Clinical, histochemical, and ultrastructural correlation in septal endomyocardial biopsies from chronic chagasic patients: Detection of early myocardial damage

In order to recognize early signs of myocardial damage, histologic, histochemical, and ultrastructural studies were performed on septal endomyocardial biopsy tissue obtained from 79 chronic chagasic patients and from 18 patients with atypical chest pain (control group). Abnormal biopsy findings were recognized in 9 of 16 (60%) chagasic patients with no clinical evidence of myocardial damage. In cases of segmental asynergy only, biopsies were abnormal in 18 of 19 patients. When signs of advanced myocardial damage were evidenced by clinical examination or ECGs, all biopsies were abnormal. Mitochondrial, nuclear, and cell membrane irregularities were consistent findings. A peculiar dilatation and filling of the T tubule system with a glycoprotein-like substance and a remarkable increase in monoamine oxidase activity were observed early in the disease and progressed in magnitude and frequency as myocardial damage became more evident by other diagnostic methods. Septal endomyocardial biopsy is a sensitive method for detection of early myocardial damage in chronic chagasic patients. Based on these findings, a modification of the currently used classification is proposed. (Am Heart J 1987; 113:716.)

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Since the discovery and description of Chagas' disease,[1] many advances have been made concerning the clinical, epidemiologic, immunologic, and therapeutic aspects of the acute phase of the disease. Less information is available regarding the chronic phase. A number of serologic tests[2] permit accurate identification of asymptomatic patients who have acquired the disease and have a significant probability of developing clinical evidence of cardiac involvement. In the last 20 years, endomyocardial biopsy has evolved as a useful low-risk research and clinical procedure.[3] When applied to chagasic (ChD) patients, this procedure offers the opportunity to obtain valuable information on the pathology and evolution of the disease.

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The objectives of the present study were: (1) the earliest recognition of histochemical and light and electron microscopic signs of myocardial involvement in ChD patients, who were adequately classified according to the degree of myocardial damage by angiographic, hemodynamic, and clinical methods, and (2) correlation of these changes with different clinical stages of the disease. The results demonstrated abnormal findings in many patients devoid of clinical signs of myocardial damage. For this reason we have proposed a modification of the current clinical classification of chronic ChD myocardial disease.

METHODS

Seventy-nine patients with positive complement fixation reactions and hemagglutination tests for Chagas' disease[2] were studied. Thirty-five of these patients were found to have positive tests results at the time of voluntary blood donation, and 44 had additional abnormal ECG or chest x-ray findings. Eighteen subjects with negative complement fixation reactions and no epidemiologic history of Chagas' heart disease were selected as a control group. Hemodynamic studies were performed in these

Table I. Clinical and left ventricular hemodynamic findings

Group	*M/F ratio*	*Age (yr)*	*EDP (mm Hg)*	*EDVI (ml/m²)*	*Ejection fraction*	*Mass Index (gm/m²)*	*Mass/EDV (gm/ml)*
Normal control (n = 18)	1.4	38 ± 14	6 ± 3	90 ± 15	0.64 ± 0.1	63 ± 11	0.7 ± 0.1
Chagas disease							
IA (n = 16)	1.3	34 ± 10	6 ± 3	93 ± 20	0.66 ± 0.1	63 ± 20	0.57 ± 0.1*
IB (n = 19)	1.4	43 ± 11	7 ± 3	131 ± 32†	0.63 ± 0.1	72 ± 18*	0.56 ± 0.1*
II (n = 27)	1.5	45 ± 12	11 ± 3	157 ± 50†	0.42 ± 0.15*	93 ± 32†	0.54 ± 0.1†
III (n = 17)	3.2	63 ± 13*	17 ± 7†	256 ± 70‡	0.20 ± 0.1†	128 ± 35‡	0.5 ± 0.1†

Abbreviations: EDP = end-diastolic pressure; EDVI = end-diastolic volume index; M/F = male to female; IA = chagasic patients with normal ECGs and left ventricular cineangiograms; IB = chagasic patients with normal ECGs and abnormal left ventricular cineangiograms; II = chagasic patients with abnormal ECGs and no signs of congestive heart failure; III = chagasic patients with abnormal ECGs and clinical signs of congestive heart failure.
*$2p < 0.05$; †$2p < 0.005$; ‡$p < 0.0005$ (values are mean ± standard deviation).

patients because of atypical chest pain; none had coronary or myocardial disease.

