

## THE CONDUCTION SYSTEM OF THE HEART IN MICE CHRONICALLY INFECTED WITH *TRYPANOSOMA CRUZI*: HISTOPATHOLOGICAL LESIONS AND ELECTROCARDIOGRAPHIC CORRELATIONS

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*Chronic focal and diffuse myocarditis with interstitial fibrosis developed in Swiss outbred mice and in the inbred AKR and A/J strains of mice which were chronically infected with several Trypanosoma cruzi strains belonging to three biological types (Type I, II and III). High incidence of electrocardiographic changes with predominance of intraventricular conduction disturbances, 1st. and 2nd. degree AV block, arrhythmias, comparable with those found in human Chagas' disease, were also present. Morphological study of the conduction tissue of the heart revealed inflammatory and fibrotic changes. The presence of inflammation in the inter-atrial septum almost always coincided with the inflammatory involvement of the ventricular conduction system. Focal inflammation was associated with vacuolization and focal necrosis of the specific fibers. Most of the lesions were seen affecting the His bundle (76.3% of the cases), the right bundle branch (73.3%), AV node (43.9%) and left bundle branch (37.5%). Correlation between morphological changes in the conduction tissue and electrocardiographic alteration occurred in 53.0 to 62.5% of the cases, according to the experimental groups.*

Key words: conduction system of the heart – *Trypanosoma cruzi* – chronic myocarditis – chronic *T. cruzi* infection in mice – electrocardiogram in mice

*Trypanosoma cruzi* infection in man is responsible for the development of a chronic cardiomyopathy with arrhythmias (Amorim et al., 1979) which can be correlated electrocardiographically to lesions in the conduction system of the heart (Oliveira et al., 1972; Andrade, 1974b) in man and in the dog, during both acute and chronic infection (Andrade et al., 1978; Andrade & Andrade, 1980; Andrade et al., 1981; Andrade et al., 1984).

The mouse has been utilized as a model for the study of chronic Chagas' disease (Federici et al., 1964; Andrade & Andrade, 1968; Kumar et al., 1969) but detailed descriptions of the alterations of the specific tissue of the heart in this model are not presented in the literature.

In the present paper we report on the lesions of the conduction system of the heart in the mouse with chronic chagasic cardiomyopathy, by means of serial sections mounted in continuous plastic tapes, a technique previously described (Andrade, 1974b; Andrade et al., 1978). An attempt was made to correlate these lesions with the electrocardiographic findings.

In the infected mice there were significant electrocardiographic alterations as well as frequent inflammatory changes in the conduction system of the heart.

### MATERIAL AND METHODS

*Experimental groups* – One hundred and twelve chronically infected mice, were utilized and distributed as follows: 22 AKR and 22 A/J inbred mice infected with either the Peruvian, the 12 SF or the Colombian strains of *T. cruzi*. The strains are respectively the prototypes of Type I, Type II and Type III strains, according to Andrade's classification (Andrade 1977a; Andrade, 1985) and isoenzymes patterns (Andrade et al., 1983). Another 68 outbred Swiss mice were infected with several strains isolated from patients of different geographical areas and classified either as Types II (São Felipe, Mambai, Firminópolis and Abadia dos Dourados-BR) or as Type III (Montalvania, Morada Nova-BR, Colombia and Bolivia).

*Inoculum* – One hundred thousand blood trypomastigotes from infected mice were used to inoculate AKR and A/J mice and  $10^5$  to  $2 \times 10^5$  were used for Swiss mice. The AKR and A/J infected with the Peruvian strain and the AKR infected with 12 SF strain were treated in the acute phase, with a suppressive dose of an anti-*T. cruzi* drug (Nifurtimox, 10 doses of 50 mg/k.b.w. each).

*Duration of the infection* – AKR and A/J

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mice were killed 180 to 360 days post-infection; Swiss mice were killed from 180 to 660 days post-infection.

