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ESSAYS ON IMMUNIZATION OF MICE WITH ULTRAVIOLET RADIATED VIRULENT AND AVIRULENT CULTURE FORMS OF *TRYPANOSOMA CRUZI*

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SUMMARY

Under our experimental conditions, ultraviolet radiation was unable to reduce the virulence of the virulent cultivated Y strain of the *Trypanosoma cruzi.*

Trypanosomes from virulent and avirulent strains when killed by long-wave ultraviolet rays have no protective effect on mice against challenge.

The presence of live parasites, virulent or avirulent, seems to be essential to obtain immunological protection.

While the injection of virulent cultivated *T. cruzi,* before challenge, gives high parasitemia and mortality to mice, that of the avirulent PF strain produces neither parasitemia nor mortality.

INTRODUCTION

Attempts to immunize laboratory animals with "vaccines" composed of trypanosomes killed by physical means have increased recently (CAPPA et al. [2], CHIARI et al. [3], GOBLE [6], SANDERS [20], STUBBS et al. [22]) on the assumption that the parasites killed in this way better retain their antigenic capacity, being able to induce efficient protection in the mammal hosts, against challenge.

SAITO [19] in 1927 studied the morphologic alterations produced in *Trypanosoma gambiense* and *Trypanosoma equiperdum* by ultraviolet radiation. COLLIER et al. [4] in 1931 verified the appearence of antigenically different forms of *Trypanosoma brucei* after radiation with a lamp of tungsten and mercury.

In 1934 and 1939, LEVADITI et al. [7, 8] studied the influence of ultraviolet rays on the virulence of *Trypanosoma evansi* and *Trypanosoma brucei,* respectively.

On the basis of these observations and in the hope of obtaining an easy and inexpensive way to produce an efficient "vaccine" with killed *Trypanosoma cruzi,* we performed the present experiments.

MATERIAL AND METHODS

A — Thirty male albino mice with 10 g of body weight were divided into three groups of ten animals, each.

The mice were from the same strain and were maintained on the same nutritional and environmental conditions.

As "vaccine", a suspension of the virulent Y strain of *Trypanosoma cruzi,* after the 12th passage in NOELLER [17] culture medium was used. The culture was 8 days old.

The flagellates were centrifuged and washed several times in saline solution until the supernatant was clear.

The final suspension had a concentration of 3 x 10^7 parasites/ml, with about 90% of live parasites, almost 2.5% being metacyclic forms.

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A.1 — In a staining dish with 45 mm of diameter 3 ml of the above suspension were placed, being later radiated by a long wave ultraviolet ray lamp *, at a distance of 10 cm, for 30 minutes.

After the radiation only 50% of the flagellates had movement. Into each of ten mice of the first group, 0.2 ml of the 30 minutes-radiated "vaccine" was injected by sub-cutaneous route.

A.2 — Three ml of the initial suspension of the virulent *T. cruzi* were radiated in the same manner for 60 minutes.

After the radiation the number of live parasites was about 40%. Ten more mice were "vaccinated" with the same dose by the same route.

A.3 — Ten animals used as controls received identical doses but of the initial non-radiated suspension of trypanosomes.

Parasitemia by the PIZZI & BRENER [1] technique was done 8 and 15 days after the "vaccination" (Table I).

The animals were observed until the 30th day, but parasitemia was not performed at this time.

B — Forty albino mice with the same characteristics as the aforementioned were divided into four groups of 10 animals each. The "vaccine" used was a saline suspension of the cultivated virulent Y strain, treated as described below.

The culture was 8 days old and the medium employed was that of NÖLLER [17]. The suspension of trypanosomes had 3.3 x 10^7 parasites/ml with about 99% of mobile flagellates and 2.5% of metacyclic forms.

B.1 — From the suspension above, 3.5 ml were mixed with 3.5 ml of a tetracycline solution ** corresponding to 50 mg of the substance. Afterwards 2.5 ml of that suspension was placed in a staining dish with 45 mm diameter and radiated from above, at a distance of 20 cm, with the same ultraviolet lamp.

