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III — THE AVIRULENCE OF THE CULTIVATED Y STRAIN OF *TRYPANOSOMA CRUZI*

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SUMMARY

The simultaneous use of successive inoculations in mice of the PF strain of *Trypanosoma cruzi* and high doses of Prednisolone was not able to restore the virulence of that strain.

INTRODUCTION

In two previous papers (MENEZES [3, 4]) we tried to demonstrate the real avirulence of the cultivated Y strain of *Trypanosoma cruzi*, (now designated PF strain — MENEZES & ALBUQUERQUE [5]) maintained for 16 years in artificial media, in the Department of Parasitology of the Faculty of Medicine, Ribeirão Prêto.

The simultaneous use of Prednisolone with the PF strain and the injection of this later into young mice, as isolated procedures, were shown to be uneffective in reducing the resistance of mice to that strain or in re — establishing its virulence (MENEZES [4]).

RUBIO [8] succeeded in enhancing the virulence of a peruvian strain of *T. cruzi* by the simultaneous use of both of the above techniques.

We tried to do the same with the avirulent PF strain, using younger animals and relatively larger doses of corticoid (Prednisolone) than that employed by that Author.

MATERIAL AND METHODS

1.0 — *Vaccine* — Trypanosomes from the 313th culture of the avirulent PF strain of *T. cruzi*, in Packchanian diphasic culture medium, 25 days old, were used as vaccine, The liquid phase of the medium was centrifuged and the sediment suspended in saline solution.

The final suspension had about 1.6 x 10^6 parasites/ml, as shown by the Petana's technique [6].

About 50% of the trypanosomes were motile and almost 10% were metacyclic forms.

Soon after the preparation, the vaccine was injected (0.1 ml) by intra-peritoneal route into 20 male albino mice from the same strain, with 10 g of body weight.

The of these animals (1.2.1) and 10 more from the same strain with identical sex and body weight (1.3.1) received daily, for 8 days, a subcutaneous injection of 1 mg of delta-hydrocortisone (+).

The remaining 10 vaccinated mice were kept as controls (1.1.1.). To prevent secondary infections all the steroid treated animals received, every two days, an i.m. injection of 25,000 IU of Penicillin G - benzetacine (++).

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(+) Hidro-decortancil. SARSA

(++) Benzetacil — Fontoura-Wyeth

1.1.1. — In the group which only received the vaccine, 3 mice died from intercurrent infections, 3, 4 and 7 days after the vaccination. The examination of the peripheral blood of these animals was negative for parasites.

The seven surviving mice were bled to death on the 8th day of the experiment. The blood serum of all the animals was centrifuged and the sediment examined for parasites.

1.2.1. — Five animals of this group died of intercurrent infections and the 5 survivors were bled to death on the 8th day and was pool of their blood serum a centrifuged and examined for parasites. Fragments of tissues were saved for histologic examination.

Into each 10 g mouse of a new group of 10 (1.2.2.), 0.15 ml of the above serum was injected, by intraperitoneal route.

1.2.2 — This group received 1 mg of Prednisolone every day by intra-muscular and 25,000 IU of Penicillin G — benzetacine every two days. Within 8 days of such treatment all the animals died.

1.3.1 — Six of these animals treated only with corticoid died before the 8th day, when the four surviving mice were bled and the blood serum was injected (0.1 ml) into each animal of a new group of 10 mice (1.3.2.).

1.3.2 — The 10 mice of this group were treated the same way as those of the preceding group and all of them died before the 8th day of treatment.

2.0 — *Vaccine — Trypanosoma cruzi* from the 315th culture of the PF avirulent strain was cultivated in liquid Warren medium. After 19 days the flagellates were centrifuged and the sediment washed several times in saline solution.

The final suspension had about 1×10^7 parasites/ml with about 80% mobile forms and 5.5% of metacyclic parasites.

This vaccine was injected (0.2 ml) by intraperitoneal route into 20 albino male mice with 10 g of body weight.

Into 10 of these animals (2.2.1.) and into 10 more with the same sex and weight (2.3.1), 1 mg of delta-hydrocortisone (+) was injected by subcutaneous route, every day.

2.1.1 — Within this group of vaccinated and non treated mice all animals were alive 7 days after the vaccination. At this time all mice were bled and the blood serum centrifuged, examined for parasites and injected (0.2 ml) into a new group of 10 g mice, by intraperitoneal route (2.1.2).

Fragments of heart liver and spleen were saved for histologic examination.

2.1.2 — As in the preceding group all the animals were alive 7 days after the blood serum injection.

