THE EFFECT OF PHENOLATED VACCINES AGAINST EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION IN MICE *

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"Vaccines" prepared from parasites of an avirulent cultivated Y strain of T. cruzi, *suspended in phenolated* 1/10.000 *saline solution, with aluminum stearate, containing alive parasites, gave high degree of protection to mice against a posterior infection with virulent blood forms of the same parasites and strain.*

The degree of protection with 1/1000 *and* 1/10.000 *phenol "vaccines", with no alive parasites, was very poor specially in the first group.*

The immunity seems to be related to the number of alive trypanosomes in the "vaccines".

INTRODUCTION

Assesment of the possibility of producing active immunization by trypanosomes killed or attenuated by physical or chemical means has been published for several years (1, 3, 10, 13, 14, 16).

As far as I know the use of phenolated "vaccines" was not mentioned, except in the work of Seneca & Peer (15) who have employed an 88% phenolated extract of *Trypanosoma cruzi*, called by them *chagastoxin*.

Endeavoring some chemicals with the aim of obtaining an effective "vaccine" with killed or attenuated parasites the phenol was the only that gave some positive results (Menezes, 12).

MATERIAL AND METHODS

Trypanosoma cruzi, Y strain, maintained in Packchanian culture medium for almost 15 years, was the antigen used in my experiments.

Flagellates from cultures 30/35 days old were washed 3 times in saline solution and separated, each time, by centrifugation at 1.500 r.p.m.

The final sediment was suspended in 1/1000 and 1/10.000 phenolated saline containning 1mg/1ml aluminum stearate.

The approximated number of parasites was $5x10^8$ per ml. in both "vaccines".

These were used 3 hours after the preparation or were kept at the refrigerator (± 2.°C) and employed 24 or more hours later.

All the flagellates of the 1/1000 phenol suspension were killed immediately but in the 1/10.000 phenol "vaccines" only 50% was alive after 3 hours, 5% after 24 hours and almost 1% at the end of 72 hours.

After this time, in one of the 1/10.000 phenol preparations, all the parasites were dead.

The alive flagellates in the 1/10.000 phenol "vaccines "showed accentuated alterations in the morphology (gross forms) and in the motility (very slow movements).

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Twenty nine albino mice with the mean weight of 10 g. received each 5 days 4 subcutaneous injections of 0,2ml of the.... 1/1000 phenolated suspension.

Twenty eight days after the first injection, all these animals and 19 others more, from the same bred stock, with the same age and weight (controls), were innoculated with 5.000 virulent parasites per gram of body weight.

These flagellates came from blood of mice at the 8th. day of innoculation with the virulent Y strain maintained in the same animal specie, for several years, in our Department.

In other experiment 10 albino mice with 10g. of mean body weight were protected with 2 subcutaneous injections of 0,2ml of the phenolated suspension with 50% of alive parasites, 10 with the same number of injections of the 1/10.000 phenolated "vaccine" with 5% of alive trypanosomes, 9 with 2 subcutaneous injections of 0,2ml of the 1/10.000 phenol suspension contaainning 1% of alive parasites and finally 10 other mice that were injected with the same amount of the 1/10.000 phenol "vaccine" with no alive flagellates.

Twenty eight days after the first injection all these animals and 39 others with the same age and weight, kept as controls, received intraperitoneally 5.000 parasites per gram of weight.

The virulent trypanosomes were obtained from the same source as described previously.

Parasitemia by the Pizzi-Brener (Brener 1) technic was done to all animals of both experiments, at the 8th, 15th, and 30th day after the virulent infection.

The percentage of mortality will be referred to these days.

Previous parasitemia had been done to all "vaccinated" mice at the 8th and 28th day after the use of the "vaccines" and was, in every case, negative.

The parasitemia and the percentage of mortality after the infection are summarised in the Graphs I to VI.

COMMENTS

Using culture forms of *Trypanosoma cruzi*, Y strain, which had lost its virulence to mice (Menezes 11), I obtained 1/10.000 phenolated "vaccines" with alive parasites, that were able to give 96% of survivors until 30 days after infection with virulent blood forms, against only 25% in the control group. (Graph IV).

