

CHRONIC MURINE MYOCARDITIS DUE TO *TRYPANOSOMA CRUZI* – AN ULTRASTRUCTURAL STUDY AND IMMUNOCHEMICAL CHARACTERIZATION OF CARDIAC INTERSTITIAL MATRIX

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In an attempt to define the mouse-model for chronic Chagas' disease, a serological, histopathological and ultrastructural study as well as immunotyping of myocardium collagenic matrix were performed on Swiss mice, chronically infected with Trypanosoma cruzi strains: 21 SF and Mambai (Type II); PMN and Bolivia (Type III), spontaneously surviving after 154 to 468 days of infection. Haemagglutination and indirect immunofluorescence tests showed high titres of specific antibodies. The ultrastructural study disclosed the cellular constitution of the inflammatory infiltrate showing the predominance of monocytes, macrophages with intense phagocytic activity, fibroblasts, myofibroblasts and abundant collagen matrix suggesting the association of the inflammatory process with fibrogenesis in chronic chagasic cardiomyopathy. Arteriolar and blood capillary alterations together with dissociation of cardiac cells from the capillary wall by edema and inflammation were related to ultrastructural lesions of myocardial cells. Rupture of parasitized cardiac myocells contribute to intensify the inflammatory process in focal areas. Collagen immunotyping showed the predominance of Types III and IV collagen. Collagen degradation and phagocytosis were present suggesting a reversibility of the fibrous process. The mouse model seems to be valuable in the study of the pathogenetic mechanisms in Chagas cardiomyopathy, providing that T. cruzi strains of low virulence and high pathogenecity are used.

Key words: *Trypanosoma cruzi* – chronic experimental *T. cruzi* infection – mouse model of chronic *T. cruzi* infection – chronic myocarditis – cardiac fibrosis – collagen immunolabelling – ultrastructural myocardial lesions

Chronic myocarditis in mice infected with *Trypanosoma cruzi* has been previously described (Federici, Abelman & Neva, 1964; Andrade & Andrade, 1968; Kumar, Kline & Abelman, 1969; Andrade & Andrade, 1976). These studies showed the possibility of utilising the mouse as a model for chronic Chagas cardiomyopathy by the use of strains with low virulence and high pathogenicity.

One difficulty in obtaining mice with chronic *T. cruzi* infection is related to the high mortality during the acute phase. Some have resorted to the use of low inoculum (Laguens, Meckert & Basombrio, 1980; Bijovsky et al., 1983; Schlemper Jr. et al., 1983) and/or drug suppression of parasitemia (Brener & Chiari, 1963; Reed, Roters & Goild, 1983), which are measures that introduce another variable in the studies. With the use of the strains of *T. cruzi*, characterized as Types II and III (Andrade, 1974) that allow a percentage of survival of infected mice, a natural evolution to a chronic phase is obtained and a detailed study of the mouse as a model for chronic Chagas' disease became possible, as recommended by the World Health Organization (WHO, 1979).

In this paper, the ultrastructural aspects of Chagas cardiomyopathy were investigated; the immunotyping of the collagen was made in mice chronically infected and submitted to serological evaluation and histopathological study.

The inflammatory infiltrate showed a predominant participation of macrophages and fibroblasts; prominent blood capillary involvement was detected and ultrastructural alterations of non-parasitized cells was described. Immunotyping of collagen in diffuse fibrosis of chronic Chagas cardiomyopathy showed a predominance of Types III and IV collagens, an aspect that seems important for the evaluation of the possibility of reversion of the fibrosis that may occurs in Chagas' disease.

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MATERIAL AND METHODS

Fourteen mice that spontaneously survived to a prolonged infection (154 to 468 days post-inoculation) with different strains of *T. cruzi* were utilized in the present study. Data concerning the duration of infection, strains of parasite and inoculum size, appear in Table I.

Seven intact control mice that were maintained under the same environmental conditions during 6 to 12 months, were utilized for comparative histopathological, ultrastructural and immunofluorescence studies of the collagen of the heart.

Strains of *Trypanosoma cruzi*: 1) 21 SF strains from São Felipe, Bahia and Mambaí strain from Goiás, Brazil both classified as type II according to morphobiological characters; Bolivia strain (from Bolivia) and PMN strain from Ceará, Brazil, both classified as type III. Briefly: infection by type II strains result in a slow increasing parasitaemia, with peaks between 12 to 20 days of infection, predominance of broad forms and myocardiotropism; while infection by type III strain leads to a slower parasitaemia increase, with peaks from 20 to 30 days of infection, predominance of broad forms, and an intense skeletal muscle tropism (Andrade, 1974).

Infected animals and seven intact controls were killed under ether anesthesia by exsanguination; blood was collected for serological tests: indirect haemagglutination and immunofluorescence. Haemagglutination was performed with red blood cells of Rh negative blood donors; the red blood cells were sensitized with *T. cruzi* antigens. Immunofluorescence was performed according Camargo (1966) with culture form antigens; sera were utilized in dilutions of 1:10, 1:20, 1:40 and 1:80; fluorescein conjugated anti-mice immunoglobulin was used in the dilution of 1:50.

Histopathological study: sections of the heart were fixed in formalin and embedded in paraffin and 5 μ m thick sections were stained with haematoxylin and eosin. The severity of myocarditis and reactive fibrosis was graded on a 3-point scale: + indicates mild and focal lesion; ++ moderate diffuse or focal lesions and +++ diffuse and severe lesion.

Ultrastructural study: for ultrastructural study, small pieces of heart tissue were immediately fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer solution, pH 7.4, followed by a post fixation with 1% Osmium tetroxide in 0.15 M cacodylate buffer solution, pH 7.4. After dehydration with ethanol, the specimens were embedded in Epon. Thick section (1 μ m) were obtained with 1% toluidine blue solution and studied by light microscopy. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and observations were performed in an EM300 Phillips microscope.

Immunotyping of collagen: sections of the heart were included in tissue Teck II O.C.T. and dropped in liquid Nitrogen. Immunolabelling of the collagen was performed by indirect immunofluorescence on frozen sections using specific and purified anticollagen antibodies.

