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Isolation of Vaccinial Immunoprecipitinogens*

By

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With 6 Figures

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Introduction

The occurrence of a multiplicity of vaccinial antigens is readily demonstrable by means of e.g. diffusion-in-gel methods (Gispen, 1955; Rondle and Dumbell, 1962; Appleyard and Westwood, 1964). Using preparations of vaccinia infected rabbit skin the present authors detected nine different vaccinial immunoprecipitinogens in material of so-called soluble antigens (Marquardt et al., 1965a). The present study describes the results obtained when separation and concentration of the different vaccinial immunoprecipitinogens were attempted; precipitation with ammonium sulfate, isoelectric precipitation and molecular exclusion chromatography were performed.

Materials and Methods

Production of soluble antigens. The production of soluble antigens by infection of rabbits with a rabbit skin-adapted strain of vaccinia virus has been described elsewhere (Marquardt et al., 1965b).

The diffusion-in-gel method. Double diffusion technique was employed using the modification introduced by Holm (1965). Ultrasonic treatment was performed on all preparations before testing. Photographic registration was made by means of Polaroid panchromatic film reproduction (Wadsworth, 1963). In all tests a reference immune system consisting of a batch of soluble antigens prepared from vaccinia infected rabbit skin (VIRS) and a vaccinia hyperimmune serum (K 225) were included to allow comparative analysis. For evidence of vaccinial specificity of the studied precipitinogens and precipitins see Marquardt et al. (1965b). Of the vaccinial precipitats studied, which were designated aA to hH, the eE-jF precipitates consisted of three lines

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developing close together, sometimes in a super-imposed position. The $dD$ precipitate formed in close connection with the $eE$-$fF$ complex of precipitates. The $aA$, $bB$ and $cC$ precipitates were found near the antiserum basin while the $gG$ and $hH$ precipitation lines were observed on the antigen basin side of the spectrum. A diagrammatic representation of the reference immune system is given in Fig. 3. In the tables the relative concentration of a particular factor, e.g. $a$ is graded "$a$", "($a$)" and "traces of $a$". The first designation indicating that the immunoprecipitate was distinct and easily detectable while "($a$)" means a faint precipitate and precipitates designated as "traces of $a$" were distinguishable only as a deviation of lines from the reference system towards the basin containing the test material.

Tests for presence of antigenic material of non-vaccinia origin in material of soluble antigens were also performed by means of immunodiffusion tests. These were done with a monkey anti-rabbit skin serum prepared by hyper-immunization of a Cynomolgus monkey with material of normal not vaccinia infected rabbit skin prepared essentially according to the method employed for the preparation of VIRS.$^1$

**Gel-filtration.** The gel-filtration on Sephadex G-100 or G-200 was performed in recycling columns (2.5 x 85 cm.) of the type described by Porath and Bennich (1962). Sample loads were kept at about 1% of the total column volume. Flow rates were regulated to 1 or 1.5 ml./h/cm$^2$. for G-200 and twice these speeds for G-100. The buffer consisted of 0.05 M sodium phosphate pH 7.0 with addition of 0.5 M NaCl in order to avoid aggregation during the separation. All experiments were performed at 3--6°C. Optical density of the fractions was read at 260 or 280 m$\mu$ on a Beckman DB spectrophotometer.

**Experiments and Results**

**Precipitation with Ammonium Sulfate**

Ammonium sulfate was added to 1.5 ml. aliquots of soluble vaccinia antigens so that levels of $(NH_4)_2SO_4$ saturation from 15 to 70% were obtained. The aliquots were then placed at 4°C overnight and centrifugated at 27,000 g for 20 min. The sediments were taken up in 1.5 ml. of a 0.05 M Tris buffer, pH 7.5, and dialysed against the same buffer. The supernatants were dialysed against Tris buffer and concentrated by dialysis against Carbowax 6000. The final volume of the supernatants was 0.5 ml. Finally, the preparations were tested for content of proteins and precipitinogenic factors.

Fifteen to 25% saturation with $(NH_4)_2SO_4$ gave sediments which contained traces only of immunoprecipitinogens. The results obtained with 30, 50 and 70% saturation, respectively, are listed in Table 1. Saturation to 30% of $(NH_4)_2SO_4$ precipitated most of the immunoprecipitinogens leaving mainly factor $d$ and the components of the $e$-$f$ antigen complex in solution. A further increase of the $(NH_4)_2SO_4$ concentration to a saturation of 50 or 70% left factor $d$ alone in a demonstrable amount. The supernatant obtained after removal of material precipitated with 70% $(NH_4)_2SO_4$ saturation contained, besides factor $d$, traces only, of one of the $e$-$f$ antigens.