After the radiation 100% of the parasites had lost their motility. A culture in Warren's[23] liquid medium proved that they were all killed. Into each of the 10 mice of the first group of this series, 0.1 ml of this radiated "vaccine" was injected by sub-cutaneous route.

B.2 — To 3.5 ml of the initial suspension 3.5 ml of a 1/10,000 Acridine Orange solution was added. This was prepared with the buffer solution of Michaelis at pH 6,5.

Ultraviolet radiation by the same method used in the previous sample was applied to this one.

Microscopic examination and culture in Warren's medium demonstrated that all the parasites were killed.

Each mouse of a second group of 10 received, by sub-cutaneous route, 0.1 ml of this "vaccine".

B.3 — To each mouse of the third group of 10, 0.1 ml of the initial, non-radiated suspension was injected by the same route.

Parasitemia by the PIZZI & BRENER [1] technique was done to each animal of the above mentioned three groups, on the 8th and 15th day of the "vaccination".

B.4 — Eight weeks later all the surviving animals of the 3 previous groups plus 10 more mice, kept as controls, were injected with blood forms of the virulent Y strain of the *Trypanosoma cruzi* (5,000 parasites/g body weight). Parasitemia was determined 8,15 and 30 days after the infection.

C — Forty six male albino mice from the same origin of the preceding groups, with 10 g of body weight, at the beginning of the experiment, were divided in four groups: 3 with nine animals each, and one control group, with 19 mice. The cultivated avirulent PF strain (a probable mutant of the cultivated Y strain) that has been already described, (MENEZES [9-16]) was used as "vaccine" in this series.

The flagellates came from a 29 days old culture in PACKCHANIAN [18] medium.

The initial saline solution suspension contained about 3.3 x 10^7 parasites/ml with almost 80% of mobile flagellates and 10% of metacyclic forms.

C.1 — Two and a half ml of the above suspension were placed in a staining dish of 45 mm diameter and radiated from above for 30 minutes with the same long-wave ultraviolet lamp, from a distance of 10 cm. After the radiation the number of trypanosomes with

* Black-Ray long-wave U.V. — Mod. B 100. Ultraviolet Products Inc. San Gabriel, California, U.S.A.

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motility was about 20%. Into each mouse of the first group of nine, 0.2 ml of this radiated "vaccine" was injected by sub-cutaneous route.

C.2 — Under identical conditions, 2.5 ml of the initial suspension of the avirulent trypanosomes were radiated for 60 minutes. After this time, only 10% of the flagellates were alive.

Nine mice of the second group each received 0.2 ml of this "vaccine" by the same route.

C.3 — A similar "vaccine" was obtained by radiating 2.5 ml of the initial trypanosomes suspension for 240 minutes. At the end of the radiation all the parasites were killed, as confirmed by a culture in liquid WARREN [23] medium.

The animals of the last group of nine were "vaccinated" as were those of the previous groups. A search for parasites by thick blood drop, was made on each animal of the 3 groups on the 8th and 15th day after "vaccination".

Four weeks after the immunization all the "vaccinated" animals plus the 19 controls were infected, by intra-peritoneal route, with virulent blood forms of the Y strain of the *Trypanosoma cruzi*, obtained from mice on the 8th day of infection.

Parasitemia was measured on the 8th, 15th and 30th day after the challenge.

RESULTS

The results are summarised in Tables I, II and III and in Graphs I, II and III.

A) Live virulent cultivated trypanosomes when radiated for 30 or 60 minutes show no significant differences in parasitemia and mortality rates when compared with the control animals.

