Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. Vol. 79 :77-83, 1984

THE CANINE MODEL OF CHAGAS' DISEASE

ZILTON A. ANDRADE

Out of the several animal species that have been utilized in the study of Chagas' disease, the dog has been the only one to reproduce the clinico-pathological picture of chronic cardiac involvement as seen in man. After a more or less prolonged and silent interval from initial infection with *Trypanosoma cruzi*, some dogs have developed cardiomegaly, arrhythmias, peripheral edema, ascites and pleural effusion due to the presence of a chronic diffuse active myocarditis (Anselmi et al., 1965; Pellegrino, 1947; Laranja, Pellegrino & Dias, 1949; Laranja, 1953; Laranja & Andrade, 1980). Since acute disease can be easily obtained in young dogs (Andrade & Andrade, 1980) and the chronic indeterminate form can also be well characterized (Andrade et al., 1981) the dog could therefore be considered as the ideal experimental model to investigate the many unknown features of Chagas' disease if it was not for the rarity, the unexpectedness and the prolonged interval with which progressive chronic cardiac disease develops in *T. cruzi* infected dogs. Recently, such a limitation has apparently been overcome when a severe chronic myocarditis appeared in dogs with latent *T. cruzi* infection soon after they were treated with low doses of cyclophosphamide, a measure assumed to destroy suppressor T-lymphocytes or their precursors (Andrade et al., 1985). In view of these recent developments, it seemed worthwhile to review herein the main features of experimental Chagas' disease in dog, considering that the coming years may show an increasing interest in the canine model.

The acute disease

To obtain a reproducible and comparable acute disease with *T. cruzi* in dogs it is necessary to use young animals, 2 to 3 months of age. Although it is possible to obtain acute myocarditis in older dogs, the majority of them would show to be quite resistant. An inoculum around 25.000 trypomastigotes per kilo is adequate, and inoculation can be performed intraperitoneally, intravenously or subcutaneously. Parasitemia can become patent from the 7th day on, but electrocardiographic and pathological evidences of severe myocarditis can be expected from the 15th to the 30th day after inoculation. Acute involvement of the heart can be monitored by electrocardiogram and these findings can be correlated with the anatomical changes (Andrade, Andrade & Sadigursky, 1980).

The large amount of information available on basic aspects of cardiac physiology and structure of the conducting system appears as a further advantage of the model (Bolton, 1975; James, 1962).

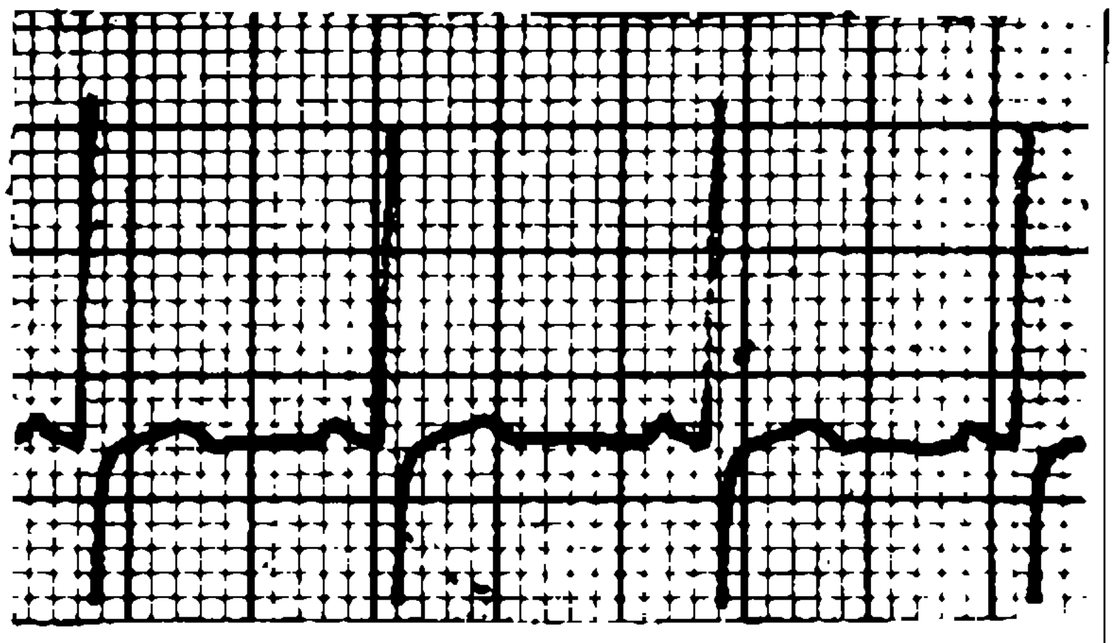
The first electrocardiographical changes to appear are alterations of T waves and ST segments (Fig. 1) but soon the evidences of atrial involvement by the inflammatory changes become prominent. Changes indicative of sinus arrhythmias, wondering pace-maker and atrial fibrillation are usually followed by cessation of all atrial electric activity and the advent of a nodal rhythm. Following specific treatment a reversal sequence of these changes can be observed (Andrade & Andrade, 1980). The later stages of fatal acute disease are characterized by the appearance of cardiac blocks (2nd and 3rd degree A-V blocks and bundle branch blocks) and severe "ischemic" changes that may simulate acute myocardial infarction (Andrade, Andrade & Sadigursky, 1980) (Fig. 2).

