

## ACTIVE IMMUNIZATION OF MICE WITH THE AVIRULENT Y STRAIN OF *TRYPANOSOMA CRUZI* AGAINST HETEROLOGOUS VIRULENT STRAINS OF THE SAME PARASITE

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### SUMMARY

Mice vaccinated with the avirulent Y strain of *Trypanosoma cruzi* became protected against infection with other virulent strains of the same parasite. This protection could be detected until 11 weeks after vaccination.

### INTRODUCTION

The cross immunization of laboratory animals with different strains of *Trypanosoma cruzi* is a well known fact<sup>1, 4, 5, 7, 8</sup> with few exceptions<sup>2</sup>. The real aim of this work is to verify whether the Y strain maintained in culture for several years and shown to be avirulent to mice<sup>6</sup> is able to protect these animals against challenge with other virulent strains of *T. cruzi*, besides the virulent Y strain.

### MATERIAL AND METHODS

Forty white mice with a mean body weight of 10 g were vaccinated with about  $3 \times 10^6$  trypanosomes by subcutaneous route.

The flagellates were obtained from a culture 35 days old, in Packchanian medium, of the Y strain maintained in this medium for 16 successive years, in the Department of Parasitology of our Medical Faculty.

The trypanosomes were washed and suspended in saline solution and the suspension used immediately after preparation.

Forty other mice of the same provenience and with the same body weight were kept as controls.

Ten mice, 4 weeks after vaccination, and 10 controls were inoculated by intraperitoneal route with 5,000 parasites/g body weight of the strain RC1729 isolated from a wild dog (*Cerdocyon thous azarae*) and maintained in mice.

The second similar group was infected with the same amount of parasites belonging to the strain M 1418 isolated from bat (*Desmodus rotundus rotundus*).

The third group with 10 mice controls and 10 vaccinated was inoculated, 11 weeks after the vaccination, with parasites of the strain "Berenice" isolated from a human chronic case of Chagas' disease.

Finally a fourth group, 11 weeks after the immunized animals had received the vaccine, was challenged with trypanosomes of the "ABC" strain isolated from a chronic human chagasic patient.

The number of inoculated parasites and the route of inoculation was the same for all the animals of the four groups and the infective parasites came from blood of mice at the 8th day of infection.

Parasitemia by thick drop was verified on the 8th, 15th and 30th day after challenge,

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except for the group of the RC 1729 strain of which the animals were killed after 3 weeks.

RESULTS

The results of all these experiments are summarized in Tables I to IV and in Graphs I to IV.

TABLE I

RC 1729 (wild dog *Cerdocyon thous*)

	Mice	Number of parasites/5 mm <sup>2</sup> of blood		
		Days after infection		
		8	15	21
CONTROLS	1	17.500	—	—
	2	14.000	—	—
	3	35.000	—	—
	4	7.875	—	—
	5	28.000	—	—
	6	—	—	—
	7	—	—	—
	8	35.000	4.375	525
	9	12.250	—	—
	10	17.500	2.730	—
	Mean	20.903	3.552	525
	Median	17.500	3.552	525
VACCINATED	1	0	0	0
	2	105	0	0
	3	70	0	0
	4	105	0	0
	5	35	0	0
	6	175	70	0
	7	70	35	0
	8	175	35	0
	9	0	0	0
	10	0	0	0
	Mean	73	14	0
	Median	70	0	0

TABLE II

M 1418 (bat, *Desmodus rotundus rotundus*)

	Mice	Number of parasites/5 mm <sup>2</sup> of blood		
		Days after infection		
		8	15	30
CONTROLS	1	10.500	—	—
	2	10.500	—	—
	3	3.920	—	—
	4	10.500	—	—
	5	4.495	—	—
	6	17.500	—	—
	7	9.450	—	—
	8	35.000	4.025	0
	9	14.000	—	—
	10	5.285	—	—
	Mean	12.115	4.025	0
	Median	10.500	4.025	0
VACCINATED	1	175	0	0
	2	280	0	0
	3	140	0	0
	4	70	0	0
	5	140	0	0
	6	70	0	0
	7	140	0	0
	8	175	0	0
	9	35	0	0
	10	70	0	0
	Mean	129	0	0
	Median	140	0	0

From the observation of all of them it can be seen that the avirulent Y strain of *Trypanosoma cruzi* gave a very good protection against challenge with virulent strains of the same trypanosome.

