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ACTIVE IMMUNIZATION OF DOGS WITH A NON VIRULENT STRAIN OF *TRYPANOSOMA CRUZI*

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SUMMARY

Dogs vaccinated with an avirulent strain (culture-forms) of *Trypanosoma cruzi* acquired a very high degree of protection against a challenge with a virulent strain (blood forms) of the same parasite.

Efficiency of induced immunity was controlled by parasitemia, ECG, hemoculture and histopathologic examination.

INTRODUCTION

In previous papers it was shown, MENEZES [2, 3] that the Y strain of *T. cruzi*, isolated from a human case of Chagas' disease, SILVA & NUSSENSWEIG [4] and kept in culture medium of Packchanian for 15 years, had become non-virulent for mice.

Dogs were immunized and submitted afterwards to an infection with the virulent forms of the same strain kept in mice, so as to verify the behaviour of the non-virulent strain in relation to other laboratory animals.

MATERIAL AND METHODS

Nine dogs, their age varying from 25 to 30 days with an average weigh of 1,100 g were divided into two groups.

The first group of five animals received subcutaneously 1 ml of a vaccine prepared in this way:

Trypanosomes of culture in Packchanian medium with 30 to 35 days, were washed and centrifuged repeatedly in saline so as to get a clear supernatant. The sediment of the last centrifugation was again suspended in saline so as to obtain suspensions with the following concentrations: $1,6 \times 18^8$; $2,3 \times 10^8$; 3×10^8 and $2,3 \times 10^9$ parasites by ml.

The vaccine was used immediately after it was prepared.

One of the dogs was vaccinated with the first suspension, another one with the second suspension and the last three with the third one.

After four weeks all the animals were inoculated with 4,000 virulent parasites of the Y strain by g of body weigh and these parasites were obtained from the blood of mice on the 8th day of infection.

The animals were submitted under anesthesia to E.C.G. before the vaccination and respectively 8, 15 and 28 days after it and 8, 15, 30 and 60 days after the virulent infection.

The parasitemia of each dog was measured through the PIZZI-BRENER technique, BRENER[1] in the same days of the E.C.G.

Hemoculture in Warren medium (heart brain infusion) was done 15 days after the vaccination and 30 days after the infection by the virulent forms (3 tubes for each animal).

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From this group, two dogs were killed 60 days after the virulent infection for a histopathologic examination, E.C.G. having been done previously.

The control group was formed of 4 dogs of the same age and same weight. All of them were submitted to E.C.G. before the infection and 8, 15, 30 and 60 days after it, except those that died before.

The control animals that died before the 60th day of infection were submitted to E.C.G. some hours before death, whenever possible.

Parasitemia was determined by the same technique and on the same days used for the vaccinated group.