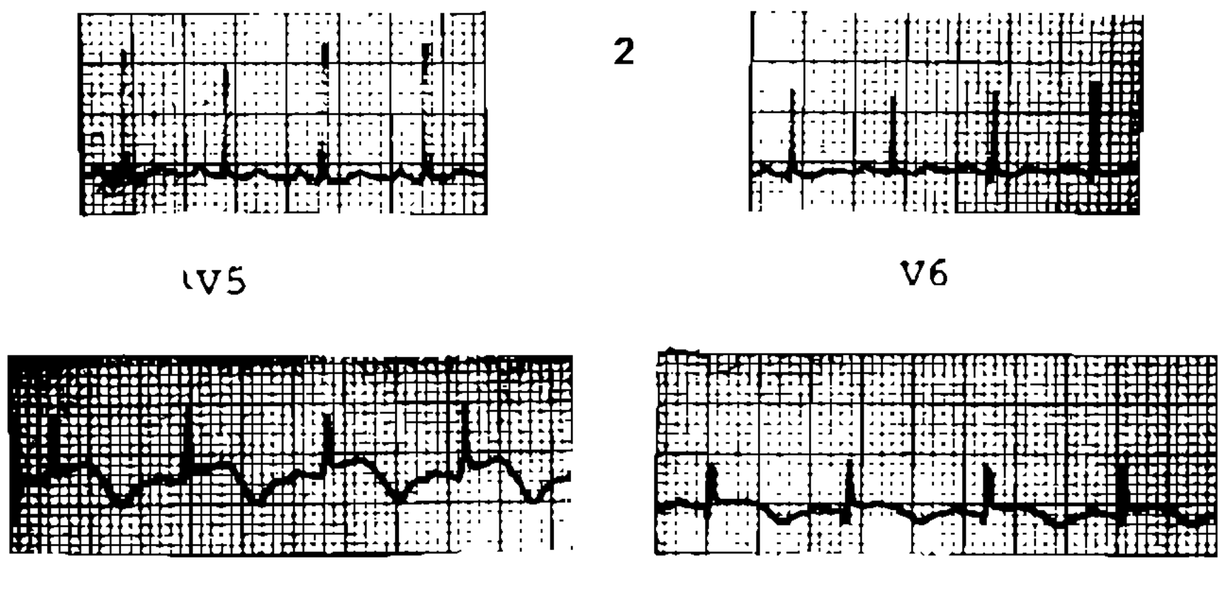
Acute myocarditis seems to start in the right atrium where the first intracellular parasites and signs of cellular infiltration may be detected from the 7th day on. Heavy atrial involvement can occur while only a few scattered inflammatory foci are present in the sections from the left ventricular wall. Sinus disfunction can be explored in dogs with acute Chagas' disease by means of a double pharmacologic blockade of the vagal and sympathetic systems by the simultaneous administration of atropine and propanolol. In dogs with 18 day *T. cruzi* infection the intrinsec sinus frequence was bradicardic in 60% of them, revealing sinus disfunction. At this time the inflammatory changes had already extended into the sinus tissue itself (Câmara et al., 1984). The right third of the intraventricular septum and the free wall of the right ventricle are the most affected areas after the atria. At first focal inflammation appears in relation to parasitized and damaged fibers, but soon interstitial edema and diffuse infiltration by mononuclear cells (macrophages, lymphocytes) sets in and dissociates the myocardial fibers. It is by this time that a striking feature takes place: necrosis of non-parasitized cells. Sometimes, even when the presence of intracellular parasites seems to decrease, the inflammation becomes more severe and single cell or focal hyaline and lytic necroses appear as an outstanding feature (Fig. 3). These are the lesions responsible for the "ischemic" changes seen in electrocardiograms and those that cause extensive damage to the conducting tissue and neurones in the autonomic plexures in the heart, and presumably, in the digestive tract. The presence of severe myocarditis with a moderate or small number of parasitized cells and extensive and frequent focal necroses turns the disease in dogs extremely similar to acute Chagas' disease in humans (Andrade & Miziara, 1983).

The pathogenesis of acute Chagas' myocarditis is incompletly known. It can be assumed that cell destruction caused by intracellular parasite growth liberates chemical mediators of inflammation. The

Centro de Pesquisas Gonçalo Moniz – FIOCRUZ, R. Valdemar Falcão, 121, Brotas, 40000 Salvador, BA, Brazil.

presence of *T. cruzi* antigen(s) and host immunoglobulin and complement as well as polymorphonuclear cell accumulation in focal lesions point out to the pathogenetic role of immune complexes. However, what causes necrosis of non-parasitized cells is not yet fully understood.