The low mortality with the "ABC" strain has been already mentioned by TAFURI & BRENER<sup>9</sup> as a peculiarity of this strain.

One vaccinated animal of this same group "ABC" was killed, by accidente, during the determination of the parasitemia on the 30th day.

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TABLE III  
"ABC" (human)

	Mice	Number of parasites/5 mm <sup>3</sup> of blood		
		Days after infection		
		8	15	30
CONTROLS	1	10.500	4.025	280
	2	17.500	—	—
	3	5.075	10.500	—
	4	5.775	2.870	175
	5	2.730	2.835	420
	6	7.280	4.375	70
	7	2.870	3.570	630
	8	4.025	3.780	525
	9	10.500	—	—
	10	5.355	7.085	455
	Mean	7.161	4.880	365
	Median	5.565	3.902	420
VACCINATED	1	0	70	0
	2	0	0	0
	3	0	0	0
	4	70	35	0
	5	0	0	70
	6	140	0	—*
	7	175	0	35
	8	0	35	0
	9	105	35	0
	10	70	0	0
	Mean	56	19	1,1
	Median	35	0	0

\* killed by accident

COMMENTS

GRAY<sup>3</sup> in his work on immunology of trypanosomiasis says that recovery from an infection with a high infective strain gives no immunity or a very low one against a latter infection with other strains.

This statement (although not explicit) seems to apply only to the African trypanosomiasis because in the South American try-

TABLE IV

"BERENICE" (human)

	Mice	Number of parasites/5 mm <sup>3</sup> of blood		
		Days after infection		
		8	15	30
CONTROLS	1	—	—	—
	2	2.520	—	—
	3	4.515	770	35
	4	2.555	—	—
	5	—	—	—
	6	—	—	—
	7	3.185	1.155	—
	8	14.000	2.170	0
	9	2.240	1.645	0
	10	1.995	735	35
	Mean	4.430	1.295	17,5
	Median	2.555	1.155	17,5
VACCINATED	1	70	0	0
	2	70	70	35
	3	280	0	0
	4	280	35	35
	5	140	70	0
	6	140	0	0
	7	105	0	0
	8	175	0	0
	9	105	0	0
	10	70	0	0
	Mean	143	17,5	7,0
	Median	122	0	0

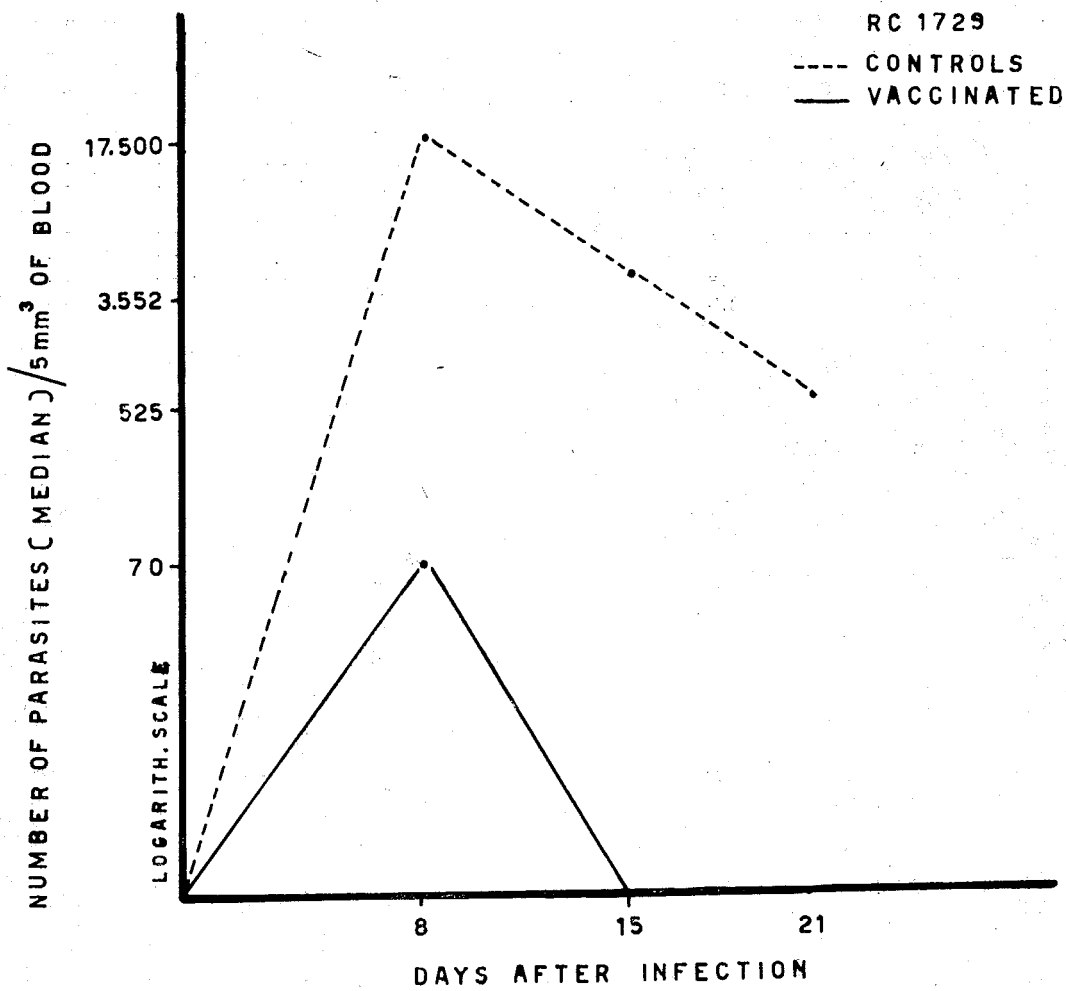
panosomiasis this is perhaps the exception and not the rule.