TABLE I

Mice "vaccinated" with radiated virulent Y strain but not challenged

Mice no	Number of parasites/5 mm³ of blood								
	Days after "vaccination" with radiated virulent strain						Controls		
	UV 30 m			UV 60 m					
	8	15	30	8	15	30	8	15	30
1	14,000	3,570	—	17,500	—	—	17,500	4,375	V
2	10,500	1,225	V	10,500	3,675	V	7,525	4,935	—
3	3,780	1,190	—	7,175	2,520	—	10,500	7,875	—
4	10,500	1,185	V	14,000	2,940	—	14,000	—	—
5	14,000	1,435	V	10,500	8,575	—	14,000	3,675	—
6	10,500	2,240	V	10,500	10,500	—	17,500	6,125	—
7	17,500	1,575	V	—	—	—	17,500	3,080	—
8	17,500	4,375	V	10,500	—	—	10,500	1,820	—
9	14,000	3,080	—	7,700	4,025	V	14,000	2,940	V
10	17,500	4,375	V	14,000	3,290	—	10,500	—	—
Mean	12,978	2,435		11,375	5,075		13,352	4,353	—
Median	14,000	1,907		10,500	3,675		14,000	4,026	
% Mortality	0	0	30	10	30	80	0	20	80

— Dead

V = Alive, no parasitemia done

m = minute

TABLE II

Mice "vaccinated" with radiated virulent Y strain in medium with fluorescent substance

Mice no.	Number of parasites/5 mm³ of blood																	
	Days after "vaccination"						Days after challenge											
	Tetrac. + U.V.		A. Orange + U.V.		Vir. culture		Tetrac. + U.V.			A. Orange + U.V.			Vir. culture			Controls infection		
	8	15	8	15	8	15	8	15	30	8	15	30	8	15	30	8	15	30
1	0	0	0	0	2,590	455	3,570	1,610	0	2,940	1,750	–	0	0	0	7,700	—	–
2	0	0	0	0	1,470	420	2,730	1,365	–	3,220	—	–	0	0	0	—	—	–
3	0	0	0	0	910	805	3,045	4,830	–	3,920	1,050	0	0	0	0	2,975	2,835	0
4	–	–	0	0	—	—	+	+	+	5,250	5,565	–	+	+	+	2,660	3,290	–
5	0	0	–	–	1,645	490	3,675	1,330	–	+	+	+	0	0	–	4,025	2,485	–
6	0	0	–	–	3,675	805	3,570	—	–	+	+	+	+	+	+	2,940	2,590	0
7	0	0	0	0	1,680	385	4,375	1,645	–	3,570	35,000	–	+	+	+	3,920	—	–
8	0	0	0	0	3,045	280	3,045	8,610	–	7,875	1,190	0	0	0	0	3,885	2,905	–
9	0	0	0	0	3,295	770	2,170	1,750	–	3,430	700	0	0	0	0	7,875	2,380	0
10	–	–	0	0	2,030	1,050	+	+	+	3,780	2,205	–	+	+	+	17,500	—	–
Mean	0	0	0	0	2,260	606	3,272	3,020	0	4,248	6,780	0	0	0	0	5,942	2,747	0
Median	0	0	0	0	2,590	490	3,307	1,645	0	3,675	1,750	0	0	0	0	3,920	2,712	0
% Mortality	20	20	20	20	10	10	0	12	87	0	12	62	0	0	13	10	40	70

