Rev. Inst. Med. trop. São Paulo
25(5):207-214, *setembro-outubro,* 1983

UDC 593.161.13
616.937.3-092.9

CHRONIC INFECTION IN MICE WITH TRYPANOSOMA CRUZI (*)

A. Tania BIJOVSKY, Marcelo V. ELIZARI, Leticia A. MULLER, Victor J. KATZIN (1) and Stella M. GONZALEZ CAPPA (2)

SUMMARY

A follow up of one year was performed in mice infected with CA-I **T. cruzi** strain. The longlasting parasitemia, non-lethal capacity and failure to stimulate neutralizing activity (NA) with stimulation of conventional serology was confirmed for this strain. Serology conversion was delayed in time evolving with a narrow peak of IgM during 4th week pi. A second IgM peak was detected on the 3rd month pi. Cross immunity between CA-I and Tul was proved when mice infected with the former survived a challenge with the latter strain. This also is an indirect indication that NA is not essential to overcome the acute lethal stage of the disease. Myositis was more severe than the myocarditis; adipose replacement was observed in the skeletal muscle during the chronic stage. The mild myocarditis was reflected in the ECG which showed a low percentage of QRS abnormalities. However, 70-75% of mice presented some ECG alterations, many times induced by the ether anesthesic effect resembling the Ajmaline effect reported for chronic Chagas in human beings.

INTRODUCTION

Most of the **Trypanosoma cruzi** strains adapted to mouse are lethal for this host during the acute stage of the infection. The number of parasites needed to kill a mouse vary with the strain of the parasite and with the strain, age and sex of the mouse. Usually, young are more susceptible than adult animals. Therefore, when adult mice are inoculated with very low doses of parasites, some of them can survive the acute infection and enter into a chronic stage [3,4,9]. During the latter stage electrocardiographic as well as histological lesions have been communicated [4,11]. On the other hand, few **T. cruzi** strains have been reported to be non lethal for mice when parasites are injected in high numbers and into young animals. Recently, we had reported the isolation of one of these strains, the CA-I, which evolved with low longlasting parasitemia [7]. As a follow-up, the humoral immune response, electrocardiogram (ECG) and histopathological patterns of mice inoculated with trypomastigotes of the CA-I strain were studied throughout one year and the results reported here.

MATERIALS AND METHODS

One hundred and twenty Rockland mice, 25 days old, 19 ± 1 g weight, were inoculated subcutaneously with $1x10^5$ CA-I trypomastigotes. This strain, whose isolation and features have been reported elsewhere [7], has been maintained by monthly transfers in mice. Trypomastigotes obtained from transfer 54 were used in this study. Sixty normal mice of the same age and weight were kept as controls. Groups of

(1) Fellowshipholder
(2) Research carrier of the CONICET — Argentina
Cátedra de Microbiología, Parasitología e Inmunología. Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 — piso nº13 — C.P. 1121. Buenos Aires, Argentina

5 infected and 3 normal mice were bled to death at different times after infection (pi) from day 5 through day 180. Sixty of the infected and 30 normal mice were left to check mortality and those surviving were killed one year pi.

Pooled serum samples obtained from day 5 to day 180 were assayed by conventional serology. It was performed by means of Direct agglutination test with and without 2 mercaptoethanol (2-MEDA and DA respectively) and by Indirect immunofluorescence test using fluorescein labelled anti-mouse-IgG (II-T-IgG) or IgM (IFT-IgM) (Cappel Laboratories) [71]. Those samples obtained on day 60 and 180 were assayed for Neutralizing activity (NA) using the method reported previously [13]. To evaluate NA parasites of the Tulahuén (Tul) strain were used and treated parasites were injected into groups of 10 young mice.