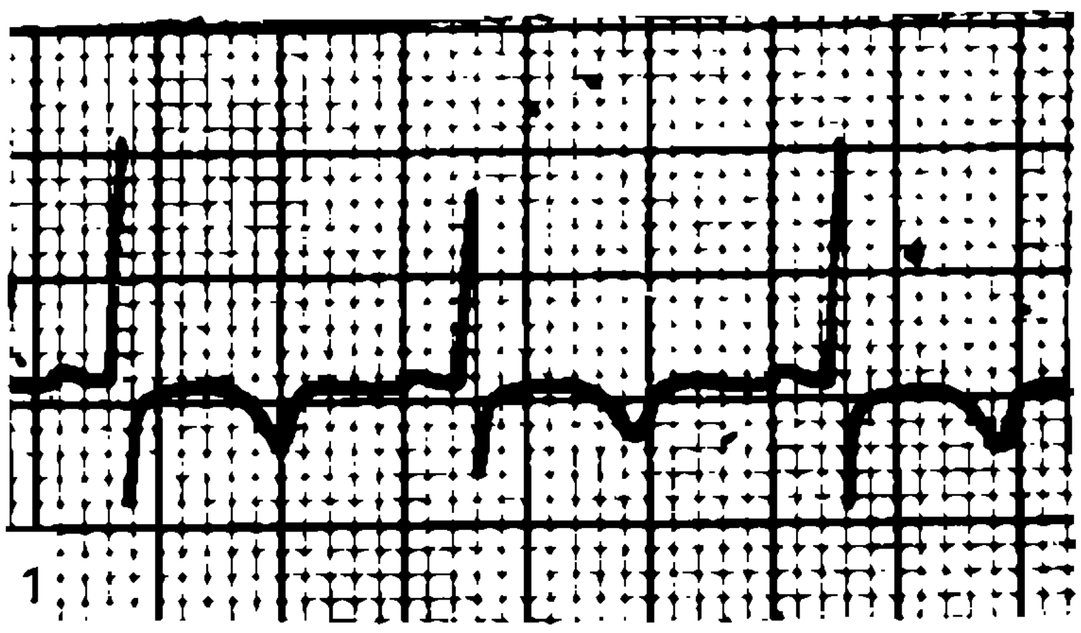


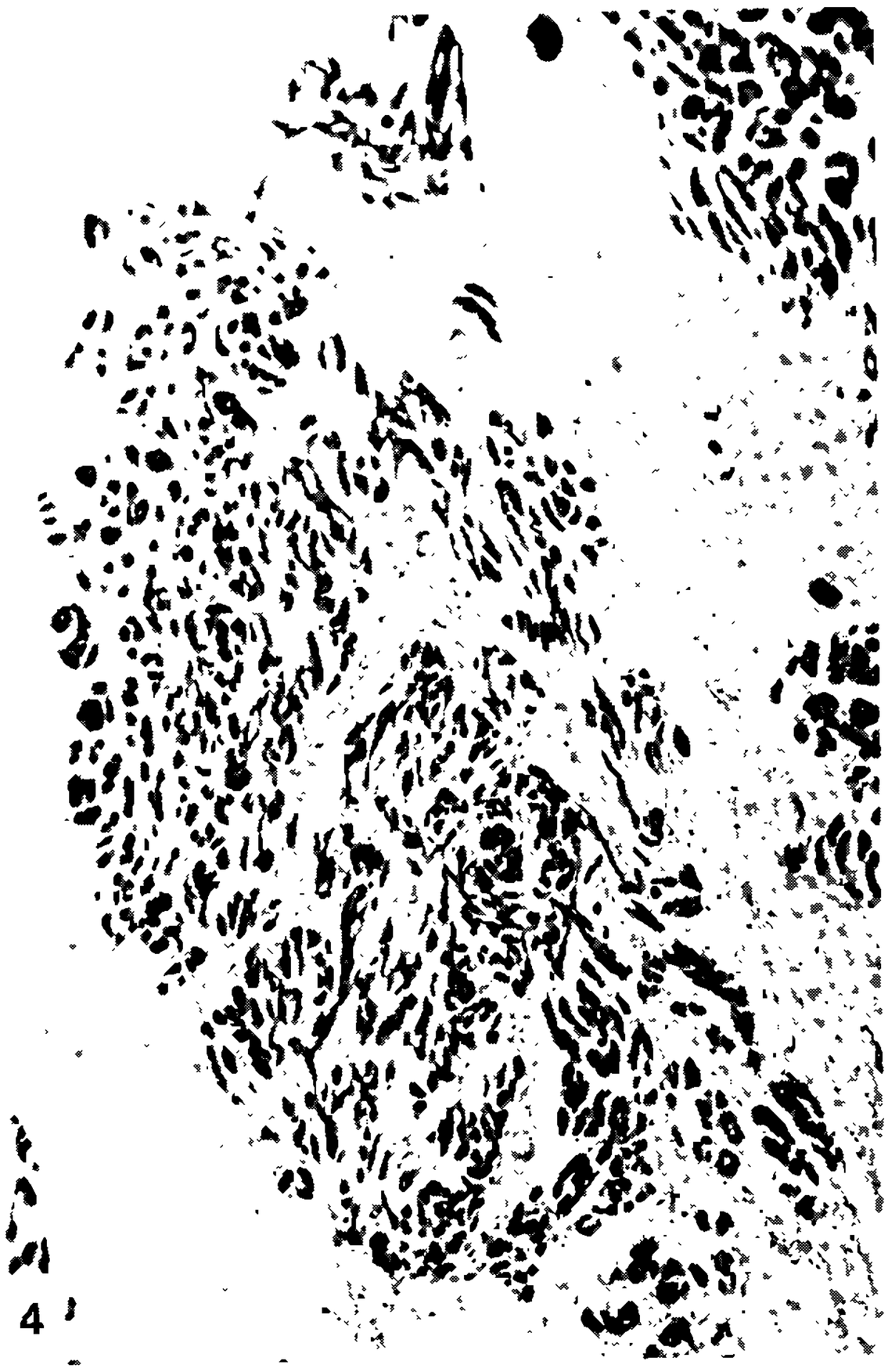
Fig. 1: ECG from a dog infected with *T. cruzi*. Above there is a normal ECG registered on the 16th day after inoculation. Two days later (below) there are inverted T waves indicative of a repolarization defect. Fig. 2: acute Chagas' disease in the dog. Above, pre-infection ECG showing normal sinus rhythm. Below, at the 17th day of infection there appear severe "ischemic" changes (alterations of the ST segments and T waves) and bradicardia. Fig. 3: acute Chagas' myocarditis with extensive necrosis of non-parasitized cells. Hematoxylin and Eosin, 100 x.

The possibility of adsorption of antigens on some cell surface followed by immunologic attack and destruction was suggested by *in vitro* observations (Ribeiro-dos-Santos & Hudson, 1981), but needs confirmation *in vivo*. Some mediators generated by the parasite-cell interaction may cause serious metabolic derangements in myocardial cells and lead them to destruction. But, especulations aside, one needs more and crucial data to understand that important aspect of acute Chagas' myocarditis. Even more important is to understand why dogs (and also men) with severe acute *T. cruzi* myocarditis may gradually and expontaneously recover. We have followed the recovery process in infected dogs either after chemotherapy (Andrade, Andrade & Sadigursky, 1980) or in untreated animals (unpublished observations). The edema is readsorbed, cellular infiltration decreases and remains as scattered foci of lymphocytic accumulations and there are stromal collapse and fibrous and fatty replacement in patchy areas. Parasites become scarse and usually can no more be demonstrate in histological sections. It has not been demonstrate whether the recovery process results solely from parasite destruction either by chemotherapy or by the immune system, or also by some sort of immunological modulation. The role of immune complexes, suppressor T lymphocytes or other suppressor mechanisms on acute myocarditis modulation has so far not been adequately explored.

