

## REPEATED XENODIAGNOSIS IN CHRONIC CHAGAS' DISEASE: EFFECT OF A SINGLE INJECTION OF PREDNISOLONE

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### SUMMARY

1) In thirty eight patients with a positive Machado-Guerreiro reaction, in the chronic or indeterminate phase of Chagas' disease, 21 per cent had a positive xenodiagnosis.

2) Two xenodiagnosis performed about one week apart in the same patients strikingly increased the percentage of positive results. A third and fourth xenodiagnosis added very little in our series.

3) Prednisolone 50 mg intravenous did not significantly increase the number of positive xenodiagnosis under the conditions of this study, but additional studies are needed before making any generalization about steroid effect in this condition.

### INTRODUCTION

A single xenodiagnosis on a patient with chronic Chagas' disease has been reported to be positive in 15-45 per cent of the cases depending upon the incidence of the disease in the endemic area and the criteria used for the selection of cases<sup>10, 11</sup>. Nevertheless, xenodiagnosis constitutes the best procedure currently available to demonstrate the presence of *T. cruzi* in the blood of patients with the chronic forms of the disease<sup>17</sup>. PIFANO<sup>14</sup> has reported that repeating xenodiagnosis periodically will increase the yield by about 10-20 per cent.

Cortisone has been shown to produce an increase in parasitemia in acute experimental Chagas' disease<sup>1, 22</sup>, although reports of an effect of cortisone in chronic Chagas' disease in laboratory animals differ<sup>4</sup>. In

man, reports on the effect of cortisone on *T. cruzi* parasitemia were unavailable.

The present study was performed to evaluate the results of serial xenodiagnosis before and after a single intravenous dose of corticosteroid in patients with chronic form of Chagas' disease.

### MATERIALS AND METHODS

Thirty eight patients from the area of Salvador, Bahia, Brasil were selected for this study on the basis of a positive Machado-Guerreiro complement fixation employing a *T. cruzi* antigen as described by FREITAS<sup>6</sup>. Nineteen patients were volunteers from the community of Brotas and thirteen from the community of Teixeira

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Mendes (T. Mendes); most of them did not have clinical signs or symptoms suggesting chronic Chagas' disease. Six patients were hospitalized at the Hospital Prof. Edgard Santos because of signs or symptoms of chronic heart disease. Two groups of patients were organized based on age and epidemiological information. One group (steroid group) consisting of nineteen cases, received one injection of 50 mg intravenous prednisolone\* and the other group (control group) of nineteen cases received 10 ml physiological saline and served as the control. Both groups had four xenodiagnosis performed on each patient, as scheduled:

Day 1 — Xenodiagnosis 1

Day 4 — Xenodiagnosis 2

Steroid group — 50 mg prednisolone IV

Control group — 10 ml physiological saline IV

Day 6 — Xenodiagnosis 3

Day 8 — Xenodiagnosis 4

Xenodiagnosis were performed as follows: Five trypanosome free laboratory breed triatomid nymphs (*Rhodnius prolixus*)\*\* were placed in a cardboard box with an opening covered with one sheet of number 00 gauze and placed on the patient's forearm secured by tape so that the triatomids could bite through the gauze for 30 minutes. This time proved sufficient for all triatomids to ingest a blood meal. The triatomids were transferred into a glass jar immediately after feeding and stored in the dark at room temperature. After 40 days the feces of the triatomid were examined by milking the bug with a pair of forceps allowing the feces to fall into a glass slide and subsequently covered with a cover slip. The slide was examined under the microscope using 45× power for the presence of the flagellated stage of the trypanosome, readily characterized by its appearance and twirling action. The test was considered positive

when at least one of the five triatomids used in each exam contained the flagellated trypanosome form of *T. cruzi*.

## RESULTS

Xenodiagnosis was positive in one patient of the steroid group before the medication was administered; two others became positive subsequent to the injection of prednisolone (Table I).

Xenodiagnosis was positive in two control patients after one test and in three different control patients after a second test four days later. Although other patients had further positive exams on the third and fourth tests, no additional patients became positive in this group (Table II).

From the eight patients with positive xenodiagnosis, three (37%) showed positive results after the first exam, and six (75%) after the second.

