Changes in electrocardiographic patterns at different stages of Chagas' heart disease in rats

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SUMMARY
1. The resting electrocardiogram was obtained from 25 Trypanosoma cruzi-infected rats 30 days after infection (phase I). The resting electrocardiogram was abnormal in 12 (group I) and normal in 13 (group II) animals. Nineteen similar but non-infected animals served as controls. Both the resting electrocardiogram and the ajmaline test were performed 120 and 350 days after infection (phases II and III, respectively).

2. With regard to the resting electrocardiogram of group I animals, left axis deviation was found in 10 of 12 (83%) in phase I, one of 12 (8%) in phase II (P<0.05) and in none in phase III (P>0.05). An intraventricular conduction delay was found in four of 12 (33%) rats in phase I, two of 12 rats (16%) in phase II (P>0.05) and six of 12 rats (50%) in phase III (P>0.05). The ajmaline test was abnormal in nine of 10 (90%) rats of group I with normal resting electrocardiogram in phase II, and in three of six (50%) animals in phase III (P>0.05).

3. An intraventricular conduction delay was found in the resting electrocardiogram of one of 13 (7%) rats of group II in phase III. The ajmaline test was abnormal in one of 13 (7%) rats in phase II and in one of 12 (8%) rats in phase III.

4. No control rat showed pathological changes. Eight of 12 (66%) rats of group I and two of 13 (15%) rats of group II showed histopathological alterations consisting of mononuclear cell infiltrate, cellular vacuolation and myocardial necrosis.

5. We conclude that different abnormal patterns may be seen in the resting electrocardiogram in the same T. cruzi-infected rat over time. The QRS axis changes predominate during acute and intraventricular conduction disturbances in the chronic stage of Chagas' heart disease in rats.

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Abbreviations: AV, atrioventricular; ECG, electrocardiogram.

INTRODUCTION
Although the 35 million people affected by Chagas' disease today are confined to Latin America [1], the pathogenesis of chronic Chagas' heart disease is of interest to basic cardiology mostly because it represents an unparalleled example of a cardioneuropathy caused by death and disappearance of the cardiac neurons [2]. Cardioneuropathies due to damaged cardiac nerves are better known [3] and experimental models can be easily obtained by sectioning cardiac nerves.

Natural Trypanosoma cruzi infection usually occurs during childhood when plenty of parasites are detected in blood and tissues. After some weeks the parasites virtually disappear and the patient dies of heart failure or suddenly after 20-30 years. This long period is usually asymptomatic or cardiac disturbances can be detected by a typical evolutive pattern of electrocardiographic changes [4]: first-degree atrioventricular (AV) block, followed by right bundle branch block and then right bundle branch block associated with left axis deviation. The electrocardiogram (ECG) may be normal in T. cruzi-infected patients, although some special procedures reveal otherwise concealed disturbances. This is the case for the ajmaline test. About 30% of asymptomatic infected patients with normal resting ECG present electrocardiographic changes not seen in normal subjects after receiving an intravenous injection of ajmaline [5].

Since experimental Chagas' heart disease may serve as a model of a peculiar type of cardioneuropathy, in the last few years we have tried to fully characterize this disease by a non-invasive procedure. We have obtained the resting ECG and performed the ajmaline test successfully
in the acute as well as in the chronic stage of *T. cruzi* infection in rats [6–8]. We have also studied the effect of isoprenaline on the heart in rats [9]. It is worth stating that *T. cruzi*-infected rats present the same degree of intrinsic heart denervation as is observed in man [10].

The electrocardiographic changes detected in *T. cruzi*-infected rats are similar to those found in human chronic Chagas’ heart disease, except for the virtual absence of right bundle branch block. Little is known, however, about the evolution of these electrocardiographic changes from the acute to the chronic stage. It is still unknown at present whether the same electrocardiographic change can be visualized at different times after *T. cruzi* infection in a given animal or whether a pathological ECG can return to normality spontaneously.

The purpose of this investigation was to study the surface ECG at different stages of *T. cruzi* infection to determine the evolutive pattern of the electrocardiographic changes from the acute to the chronic stage, as well as to determine the possible reproducibility of these electrocardiographic changes during *T. cruzi* infection.

**METHODS**

**Animals**

Twenty five male albino rats were infected with *T. cruzi* (2000 parasites/g body weight, intraperitoneally) soon after weaning. Light microscopy showed that all rats had circulating parasites in the bloodstream 1 week later. Nineteen similar but non-infected animals served as controls. All the animals were kept in wire cages, with free access to Purina rat food and water.