Acute Chagas' disease changes in dogs predominate in the heart, but the central nervous system is invariably involved. Lesions occurs in the brain, cerebellum, meninges, and spinal cord and are represented by focal inflammation related to parasite-induced cell damage. Sketetal and smooth muscles also show frequent inflammatory foci similar to those seen in the central nervous system.

Latent infection (Indeterminate form of Chagas' disease)

Puppies are very susceptible to *T. cruzi*. The majority of them will die in the acute stage of the infection even with inocula as low as 25.000 trypomastigotes per kilo. Suppressive treatment with current anti-Chagas drugs may be necessary to maintain them alive and to let them evolve toward chronic infection. However, some dogs with severe acute disease will expontaneously recover. They can then remain in apparent


4


5

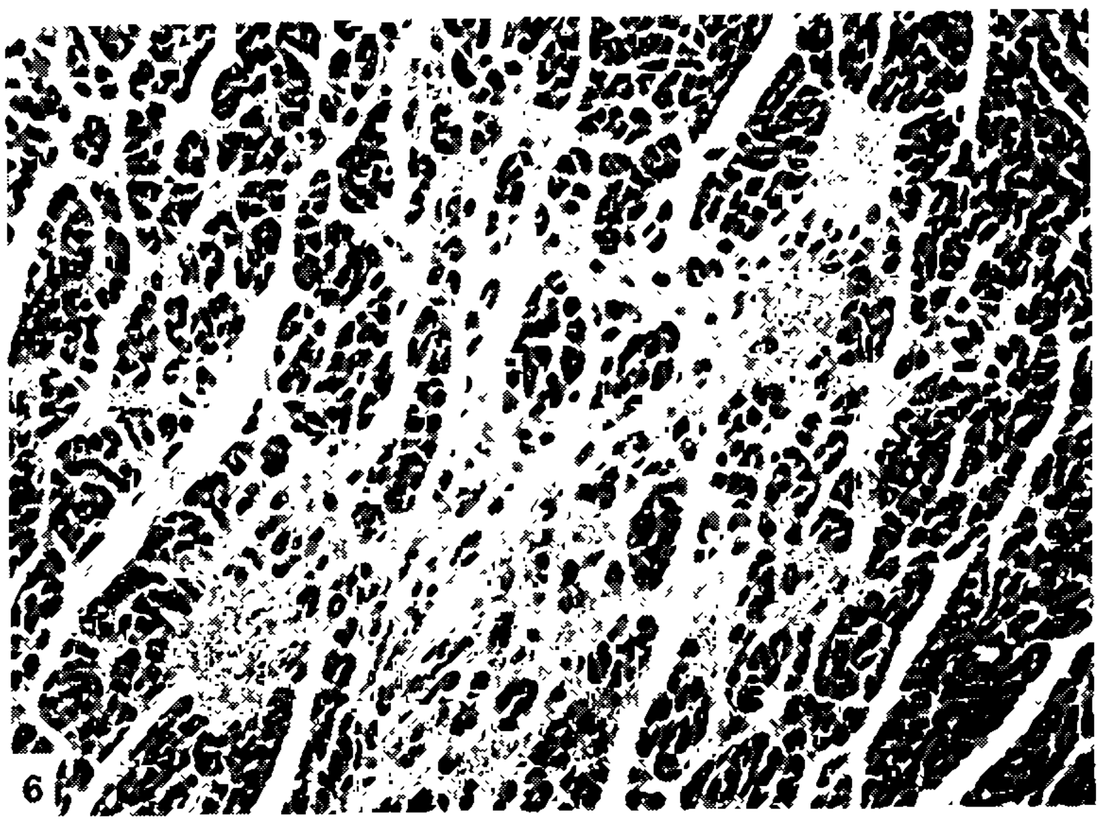

6

Fig. 4: a lateralized His main bundle in a dog with the chronic indeterminate form of Chagas' disease. There are focal and interstitial fibrosis and a mild lymphocytic infiltration. Hematoxylin and Eosin, 120 x. Fig. 5: chronic Chagas' myocarditis. Inflammatory cells are seen around and within myocardial fibers representing an invasive-destructive lesion. Hematoxylin and Eosin, 120 x. Fig. 6: chronic myocarditis in a dog chronically infected with *T. cruzi* and treated for 3 weeks with low dose cyclophosphamide. There are mild fibrosis and focal and diffuse mononuclear cell infiltration. Hematoxylin and Eosin, 100 x.

good health, with repeated normal electrocardiograms, although with the persistence of anti-*T. cruzi* circulating antibodies and an occasionally positive xenodiagnosis (Andrade et al., 1981). This correspond to the chronic indeterminate form of Chagas' disease as observed in humans. Here again many investigations about this form of the disease can be advantageously investigated with the canine model. One of the main aspect is probable the demonstration of what kind of lesions could explain the cardiologist findings of mild cardiac disfunction observed when refined pharmacological (Moleiro et al., 1982; Chiale et al., 1982; Carrasco et al., 1982), hemodynamic (Acquatella et al., 1980; Marins et al., 1981) and electrophysiological (Pimenta, Miranda & Pereira, 1983) methods of investigation are applied to assymptomatic *T. cruzi* infected individuals.

Dogs which recovered from acute infection show mild myocardial changes when examined after more or less prolonged intervals. There are scattered microscopic foci of fibrosis and lymphocytic infiltrations (Goble, 1952) a picture similar to that observed in human material (Lopes et al., 1975; 1981).

