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I — THE AVIRULENCE OF THE CULTIVATED Y STRAIN OF *TRYPANOSOMA CRUZI*

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SUMMARY

The Author tries to demonstrate through a series of experiments in dogs and mice that the cultivated Y strain of *Trypanosoma cruzi* loses its disease-producing capacity.

INTRODUCTION

After the presentation of our previous papers [5, 6, 7] on the subject, the principal pertinent objection raised was that we were working with live trypanosomes.

The possibility of an exacerbation of the virulence would be a permanent danger and, in addition, rather than controlling the disease we could be spreading it.

By means of a series of experiments we will try to demonstrate the real avirulence of the strain we use to "vaccinate" our animals.

This paper will be the first on such subject.

MATERIAL AND METHODS

1) Four dogs, 2 males and 2 females, with a body weight from 450 g to 2,300 g (mean 1,037 g), have had an ECG and a search for trypanosomes in "thick drops" of the peripheral blood.

Later they were "vaccinated" with $1,6 \times 10^8$ to $2,3 \times 10^9$ live cultivated trypanosomes, suspended in saline [5, 7].

Parasitemia and ECG were repeated on the 8th, 15th and 28th day after the "vaccination".

Hemoculture in liquid Warren medium was done on two of the dogs on the 8th day and on the two others (that received $2,3 \times 10^9$ parasites) on the 15th day of the "vaccination".

2) A group of 6 dogs, 3 males and 3 females with a body weight from 830 g to 2,500 g (mean 1,731 g) have been submitted to the same procedure as the animals of the preceeding group.

At the end of the 30th day after "vaccination", xenodiagnosis (4 nymphs for each animal) was done.

3) A lote of 9 white mice with 10 g of body weight received by intracardiac route $4,5 \times 10^7$ live trypanosomes, suspended in saline solution as previously described [5, 7].

After 60 days the animals were bled to death and hemoculture was performed in liquid medium of Warren (3 tubes for each animal).

Parts of the heart, liver and spleen were triturated in saline solution containing 100 UI of Penicillin and 100 mg of Streptomycin per ml and cultivated in the same medium (3 tubes for each animal).

Histologic examination was made of parts of the heart, and liver. The material was

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fixed in 10% formalin and paraffin sections were stained by Hematoxylin-Eosin.

4) Five white mice of 2 g body weight were "vaccinated" each with 1,700 cultivated live parasites, by intraperitoneal route.

Search for parasites was made, by blood thick drop, 8,15 and 30 days after the "vaccination".

5) Eight albino mice with 2 g of body weight received, each, intraperitoneally, 900,000 live cultivated trypanosomes of the Y strain, suspended in saline solution.

Parasitemia by the same method as in the previous group was done 8,15 and 30 days after the "vaccination".

30 days later, i.e., 60 days after the animals received the "vaccine" they were killed and fragments of the heart and liver were fixed in 10% formalin for histologic examination.

RESULTS

1) All the animals have had normal ECG before and after the "vaccination" (Fig. 1).

The parasitemias were consistently negative during the experiment. The hemoculture gave negative results in the animals examined on the 8th day after "vaccination" but was positive in the dogs on the 15th day after the immunization with 2,3 × 10^{9} trypanosomes.

Sub-inoculation of young mice with the cultivated parasites gave negative results.

2) The electrocardiographic studies of all the dogs before and after the "vaccination" were normal (Fig. 2).

The parasitemias and the xenodiagnosis (on the 30th day) were equally negative.

3) The cultures of blood and of the viscerae were negative.

The histopathology has shown only very small and scanty foci of lympho-histiocytic infiltration especially in the atria, in 56% of the animals (Fig. 3). In the liver 89% have shown parenchymatous hystio-lymphocytic nodules without parasites (Fig. 4).

4) Only in one out of five mice (20%) small and few lympho-histiocytic infiltration foci were seen in the heart. No parasites were identified. In the liver neither cellular infiltration nor parasites were observed.

5) The parasitemias were negative in all the animal of this series and the most important histopathological finding was a