— Died

\+ Died after challenge and before the first parasitemia

TABLE III

Mice "vaccinated" with radiated non-virulent strain PF

Mice no.	Number of parasites/5 mm³ of blood											
	Days after challenge									Controls		
	U.V. 30 m			U.V. 60 m			U.V. 240 m					
	8 days	15 days	30 days	8 days	15 days	30 days	8 days	15 days	30 days	8 days	15 days	30 days
1	280	70	0	525	70	0	4,725	980	0	1,365	280	0
2	35	140	0	315	35	0	3,080	—	—	1,400	1,015	0
3	0	70	0	0	35	0	3,640	1,610	35	1,925	770	0
4	0	105	0	245	35	35	3,255	1,785	0	2,800	455	0
5	700	350	0	0	0	35	2,590	525	0	3,605	105	0
6	70	35	0	385	280	0	2,730	2,976	—	9,135	10,080	—
7	105	210	0	70	35	0	5,285	1,820	0	10,780	1,190	—
8	0	35	0	175	105	0	5,180	1,780	—	16,135	140	—
9	35	70	0	0	0	0	5,250	1,190	—	17,675	—	—
10										1,785	1,785	35
11										2,660	4,880	—
12										3,045	1,085	0
13										3,045	1,470	70
14										3,570	3,570	—
15										4,025	2,660	0
16										4,025	1,645	—
17										4,725	2,835	—
18										5,935	1,680	—
19										6,300	1,330	—
