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EXPERIMENTAL CHAGAS' DISEASE IN DOGS

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This paper describes the development of experimental Chagas' disease in 64 out-bred young dogs. Twenty-nine animals were inoculated with the Be-62 and 35 with Be-78 Trypanosoma cruzi *strains. Twenty-six were infected with blood trypomastigotes by different inoculation routes and 38 with metacyclic trypomastigotes from the vector via the conjunctival route.*

Twenty of the 26 dogs infected with blood trypomastigotes were autopsied during the acute phase. Eleven died spontaneously and nine were sacrificed. Six remained alive until they died suddenly (two) or were autopsied. (four). Twelve of the 38 dogs infected with metacyclic trypomastigotes evolved naturally to the chronic phase and remained alive for 24-48 months.

The parasitemia, clinical aspects and serology (IgM and IgG) as well as electrocardiogram, hemogram and heart anatomo-histopathologic patterns of acute and chronic cardiac forms of Chagas' disease as seen in human infections, were reproduced.

The most important finding is the reproductibility of diffuse fibrosing chronic chagasic cardiopathy in all dogs infected with Be-78 T. cruzi *strain autopsied between the 90th and 864th days of infection.*

Thus, the dog can be considered as a suitable experimental model to study Chagas' disease according to the requisites of the Word Health Organization (1984). Futhermore the animal is easily obtained and easy to handle and maintain in experimental laboratory conditions.

Key words: Chagas' disease – experimental model – dog

Chagas' disease remains a serious public health problem in Latin America. The identification of an experimental model that reflects the human disease is of considerable importance and contitutes one of the priorities of the SWG (Scientific Working Group) on Chagas' disease (WHO, 1984). In this context, several mammals, including mice (Andrade & Andrade, 1976), rabbits (Teixeira et al., 1983; Ramirez, 1984), primates (Marsden et al., 1976; Falasca et al., 1986; Bonecini-Almeida et al., 1990), rats Alcântara, 1964; Revelli et al., 1980), guinea-pigs (Kosma et al., 1960; Lopes et al., 1969) and dogs (Andrade & Andrade, 1980) have been proposed. The canine model has some advantages since all phases of the disease have been reproduced in this animal (Johnson, 1938; Laranja, 1953; Köeberle, 1957; Okumura & Corrêa Neto, 1961; Anselmi et al., 1966). However, the development of the disease has not been reported to be typical of that which occurs in man (Andrade & Andrade, 1980; Andrade, 1984). Most importantly the diffuse fibrosing myocarditis, the most characteristic lesion in human chronic chagasic cardiopathy, has not been well reproduced spontaneously in dogs (Laranja, 1953; Andrade & Andrade, 1980).

In this paper, the natural development of Chagas' disease is studied in dogs over a extended period of time. The acute, indetermi-

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nate and chronic phases have been reproduced and special attention has been given to diffuse fibrosing cardiopathy.

Parasitological, clinical, electrocardiographic, serological and histopathological data were recorded. The results show that the dog is a suitable model to study Chagas' disease according to criteria of WHO (1984).

MATERIALS AND METHODS

Trypanosoma cruzi strains – Berenice-62 (Be-62) was isolated by Salgado et al. (1962) and Berenice-78 (Be-78) by Lana & Chiari (1986) 16 years later from the same patient who is considered to be the first clinical case of Chagas' disease described by Chagas (1909).

Animals – Sixty-four young out-bred dogs (five to 120 days old), born and maintened in laboratory conditions, were used. All dogs were negative for anti-*T. cruzi* antibodies and had normal hemogram and electrocardiographic tracings. Sixteen normal control dogs were observed in parallel.

Inoculum and route of inoculation – Twenty-six dogs were inoculated in different conditions with blood trypomastigotes isolated from albino mice. Thirt-eight dogs were inoculated with metacyclic trypomastigotes obtained from nymphs of *Dipetalogaster maximus,* via the conjunctival route. Thirty of them were inoculated with 2,000 trypomastigotes/kg body weight.

Fresh blood examination – From the first week of infection all dogs were examined daily for detection of flagellates. Parasitemia was quantified according to Brener (1962). Blood was collected from the marginal ear vein.

Hemoculture and Xenodiagnosis – These examinations were undertaken in dogs infected with metacyclic trypomastigotes at three-monthly intervals throughought the infection.

Hemoculture was performed according to Chiari et al., (1989) with 10 ml of heparinized blood collected asseptically.

Xenodiagnosis was carried out with ten 2nd instar nymphs of *D. maximus.* Nymphs were kept at 27 °C with 60% humidity. The whole intestinal contents were collected after 40 days in 0.2 ml of PBS (Phosphate Buffered Saline) 0.1 M, pH 7.2, and examined. If the result was negative, the material was seeded in LIT medium (xenoculture) according to Bronfen et al. (1989).

All dogs were observed daily in order to record clinical signs, rectal temperature, variation of body-weight and behaviour. Dogs inoculated with metacyclic trypomastigotes were evaluated weekly by electrocardiogram (ECG), hemogram and serological tests. Blood was collected by puncture of the femoral vein.

Electrocardiographic records – The ECG was recorded from dogs anesthesied with 15 mg/kg weight of thionenbutal via the intravenous route. An electrocardiograph model ECG-04 FUNBEC, São Paulo, Brazil, was used. Electrocardiographic tracings were recorded with a standard electrocardiograph that measures cardiac vectors recorded on the limb leads (aVR, aVL, aVF) and V_{10} lead which explores thoracic lead. Traces were made at 50 mm/s and with a voltage of 1 mV standardized to 1 cm.

Serological tests – Indirect Immunofluorescence (IF) and Enzime Linked Immunosorbent Assay (ELISA) were carried out. The antigens employed in both techniques were obtained from the Y strain of *T. cruzi* cultivated in LIT medium and collected in the exponential phase of growth.

For IF tests the flagellates were fixed with 2% formalin. Dog IgM and IgG were obtained from Cappel Laboratories, USA. Anti-dog-gamaglobulins (IgM and IgG) were labelled with fluorescein isothiocyanate (Sigma Co.). Sera were diluted from 1/10 onwards, in sucessive two-fold dilutions. Alkaline antigen, extracted with 0.15% NaOH solution was used in ELISA tests. Conjugate was labelled with peroxidase (Sigma Co.). Sera were diluted from 1:320 and the results expressed as absorbance at 492 nm (Biorad, 2550).

Assessment of pathology – All dogs infected with blood trypomastigotes were sacrificed and autopsied in different periods of the acute or chronic phases (3rd, 9th, 11th, 18th, 28th and 29th months). Dogs infected with metacyclic trypomastigotes were autopsied only when they died spontaneously. Twelve remained alive and were evaluated at regular intervals of three months.

Complet autopsies were performed but only heart material will be considered in this paper. The organ was totaly fixed in 10% buffered formalin, pH 7.2, then washed with saline. The atrium, ventricle fragments and interventricle septa were processed by routine methods. Sections were 4μ thick and stained by hematoxilin-eosin and Gomory's trichromic. Some fragments were stained by sirius-red and examined with polarized light microscopy to differentiate collagen types (Junqueira et al., 1979; Montes et al., 1980).