In 9 dogs that survived acute disease and were later sacrified after periods from 8 months to 3 years, several degrees of cicatricial lesions were found in the conducting tissue of the heart and in intracardiac vagal neurones (Andrade, Andrade & Sadigursky, 1984). Such lesions were represented by fibrous replacement, fatty infiltration and sclero-atrophy and were seen disseminated along the sinus node, A-V node, His bundle and its branches (Fig. 4). Thus, it was apparent that the destructive changes occurring during acute infection and involving structures that can not regenerate will leave cicatricial areas in the heart and, presumably, elsewhere. Will such cicatricial lesions in the conducting tissue and autonomic nervous system be responsible for the positivity of the refined test when cardiac function is explored in asymptomatic Chagas' patients? The answer is probably affirmative but further studies on this subject are needed. Even apical aneurysm has been observed in subjects with indeterminate Chagas' disease, some of them presenting severe paroxysmal arrhythmias (Marins et al., 1981) that can be cured by aneurysmectomy (Pinke et al., 1979; Marins et al., 1981a). It has been suggested that apical aneurysm in Chagas'

disease may result from interference with the conducting tissue responsible for activation of the left ventricle apical region (Andrade, 1974). The resulting discinesia could lead to compression atrophy and dilatation of the apical region by means of the intermitent intraventricular systolic pressure.

Chronic cardiac disease

The appearance of the chronic progressive myocardial failure after a more or less prolonged and silent interval from initial infection with *T. cruzi* is a crucial point to be fulfilled if an experimental model of Chagas' disease is to be considered as capable of reproducing the human disease. Although some prolonged observations on dogs with chronic *T. cruzi* infection failed to reveal progression to active myocarditis (Johnson, 1938; Goble, 1952; Magalhães & Freire, 1945; Andrade et al., 1981), others have documented the occurrence of cardiomegaly, generalized chronic passive congestion, arrhythmias, including right bundle branch block with left anterior hemiblock (Laranja, Pellegrino & Dias, 1949; Pellegrino, 1947; Anselmi et al., 1965; Laranja & Andrade, 1980). In these latter cases, microscopically the heart showed chronic diffuse myocarditis with focal and interstitial fibrosis, with the intracellular parasites being scarce and difficult to be detected in histological sections. Again, that is a picture reminiscent of the chronic cardiac form of Chagas' disease such as can be observed in man.

The long silent interval between acute infection and late cardiac manifestations of Chagas' disease has not been adequately explained. When chronic active myocarditis starts, it apparently assumes a self perpetuating mechanism and goes on until the death of the host. The severity of the inflammatory changes present is out of proportion to the number of parasites within cardiac fibers, which usually are extremely rare. Although it is not known which are the antigen(s) responsible for the main lesions, there is a general assumption that they result from hypersensitive mechanisms. The main reasons for that are as follows: a) The morphology of the myocardial changes, with infiltration of immunologically competent cells, which show invasive-destructive features toward myocardial fibers (Fig. 5), a perivenular distribution and a fibrosing tendency is compatible with an immune delayed-type pathogenesis; b) Several types of auto-antibody can be demonstrated, such as those against cardiac and skeletal muscles (Szarfman et al., 1981), nerve fibres (Khoury et al., 1979), neurons (Ribeiro-dos-Santos & Hudson, 1981) and laminin (Szarfman et al., 1982); c) Lymphoid cells from infected host can be shown to adhere to and destroy allogeneic cardiac cells, but not kidney cells *in vitro* (Santos Buch & Teixeira, 1974); d) Other evidence of delayed-type hypersensitivity to *T. cruzi* antigens, such as lymphocyte blastogenesis and cutaneous reactions, can also be observed in infected host (Brener, 1980).

In conclusion, all the markers of hypersensitivity are found in hosts that present with either chronic asymptomatic infection or the cardiac form of Chagas' disease. One is therefore tempted to suggest that differences in the two conditions may lie in the state of the immunological suppressive factors which, while they seem to be successful during the indeterminate stage, appear to have been overcome to allow for the development of chronic progressive myocarditis. Scott (1981) has recently shown in the mouse with chronic *T. cruzi* infection a specific suppression of delayed hypersensitivity to *T. cruzi* antigens but not to an unrelated antigen. Further studies have demonstrated a persistent suppression of responses by immunoglobulin G antibody, in mice with chronic *T. cruzi* infection, which is mediated by suppressor T lymphocytes (Reed, Inverso & Roters, 1984).

These considerations led us recently to investigate the influence of low doses of cyclophosphamide on the course of chronic latent *T. cruzi* infection in dogs. Cyclophosphamide in larger doses are known to cause immunodepression by destroying immune cells and therefore is able to turn chronic infection into an acute one, with *T. cruzi* being easily detected in circulating blood (Brener & Chiari, 1971). However low doses of cyclophosphamide will only destroy the most sensitive lymphocytes, which happen to be those belonging to the immune suppressor network (Schwartz, Askanase & Gershon, 1978; Turk, Parker & Poulter, 1972). Cyclophosphamide was administered intraperitoneally 3 times a week, during 3 weeks, in the dosis of 50 mg per m^2 of body surface into 8 dogs chronically infected with *Trypanosoma cruzi* (Andrade et al., 1985). The animals has been infected from 4 to 11 months, had presented acute disease soon after inoculations but were now in apparently good health.

They presented normal electrocardiograms, direct parasitemia was negative and anti-*T. cruzi* serum antibodies were high. They could then be considered as presenting the chronic indeterminate form of Chagas' disease. Four were previously vaccinated with a live non-replicating vaccine (Enders et al., 1982) and two of them were later challenged with virulent *T. cruzi* trypomastigotes. After cyclophosphamide administration parasitological and immunological parameters were unaltered, but some minor electrocardiographic changes were detected, especially after ajmaline administration. Ajmaline is a conduction depressor drug and can unmask subpatent conduction defects in patients with Chagas' disease (Chiale et al., 1982).