Anticollagen antibodies: specific antibodies against type I, III, pro-III and IV collagens were isolated from immunized New Zealand rabbits or goats, with the following antigens: collagen type I and III extracted from fibrotic human liver (Grimaud et al., 1980) and pro-III from calf skin by limited pepsin digestion and fractional precipitation with sodium chloride (Rhodes & Miller, 1978); type IV collagen from bovine lens obtained using the technique of Dehm & Kefalides (1978). Monospecificity of the antibody against a specific type of collagen was achieved by affinity chromatography on CNBr-activated Sepharose 4B according to the method of Grimaud et al. (1980). Cross reacting antibodies were removed by adsorption after repeated reciprocal passages on the different collagen types bound to CNBr-activated sepharose.

The monospecificity of each purified antiserum was then controlled by ELISA according to Takiya et al. (1983) using microplates coated with the different types of pure collagens. Using the same assay, no cross-reaction was detected between the anti-type IV and laminin (kindly given by G. Martin).

Recognition of the pN antigenic determinant on the procollagen type III molecule, was performed with radioimmunoassay (RIA) (courtesy of J.M. Foidard, Belgium).

The cross-reactivity of the anti-type I and anti-type III antisera with mouse collagen was demonstrated by an ELISA on microplates coated with purified mouse collagen of type I and III (extracted from the skin and controlled by SDS-PAGE). The interspecies cross-reactivity to the anti-type IV antiserum was also tested by an ELISA on macroplates coated with mouse type IV (kindly provided by J.M. Foidard).

Immunodetection: indirect immunofluorescence was performed on 6 μ m thick cryostat sections of the heart, treated with the purified antibodies: rabbit anti-human type I, goat anti-

human type III, rabbit anti-calf type III procollagen and rabbit anti-bovine type IV (0.005 to 0.002 mg/ml) and a fluorescein isothiocyanate (FITC) labelled sheep anti-rabbit or rabbit anti-goat IgG globulin (Institut Pasteur, France, code 74561 and Nordic, RAG-FITC). All readings were performed in a Leitz-Dialux fluorescence microscope fitted with a ploom incident illuminator and a CSI Philips lamp.

The specificity of the reaction for all types of collagen and procollagen was demonstrated by extinction of the immunofluorescence after pre-incubation of the antisera with the corresponding antigen.

RESULTS

Data concerning direct parasitemia, immunofluorescence and haemagglutination test from each animal, are shown in Table I.

TABLE I

General data on mice chronically infected with different strains of *T. cruzi**

No.	<i>T. cruzi</i> Strains**	Duration of Infection (Days)	Inoculum: No. Trip. 0.2 ml	Direct Parasitaemia	Serological Examination	
					Indirect Immuno-fluores.	Haemagglutination
1	21 SF	270	283.000	Neg.	1:80	1:80
2	21 SF	248	221.840	Neg.	1:160	1:80
3	21 SF	210	221.820	Pos.	1:80	1:40
4	21 SF	180	184.240	Neg.	1:80	1:160
5	21 SF	180	255.680	Neg.	1:320	1:40
6	21 SF	180	255.680	Neg.	1:160	1:80
7	21 SF	180	255.680	Neg.	1:320	1:20
8	21 SF	154	345.000	Neg.	1:80	1:20
9	Mambai	475	90.240	Pos.	1:640	1:40
10	Mambai	403	90.240	Pos.	1:80	1:40
11	PMN	345	206.000	Pos.	1:160	1:20
12	PMN	327	52.640	Pos.	1:40	1:20
13	Bolivia	468	282.000	Pos.	1:80	1:40
14	Bolivia	275	319.600	Pos.	1:640	1:80

* Mice from different experimental groups, that survived spontaneously to *T. cruzi* inoculation.

** 1 to 10 - type II strains; 11 to 14 - type III strains.

Light microscopy (Fig. 1, a, b, c, d): the hearts from chronically infected mice, disclosed diffuse and focal inflammatory infiltration with macrophages, lymphocytes and plasma cells. Changes were most marked in the atrium and varied in intensity and extension from case to case both in the atria and ventricles (Table II). Parasites were present within myocardial fibers in three animals infected with type III strains, PMN and Bolivia (Table II). In such instances, the presence of *T. cruzi* amastigotes was coincident with myocardial fiber degeneration and focal mononuclear infiltration. Isolated hyaline necrosis of non-parasitized myocardial fibers was also seen. Slight to dense fibrous thickening of the interstitial tissue was observed predominantly in the atria and more dense in the subepicardial areas, around blood vessels, usually associated with mononuclear cell infiltration. Besides diffuse fibrosis, focal scars were present in atria and ventricles. Fibrinoid necrosis of the media of small arteries occurred frequently and in general these necrotic arteriolar lesions were accompanied by infiltration with macrophages, lymphocytes, plasmocytes and polymorphonuclear eosinophils. The conduction tissue of the heart was identified in several cases (the AV node, and His bundle and its bundle branches) and showed diffuse or focal mononuclear infiltration, necrosis of the specific fibers and slight degree of fibrosis.

Control mice showed normal histopathological myocardial structure.

Electron microscopy (Figs. 2, 3, 4, 5, 6): macrophages were the main components of the foci of inflammatory infiltration with two distinct coexisting populations (Fig. 2b): 1) small round mononuclear cells with regular plasmic membrane, few small lysosomes, normal organelle components and absence of cell to cell contact; 2) large mononuclear cells, showing intense phagocytic activity associated with a large and active lysosomal component, irregular plasmic membrane with cytoplasmic expansions and numerous peripheral pinocytic vesicles, some of which contained