RESULTS

Clinical aspects – The following were observed: elevation of rectal temperature (sometimes up to 40 °C), diarrhea, loss of bodyweight, anorexia, ascites, enlargement of lymphnodes, hepatomegaly, splenomegaly, dyspnea and paraplegia. Some dogs showed total paralysis which was sometimes, reversible. Signs of heart involvment were confirmed by ECG. Seventeen of the 38 dogs infected with metacyclic trypomastigotes by the conjunctival route, showed uni or bilateral ocular edema with lacrimation. All signs disapeared after two or three months of infection, except in one dog that displayed a permanent clinical picture of congestive heart failure and which was autopsied on the 90th day of infection. The remaining infected dogs (12) were apparently in good health during the later phases of infection.

Electrocardiographic changes – During the acute phase, 80% of infected dogs displayed ECG alterations when compared to normal controls. The following prevalence was recorded: decrease voltage of the QRS complex (65.7%); disturbance of repolarization (57.1%); axis deviation, supra ventricular indeterminate rhythm and grade I AV block (11.4%); displacement of J point (8.6%); complete right bundle branch block (CRBBB) with left anterior hemiblock (LAH) (2.3%). Approximatelly three months after infection, the ECG showed tendency to normalize. Four of the twelve dogs (33%) showed ECG patterns compatible with cardiac lesions six displayed slight alterations and two showed normal ECG in the later phase of the infection. During the chronic phase, 100% of the dogs displayed ECG alterations: 50% with displacement of J point; 41.7% with supraventricular indeterminate rhythm and grade I AV block; 33.3% with sinus arrythmia and a decrease of voltage of the QRS complex; 8.3% multifocal and unifocal ventricular extrassystoles and CRBBB with LAH (Fig. 1). These ECG changes showed a higher prevalence in dogs infected with Be-78 T. cruzi strain and usually were correlated with clinical evolution (Fig. 2).

Hematological changes – Alterations were observed only during the acute phase of infection. However they were slight in general and occured in both infected and control dogs.

Parasitemia and mortality – All dogs inoculated with either *T. cruzi* strain displayed

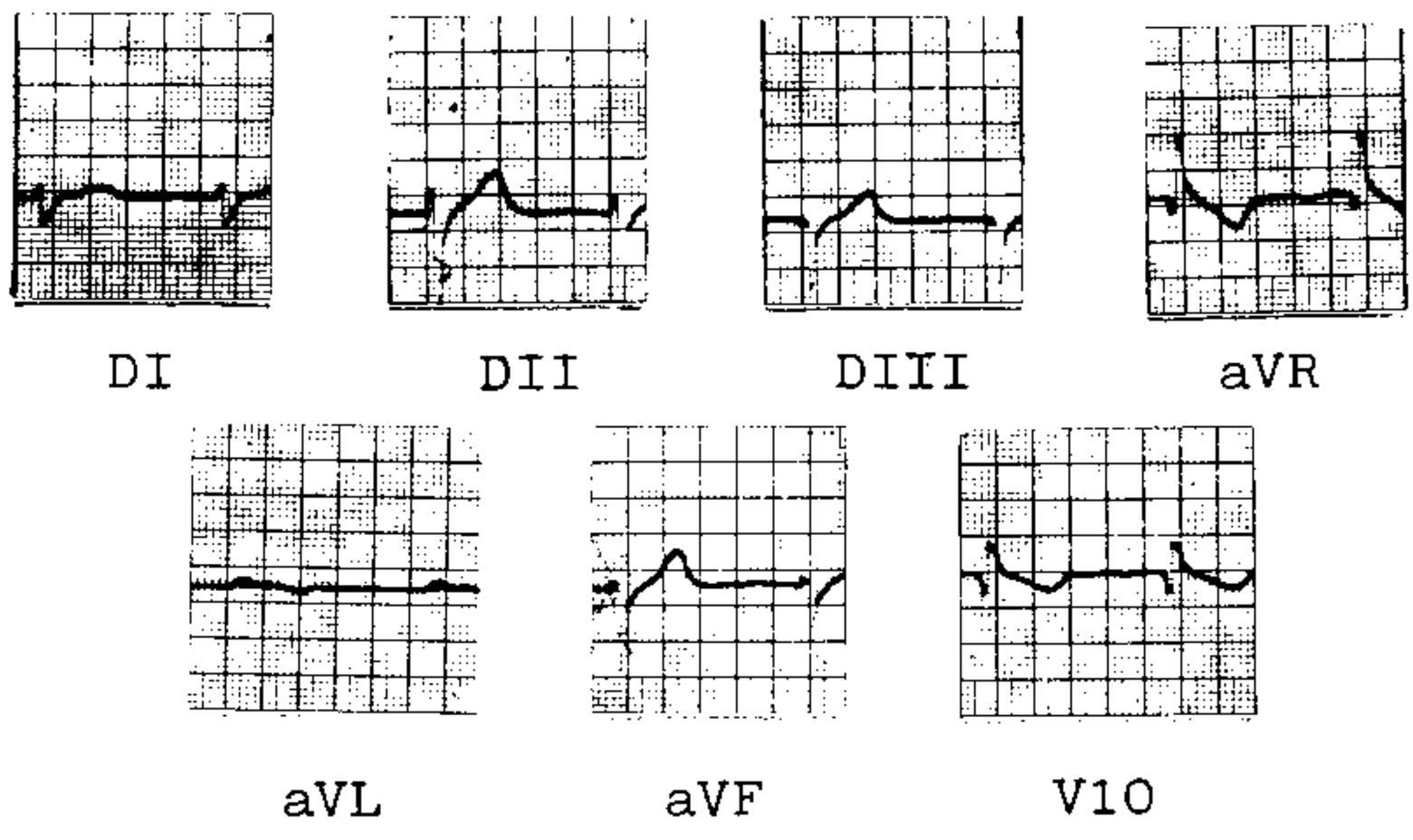

Fig. 1: ECG of dog 56, infected with Be-78 *Trypanosoma cruzi* strain showing left axy deviation, right bundle branch block (RBBB) with left anterior hemiblock (LAH). These alterations appeared in the 15th month of infection and remained present in all examinations.