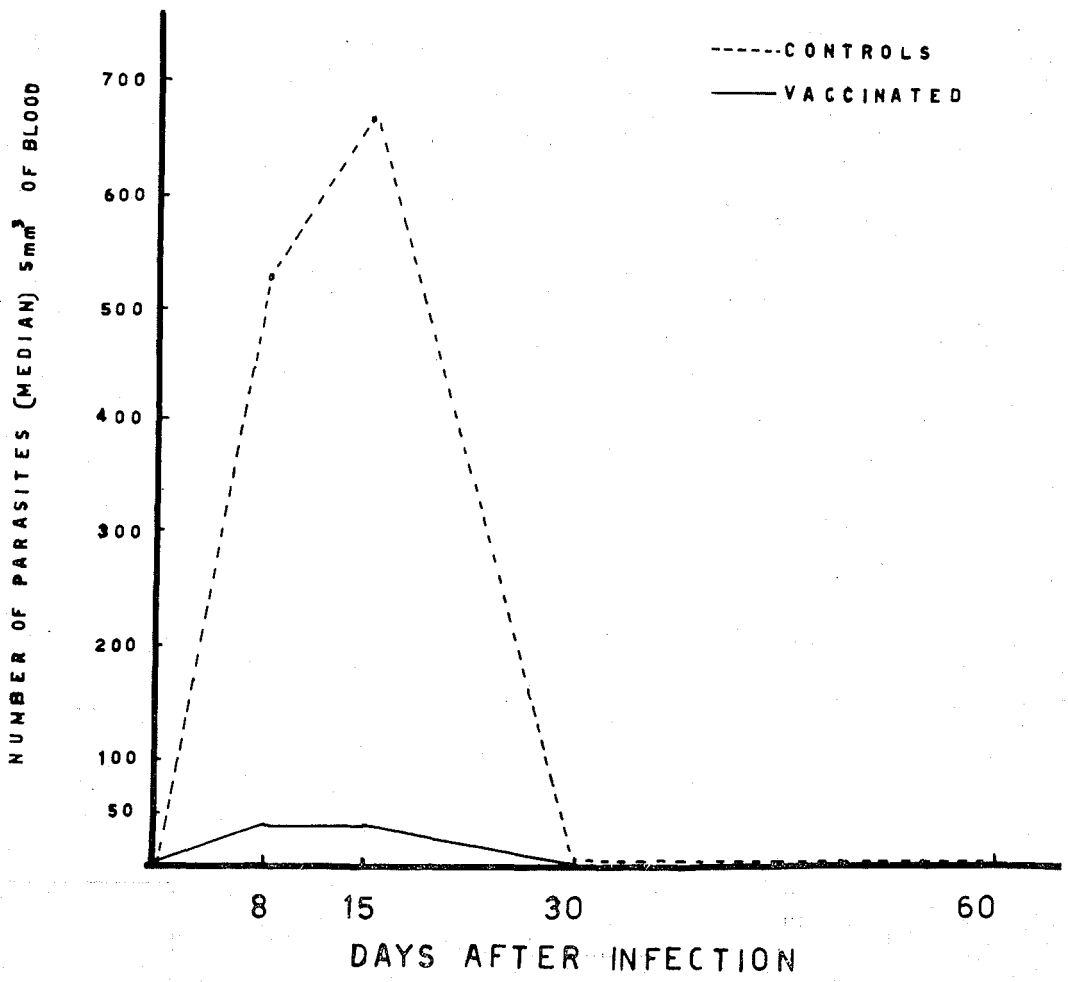
Two dogs, with the same characteristics of those used in the two previous groups, were vaccinated with 3×10^8 parasites and were used only for histologic control. One of these animals was killed 15 days after the vaccination and the other one 8 days after the virulent infection.

RESULTS

The parasitemic curve of the first two groups is shown in Graph I and in Table I.

The percentage of mortality is presented in Graph II, where one can verify that all the animals vaccinated survived until the 60th day of virulent infection whereas only one of the four control animals survived.

GRAPH I



This last one, however, did not tolerate the dose of Nembutal (30 mg/kg) after having received it. It is worth noticing that the same anesthesia with the same dose was well tolerated by vaccinated animals.

The other 3 control dogs died on the 19th, 21st and 22nd day after the infection.

None of the vaccinated animals presented any electrocardiographic alteration 8, 15 and 28 days after receiving the vaccine (Fig. 1 and 2).

Two dogs from this group had slight anomalies (in one of them absence of P. wave was noted and in another presence of T. negative waves, symetrics, from V_1 to V_6 were observed). This occurred at the end of the 15th day of virulent infection but disappeared at the end of the 30th day.

The control animals died presenting deep electrocardiographic alterations, specially total A.V. blocking (Fig. 3).

The control that died on the 60th day presented discrete alterations of the S.T. segment in DII, DIII and AVF and disturbances of ventricular repolarization in the anterior and diaphragmatic sides.

Among the vaccinated animals that received the vaccine with higher concentration of parasites ($2{,}3 \times 10^9$) two had positive hemoculture on the 15th day of vaccination. The sub-inoculation of cultivated trypanosoma in two young mice was negative.

