THE VACCINATION OF HUMAN BEINGS WITH LIVE AVIRULENT VACCINE OF TRYPANOSOMA CRUZI. A TWO-YEAR FOLLOW-UP OF THE FIRST TWO CASES.

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A two year follow-up of the first two volunteers vaccinated with the live PF Trypanosoma cruzi *strain demonstrated that the parasitological and clinical test were negative during and after that period.*

Of the serological tests employed, the CFT presented, in only one case, conflicting results, particulary in one laboratory and among different laboratories. However negative results were greater than all doubtful and positive one combined.

The IFT were negative in both patients.

Some comments are made about the sensibility and specificity of these tests.

The author concluded that the vaccine, in spite of the very large dose used in these cases, seems to be safe for human beings.

INTRODUCTION

The probable mutant of the *Trypanosoma cruzi* Y strain that has been demonstrated to be avirulent for laboratory animals (Menezes, [10, 11, 12, 14], Santos,[16] and Hungerer[6]) seems to be avirulent for human beings as well.

In a preliminary report (Menezes[13]) the follow-up studies on the 2 volunteers, within the first 3 months after vaccination, are referred to.

In this paper we will present the 2 year post-vaccination observations.

MATERIAL AND METHODS

Two male volunteers, one 52 years old (Patient 1) and the other 32 years old (Patient 2) were vaccinated with two doses of the live *T. cruzi* vaccine, PF strain. The two doses were separated by a forty day interval. The first had almost $5x10^4$ parasites and the second $3x10^7$ flagellates, and both were injected subcutaneously (Menezes[13]).

Before vaccination, and 8, 15 and 30 days after each vaccine, and almost once a month thereafter, for two years, ECG, peripheral blood-search for parasites, blood culture for trypanosomes, CFT (Guerreiro & Machado test), Imunnofluorescence Test (IFT) for *T. cruzi*, Xenodiagnosis and blood innoculations in young mice were carried out (Tables 1 & 2).

Other tests were performed during the first 3 months, as already mentioned (Menezes[13]).

Hemogram and blood sedimentation rate (BSR) tests were done 8, 15 and 30

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days after each vaccination and every month for 12 months, and after the second year of observation.

Patient 1 had xenodiagnosis in the 5th, 6th and 7th months by the Schenone technique (Schenone et al.[9]).

At the same time, a blood culture was performed with 14 test tubes of Warren medium, each day, for successive 3 day periods, once a month for 3 successive months, following the technique described by Albuquerque et al.[1].

The triatominae bugs used for the Schenone's xenodiagnosis were *Triatoma infestans* and those employed in the other tests were *Rhodnius prolixus*, safe when specifically reported (Table1).

The inoculations in very young mice were of centrifuged blood serum, following the Strout technique (Strout[18]).

The serological tests, until the fourth month, were made in three different laboratories and in four laboratories thereafter (Tables 1 & 2).

Sometimes two or more CFT were carried out in the same laboratory with the same blood sample.

RESULTS AND DISCUSSION

Patient 1, besides all negative parasitological tests, presented few positive CFT, at different times, in two of the four laboratories where the majority of these tests were performed.

Considering the total number of CFT in all the laboratories, the percentage of positive results was 4,9%, that of doubtful, 18,6% and finally, 76,5% negative results.

It must be emphasized that with almost all positive and doubtful results, when the test was repeated, as a blind test, in the same laboratory, with the same blood sample, the results were divergent (Table 1).

Special mention must be made of the CFT in the 4th month of the second vaccination. Laboratory 1 presented, with the same blood sample, two positive results, one doubtful and two negative, while the same blood serum gave negative tests in the other three laboratories, even after repetition (Table 1).

Another surprising and unexpected result was obtained in laboratory 2 on the 23th month of vaccination, when two tests gave negative results and a third with the same blood, was positive several days later.

In consequence of this discrepancy, in the test realized at the end of the second year of vaccination, we used a positive and a negative control serum.

The first one was a pool of sera of patients with several positive CFT, and the second the blood serum of a young man who always lived in the city, never had contact with triatominae bugs, and had two previous negative CFT.