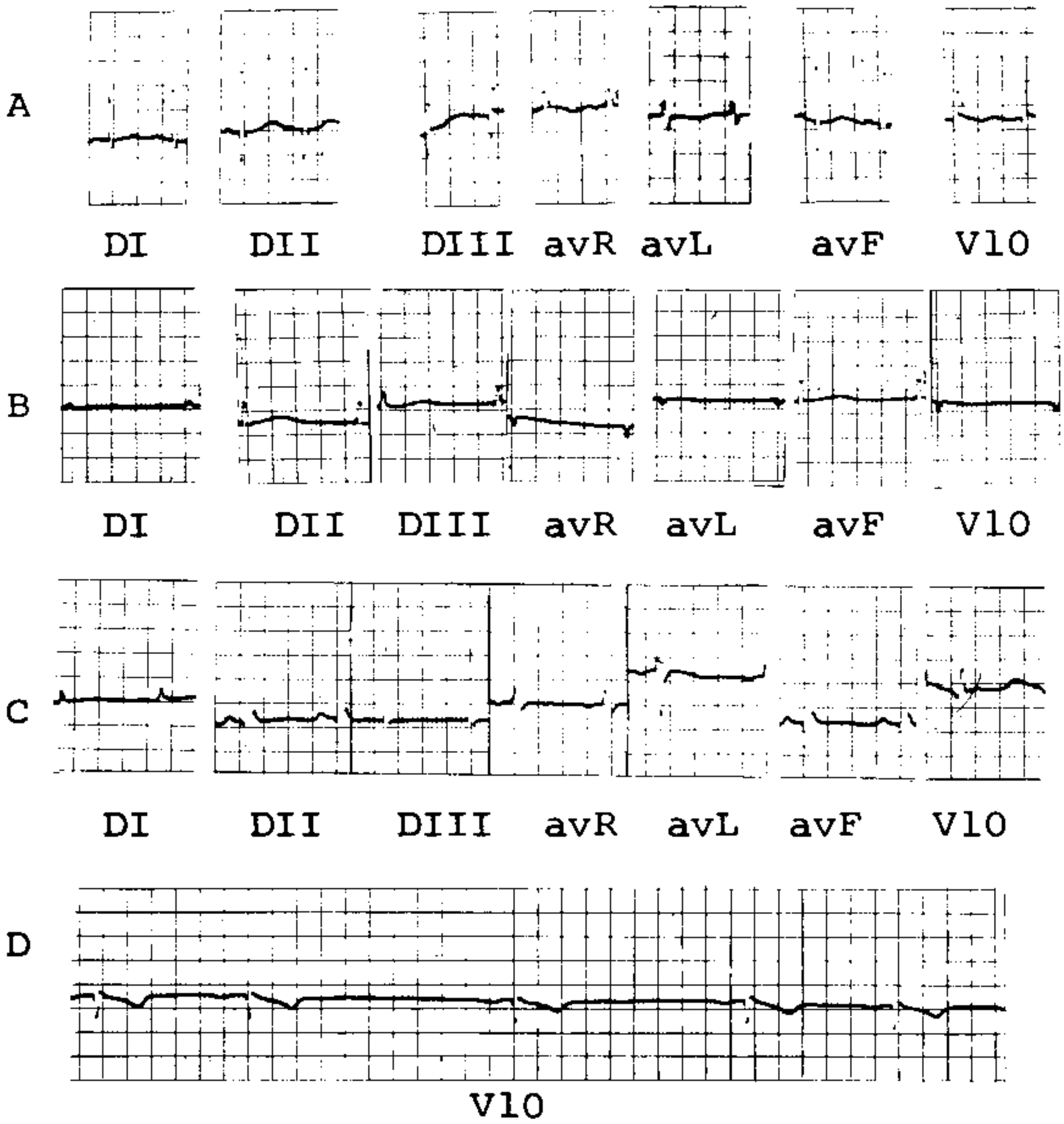

Fig. 2: ECG tracings of one dog before and after different periods of infection with the Be-62 *Trypanosoma cruzi* strain. A – Before infection: normal tracings. B – 28th day of infection: the more common alterations were disturbance of ventricular repolarization, left axis deviation, decrease of the QRS complex and decrease of heart rate. C – 9th month of infection: there is a tendency towards normality with improvement of voltage of the QRS complex and ventricular repolarization, sinus rhythm. D – 18th month of infection: evolution of alterations with signs of sinus arrythmias.

patent parasitemia in the acute phase of the disease. It was observed that dogs infected with Be-78 had a longer patent-period than those infected with Be-62 strain (Fig. 3).

It was not possible to assess accurately mortality rates of dogs infected with blood trypomastigotes because 13 of them were sacrificed at different times during the acute and chronic phases of infection for anatomical and histopathological observations. Two of the five dogs infected with Be-78 *T. cruzi* strain died suddenly in the 11th and 28th months of infection.

Dogs infected with metacyclic trypomastigotes were inoculated under the same conditions. The curves of parasitemia and rates of mortality during the acute phase are shown in Fig. 4. Mortality rates were higher in dogs infected with Be-78 strain (53.8%) than in animals infected with Be-62 (26.3%).

Humoral antibodies – Both tests detected IgM and IgG in all infected dogs in the first (ELISA) and fourth (IF) weeks of infection. The levels of these antibodies increased rapidly at the begining of infection. IgM began to fall earlier and disappeared in the 21st month or later. IgG decreased later and tended to stabilize two years after infection (Fig. 5). All groups of dogs showed similar profiles of immunoglobulins in both tests. Sera of control