On days 5, 10, 15, 20, 25, 30, 60, 90, 120, 180, 270, 370, each group of mice were bled to death and samples of skeletal muscle (hind leg), intestine (cecum) and the heart were fixed in 10% formaldehyde, embedded in paraffin and 5 μm sections were stained with hematoxylin-eosin. In the group to be killed one year pi, electrocardiograms (ECG) were obtained after 8 and 12 months pi. The mice were anesthetized with ethylic ether and leads I, II, III, aVR, aVL, and aVF of the ECG were recorded in a three channel machine at 50 mm/seg paper speed.

In order to establish whether the infection with the CA-I strain conferred any cross protection against other **T. cruzi** strains, 3 additional groups of 10 mice each, inoculated 90 days earlier with 0.1, 1.0 and 10.0×10^5 CA-I trypomastigotes were challenged with 5×10^5 Tul strain trypomastigotes whose lethal activity has already been reported [8]. Parasitemia was compared with a control group of similar age but only infected with the Tul strain, and their survival time was recorded during 60 days after challenge.

RESULTS

All the infected animals revealed patent parasitemia after infection and by the end of the experiment it was still detectable in about 50% of the animals by direct examination of a fresh blood preparation. Deaths rarely occurred during the first six months pi (7% and 13% for normal and infected mice respectively) but after 12 months 55% of the infected and 30% of the normal mice were dead.

Conventional serology became positive as from day 25 pi. At this time and although the antibody titers measured by DA test were low, a significant drop was registered when the sera were previously treated with 2-ME, indicating the IgM nature of these antibodies. This IgM peak was not detected by IFT-IgM, but 3 months pi a second peak of IgM was found by this technique. Simultaneously with the second IgM peak a slight increase in DA titers was registered (Fig. 1). On the other hand, IgG antibodies were persistantly detected by both techniques after proving positive on day 25 (IFT-IgG) and 30 (DA) pi (Fig. 1). The CA-I **T. cruzi** strains failed to stimulate NA; therefore, parasites showed similar virulence for mice both when incubated with the pooled serum samples obtained on day 60 or 180 pi, and with normal sera.

The ECG patterns for control mice, even under the deepest anesthesic effect, were normal. Conversely, ECG alterations were detected in 70-75% of the infected animals (Table I). Two of the signs more frequently observed were ST elevation (injury), 1st or 2nd degree atrioventricular block and QRS widening; bradicardia and arrythmias were also present. In some of the animals these alterations were clearly induced by anesthesia because in these cases pathological patterns reverted to normality when the effect of the ether wore off, as illustrated in Fig. 2A. In 30-50% injury signs and blocks disappeared while bradicardia and arrythmias reverted in every case. The percentage of these signs was practically the same after 8 or 12 months pi. Besides, abnormal QRS were registered in few mice (widening over 0.04 and/or morphologic changes of the QRS); these signs proved constant and irreversible (Fig. 2B). In the present experiments abnormal QRS were only registered in 8.57% of the animals 8 months pi, rising to 25.93% after 12 months pi (Table I).

Signs of myocarditis were first observed in mice killed 45 days pi as a discrete mononuclear infiltration in the auricles and ventricular bases (Fig. 3A). Myocarditis was more severe in those mice killed 4 months pi than previously, located mostly in auricles and ven-