As can be seen in Table 1, the results of these controls were concordant in 3 different laboratories but gave both positive results in laboratory 2.

Over 19 occasions that the CFT was performed on Patient 1, only in 9 (47%) where there 100% agreement (doubtful and negative results) among the different laboratories.

The IFT, except for two positive results (at very low titers), each one in a different periods, were negative in 94,3% of the time.

Patient 2 had only 2 doubtful CFT in one laboratory, representing 2,6% of the total tests realized, or 97,4% negative results. The IFT in the blood of the same patient gave 100% negative results (Table 2).

All the ECG were within the normal limits in both cases.

The Hemogram and BSR presented no abnormalities.

Finally, no clinical or laboratory data demonstrated any sign of disease during the whole two-year period. Both volunteers are living a normal life in apparent good health.

The serological tests of Patient 1 raise some discussion about the specificity and sensibility of the CFT and IFT in the diagnosis of Chagas' disease.

It is not our intention to go into this controversial field. Let us only quote the opinions of some experts on the subject.

The IFT is considered as the most sensitive of the standard test for serological diagnosis of parasitic diseases (Fife Jr.[5]), particularly in Chagas' disease where it gives earlier positive results and shows the greatest sensibility (100%) after the 9th month of infection (Cerisola[4]). This test, besides its high degree of sensibility in comparison with the CFT, shows no anti-complementary effects and no doubtful results (Araujo & Batista[4]).

In Patient 1, during the whole 2 years, only 2 out of 35 IFT gave positive results, but in both, the serum dilution was too low to be considered of clinical value.

Laboratory 3 used the methanolic antigen in the CFT; following Almeida et al.[2] it is with this antigen that the higher titers are observed in the positive cases.

The CFT of Patient 1 gave only 3 doubtful results in that laboratory after the 18th month of the second vaccination, and all the IFT were negative.

In chronic Chagas disease, when several CFT are realized, the tests show consistent positive results with only fortuitous accentuated titer variations and transitory negative results (Rassi et al.[15]).

In Patient 1, the tests show consistent negative results with only four positive cases, with the discrepancies already mentioned.

The parasitological tests, even sophisticated ones as such as Schenone's xenodiagnosis,[17] blood culture by the Albuquerque et al.[1] technique and serum inoculation in young mice (Strout's technique[13]) were always negative in both patients during the two-year period of observation.

It seems to us that the PF strain is avirulent for man as well as for laboratory animals.

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RESUMO

O autor apresenta o seguimento de dois casos humanos vacinados com vacina viva avirulenta de Trypanosoma cruzi.

Os resultados dos exames ditos específicos e também alguns inespecíficos demonstraram que não houve, no período de dois anos, qualquer sinal de infecção-doença.

Em um dos voluntários houve acentuada discrepância nos resultados da reação de Guerreiro & Machado, não só em um mesmo laboratório, com a mesma amostra de soro, mas também entre diferentes laboratórios, na mesma e em diferentes oportunidades.

Apesar de toda essa confusão, os resultados POSITIVOS não foram além de 4,9% (em dois laboratórios apenas), os DUVIDOSOS não ultrapassaram 18,6% e finalmente os casos NEGATIVOS atingiram 76% de todas essas reações.

Os testes de imunofluorescência para T. cruzi *deram 94,3% de resultados NEGATIVOS no Paciente 1 e 100% no Paciente 2.*

Hemoculturas, xenodiagnósticos e sub-inoculação em camundongos jovens foram sempre negativos em ambos os pacientes.

Conclue o autor que a cepa PF parece ser avirulenta para o homem tal como ele e outros (Santos[16] e Hungerer[6]) já demonstraram ser para animais de laboratório.