Clinical protocol. As required by our research council (CDCHT-ULA), a detailed explanation of the objectives, procedures, and potential risks of this research project was given to each patient.[4] Each patient was then subjected to an extensive examination in order to obtain an accurate clinical history. ECG recording, routine laboratory analysis, chest x-ray examination, noninvasive studies, and cardiac catheterization were also performed as part of our prospective protocol on Chagas' disease. Cardiac catheterization consisted of right and left hemodynamic and ventriculographic studies, coronary arteriography, and segmental wall motion analysis. Details of these procedures have been reported elsewhere.[5]

Biopsy studies. Septal endomyocardial biopsies were obtained at the beginning of the hemodynamic studies in each patient, by means of percutaneous right jugular venous approach and 8F Stanford bioptome.[3] A minimum of four tissue fragments for histochemical, light and electron microscopic, and immunofluorescence studies were obtained. Immunologic results have been reported separately[6] and will not be presented here.

Light microscopic and ultrastructural analysis. Immediately after removal, the myocardial fragments were placed in a fixative solution maintained at 4° C; the solution consisted of a mixture of 3% glutaraldehyde and 3% formaldehyde in 0.1M sodium cacodylate buffer, pH 6.3.[7] The fragments were fixed for 2 hours, then washed in the same buffer, and transferred to a 1% buffered osmium tetroxide solution and postfixed for 10 hours. After washing, the samples were dehydrated in ethyl alcohol and propylene oxide, and embedded in Epon 812. Thin sections (9 nm average) were stained according to a modified Reynold's method.[7] Some samples were incubated during dehydration in uranyl acetate prepared in 70% alcohol solution. One-micrometer sections were stained according to a procedure consisting of a series of metallic impregnations (unpublished data). For light and electron microscopic analysis, a tabulator[8] was made which consisted of the following morphologic components: contractile system (myofibers, Z bands), subcellular organelles (mitochondria, nuclei, sarcoplasmic reticulum, and T tubule system), cellular deposits (glycogen, liposomes, dense bodies), and extracellular components (nerve, blood vessels, interstitial cells, fibrosis). Each of these structures was evaluated on a scale of 0 to 4 points, according to the level of alteration. The tabulator included 244 normal morphologic characteristics of myocardial cells, which constituted the maximal theoretic scoring of myocardial damage. Individual biopsy scoring was reported as a percentage of this maximal theoretic value.

In an attempt to recognize any selective alteration pattern, the proportional degree of damage for each cellular component considered in our cytogram was calculated for the study population as a whole (cell component abnormality rate) and for each group of patients (relative abnormality rate). Although myocellular changes indicative of hypertrophy were included as a form of contractile system abnormality in our cytogram, no morphometric characterization of these biopsies was done.

Histochemical studies. Myocardial fragments were immediately frozen in dichlorodifluoromethane (Freon 12 or Arcton 12) cooled in liquid nitrogen. They were subsequently subjected to routine hematoxylin-eosin and trichromic of van Gieson staining, and to histochemical techniques[9] for determination of activity of succinate dehydrogenase, acid phosphatase, myosine ATPase and monoamine oxidase and assessment of deposits of polysaccharides and lipids. A semiquantitative scoring system of 0 to 4 points was also applied to determine the degree of activity for each enzyme studied.[10] Individual histochemical biopsy scores were composed of a summation of partial scores calculated for each enzyme activity and were reported as percentage of the maximal theoretic histochemical score (24 points).

Clinical classification. Chagasic patients were classified into one of the following four groups, according to their

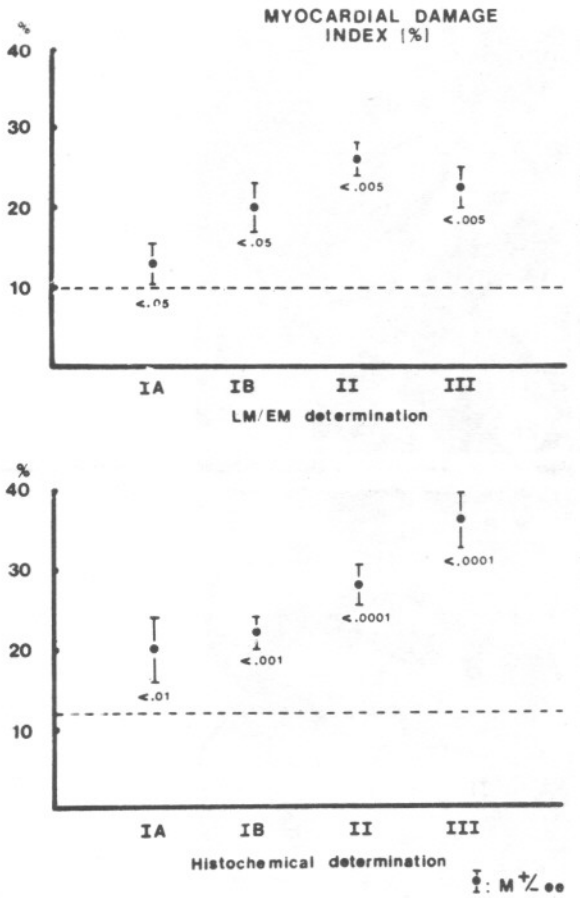


Fig. 1. Percentage of myocardial damage calculated for each group of chagasic patients. Maximal values observed in control group are represented by broken horizontal line. Light and electron microscopic *(LM/EM)* and histochemical scores are significantly elevated, even in patients without any other evidence of heart disease (group IA) and become higher as signs of segmental asynergy appear in left ventricular cineangiograms (group IB). Maximal LM/EM scores are seen in group II, with advanced myocardial damage, but are decreased in patients with congestive heart failure (group III). These end-stage patients showed maximal values for histochemical abnormalities. Their LM/EM scores are reduced because there are less cell components to be quantified.