Four days after completion of cyclophosphamide administration the animals were killed (8 with chronic *T. cruzi* infection and 2 vaccinated but not challenged). Unlike what one usually sees in chronically infected dogs, 5 of them presented focal and diffuse myocarditis similar to that seen in humans with the cardiac form of Chagas' disease (Figs. 6, 7). The animals which were vaccinated only did not show any myocardial lesion, although they presented a fair titre of anti-*T. cruzi* serum antibodies. So, the vaccine

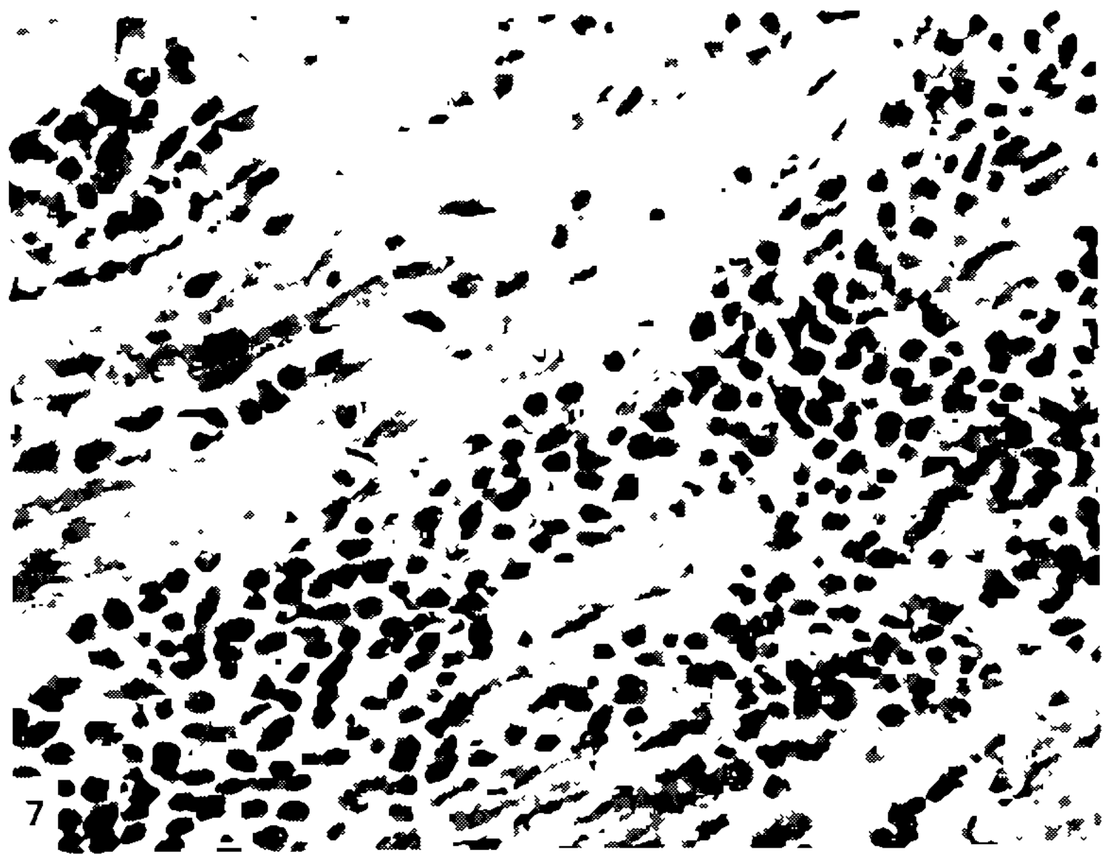


Fig. 7: chronic Chagas' myocarditis evoked in a dog with latent *T. cruzi* infection and low dose cyclophosphamide treatment. In this area, as frequently occurs in the dog with chronic Chagas' disease, plasma-cells are prominent among the inflammatory cells. Hematoxylin and Eosin, 150 x.

TABLE I

General data on 10 dogs which were either infected with *Trypanosoma cruzi*, vaccinated and challenged or vaccinated, and later on treated with low dose cyclophosphamide.

Dog nº	Sex	Duration of Infection	Final Antibody Titres	Experimental Status	Myocarditis	
					Focal	Diffuse
73	F	11m, 5d.	1:1280	Infection only	+	0
74	F	Idem	1:1280	Idem	+++	+++
75	F	Idem	1:2560	Idem	++	0
82	F	9m, 4d.	1:2560	Idem	+++	++
86	F	4m.	1:5120	Vaccinated and Challenged	++	+++
87	M	Idem.	1:5120	Infection only	+++	+++
90	F	0	1:1280	Vaccinated only	0	0
91	F	0	1:640	Vaccinated only	0	0
92	F	4m.	1:2560	Vaccinated and Challenged	++	+
93	F	4m.	1:2560	Infection only	+++	+++

*Treated with nitro imidazole (Rochagan) plus corticoid (Betamethasone) from the 28th through the 35th day after infection.

used did not protect the animals against the development of chronic disease, although it did not induce hypersensitivity by itself (Table I).

These findings point to a way for the experimental production of chronic Chagas' myocarditis in dogs, suggest that such a proccess depends on a delayed-type hypersensitive mechanism, and offer a method to investigate whether a given vaccine may be causing adverse host sensitization.

The possibility of obtaining the chronic cardiac form practically at will turns the canine model more complete for further studies on Chagas' disease.