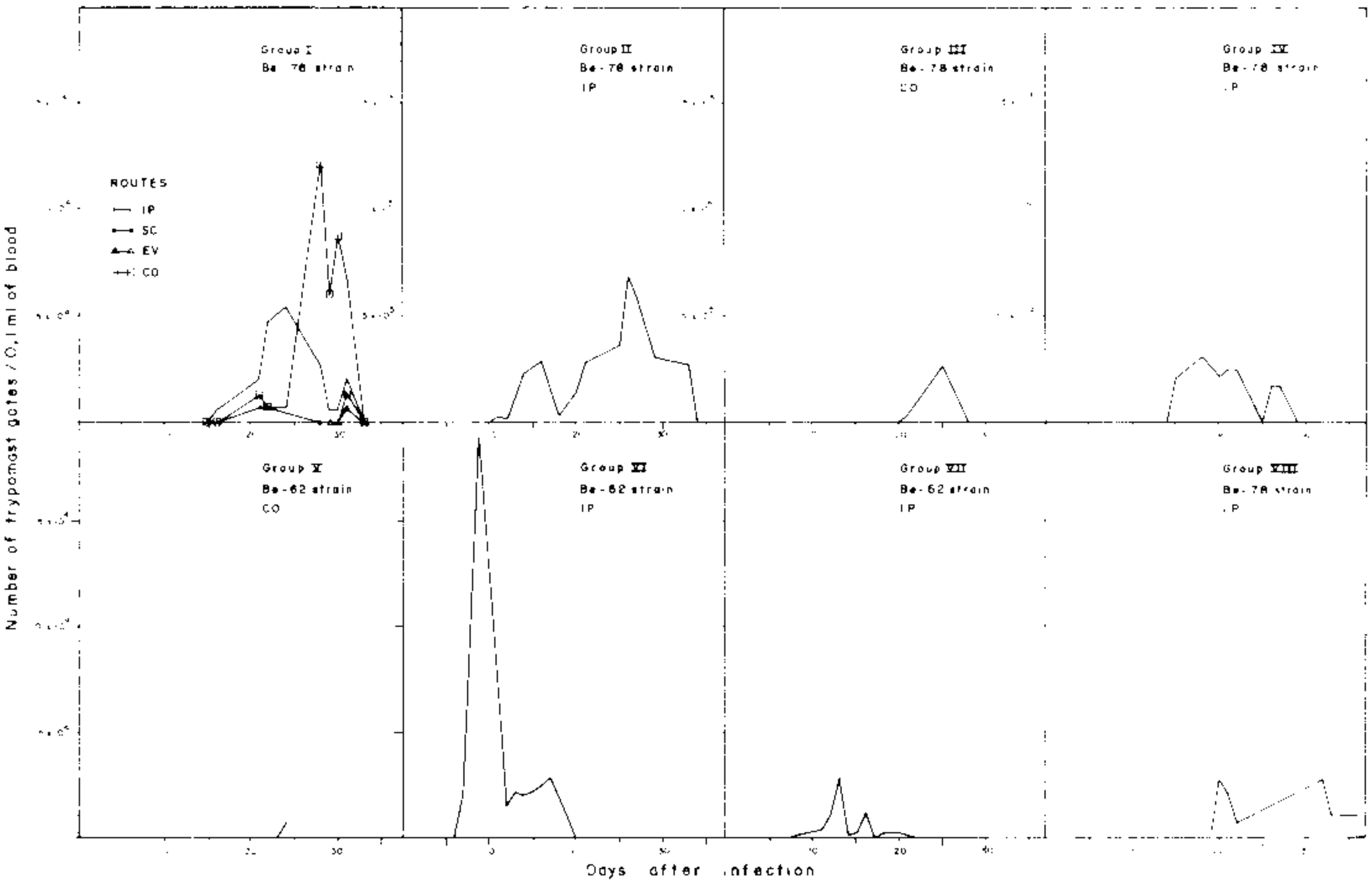

Fig. 3: curves of parasitemia of eight groups of dogs infected with blood trypomastigotes of the Be-62 and Be-78 *Trypanosoma cruzi* strains.

dogs were always negative. ELISA showed more sensitive than IIF test in detecting higher levels of IgM and IgG up two years of infection.

Autopsy findings – During the acute phase, dogs infected with blood trypomastigotes from Be-62 strain showed cardiomegaly, congestion, edema, dilatation of cardiac chambers (specially the right one) with consequent functional insuffiency of cardiac valves. In the chronic phase, the hearts showed slight congestion without signs of functional insufficiency. Dogs infected with Be-78 strain showed cardiomegaly, heart with globular shape with a tip formed by both ventricles, congestion, dilatation of cardiac cavities and atrio-ventricular connections with consequent functional insufficiency of cardiac valves. Hidropericarditis was generally moderate but intense in some cases. In the chronic phase, cardiomegaly and congestion were observed. In some dogs were present: chronic nodular epicarditis through the coronary arteries; fibrosing plaques in the epicardium, atrium and ventricles (Fig. 6); moderate hidropericarditis with intense dilatation of heart cavities and functional insufficiency of the cardiac valves.

Histopathological findings – During the acute phase, dogs infected with Be-62 strain showed moderate myocarditis in regular foci with predominance of mononuclear cells in the exudate, slight parasitism, moderate periganglioitis, neuritis and perineuritis of the cardiac plexus. In the chronic phase, no important heart histopathological changes were observed.

Dogs infected with Be-78 strain showed during the acute phase, myocarditis of variable degrees, intense parasitism of the myocardium and slight parasitism of the autonomous nervous system. The inflammatory exudate was diffuse or focal separating the muscular cells sometimes with phlogistic aspect. Foci of pericarditis and ganglionitis were present (Figs 7A, B). In the chronic phase, focal and diffuse productive chronic myocarditis, exudative and productive phenomena with intense epicarditis (Figs 8A, B) were seen. Sporadic presence of giant cells next to inflammatory nodules (Fig. 9A), ganglionitis, periganglionitis, neuritis, diffuse and focal periganglionitis with mononuclear cells, intense regressive phenomena of neurons and nervous fibers (Figs 9B, C) and Magarinos-Torres' lesions were present (Fig. 9D).