*Methods of study* – Parasitemia was determined by direct examination of peripheral blood or by xenodiagnosis. Electrocardiographic study was performed in 60 out of the 112 animals. Pentobarbital anesthesia (20 mg/k.b.w.) was used. Peripheral leads (D1, D2, D3, avR, avF and avL) and two precordial leads: A (right) and D (left) were utilized. As controls for the electrocardiographic study, 15 Swiss mice, 10 AKR and 10 A/J inbred normal mice, paired for age and sex with the infected ones, were used. The tracings were obtained at 50 or 90mm/second rates, 1 mV (N) or 2 mV (2N) in a single canal ECG apparatus (Funbec, São Paulo).

*Serological study* – Was performed by the indirect immunofluorescence test, according with Camargo (1966), using culture forms of *T. cruzi* as antigens and fluoresceinated goat anti-mice IgG (Cappel Laboratory) in the dilution of 1:40.

*Histopathological study of the heart* – Was performed after sagittal section and fixation of the heart in 10 per cent formalin followed by paraffin inclusion. In 20 mice, 100 serial section of each heart, mounted in transparent plastic tapes were obtained by the method of Pickett & Sommer (1960). The sections were stained by the Masson's trichrome method. In addition, serial section of the heart from 18 mice and stepped sections of the heart from 74 mice were examined in glass slides after staining with hematoxylin and eosin.

## RESULTS

All the chronically infected mice showed positive parasitemia either by direct examination of peripheral blood or by xenodiagnosis. Positive specific immunofluorescence test was observed in all the animals (titres of 1:10 to 1:640).

*Electrocardiographic studies* – (Fig. 1) In normal control mice the electrocardiographic tracings were comparable with the parameters of the human ECG, with well marked P wave and P-R intervals, but the T wave appeared fused with the QRS complex. The electric axis position and cardiac frequency differed in the mice of different strains. Mean cardiac frequency in Swiss mice was of  $503/m \pm 33$  and in the A/J mice,  $409/m \pm 55$ . The patterns for the normal ECG tracings were established elsewhere (Sadigursky & Andrade, 1986).

*Chronically infected mice* – (Fig. 1) The main alterations in the ECG were: intraventricular conduction disturbance, with configuration of either right or left bundle branch block; sinus tachycardia or bradycardia, sinus arrhythmia; left and right ventricle overload; 1st and 2nd degree AV block. Detailed description of the electrocardiographic changes by Sadigursky & Andrade (1986) appears elsewhere. Fig. 1 shows several aspects of the normal and altered ECG in mice.

The incidence of electrocardiographic alterations in mice of the different experimental groups is showed in Table I. In the AKR mice, the same incidence of ECG alterations was seen in the infection with the three *T. cruzi* strains. In the A/J mice a significant difference in the incidence was seen between the animals infected with the Colombian and the Peruvian strains (100 per cent) and those infected with the 12 SF strain (26.1 per cent). The Swiss mice showed higher incidence of electrocardiographic alterations in the infection with Type III strains (71.4 per cent), as compared with the type II strains (53.3 per cent).

Considering the patterns of electrocardiographic alterations, there was a predominance of 1st degree AV block and of intra-ventricular conduction disturbances in all the groups, with the exception of the A/J mice infected with the Colombian strain that showed sinus arrhythmia and bradycardia.

*Histopathology of the myocardium* – Chro-

TABLE I

Incidente of electrocardiographic disturbances in mice chronically infected with *Trypanosoma cruzi*

Mouse strains	Type I	<i>Trypanosoma cruzi</i> strains	
		Type II	Type III
AKR	87%	80%	83.3%
A/J	100%	26.1%	100%
Swiss*	**	53.5%	71.4%

\* Infected with strains from several geographical areas.

\*\* No surviving mice inoculated with type I strains.