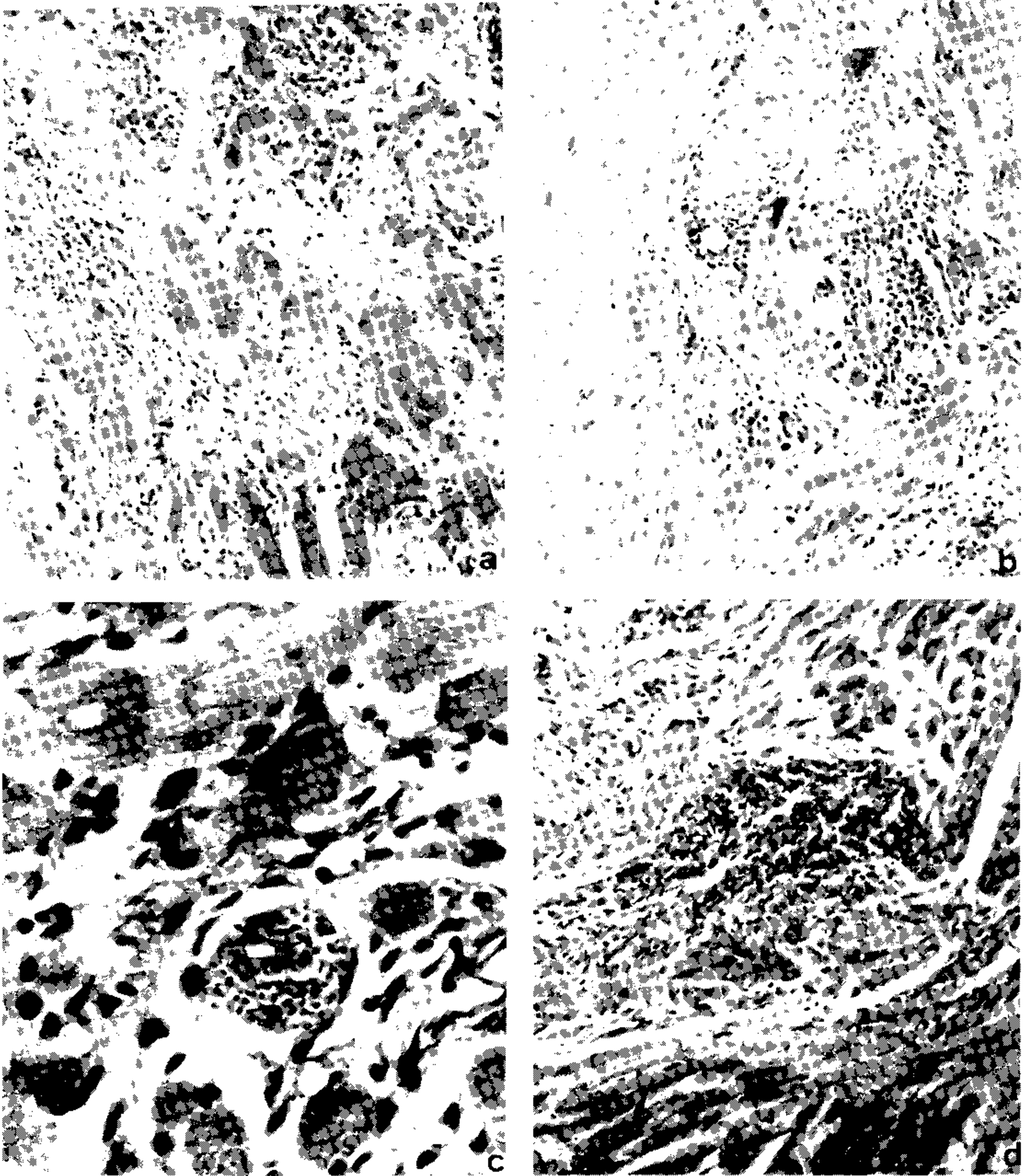


Fig. 1: *a* — chronic myocarditis with fibrosis; *b* — arteriolar lesion with perivascular mononuclear infiltration; *c* — amastigotes of *T. cruzi* into myocardic fiber; myocytolysis and mononuclear infiltration; *d* — necrosis and mononuclear infiltration in the conduction tissue of the heart (His bundle) (arrow). H. & E. — *a*, *b*, *d*: 100x; *c*: 630 X.

TABLE II

Histological features of the cardiac lesions due to chronic *T. cruzi* infection in mice

No.	Strain* Ident.	Presence of Parasites	Inflammat. Process	Fibrosis
1	21 SF	-	++	+
2	21 SF	-	++	++
3	21 SF	-	+	+
4	21 SF	-	+	+
5	21 SF	-	+	+
6	21 SF	-	+	+
7	21 SF	-	+++	+
8	21 SF	-	+	+
9	Mambai	-	+	+
10	Mambai	-	+++	+
11	PMN	+	+++	++
12	PMN	+	+	+
13	Bolivia	+	+	+
14	Bolivia	-	+	+

* 1 to 10 - type II - strains; 11 to 14 - type III - strains.

degenerated amastigotes of *T. cruzi* in the cytoplasmic phagosomes (Fig. 3a, b). Lymphocytes and plasma cells were rarely seen. Numerous fibroblasts with signs of intracellular synthesis (endoplasmic reticulum dilatation and transport vesicles) were present. Myofibroblasts were occasionally seen with evident basal membrane and cytoplasmic microfilaments (Fig. 5a, b). The enlarged interstitial space contained inflammatory cells which appeared oedematous and with collagen deposits. Two different aspects of the interstitial matrix were seen: 1) abundant microfibrils occasionally associated with isolated collagen fibrils or small collagen bundles. 2) irregular microfibrils with dense amorphous irregular matrix component, that suggested a degradation of matrix deposit. In these areas macrophages containing intraphagosomal collagen fibrils were seen. A newly formed extracellular matrix showed a large predominance of a loose connective matrix organization (LCMO) where collagen is present in small bundles of thin fibers mixed with electron dense basement membrane-like deposit (Grimaud et al., 1980).

Vascular alterations were prominent and some vessels exhibited a fibrinoid necrosis of their walls and extravascular fibrin deposition. Capillaries were frequently altered with perivascular oedema and irregular basement membrane, loss of intercellular junctions and prominent endothelial cells. Platelets aggregation was noted inside small interstitial vessels.

Myocardial cells showed different degrees of injury, associated or adjacent to inflammatory infiltration including moderate myofibril disorganization, mitochondria with clear matrix and/or fragmented cristae, endoplasmic reticulum vesiculation, increase of lysosomal or residual bodies and accumulation of pseudo-myelinic figures (Fig. 6b). Nuclear changes with nucleolar hypertrophy, irregular pattern of dense chromatin and intranuclear inclusions were present (Fig. 6a).

Ultrastructural study of the heart in normal controls failed to show any abnormality.

