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HUMAN PRECIPITINS AGAINST A MICRO-ORGANISM (*CANDIDA ALBICANS*) DEMONSTRATED BY MEANS OF QUANTITATIVE IMMUNOELECTROPHORESIS

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SUMMARY

By means of the antigen–antibody crossed electrophoresis of Laurell twenty-two different *Candida albicans* precipitins were demonstrated in a serum from a 56-year-old woman suffering from idiopathic hypoparathyroidism and chronic candidiasis. This precipitin response is remarkably strong, but a detailed evaluation of the finding must await further investigations.

It is mentioned how the human precipitins may be quantitated and the titres expressed in arbitrary rabbit precipitin units.

The antigen–antibody crossed electrophoresis may be useful in other infections, and possibly in certain autoimmune diseases, in order quantitatively to screen patient sera for a multitude of precipitins without purification of the individual antigens.

INTRODUCTION

By means of rabbit antisera the antigen–antibody crossed electrophoresis (Laurell, 1965) has been used in quantitative studies of serum proteins (Clarke & Freeman, 1968; Weeke, 1970). However, the purpose of study may be reversed, and the method used for detection and quantitation of precipitins by means of a standard mixture of antigens. In the present article the method was used in order to demonstrate *Candida albicans* precipitins in a human serum.

From a clinical point of view the study of human precipitins against *Candida albicans* may be justified, since the diagnostic value of some Candida precipitins has been shown to be high in systemic candidiasis (Taschdjian *et al.*, 1969). Furthermore precipitin-mediated reactions may be clinically relevant in some cases of asthma and pulmonary eosinophilia (Pepys *et al.*, 1968).

From an immunological point of view it might be of interest to study the specificity and titre of Candida precipitins in cases of chronic mucocutaneous candidiasis with and without cellular immune defects.

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MATERIALS AND METHODS

Serum was obtained from a 56-year-old woman suffering from idiopathic hypoparathyroidism and chronic candidiasis. The case has been reported earlier (Svane-Knudsen, 1959). The patient has a positive Mantoux reaction.

The strain of *Candida albicans A* and the antigen preparation procedure will be described elsewhere (Axelsen, in preparation).

The immunoelectrophoreses were run according to Laurell (1965) using 1% agarose gel (Litex, Glostrup, Denmark) in barbital buffer pH 8·6 and ionic strength 0·02. The first dimension electrophoresis of 1 and 10 μl Candida antigen were run for 50 min at 10 V per cm. The second dimension electrophoreses were run for 22 hr applying 3 V per cm. The second dimension gel contained 10% patient serum (10 μl per cm^2). Thickness of gel: 1 mm,

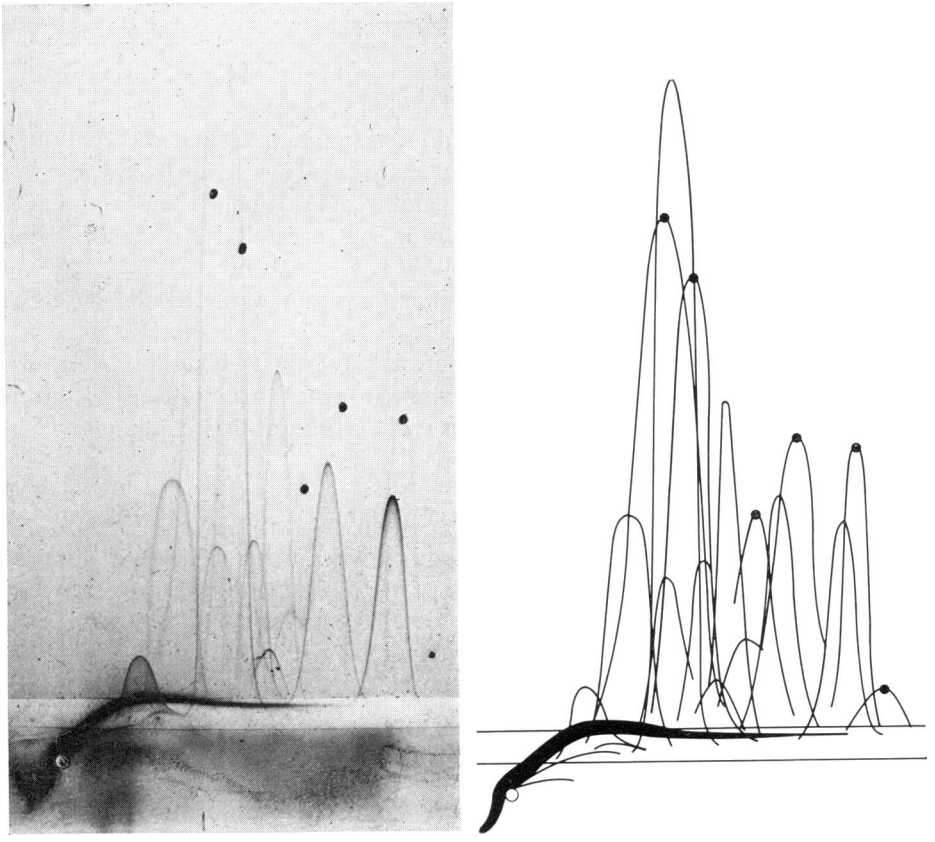

FIG. 1. Antigen–antibody crossed electrophoresis. The *Candida albicans* antigens were electrophoretically separated (1. dimension electrophoresis, anode to the right) and subsequently forced through a gel containing serum from the patient (2. dimension electrophoresis, anode at the top). The dots indicate peaks of weak precipitates. Nineteen precipitates could be counted, (see drawing, right). Dimension of the plate: 7·6 × 13·8 cm. Staining: Coomassie brilliant blue R, Michrome. (Drawing (right) of the antigen–antibody crossed electrophoresis of left figure).

dimension of the plates: 10 × 20 cm. After the run the plates were washed, dried and stained with Coomassie brilliant blue R (Michrome).

RESULTS

By using 1 μl of the Candida antigen for the immunoelectrophoresis nineteen precipitins could be demonstrated in the patient serum (see the Fig. 1). Three additional precipitins were revealed in another immunoplate using 10 μl Candida antigen instead of one μl. Thus a total number of twenty-two precipitins were demonstrated in the patient serum.

DISCUSSION

The area limited by a precipitate is in this method inversely proportional to the precipitin titre: The area is small if the precipitin titre is high, and vice versa. Therefore the method may be used for quantitation of a multitude of precipitins in one immunoelectrophoretic run. A precipitin titre may be expressed in arbitrary rabbit antibody units if the area obtained with the human serum is compared to the corresponding area of standard immunoelectrophoreses with rabbit antibodies.

The number of human precipitins against *Candida albicans* demonstrated in this study is considerable, as previously seven precipitins seem to be the highest number demonstrated in a human serum (Coudert *et al.*, 1968). However, the antigen preparations, the immunochemical techniques, and probably also the patients are not comparable.

A detailed clinical evaluation of the finding in this patient must await further investigations of sera from normal persons and patients suffering from various forms of candidiasis. However, it can be said that the precipitin response was very strong, as several rabbits injected intracutaneously with the Candida antigen, suspended in Freund's incomplete adjuvant, in this laboratory have had precipitin responses equal to that of the patient.

The demonstration of human precipitins with the antigen–antibody crossed electrophoresis may be useful in other infections and possibly in certain autoimmune diseases, in order quantitatively to screen patient sera for a multitude of precipitins in a few immunoelectrophoretic runs. The main advantage being that precipitin titres may be determined without purification of individual antigens.

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