Besides not one of the animals showed any electrocardiographic alteration and the parasitemia was persistently negative in one of them 8, 15, 30 and 60 days after the infection

TABLE I

Parasitemia in dogs vaccinated with avirulent cultures of *T. cruzi* and in dogs used as controls, at different intervals after inoculation of blood with virulent forms of the parasite

Group of dogs	Dog no.	Number of parasites per 5 mm³ of blood, 8, 15, 30 and 60 days after inoculation			
		8	15	30	60
Vaccinated	1	35	105	0	0
	2	0	0	0	0
	3	35	35	0	0
	4	105	35	0	0
	5	0	0	0	0
	Average	35	35	0	0
	Median	35	35	0	0
Controls	1	1,120	1,125	—	—
	2	770	595	—	—
	3	280	35	0	0
	4	210	735	—	—
	Average	595	625	0	0
	Median	525	665	0	0

and very low in the other on the 8th and 15th days (35 parasites/5 mm³ of blood), being negative on the 30th day.

The hemoculture of all the five vaccinated dogs was negative at the end of 30 days of infection.

The animal killed 15 days after the vaccination as well as the vaccinated and infected ones which were killed 8 and 60 days after they had been infected, did not show any histologic alteration of the myocardium (Fig. 1 and 2). By the other side, the control

GRAPH II

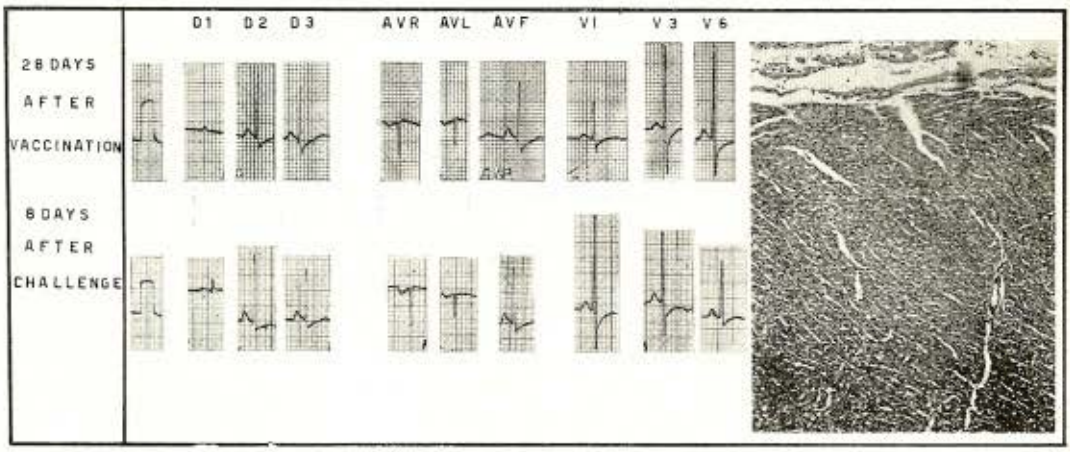


Fig. 1 — Dog vaccinated and killed 8 days after challenge

animals that died before the 30th day showed intense parasitism of the myocardium, diffuse inflammatory infiltration and necrosis in accordance with the electrocardiographic alterations observed (Fig. 3).

The control animal that died on the 60th day showed as well a great inflammatory infiltrate of the myocardium and presence of pseudo-cysts.

DISCUSSION

The protection given to the vaccinated dogs with the non-virulent Y strain of *T. cruzi* was simular to that given to mice, as previously referred, MENEZES [2, 3].

In spite of electrocardiographic examination, determination of parasitemia, of hemoculture and histopathologic examinations, no evolutive infectious process was observed in the vaccinated animals.

These, when submitted to an intense virulent infection only showed a slight infection, with low parasitemia and occasional electrocardiographic alterations which disappeared at the end of 30 days.

The appearance of positive hemoculture in two of the vaccinated animals can be explained by the great number of parasites inoculated with the vaccine.