This is a crucial point for the success in any attempt to obtain a vaccine against the disease.

The specificity of the vaccine being of strain and not of species it would be necessary to have a great mixture of antigens and would make the vaccination very difficult or even impossible.

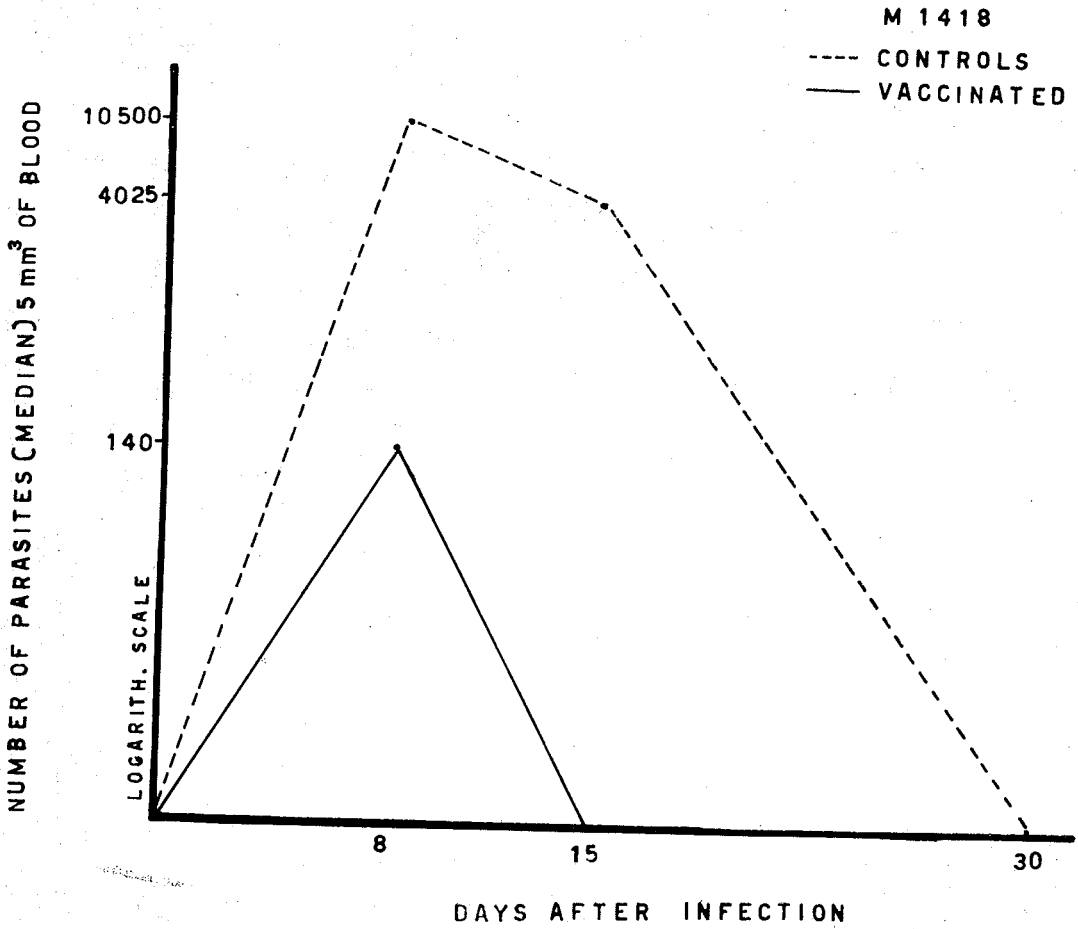
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GRAPH I