The degree of resistence, if we consider the parasitemia as an expression of this later, seems related to the number of alive parasites injected.

Animals protected with "vaccine" containing almost 50% of alive flagellates gave a persistent negative parasitemia during the period of the experiment, while those that received 5% and 1% gave positive parasitemia that was higher in the later group (Graph I).

In my own experience there is no significant relationship between parasitemia and surviving time of the infected animals. So I prefer the percentage of survivors as the best indication of protection by drugs or "vaccines".

This point of view is very well exemplified in the experiment with the use of .... 1/10.000 phenolated "vaccine" without alive parasites.

This preparation reduced significantly the parasitemia of the animals but the mortality rate was practically the same, as that of the control group, at the end of the 30th day (Graphs II-V).

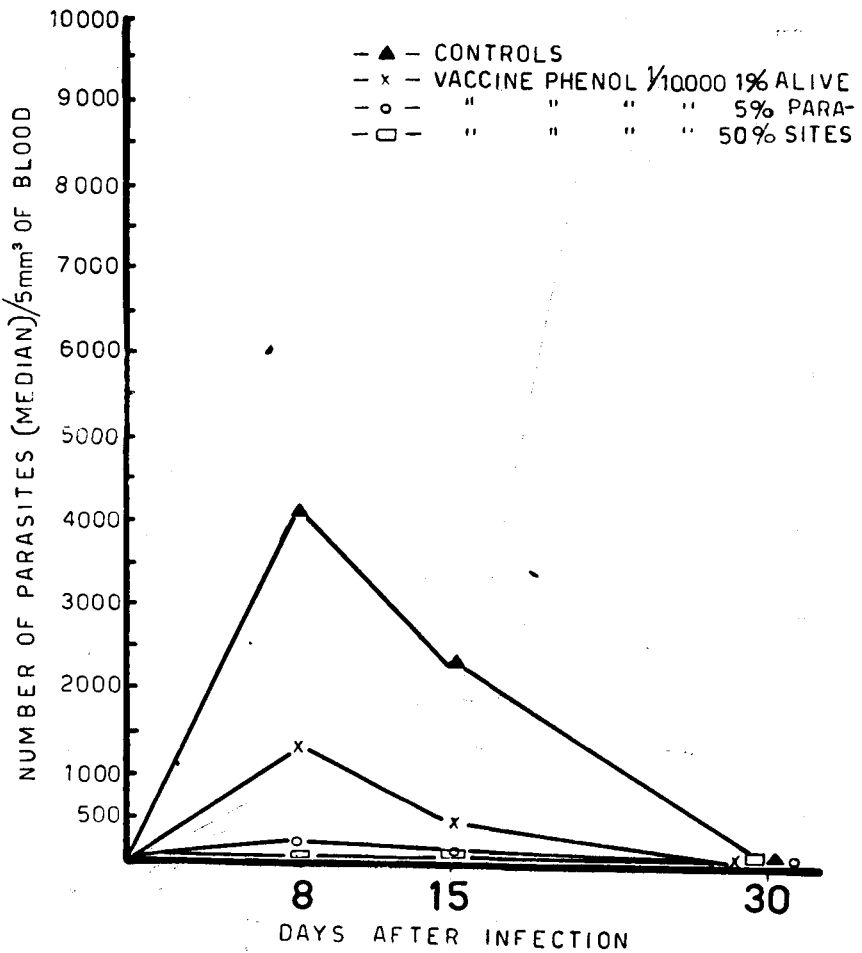
The same thing, in a much lower degree, was seen with the animals injected with the 1/1000 phenol "vaccine" (Graphs II-VI).

I was unable, in successive experiments, to reproduce the good results with the.. 1/1000 phenol "vaccine" I had communicated previously (Menezes 12). I am sure, now, that certainly I had injected some alive parasites in the animals of that experiment.