## DISCUSSION

In the chronic phase of Chagas' disease, it is usually difficult to detect the infecting agent by the usual procedures. Patients from endemic areas have only serological evidence of the chronic disease, namely a positive Machado-Guerreiro reaction. FREITAS<sup>6</sup> demonstrated that this complement fixation test employing the Davis antigen, because of its very high sensitivity and specificity, was the best method for the diagnosis of chronic forms of the disease. He obtained a positive Machado-Guerreiro reaction with a titer of 2.0 or greater in over 95 per cent and PIFANO<sup>14</sup> in 90 per cent of cases with chronic Chagas' disease proven by xenodiagnosis. The Machado-Guerreiro reaction, however, while specific for the presence of complement fixation antibodies, is not specific for the presence of the blood parasite at the time the patient presents with symptoms of chronic Chagas' disease. In addition, in the few cases when the Machado-Guerreiro reaction is negative, a diagnosis of chronic Chagas' disease might be made on the basis of xenodiagnosis. PRATA<sup>16</sup> in-

\* Supplied as "Hidrocortancyl Intravenoso" (hemisuccinate salt), by Lab. Silva Araújo, Roussel, S.A.

\*\* The triatomids were supplied through the generosity of Professors Aluizio Rosa Prata and Air Colombo Barreto

TABLE I

Xenodiagnosis before and after a single IV inoculation of Prednisolone (steroid group)

Patient	Sex	Age (yrs)	Area	Xenodiagnosis			
				Before steroids		After steroids	
				1	2	3	4
B.A.R.	♀	32	Brotas	N	N	N	N
A.S.C.	♀	52	Brotas	N	N	N	N
M.P.S.	♀	15	Brotas	N	N	N	N
I.A.N.	♂	29	Brotas	N	N	N	N
M.D.S.	♀	30	Brotas	N	N	N	N
C.C.S.	♀	56	Brotas	N	N	N	N
M.J.S.	♀	42	Brotas	N	N	N	N
M.C.	♂	10	Brotas	N	N	N	N
M.P.O.	♀	49	Brotas	N	N	N	N
I.A.F.	♀	12	Brotas	N	N	N	N
C.B.C.	♂	12	T. Mendes	N	N	N	N
A.B.C.	♀	13	T. Mendes	N	N	N	N
M.B.	♀	15	T. Mendes	N	N	N	N
M.S.	♀	15	T. Mendes	N	N	N	N
C.S.	♀	34	T. Mendes	N	N	Pos.	N
H.P.	♂	42	T. Mendes	Pos.	N	Pos.	N
M.F.S.	♂	32	H.P.E.S.	N	N	N	N
O.R.A.	♀	34	H.P.E.S.	N	N	N	N
M.T.S.	♀	30	H.P.E.S.	N	N	N	Pos.

dicated, for instance, that in 68 patients with megaesophagus probably secondary to Chagas' disease, 13 had negative Machado-Guerreiro reactions and of these 3 had positive xenodiagnosis.

The incidence of positive xenodiagnosis in chronic Chagas' disease varies with several factors. In patients from the state of Bahia with positive Machado-Guerreiro

reactions and megaesophagus, PRATA<sup>16</sup> reported 23 per cent positive xenodiagnosis, and in a similar series from the state of Minas Gerais, MARTINS et al.<sup>11</sup> reported 45 per cent positive results.

In an attempt to increase the yield of positive xenodiagnosis, in the present study a single IV dose of prednisolone was used. The deleterious effects of cortisone in some

TABLE II  
Xenodiagnosis before and after IV inoculation of saline (control group)