Mean	136	120	0	190	66	7	3,970	1,583	7	5,470	2,054	11
Median	35	70	0	175	35	0	3,640	1,695	0	3,605	1,400	0
% Mortality	0	0	0	0	0	0	0	11	44	0	5	52

One exception was found in the 30 minute-radiated group with regard to the mortality rate. On the 30th day this rate was only 30% while in the 60 minute-radiated group and in the control it was 80%. We have no explanation at present for this difference. By virtue of the very high parasitemia presented by all the animals of this series we did not challenge it.

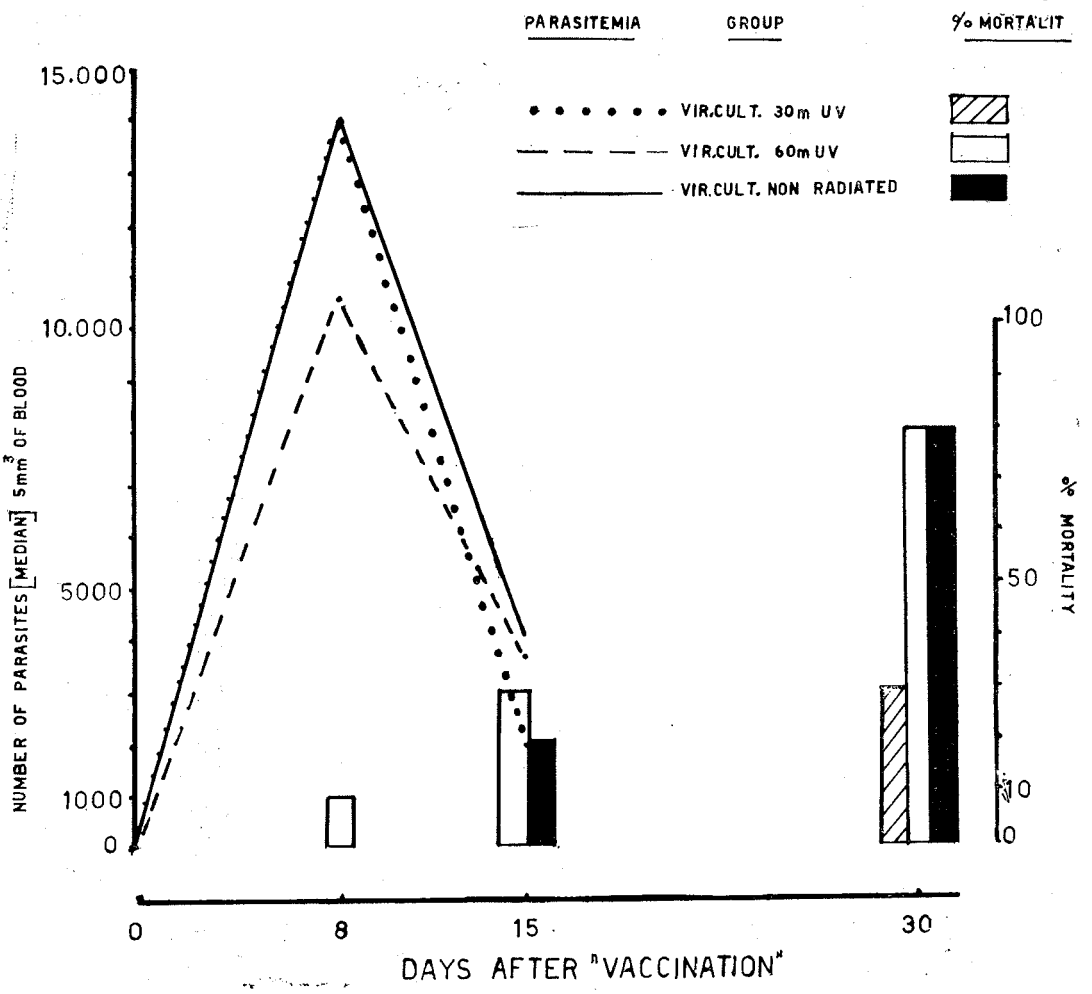
B) The addition of a fluorochrome to the trypanosoma suspension before radiation, which has already been observed by LEVADITI et al. [8] to promote the reduction of the virulence of *Trypanosoma brucei*, kills all the virulent *Trypanosoma cruzi* and affords no protection to the animals injected with the dead parasites. Protection against challenge was obtained only with the virulent non-radiated culture that gave a high parasitemia, i.e., infected the mice (Table II).