clinical, ECG, hemodynamic, and left ventricular angiographic findings[5]: IA = asymptomatic, normal ECG, no hemodynamic or cineangiographic findings of heart disease; IB = asymptomatic, normal ECG, evidence of segmental myocardial damage demonstrated by cineangiography (early myocardial damage); II = asymptomatic or symptomatic, with abnormal ECG and evidence of widespread myocardial damage at hemodynamic and cineangiographic studies, but without signs of congestive heart failure (advanced myocardial damage); III = clinical evidence of congestive heart failure, abnormal ECG, and cineangiographic evidence of severe myocardial damage (congestive cardiomyopathy). In the present study, as in our other previously reported studies,[5] incomplete bundle branch block, first-degree atrioventricular block, and nonspecific ST-T changes were considered normal ECG findings.

Statistical analysis. Two-tailed Student's t test for unpaired comparison and chi square analysis were applied as indicated for assessing statistical significance. Unless otherwise specified, all data are shown as mean ± standard deviation. Biopsy analyses were performed without knowledge of clinical findings or diagnosis.

RESULTS

No complications were observed from the procedure for obtaining septal endomyocardial biopsies.

Clinical and hemodynamic findings. Table I summarizes pertinent findings, including those related to left ventricular function. More detailed information on these patients has previously been reported.[5] Patients included in the control group had normal hemodynamic and angiographic findings, including coronary angiographic findings. Group IA ChD patients also had normal hemodynamic findings except for an early but significant ($p < 0.05$) reduction in left ventricular mass when related to corresponding end-diastolic volume. Group IB patients had segmental left ventricular asynergy[5] in the presence of normal ECG findings. In addition, these patients had an increase ($p < 0.05$) in both left ventricular end-diastolic volume and mass. The increase in mass was inadequate for the amount of left ventricular dilatation observed, and thus the mass/volume relationship decreased further in this group ($p < 0.05$). No other hemodynamic abnormalities were found.

Group II patients had abnormal ECG and left ventricular angiographic findings, but symptoms of dyspnea or palpitations were present in only 50%. Increased left ventricular volume and mass and reduced mass/volume relationships were even more pronounced ($p < 0.01$) in this group. Ejection fraction was depressed ($p < 0.05$), indicating a more advanced degree of myocardial damage.

Group III patients were older ($p < 0.05$) than the rest of the ChD patients and manifested the end stage of the disease. Their clinical findings were those of congestive heart failure with abnormal ECGs, severe biventricular dilatation, apical aneurysm, and mitral valve regurgitation. All parameters of ventricular function were abnormal. Mass/volume relationships demonstrated an overt predominance of dilatation (Table I). Coronary arteriography did not demonstrate significant obstruction in any patient.

Light and electron microscopic findings. Samples taken from the control group had few alterations.