Patient	Sex	Age (yrs)	Area	Xenodiagnosis			
				Before saline		After saline	
				1	2	3	4
I.L.S.	♀	58	Brotas	N	N	N	N
C.F.C.	♀	27	Brotas	N	N	N	N
J.F.C.	♀	21	Brotas	N	Pos.	Pos.	Pos.
M.A.N.	♀	15	Brotas	N	N	N	N
M.H.S.	♀	15	Brotas	N	N	N	N
M.L.C.	♀	32	Brotas	Pos.	N	Pos.	Pos.
J.B.S.	♀	60	Brotas	N	Pos.	N	N
M.A.C.S.	♀	39	Brotas	N	Pos.	N	N
M.I.A.	♀	30	Brotas	N	N	N	N
R.C.	♂	12	T. Mendes	N	N	N	N
R.B.	♂	13	T. Mendes	N	N	N	N
D.C.	♀	10	T. Mendes	N	N	N	N
A.C.F.	♂	15	T. Mendes	N	N	N	N
J.M.Z.	♂	8	T. Mendes	N	N	N	N
V.S.S.	♂	13	T. Mendes	N	N	N	N
M.S.M.	♀	29	T. Mendes	N	N	N	N
A.N.S.	♀	38	H.P.E.S.	N	N	N	N
D.P.S.	♂	30	H.P.E.S.	N	N	N	N
A.J.R.	♂	43	H.P.E.S.	Pos.	N	N	N

infections are well established, and for this reason only a single injection of cortisone was used. Based on observations in laboratory animals<sup>1, 22</sup>, it was felt that an increase in transient parasitemia might be produced without harming the host. Fifty milligrams of prednisolone was used to give a "pharmacological" blood level for one day<sup>13</sup>.

The number of patients with positive xenodiagnosis in the control group was five

and the number in the steroid group only three. Although two of the three positive results in the steroid group occurred only after the medication, these data do not support a significant cortisone effect. There was however a striking increase in the number of patients with positive results when a second xenodiagnosis was performed (from three to six). From the second test on, additional xenodiagnosis increased only very

little the number of patients with positive results (only two new positive results, in the third and fourth test), but confirmed previous positives in three patients. There was a difference in the total number of positive xenodiagnosis in the steroid group (3 cases in 19, or 15%) as compared to the control group (5 cases in 19, or 26.3%). This difference cannot be explained by obvious population bias since sex distribution was similar and the mean and modal ages of both groups were about the same.

Possible reasons for failure to see a steroid effect in the present study are innumerable. A higher and sustained blood level of steroids might be needed to influence the xenodiagnosis. The time sequence of steroid injection and xenodiagnosis might have missed any transient parasitemia. Furthermore, a second examination of the triatomid could have been performed after 60 days<sup>13</sup>.

It is of interest that the number of positive xenodiagnosis in the total series was 21 per cent, similar to others reported in the literature<sup>10, 11</sup>.

#### RESUMO

##### *Xenodiagnóstico repetido na moléstia de Chagas crônica: efeito de uma única injeção de Prednisolona.*

1) Em 38 pacientes apresentando a forma crônica ou indeterminada da doença de Chagas, todos com reação de Machado-Guerreiro positiva, a positividade do xenodiagnóstico foi de 20%.

2) A repetição do xenodiagnóstico, cerca de uma semana após o primeiro, resultou em aumento acentuado do índice de positividade; a realização de um terceiro, e um quarto xenodiagnósticos, no mesmo doente, aumentou muito pouco o número de resultados positivos.

3) A aplicação de 50 mg de prednisolona, por via intravenosa, não pareceu aumentar significativamente a positividade de xenodiagnóstico nestes pacientes com doença de Chagas. Estudos adicionais, entretanto, serão necessários para uma conclusão mais definitiva sobre o efeito de corticóides sobre a parasitemia nesta condição.