Limitations

It would not be fair to close up this paper without saying something on the limitations presented by the canine model.

a) Some refined immunological investigations would require isogenic animals. There will be some time before isogenic, and not too expensive dogs, are available for research.

b) Occasionally a dog which had been infected for a prolonged time may present with negative serological and parasitological testes. Pathological examination may reveal essentially normal organs, with no trace of myocarditis. This is suggestive of an auto-cure and could well be a peculiarity of the canine model.

c) Although digestive megas, especially megaesophagus, have been documented in dogs with natural *T. cruzi* infection (Koberle, 1957; Okumura & Correa Neto, 1961) in our 10-year experience with the canine model such megas have never been recognised. Neither have digestive megas been mentioned

in other papers related to chronic Chagas' disease in dogs. Probably, dogs are not a good model for the reproduction of the digestive pathology associated with Chagas' disease.

d) Cardiac left ventricle apical aneurysm, a characteristic anatomical feature of chronic Chagas' disease has apparently never been reported in dogs. At least we have no example of such lesion in our own material.

e) The large predominance of plasma cells in the chronic myocarditis, as well as the mild fibrosis, seem also to be peculiar features of Chagas' disease in dogs, and are not usually observed in human material.

Actually, none of the limitations listed above are absolute and at least some of them may be related to *T. cruzi* strains, to the host genetic background and to lack of proper investigation.

REFERENCES

ACQUATELLA, H.; SCHILLER, N.B.; PUIGBÓ, J.J.; GIORDANO, H.; SUAREZ, J.A.; CASAL, H.; ARREAZA, N.; VALENCILLOS, R. & HIRSCHHAUT, E., 1980. M-mode and two-dimensional ecochardiography in chronic Chagas' heart disease. A clinical and patologic story. *Circulation, 62* :787-799.

ANDRADE, S.G.; ANDRADE, Z.A. & SADIGURSKY, M., 1980. Combined treatment with a nitrofuranic and a corticoid in experimental Chagas' disease in the dog. *Am. J. Trop. Med. Hyg., 29* :766-773.

ANDRADE, Z.A., 1974. A patologia do sistema excito-condutor do coração na miocardiopatia chagásica. *Rev. Pat. Trop., 3* :367-428.

ANDRADE, Z.A. & ANDRADE, S.G., 1980. A patologia da doença de Chagas experimental no cão. *Mem. Inst. Oswaldo Cruz, 75* :77-95.

ANDRADE, Z.A.; ANDRADE, S.G. & SADIGURSKY, M., 1984. Damage and healing in the conducting tissue of the heart (an experimental study in dogs infected with *Trypanosoma cruzi*). *J. Path., 143* :93-101.

ANDRADE, Z.A.; ANDRADE, S.G.; SADIGURSKY, M.; ENDERS, B. & WEIGAND, K., 1985. Progressive chronic myocarditis in *Trypanosoma cruzi* infected dogs treated with low dose cyclophosphamide (in press).

ANDRADE, Z.A.; ANDRADE, S.G.; SADIGURSKY, M. & MAGUIRE, J.H., 1981. Experimental Chagas' disease in dogs. A pathologic and ECG study of the chronic indeterminate phase of the infection. *Arch. Path. Lab. Med., 105* :460-464.

ANDRADE, Z.A. & MIZIARA, H.L., 1983. Alterações do sistema de gênese e condução do estímulo cardíaco na fase aguda da doença de Chagas humana. *Arq. Bras. Cardiol., 40* :233-237.

ANSELMI, O.A.; PIFANO, F.C.; SUAREZ, J.A.; DOMINGUES, A.; VÁSQUES, A.D. & ANSELMI, G., 1965. Experimental *Schizotrypanum cruzi* myocarditis. *Am. Heart. J., 70* :638-656.

BOLTON, G.R., 1975. Handbook of canine electrocardiography W. B. Saunders Co., Philadelphia.

BRENER, Z., 1980. Immunity to *Trypanosoma cruzi. Advances in Parasitology, 18* :247-292.

BRENER, Z. & CHIARI, E., 1971. The effects of some immunosuppressive agents in experimental chronic Chagas' disease. *Trans. Roy. Soc. Trop. Med. Hyg., 65* :629-636.

CÂMARA, E.J.N.; SADIGURSKY, M.; ANDRADE, S.G. & ANDRADE, Z.A., 1984. Disfunção do nódulo sinusal na miocardite chagásica aguda do cão. Pesquisa básica em Doença de Chagas. Caxambu, MG, novembro.

CARRASCO, H.A.G.; MORA, R.G.; INGLESSIS, G.; CONTRERAS, J.M.; MARVAL, J. & FUENMAYOR, A., 1982. Estudio de la función del nodo sinusal y de la conducción atrioventricular en pacientes con enfermedad de Chagas. *Arch. Inst. Cardiol. Méx., 52* :245-251.

CHIALE, P.A.; PRZYBYLSKY, J.; LAINO, R.A.; HALPERN, M.S.; SÁNCHEZ, R.A.; GABRIELI, A.; ELIZARI, M.V. & ROSENBAUM, M.B., 1982. Electrocardiographic changes evoked by Aymaline in chronic Chagas' disease without manifest myocarditis. *Amer. J. Cardiol., 49* :14-20.

ENDERS, B.; WEINMANN, E.; NAGLE, C.A.; HEIN, B. & ZWISLER, O., 1982. Experimental Chagas' disease in primates *Macaca fascicularis* and *Cebus apella* with special reference to preclinical trials and the evaluation of a living non replicating *T. cruzi* vaccine. *Behring Inst. Mitt., 71* :132-156.

GOBLE, F.C., 1952. Observations on experimental Chagas' disease in dogs. *Am. J. Trop. Med. Hyg., 1* :189-204.

JAMES, T.N., 1962. Anatomy of the sinus node of the dogs. *Anat. Rec., 143* :251-265.

JOHNSON, C.M., 1938. Cardiac changes in dogs experimentally infected with *Trypanosoma cruzi. Am. J. Trop. Med., 18* :197-206.