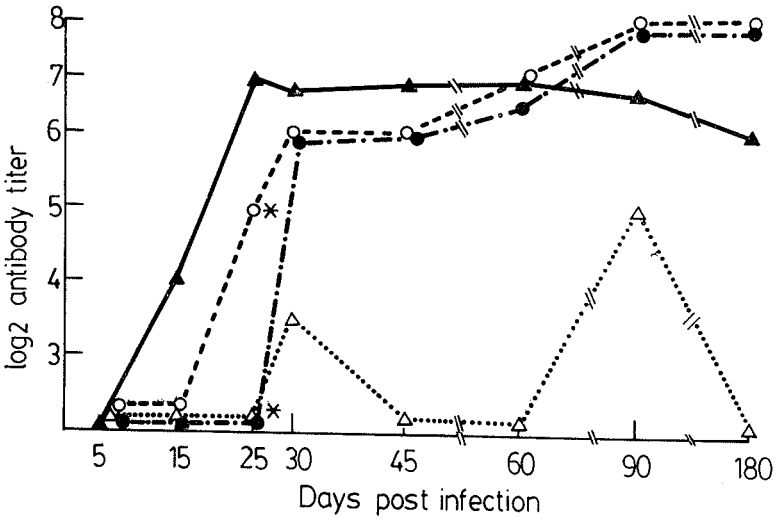

Fig. 1 — Antibody patterns as measured by conventional serologic tests
* Significant titer drop after 2ME serum treatment.
▲—▲ IFT-IgG
△···△ IFT-IgM
—.— DA
○- - -○ 2-MEDA
Antibody titer is expressed as the reciprocal of the serum dilution.

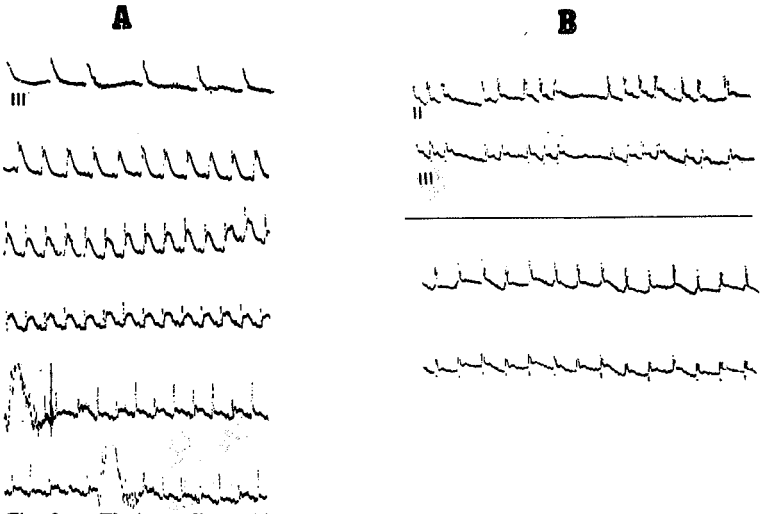

Fig. 2 — Electrocardiographic recording showing the anesthesic effect in infected mice
A) Effect of anesthesia upon sinus rythm and ventricular repolarization (lead III). First strip: marked sinus bradycardia and ST segment elevation during maximum effect of anesthesia. From strip 2 to 4, sinus rate increase and ST segment elevation decreases when the effect of ether is desappearing. The last 2 strips were recorded 50 to 60 seconds later than strip 1, when the animal was practically awaken only a slight elevation of the ST segment persists. B) Simultaneous recording of leads II and III from other animal showing the effect of anesthesia upon atrioventricular conduction. Top panel: second degree atrioventricular block and marked sinus arrythmia during deep anesthesia. Bottom panel: A few seconds later, when the effect of anesthesia is vanishing, sinus rythm and atrioventricular conduction reverted to normality. Conversely, the widening and morphologic changes of the QRS interval remain unchanged throughout the anesthetic period indicating a constant and persistent abnormality.

tricular bases (Fig. 3B); in 2 out of 5 animals nests of amastigotes were found. Similar lesions were detected in those groups killed 6 or 9 months pi, except that parasites were hardly observed. One year pi 14 out of 27 of the surviving animals showed significant alterations in the myocardium when compared with the control group: half of them exhibited focal and moderate myocarditis, specially located in auricles without detectable parasites, while in the

other half mononuclear infiltration was minimum (Fig. 3C).