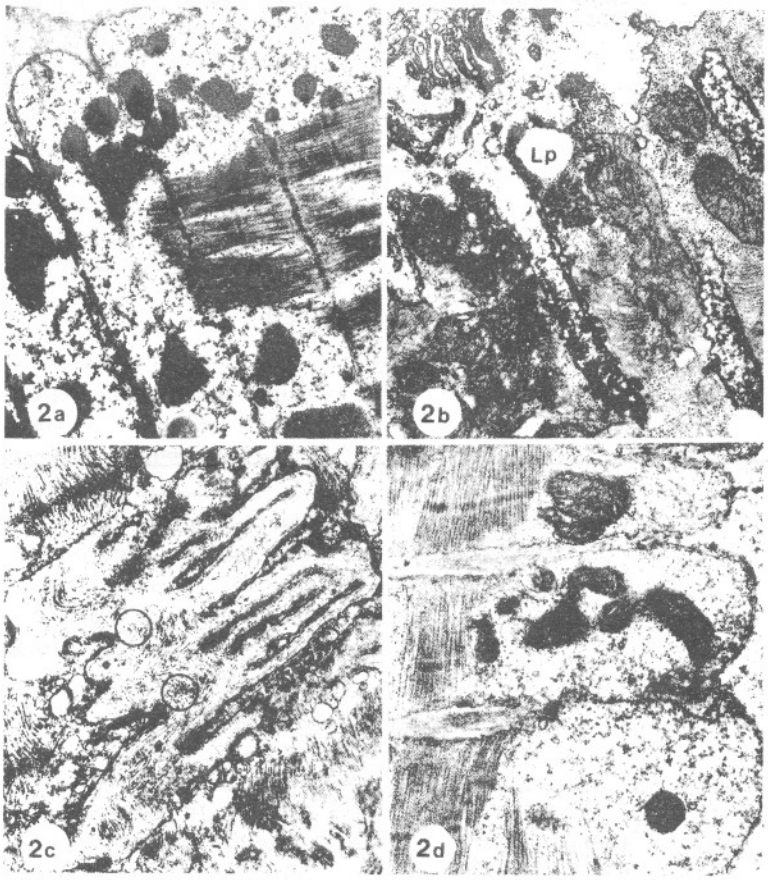
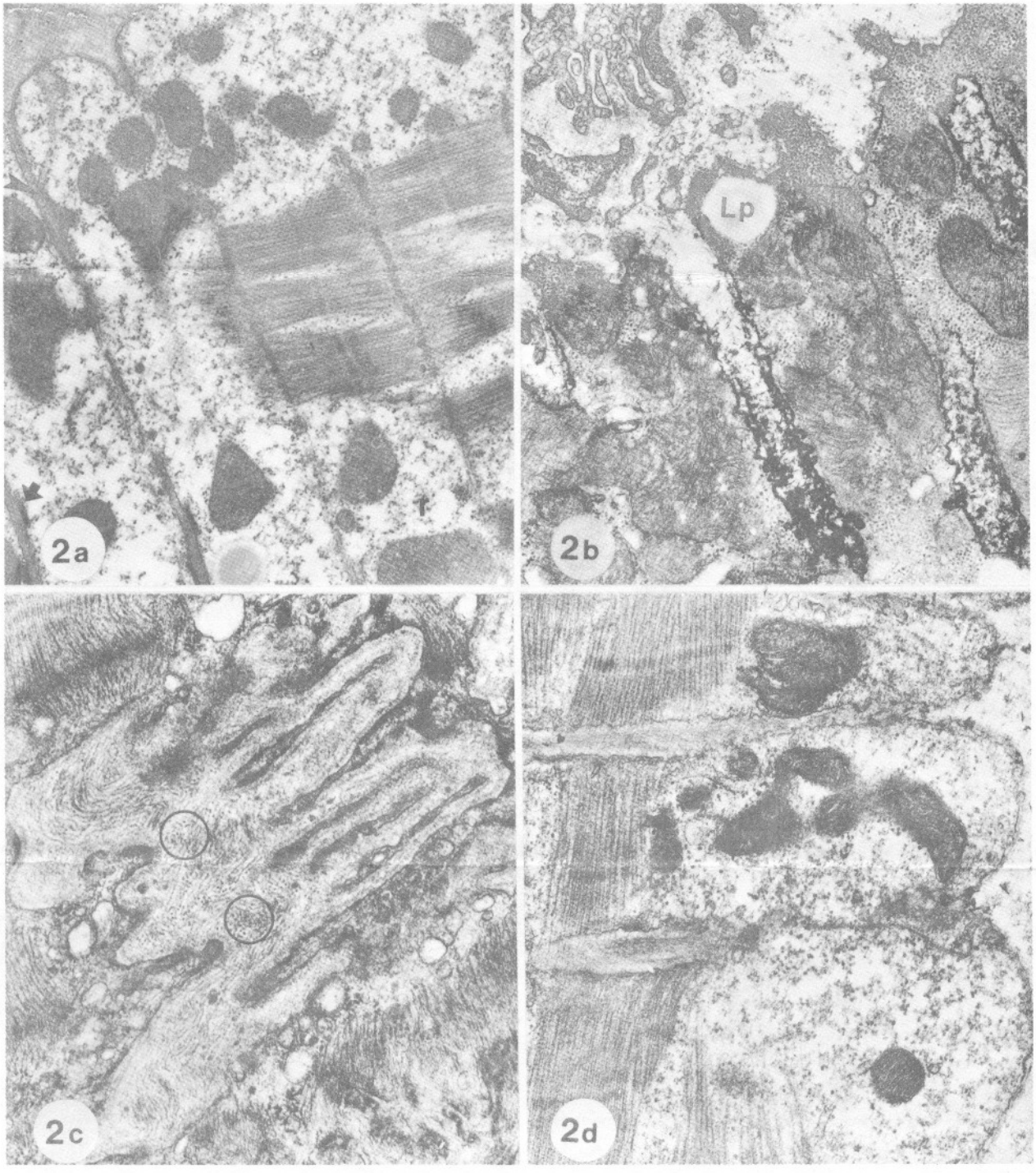


Fig. 2. Examples of ultrastructural abnormalities. *A*, Group IA patient. T tubules show incipient dilatation and filling with an electron-dense substance *(arrowhead)*. Mitochondria are regularly shaped. Sarcoplasmic reticulum cisternae are normal *(arrow)*. (×15,000). *B*, Group IB patients. Uranyl acetate reacts with intratubular glycoprotein-like substance. T tubules are irregular and dilated. *Lp* = liposome. (×18,000.) *C*, Group II patient. T tubules joined together to form large vacuolar spaces filled with microfilamentous substance *(circles)*. (×22,000.) *D*, Group III patient. Severe contractile system lysis. T tubules and sarcoplasmic reticulum terminal cisternae are filled with glycoprotein-like substance. (×22,000.)