KHOURY, E.L.; RITACCO, V.; COSSIO, P.M.; LAGUENS, R.P.; SZARTMAN, A.; DIEZ, C. & ARANA, R.M., 1979. Circulating antibodies to peripheral nerve in American trypanosomiasis (Chagas' disease). *Clin. Exp. Immunol., 36* :8-15.

KOBERLE, F., 1957. Patogenia da moléstia de Chagas. Estudo dos órgãos musculares ôcos. *Rev. Goiania Med., 3* :155-180.

LARANJA, F.S., 1953. Aspectos clínicos da moléstia de Chagas. *Rev. Bras. Med., 10* :482-491.

LARANJA, F.S. & ANDRADE, Z.A., 1980. Forma crônica da doença de Chagas no cão. *Arq. Bras. Cardiol., 35* :377-380.

LARANJA, F.S.; PELLEGRINO, J. & DIAS, E., 1949. Experimental Chagas' disease. *Am. Heart. J., 4* :646.

LOPES, E.R.; CHAPADEIRO, E.; ALMEIDA, H.O. & ROCHA, A., 1975. Contribuição ao estudo da anatomia patológica dos corações de chagásicos falecidos subitamente. *Rev. Soc. Bras. Med. Trop., 9* :269-282.

LOPES, E.R.; CHAPADEIRO, E.; ANDRADE, Z.A.; ALMEIDA, H.O. & ROCHA, A., 1981. Anatomia patológica de corações de chagásicos assintomáticos falecidos de modo violento. *Mem. Inst. Oswaldo Cruz, 76* :189-197.

MAGALHÃES, B.F. & FREIRE, S.A., 1945. O eletrocardiograma na doença de Chagas experimental. *Mem. Inst. Oswaldo Cruz, 42* :243-261.

MARINS, N.; MIZIARA, H.L.; DUARTE, L.C.; SOBRAL NETO, J.; MATOS, J.V.; DUMARESQ, L.V. & SOUZA, R., 1981. Taquirritimias paroxísticas em chagásicos com lesão da ponta. Correlação anátomo-eletrocardiográfica. *Folha Médica, 83* :175-178.

MARINS, N.; SILVA, C.T.; MOTTA, V.P.; SCIANNI, C.C.P.; ARCALDI, N. & MATOS, N.V., 1981 a. Estudo hemodinâmico em indivíduos chagásicos sem cardiopatia aparente. *Arq. Bras. Cardiol., 37* :463-466.

MOLEIRO, F.; MENDOZA, I.; OCTAVIO, J.A.; ANSELMI, A. & CASTELLANOS, A., 1982. Los trastornos de conducción intraventricular y la prueba de ajmalina en pacientes con infección chagásica. *Rev. Lat. Cardiol., 3* :414-422.

OKUMURA, M. & CORREA NETO, A., 1961. Produção experimental de "megas" em animais inoculados com *Trypanosoma cruzi. Rev. Hosp. Clin.* (São Paulo), *16* :338-341.

PELLEGRINO, J., 1947. O eletrocardiograma na doença de Chagas experimental no cão. *Brasil-Médico, 51* :3-10.

PIMENTA, J.; MIRANDA, M. & PEREIRA, C.B., 1983. Electrophysiologic findings in long-term asymptomatic chagasic individuals. *Am. Heart. J., 106* :374-380.

PINKE, R.F.; SILVA, P.H.B.; SILVA, C.L.; FIRMINO, L.F.; TAVARES, I.P.; MARINS, N.B.C.; VEIGA, I.P.; MOTA, V.P.; TORQUATO, C.; SCIANNI, C.C.; PORTELA FILHO, R.; RIBEIRO, H.E.; MARTINELLI, J.G.; CAMPOS, G.P. & FRANCESCHINI, I.R., 1979. Aneurismectomia de ventrículo esquerdo em chagásicos. *Arq. Bras. Cardiol.* (Supl.), :308-313.

REED, S.G.; INVERSO, J.A. & ROTERS, S.B., 1984. Heterologous antibody responses in mice with chronic *T. cruzi* infection: depressed T helper function restored with supernatants containing interleukin 2. *J. Immunol., 133* :1558-1563.

RIBEIRO-DOS-SANTOS, R. & HUDSON, L., 1981. Denervation and the immune response in mice infected with *Trypanosoma cruzi. Clin. Exp. Immunol., 44* :349-354.

SANTOS BUCH, C.A. & TEIXEIRA, A.R.L., 1974. The immunology of experimental Chagas' disease, III: Rejection of allogeneic heart cells in vitro. *J. Exp. Med., 140* :38-53.

SCOTT, M.T., 1981. Delayed hypersensitivity to *Trypanosoma cruzi* in mice: specific suppressor cells in chronic infection. *Immunology, 44* :409-417.

SCHWARTZ, A.; ASKENASE, P.W. & GERSHON, R.K., 1978. Regulation of delayed-type hypersensitivity reactions by cyclophosphamide sensitive T cells. *J. Immunol., 121* :1573.

SZARFMAN, A.; LUQUETTI, A.; RASSI, A.; REZENDE, J.M. & SCHMUNIS, G.A., 1981. Tissue reacting immunoglobulin in patients with different clinical forms of Chagas' disease. *Am. J. Trop. Med. Hyg., 30* :43-46.

SZARFMAN, A.; TERRANOVA, V.P.; RENNARD, S.J.; FOIDART, J.M.; LIMA, M.F.; SCHEINMAN, J.I. & MARTIN, G.R., 1982. Antibodies to laminin in Chagas' disease. *J. Exp. Med., 155* :1161-1171.

TURK, J.L.; PARKER, D. & POULTER, L.W., 1972. Functional aspects of the selective depletion of lymphoid tissue by cyclophosphamide. *Immunology, 23* :493.