T A B L E I

ECG lesions in mice infected with CA-I strain after 8 or 12 month pi

ECG lesion	Normal mice		Infected mice	
	8 m*	12 m**	8 m°	12 m°°
Injury	0%	5%	54,28%	33,33%
Block	0%	0%	31,42%	44,44%
Bradicardia	0%	0%	11,43%	18,52%
Arrythmia	0%	0%	2,86%	7,41%
Abnormal QRS	0%	5%	8,57%	25,93%
Normal ECG	100%	95%	28,57%	25,92%

*: 22 mice
**: 20 mice
°: 35 mice
°°: 27 mice

At 15 days pi moderate to intense myositis was present: amastigote nests and unrelated mononuclear infiltration were seen in all the animals (Fig. 4A); polymorphonuclear leucocytes were also present in those areas where a recent fibrilar necrosis was determined by nest disruption. This picture was practically the same until day 45 pi, when the inflammatory signs decreased, remaining moderate, and parasites were found in 3 out of the 5. In mice killed between 2-4 months pi a moderate mononuclear infiltration persisted in skeletal muscle as well as signs of incipient adipose tissue replacement (Fig. 4B). Perivascular mononuclear infiltration was observed. In those killed 6-9 months pi the adipose replacement increased (Fig. 4C). Although nests of parasites were

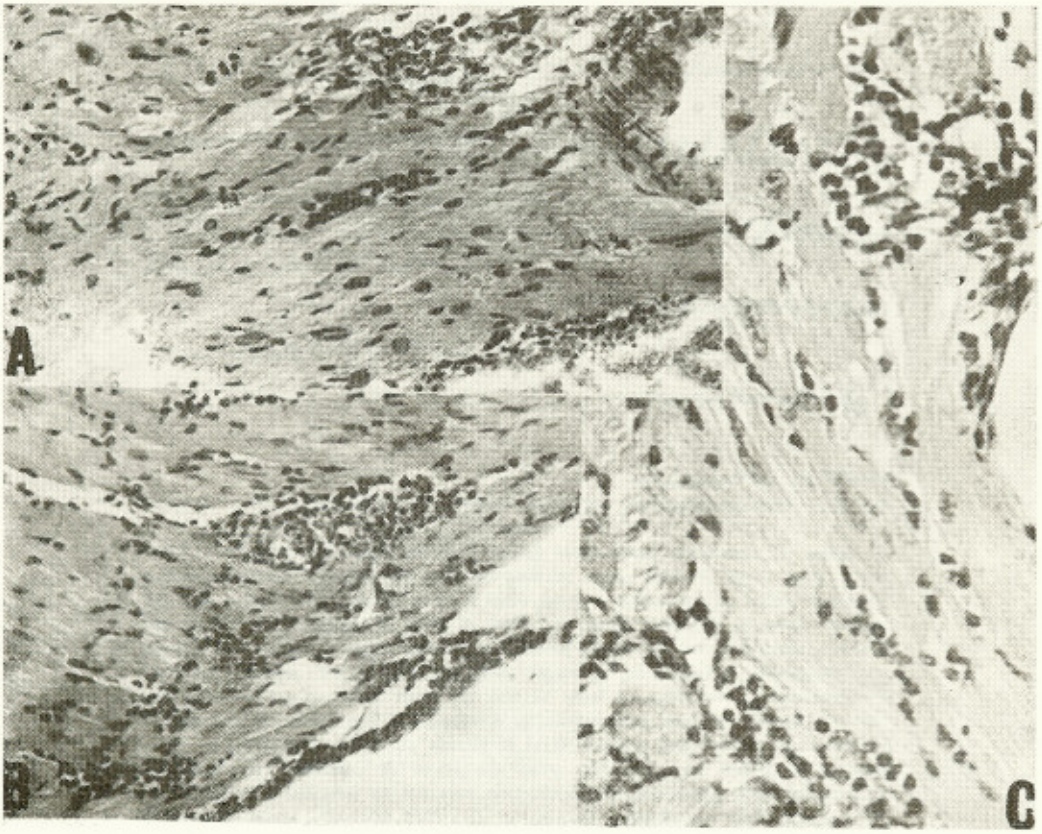

Fig. 3 — Signs of myocarditis in mice infected with CA-I T. cruzi strain.
A) Scarse mononuclear infiltration in the ventricular base; mouse killed 45 days pi (64 ×). B) Moderate myocarditis signs in ventricle; mouse killed 3 months pi (64 ×). C) Mononuclear infiltration in auricle; mouse killed one year pi (102,4 ×).

also detected, numbers were lower than during the first 45 days pi. Twenty four out of the 27 mice killed one year pi still showed some mononuclear infiltration in skeletal muscle

with a variable degree of adipose replacement (Fig. 4D, E). Myositis was severe in few cases and in three parasites were also seen. This severe myositis and the adipose replacement might be the cause of the macroscopic atrophy shown by part of the mice in the hind legs (Fig. 5).