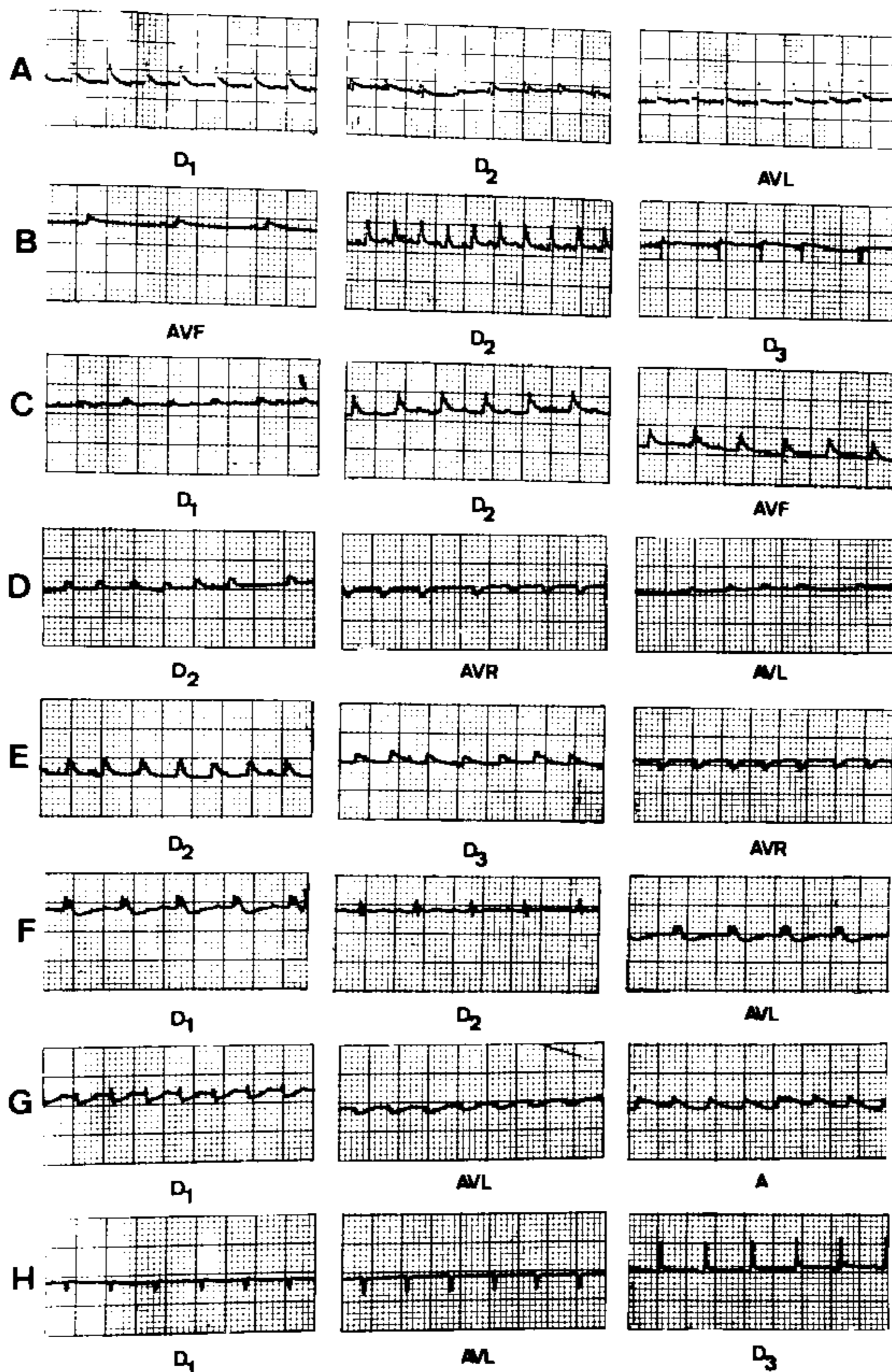


Fig. 1: A - Normal electrocardiographic tracing in Swiss control mouse. B to H - Electrocardiographic tracing representatives of the different disturbances detected in mice chronically infected with *T. cruzi* as follows: B - sinus bradycardia (aVL), sinus tachycardia (D2) and sinus arrhythmia (D2). C - 1st degree A V block. D - 2nd degree A V block, Weckenbach type. E - A V dissociation with junctional rhythm. F and G - intraventricular conduction disturbances. H - right ventricle overload.



Fig. 2. a to d -- Different sections of the inter-atrial and interventricular septum, showing the His bundle (double arrows) and the fibrous body of the heart (H & E staining 100X). a -- His bundle and bifurcation of right (RB) and left (LB) bundle branches, with mild and diffuse mononuclear infiltration. b -- inter-atrial septum with peri-capillary infiltrate extending to His bundle with focal destruction of the specific tissue. c -- His bundle and subendocardial portion of the right bundle branch with diffuse mononuclear infiltration and intramyocardial segment of the right bundle branch (RB).

