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ANTIGENEMIA IN PARACOCCIDIOIDOMYCOSIS. PROBABLE DEMONSTRATION OF CIRCULATING ANTIGEN BY COUNTERIMMUNOELECTROPHORESIS TEST

PRELIMINARY REPORT (1)

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Paracoccidioides brasiliensis presents as other dimorphic fungi a very complex antigenic structure. **In vitro** studies of STANDARD & KAUFMAN [17], and KAUFMAN et al. [6] on mycelial form cultures of that fungus demonstrate the production of three specific exoantigens designated 1, 2 and 3, able to react with precipitin-positive sera of patients with paracoccidioidomycosis. Peptide-polysaccharides containing mainly galactomannan were isolated from culture medium of the yeast form of **P. brasiliensis** (RODRIGUEZ V. et al. [15]). By the application of immunoenzymatic techniques and electron microscopy study, CAMPO-AASEN et al. [2] have shown the production and localization of the antigen E_2 in yeast cells of **P. brasiliensis.** This antigen seems to be an important factor in the modulation of the host immune response. Circulating antigens have been detected in several experimental and human mycoses, as in cases of disseminated candidiasis (MILLER et al. [9], WEINER & YOUNT [20], WARREN et al. [18], RIPPON & ANDERSON [14], SEGAL et al. [16], MECKSTROTH et al. [8], NEGRONI et al. [10]), and in invasive aspergillosis (LEHMAN & REISS [7], REISS & LEHMAN [12], RICHARDSON et al. [13], WEINER [19]). In the diagnosis of cryptococcosis, the detection of **C. neoformans** capsular polysaccharide antigen in body fluids is considered a useful routine test (KAUFMAN & BLUMER [5], KAUFMAN [4]). It is valuable for starting specific therapy, when positive, even before the fungus is detected. Circulating immune complexes were detected and characterized in coccidioidomycosis (YOSHINOYA et al. [21]), and in cases of paracoccidioidomycosis (RAMOS e SILVA et al. [11], ARANGO et al. [1], CARVALHO et al. [3]). Indirectly, the presence of these immune complexes demonstrates the occurrence of circulating antigens in those mycoses.

In the evaluation of results of immunodiffusion test for demonstration of antibodies in sera from suspect cases of paracoccidioidomycosis we have verified the presence of precipitin lines at different positions as compared with those usually seen with precipitin-positive human sera. Some sera placed in the peripheral wells of the agar gel immunodiffusion slides with 2 seven-well patterns produced precipitin lines with the adjacent peripheral well. Apparently inespecific, these lines were not changed by wash in sodium citrate. This fact, with the additional presence of identity lines with the **P. brasiliensis** yeast form culture filtrate antigen placed in the center well suggested the presence of probable antigen in some of the studied sera.

In the last few years, counterimmunoelectrophoresis test has been widely used in the serodiagnosis of systemic mycoses. Once standardized in our Laboratory, it has been a routine test in mycoserology. Due to its sensitivity, specificity and prognostic value, it was chosen as a reliable method for the present study. Proper 6 three-well patterns were used for the arrangement of the sera, in a trial to study the simultaneous presence of antibodies and antigens. Control antisera yet in preparation were replaced by precipitin-positive sera from patients with paracoccidioidomycosis, as mentioned in studies on other mycoses (WEINER & YOUNT [20], STANDARD & KAUFMAN [17], KAUFMAN et al. [6]). The results of the counterimmunoelectrophoresis correlated with those initially mentioned in the immunodiffusion test. The presence of precipitin lines has shown the occurrence of an

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anodic migration from the sera in study. This fact emphasizes the probable presence of antigen in the sera, as also suggested by the identity lines we have mentioned concerning the initial results of the immunodiffusion test (Fig. 1).

At the present we have studied ten samples of sera showing probable presence of antigen, one of which seemed to present antigen and antibody as reacting with other sera.

Antibody response do not always reflect active disease; they are variable and may be absent in certain compromised patients (KAUFMAN [4]). Although our study is yet in an early stage, next step is the examination of negative sera from patients with clinically suspect relapse or active disease. High titer precipitin-positive sera were used as control antisera. From six negative sera studied, one has reacted as antigen with five of nine positive sera.

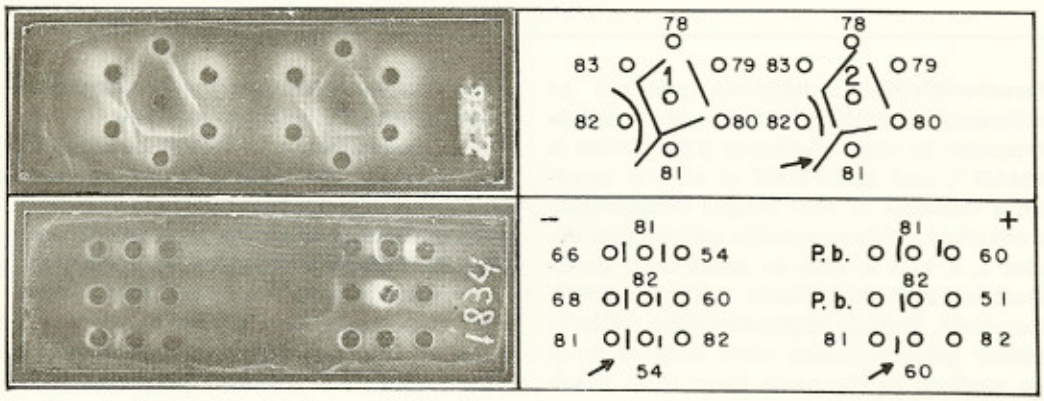

Fig. 1 — Serological tests showing precipitation lines between sera from patients with paracoccidioidomycosis (arrows) suggestive of presence of soluble antigen. A) Immunodiffusion test: 1. **P. brasiliensis** culture filtrate antigen; 2. **P. brasiliensis** antigen diluted at 1:4; 78 to 83: patients sera. B) Counterimmunoelectrophoresis; 51 to 83: patients sera.

We did not establish yet the correlation of the probable presence of circulating antigen and the patients clinical data, forms of disease and therapy. Further studies with the addition of polyetilenoglycol 6000 (PEG) to the agar or agarose gel in order to improve the sensitivity of precipitin tests (ZAROR et al. [22]) will be performed. The immunochemical characterization of the supposed antigen also must be accomplished by proper methods. We consider counterimmunoelectrophoresis test to be a reliable and useful method also for the detection of circulating antigen in paracoccidioidomycosis.

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