Scarce mononuclear infiltration was seen in the muscular intestine wall, starting on day

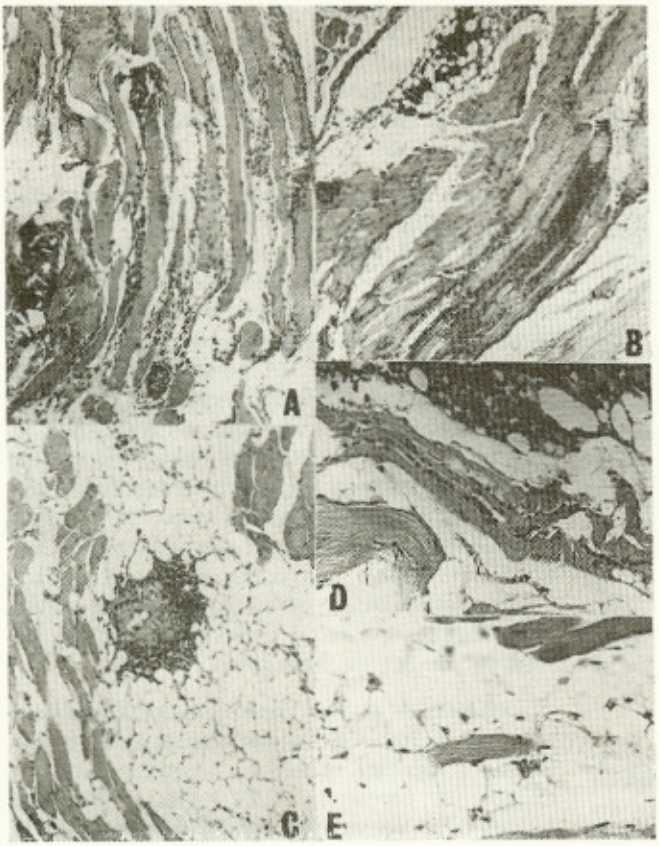

Fig. 4 — Skeletal muscle lesions (hind leg) in mice infected with CA-I **T. cruzi** strain. A) Myositis with mononuclear infiltration after 15 days of infection (16 ×). B) Mononuclear infiltration and incipient adipose replacement after 3 months of infection (16 ×). C) Perivascular mononuclear infiltration and adipose replacement after 9 months pi (49,6 ×). D) Mononuclear infiltration and moderate adipose replacement after one year pi (64 ×). E) Replace of muscle fibres by adipose tissue; mouse killed one year pi (64 ×).

Fig. 5 — Hind legs' atrophy shown by part of mice

45 pi and persisting in all the groups until the end of the experiment.

Histological alterations were entirely lacking in controls.

None of the mice previously infected with the CA-I strain died after being challenged with lethal doses of the Tul strain. Fifteen out of the 30 developed patent parasitemia, never higler than $4x10^4$/ml, detected by direct examination of a fresh blood drop. The control group died between days 13 and 16 post challenge and parasitemia reached $2.3x10^6$/ml by day 10 for this group.

DISCUSSION

We have proved that NA is involved in parasitemia control [13]. The longlasting and easily detectable parasitemia during the chronic infection with the CA-I strain, might be determined by its failure to induce NA. In contrast, chronic infections induced in mice with low doses of Tul trypomastigotes, a strain able to stimulate NA, usually evolved with easily detectable parasitemia during the 1st month pi, but was hardly ever detected later [4]. The cause of NA non-stimulation is unknown at present and might be related to the surface antigens of the parasite.

