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# CROSSING OVER IMMUNOELECTROPHORESIS APPLIED TO THE STUDY OF IMMUNOLOGY OF SOUTH AMERICAN BLASTOMYCOSIS — PREVIOUS NOTE *

WILLIAM BARBOSA ** EDITH BLAU *** JOANA ROSA MENDONÇA **** RAQUEL LOPES DE OLIVEIRA ****


## SUMMARY

Crossing over immunoelectrophoresis was used for detection of antibodies of P. brasiliensis in 31 patients with several clinical forms of South American blastomycosis. Positive results were obtained in 24 of these cases. (77,4% positivity). Furthermore it was observed that precipitins were not detected in all serial serum specimens investigated and that its presence or absence could not be correlated with the period of the disease, treatment or clinical evolution. Further studies are necessary to assess the value of the method in the study of this disease.


## INTRODUCTION

The results obtained with an antigen prepared from cells of the yeast phase of P. brasiliensis in the study of immunology of South American blastomycosis by the double gel diffusion technique and the observation that fractions of this antigen travel electrophoretically to the anode, have led us to use the crossing over immunoelectrophoretic technique for the detection of antibodies in that mycosis.

Previously we have used the same technique for the detection of Australia antigen and for the study of circulating antigens in human malaria. As it is known, this technique has recently gained widespread acceptance for detecting a hepatitis — associated antigen. [1, 2, 3]

## MATERIALS AND METHODS

Serial serum specimens were obtained from 31 patients with blastomycosis who were hospitalized at the Department of Tropical Medicine of the Instituto de Patologia Tropical, Federal University of Goiás and remained under observation during the

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** Professor — Department of Tropical Medicine (IPT) — Federal University of Goiás.
*** Assistant Professor — Department of Pathology (IPT) — Federal University of Goiás.
**** Technical Assistants — IPT — UFGo.

years 1971 and 1972. Diagnosis was based on the finding of the parasite. The clinical course and some immunologic aspects were extensively investigated. [1]

The antigen was obtained by freezing and thawing cultures of P. brasiliensis isolated from patients with South American blastomycosis according to the method previously described. [1]

A buffered solution containing 50 mg of the lyophilized parasite per milliliter was used.

Three inches by one inch microscope slides were covered with three milliliters of a 1% buffered solution (barbital buffer, pH 8,6). After the agar had solidified, 12 wells (3 mm diameter) were punched out. The distance between the antigen and antibody wells was about 3 mm. The wells were completely filled with about 0,05 ml antigen and serum samples, using capillaries. Antigen was placed in the hole of the cathode and serum in the hole of the anode. Undiluted sera were tested in all the cases; besides, some sera were used at threefold concentration and, in some samples, dilutions from 1:4 to 1:16 were tried.

Electrophoresis was performed in a Shandon chamber. 220 volts were applied, the power supply was adjusted to deliver 15 ma. per slide. Time of electrophoresis — 20 minutes. Appearance of precipitin lines was checked immediately after electrophoresis and confirmed after washing and staining the slides. (Fig. 1)

## RESULTS

Sera from 31 patients were tested, 24 of which gave positive results (77,4% positivity). 167 samples of the 31 sera of these patients were investigated, of which 85 gave positive results and 82 negative results — two samples of the latter, after threefold concentration turned positive with the appearance of one precipitin line.

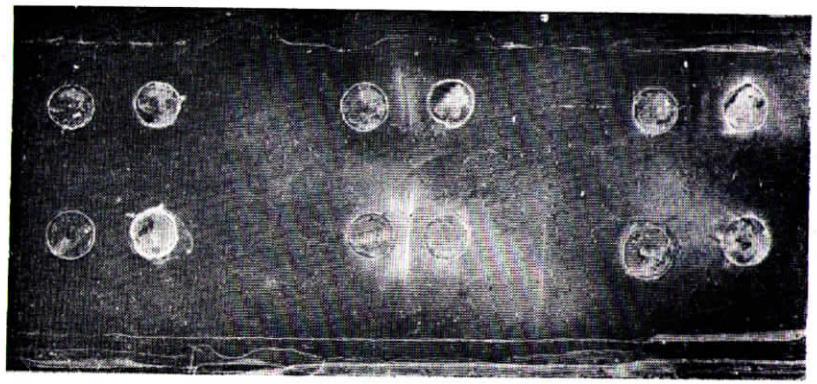
As to sensitivity, it was shown that dilutions up to 1:16 gave precipitin lines.

When the results of crossing over immunoelectrophoresis of all sera were compared (3 to 16 samples — with an average of 6 samples), it was not possible to correlate the value of the reaction with the clinical course of the disease.

Of the 7 negative results — 4 were observed in patients with chronic disseminated lymphatic cutaneous visceral (pulmonary) form; one patient with chronic disseminated lymphatic visceral (intestinal) form who was clinically cured; 1 patient with chronic disseminated lymphatic cutaneous form and one patient with acute dissseminated form who died during the first month of hospitalization.

No absolute correlation was found between the results of crossing over immunoelectrophoresis and double gel immunodiffusion. Some sera gave positive results in one or another of the samples, although, in some cases where the reactions were repeated after diluting or concentrating the sera for crossing over immunoelectrophoresis, sometimes the reactions became positive. We don't know why these discrepancies occur, however we believe that the presence of lower concentrations of antibodies may be more easily detected by crossing over immunoelectrophoresis.

FIGURA 1





## DISCUSSION

The possibility of adapting crossing over immunoelectrophoresis to the study of South American blastomycosis may increase the speed of diagnosis of this mycosis by the indirect technique, as the time required for one determination is not more than 30 minutes. Thus this method may be employed as a screening technique in epidemiological surveys and in the diagnosis of these patients.

Although no specificity tests of the reaction were repeated, in view of previous results with double immunodifusion, we believe that the method is fairly specific. The fact that antibodies may be detected in dilutions up to 1:16 shows that its sensitivity is as good or better than that of immunodiffusion.

The appearance of secondary precipitin lines possibly corresponds to several antigenic components.

## RESUMO


### IMUNOELETROFORESE POR "CROSSING-OVER" APLICADA AO ESTUDO DA BLASTOMICOSE SUL-AMERICANA — NOTA PRÉVIA

Os autores empregaram a técnica de crossing over imunoeletroforese para pesquisa de anticorpos de P. brasiliensis em 31 pacientes de diversas formas clínicas de blastomicose sul americana. Constataram resultados positivos em 24 dos 31 soros examinados (77,4%). Observaram, ain-

da, que no decurso evolutivo da doença as precipitinas não foram detectadas em todas as amostras estudadas e que sua presença ou ausência, aparentemente, não apresentava correlação com o período da doença, o tratamento, sequer com a evolução clínica. Admitem que um estudo mais apurado deva ser ensaiado para que os resultados permitam situar definitivamente o valor do método no estudo desta doença.

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