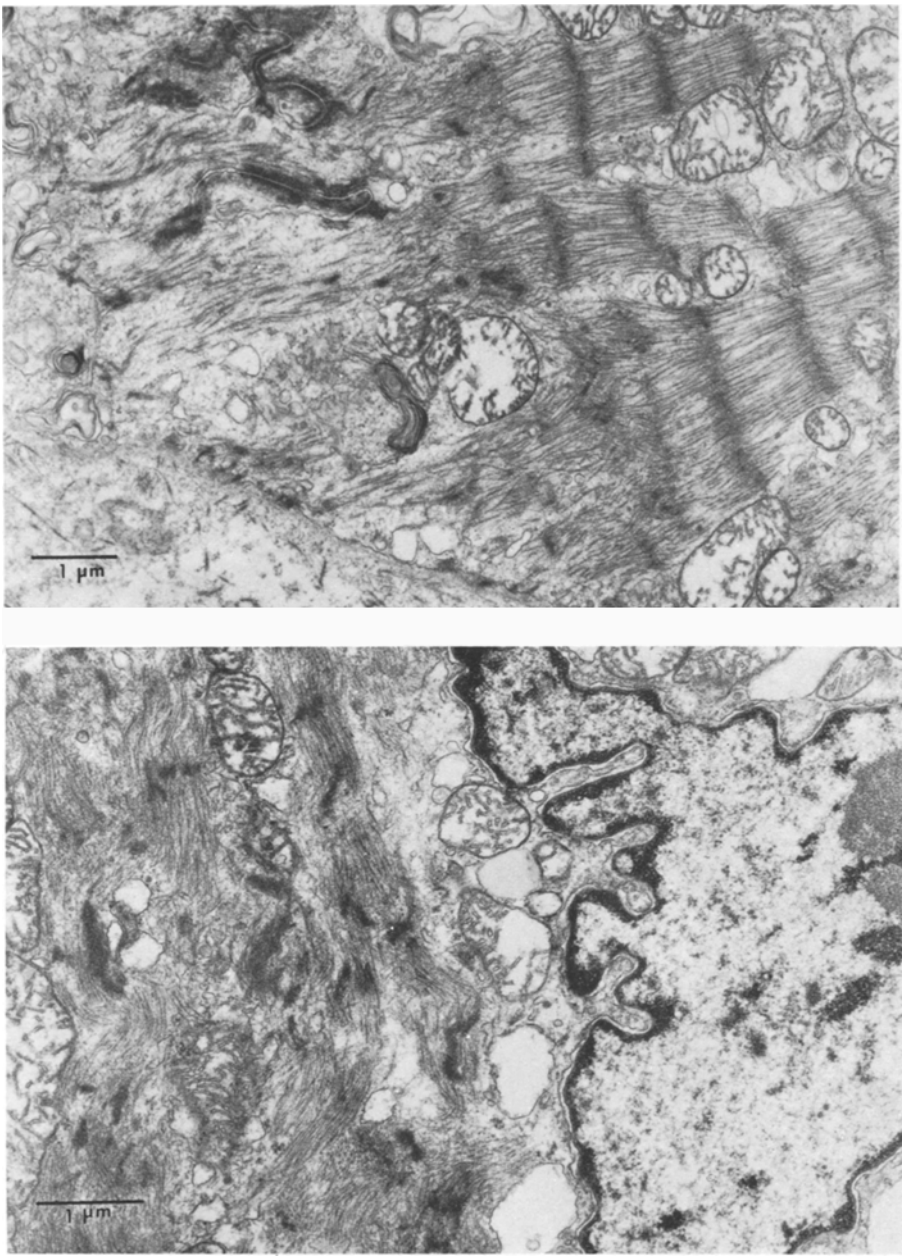

Fig. 3. a Focal fibrillolysis bordering an intercalated disc and the sarcolemma. No sarcomeric disposition is recognized inside the lesion. Case No 11, heart weight 640 g. ×8,000. **b** Myofibrillar disruption and sarcomeric rarefaction. Same case. ×10,000