Collagen immunotyping - control animals: type I collagen (Fig. 7e) pattern distribution appeared as a thin interstitial homogeneous and regular deposit with a sarcolemmal and perivascular distribution; type III and pro-III collagen appeared as a thin peri-muscular deposit with low intensity fluorescence. Type IV collagen (Fig. 8a) was distributed around vessels, lining endothelium and adventitia as well as thin delicate deposits lining the myocardial cells.

Chronically infected mice: type III collagen (Fig. 7a, b, c, d) revealed an interfascicular distribution with focal condensation and variable degree of fluorescence; using antiprocollagen III an intense fluorescence in the interstitial space, with dense and homogeneous deposits was seen in arteriolar walls, as well as in focal areas of cardiac fibrosis. Type IV collagen (Fig. 8b, c, d, e, f) showed a thin and diffuse interstitial distribution with focal areas of thickening. Arteriolar and venous vessels showed intense parietal fluorescence, with focal deposits in the media of some arteriolar sections. Conduction tissue of the heart showed a diffuse deposit of type IV collagen in some animals (Fig. 8c, d).

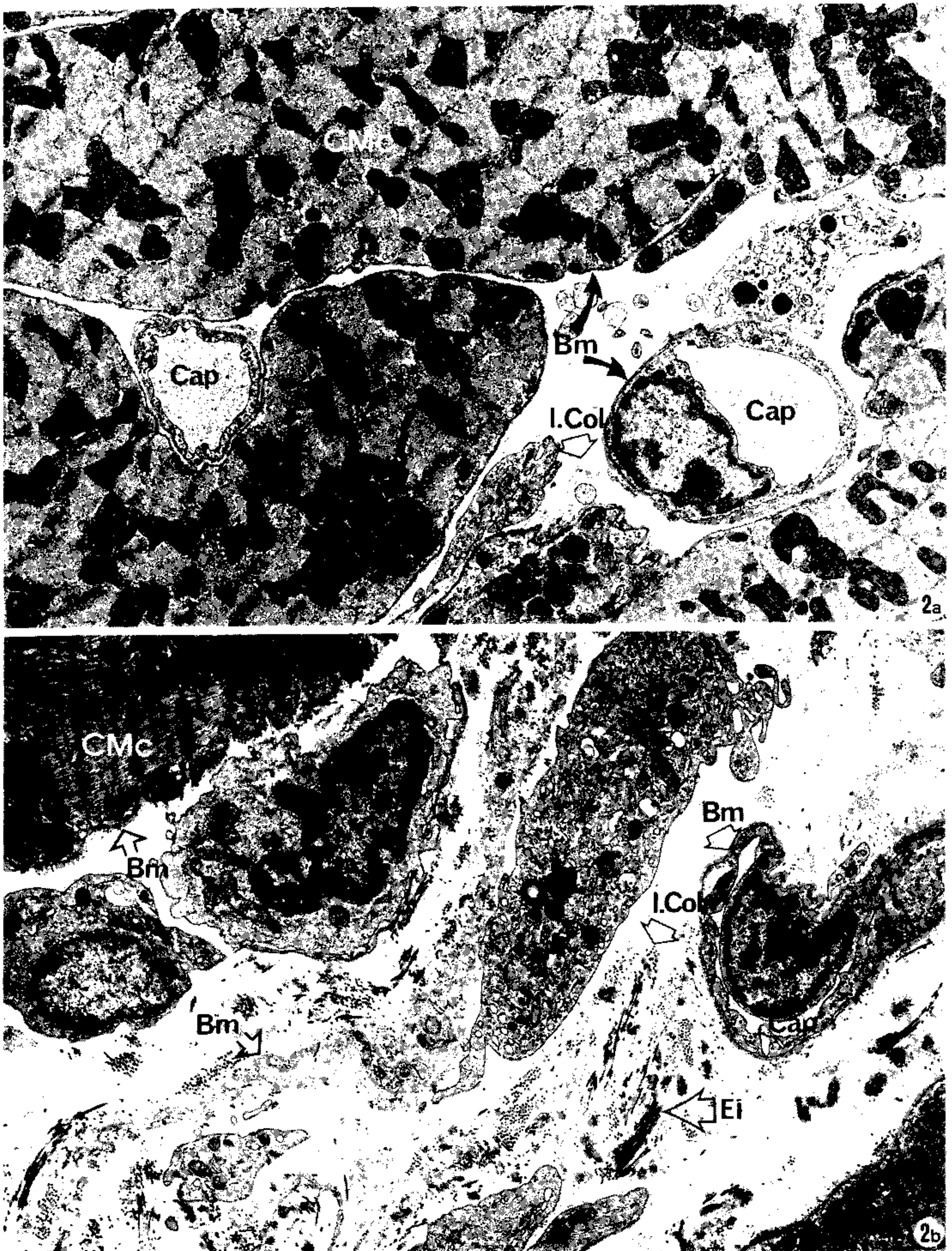


Fig. 2a: electron microscopic view of normal cardiac muscle cell (CMc) showing interstitial space with normal capillary (Cap); interstitial collagen (I. Col.) and well preserved basal membrane, both in capillary wall and cardiac cell (Bm). 2670 X. Fig. 2b: interstitial myocardial space in chronically infected animal, showing dissociation of cardiac myocells by mononuclear inflammatory cells. Dissociation of muscle basal membrane (Bm) and presence of inflammatory cells in the space between the muscle and the basement membrane. I. Col. Interstitial collagen deposit. 3250 X.