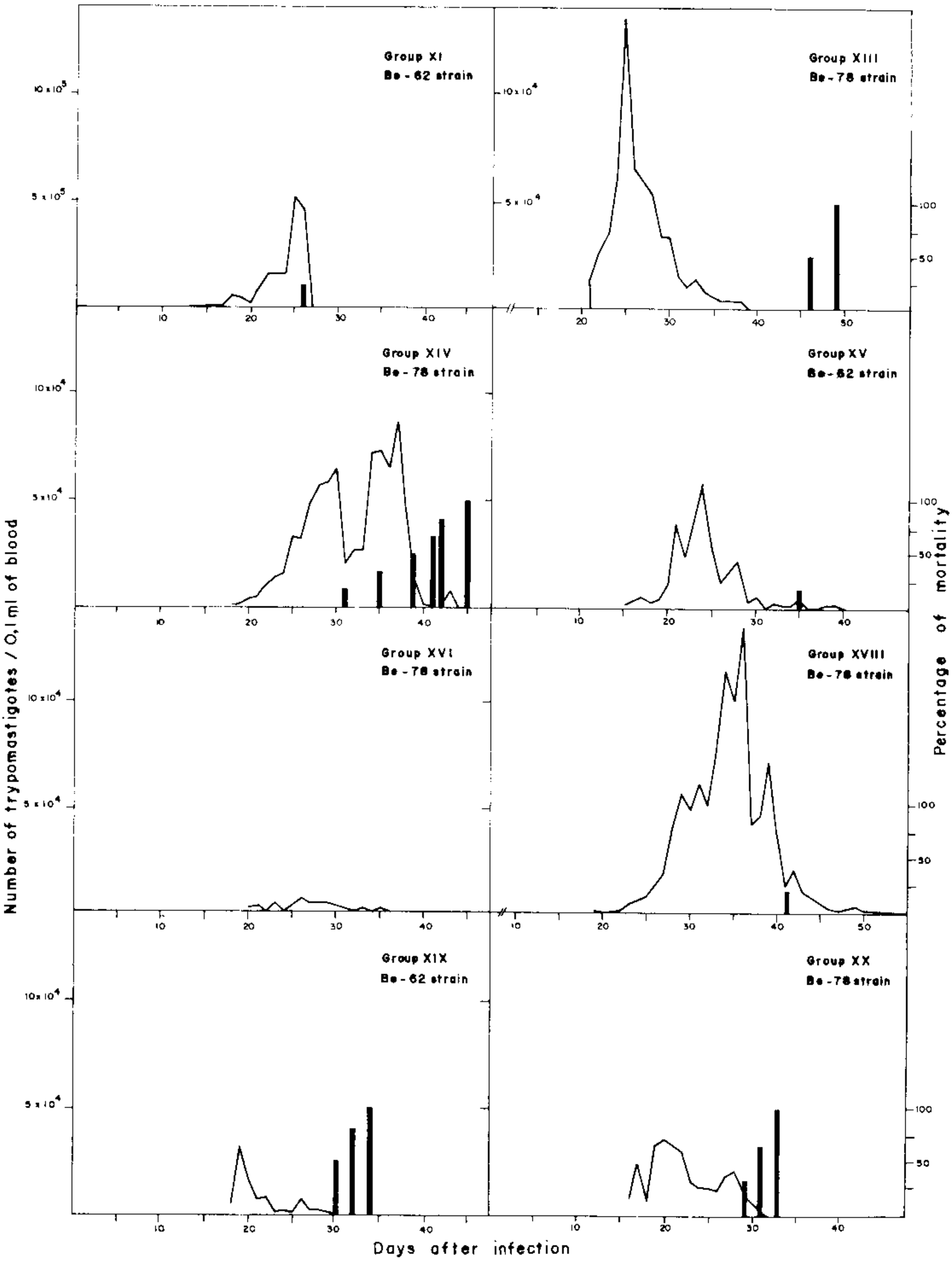

Fig. 4: curves of parasitemia and cumulative rates of mortality of eight groups of dogs infected with metacyclical trypomastigotes of the Be-62 and Be-78 *Trypanosoma cruzi* strains. Inoculum: 2000 trypomastigotes/kg body weight. Route: conjunctival.

The bundles of muscular cells were separated by fibrosis which showed predominance of collagens types I and III. This fibrosis was observed in all dogs (seven animals) infected with Be-78 strain autopsied between three and 29 months of infection, inoculated with either

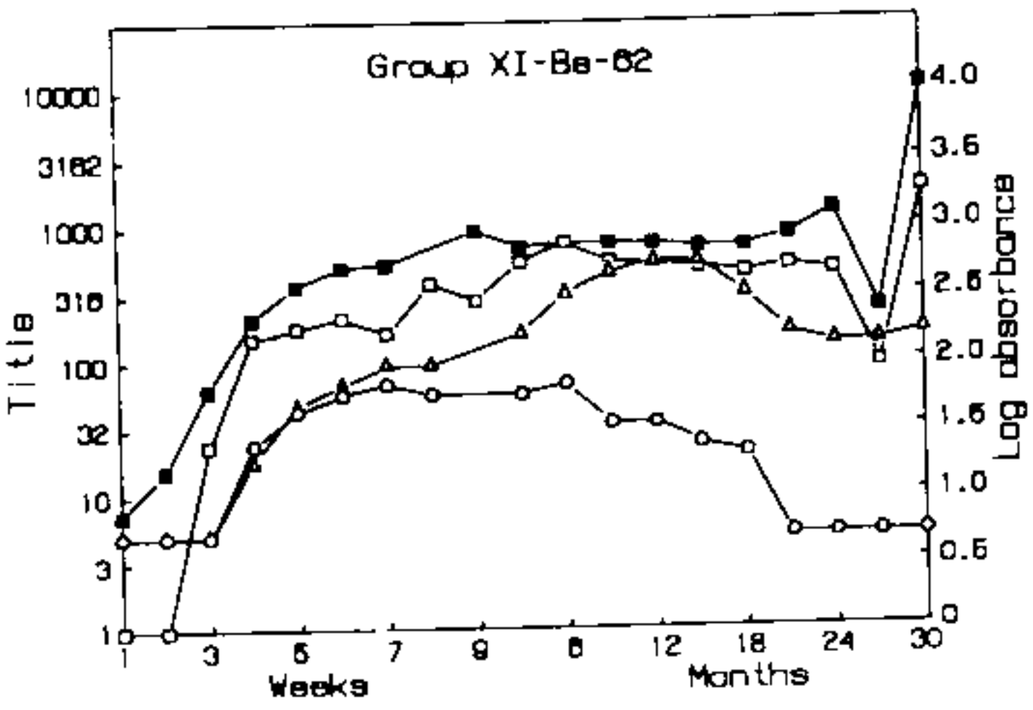

Fig. 5: IgM and IgG profiles of dogs experimentally infected with metacyclic trypomastigotes of the Be-62 and Be-78 *Trypanosoma cruzi* strains.

blood (five dogs) or metacyclic trypomastigotes (two dogs).

Parasitological examinations during the chronic phase – During the chronic phase xenodiagnosis did not detect parasites in dogs infected with Be-62 strain (0/7) but was positive in all animals infected with Be-78 strain (5/5) with different numbers of positive exams. However, the hemoculture done in parallel, was always negative in all dogs infected with either strains. The xenoculture detected