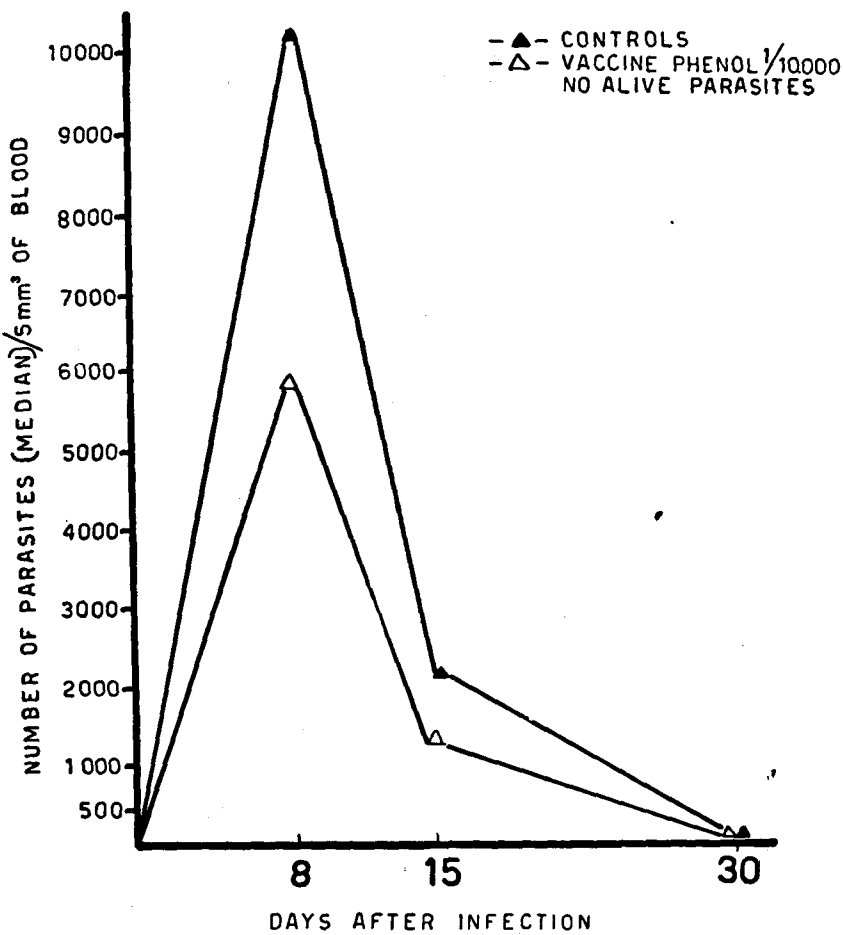
My present results are in accordance with the opinion of Collier (2) who ascribes the acquired resistance to the persistence of the primary infection.

In mice this infection produced by the "vaccine" is insignificant, clinically inapparent and demonstrated only by histological means as I will communicate later in an other paper.