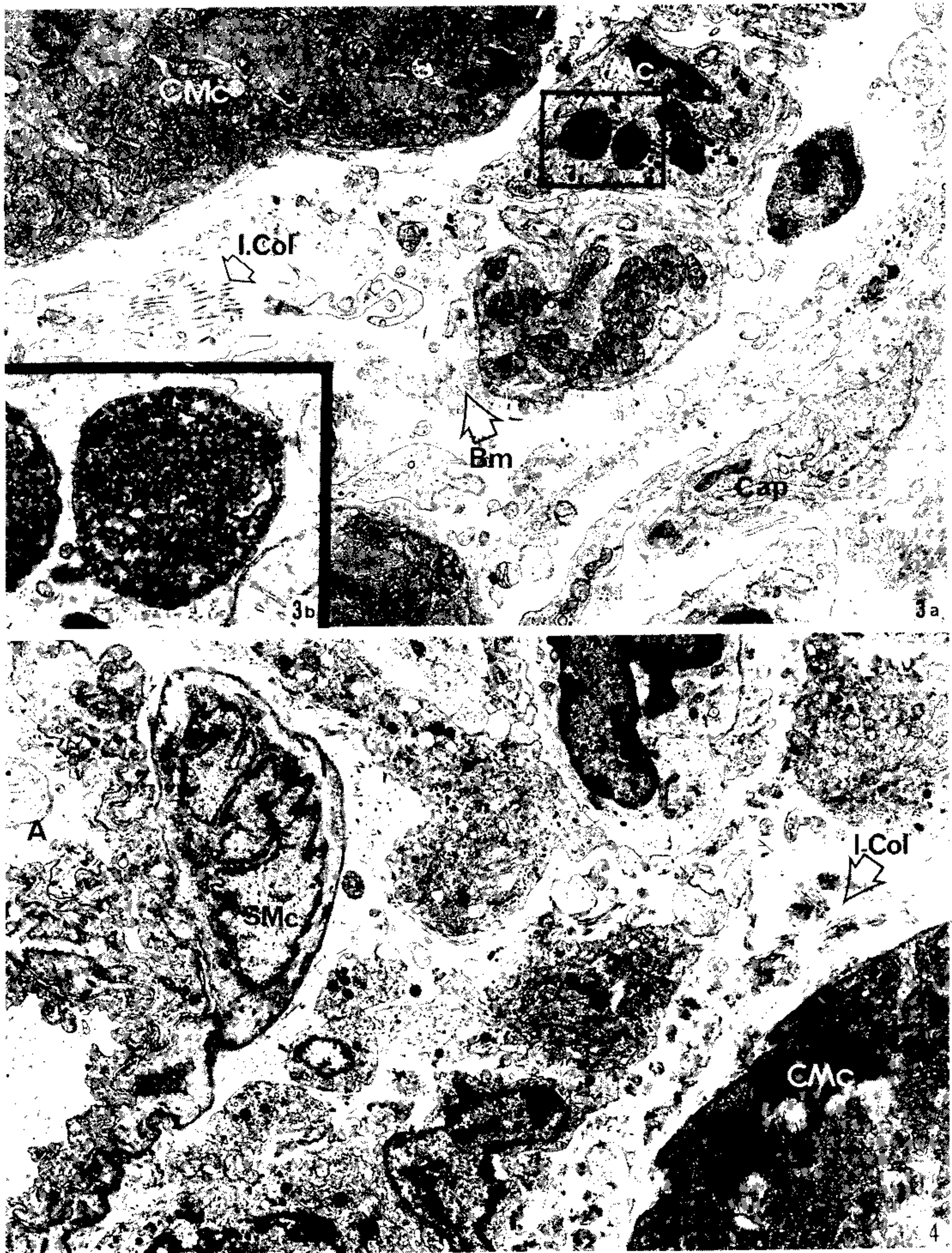


Fig. 3a: dissociation of cardiac myocells by cellular accumulation and collagen deposit (I. Col.); macrophage (Mc) showing phagocytosed amastigotes (insert). Thickening and duplication of basal membrane (Bm). 4.000 X. Fig. 3b: detail of phagolysosome with degenerate amastigotes. 21.500 X. Fig. 4: enlargement of the interstitial space of myocardium in chronically infected mouse: Perivascular infiltration with macrophages, fibroblasts and collagen deposit (I. Col.); Cardiac cell (Cmc) with densification of myofibers; Smc: smooth muscle cell of arteriolar wall; A – arteriolar lumen. 5.000 X.

