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CHICKENPOX AND HERPES ZOSTER

II. OUCHTERLONY PRECIPITATION STUDIES

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THE gel-diffusion precipitation technique has not hitherto been used to study the relationship of chickenpox and zoster viruses, although the methods described by Oudin (1948) and Ouchterlony (1948) have been widely used in virus studies. Thus Jensen and Francis (1953) were the first to use both methods to indicate immunological differences and relationships between several strains of influenza virus and more recently Datt and Orlans (1958) found that a minor antigenic constituent common to both vaccinia and pig pox virus could be demonstrated only by agar diffusion techniques.

It seemed that the Ouchterlony agar diffusion technique might be of value in augmenting the results obtained by complement fixation in serological studies of chickenpox and zoster antisera and antigens (Taylor-Robinson and Downie, 1959). The results of such an investigation are given in this paper.

MATERIALS AND METHODS

Antigens.—Vesicle fluids from chickenpox and zoster patients were diluted at the time of collection from $\frac{3}{5}$ to $\frac{1}{5}$ with either phosphate-buffered saline, Hanks' solution, or a fluid containing 10 per cent horse serum, 10 per cent tryptic digest broth and 80 per cent Hanks' solution. The vesicle fluids were diluted in this way so that if required they could be used for tissue culture experiments. The tissue culture fluids were supernatant media from amnion cell cultures infected with chickenpox and zoster viruses. All fluids were stored at —70° in hard-glass tubes. Concentration of fluids of 5- and 25-fold was achieved by drying from the frozen state and reconstitution of the dried material in an appropriate volume of distilled water. Some fluids were freed from salt by dialysis against distilled water before concentration.

Sera.—The sera were those obtained from cases of chickenpox and zoster and previously used in complement-fixation tests (Taylor-Robinson and Downie, 1959). They were stored at —20° in hard-glass tubes and were not inactivated or diluted before use. In several indicated cases chickenpox sera concentrated fivefold were used. Sera to be concentrated were freed from lipid by centrifugation for 30 min. at 3,000 r.p.m., dried from the frozen state, and reconstituted in an appropriate volume of water.

Agar-diffusion technique.—The technique of Crumpton and Davies (1956) was followed except that the reservoirs were 5 mm. in diameter and their centres 8 mm. apart; they were punched out with a cork borer.

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RESULTS

Experiments using Vesicle Fluids as Antigen

Zoster vesicle fluid and zoster sera

Fig. 1 shows the reaction between 6 convalescent zoster sera and a zoster vesicle fluid. The lines of precipitation were discernible at 18 hr. and clearly visible after 48 hr. It can be seen that the lines of precipitation in one antigen-antibody system are contiguous with those of the neighbouring systems and that in at least 2 cases a minimum of 3 components can be identified. Similar line patterns were given by 21 convalescent zoster sera that were tested.

Sera collected from zoster cases during the first week of the rash were similarly tested against zoster vesicle fluid. Of 22 acute stage sera, 11 gave lines of precipitation, a two-component system being found in 3 cases and a one-component system in the other 8 cases.

Chickenpox vesicle fluid and zoster sera

Convalescent zoster sera gave lines of precipitation when tested also against a suitable chickenpox vesicle fluid and the reaction of chickenpox and zoster vesicle fluids with such sera is shown in Fig. 2. It can be seen that the lines of precipitation formed between convalescent zoster sera and the chickenpox vesicle fluid are continuous with those formed between these sera and zoster vesicle fluid. This finding suggests that with respect to convalescent zoster sera, chickenpox vesicle fluid contains the same antigens as zoster vesicle fluid. Four reservoirs in the experiment shown in Fig. 2 contained serum collected from 2 cases of chickenpox 11 weeks after onset; the sera failed to react with either antigen.

Vesicle fluids and convalescent chickenpox sera

In addition to the 2 chickenpox sera shown in Fig. 2, 21 convalescent chickenpox sera (collected between 22 and 25 days after the onset of the rash) were tested with zoster vesicle fluid. Lines of precipitation did not form. Chickenpox vesicle fluid was not available in sufficient quantity to test all the convalescent chickenpox

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EXPLANATION OF PLATES.

