THE VACCINATION OF HUMAN BEINGS WITH A LIVE AVIRULENT STRAIN OF TRYPANOSOMA CRUZI.

A new series of volunteers.

Humberto Menezes *

Five human volunteers were vaccinated with a live avirulent strain of Trypanosoma cruzi *and followed-up for one year. Except for a few cases of questionable results presented by only one Laboratory, all the other clinical, parasitological and serological tests remained negative during that period.*

Through several experiments (2-9) an avirulent strain of *Trypanosoma cruzi* has been studied that is supposed to be a mutant of the virulent Y strain, and for this reason named it PF strain (10).

In 1971 a previous note concerning the first two human beings vaccinated with such avirulent trypanosome was published (11). Later the two-year follow-up on the same volunteers was described (12).

It will be presented in this paper a few more arguments confirming the non pathogenicity of that strain, describing the one-year follow-up of a new series of human beings injected with the PF trypanosome.

MATERIAL AND METHODS

Five adult males, convicts at the Prison of Ribeirão Preto, presented themselves as volunteers to be vaccinated. After the legal procedures, clinical and laboratorial examinations on the five patients were carried out.

The complement fixation test (CFT), the immunofluorescence test (IFT) and the electrocardiogram (ECG) were performed before the vaccination.

Five days later each volunteer received by subcutaneous route 0.2ml of a suspension of the PF trypanosome harvested in Warren medium for 18 days. The culture medium was centrifuged at 1.500 r.p.m. and the sediment washed several times in saline solution. The final suspension had almost 75% of mobile parasites with a total of 10^6 flagellates per ml with about 4% of metacyclic trypomastigotes. This same vaccine was injected, subcutaneously in 15, 20 day-old mice.

* Associate Professor, Departamento de Genética e Matemática Aplicada à Biologia, Faculdade de Medicina. USP. 14100 Ribeirão Preto, Est. S. Paulo, Brasil.

Submitted to publication on 11.22.1972.

The blood of these animals was searched for parasites 6, 8, 15 and 30 days after vaccination.

The five human volunteers were observed daily during the first month and their pulse and temperature recorded every day (Tables 1-5).

After the first, second, third, fourth, fifth, seventh and twelveth month, CFT, IFT, ECG, blood cultures, xenodiagnosis and blood inoculations in young mice were performed. The blood cultures were done in Warren medium and examined after 30 days. The xenodiagnosis were made with at least 5 nymphs of *Rhodnius prolixus* in the 5th instar. The triatominae bugs were examined on the 30th day. The blood sera of the patients, after centrifugation, had the sediment inoculated in a minimum of five young mice that had blood search for parasites 8, 15 and 30 days after the blood infection. The serologic tests were performed by more than one laboratory (Tables 1-5).

RESULTS

Patients results were summarized in the Tables 1-5.

The parasitologic examination of the 15 mice inoculated with the vaccine were all negative.

COMMENTS AND CONCLUSIONS

The five patients received only one dose of the vaccine.

Three of them were observed for twelve months and two for only seven months.

One of the two latter was liberated on the seventh month and moved to another city and the other was transfered to a Prison in another State.

In all the cases, the clinical and parasitological tests were negative.

The serological tests were almost all negative, except for some results of one laboratory that had in the six previous months presented concordant results with the other laboratories (Tables 2-5).

It must be emphasized that with the same blood sample the Laboratory B gave opposite results (Tables 2, 3).

As a consequence of this, on the twelveth month a blind test was performed using a positive and a negative control that presented POSITIVE results in both cases in Laboratory B while in the Laboratories C, D and E the results were what had been expected. Thus, discarding these last discrepant tests done by Lab. B, it is concluded that all the tests performed on the volunteers were negative, giving us the conviction that the PF strain, in this experiment, demonstrated to be avirulent for men as it had been already shown for laboratory animals (2-9, 1, 13-14).

Acknowledgments — To the several people that directly or indirectly help me in doing this experiment, specially to the five volunteers João B.; José R.; Osvaldo F.; Edson M. e José C.