SCHMUÑIS et al. [16] had reported that Rockland mice inoculated with high doses of trypomastigotes of the Tul strain showed a depressed response for sheep red blood cells late during the infection, but the immunoresponse was stimulated in early stages. CUNNINGRAM[5] using Brasil strain trypomastigotes had found similar initial enhacement for $C_{57}BL/_6$ but not for C_3H mice (high and low resistant mouse strains, respectively). This early immune stimulation for unrelated antigens, resulting from the interaction between the natural resistance of the mouse strain and the degree of infection induced by a **T. cruzi** strain, might also be effective for homologous antigens. Therefore, if low number of parasites of a lethal strain are injected, the resultant infection may be mild with survival of the mice and late serology conversion [12,17]; otherwise, humoral immunoresponse is detected earlier [19]. However, with the CA-I strain the response was delayed in spite of the number of parasites injected. This late serology conversion and the short period of specific IgM stimulation identify this strain as one of "low virulence".

It has been reported passive serum transfer is able to control the parasitemia induced by lethal doses of parasites resulting in a longer survival of the receptors provided the IS is positive for NA [13]. The fact that the CA-I strain induced chronic infection in mice but failed to stimulate NA, indicates that NA participation is not essential to overcome the acute infection in all strains.

Cross immunity among **T. cruzi** strains had been widely reported [3,18]; cross immunity between CA-I and Tul developed without signs of positive NA in the animals at the time of challenge, proving that, even with those **T. cruzi** strains where the acute infection is controlled by sera with positive NA, as is the case of Tul strain, humoral immune response is not the only mechanism to overcome the acute and lethal infection. The role of T cell in controlling this stage had been implied by infection of surgically or naturally athymic mice [10,14,15]. Thus, several mechanisms may be evoked by the parasite to survive the acute infection and the type of stimulation seems to be strain dependent. Studies related to the latter subject are in progress in our laboratory.

PERALTA et al. [12] had communicated late serology conversion with persistant IgM response (up to the 3rd. month pi) injecting very low number of parasites. VATTUONE et al. [19] had reported a longer IgM antibody response in mice inoculated with low doses of trypomastigotes of several lethal **T. cruzi** strains, compared with the response obtained when epimastigotes of the same strains were injected. The IgM response detected in this study for the CA-I strain resembles that obtained by the latter authors for epimastigotes. They had suggested a correlation between active intracellular parasite duplication and longer IgM persistance. If this is true, CA-I strain may possess a lower duplication rate as compared to other **T. cruzi** strains, at least **in vivo.** In the present report, the second IgM peak registered might indicate parasite duplication enhacement. We have seen a similar peak in rabbits immediately after a second inoculation of **T. cruzi** trypomastigotes (unpublished data).

ANDRADE et al. [2] and YANOVSKY et al. [20] had been able to induce histological lesions in

infected mice. Recently, LAGUENS et al.[11] had reported that **T. cruzi** infected mice can develop chronic Chagas' disease with ECG disturbances as well as histological lesions. In all cases signs of myocarditis were mild and located mostly in the auricles.

In the present report myocarditis was also mild and it was reflected in the low numbers of abnormal ECG patterns with permanent alteration. At any rate, the high sensitivity of the infected mice to anesthesia suggests incipient tissue damage induced by the parasites. The anesthesic effect of the ECG patterns resembles that produced by Ajmaline injection in human beings; this drug had proved useful to forcast myocardial damage in chronic Chagas' disease before any sign could be detected by an ECG in basic conditions[6]. However, in the absence of reports concerning the electrophysiological effects of ethylic ether, it is unknown whether the above described sequence of ECG alterations are the result of the direct effect of the drug or the consequence of anoxia induced by the deeper level of anesthesia.