Table II. Abnormal ultrastructural findings

	Intracellular				*Extracellular*		
	Subcellular organelles	*Contractile system*	*Cell membranes*	*Deposit elements*	*Fibrosis, Infiltrates*	*Abnormal Capillaries*	*Nerve Endings*
Cell com[illegible] ab[illegible]	36	17	16	13.4	10	6	1.5
[illegible]							
[illegible]	9	10	10	2	10	9	0
[illegible]							
	21*	19	20*	20*	15	15	27*
	22*	26*	23*	24*	19	24*	33*
	23*	29*	24*	25*	22*	24*	27*
[illegible]	25*	16	23*	29*	34*	28*	13*

Abbreviations [illegible] Table I.
Cell component abnormality rate = total observed scoring for each cell component/total observed scoring for all biopsies.
Relative abnormality rate = % of cellular component abnormality scoring observed in each group of patients.
*$2p < 0.05$ (X2).


Lp
f
2a
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2c
2d

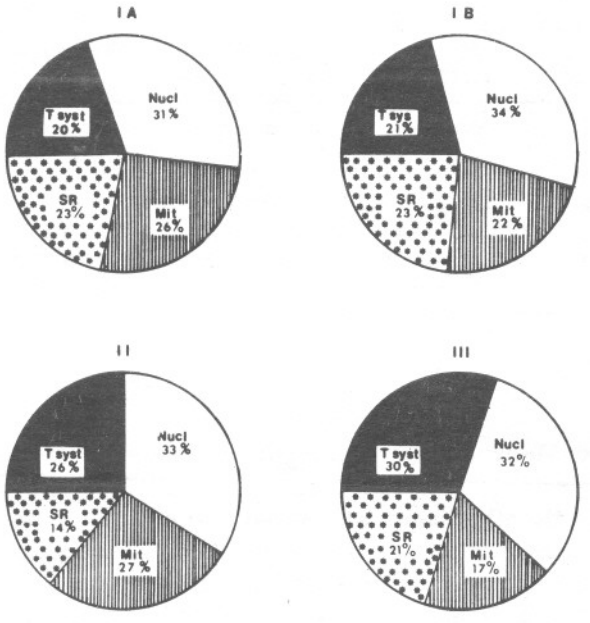

Fig. 3. Relative abnormality rates (%) among subcellular organelles. Abnormalities of subcellular organelles were predominantly seen in nuclear *(Nucl)* and mitochondrial *(Mit)* structures. Sarcoplasmic reticulum *(SR)* seemed less affected. Damage to T tubules (T *Syst*) increased as the disease progressed from biopsy signs of heart disease *(IA)* to end-stage congestive heart failure *(III)*.

The quantification of myocardial damage in this group was always less than 10% in our cytogram (Fig. 1). Abnormalities consisted of minor degrees of cell membrane modifications, slight increases in lipofuscin or other deposited elements, mitochondrial and contractile system irregularities, and occasional extracellular infiltrates (Table II). The sarcoplasmic reticulum and T tubule system were always devoid of any intratubular deposits.

In 9 of 16 (60%) group IA ChD patients without any other evidence of myocardial damage, light and electron microscopic studies demonstrated degenerative abnormalities ($p < 0.05$). The nuclei increased in size; lipofuscin, liposome bodies, and nerve endings were frequently seen; cell membranes had more evidence of alteration, and mitochondria showed several degrees of atrophy and edema. Many T tubules had incipient dilatation and some intratubular deposits of microfilamentous granular substance, which reacted to uranyl acetate as glycoprotein-like material (Fig. 2). When a relative assessment of subcellular organelle damage was done, nuclei and mitochondria were slightly more affected in this stage than the T tubule system or the sarcoplasmic reticulum (Fig. 3). The contractile system was almost normal and no extracellular abnormalities were found (Table II). The light and electron microscopic myocardial damage index was 13 ± 5% (mean ± SD; $p < 0.05$).

Group IB patients had angiographic findings of segmental asynergy. Light and electron microscopic evidence of myocardial damage was found in 18 patients (90%), which accounted for an index of 21 ± 7% ($p < 0.005$; Fig. 1). All samples from these patients showed atrophy and invagination of nuclear outlines, mitochondrial atrophy and edema, minor irregularities of the contractile system, and early signs of focal myofibrillar lysis (Table II). The T system was extremely affected, with irregular outlines and more obvious intratubular deposits (Fig. 2). Damage to subcellular organelles was more prominent in nuclear and mitochondrial structures (Fig. 3). Atrophic but functioning capillaries and numerous nerve endings were also seen.

