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PROTECTIVE EFFECT OF AN AVIRULENT (CULTIVATED) STRAIN OF *TRYPANOSOMA CRUZI* AGAINST EXPERIMENTAL INFECTION IN MICE

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SUMMARY

"Vaccine" prepared with living *Trypanosoma cruzi* from a culture maintained for 15 years gives a high degree of protection to mice against a subsequent infection with virulent blood forms of the same parasite.

INTRODUCTION

It is known that cultivated *Trypanosoma cruzi* can have its virulence to laboratory animals attenuated or even abolished (CARVALHEIRO [4]).

PIZZI & PRAGER [9] injecting mice with *T. cruzi* cultivated in artificial media for 6 years, observed a complete immunity against reinfection with a virulent strain, 3 weeks later.

The injection of avirulent blood flagellates also gives an effective protection to laboratory animals (NORMAN & KAGAN [7]).

These observations seem to have been abandoned without a more extensive and intensive research to get all the possible profits that could be obtained from such results.

I intend to return to the problem and here are my first results.

MATERIAL AND METHODS

Trypanosoma cruzi, Y strain, isolated in 1953 by NUSSENZWEIG (in SILVA & NUSSENZWEIG [10]) from a human case and since then maintained in culture media in the Departamento de Parasitologia da Faculdade de Medicina de Ribeirão Prêto, was used in this experiment.

The flagellates were taken from 30/35 days old culture in Packchanian media and suspended in saline solution.

A suspension of about 5 x 10^8 parasites per ml was kept in the refrigerator ($\pm$ 2°C) for 24 hours. Before being used, it was examined at the microscope to certify that it contained living parasites.

Sixty eight albino mice of the same strain, 20/25 days old and with a mean weight of 10 g were divided in 3 groups: one with 29 named "vaccinated" (VV), other with 10 animals designated "vaccine control" (VC) and another with 29 mice used as "control of the infecting virulent strain" (SC).

Each mouse of groups VV and VC received, per intraperitoneal route, 0,4 ml of the "vaccine".

After 8 and 15 days a microscopic examination was made to ensure that the blood of the animals was free of parasites.

Four weeks after the "vaccination" each mouse of groups VV and SC was inoculated, intraperitonealy, with 5,000 virulent parasites per g body weight. The infecting virulent trypanosomas came from the blood of mice at the 8th day of infection with Y virulent strain of *T. cruzi* maintained in the "Departamento de Patologia" through successive inoculations in mice.

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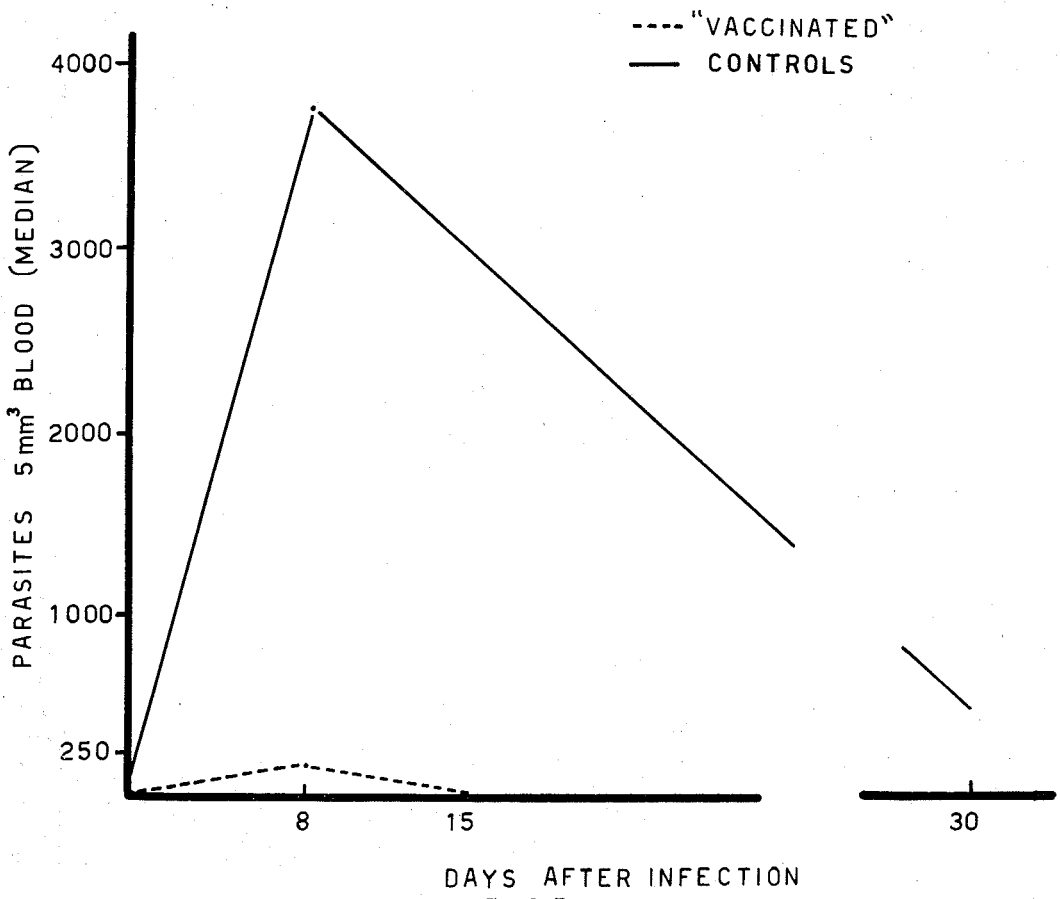


Graph I

The parasitemia in both groups was determined by the PIZZI-BRENER [2] technic, 8, 15 and 30 days after the infection.