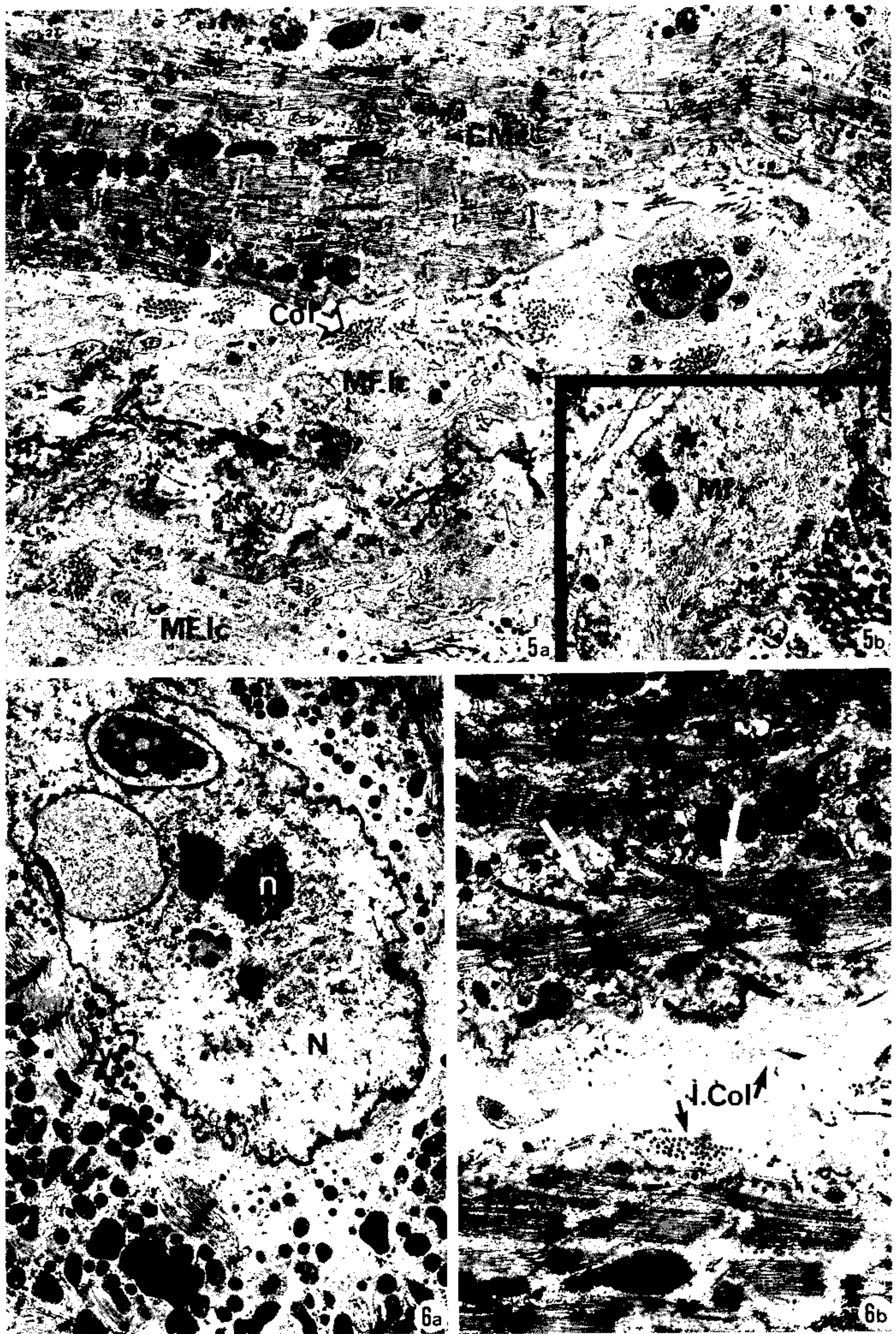


Fig. 5a: dissociation of cardiac myocells (CMc) by the presence of the cellular expansions of a myofibroblast containing numerous microfilaments in the cytoplasm (MF.c) and extracellular deposits of fibrin and collagen (Col.). 3250 X. Fig. 5b: details of the myofibroblast cytoplasm (Mf). 6300 X. Fig. 6a: cardiac myocell showing nuclear (N), alterations, with dense nucleoli (n), irregular membrane and intra nuclear pseudo inclusions. Dilatation of perinuclear area with increased number of lysosomes. 5000 X. Fig. 6b: cardiac cell alterations, with disorganization of myofibrils (arrows) and dilatation of endoplasmic reticulum; interstitial deposits of fibrilla collagen (arrows). 6300 X.

Distribution of type I collagen was not generally affected in chronically infected mice. However, a slight interstitial deposit of type I collagen was noted in small foci and/or associated with vascular areas (Fig. 7f).

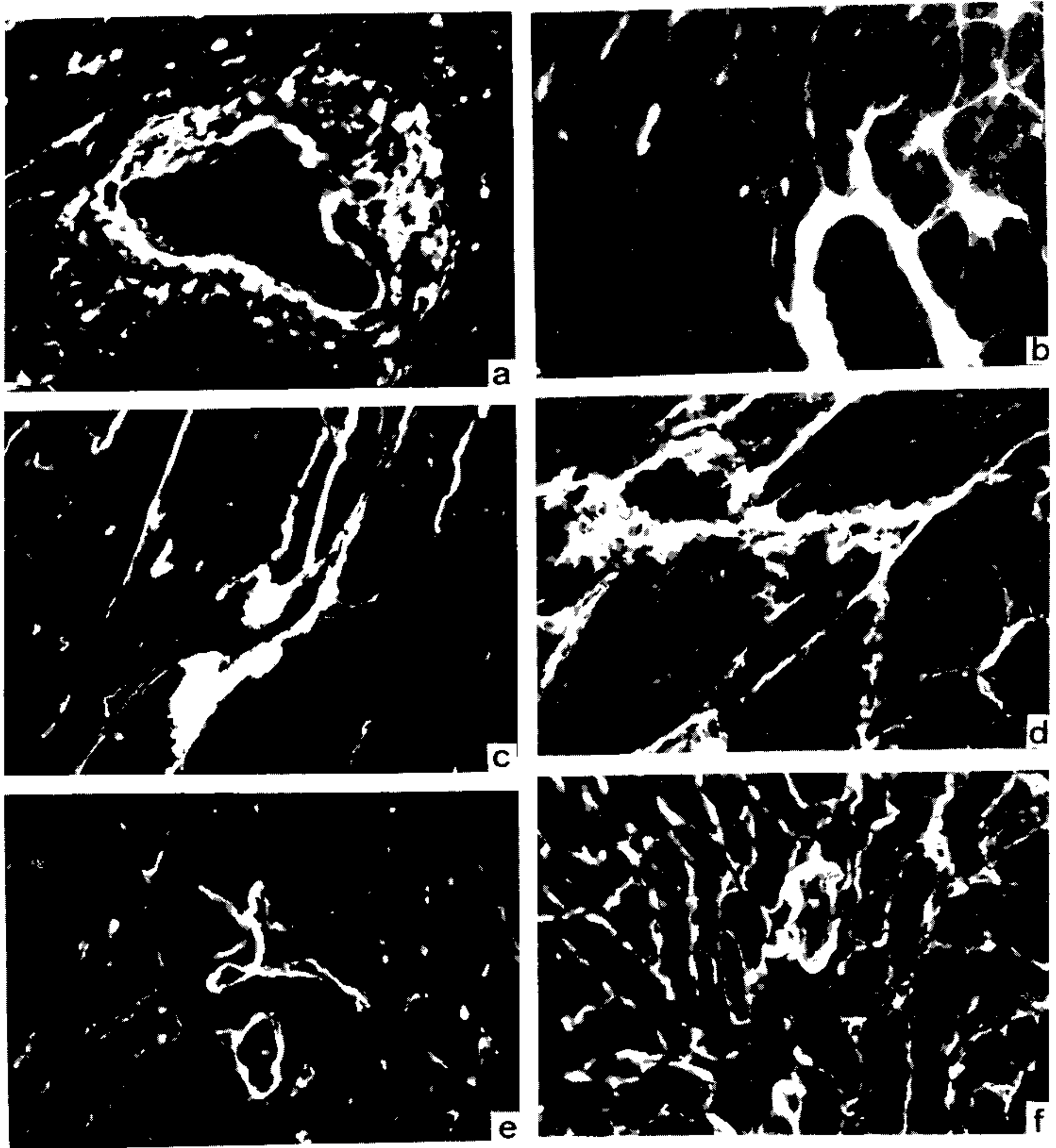


Fig. 7a: Pro III – collagen as an intraparietal deposit in a small artery in an infected mouse. 400 X. Fig. 7b: small vein showing intraparietal Pro III collagen; cardiac muscle of infected mouse. 400 X. Fig. 7c: interstitial dense deposit of Pro III collagen in chronically infected mouse. 250 X. Fig. 7d: interstitial loose distribution of Pro III collagen in cardiac muscle of infected animal. 400 X. Fig. 7e: type I collagen labelling in a control mouse. 250 X. Fig. 7f: focus of slight deposit of type I collagen in infected animal. 250 X.