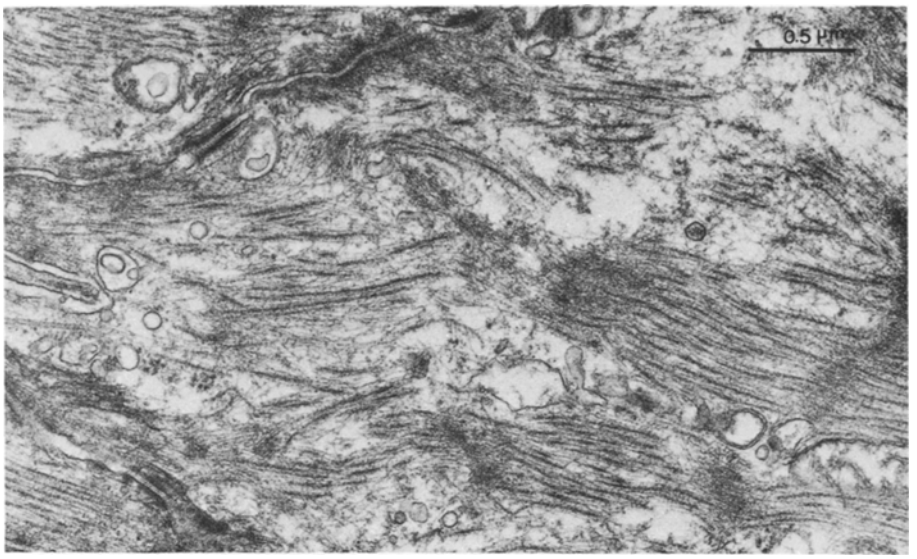

Fig. 4. Focal fibrillolysis at an intercalated disc. Two sarcomeres which border the lesion show rarefaction due to preferential loss of thick filaments, and no H or M discs are distinguished. Case No 10, heart weight 595 g. × 20,000

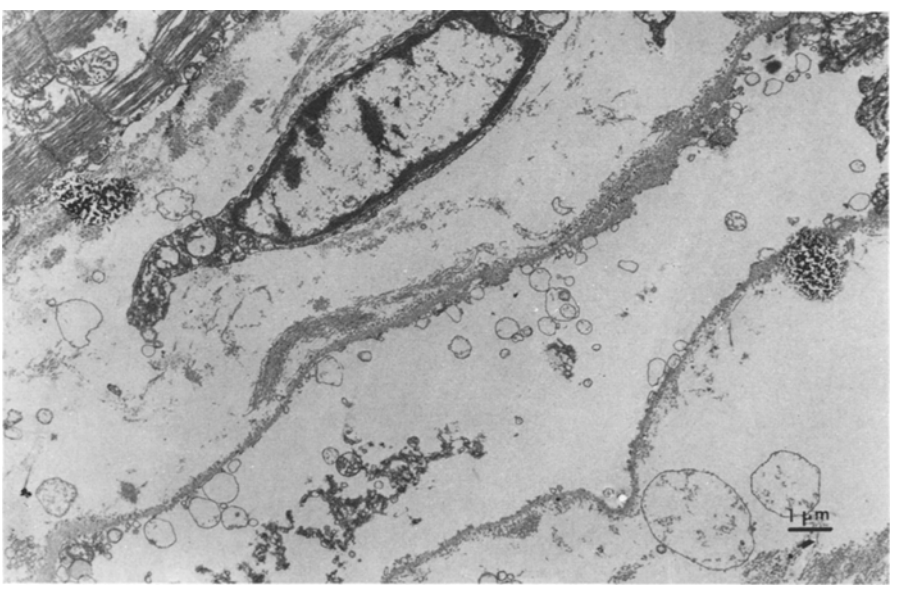

Fig. 5. Myocytolysis. Note at the upper right the presence of an intercellular border. Case No 9, heart weight 590. × 4,000

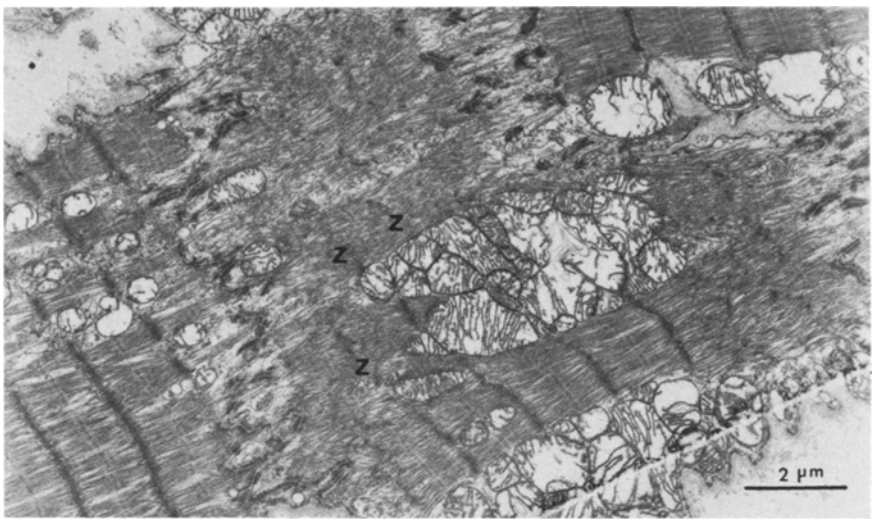

Fig. 6. Focal disgregation of myofibrils. Note that *Z* discs inside the lesion maintain the same spacing as the one seen on the well preserved myofibrils. Case No 16, heart weight 251 g. × 5,000