We had found that the inflammatory lesions seen at the skeletal muscles were more severe than the myocarditis. Pronounced myositis had been reported by ANDRADE & ANDRADE[1] for the Columbian strain, which is a "low virulence" strain for mice, like CA I. Lesions of similar severity at the skeletal muscles as shown here were reported by CABEZA MECKERT et al. only for those animals repeatedly infected with Tul strain[4]. These Authors had communicated that with Tul strain each trypomastigote inoculation was followed by a short period of patent parasitemia whereas a constant patent parasitemia is known to be characteristic of the CA-I strain. The longlasting relatively high parasitemia quite likely determines a constant muscular invasion with direct tissue damage and continuos antigenic liberation which might enhance the initial immunologic stimulation.

The results presented here confirm the mouse is a host capable of developing chronic lesions by **T. cruzi** infection. Although the lesions are mostly mild, this host is up to now the only chronic experimental model reported, capable of being replicated by different workers.

RESUMEN

Infección crónica en ratones por Trypanosoma cruzi

La cepa CA-I, de escasa o nula letalidad para el ratón, evoluciona con una parasitemia baja pero persistente. En este trabajo se estudió, en este huésped, la respuesta inmune humoral, el trazado ECG y el cuadro histopatológico, durante un año de infección. Se confirmó la persistencia de la parasitemia fácilmente detectable en el período crónico de la infección y la positivización de la serología convencional sin estimulación de la actividad neutralizante.

La respuesta inmune humoral se positivizó retardada en el tiempo en relación a la cantidad de parásitos utilizados para la infección del ratón. Se detectó un estrecho pico de IgM durante la cuarta semana pi y un segundo pico en el tercer mes. Se comprobó que esta cepa es capaz de inducir resistencia cruzada con la cepa Tul protegiendo a los ratones contra dosis desencadenantes letales de esta última cepa. Esto además es una indicación indirecta de que no es esencial la existencia de actividad neutralizante evidente para sobrellevar el período agudo letal de la infección por **T. cruzi.**

La miositis fue más severa que la miocarditis, observándose reemplazo de tejido muscular periférico por adiposo durante la infección crónica. La falta de severidad de la miocarditis se reflejó en el bajo porcentaje de ECG que presentaron anormalidades en el QRS. De todos modos, 70-75% de los ratones infectados presentaron alteraciones ECG, muchas veces inducidas por los efectos anestésicos del éter, recordando el efecto comunicado para la Ajmalina en el hombre con infección chagásica crónica.

ACKNOWLEDGMENT

This investigation received finantial support from the UNDP/World Bank/WHO (Special Programme for Research an Training in Tropical Diseases) and from Secretaría de Estado de Ciencia y Tecnología from Argentina.

REFERENCES

1. ANDRADE, S. G. & ANDRADE, Z. — Patologia da Doença de Chagas experimental de longa duração. **Rev. Inst. Med. trop. São Paulo 10:** 180-187, 1968.

2. ANDRADE, S. G.; FIGUEIRA, R. M.; CARVALHO, M. L. & GORDINI, D. F. — Influencia da cepa do **Trypanosoma cruzi** na resposta a terapeutica pela Bay 2502 (Resultados de tratamiento a largo plazo). **Rev. Inst. Med. trop. São Paulo 17:** 380-389, 1975.

3. BRENER, Z. — Immunity to **Trypanosoma cruzi. Adv. Parasitol. 18:** 247-292, 1980.

4. CABEZA MECKERT, P. & LAGUENS, R. P. — Chronic Chagas disease in the mouse: III Absence of concomitant immunity after repetitive infections. **Medicina (Bs.As.) 41:** 543-548, 1981.

5. CUNNINGHAM, D. S.; KHUN, R. S. & ROWLAND, E. C. — Suppression of humoral responses during **Trypanosoma cruzi** infections in mice. **Infect. Immun. 22:** 155-160, 1978.

6. CHIALE, P. A.; PRZYBYLSKI, J.; LAIÑO, R. A.; HALPERN, M. S.; SANCHEZ, R. A.; GABRIELLI, A.; ELIZARI, M. V. & ROSENBAUM, M. B. — Electrocardiographic changes evoked by ajmaline in chronic Chagas disease without manifest myocarditis. **Am. J. Cardiol. 49:** 14-20, 1982.