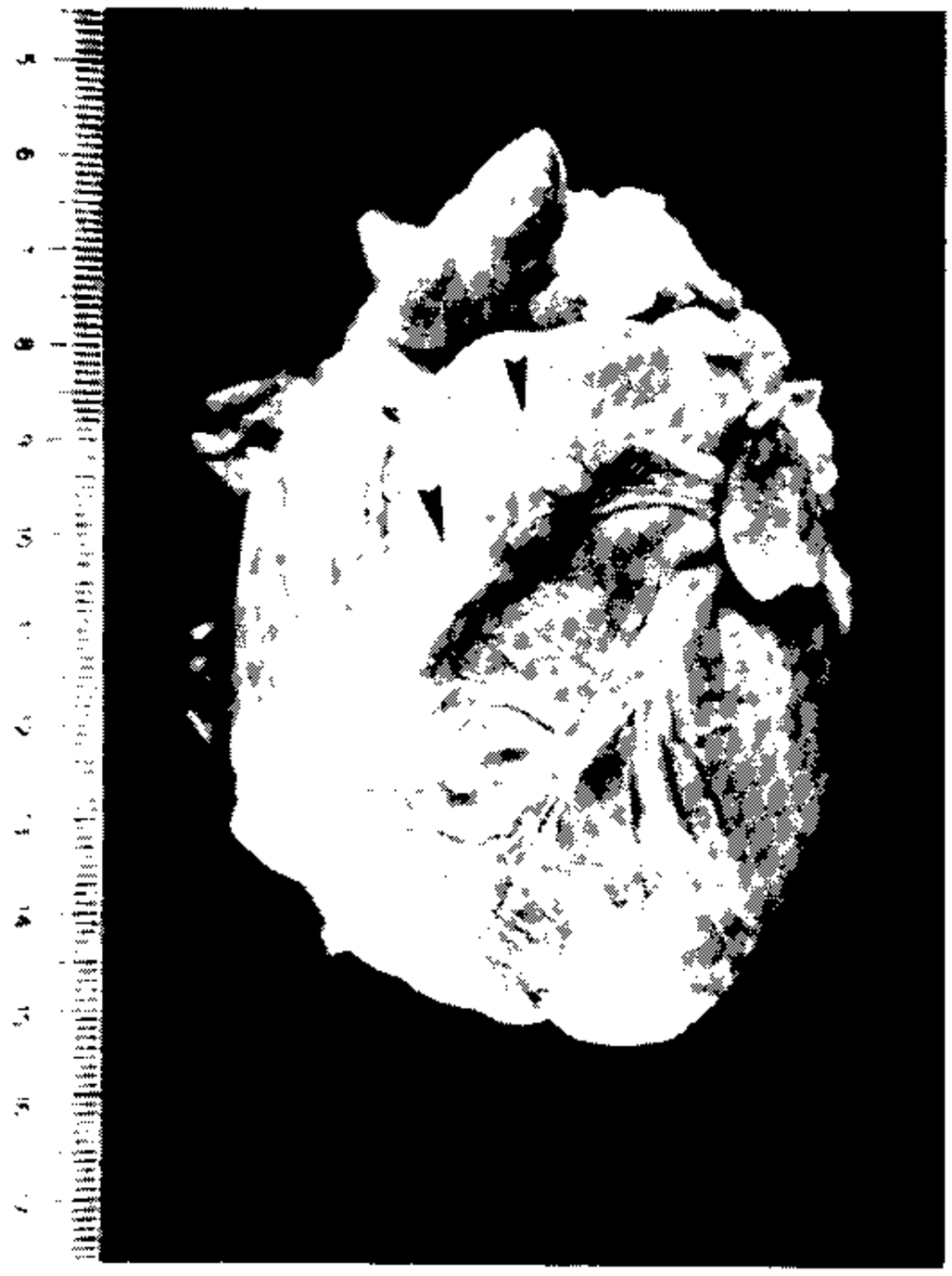

Fig. 6: heart of a dog infected with blood trypomastigotes of the Be-78 *Trypanosoma cruzi* strain autopsied in the 13th month of infection: cardiomegaly, congestion, epicardium granulous and with plaques (arrows).

parasites in only one dog infected with Be-62 in the third month of infection, and in all dogs infected with Be-78 strain with different numbers of positive exams.

DISCUSSION

The present study shows that acute Chagas' disease is easily reproduced in young dogs. The results agree with those of Goble (1952), Laranja (1953), Andrade & Andrade (1980). Parasitemia was detected by blood fresh examination in all dogs. Typical clinical signs of human acute phase of the disease were recorded in pratically all cases between the 20th and 45th months of infection. Hemograms showed slight and inconsistant alterations, not correlated with the infection.

Some dogs developed a severe acute disease and died after a few days. Electrocardiograms showed alterations correlated with the clinical picture observed by Laranja et al. (1948) in man and Laranja (1953), Anselmi et al. (1966), Andrade & Andrade (1980) in dogs. The ECG alterations were present in 80% of the dogs during the acute phase. If the dog did

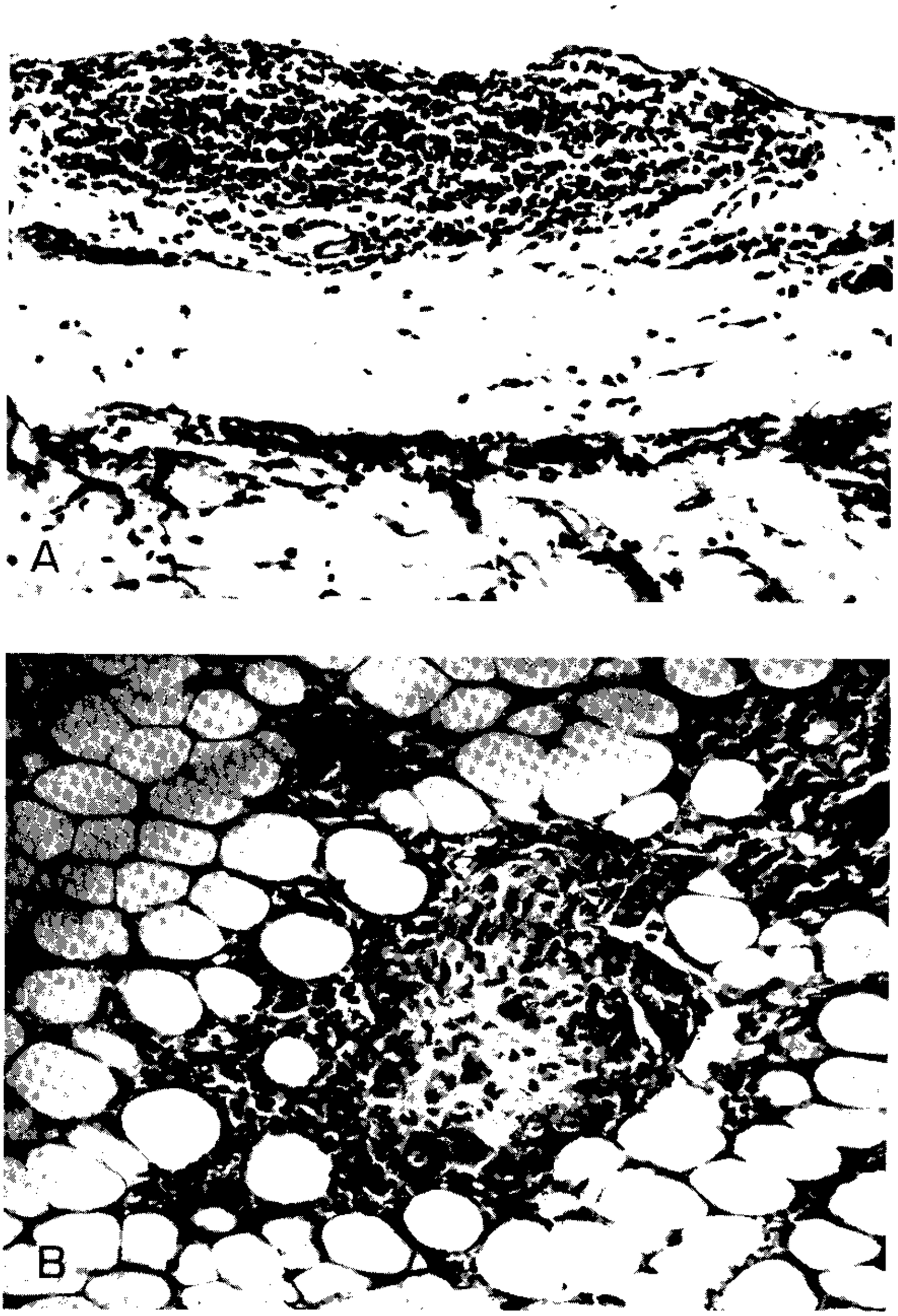

Fig. 7: epicardium of a dog infected with blood trypomastigotes of the Be-78 *Trypanosoma cruzi* strain autopsied in the acute phase. A – Chronic epicarditis in multiple foci, periganglionitis, intense exudation of mononuclear cells. B – Details from figure 6A showing predominance of lymphocites in the exudate. Normal neurons.

not die, the ECG tended to normalize characterizing the indeterminate phase of the disease as described by Andrade et al. (1981). Thirty-three percent of the dogs displayed ECG alterations indicative of cardiac lesions present in chagasic cardiopathy.