C) The ultraviolet radiation does not alter the protective effect of the avirulent "vaccine" since some percentage of live trypanosomes were present in it. Radiation for 240 minutes killed all the flagellates and abolished the protective action of the "vaccine".

DISCUSSION AND CONCLUSIONS

In spite of having an efficient live avirulent "vaccine" in laboratory animal (MENEZES [9-11]) we think the ideal would be to have the same protection with killed parasites.

GRAPH I

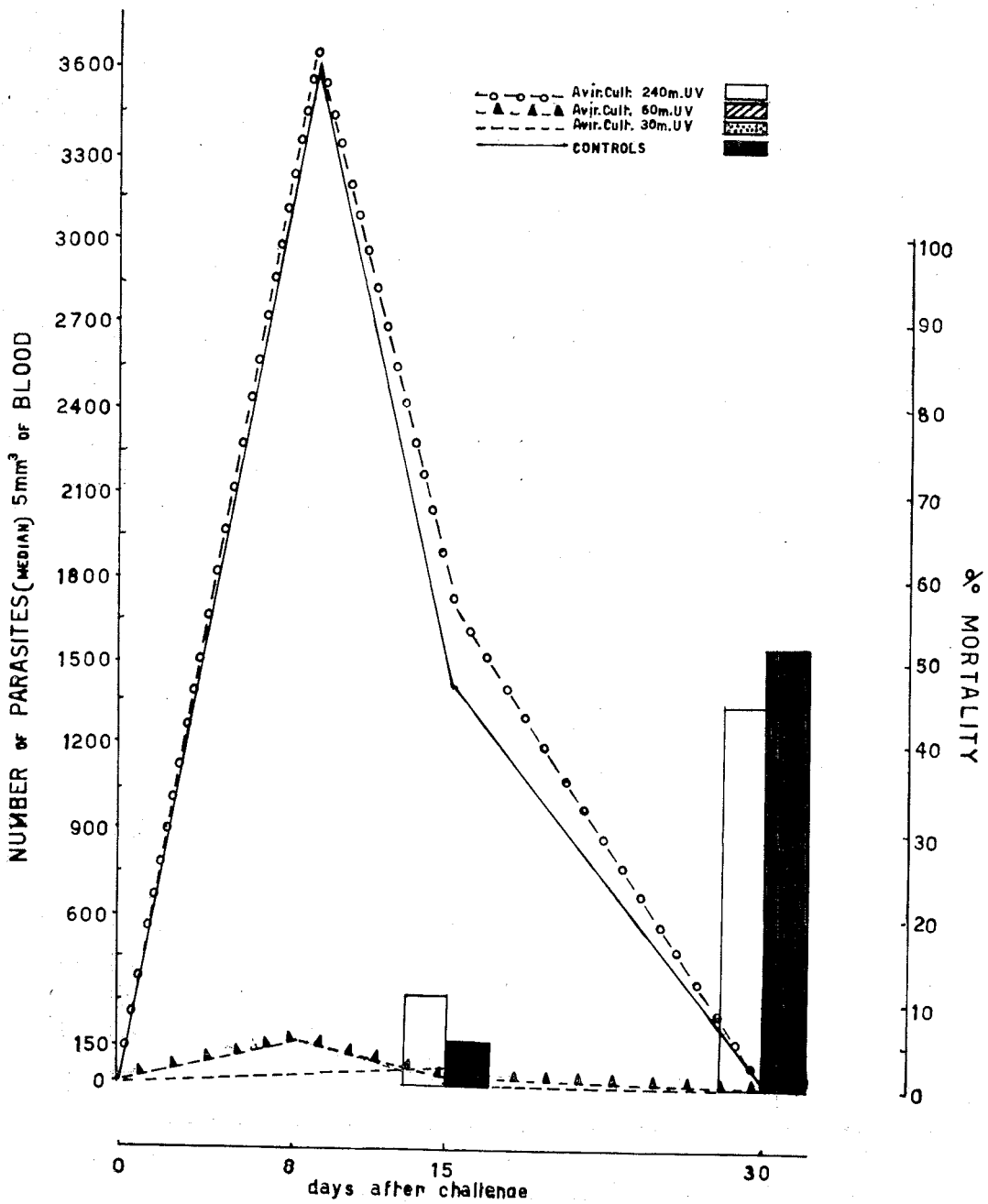


used in an attempt to obtain efficient "vaccine" against trypanosome infections, all with negative results.

Ultraviolet radiated trypanosomes have been used to observe morphologic alterations (LEVADITI et al. [7, 8], SAITO [19]) loss of virulence (COLLIER [4], STUBBS et al. [22], EMMET [5]), but not, to our knowledge, as a means of obtaining a "vaccine".

Our results have shown that under the experimental conditions described, the ultraviolet radiation was unable to reduce the