RESUMO

Cinco voluntários humanos foram vacinados com tripanosomas vivos de uma cepa avirulenta de T. cruzi *e seguidos durante um ano.*

Exceto alguns poucos resultados serológicos conflitantes apresentados por um mesmo Laboratório, exames idênticos realizados simultâneamente em outros Laboratórios deram resultados negativos.

Os exames clínicos e parasitológicos se mantiveram uniformemente negativos no decorrer de toda a experimentação.

REFERENCES

1. HUNGERER, K. D. — The immunology of experimental Chaga's disease. Conference at The Depat. Doenças Infect. Fac. Med. USP. Feb. 12, 1972.

2. MENEZES, H. — Protective effect of an avirulent (cultivated) strain of *Trypanosoma cruzi* against experimental infection in mice. Rev. Inst. Med. Trop. S. Paulo *10:* 1-4, 1968.

3. —— Active immunization of dogs with a non virulent strain of *T. cruzi*. Rev. Inst. Med. Trop. S. Paulo, *11:* 258-263, 1969.

4. —— I. The avirulence of the cultivated Y strain of *Trypanosoma cruzi*. Rev. Inst. Med. Trop. S. Paulo, *12:* 64-68, 1970.

5. —— II. The avirulence of the cultivated Y strain of *Trypanosoma cruzi*. Rev. Inst. Med. Trop. S. Paulo, *12:* 129-135, 1970.

6. —— III. The avirulence of the cultivated Y strain of *Trypanosoma cruzi*. Rev. Inst. Med. Trop. S. Paulo, *13:* 14-17, 1971.

7. —— IV. The avirulence of the cultivated Y strain of *Trypanosoma cruzi*. Rev. Soc. Bras. Med. Trop. *5:* 214-233, 1971.

8. MENEZES, H. & RIBEIRO, R. — V. The avirulence of the cultivated PF strain of *T. cruzi*. The evaluation of parasitologic tests after vaccination of different animal species. Rev. Soc. Bras. Med. Trop. *6:* 41-48, 1972.

9. MENEZES H. — VI. The avirulence of the cultivated PF strain of *Trypanosoma cruzi*. Induced immunotolerance in mice by a very high inoculum. Rev. Soc. Bras. Med. Trop. *6:* 79-84, 1972.

10. MENEZES, H. & ALBUQUERQUE, R. D. R. — Imunização de camundongos com vacina viva avirulenta de *Trypanosoma cruzi*. III. Vacinação do meio de cultura. Rev. Soc. Bras. Med. Trop., *4:* 69-74, 1970.

11. MENEZES, H. — Aplicação de vacina viva avirulenta do *Trypanosoma cruzi* em seres humanos. Nota prévia. Rev. Inst. Med. Trop. S. Paulo, *13:* 144-154, 1971.

12. MENEZES, H. — The vaccination of human beings with live avirulent vaccine of *Trypanoroma cruzi*. A two year follow-up of the first two cases. Rev. Soc. Bras. Med. Trop. In press.

13. RIBEIRO DOS SANTOS, R. — Personal communication. 1972.

14. RIBEIRO DOS SANTOS, R. — Imunização passiva de camundongos isogênicos por linfócitos sensibilizados com a cepa PF de *Trypanosoma cruzi*. Meeting at the Dept. Trop. Dis. USP. S. Paulo, Jume 16, 1972.

TABLE 1

PATIENT — João B... O...; Age — 20 years; Body Weight — 65 Kg.