**ECG**

A surface ECG was obtained from each rat with a FUNBEC-ECG 3 model apparatus which was modified to record the ECG at an amplitude of 2N and a paper speed of 100 mm/s. A Faraday cage was connected to the electrocardiograph to decrease electrical interference. All rats were lightly anaesthetized with ether and placed in the Faraday cage resting on their back. Small-needle electrodes were placed subcutaneously to obtain classical (I, II, III, AVR, AVL and AVF) and precordial leads as follows: VA= electrode placed at the fourth right intercostal space on the hemiclavicular line; VB= electrode placed at the fifth left intercostal space on the hemiclavicular line; VC= electrode placed at the fifth left intercostal space on the anterior axillary line.

The resting ECG was obtained from each rat after 30 (phase I), 120 (phase II) and 350 (phase III) days of infection. Rats with an abnormal resting ECG at phase I formed group I, while rats with a normal resting ECG at phase I formed group II. Uninfected rats formed the control group.

After obtaining the resting ECG, each animal was submitted to the ajmaline test. Ajmaline is a type I antiarrhythmic drug which increases both the refractory period and the duration of the action potential in all cardiac tissues. The action of ajmaline depends on the extent of fascicular damage to reveal latent bundle branch block. For this reason, we administered ajmaline to *T. cruzi*-infected rats with normal resting ECG to unmask cardiac disease [7, 8]. The ajmaline test consists of a bolus injection of ajmaline aspartate (Kalickemie AG; 1 mg/kg body weight) into the dorsal vein of the penis, followed by complete ECG tracing 30 s later.

The following electrocardiographic variables were analysed before and after the ajmaline test: P wave duration in II, III, AVF and VB leads; PR interval in VB lead; QRS duration in all leads; QRS axis; Q wave in I, AVL and VC leads; J point in all leads.

Criteria for determining the abnormality of the resting ECG as well as the ajmaline test have been described elsewhere [6–9]. Briefly, the 5th and 95th percentiles for each electrocardiographic variable for control rats were determined at different stages of the experiment. Values higher than the 95th percentile or lower than the 5th percentile for control rats were indicative of heart disease. On the basis of these determinations, the following definitions were obtained.

1. Intra-atrial conduction delay: P wave duration longer than the 95th percentile for control rats in at least two leads.
2. First-degree AV block: PR interval longer than the 95th percentile for control rats.
3. Intraventricular conduction delay: QRS duration longer than the 95th percentile for control rats in at least two leads.
4. Left axis deviation: QRS axis between −45° and −90°.
5. Right axis deviation: QRS axis between +120° and +180°.
7. Pathological Q wave: Q wave duration longer than 10 ms in I, AVL, VC leads, or its presence in II, III and AVF leads.
8. J point elevation: elevation of the J point > 1 mm in I, AVL or VC leads or > 2 mm in II, III and AVF leads.
9. J point depression: depression of the J point > 3 mm in I, AVL or VC leads or > 2 mm in II, III, AVF and VB leads.

All of these electrocardiographic criteria were applied to the resting ECG. However, for the ajmaline test, only intra-atrial conduction delay, intraventricular conduction delay, first-degree AV block and indeterminate axis were considered to be indicative of latent Chagas’ heart disease in rats because these alterations have a high positive predictive value for myocardial disease when compared with histopathological findings.

**Pathological study**

All animals were killed by excess ether anaesthesia at the end of phase III. The heart was excised, blotted, weighed and immersed in formaldehyde. A transversal
section was cut at the AV groove in order to separate atria from ventricles. A biventricular section was cut 2 mm below the AV groove and another 2 mm from the cardiac apex. This fragment was then cut longitudinally. Two histological sections (7 μm each) from each fragment were obtained and routinely stained with haematoxylin/eosin.

Criteria for determining histological abnormalities have been described elsewhere [11]. Briefly, myocardial lesions (polymorphonuclear cell infiltrate, mononuclear cell infiltrate, necrosis, vacuolation and fibrosis) were scored as follows for intensity: light +, moderate ++ and severe ++++, and for extension: focal +, confluent + + and diffuse +++. The total score for each animal was obtained by summing the partial scores. A score >4 was considered to be indicative of heart disease.