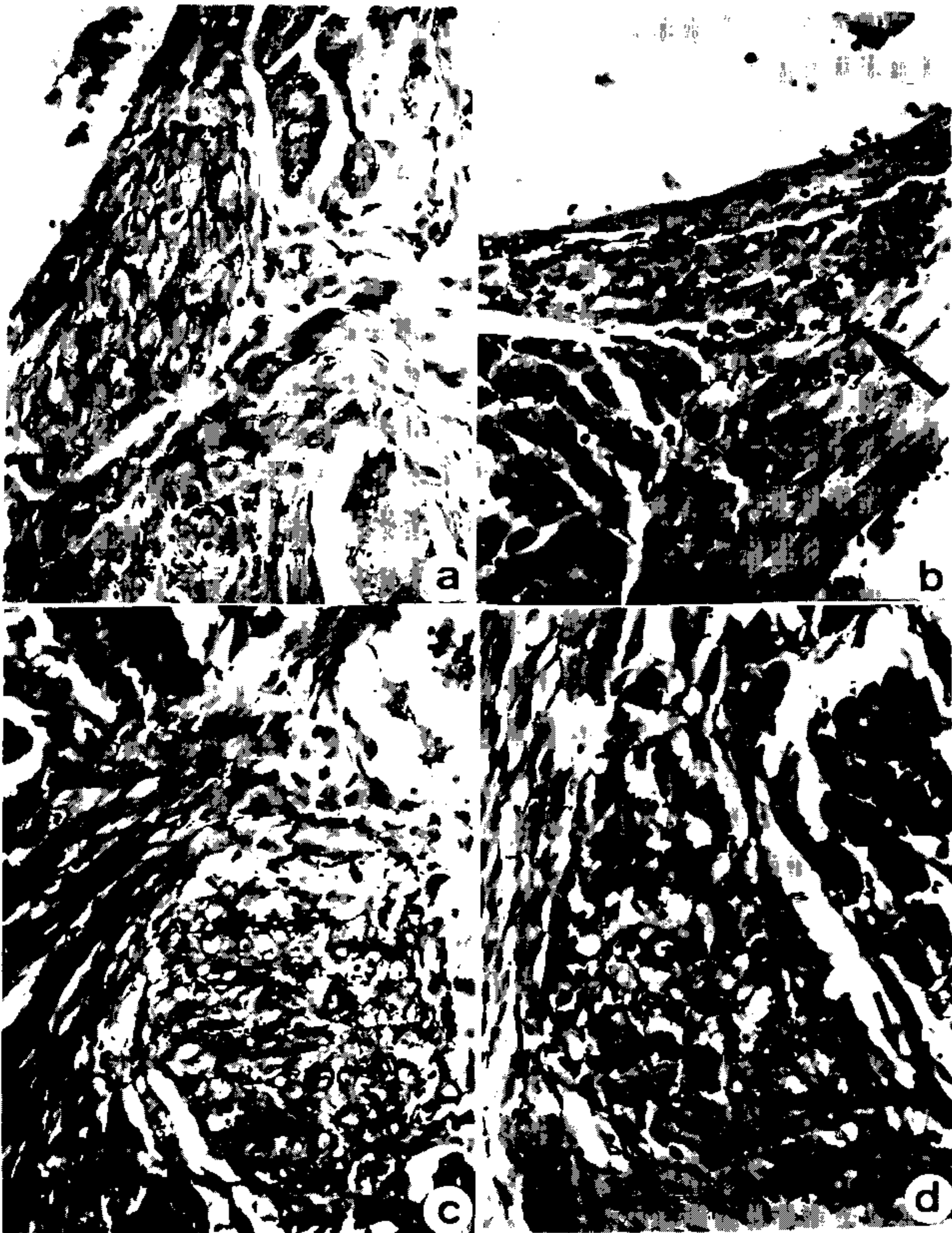


Fig. 3. a - Histologically normal A V node subendocardially localized (H & E, 250X). b - A V node with dense mononuclear infiltration and focal destruction of the specific tissue fibers. c - undivided portion of the His bundle. cytoplasmic vacuolization and interstitial collagen deposits (trichromic of Masson 250X). d - His bundle and the origin of the left bundle branch (arrow) vacuolization and focal cells destruction, interstitial edema and fibrosis, (trichromic of Masson, 250X).