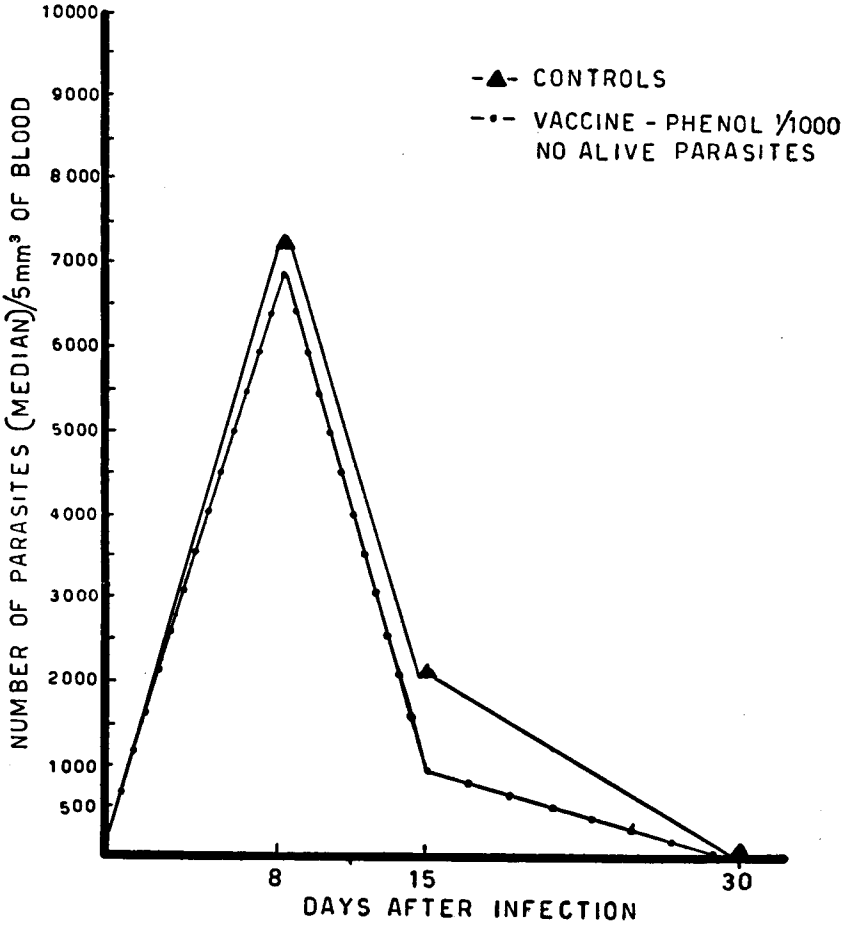
GRAPH I


10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
500
NUMBER OF PARASITES (MEDIAN)/5mm³ OF BLOOD
— ▲ — CONTROLS
— x — VACCINE PHENOL 1/10000 1% ALIVE
— o — " " " " 5% PARA-
— □ — " " " " 50% SITES
8
15
30
DAYS AFTER INFECTION

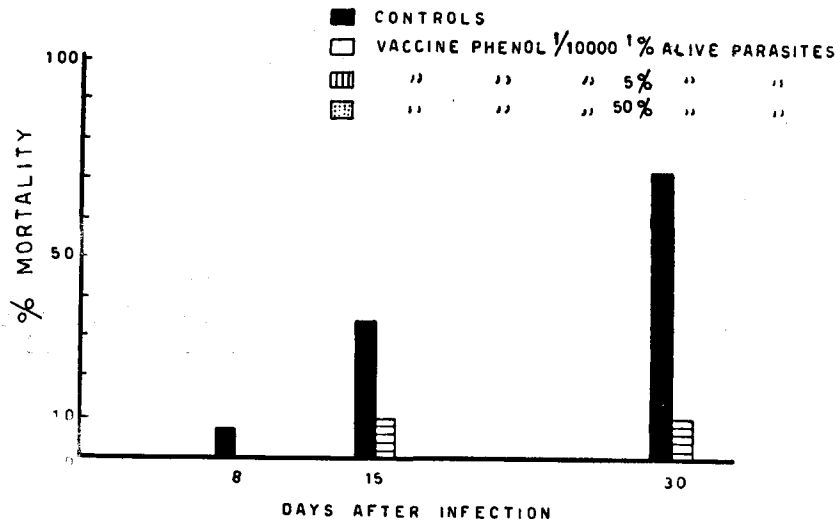
GRAPH II


CONTROLS
VACCINE PHENOL 1/10000
NO ALIVE PARASITES
NUMBER OF PARASITES (MEDIAN)/5mm³ OF BLOOD
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
500
8
15
30
DAYS AFTER INFECTION