All group II patients had ECG, hemodynamic, angiographic, and light and electron microscopic abnormalities. The corresponding damage index was the highest observed (26 ± 5%, $p < 0.005$; Fig. 1) and most cellular components were affected (Table II). Dense bodies, lipofuscin, active lysosomes, liposomes, and a great variety of mitochondrial irregularities were present. Larger amounts of extracellular lipid deposits, nerve endings, abnormal capillaries, focal fibrosis, myofibrillar lysis, and active fibrocytes were also present (Table II). The dilated T tubules frequently joined together to form large vacuolar spaces filled with glycoprotein-like deposits (Fig. 2) and were relatively more affected than the nuclei and mitochondria (Fig. 3). Few mononuclear infiltrates[11] were recognized in some samples.

In patients with end-stage disease (group III), the most important morphologic findings were the extensive areas of lysis of the contractile system and an important reduction in the number of subcellular organelles. These changes may explain the relative decrease in the myocardial damage index (22 ± 6%, $p < 0.01$; Fig. 1), since there were fewer cell components to be quantified. The T system and sarcoplasmic reticulum were always dilated and filled with glycoprotein-like deposits (Fig. 2); the remaining mitochondria and nuclei were relatively less altered than the T system in this stage (Fig. 3). Abundant scar tissue, atrophic capillaries, and mononuclear infiltrates completed the well-known histologic picture of chronic chagasic myocarditis.

Histochemical findings. Biopsies taken from normal control subjects showed a slight degree of activity of acid phosphatase; all other histochemical findings were irrelevant (Table III). The histochemical index of myocardial damage was 6 ± 6% (Fig. 1); values

Table III. Histochemical scores in endomyocardial biopsies

Groups	*Lipids*	*Polysaccharide deposits*	*Acid phosphatase*	*Myosine ATPase*	*Succinate dehydrogenase*	*Monoamine oxidase*
Normal control (n = 18)	0.6 ± 0.1	0.1 ± 0.3	0.8 ± 0.2	−0.2 ± 0.9	−0.2 ± 0.9	0.2 ± 0.9
Chagas disease						
IA (n = 16)	1.1 ± 1.7	0.12 ± 0.3	1.5 ± 0.8	−0.5 ± 1	−0.6 ± 1	1.7 ± 1*
IB (n = 19)	1.1 ± 1.8	0.16 ± 0.5	1.5 ± 0.9	−1.1 ± 0.5*	−1.1 ± 0.5*	1.7 ± 0.9*
II (n = 27)	1.2 ± 1.5	0.68 ± 1*	1.8 ± 0.4*	−1.3 ± 0.6*	−1.2 ± 0.8*	2.1 ± 0.6*
III (n = 17)	1 ± 1	1 ± 1.3*	2.7 ± 1.1*	−1.6 ± 0.8*	− 2 ± 1.5*	2.9 ± 0.7*

Abbreviations as in Table I.
*$2p < 0.05$.

over 12% were never observed. Eight of 16 group IA ChD patients had a remarkable increase in monoamine oxidase activity without any other significant histochemical changes (Table III). All but one patient with abnormal light and electron microscopic findings had abnormal histochemical findings. The calculated histochemical myocardial alteration index rose to 20 ± 10% ($p < 0.01$; Fig. 1). Augmented activity of monoamine oxidase was again a prominent histochemical finding in 18 of 19 group IB patients, who also had reduction in succinate dehydrogenase ($p < 0.01$) and myosine ATPase ($p < 0.01$) activities, indicating altered mitochondrial and myofibrillar functions. The histochemical alteration index was 23 ± 9% ($p < 0.001$; Fig. 1).

In all group II patients, the increment of lysosomal acid phosphatase activity added to the even more accentuated, previously described pattern of abnormal monoamine oxidase, succinate dehydrogenase, and ATPase activities (Table III). Polysaccharide deposits were also demonstrated in larger amounts, all of which accounted for a histochemical alteration index of 28 ± 10% ($p < 0.001$; Fig. 1).

Group III patients demonstrated the highest histochemical myocardial alteration index (36 ± 12%, $p < 0.0001$; Fig. 1). All enzymes studied in this group attained their maximal observed degree of altered activity along with polysaccharide deposits; lipids were not significantly modified (Table III).