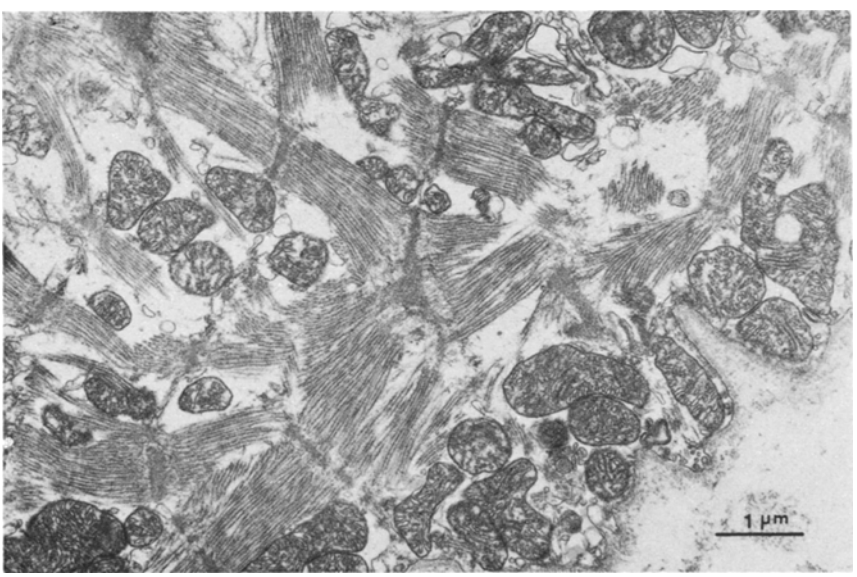

Fig. 7. Myofibrillar disarray and branching. At the centre an abnormal *Z* disc. Case No 17, heart weight 267. × 8,000

foci were dense structures consisting mainly of abundant disgregated material among which altered Z discs were recognizable; these discs showed a spacing comparable to normal sarcomeres. Electrophoresis showed a reduction of myosin values. *Case No 17:* areas presenting myofibrillar disarray were seen (Fig. 7). Myofibrils showed a clear sarcomeric structure but were

oriented irregularly and often branched from deformed Z discs. Electrophoresis was within normal values. *Case No 18:* this case showed incompletely formed contraction bands; these involved 3–4 clearly recognizable sarcomeres approximately 1 μm in length delimited by wide Z discs whose material appeared partially disgregated. H and M discs were visible. Electrophoresis was normal.

The findings are summarized in Table 2.

Discussion

Since the lesions observed are focal in nature we shall first consider whether the tissue sample examined microscopically is representative of the sample employed in electrophoretic analysis. In order to reduce any possible dissimilarities between the samples to a minimum, the samples for both procedures were taken from a tissue block not exceeding 1 ml in volume. In fact, the two cases in group II which showed ultrastructural preservation of the cell contractile apparatus (excepting the presence of abnormal Z disc material) possessed electrophoretic values comparable to the reference group (cases No 6 and 13). The representativity alluded to above would be doubtful if some case had shown normal ultrastructure together with altered electrophoretic values.

The electrophoretic data for the reference group seem well-founded. Little variation is seen among them. With the same procedure, electrophoretic determination of actin and myosin was carried out on samples of rat myocardium. The values obtained were quite comparable to those seen on normal human myocardium.

It appears highly improbable that the statistically significant myosin reduction found in five cases be due to technical error; the electrophoretic study of all the samples was carried out in the same conditions and the results of duplicate runs always coincided. Technical error in these conditions should be randomly distributed rather than concentrated in any five cases.

The myolytic alterations and the abnormal Z disc material found in cases of group II have been described in detail by several workers in biopsy material obtained from cases of myocardial hypertrophy due to different causes (Ferrans 1977 and 1978; Maron and Ferrans 1978; Maron et al. 1975a and b; Schaper et al. 1978; Themann et al. 1980) and have also been found in idiopathic cardiomyopathies (Bulloch and Pearce 1977; Doerr et al. 1979; Knieriem 1978; Kuhn et al. 1978 and 1980; Kunkel et al. 1978 and 1980). The tissue examined in the present study shows that autolytic phenomena do not affect the structure of the cell contractile apparatus importantly, at least during the first 3 h post mortem. This study, carried out on necropsy material clearly shows that myolytic lesions may already be present in hypertrophic hearts less than 500 g in weight (cases No 8, 14 and 15) and may apparently be absent in hearts with great hypertrophy (case No 12). Necropsy material, on the other hand, may reflect other patho-

logic phenomena, in particular those derived from hypoxia. The dehiscence of intercalated discs (case No 14) has been described in hypoxia (Hasper 1964; Heggtveit 1969; Büchner and Onishi 1968) as well as the disgregation of myofilaments observed in case No 16 (Büchner and Onishi 1968). The cytolysis seen in case No 9, whose ultrastructural aspects we have not seen described in the literature, may be related to hypoxic or toxic phenomena (Schlesinger and Reiner 1955) and in particular to ischaemic processes (Baroldi 1973). In none of the cases studied have we found in the neighborhood of myofilaments or rough endoplasmic reticulum membranes the free polysomes that have been interpreted by Saetersdal et al. (1976) as signs of myofilament regeneration.