Statistical analysis

The Cochran test was used to compare the proportion of a given electrocardiographic change between phases I and II and between phases II and III for each group of animals studied. The McNemar test was applied to compare the proportion of all electrocardiographic changes for each group among the three phases of the experiment. A P value less than 0.05 was considered to have statistical significance.

RESULTS

Electrocardiographic study

Phase I. Control rats. There was no electrocardiographic change in any of these animals.

Group 1. The abnormalities in the resting ECG were as follows: left axis deviation in 10 of 12 rats (83%), intraventricular conduction delay in two of 12 rats (16%), indeterminate axis in one of 12 rats and intra-atrial conduction delay in one of 12 rats (8%). The data are summarized in Table 1.

Group 2. There was no electrocardiographic change in this group of animals (Table 2).

Phase II. Control rats. Both the resting ECG and the ajmaline test were normal in these animals.

Group 1. Abnormal resting ECG was observed in two of 12 rats (16%); intraventricular conduction delay in two of 12 rats (16%) and left axis deviation in one of 12 rats (8%).

The proportion of left axis deviation was statistically higher in phase I than phase II (P< 0.001), but the proportion of intraventricular conduction delay did not differ statistically (P> 0.10) between the two phases.

The ajmaline test was abnormal in nine of 10 rats (90%) with normal resting ECG; intra-atrial conduction delay was detected in three of 10 rats (30%), first-degree AV block in two of 10 rats (20%) and intraventricular conduction delay in nine of 10 rats (90%).

Group 2. There was no abnormal resting ECG, but the ajmaline test was abnormal in one animal (first-degree AV block).

Phase III. Control rats. Both the resting ECG and ajmaline test were normal in these animals.

Group 1. Six of 12 rats (50%) presented an abnormal resting ECG, and intraventricular conduction delay was observed in all of them.

The ajmaline test was abnormal in three of the remaining six rats which presented a normal resting ECG; intra-ventricular conduction delay was observed in all of them.

The proportion of abnormal resting ECG was lower in phase III than in phase I (P< 0.001). However, there was no difference in the frequency of abnormal ajmaline tests between phases II and III (P> 0.50). Importantly, there was also no difference in the frequency of total electrocardiographic changes (resting ECG plus ajmaline test) among the three phases of the experiment (P> 0.10).

Group 2. One of 13 rats (7%) showed first-degree AV block associated with intraventricular conduction delay. One of the remaining 12 rats (8%) with a normal resting ECG showed first-degree AV block after ajmaline injec-

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Table 1. Electrocardiographic changes observed at different intervals after Trypanosoma cruzi infection

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Histopathological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LAD</td>
<td>R: IVCD</td>
<td>R: IVCD</td>
<td>MNC, V, N, S=11</td>
</tr>
<tr>
<td>2</td>
<td>LAD, IVCD</td>
<td>R: LAD, IVCD</td>
<td>R: IVCD</td>
<td>MNC, V, N, S=12</td>
</tr>
<tr>
<td>3</td>
<td>LAD, IVCD</td>
<td>A: IACD, IVCD</td>
<td>A: IVCD</td>
<td>MNC, V, N, S=12</td>
</tr>
<tr>
<td>4</td>
<td>LAD</td>
<td>R: IVCD</td>
<td>R: IVCD</td>
<td>MNC, V, N, S=10</td>
</tr>
<tr>
<td>5</td>
<td>IA</td>
<td>A: IVCD</td>
<td>A: IVCD</td>
<td>MNC, V, N, S=12</td>
</tr>
<tr>
<td>6</td>
<td>LAD</td>
<td>A: IVCD</td>
<td>Normal</td>
<td>MNC, S=2</td>
</tr>
<tr>
<td>7</td>
<td>IACD, LAD</td>
<td>A: IVCD</td>
<td>R: IVCD</td>
<td>MNC, V, N, S=12</td>
</tr>
<tr>
<td>8</td>
<td>LAD, IVCD</td>
<td>A: 1st-degree AV block, IVCD</td>
<td>R: IVCD</td>
<td>MNC, PNC, V, N, S=10</td>
</tr>
<tr>
<td>9</td>
<td>LAD</td>
<td>A: 1st-degree AV block, IVCD</td>
<td>Normal</td>
<td>MNC, S=2</td>
</tr>
<tr>
<td>10</td>
<td>LAD</td>
<td>Normal</td>
<td>A: IVCD</td>
<td>MNC, S=2</td>
</tr>
<tr>
<td>11</td>
<td>IVCD</td>
<td>A: IACD, IVCD</td>
<td>A: IVCD</td>
<td>MNC, PNC, V, N, S=10</td>
</tr>
<tr>
<td>12</td>
<td>LAD</td>
<td>A: IVCD</td>
<td>Normal</td>
<td>S=0</td>
</tr>
</tbody>
</table>
tion. There were no differences in the frequency of either abnormal resting ECG or abnormal ajmaline test between phases II and III (P > 0.20).