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TABLE 1

PATIENT 1

SEROLOGICAL TESTS

Laboratories

1 CFT	1 IFT	2 CFT	2 IFT	3 CFT	3 IFT	4 CFT	4 IFT	5 CFT	5 IFT	6 CFT	6 IFT	6 IHT	ECG	XENO Nº Bugs	XENO Res.	BLOOD CULTURE Nº Tubes	BLOOD CULTURE Res.	[illegible] Nº	[illegible] Res.
+		+		++		+		+		+									
									—				N	5		5		[illegible]	
									—				N	5		3		[illegible]	
									—				N	5		1		[illegible]	
									—				N	5		3		[illegible]	
									—				N	5		3		[illegible]	
		1,6							—				N	5		3		[illegible]	
		1,8																	
1,5		1,7							—				N	5	—	3		[illegible]	
						1,8	1/20							5	—	3		6	
2,8																			
2,5	1/40																		
				—	—	—	—						N	5		3		[illegible]	
1,9					—	—	—												
1,5				—	—	—	—						N	42+++	—	42		[illegible]	
				—															
				—	—	—	—						N	42+++	—	42		[illegible]	
1,7				—	—	—	—						N	42+++	—	42		[illegible]	
1,6				—	—	—	—						N	5	—	5		[illegible]	
				—	—	—	—						N	5+++	—				
													N			5		[illegible]	
1,6				[illegible]	—	—	—												
				—	—	—	—												
1,5		1,7		+	—	—	—												
Ac		Ac		Ac		—	—							5	—	5		[illegible]	
					—	—	—												
		3,0																	
										—	—	—							
		2,3		—	—	—	—			—	—	—	N	5+++	—	10		5	
										1,2									
		3,0		—	—	—	—			—	—	—							
		3,0		+	+	>3,0	+++			>3,0	+++	+++							
2		2																	
7	1	1		3		1	1												
17		20		9	12	13	13		6	3	2	2		191		131		80	

CONTROL NEGATIVE
CONTROL POSITIVE

	4		0	
CFT	15	IFT	2	IHT
	62		33	
Total	81		35	

[illegible] (Wadsworth, Maltaner & Maltaner) Positive 1,9
[illegible]
[illegible]
[illegible]

TABLE 2

PATIENT 2

ERIOD	Serological tests Laboratories 1 CFT +	1 IFT	2 CFT +	2 IFT	3 CFT ++	3 IFT	4 CFT +	4 IFT	5 CFT	5 IFT	6 CFT +	6 IFT	IHT	ECG	Parasitological tests XENO Nº Bugs	Res.	BLOOD CULTURE Nº Tubes	Res.	[illegible]	Res.
accine			—							—				N	5		5		[illegible]	
after 1st. vac.			—							—				N	5		4		[illegible]	
..			—							—				N	5		4		[illegible]	
			—							—				N	5		3		[illegible]	
2nd. vac.			—							—				N	5		3		[illegible]	
..										—				N	5	—	3		[illegible]	
..			1,7											N	5		3		[illegible]	
..			1,7							—										
ths. ..							—	—							5	—	3		[illegible]	
..					—	—	—	—						N	5	—	3		[illegible]	
.					—	—	—	—						N	9	—	10		[illegible]	
..					—	—	—	—						N	5	—	3		[illegible]	
..					—	—	—	—						N	5	—	3		[illegible]	
..					—	—	—	—						N	6	—	5		[illegible]	
AR ..			—		—	—	—	—						N	5		5		[illegible]	
aths. ..			—		—	—	—	—						N			5			
..					—	—	—	—												
..					—	—	—	—												
..					—	—	—	—												
..					—	—	—	—							5 [illegible]		5		5	
..					—	—	—	—												
EARS					—	—	—	—			—	—		N	5 [illegible]		10	—	5	
years			>3,0		—	—	—	—			—	—								
			3,0		+	+	>3,0	+++			>3,0	+++	+++							
TIVE																				
BTFUL			2																	
ATIVE	22		24		13	13	14	14		7	1	1	1		85		77		80	

CONTROL NEGAT
CONTROL POSIT
| 0 0
CFT [illegible] IFT [illegible]
73 15
Total 75 15

Quantitative test (Wadsworth, Maltaner & Maltaner) Positive >1,9
Qualitative test
· *Triatoma infestans*
Normal
Anticomplementar