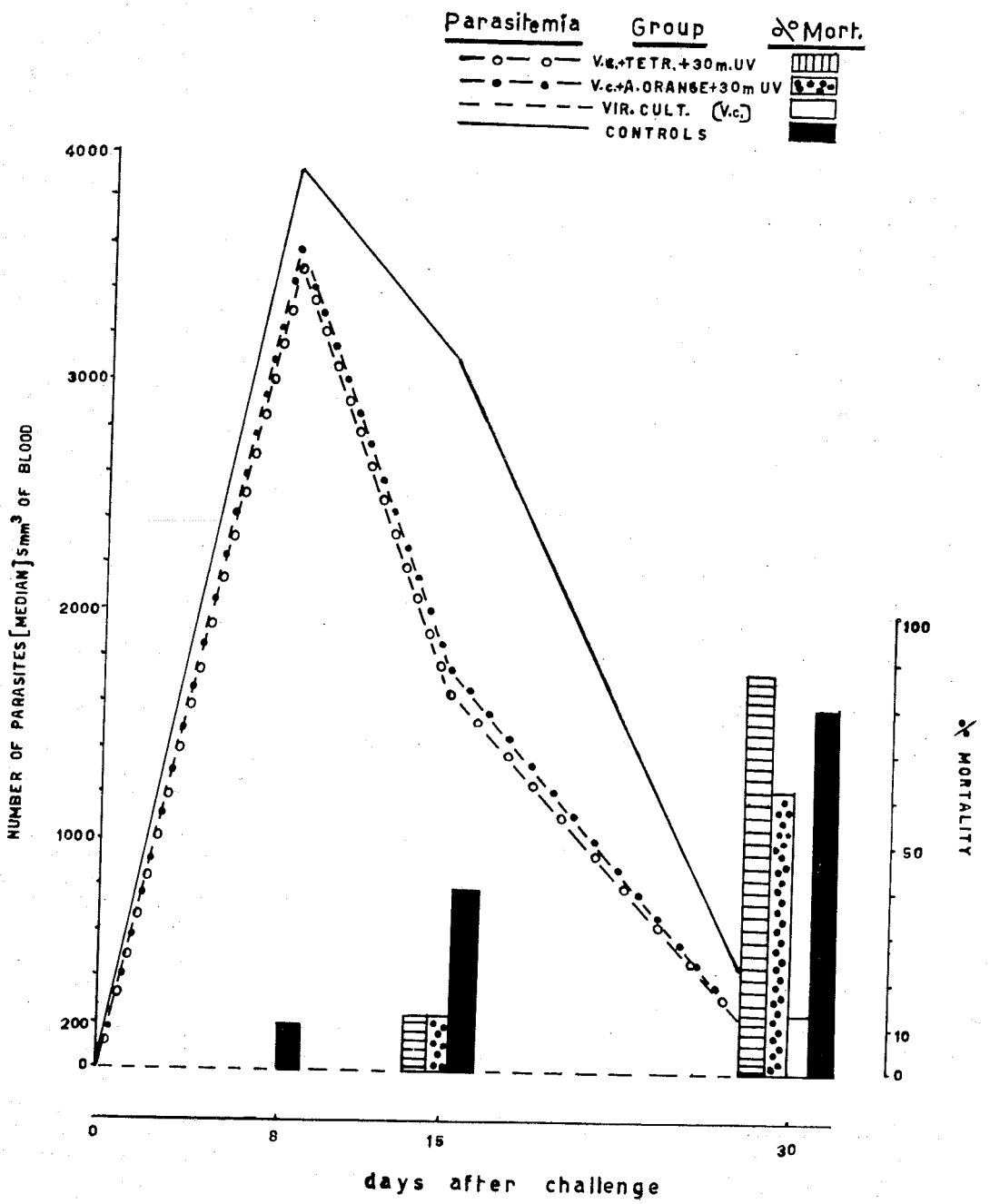
GRAPH III



Stimulated by the work of GOBLE [6] who reports good results with untrasonicated trypanosomes, of SENECA [21] who isolated a lipopolysaccharide fraction from the *T. cruzi* with good antigenic activity, and those of CAPPA et al. [2] using as "vaccine" trypanosomes killed by high pressure, I tried this experiment with ultraviolet radiation.

Roentgen rays (SANDERS [20]) and Gamma radiation (CHIARI et al. [3]) have been already

GRAPH II



virulence of a virulent strain of *Trypanosoma cruzi*, that the injection of virulent or avirulent parasites killed by U.V. radiation does not confer any protection on mice against challenge and that this protection is achieved only when a certain number of virulent or avirulent trypanosomes are present in the innoculum.

The virulent flagellates always gave patent parasitemia with high degree of mortality (Tables I, II), while the avirulent PF strain confirms its ability to protect laboratory animals without producing a detectable infection (Table III, Ghaph III).

RESUMO

Tentativa de imunização de camundongos com formas virulentas e avirulentas de Trypanosoma cruzi *submetidas à radiação ultravioleta*

Nas condições experimentais descritas, a radiação ultravioleta se mostrou incapaz de reduzir a virulência de formas de cultura, virulenta, da cepa Y do *Trypanosoma cruzi*.

Os tripanosomas tanto da forma virulenta (Y) como da avirulenta (PF) quando mortos pela radiação perdem seu efeito protetor sôbre os camundongos, contra ulterior infecção.

A presença de parasitas vivos, virulentos ou avirulentos, parece ser essencial para obter a proteção imunológica.

As formas virulentas quando injetadas com objetivo profilático produzem sempre alta parasitemia e elevada mortalidade enquanto a forma não virulenta (PF) não produz nem parasitemia nem mortalidade.

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