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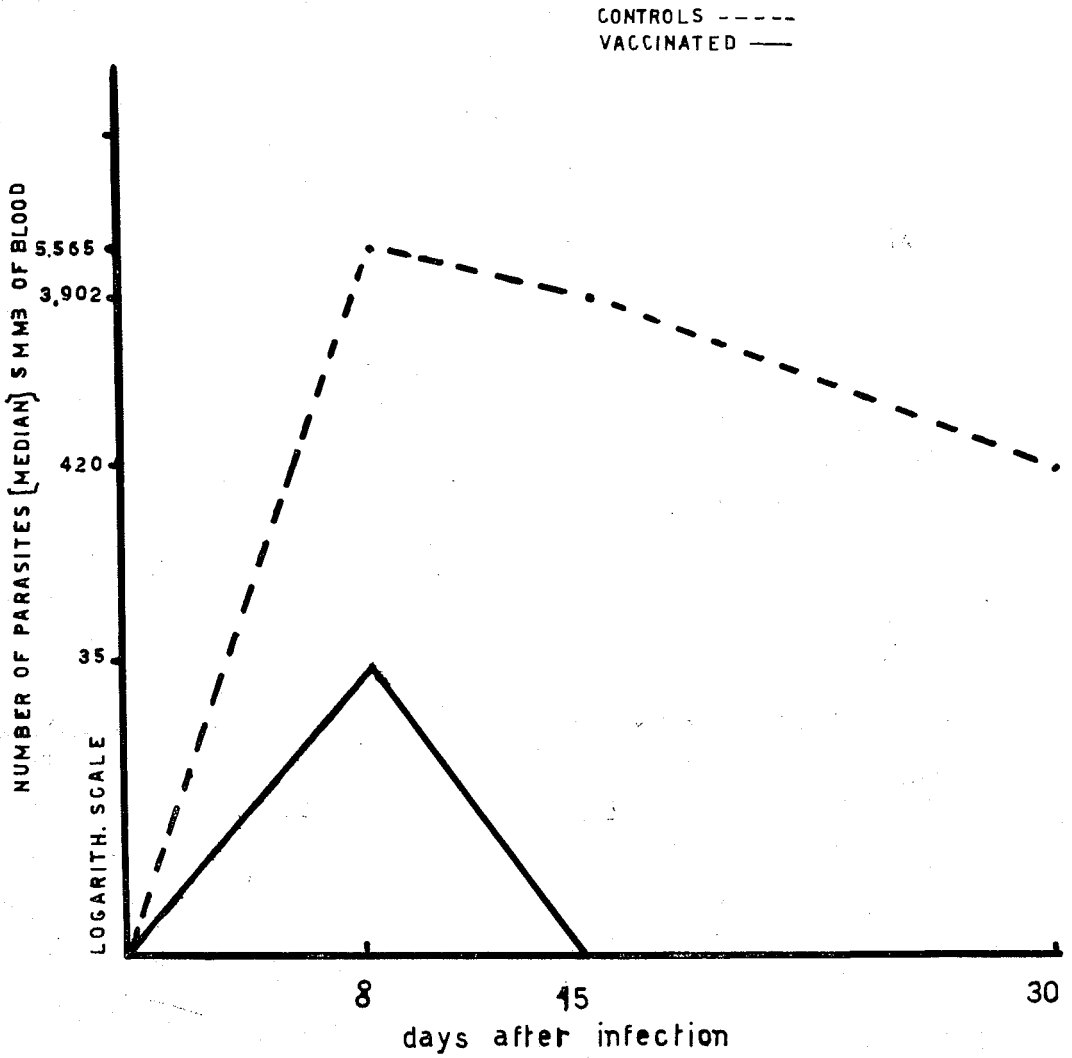
GRAPH II