TABLE II

Topographic distribution of histopathological lesions of the conduction system in mice chronically infected with *T. cruzi*\*

Conduction system	No. of cases	Frequency of histopathological lesions			
		Inflammatory infiltrate	Interstitial fibrosis	Focal necrosis	Conduction tissue vacuolization
His bundle	30/30	76.3%	26.6%	26.6%	43.3%
AV node	7/30	43.0%	14.2%	—	—
Right bundle branch	15/30	73.3%	13.3%	13.3%	6.6%
Left bundle branch	8/30	37.5%	12.5%	12.5%	25.0%

\* Serial sections study.

nically infected mice of the several experimental groups showed diffuse and focal inflammatory infiltrate most intense in the atria and in the interatrial septum (Figs. 2a, b) with predominance of macrophages and some lymphocytes. Ventricular lesions were also frequent and most intense in the highest portion of the interventricular septum and around the great vessels and in the wall of the right ventricle. Focal and diffuse fibrosis was also present. The intensity of the inflammatory and fibrotic process was variable from case to case being consistently most intense in the animals infected with Type III strains.

*Histopathology of the conduction tissue of the heart* — In serial sections of the heart of both control and chronically infected mice the segments of the conduction tissue of the heart were identified; the AV node was located in the subendocardium, above the tricuspid valve insertion (Figs. 3a, b) and was characterized as thin myocardial fibers, irregularly arranged. The penetration into the fibrous skeletal body of the heart, forming the undivided portion of the His bundle, was identified. The His bundle was thus seen as one undivided portion into the fibrous body (Figs. 2a, b, c) and in its bifurcation, giving origin to the right and left branches. The right branch of the His bundle appeared well defined, with intramyocardial and subendocardial tracts (Figs. 2a, c, d). The left branch of His bundle can be followed from the bifurcation until the left side of the interventricular septum (Figs. 2a, 3d).

Histopathological lesions of the conduction tissue, in chronically infected mice, was represented by edema that separates the myocells, focal or diffuse mononuclear infiltrations re-

lated to focal necrotic lesions (Figs. 2a, b, c, d) and vacuolization of the conduction tissue fibers, sometimes with the aspects of lipid deposits (Figs. 3c, d). In several cases, inflammatory infiltration of the inter atrial septum extended into the conduction tissue (Figs. 2a, b) with a perivascular distribution. Thin strands of fibrous tissue were seen in the interstitium of the conduction tissue, revealed by Masson's trichromatic staining method (Figs. 3c, d).

Topographically, the lesions were more frequent in the His bundle (76.3 per cent of the cases) and the right bundle branch (73.3 per cent) — Table II.

Considering the AKR and A/J chronically infected mice, a correlation between the presence of histopathological lesions of the conduction tissue and of electrocardiographic alterations, occurred in 55.5 per cent of the mice infected with the Peruvian strain, in 62.5 per cent of those with the 12 SF strain and in 60 per cent of those infected with the Colombian strain. In the Swiss mice there were coincident ECG alterations and histopathological lesions of the conduction tissue in 53 per cent of the animals infected with Type II strain and in 55 per cent of those infected with Type III strains.

#### DISCUSSION

According to several authors (Davies, 1971; Truex & Smythe, 1965), there is a certain degree of uniformity in the anatomical distribution and in the histological structure of the conduction system of the heart in different species of mammals. However the murine model has been scarcely studied, with the ex-

ception of the detailed description done by Nomura (1952).

In the present paper, we could identify the several structures of the conduction tissue in the mouse and disclose its correspondence with the aspects that have been described in other mammals. In the chronically infected mice the lesions of this specific tissue were comparable with those previously described in the man and in dogs (Andrade, 1974b; Andrade et al., 1978; Andrade et al., 1981).