The proof that the parasites were non-virulent is in that they cannot infect young

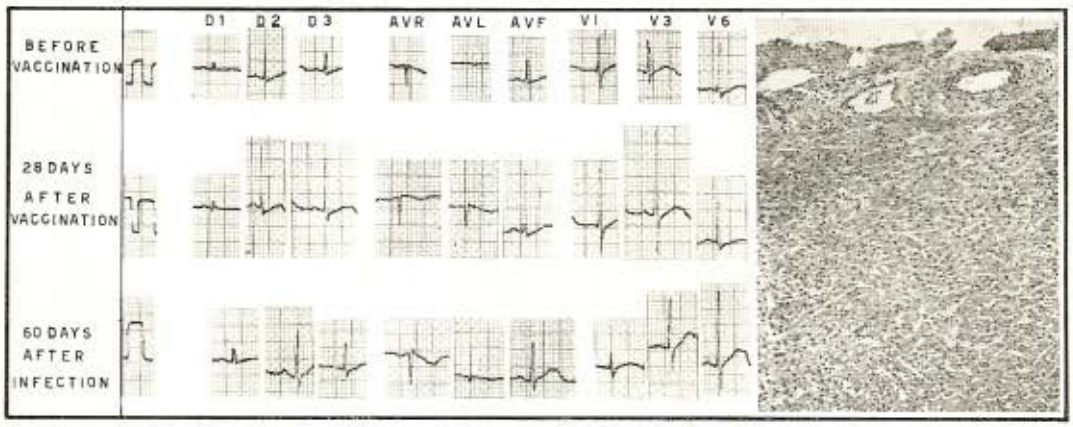


Fig. 2 — Dog vaccinated and killed 60 days after challenge

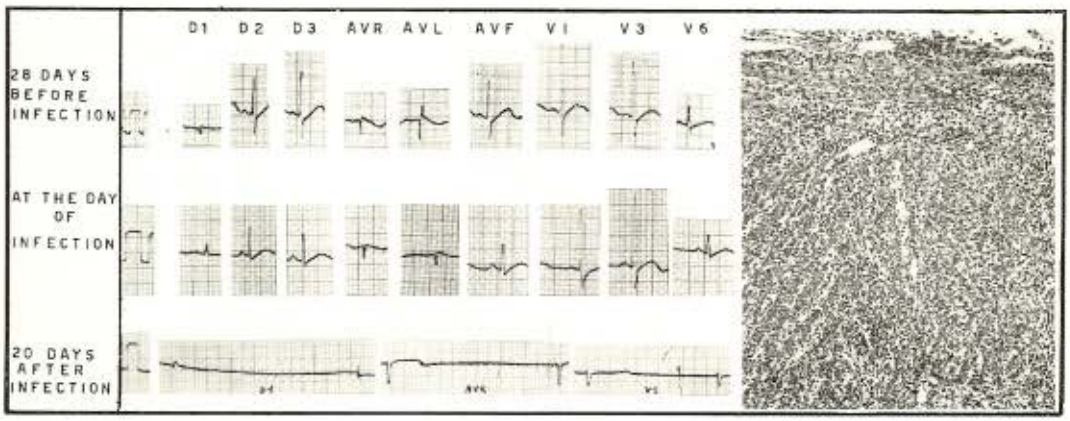


Fig. 3 — Dog control-died 20 days after infection

mice and cannot bring about an evolutive infection in the dogs themselves.

The absence of parasites and of inflammatory infiltrates in the myocardium among the vaccinated animals killed on the 60th day sharply contrasts with the persistence of low parasitism and of accentuated myocarditis in the control that died in the same period.

RESUMO

Imunização ativa de cães com uma cepa não virulenta do Trypanosoma cruzi

Vacinação de cães com a cepa Y do *T. cruzi*, mantida em cultura por longos anos, conferiu aos animais alto grau de proteção.

O caráter não evolutivo da infecção com vacina foi demonstrado através de parasitemia, ECG, hemocultura e exame histopatológico do miocárdio.

Uma vez injetados, com formas sangüíneas virulentas, os animais vacinados adquiriram uma leve infecção da qual logo se recuperam, em contraposição com os contrôles que morrem num percentual de 75%.

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