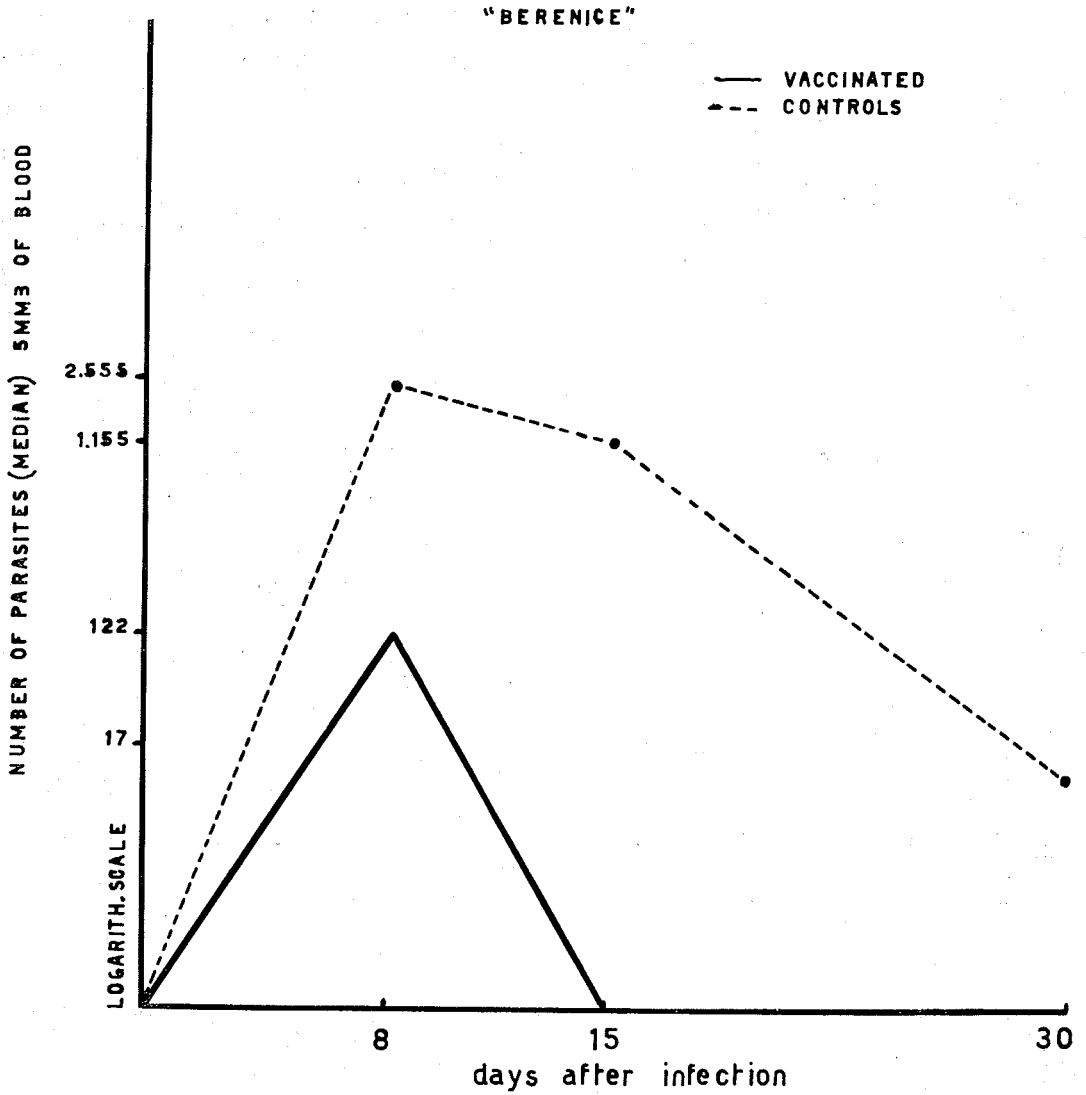
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GRAPH III

“ABC”



GRAPH IV



Our experiments show that the avirulent Y strain keeps the ability to give cross protection against different virulent strains of *T. cruzi*.

#### RESUMO

*Imunidade cruzada de camundongos vacinados com cepa avirulenta de T. cruzi contra outras cepas, virulentas, do mesmo parasita*

A cepa Y, avirulenta, confere a camundongos, imunidade cruzada contra infecção por várias cepas virulentas do *Trypanosoma cruzi*.

Essa imunidade foi determinada até 11 semanas após a vacinação.

#### ACKNOWLEDGMENTS

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