DATE	PULSE	TEMP. C°	LABORATORIES								ECG	VACCINE	BLOOD CULT. Number tubes ( )	XENO Number bugs ( )	BLOOD INOC. Number mice ( )	OBS.
			A++	B++	C'		D		E							
			CFT	CFT	CFT+	IFT	CFT++	IFT	CFT++	IFT						
11-06-71			—	—	—	—					N					
21-06-71	80	36,4										$2x10^5$				
22-06-71	82	36,7														
23-06-71	80	36,0														
24-06-71	80	36,0														
25-06-71	80	36,0														
26-06-71	80	36,0														
27-06-71	78	36,0														
28-06-71	78	36,0														
29-06-71	78	36,0														
30-06-71	78	36,0														
02-07-71	76	36,0														
05-07-71	78	36,0														
06-07-71	72	36,2														
07-07-71	78	36,2														
08-07-71	80	36,0														
09-07-71	78	36,0														
10-07-71	76	36,0														
12-07-71	78	36,2														
15-07-71	78	36,0														
21-07-71	76	36,0	—	—	—	—					N		— (5)	— (5)	— (5)	
21-08-71	78	36,0	—	—	—	—					N		— (5)	— (5)	— (5)	
					—	—										
19-09-71	76	36,5	—	—	—	—					N		— (5)	— (5)	— (5)	
18-10-71			—	—	—	—										
21-11-71			—	—	—	—										
08-01-72	76	36,0		—							N		— (5)	— (5)	— (5)	
				—	—	—										
				—												
				a.c.												
27-06-72					—	—	—	—	—	—	N		— (5)	— (5)	— (5)	

\+ Qualitative test ++ Quantitative test (Waldsworth, Maltner & Maltner) Positive > 1,9 a.c. anticomplementary N normal — negative

TABLE 2

PATIENT — José R... B... F...; Age — 23 years; Body Weight — 75 Kg.

DATE	PULSE	TEMP. C°	LABORATORIES				ECG	VACCINE	BLOOD CULT. Number tubes ( )	XENO Number bugs ( )	BLOOD INOC. Number mice ( )	OBS.
			A	B++	C							
			CFT	CFT	CFT+	IFT						
11-06-71			—	—			N					
21-06-71	76	36,5						$2x10^5$				
22-06-71	76	36,5										
23-06-71	78	35,8										
24-06-71	78	36,2										
25-06-71	88	35,8										Test after exercise (Foot-ball)
26-06-71	76	36,0										
27-06-71	78	36,0										
28-06-71	78	35,9										
29-06-71	76	35,8										
30-06-71	76	36,0										
02-07-71	76	36,0										
05-07-71	92	35,8										Test after exercise (Foot-ball)
06-07-71	78	35,6										
07-07-71	76	36,0										
08-07-71	78	36,0										
09-07-71	78	36,0										
10-07-71	76	36,0										
12-07-71	76	35,8										
15-07-71	76	36,0										
21-07-71	76	36,0	—	—	—	—	N		— (5)	— (5)	— (5)	
20-08-71			—	—	+	—	N		— (5)	— (5)	— (5)	
18-09-71	76	35,8	—	—	—	—	N		— (5)	— (5)	— (5)	
18-10-71			—	—	—	—						
21-11-71			—	—	—	—						
08-01-72	74	36,4		—	—	—	N		— (5)	— (5)	— (5)	
				1,6								
				>3,0								

Liberated

\+ Qualitative test ++ Quantitative test (Waldsworth, Maltner & Maltner) Positive > 1,9 N normal — negative

TABLE 3

PATIENT — Osvaldo F... R...; Age — 33 years; Body Weight — 56Kg.

DATE	PULSE	TEMP. C°	LABORATORIES				ECG	VACCINE N^{er} parasito	BLOOD CULT. Number tubes ( )	XENO Number bugs ( )	BLOOD INOC. IN MICE Number mice ( )	OBS.
			A	B	C							
			CFT	CFT	CFT	IFT						
11-06-71			—	—	—	—	N					
21-06-71	80	36,7						2x10^5				
22-06-71	78	36,2										
23-06-71	78	36,2										
24-06-71	80	36,0										
25-06-71	76	36,0										
26-06-71	76	36,0										
27-06-71	76	36,7										
28-06-71	78	36,0										
29-06-71	78	35,8										
30-06-71	78	36,5										
02-07-71	78	36,3										
05-07-71	76	35,8										
06-07-71	78	36,4										
07-07-71	78	36,2										
08-07-71	78	36,0										
09-07-71	76	36,0										
10-07-71	76	36,0										
12-07-71	78	36,0										
15-07-71	78	36,0										
21-07-71	76	36,0	—	—	—	—	N		— (5)	— (5)	— (5)	
21-08-71	76	36,0	—	—	—	—	N		— (5)	— (5)	— (5)	
					—	—						
19-09-71	78	36,2	—	—	—	—	N		— (5)	— (5)	— (5)	
18-10-71			—	—	—	—						
21-11-71			—	—	—	—						
08-01-72	78	36,5		—								
				—								
				—	—	—	N		— (5)	— (5)	— (5)	
				2,4								

Transfered to a Prision in another State.