7 GONZALEZ CAPPA, S. M.; CHIALE, P.; Del PRADO, G. E.; KATZIN, A. M.; MARTINI, G. W. J.; ISOLA, E. L. D. de; ABRAMO ORREGO, L. & SEGURA, E. L. — Aislamiento de una cepa de **Trypanosoma cruzi** de un paciente con miocardiopatía chagásica crónica y su caracterización biológica. **Medicina (Bs.As.) 40** (Supl. 1): 63-68, 1980.

8 GONZALEZ CAPPA, S. M.; KATZIN, A. M.; AÑASCO, N. & LAJMANOVICH, S. — Comparative studies on infectivity and surface carbohydrates of several strains of **Trypanosoma cruzi. Medicina (Bs.As.) 41:** 549-555, 1981.

9. GONZALEZ CAPPA, S. M. & SEGURA, E. L. — Enfermedad de Chagas. **Adelantos en Microbiología y Enfermedades Infecciosas 1:** 51-102, 1982.

10. KIERSZEMBAUM, F. & PIERIKOWSKI, M. M. — Thymus dependent control of host defense mechanisms against **Trypanosoma cruzi** infections. **Inf. Immun. 24:** 117-120, 1979.

11. LAGUENS, R. P.; CABEZA MECKERT, P. & GELPI, R. J. — Chronic Chagas' disease in the mouse. I. Electrocardiographic and morphological patterns of the cardiopathy. **Medicina (Bs.As.) 41:** 35-39, 1981.

12. PERALTA, J. F.; FILARDI, L.; LOURDES, M. A. & TORRES, S. — **Trypanosoma cruzi:** antibodies in mice infected with different strains of **T. cruzi. J. Parasitol. 66:** 342-343, 1980.

13. SANCHEZ, D. O. & GONZALEZ CAPPA, S. M. — Neutralizing activity in **T. cruzi** infection. **Medicina (Bs.As.)** (in press).

14. SEGURA, E. L.; ESTEVA, M.; QUINTANS, C. J.; MANTOVA, L. S. & WEISSENBACHER, M. C. — Infección con **Trypanosoma cruzi** en ratones congénitamente atímicos. **Medicina (Bs.As.) 41:** 328-332, 1981.

15. SCHMUÑIS, G. A.; GONZALEZ CAPPA, S. M.; TRAVERSA, O. C. & YANOVSKY, J. F. — The effect of immuno-depression due to neonatal tymectomy of infections with **Trypanosoma cruzi** in mice. **Trans. Roy. Soc. Med. Hyg. 65:** 89-94, 1971.

16. SCHMUÑIS, G. A.; SZARFMAN, A.; PESCE, U. & GONZALEZ CAPPA, S. M. — The effect of acute infection by **Trypanosoma cruzi** upon the response of mice to sheep erythrocytes. **Rev. Inst. Med. trop. São Paulo 19:** 323-331, 1977.

17. SCHMUÑIS, G. A.; SZARFMAN, A. & VATTUONE, N. — Direct agglutination test of anti **T. cruzi** antibodies in mice. **J. Parasit. 58:** 1006-1007, 1972.

18. TEIXEIRA, A. R. L. — Perspectivas de vacinação contra Doença de Chagas. En **Progressos na Imunologia das Parasitoses.** C.E. Tosta (ed). Brasília, p. 106-137, 1977.

19. VATTUONE, N. H.; GONZALEZ CAPPA, S. M.; MENES, S. & SCHMUÑIS, G. A. — Cell mediated and humoral immune response in mice infected with **T. cruzi. Tropenmed. Parasit. 25:** 267-277, 1974.

20. YANOVSKY, J. F.; TRAVERSA, O. C.; TARATUTO, A.; SCHMUÑIS, G. A.; GONZALEZ CAPPA, S. M. & PARODI, A. S. — **Trypanosoma cruzi:** experimental immunization of mice. **Exp. Parasit. 26:** 73-85, 1969.

Recebido para publicação em 8/7/1982.