It was impossible to distinguish the behaviour of Be-62 and Be-78 strains in dogs by examining only curves of parasitemia and mortality rates. Both strains caused the development of acute disease in dogs with death of several animals. These data are very different from those observed in albino and C_3H mice where Be-62 strain killed all animals by the 13th of infection with high parasitemias while

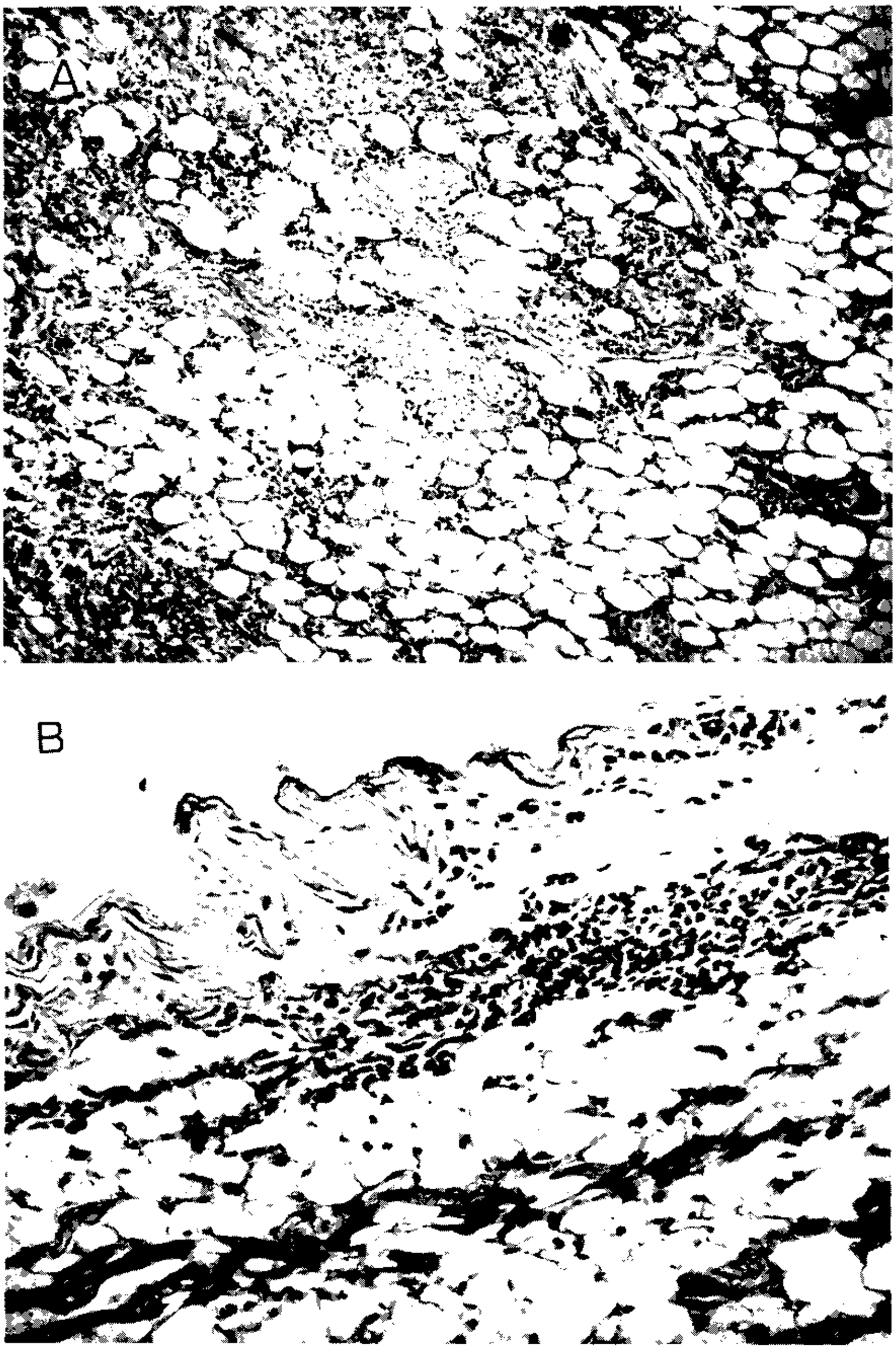

Fig. 8: epicardium of a dog infected with blood trypomastigotes of the Be-78 *Trypanosoma cruzi* strain autopsied in the chronic phase. A and B – Multifocal exudative chronic epicarditis.

Be-78 strain did not kill any animals. Be-78 develops a low parasitemia in mice that became subpatent around 40th day of infection (Lana & Chiari, 1986).

The histopathological observations clearly show that the Be-62 *T. cruzi* strain is less pathogenic to dogs than the Be-78 strain. The myocardium of all dogs infected with blood or metacyclic trypomastigotes of the Be-78 strain, autopsied in the acute or chronic phase, showed more damage than the myocardium of dogs infected with Be-62 strain (Lana et al., 1988). This fact was also demonstrated when these strains were studied in some strains of mice (Lana et al., 1989).