The mice were bled, the blood serum searched for parasites and injected (0.1 ml) into a new group of 10 g albino mice (2.1.3.).

Fragments of several organs were fixed in 10% formalin for histologic examination.

2.1.3 — All the animals of this group were alive 7 days after the serum injection.

The search for parasites in the sediment of the centrifuged blood serum of all animals was negative.

Fragments of heart, liver and spleen were fixed for histologic examination.

2.2.1. — This group was composed of the vaccinated and corticoid treated mice.

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Seven days after the vaccination the animals were bled and the blood serum, centrifugation and search for parasites, was injected (0.15 ml) into a new group of 10 albino mice (2.2.2).

2.2.2 — Prednisolone 1 mg was injected daily into each animal of this group until the 7th day.
At this time 3 mice had died with no detectable trypanosomes in the blood.
The blood serum of the surviors was injected into another group of 10 mice (2.2.3.).

2.2.3. — The same corticoid treatment was applied to the mice of this group and after 7 days only one animal was alive. Its blood was negative for parasites. The animal was killed and fragments of the heart, liver and spleen were saved for microscopic examination.

2.3.1 — In this group, composed of the control mice that received only Prednisolone, one animal died before the 7th day.
At this time, the remaining 9 mice were bled and the blood serum injected (0.2 ml) into a new group of 10 mice (2.3.2.).

2.3.2 — The corticoid treatment was continued in the mice of this new group and only 7 survived until the 7th day.
The survivors were bled and the blood serum was injected (0.15 ml) into the following group.

2.3.3 — Ten albino mice with 10 g of body weight, injected with the blood serum of the preceding animals, were treated with 1 mg of Prednisolone daily.
At the end of the 7th day there were no survivors.

RESULTS

The blood search for parasites by the technique of STROUT [11] and the histologic examination of heart, liver and spleen of the mice in the vaccinated group and of the group vaccinated and treated with Prednisolone were negative for trypanosomes.

Tables I and II give a summary of the mortality rate and/or parasitemia in each group.

The percentage of dead animals was generally greater in the group treated only with corticoid than in the other two groups.

TABLE I

Experiment 1
Parasitemia and/or mortality rate of mice of different groups, after 8 days. Ten mice in each group

Group no.	Condition	Parasitemia	Mortality %
1.1.1.	Vaccinated	—	30
1.2.1.	Vaccinated + Prednisolone	—	50
1.2.2.		—	100
1.3.1.	Prednisolone	X	60
1.3.2.		X	100

TABLE II

Experiment 2
Parasitemia and/or mortality rate of mice of different groups, after 7 days. Ten mice in each group

Group no.	Condition	Parasitemia	Mortality %
2.1.1.	Vaccinated	—	0
2.1.2.		—	0
2.1.3.		—	0
2.2.1.	Vaccinated + Prednisolone	—	0
2.2.2.		—	30
2.2.3.		—	90
2.3.1.	Prednisolone	X	10
2.3.2.		X	30
2.3.3.		X	100

COMMENTS AND CONCLUSIONS

Is is unquestionable that corticosteroids enhance acute trypanosoma infections of laboratory animals (AGOSIN [1], RUBIO [8, 9]).

The mechanism through which this aggravation takes place is still unknown. However, it is believed to be due more to an interference with the immunologic defense of the host than to a direct action upon the parasites (SHERMAN & RUBLE [10]). Trypanosomes from the blood of cortisone-treated animals, injected into non-treated ones, do not present any increased virulence (RUBIO [8]).

The increase in virulence of a low virulent strain is usually obtained by its successive injection into young mice. Even the Y strain (not the PF mutant) maintained in artificial culture medium for 15 years, in another Laboratory, regains its virulence after two successive injections into mice (FERNANDES [2]).

We have already shown (MENEZES [3,4]) that neither of the above procedures was able to induce a parasitemia or a tissular invasion in mice with the avirulent PF strain of *Trypanosoma cruzi*.

The negative results of the present work is an additional proof of the real avirulence of that strain, which however maintains its immunogenic activity.

RESUMO

III — *A avirulência da cepa Y cultivada de* Trypanosoma cruzi

O Autor procura demonstrar que o mutante PF, avirulento, da cepa Y mantida em cultura por 16 anos do Departamento de Parasitologia da Faculdade de Medicina de Ribeirão Prêto, permanece avirulento mesmo quando se faz uso simultâneo de sub-inoculações em camundongos jovens com injeções de altas doses de Prednisolona.

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