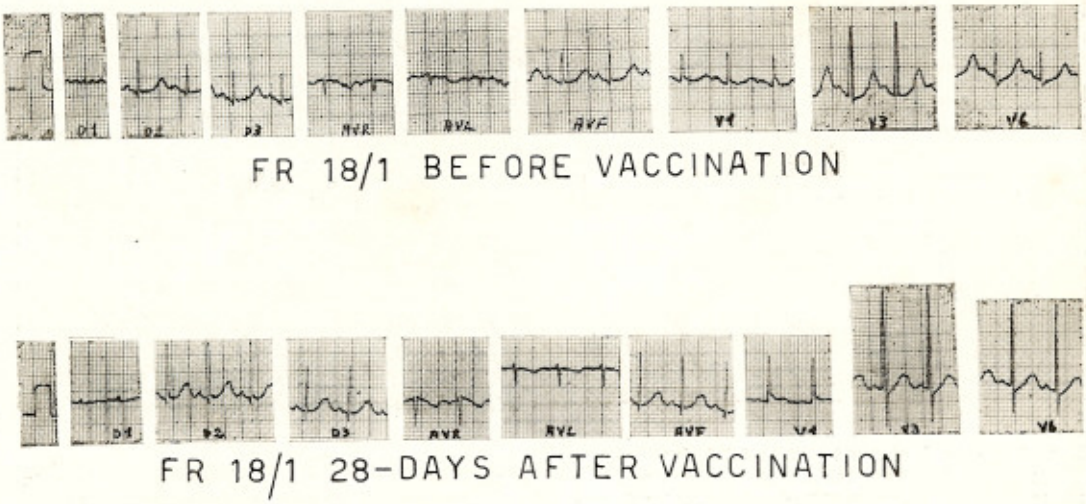

Fig. 1 — ECG of a dog "vaccinated" with 2,3 × 10^{9} live avirulent trypanosomes

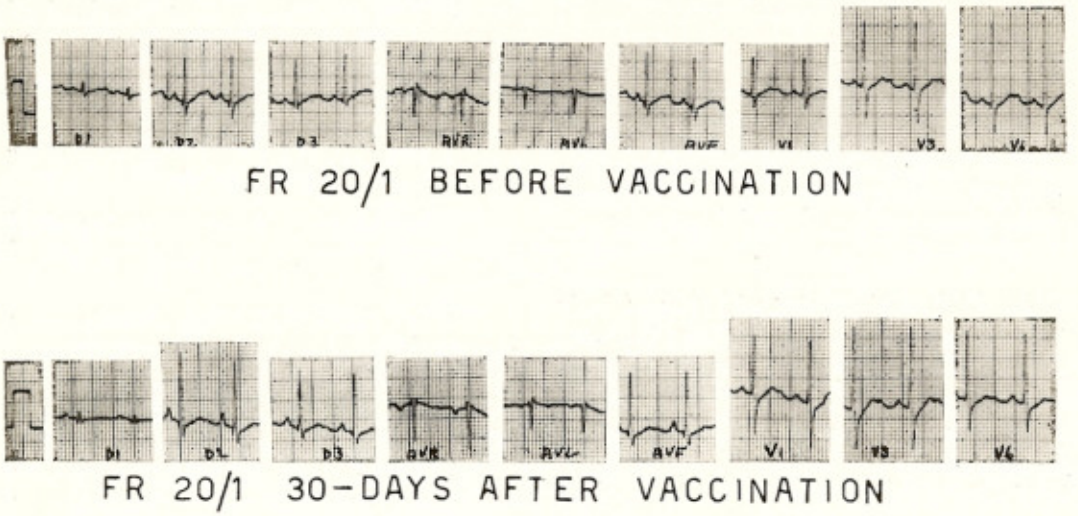

Fig. 2 — ECG of a dog "vaccinated" with $1{,}6 \times 10^5$ live parasites

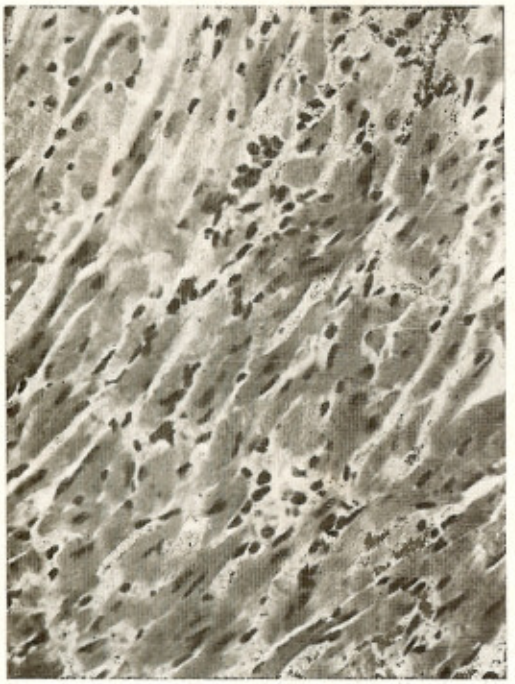

Fig. 3 — Insignificant intersticial hystio-lymphocytic infiltration in the myocardium of a mouse that received 9×10^5 parasites by intracardiac route. 550 ×