It is currently accepted that in the fibrillolytic alterations found in cardiac hypertrophy lysis affects preferentially the thick filaments (see previously cited authors), a fact which is confirmed in this study. According to Ferrans (1978) it is not yet known if fibrillolytic alterations in cardiac hypertrophy are mediated by an inhibition of the synthesis of myofilaments, by an acceleration of the degradation of myofilaments and contractile proteins or by a change in the state of aggregation of these proteins from a filamentous form to a submicroscopic nonfilamentous one.

In fibrillolytic lesions seen in hypoxia, lysis predominantly affects thin filaments; this results in an enhanced image of the M disc (Hasper 1964). Preferential lysis of thin filaments is also seen in hypokalemia and certain intoxications (Ferrans et al. 1973).

In group IIb electrophoretic analysis shows: a) actin values similar to normal reference values in all cases; b) normal myosin values in 3 cases (No 10, 11 and 15), and c) myosin reduction in 3 cases (No 8, 9 and 14). Normal actin values cannot be explained by complete preservation of thin filaments since the lytic process undoubtely affected them to a greater or lesser degree. However, normal electrophoretic values may be explained either by the presence of an increased pool of monomeric actin or by the presence of functionally denatured actin whose electrophoretic mobility remains unaltered.

This question cannot be answered satisfactorily with the method employed. The same considerations are also valid for three cases in which no reduction in myosin was found. It has however been shown that at least in some cases of cardiac hypertrophy with fibrillolysis myosin values are reduced. Such reduction appears well documented since in the electrophoretic analysis carried out, the overall qualitative pattern was normal, with no additional bands present which might have altered total protein values significantly. In this sense the sensitivity of the method is adequate, since a clathrin band was detected in case No 12.

In the cases examined up to the present time we have not been able to find a clear correlation between ultrastructure and electrophoretic analysis. These preliminary results do not enable us to interpret the finding that in some cases of cardiac hypertrophy with fibrillolysis myosin is reduced, while in others, with similar morphological alterations, contractile protein values appear to be normal. Finally, if these normal values are not due

to the presence of "denatured" contractile proteins, the possibility of an increased synthesis of monomeric proteins of this type must be considered.

In relation to group IIa and III, the findings of case No 12 should be emphasized. This case showed a heart weight of 747 g; no fibrillolysis was seen in the material examined. Abundant coated vesicles were present; electrophoresis showed normal actin and myosin values, with the presence of an identifiable clathrin band. We have not been able to find references on the discovery of such vesicles in the myocardium. Coated vesicles participate, according to our present knowledge in several processes involving directional transport of subcellular components, especially of membranes (Pearse 1975). We do not believe that the contraction bands found in cases 7 and 18 are artifacts, since this alteration was seen in no other case, including those with post mortem periods of 60 min (cases 3, 10, 16 and 17) or shorter (cases 4, 8, 12 and 15). The electrophoretic results seem to indicate that when contraction bands are well-developed, myosin content is reduced; on the contrary, incomplete contraction bands are related to normal actin and myosin values. The morphological aspect of the lesions in case No 16 (partial myofilament disgregation) seems to show that as in the case of contraction bands, we see a violent destruction of the cell contractile apparatus which is reflected in a myosin decrease on electrophoresis. The myofibrillar disarray seen in case No 17 has been pointed out as a frequent finding especially in idiopathic hypertrophic cardiomyopathies (see previously cited authors). The alteration in itself is non-specific (see also Doerr et al. 1976 and Olsen 1980). The finding of this alteration in case No 17 in the absence of a history of cardiovascular disease and with apparently normal heart weighting 267 g emphasizes its non-specific character. This alteration may represent the result of an aberrant regeneration of myofibrils following partial destructive processes which affect the cell contractile apparatus; examples of these processes may be the myofilament disgregation encountered in case No 16 or the result of the action of mechanical factors (Ferrans et al. 1973). The normal structure of myofibrils may be envisaged as the product of a dynamic equilibrium in which both synthetic and lytic processes continually occur (Maron et al. 1978; Morkin 1974). Evidence has been put forward implicating Z disc material in myofibril synthesis (Legato 1972). In this sense, the myofibril can be compared to the bone trabecula, permanently remodelled and subject to the action of possible microtrauma. However this may be, the examination of this case indicates that microarchitectural distortion does not necessarily involve electrophoretically demonstrable protein loss.

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