Inflammatory and fibrotic changes involved the conduction tissue of the heart and there was a clear cut correlation between the peri-vascular inflammatory infiltration in the interatrial septum and its extension to the segments of the specific tissue, suggesting the important role of this peri-vascular infiltration in the topographic distribution of the lesions. In some instances, focal inflammation was associated with vacuolization and focal necrosis of the specific fibers. One interesting feature was the predominance of lesions in the His bundle, in the right bundle branch and in the AV node, comparable with previous observations made on similar materials from man and dog (Andrade, 1974b; Andrade et al., 1978; Andrade et al., 1984).

In the evaluation of the electrocardiographic study one important aspect to be taken into account is the type of anesthesia that is employed. Electrocardiographic alterations determined by the use of ether anesthesia has been referred by Bijovsky et al. (1983) and can have interfered in the results obtained by other authors (Laguens et al., 1981; Bolomo et al., 1982). In the present investigation the electrocardiographic tracings obtained from normal mice under adequate anesthesia with Sodium Pentobarbital has provided a good control of the normal ECG in mice, as previously described (Sadigursky & Andrade, 1986). A high incidence of electrocardiographic disturbances could be detected in chronically infected mice, when compared with the control mice. The peculiarities of the electrocardiographic tracings in the mouse do not allow a precise definition of the branch blocks considering that there normally is a fusion of the T wave with the QRS complex and it is difficult to obtain precise tracing of the precordial leads. However, some definite alterations were seen which could be compared with the right and left bundle branch blocks here designated as "disturbances of the intraventricular conduction". Differences in the incidence of electrocardiographic alterations were detected in isogenic mice of the same strain but infected with different strains of *T. cruzi* as was seen in the A/J mice, with high incidence of ECG disturb-

ances in the infection with the Peruvian and Colombian strains and a relatively low incidence in those infected with 12 SF strain. In the Swiss mice the electrocardiographic disturbances were most frequent in those infected with the strains of *T. cruzi* characterized as Type III. This observation indicates that parasites are more important in the development of the electrocardiographic alterations than the mouse strain, and this is in accordance with previous studies on the behaviour of six inbred strains of mice infected with three different strains of *T. cruzi* (Andrade, et al., 1985). It is important to stress the significance of the lesions that occurs in the acute phase in the mice infected with a highly pathogenic strain such as the Peruvian strain, surviving after a suppressive dose of anti-*T. cruzi* chemotherapeutic, fibrous scars of the myocardium and of the conduction tissue in the chronic phase could explain the electrocardiographic alterations, as was described in dogs (Andrade et al., 1984).

It should be pointed out that in a high percentage of cases there was a good correlation between the presence of morphological changes in the conduction tissue and the electrocardiographic alterations. This aspect may be of interest for studies in which one needs to monitor the effects of a given therapy, or to evaluate the evolution of the chronic cardiomyopathy utilizing the murine model.

#### RESUMO

Em camundongos suíços não isogênicos e em camundongos isogênicos das linhagens AKR e A/J, cronicamente infectados com cepas do *Trypanosoma cruzi* representativas de três tipos biológicos (Tipos I, II e III) foi observada uma miocardite crônica difusa e focal com graus variáveis de fibrose intersticial. Observou-se alta incidência de alterações eletrocardiográficas com predominância de distúrbios da condução intraventricular, bloqueios A-V de 1º e 2º graus e arritmias, comparáveis às encontradas na doença de Chagas humana. O estudo histopatológico do sistema de condução do coração mostrou alterações inflamatórias e fibroticas. A presença de inflamação no septo inter-atrial em geral coincidiu com o envolvimento do sistema de condução pelo processo inflamatório. Os infiltrados inflamatórios estavam em geral associados com vacuolização e necrose focal das fibras do tecido específico. As lesões foram mais frequentes no feixe de His (76,3% dos casos), no ramo direito do feixe de His (73,3%), no nódulo AV (43,0%) e no ramo esquerdo do feixe de His (37,5%). Houve coincidência entre a presença de lesões do sistema de condução e de alterações eletrocardio-

gráficas, em 53 a 62,5% dos casos, de acordo com o grupo experimental.

Palavras-chave: sistema de condução do coração – *Trypanosoma cruzi* – miocardite crônica – camundongos cronicamente infectados com *Trypanosoma cruzi* – eletrocardiograma em camundongos

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