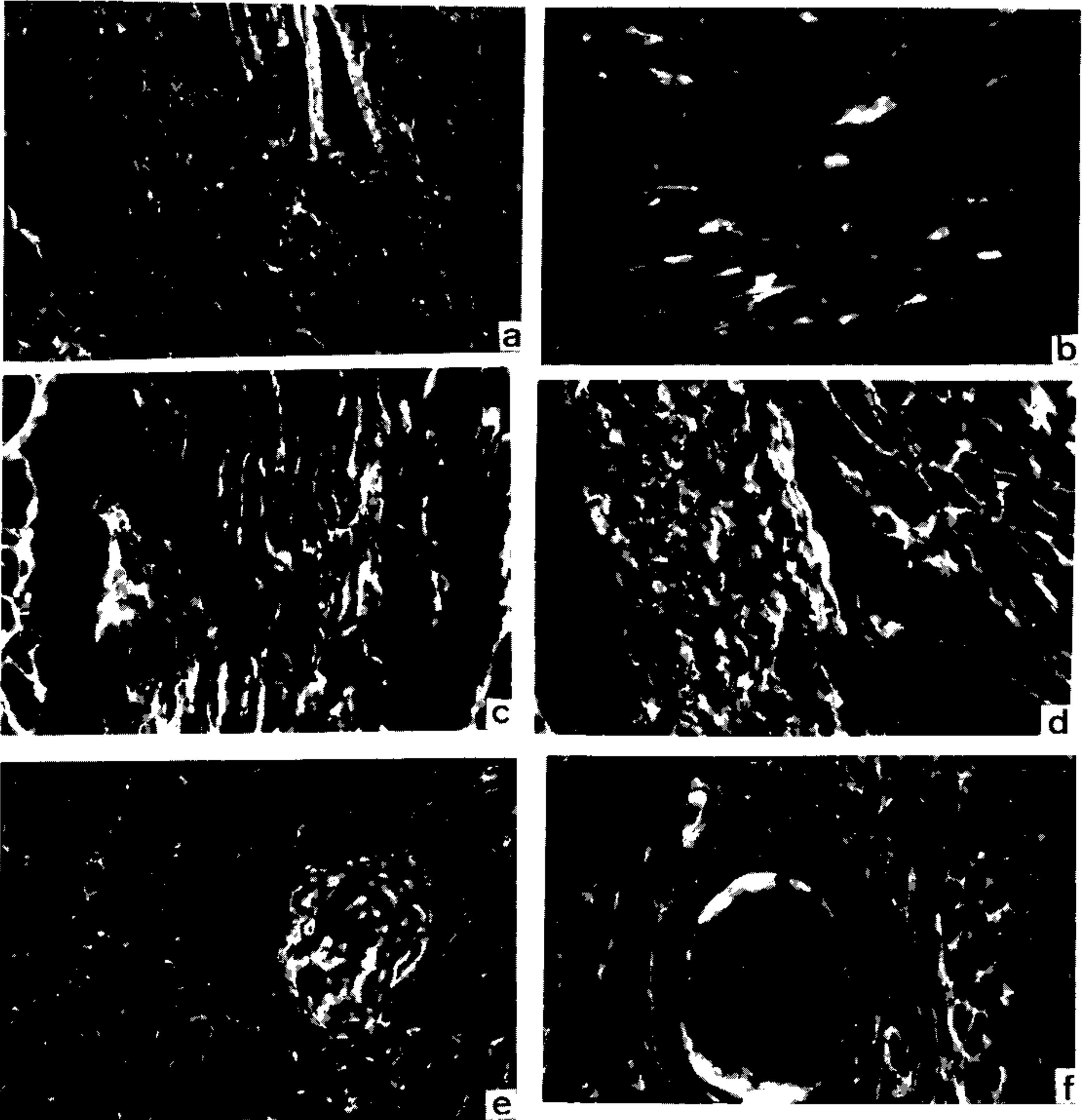


Fig. 8a: control mouse type IV collagen immunotyping of cardiac muscle. 100 X. Fig. 8b: chronically infected mouse – Heart – Type IV collagen deposit on pericapillary areas and interstitial spaces. 250 X. Figs. 8c-d: type IV collagen deposit around myofibers of the conduction tissue in chronically infected mouse. 250X. Fig. 8e: small arteriole showing a diffuse intraparietal type IV collagen labelling. (Cardiac muscle – chronically infected mouse) 100 X. Fig. 8f: small intramyo-cardial vein with dense irregular deposits labelled with anti-type IV collagen antibodies, in chronically infected mouse. 100 X.

COMMENTS

The spontaneous survival of several infected mice, with high inocula of virulent strains of *Trypanosoma cruzi*, provides an example of the chronic phase of Chagas' disease, thus confirming previous studies indicating that apparently healthy animals, previously infected, actually develop a progressive disease during prolonged infection (Andrade & Andrade, 1968; Andrade & Andrade, 1976). In spite of low parasitemias, all animals showed high titres of specific antibodies. The description by Federici et al. (1964) of the lesions produced in mice by the Colombian strain of *T. cruzi* has shown that prolonged survival of infected mice can be obtained by the use of strains biologically adapted to a progressive evolution in the experimental animal. With the same model, Kumar et al. (1969) gave a detailed description of the pathological lesions determined in C3H mice by the Colombian strain, with cardiac dilatation, intracardiac thrombosis, myocarditis and

fibrosis, and compared these aspects with those from human hearts. Rossi, Gonçalves & Ribeiro dos Santos (1984) using this same strain of *T. cruzi* as a challenge, in mice previously inoculated with epimastigotes of the PF strain, showed a cardiomyopathy with focal areas of myocytolytic necrosis, inflammatory infiltration and interstitial fibrosis. The Colombian strain, used by us in previous studies of the chronic infection (Andrade & Andrade, 1968; Andrade & Andrade, 1976), was characterized morphobiologically as Type III, the same type as PMN and Bolivian strains, used in this study. Type II strains (Mambáí and 21 SF) proved to be also adapted to provide a prolonged survival of infected mice, since, characteristically they determined a slow course of parasitemia and relatively low mortality, in the acute phase of the infection.