DISCUSSION

Light and electron microscopic observations. The previously described results demonstrate that the combined light and electron microscopic and histochemical studies of septal endomyocardial biopsies are a very sensitive method for detecting earliest signs of myocardial damage. As has been mentioned, 60% of patients without any other evidence of myocardial dysfunction had degenerative changes in their biopsies. Similar findings have been reported by Mady et al.[12] in a light microscopic study of biopsies from a comparable group of ChD patients. On the other hand, we learned that in cases in which evidence of myocardial damage can be detected by conventional invasive (group IB) or noninvasive (groups II and III) methods (Fig. 4), myocardial biopsy reveals advanced degrees of degenerative changes.

Before entering into further discussion it is important to point out that in spite of the existence of some reports[13, 14] describing abnormal biopsy findings and a reduced capacity for coronary vasodilatation in patients with atypical chest pain such as our control group, the present light and electron microscopic results and those of Unverferth et al.[15] showed minor abnormalities which were interpreted as being within normal limits.

Biopsy studies of advanced dilated cardiomyopathy[16] have uniformly shown a pattern of nonspecific nuclear abnormalities, mitochondrial damage, severe myofibrillar lysis, and variable amounts of fibrous tissue and cellular infiltrates[3, 14, 15, 17, 18]; however, few reports have been made on the early stages of this syndrome.[17-20] In Chagas' disease, a specific myocardial disease[16] which evolves similarly to congestive cardiomyopathy, it is possible to study all stages of myocardial damage. Group IA represents the earliest stage that can be studied; although it is not possible to recognize myocardial damage by means of the conventional invasive and noninvasive diagnostic techniques currently in use,[5] light and electron microscopy demonstrated abnormal degenerative changes affecting mainly the nuclei and mitochondria in 60% of these patients (Table II; Figs. 1 and 3). As the disease progresses, degenerative changes become more severe (Table II; Fig. 3). Group IB patients showed initial alterations of the contractile system probably indicative of the observed hemodynamic (Table I) and cineangiographic[5] impairment of myocardial function. Inade-

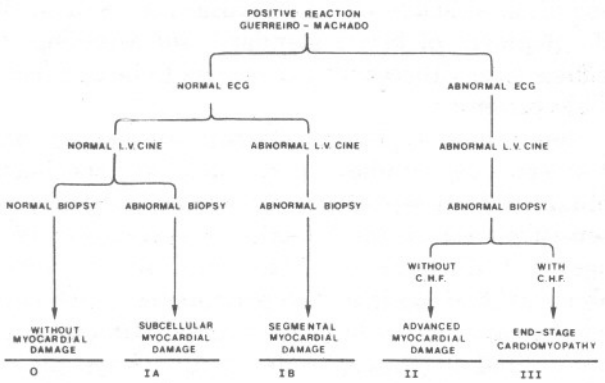

Fig. 4. Classification of chronic chagasic heart disease. The combination of findings in biopsy studies, cineventriculograms *(LV cine)*, ECGs and clinical examinations enables adequate classification of chronic chagasic patients based on degree of myocardial damage. *CHF* = congestive heart failure.

quate hypertrophy is the more prominent functional feature in this group, along with left ventricular dilatation and apical segmental asynergy (Table I). In contrast with other reports,[12] inflammatory infiltrates were not found in groups IA and IB.

Group II patients had some clinical features which resembled the so-called "early stage" cardiomyopathies found in patients with ventricular arrhythmias, normal coronary arteries, and normal or moderately impaired left ventricular function.[17-20] It is obvious that the term "early stage" alludes only to the fact that this stage has few easily recognizable signs of heart disease. Biopsy studies demonstrated a substantial amount of degenerative changes in these cases,[17-20] indicating a rather advanced stage in the evolution of a previously undiagnosed myopathic process. Aside from differences in involvement of the conduction system and in segmental asynergy,[5, 20] biopsies from group II patients demonstrated a lesser degree of hypertrophy and a higher degree of damage than dilated idiopathic cardiomyopathy without signs of congestive heart failure.[20] These findings are also reflected in the more accentuated tendency toward inadequate hypertrophy in ChD patients (Table I).[20]

None of the studies performed in patients with dilated cardiomyopathy has described filling of the T tubule system with a glycoprotein-like substance, as was seen in our biopsies. We believe this finding deserves more careful investigation, since it may represent a more general consequence of inflammatory myocardial processes rather than be a specific characteristic of ChD. Palacios-Prü et al.[20] proposed that this glycoprotein originates in the healing process of the acute phase, perhaps as a precollagen product elaborated by fibrocytes. The occupation of T tubules interferes with the nutrition of the cells, the propagation of the action potential, and the transport of calcium ions to the sarcoplasmic reticulum,[20, 21] thus altering myocellular performance. Sarcoplasmic reticulum Ca^{2+} uptake and release have been reported to be abnormal in early subclinical stages of naturally occurring round heart turkey cardiomyopathy,[22] long before any other abnormality can be detected. As a consequence of altered T tubule system function, impairment of development of an adequate degree of hypertrophy (Table I) could result in an early hemodynamic manifestation. We have been unable[6] to confirm previous findings of immune deposits in biopsies of ChD patients.[23]