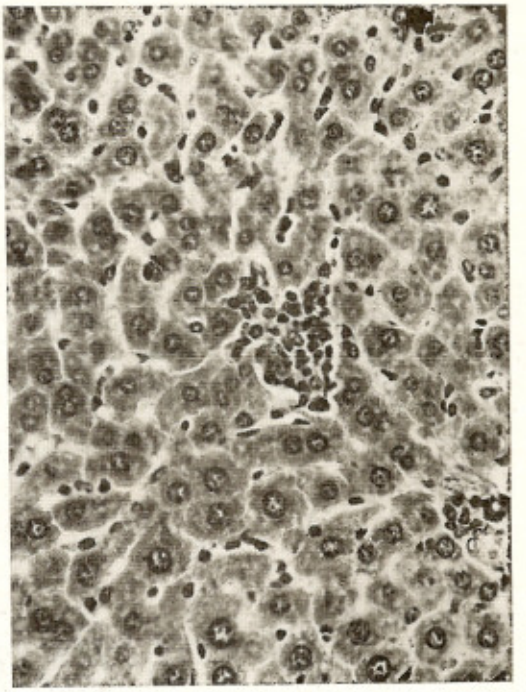

Fig. 4 — Small hystio-lymphocytic nodule in the liver of the same animal. 550 ×

small focal lympho-histiocytic infiltration without parasites, in the heart of 4 of the 8 mice. In only one case the liver presented several small lympho-hystio-neutrophilic nodules, without parasites.

DISCUSSION AND CONCLUSIONS

Avirulence as we have been using it in this and in previous papers [5, 6, 7] means the disease producing incapacity of a microorganism.

In this sense we mean that the "vaccinated" animal is infected. But the important point is that this infection is not progressive (i.e., does not lead to disease). We believe that the parasite penetrates the animals cells but is not able to divide inside them. We were unable to demonstrate pseudo-cysts in the several histologic sections examined and the negativity of the hemocultures, the cultures of organs triturate and the xenodiagnosis seems to offer more evidence in support of this fact.

We know that histologic examination is not a very good means to detect infection, especially in the chronic phase of trypanosomiasis, but in the acute phase the finding of pseudo-cysts is very common. In "vaccinated" animals, in the period corresponding to the acute phase, the histologic examination has been negative [8, 9].

The same restriction can be made concerning the parasitemia by blood thick drop, but in the acute period of the infection this technique is used with good results.

All our animals had been examined for parasites in the peripheral blood during the probable acute phase of the infection always with negative results.

The hemoculture in Warren medium has given very good results (61.7% positivity) in the diagnosis of chronic cases in experimental trypanosomiasis. This percentage increases (83.3%) with the culture of a triturate of heart [1].

The existence of two positive hemocultures doesn't invalidate our hypothesis of the loss of virulence by the cultivated Y strain. The dogs were "vaccinated" with a very great number of parasites and the sub-inoculation of very young mice was negative.

Our interpretation of this phenomenon was that the cultivated trypanosomes were the same inoculated as the "vaccine" that remained in the blood stream.

We never find pseudo-cysts in "vaccinated" animals [7, 8].

We dont' know any reference on the surviving time of the metacyclics forms of the *Trypanosoma cruzi* in the blood stream of chronic infected vertebrate, but *Trypanosoma lewisi* can remain in the blood of rats for several weeks or months as non reproducing adults [10].

We have no data about the sensibility of the ECG in detecting small heart lesions in cases of chronic Chagas' disease in animals, but the hearts of dogs infected with the Y virulent strain of *T. cruzi* have shown great electric alterations, especially when near death [7].

The value of xenodiagnosis as the most important parasitological test to detect the chronic cases of the parasitosis, seems beyond doubt [4].

BARRETTO [2] observed that trypanosomes isolated from wild animals if cultivated in an artificial medium became non-infective in mice. However when these cultivated flagellates were injected by intracardiac route they regain their virulence.

Since the work of Chagas (*in* 3) it is known that the very young mouse is an excellent animal for experimental infection with *Trypanosoma cruzi*.

The injection of large doses of "vaccine" in mice of 2 g of body weight, as described, gave negative parasitemias and culture in a triturate of organs.

We believe that all these negative results allow us to assume that the Y cultivated strain of *T. cruzi* has lost its virulence.

In a future paper we will bring more data in support of our point of view.

MENEZES, H. — I — The avirulence of the cultivated Y strain of *Trypanosoma cruzi*. *Rev. Inst. Med. trop. São Paulo* **12**:64-68, 1970.

RESUMO

I — *A avirulência da cepa Y (cultivada) do* Trypanosoma cruzi

O Autor procura através de uma série de experimentos em cães e camundongos demonstrar que a cepa Y (cultivada) do *T. cruzi* tornou-se avirulenta para êsses animais.

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