In the present study, both Types II and III strains led to varying degrees of myocardial inflammation in the infected animals, that varied from mild to intense, independently of the presence of tissue parasitism. Focal lesions dependent on the rupture and necrosis of parasitized fibres, seem to be an important factor in the intensity of the inflammatory process and could be considered as part of the pathogenic mechanism of chronic myocarditis. Some other findings indicate that a delayed type hypersensitivity mechanism is involved, as has been suggested by several authors (Andrade & Carvalho, 1969; Montufar et al., 1977; Roberson, Hanson & Chapman, 1973; Schimunis et al., 1971; Scott, 1981). Chronic myocarditis can be associated with an immunocellular mechanism as shown by the parasite independent diffuse and focal mononuclear infiltration the cellular composition with predominance of macrophages and the association with ultrastructural alterations of non-parasitized cardiac myocells.

The irregularity of the inflammatory process, even in animals infected with the same strain, indicate that individual response is important in determining the outcome of the hypersensitivity reaction. Scott (1981) showed that in mice chronically infected with *T. cruzi*, there are antigen-specific suppressor cells which inhibit a delayed hypersensitivity response to *T. cruzi* antigens but not to an unrelated antigen. According to this author, intracellular parasites, persistent in chronic infection, induce suppression of cell mediated reaction. This could represent a mechanism whereby intracellular parasites avoid immune destruction and could be compatible with prolonging parasite survival and reducing pathological lesions in the host. By treating with low doses of cyclophosphamide, a procedure assumed to destroy suppressor T cells, Andrade et al. (1984) demonstrated severe chronic diffuse and progressive myocarditis in dogs in the asymptomatic phase of the infection.

The humoral response is also intense in those mice as indicated by the high titers of haemagglutination and by immunofluorescence antibodies. Independently of the immunological response, the interstitial infiltration, oedema and collagen deposits dissociates the cardiac cell from the capillary wall and, together with the necrotic arterial lesions contribute to the intensification of the cardiac lesions. Platelet thrombi were rarely seen in our cases, but, according to other authors (Rossi, Gonçalves & Ribeiro dos Santos, 1984) they can represent a factor in the myocardial lesion.

Collagen deposits were a constant finding in the presence of fibroblasts with endoplasmic reticulum hyperplasia. Immunolabelling for type III, pro-III collagen and type IV, appears as a constant finding. It is known that a marked difference exists in the susceptibility to enzymatic attack, between collagen types, i.e. type I collagen is resistant to proteolytic attack by non-specific proteases while collagens types III and IV contain sites susceptible to non-collagenolytic enzymes (Rojkind, Giambone & Takahashi, 1982). It is probable that the newly deposited matrix in chronic murine Chagas' disease appears less stable and more susceptible to the post inflammatory remodeling process. On the other hand, the importance of a permanent post inflammatory fibrous scar can not be evaluated in our model, since numerous inflammatory cells are still present. The active process of fibrosis seems to be correlated with this inflammatory process and the morphological aspects of the myocarditis indicates that the macrophages are the main cells involved in the fibroblastic stimulation. The influence of macrophages on fibroblastic proliferation during wound repair has been demonstrated *in vivo* by depleting the macrophages in the inflammatory process with anti-macrophage serum (Leibovich & Ross, 1975) and *in vitro* by demonstration of a macrophage-fibroblast stimulating factor (Leibovich & Ross, 1976). It has been demonstrated that in the delayed hypersensitivity process there is an enhanced collagen synthesis (Boros, Landle & Carrick, 1981) and according to Wahl, Wahl & MacCarthy (1978), lymphocytes may produce lymphokines, that stimulate proliferation and collagen synthesis by fibroblasts.

The fibrous matrix, seen at the ultrastructural level appeared similar to the "loose connective matrix organization" described by Grimaud et al. (1980). It is characteristically formed by young and reversible collagen and there was evidence of degradation and phagocytosis by macrophages (Perez-Tamayo, 1978).

From the morphological standpoint, chronic cardiac lesions produced in mice by *T. cruzi* infection, seem to fulfill the requirements for a suitable model of chronic Chagas' disease, since they are a reproduction of the main aspects described in the human cardiomyopathy (Andrade, 1983). Further studies are necessary to clarify the immunological and pathogenetic aspects of this chronic model.

RESUMO

Utilizando o modelo experimental do camundongo, foi realizado um estudo sorológico, histopatológico e ultraestrutural bem como a imunotipagem do colágeno na matriz conjuntiva do miocárdio em camundongos suíços cronicamente infectados com as cepas 21 SF e Mambai (Tipo II), PMN e Bolívia (Tipo III) por períodos de 154 a 468 dias. Os testes sorológicos e de imunofluorescência indireta mostraram altos títulos de anticorpos específicos. O estudo estrutural definiu melhor a constituição celular do infiltrado inflamatório, mostrando a predominância de monócitos e de macrófagos com intensa atividade fagocítica, fibroblastos em atividade de síntese e miofibroblastos bem como abundante matriz colagênica sugerindo uma associação entre o processo inflamatório e a fibrogênese na cardiomiopatia chagásica crônica. A imunotipagem do colágeno mostrou a predominância dos tipos III e IV. Alterações dos capilares sanguíneos e de arteríolas e sua dissociação das miocélulas, pelo infiltrado inflamatório, se relacionam com alterações ultraestruturais em miocélulas cardíacas não parasitadas. Havia intensificação do processo inflamatório em áreas focais correspondentes à rotura de fibras cardíacas parasitadas. Os achados do presente trabalho sugerem que o modelo do camundongo é adequado para o estudo dos mecanismos patogênicos na doença de Chagas, pela utilização de cepas do *T. cruzi* com baixa virulência e alta patogenicidade.

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