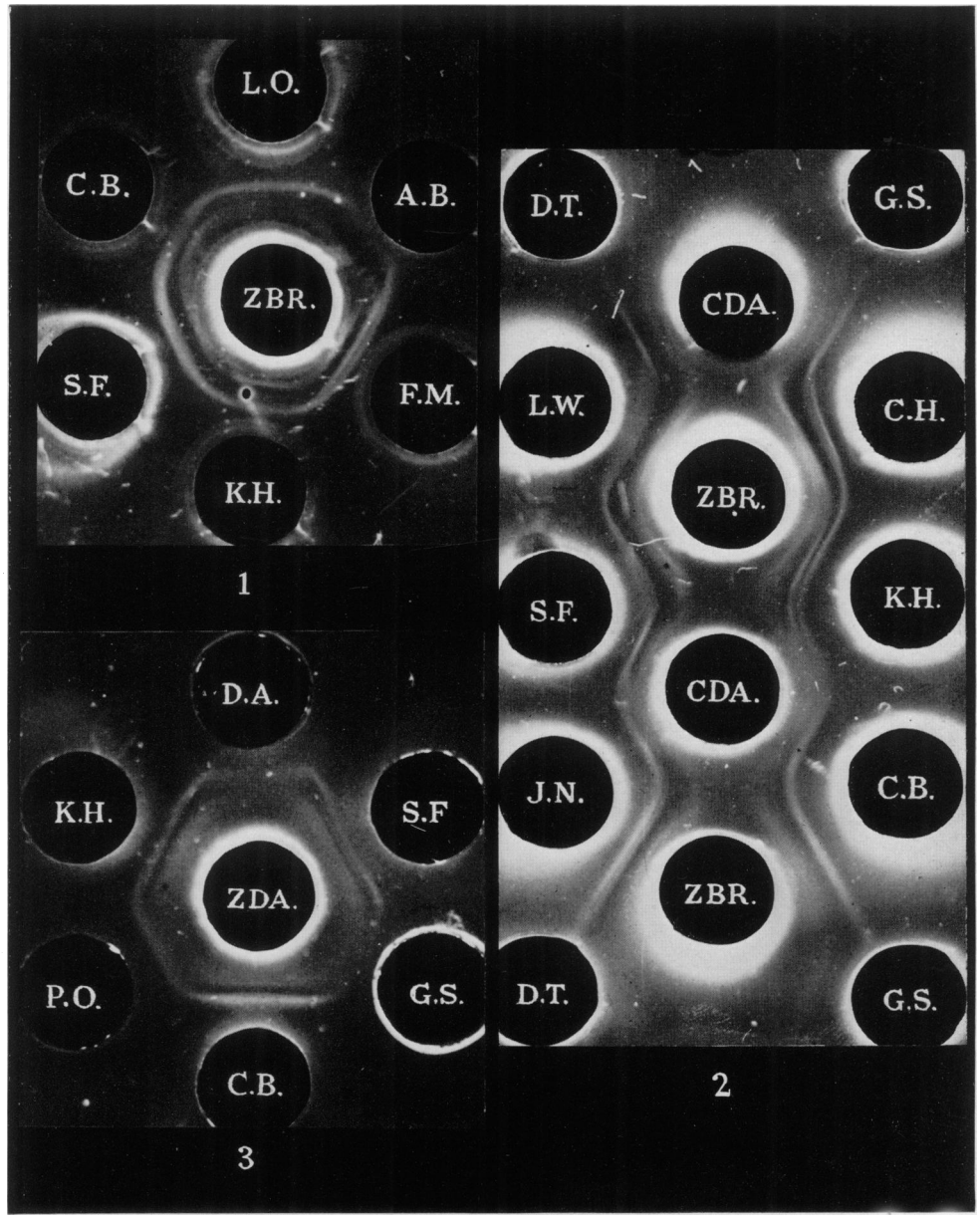
FIG. 1.—The reaction between convalescent zoster sera and zoster vesicle fluid.
Convalescent zoster sera: K.H., F.M., A.B., L.O., C.B., S.F. Zoster vesicle fluid: ZBR.

FIG. 2.—The reaction of convalescent zoster sera with zoster and chickenpox vesicle fluids.
Convalescent zoster sera: C.B., K.H., C.H., L.W., S.F., J.N. Zoster vesicle fluid: ZBR. Chickenpox vesicle fluid: CDA. D.T. and G.S. are unconcentrated convalescent chickenpox sera.

FIG. 3.—The reaction of zoster vesicle fluid with convalescent zoster sera and concentrated convalescent chickenpox sera.
Zoster vesicle fluid: ZDA. Convalescent zoster sera: C.B., S.F., K.H. Concentrated convalescent chickenpox sera: G.S., D.A., P.O.

FIG. 4.—The reaction of convalescent zoster sera and concentrated convalescent chickenpox sera with zoster and chickenpox vesicle fluid.
Convalescent zoster sera: K.H., L.O., A.B. Concentrated convalescent chickenpox sera: D.A., A.P., P.O. Zoster vesicle fluid ZM. Chickenpox vesicle fluid CW.