The highest degree of myocardial alteration was found in group III, including congestive heart failure patients. It is only in this end stage of Chagas' disease that significant and extensive fibrotic areas were recognized in our patients. This is the classic histopathologic picture of chronic ChD myocarditis[1, 11, 12, 20] and also of dilated cardiomyopathies with signs of congestive heart failure.[15, 17, 18] However, the electronic microscopic findings of a dilated and obstructed T tubule system, the lack of overt myocellular hypertrophy, and the corresponding low mass/volume relationship remain characteristics of group III ChD patients, when compared to dilated cardiomyopathies.[20]

Histochemical findings. Aside from a wide variation of subjectively assessed activity of acid phosphatase (Table III), not matched by electron microscopic signs of lysosomal activity, no clear histochemical abnormality was found in our control group. Group IA demonstrated a remarkable increase in monoamine-oxidase activity (Table III). In advanced dilated cardiomyopathy, Kawai and Yui[24] have reported a reduction in the amount of tissue norepinephrine, which should be searched for in earlier stages. It might be related to the increased activity of monoamine oxidase or it could be a nonspecific consequence of congestive heart failure. We did not find anyother biopsy histochemical studies in ChD patients to compare with our results; however, Mello[25] reported similar findings in experimental models of chronic ChD myocarditis. Malcom et al.[10] also reported an augmented activity of biochemically determined monoamine oxidase in left ventricular biopsies from patients with mitral valve prolapse not related to abnormal myocardial function. Since the activity of another mitochondrial enzyme, succinate dehydrogenase, is not altered in the early stages of the disease, a decrease in mitochondrial function cannot be proposed as the explanation for this

finding. The increased amount of sympathetic nerve endings observed in these biopsies (Table II) could be related to a higher level of catabolic activity of catecholamines and a greater activity of monoamine oxidase. Whatever the intrinsic mechamism might be, it cannot be discerned by our current data.

Group IB patients showed a clear decrease in the activities of myosine ATPase and succinate dehydrogenase (Table III). In dilated cardiomyopathies, this indicator of mitochondrial dysfunction has been reported to be reduced[26] or normal,[27] and coupled with inverse changes in the activity of lactate dehydrogenase. These changes are more comparable to those of our group II or group III patients, who have far more advanced evidence of myocardial damage than group IB patients. However, augmented activity of acid phosphatase and more elevated levels of activity of monamine oxidase were detected in biopsies from ChD patients, none of which have been found in dilated cardiomyopathies.[10, 26, 27] Aerobic mitochondrial and contractile protein enzymes demonstrated a further reduction in activity (Table III) as expected from the hemodynamic findings of depressed contractility (Table I).

Semiquantification of the histochemical damage index demonstrated that group III, the end-stage patients, had the highest degree of derangement, a manifestation of the abnormal functioning of the remaining cellular components, and the activity of the repair process. All changes observed were in the same direction but of higher magnitude than those observed in group II patients. Lipid deposits did not show significant alteration in any group studied (Table II).

Clinical relevance of biopsy findings. Based on these biopsy findings, we propose a modification of the current clinical hemodynamic classification of chronic ChD myocardiopathy.[5] In this new classification (Fig. 4), patients with normal findings in all studies are considered to be free of heart disease (group 0). Those patients with early evidence of degenerative changes appearing only on endomyocardial biopsy are regarded as patients with "subcellular" myocardial damage (group IA), whereas those who also exhibit areas of asynergy on the left ventriculogram are considered to have segmental myocardial damage (group IB). In group II and group III patients, biopsy findings did not add much more information than what could be collected from clinical, ECG, and angiographic evaluations; therefore, classification of these two groups with advanced myocardial damage is not altered by biopsy findings. This new classification may contribute to the study of the evolution of early myocardial damage, to the recognition of evolving signs of active chronic myocarditis, and to the development of better standards for assessing the effects of any therapeutic measures to be applied to ChD patients.

Conclusions. Light and electron microscopic and histochemical studies, performed on specimens obtained from septal endomyocardial biopsies, are sensitive methods for detection of myocardial damage in ChD patients. These methods identified abnormal findings in 9 of 16 patients (60%) who had no clinical evidence of heart disease. Mitochondria, nuclei, and the sarcoplasmic T tubule system are affected early by the degenerative process. Dilatation and filling of the T tubules and sarcoplasmic reticulum with a glycoprotein-like substance is a prominent feature in all stages of the disease, along with a marked early increase in activity of monoamine oxidase. Histochemical signs of mitochondrial and contractile protein dysfunction appear later in their evolution. Late stages are characterized by activation of lysosomal enzymes and accumulation of polysaccharide waste deposits, in addition to fibrosis and mononuclear extracellular infiltrates. Further characterization and follow-up of these abnormal biopsy findings should enable us to recognize early and evolving signs of active chronic myocarditis.

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