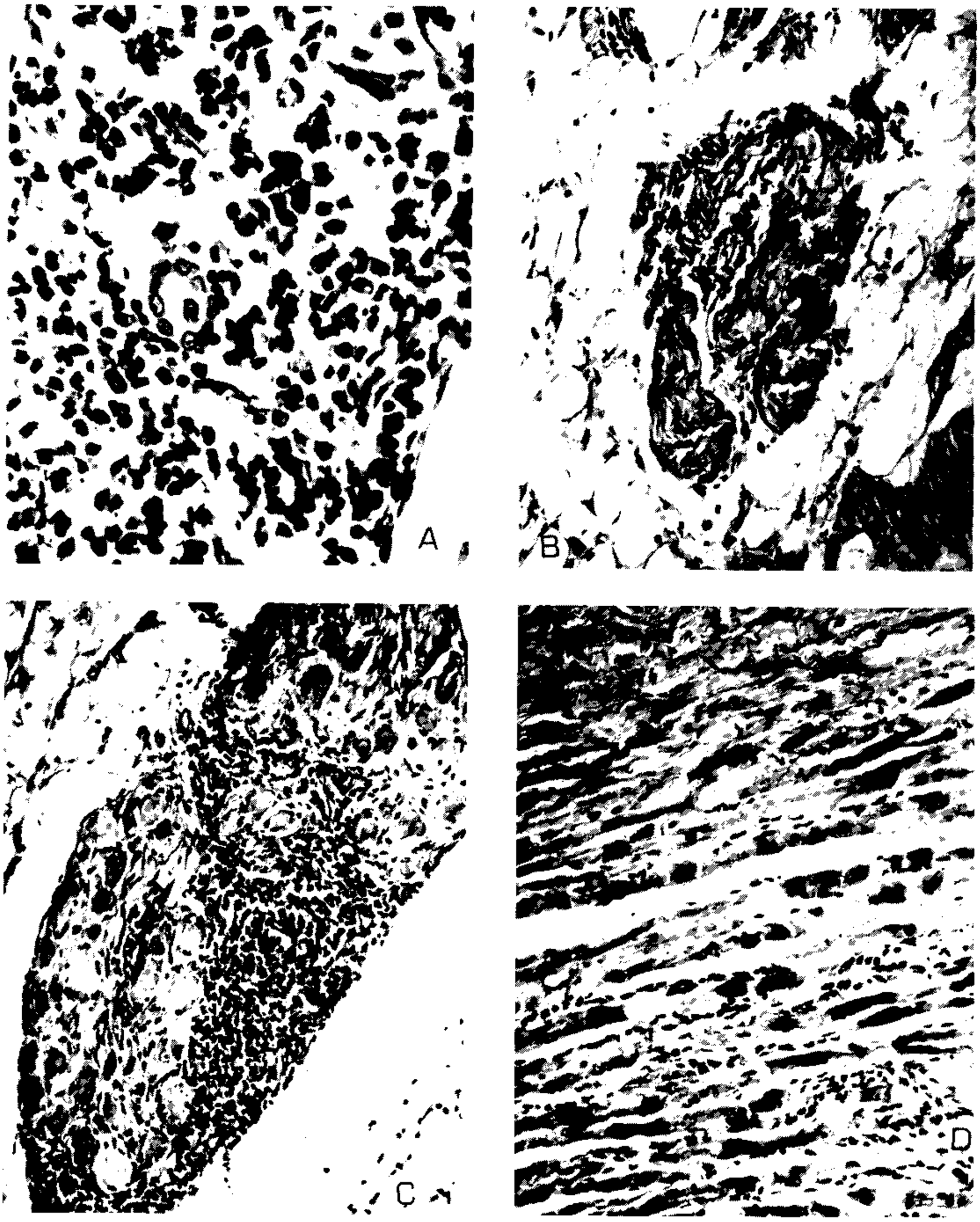

Fig. 9: dog infected with blood trypomastigotes of the Be-78 *Trypanosoma cruzi* strain autopsied in the chronic phase. A – Myocardium with focal myocarditis and presence of giant cell (arrow). B – Pericardium with focal ganglionitis, intense lymphocitic exudate and regressive phenomena of neurons. C – Chronic pericarditis with intense exudation of mononuclear cells. D – Myocardium with Magarinos Torres' lesion.

The most important finding from the histopathological observations is the fibrosing capacity of Be-78 strain. All dogs autopsied between 70th day and 29th month of infection displayed focal (two dogs) or diffuse fibrosis (seven dogs) in the myocardium. Fibrosis was absent in many strains of mice and was intense only in AKR mice (Lana et al., 1989). The aspect of the cardiac fibrosis in dogs, the local and general reactions are very similar to

those observed in man. Although the methodology here employed did not involve immunochemistry methods using monoclonal antibodies (Andrade & Grimaud, 1986), the results indicated predominance of collagen types I and III.

As far as we know there is no cardiopathy similar to fibrosing chronic chagasic cardiopathy in man caused by another etiology (Tafuri, 1987). In the present study, this type of pathology occurred naturally with all the signs of congestive heart failure. Possibly, the dog could be considered the ideal model to study the mechanisms of immunity responsible for the alterations related to local and general host reactions to parasites or host cells antigens. Futhermore, these studies could improve understanding of the mechanisms related to diffuse fibrosis and their modulation (neoformation and modulation of the collagen) as discussed by Tafuri (1985, 1987).

Fibrosis not directly related to repair mechanism of inflammation but possibly concerned with immunologic mechanisms, constitutes a new and more important pathogenic factor of chronic Chagas' disease. This factor is that chiefly responsible for the destruction of muscle fibres of the hole organs with consequent alterations of their physiology and anatomy. The physiopathology of the disease may be better understood in the light of these results (Tafuri, 1987).

Several authors have tried to reproduce the diffuse chronic chagasic cardiopathy in different experimental models but without satisfactory results (Laranja, 1953; Anselmi et al., 1966; Marsden et al., 1976; Andrade & Andrade, 1980; Andrade et al., 1981; Ramirez, 1984). Andrade et al. (1987) obtained cardiac fibrosis similar to that observed in human Chagas' disease, only when treated infected chronic dogs, apparently with good health, with low doses of cyclophosphamide. Some of these dogs then showed severe and diffuse myocarditis. These authors admitted that the drug interferes with the immunologic supressor network that maintains the chronic indeterminate phase of *T. cruzi* infection.

IF and ELISA tests detected IgM and IgG antibodies in all dogs infected with the Be-62 and Be-78 *T. cruzi* strains from the begining of infection. Both antibody classes increased rapidly when the dogs displayed patent parasitemia and signs of acute disease. The ELISA test was more sensitive in detecting higher levels of both antibody classes. The serological profile of dogs infected with *T. cruzi* resembles that of man (Magnani et al., 1973; Vattuone et al., 1973; Camargo & Amato Neto, 1974). Antibody levels in the infected dogs did not correlate with the strain of parasite, clinical aspects, curves of parasitemia or parasitological examinations during the chronic phase (hemoculture, xenodiagnosis and xenoculture). These results agree with observations in man (Maekelt, 1973; Vattuone et al., 1973; Camargo & Amato Neto, 1974), rabbits (Ramirez, 1984) and monkeys (Miles et al., 1979).

In this paper, the detection of parasites during the chronic phase of the disease was possible only in dogs infected with the Be-78 strain when xenodiagnosis and xenoculture were used. The hemoculture was always negative in all dogs infected by either strains. The results differ from those reported in dogs infected by the Colombian strain, after eight years of infection, when four of the five dogs showed positive parasitological examinations (Lana et al., 1988) and from those reported by Chiari et al. (1989) and Bronfen et al. (1989) when the association of xenodiagnosis and hemoculture gave the best results.

The difference between rates of positivity of xenodiagnosis in dogs infected with Be-62 and Be-78 strains could be related to the difference of polymorphism of these two strains since Pereira da Silva (1959) demonstrated that triatomines are more suceptible to infection with strains that show predominance of broad or stout forms than slender ones. The Be-78 strain displays predominance of broad forms (Lana & Chiari, 1986) and only dogs infected with this strain exhibited positive xenodiagnosis throughout the chronic infection.

The results presented show that the dog conforms with the requirementes for a good experimental model according to the SWG on Chagas' disease of WHO (1984). Moreover, the dog has a longer natural life-span than other laboratory animals, except monkeys, which are very resistent to infection and difficult to handle (Torres & Tavares, 1958; Marsden et al., 1970). Thus, the dog model may permit studies on the natural evolution and other clinical aspects observed many years later in human Chagas' disease such as

megacolon and megaesophagus described by Okumura & Corrêa Neto (1961) in this animal. Additionally, the dog is easily obtainable, easily handled and maintained.

The knowledge of all these aspects of experimental Chagas' disease in dogs enables this experimental model to be used in studies concerning other aspects of the disease: mechanisms and efficacy of chemotherapeutic agents, immunological mechanisms during the evolution of the disease, host resistence, attempts at vaccination, autocure phenomena, criterion for cure, etc.

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