FIG. 5.—The reaction between a concentrated zoster tissue culture fluid and convalescent zoster sera.
Concentrated zoster tissue culture fluid: ZG. Convalescent zoster sera: C.B., M.M., C.H., C.F., K.H. G.S. is an unconcentrated chickenpox serum.



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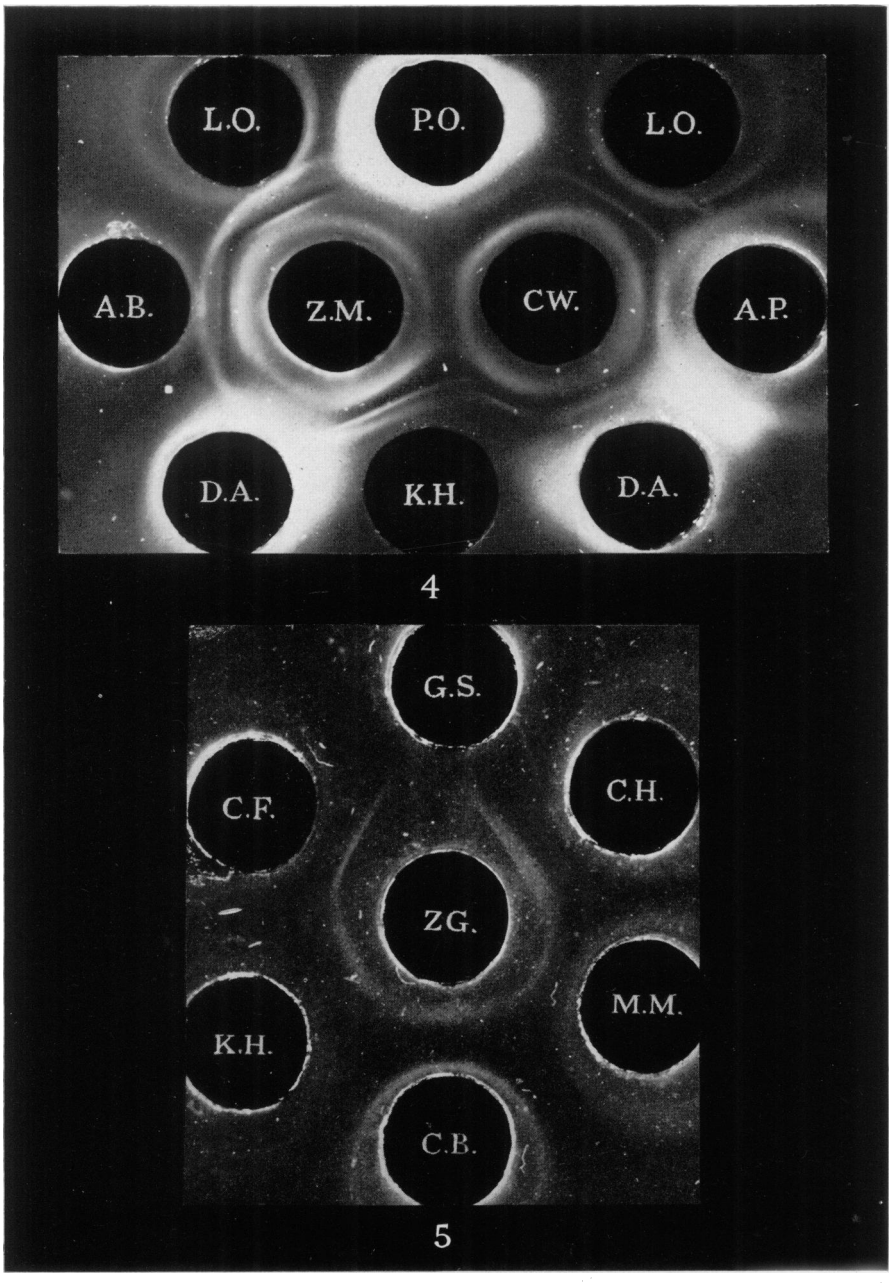

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sera, but 2 chickenpox fluids which gave marked lines of precipitation with convalescent zoster sera were tested against 8 convalescent chickenpox sera. Four of these sera at dilutions of $\frac{1}{4}$ or $\frac{1}{8}$ fixed complement with standard zoster antigen but none of the sera gave lines of precipitation with chickenpox vesicle fluid.