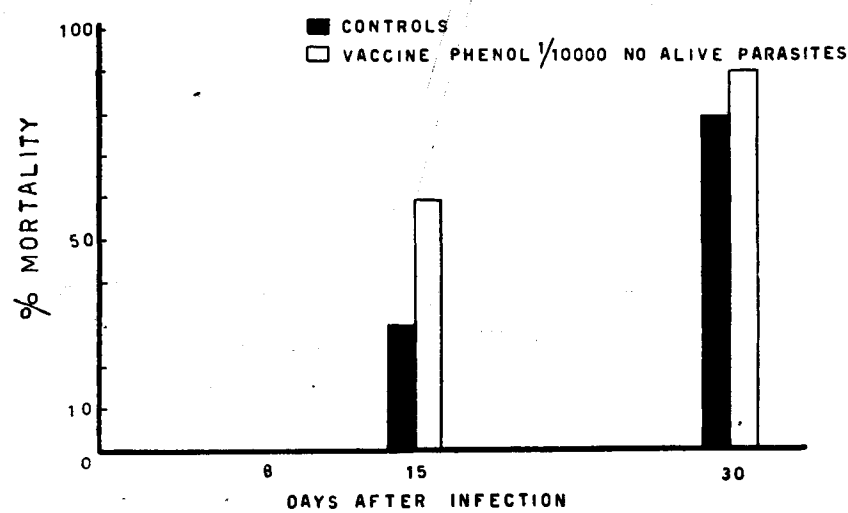
GRAPH III


10000
9000
8 000
7000
6000
5000
4000
3000
2000
1000
500
NUMBER OF PARASITES (MEDIAN)/5mm³ OF BLOOD
-▲- CONTROLS
-•- VACCINE - PHENOL 1/1000
NO ALIVE PARASITES
8
15
30
DAYS AFTER INFECTION

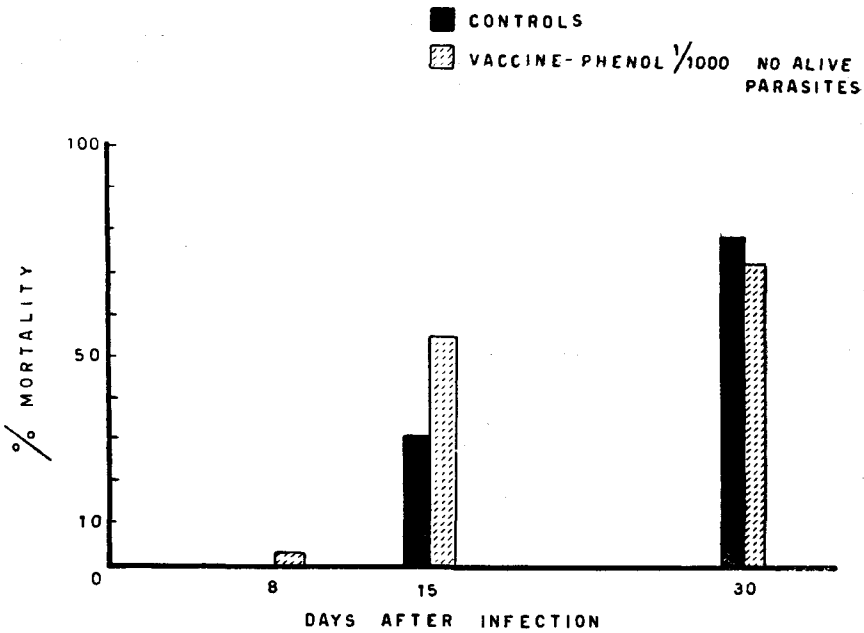
GRAPH IV



GRAPH V



GRAPH VI



CONCLUSIONS

This work so far indicates that it is possible to produce immunity against a high infecting dose of *Trypanosoma cruzi* by phenolated "vaccines", since these contain alive parasites.

I have no elements to indicate that the phenolated "vaccines" are better than the simple suspension of the avirulent tripanosomes (Menezes 11) but since the phenol at 1/10.000, acting for less than 72 hours, reduces the number of alive flagellates and modify their morphology and motility it is reasonable to suppose that the first "vaccine" is safer than the latter.

R E S U M O

Suspensões salinas fenoladas de Trypanosoma cruzi, *cêpa Y, mantidas por 15 anos em meio de cultura artificial, desde que contenham alguns tripanosomas vivos, constituem bom meio de proteção, para camundongos, contra ulterior infecção com cêpa virulenta.*

O fenol a 1/10.000 determina alteracões da forma e da motilidade dos parasitas o que faz supor uma redução maior ainda de sua capacidade infectante.

O grau da imunidade parece relacionado com o número de parasitas vivos contidos nas "vacinas".

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