TABLE 4

PATIENT — Edson M... S...; Age — 23 years; Body Weight — 60 Kg.

DATE	PULSE	TEMP. C°	LABORATORIES								ECG	VACCINE	BLOOD CULT. Number tubes ( )	XENO Number bugs ( )	BLOOD INOC. Number mice ( )	OBS.
			A	B	C		D		E							
			CFT	CFT++	CFT+	IFT	CFT++	IFT	CFT++	IFT						
11-06-71			—	—	—	—					N					
21-06-71	78	36,0										$2x10^5$				
22-06-71	74	35,8														
23-06-71	76	35,8														
24-06-71	76	36,0														
25-06-71	74	36,0														
26-06-71	74	36,5														
27-06-71	78	36,0														
28-06-71	78	36,0														
29-06-71	76	36,2														
30-06-71	76	36,0														
02-07-71	78	36,0														
05-07-71	76	36,0														
06-07-71	80	36,0														
07-07-71	80	36,5														
08-07-71	76	36,2														
09-07-71	76	36,2														
12-07-71	76	36,0														
15-07-71	76	36,0														
21-07-71	76	35,8	—	—	—	—					N		— (5)	— (5)	— (5)	
20-08-71			—	—	±	—					N		— (5)	— (5)	— (5)	
					—	—										
19-09-71	78	36,5	—	—	—	—					N		— (5)	— (5)	— (5)	
18-10-71			—	—	±	—										
21-11-71			—	a.c.	—	—										
21-03-72				>3,0	—	—	—	—								
27-06-72					—	—	—	—	—	—	N		— (5)	— (5)	— (5)	

\+ Qualitative test ++ Quantitative test (Waldsworth, Maltner & Maltner) Positive > 1,9 a.c. anticomplementary N normal — negative ± doubtful

TABLE 5

PATIENT — José C... S... M...; Age — 23 years; Body Weight — 78 Kg.

DATE	PULSE	TEMP. C°	LABORATORIES A CFT++	B CFT++	C CFT+	C IFT	D CFT++	D IFT	E CFT++	E IFT	ECG	VACCINE	BLOOD CULT. Number tubes ( )	XENO Number bugs ( )	BLOOD INOC. Number mice ( )	OBS.
11-06-71			—	—	—	—					N					
21-06-71	78	36,1										2x10⁵				
22-06-71	78	36,5														
23-06-71	78	36,0														
24-06-71	76	36,3														
25-06-71	84	36,0														Test after exercise (Foot-ball)
26-06-71	76	36,8														
27-06-71	76	36,5														
28-06-71	76	36,0														
29-06-71	76	36,5														
30-06-71	78	36,2														
02-07-71	78	36,4														
05-07-71	78	36,1														
06-07-71	78	36,5														
07-07-71	76	36,5														
08-07-71	76	36 6														
09-07-71	78	36,0														
10-07-71	78	36,5														
12-07-71	78	36,0														
15-07-71	76	36,0														
21-07-71	78	36,5	—	—	—	—					N		— (5)	— (5)	— (5)	
21-08-71	76	37,0	—	—	—	—					N		— (5)	— (5)	— (5)	
18-09-71			—	—	—	—					N		— (5)	— (5)	— (5)	
18-10-71			—	—	—	—										
21-11-71			—	—	—	—										
08-01-72	78	36,3		>3,0	—	—	—	—								
					—	—	—	—			N		— (5)	— (5)	— (5)	
				>3,0	±	—										
				>3,0												
13-01-72				>3,0												
				>3,0	—	—	—	—	1,8	—			— (10)	— (10)		Lab. E:
27-06-72					—	—	—	—	—	—	N		— (5)	— (5)	— (5)	IHT —; IT —

\+ Qualitative test ++ Quantitative test (Waldsworth, Maltner & Maltner) Positive > 1,9 LT latex test IHT indirect hemaglutination test N normal — negative