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APPLICATION OF ELECTROIMMUNODIFFUSION TEST FOR DETECTION OF ANTIGENIC RELATIONSHIP BETWEEN SHEEPPOX AND GOATPOX VIRUSES

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Summary. — Electroimmunodiffusion (EID) test was adapted to detect the antigenic relationship between sheeppox and goatpox viruses. Seven and five precipitation peaks were detected with sheeppox and goatpox homologous antigen — antibody system, respectively. The cross reactions revealed a serological relationship between the two viruses in three common antigens.

Key words: *poxviruses*; *antigenic relationship*; *electroimmunodiffusion test*

Soluble antigens of poxviruses have generally been studied by immunodiffusion and immunoelectrophoretic tests (Wilcox and Cohen, 1969; Pandey and Singh, 1972). Electroimmunodiffusion (EID) test (Laurell, 1966; Merril *et al.*, 1967) was judged superior to immunodiffusion in its resolution capabilities and to detect antigenic relationships among microorganisms (Sweet *et al.*, 1973, 1979). The resolution of antigens in EID is based on the charge differences of the antigen-antibody complex rather than on the charge differences of the antigens alone as it is the case in immunoelectrophoresis test. However, the ability of EID test to determine the antigenic relationships among poxviruses has not been explored. The present report describes the application of EID test for determining antigenic relationship between sheepox and goatpox viruses.

The "Ranipet" strain of sheeppox virus and "Poona" strain of goatpox virus used in this study were described previously (Subba Rao and Malik, 1979). Sheeppox and goatpox virus soluble antigens were prepared from skin scabs harvested on 8th to 10th day of animal inoculation. Twenty per cent (w/v) scab suspensions in 0.01 mol/l phosphate buffered saline (PBS) pH 7.2 were centrifuged at 1,500 rev/min for 10 min to remove cell debris. This was followed by centrifugation at 18,400 rev/min (International centrifuge, Model, PR-2, with multispeed attachment) for 30 min at 4 °C. The supernatants were concentrated ten-fold by dialyzing against polyethylene glycol 20 M (Kohn, 1959) and used as soluble antigens.

Antiserum against sheeppox virus was prepared in three young healthy rabbits. The rabbits were given eight consecutive intramuscular injections at weekly intervals. Each injection con-

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sisted of 0.5 ml of 20% (w/v) sheeppox scab suspension emulsified with an equal volume of Freund's complete adjuvant. The rabbits were bled one week after last injection. Antiserum to goatpox virus was obtained similarly in rabbits. The antisera were adequately absorbed with uninfected sheep and/or goat skin antigens to remove the antibodies against normal host skin antigens (Rodriguez-Burgeos *et al.*, 1966). The pooled absorbed antisera were stored at 4 °C in small aliquote after adding merthiolate (1 : 10,000) until used.

EID test, as described by Sweet *et al.*, (1973), was performed on 75 × 25 nm glass slides overlaid with 2 ml of agar-antiserum mixture. Agar gel contained 1% Bacto-agar (Difco) in sodium veronal buffer of pH 8.2 and ionicity 0.05. The antigen was placed in a 3 mm diameter well located at a distance of 1.5 cm from the cathodic end of the slide, and electrophoresed in the presence of sodium veronal buffer at 150 volts at 4 °C. When two antigens were to be compared they were placed in wells 1 mm apart in a lie perpendicular to the direction of migration. Finally, the slides were washed, dried and stained with amidoblack 10B.

EID test was standardized using sheeppox soluble antigen-antibody system. For standardizing the test, the amount of antiserum to be incorporated in the agar-antiserum mixture and the duration of electrophoretic run were determined. Various amounts of sheeppox antiserum, namely 0.1, 0.2 and 0.3 ml were separately incorporated in 2 ml of agar gel and each slide was overlaid with 2 ml of agar-antiserum mixture. Sheeppox virus soluble antigen was electrophoresed into agar-antiserum mixture for varying periods of time viz. 4, 6 and 12 hr. In these tests, it was observed that the slides prepared with antiserum-agar mixture containing 0.1 and 0.2 ml of sheeppox antiserum did not result in proper precipitation peaks and the migration of peaks was very poor at any duration of the electrophoretic runs tested. The agar gel containing 0.3 ml of antiserum also resulted in poor heights of precipitation peaks when the electrophoretic run was only for 4 hr. However, the precipitation pattern resolved into at least seven identifiable peaks when the electrophoresis was carried out for 6 hr. Because the resolution of the peaks was considered unsatisfactory due to poor heights and fusion of peaks at the top of the precipitation pattern, the duration of the electrophoretic run was increased to 12 hr. This resulted in an adequate resolution of the peaks, but in a comparatively fainter precipitation reaction than at 6 hr, probably due to dilution of the antigen during electrophoretic run. Hence, in the study that followed, electrophoresis was carried out for 12 hr incorporating 0.3 ml of sheeppox antiserum in each agar slide. Still higher concentration of antiserum in agar gel was not tried.

The antigenic relationship between sheeppox and goatpox viruses was determined by electrophoresing the two antigens simultaneously into agar gel containing sheeppox antiserum. The EID pattern revelaed that sheeppox soluble antigen produced at least seven precipitation peaks. This was also confirmed by the test conducted with sheeppox soluble antigen alone. Goatpox soluble antigen produced three peaks with sheeppox antiserum. The test was also performed by electrophoresing the two antigens into the agar gel containing goatpox antiserum. The EID pattern (Fig. 1) obtained with goatpox antiserum revelaed five precipitation peaks with goatpox antigen and three common precipitation peaks with sheeppox antigen. However, some of the precipitation peaks were of weaker intensity. These observations clearly showed a relationship between sheeppox and goatpox viruses in three common antigens. Similar results were observed in cross reactions of sheeppox and goatpox antigens with their antisera in immunodiffusion and immunoelectrophoretic tests (Subba Rao, 1979). However, the results observed in EID test are more clear-cut than those with immunodiffusion and immunoelectrophoretic tests, because immunodiffusion showed considerable overlapping of the precipitation lines and the margining of the lines was not very clear. In immunoelectrophoretic test, it was not possible to establish any relationship as the nature of the test does not permit margining of identical lines with each other. Thus the results of the present study suggest the superiority of EID test over immunodiffusion and immunoelectrophoretic tests in establishing the relationship between sheeppox and goatpox viruses.

It was also observed that the peaks formed by heterologous antigen-antibody reactions were of less height than those of homologous reactions. These results suggest possible quantitative differences among the antigens shared by the two viruses. Though there is a quantitative aspect to EID test, in that the height of precipitation peak is proportional to the antigen concentration (Laurell, 1966; Merril *et al.*, 1967; Sweet *et al.*, 1973), it is difficult to conclude at this stage on these quantitative differences since the antigen concentrations employed in the present study were not standardized in terms of their protein content.

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Explanation of the Figure (Plate XXIV):

Fig. 1. Precipitation peaks in the EID test. Well 1 — goatpox antigen; well 2 — sheeppox antigen; agar gel contained hyperimmune serum to goatpox virus.