#### REFERENCES

1. AGOSIN, M. — Cortisona y enfermedad de Chagas experimental. *Biológica* 14:29-54, 1951.
2. BOTAFOGO, G. N. — Ação de corticosteróides sobre a infecção experimental pelo *T. cruzi*. *An. Cong. Intern. sobre doença de Chagas*. Rio de Janeiro, 25, 1959.
3. BRENER, Z. — *Contribuição ao estudo de terapêutica experimental da doença de Chagas*. Belo Horizonte, 1961.
4. BRENER, Z. & CHIARI, E. — Observações sobre a fase crônica da doença de Chagas experimental no camundongo. *Rev. Inst. Med. trop. São Paulo* 5:128-132, 1963.
5. FREITAS, J. L. P. — Observações sobre o tempo ótimo para exame de triatomídeos empregados em xenodiagnóstico. *Folia Clin. Biol.* (São Paulo) 16:180-185, 1950.
6. FREITAS, J. L. P. — Reação de fixação do complemento para diagnóstico de moléstia de Chagas pela técnica quantitativa. *Arq. Hig.* (São Paulo) 16:55-94, 1951.
7. GOBLE, F. C. — Experimental therapeutics of Chagas' disease. *An. Cong. Intern. sobre doença de Chagas*. Rio de Janeiro, 613-633, 1961.
8. GONÇALVES, B.; CARMO, A. & TAVARES, I. — Chemotherapy of Chagas' disease. *Abstr. 6th Intern. Cong. Trop. Med. & Malária* (Lisboa), p. 76, 1958.
9. JARPA, A.; AGOSIN, M.; CHRISTEN, R. & ATIAS, V. — Ensayos de quimioterapia de la enfermedad de Chagas. *Biol. Inform. Parasit. Chilenas* 6:25-27, 1951.
10. LARANJA, F. S.; DIAS, E.; NOBREGA, G. & MIRANDA, A. — Chagas' disease. A clinical, epidemiological and pathological study. *Circulation* 14:1035-1060, 1956.
11. MARTINS, A. V.; VERSIANI, V. & TUPI-NAMBÁ, A. A. — Estudo sobre a moléstia de Chagas no Estado de Minas Gerais. II — Sobre 156 xenodiagnósticos feitos em Belo Horizonte. *Arq. Inst. Químico Biol. Estado de Minas Gerais* 1:63-70, 1945.
12. NEGhme, A. — Acción de la cortisona sobre la trypanosomiasis chagastica experimental del ratón suprarrenal. *Bol. Inform. Parasit. Chilenas* 7:4-6, 1952.
13. PETERSON, R. E. — Adrenocortical steroid metabolism and adrenal cortical function in liver disease. *J. Clin. Invest.* 39: 320-331, 1960.
14. PIFANO, F. C. — Evaluation de los procedimientos de laboratorio diagnóstico de

- la enfermedad de Chagas. *Bol. Ofic. Sanit. Panamer.* 44:563-571, 1960.
15. PIZZI, T. P. — Acción de la cortisona sobre la infección experimental de la rata por *T. cruzi*. *Biológica* 21:31-58, 1955.
16. PRATA, A. — Relação etiológica entre doença de Chagas e megacófago. *An. Cong. Intern. sobre doença de Chagas*, 1317-1336, 1959.
17. REPORT OF THE ADVISORY GROUP ON MEDICAL RESEARCH IN CHAGAS' DISEASE. PAN AMERICAN HEALTH ORGANIZATION — ADVISORY COMMITTEE ON MEDICAL RESEARCH. Washington, D.C., 12 June 1962.
18. ROBLES, G. J. & PERRIN, M. — Nota preliminar del estudio experimental sobre la acción de la hormona adrenocorticotrópica de la hipófisis en la enfermedad de Chagas. *Arch. Inst. Cardiol. Mex.* 20:314-326, 1950.
19. ROBLES, G.; PERRIN, J. & BALCAZAR, J. — Actions of ACTH hormone in Chagas' disease. An experimental study. *Proc. 2nd Clin. ACTH Conf.* 1:468-477, 1951.
20. RUBIO, M. D. — Estudio de los factores que intervienen en la virulencia de una cepa de *T. cruzi*. I — Acción de la cortisona en la capacidad de invasión y multiplicación del parásito. *Biológica* 20:89-125, 1954.
21. RUBIO, M. D. — Influencia del acetato de cortisona sobre la virulencia y localización tisular de una nueva cepa de *T. cruzi*. Estudio de la persistencia de los cambios observados. *Biológica* 21:75-89, 1955.
22. SENECA, H. — Fatal T.C. infections in white baby rats with cortisone. *Science* 116:14-16, 1952.
23. SENECA, H. & IDES, D. — The effect of exysteroids en *T. cruzi* infections in mice. *Amer. J. Trop. Med. & Hyg.* 4:833-836, 1955.
24. THIERMANN, I. & CHRISTEN, R. A. — Influencia del acetato de cortisona y de la aureomicina como agente agravador de la infección chagásica atenuada. *Bol. Inform. Parasit. Chilenas* 7:53-55, 1952.

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