At the 30th day all the surviving mice of the 3 groups were bled to death. Heparinized blood of those with negative parasitemia was inoculated (0,5 ml) into mice of 10 g body weight.

The blood examination for flagellates was performed at the 8th day of the sub-inoculation.

RESULTS

The whole group VC survived until the 30th day and never presented any parasitemia even in the sub-inoculated animals.

The parasitemia and the percentage of mortality of the VV and SC groups are shown in the Graphs I and II.

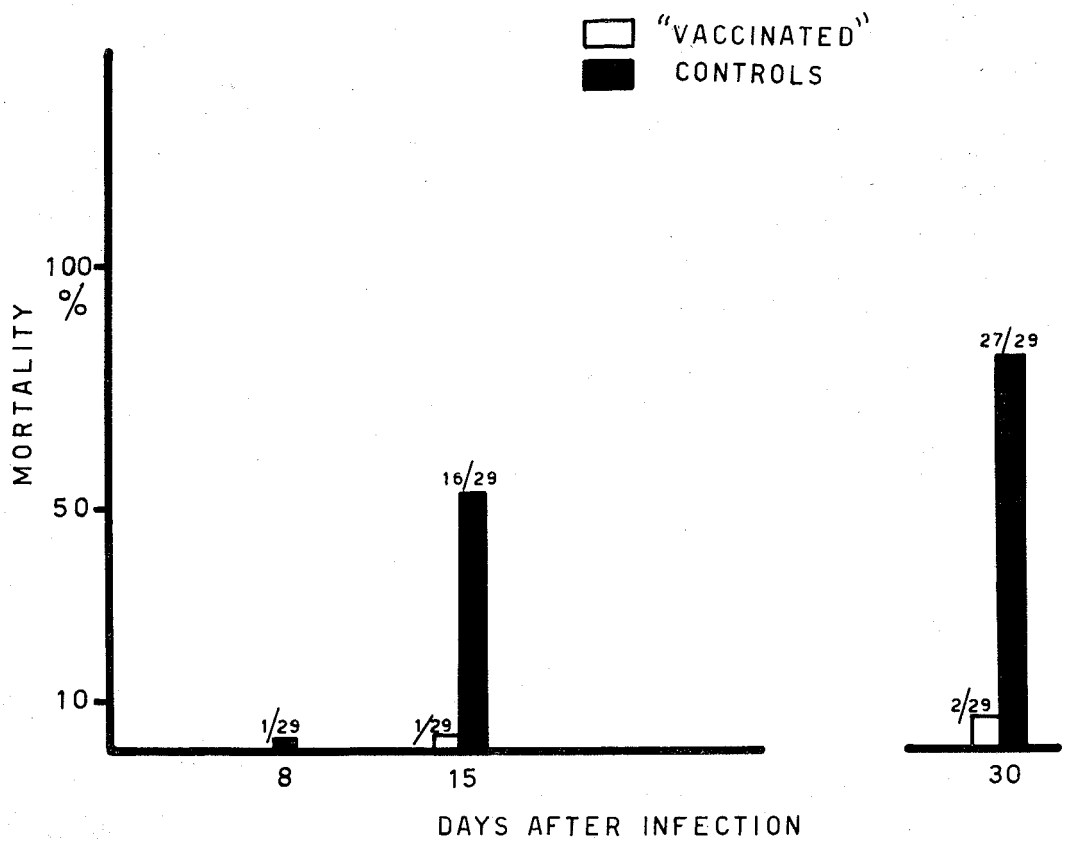
Low parasitemia (mean: 27 parasites/5mm3 blood) was verified in 17% of sub-inoculated cases of the "vaccinated" (VV) animals.

All the survivors of the group "control of the infecting virulent strain" (SC) gave positive sub-inoculations.

The mortality of 10% among the "vaccinated" (VV) animals in comparison with 90% among the controls (SC) is the best demonstration of the high degree of protection offered by the "vaccine".

COMMENTS

Since the works of BLANCHARD [1], BRUMPT [3] and MAYER & ROCHA LIMA [6], we know that an initial infection confers immunity against a reinfection.



Graph II

COLLIER [5] ascribes the acquired resistance to the persistence of the primary infection.

The problem of the immunization with living *Trypanosoma cruzi* is the same of all vaccination with living microorganisms: to have an avirulent strain unable to induce an active infection in the vaccinated animal.

The cultivated flagellates used in this experiment seem to have lost their virulence to mice retaining however the ability to protect the animals against a virulent infection.

The parasitemia and the sub-inoculations consistently negative in all mice of the VC group permits the assumption that the "vaccine" does not induce in the mice an evolutive infection, with tissular reproduction of the parasites.

It must be settled now if this non virulence is permanent and valid to other species of mammals,

RESUMO

Ação protetora de uma linhagem atenuada (cultivada) de Trypanosoma cruzi *contra infecção experimental em camundongos*

Trypanosoma cruzi, cepa Y, mantido em cultura por 15 anos quando injetado em camundongos albinos foi capaz de conferir um elevado grau de proteção contra ulterior infecção com uma cepa altamente virulenta do mesmo parasita.

Os tripanossomos cultivados não foram capazes de induzir, em camundongos, infecção evidenciável por parasitemia direta e por sub-inoculação em camundongos jovens.

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