Pathological study

Control rats. No rat showed a score of histological changes greater than 4.

Group 1. In eight of 12 rats (66%) the histological changes were scored as > 4 (Table 1). Mononuclear cell infiltrate, cellular vacuolation and myocardial necrosis were detected in all animals.

Group 2. Two of 13 rats (15%) presented histopathological alterations similar to those observed in group 1 (Table 2).

DISCUSSION

It is apparent from this investigation that different changes in the resting ECG can be detected at the various stages of T. cruzi infection.

At the end of the acute stage (phase I), left axis deviation was the electrocardiographic abnormality most frequently observed. This fact is in accordance with previous work from this laboratory [6] in which we observed QRS axis alterations in several rats with Chagas’ heart disease. Left axis deviation is the electrocardiographic manifestation of severe bundle branch involvement in man [12]. Since the atria and intraventricular septum are the areas preferentially affected in mice [13] at the end of the acute stage, it is possible that a similar mechanism is the cause of the high frequency of left axis deviation observed in this work during phase I.

At the onset of the chronic stage (phase II), most rats of group I (83%) showed a normal resting ECG, in contrast to what had been observed in phase I. In this respect, the evolutive pattern of these electrocardiographic changes is similar to that found in human Chagas’ disease, since most patients are found to have a normal resting ECG at the onset of the chronic stage [4].

An intraventricular conduction delay was observed at higher frequency at the end of the chronic stage of T. cruzi infection (phase III). This electrocardiographic abnormality reflects pronounced myocardial disease in both chronic human [14] and rodent heart disease [6, 7, 8, 15–18]. Therefore, QRS axis abnormalities precede the intraventricular conduction delay in the setting of evolving Chagas’ heart disease in the rat, as suggested morphologically by Laguens et al. [13].

It is important to stress that there was a reduction in total electrocardiographic changes in phase III when compared with phase II in group I. Moreover, there was no increase in the proportion of electrocardiographic changes in animals whose ECG was normal in phase I (group II). Taken together, these data suggest, at least in part, that chronic Chagas’ heart disease in the rat may not be a progressive myocardial disease. Rather, they are consistent with the concept of a chronic disease defined during the initial phase of the infection [19].

Another interesting finding obtained in this investigation is that the total number of electrocardiographic alterations (resting ECG associated with the ajmaline test) remained unchanged throughout the two later phases of the experiment. Therefore, although the same electrocardiographic change cannot be detected repeatedly in the course of T. cruzi infection in a given rat, the association of the resting ECG with the ajmaline test will detect an abnormal ECG in this animal. In other words, an abnormal surface ECG can be reproduced in the course of T. cruzi infection.

The absence of reproducibility of the same electrocardiographic change in the resting ECG during the course of Chagas’ heart disease in the rat may be the consequence of the pathophysiological peculiarity of the disease [13], inasmuch as Normann et al. [20] have not detected the same phenomenon in the setting of ischaemic heart disease in the rat.

It is apparent that the proportion of abnormal ajmaline tests remains the same during the two later phases of the experiment. In this sense, therefore, the ajmaline test
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appears to be useful to unmask cardiac disease at different times of T. cruzi infection.

The histopathological study showed cardiac involvement similar to that previously detailed [11]. These alterations are essentially compatible with chronic cardiac disease, with a virtual absence of the parasite in the myocardium. In addition, these myocardial lesions also correlated well with an abnormal surface ECG, as previously demonstrated by our group [6, 9].

In conclusion, this investigation shows that different electrocardiographic patterns can be observed in the various stages of Chagas' heart disease in the rat. It emerges that an abnormal surface ECG, detected by either the resting ECG or the ajmaline test, can be observed in the same T. cruzi-infected rat over time. This fact may be useful for the screening of T. cruzi-infected rats, as we have recently shown [21].

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