It seemed possible that the failure of convalescent chickenpox sera to produce lines of precipitation with vesicle fluids giving satisfactory line patterns with zoster sera might be due to insufficient concentration of antibody in the chickenpox sera. Three chickenpox sera concentrated fivefold and 3 unconcentrated zoster sera were tested therefore against a zoster vesicle fluid. The result is shown in Fig. 3. It is apparent that, although the chickenpox sera react less strongly than the zoster sera, the lines of precipitation formed by the different sera are continuous, suggesting that the precipitating antibodies demonstrable by concentration of the chickenpox sera are similar to those present in zoster sera. Fig. 4 shows the reaction of convalescent zoster and concentrated convalescent chickenpox sera with zoster and chickenpox vesicle fluids. Although the reactions with the chickenpox fluid are rather weak, they do not indicate any qualitative difference between the antigens in vesicle fluids from chickenpox and zoster cases.

Experiments Using Tissue Culture Fluid as Antigen

Doubling dilutions of vesicle fluids were tested against undiluted zoster convalescent sera. It was found that good fluids gave lines of precipitation up to a dilution of 1/6 but not at 1/12. The same antigens fixed complement with convalescent zoster sera (diluted $\frac{1}{4}$ or $\frac{1}{8}$) up to dilutions of 1/80 or even 1/160. It appeared therefore that with the techniques employed, a 10- to 20-fold greater concentration of vesicle fluid was necessary for the demonstration of precipitation than would suffice to fix complement.

Tissue culture fluids which fixed complement did so only when undiluted. It was not to be expected therefore that such fluids would give precipitation with convalescent sera and even after 5-fold concentration lines of precipitation were not obtained. Tissue culture fluids which were shown to fix complement undiluted were found, however, to give line patterns when concentrated 25 times. Fig. 5 shows the reaction obtained between a twenty-five fold concentrated zoster tissue culture fluid and 5 convalescent zoster sera. The sixth serum was from an early case of chickenpox.

In a further experiment concentrated zoster and chickenpox tissue culture fluids were tested against 10 convalescent zoster sera and 2 concentrated convalescent chickenpox sera. Lines of precipitation were observed which appeared to be continuous between the various sera and the 2 tissue culture fluids. The line pattern was unsuitable for photographic reproduction however, being obscured by non-specific zones of opacity around the antigen reservoirs.

The Complement-fixing and Precipitating Systems

All the sera examined by the agar diffusion technique had been tested by complement fixation. A comparison of the results observed with convalescent zoster sera showed a rough parallelism in that those sera possessing a high complement-fixing titre usually gave more sharply defined lines of precipitation than those exhibiting a low complement-fixing titre. Of 22 acute zoster sera, collected

within 7 days of the onset of the rash, 11 gave lines of precipitation with zoster vesicle fluids; these reactions were adjudged weaker than those produced by convalescent sera from the same patients. Of these 11 sera, only 7 fixed complement in dilutions of $\frac{1}{4}$ or higher. Of the 11 acute zoster sera which failed to give lines of precipitation, one fixed complement at a serum dilution of $\frac{1}{4}$. On the other hand, of 21 convalescent chickenpox sera none gave lines of precipitation when tested against zoster vesicle fluid known to contain precipitable antigen, although 8 of them fixed complement in a serum dilution of $\frac{1}{4}$ or $\frac{1}{8}$. Similarly 8 convalescent chickenpox sera failed to precipitate with a chickenpox vesicle fluid which gave well marked lines of precipitation with zoster sera although 3 or these 8 sera fixed complement with zoster or chickenpox vesicle fluid. Moreover of 2 zoster vesicle fluids one had a complement-fixing titre of 1/40 and gave lines of precipitation at a dilution of $\frac{1}{6}$ while the other fluid had a complement-fixing titre of 1/80 and gave lines of precipitation when diluted $\frac{1}{3}$ but not $\frac{1}{6}$. The zoster serum was used at a dilution of $\frac{1}{8}$ in the complement fixation tests and was used undiluted in the agar diffusion experiments. The antigen-antibody reactions apparent in agar diffusion tests are not necessarily the only reactions on which fixation of complement depends and strict parallelism of the results from the two techniques is not, perhaps, to be expected.

SUMMARY

In agar diffusion experiments lines of precipitation were observed between zoster vesicle fluids and 21 convalescent zoster sera. Line patterns could be resolved into at least 3 components, presumably representing at least 3 antigen-antibody pairs in the zoster-antizoster precipitating system.

Chickenpox vesicle fluid also produced lines of precipitation with convalescent zoster sera. These lines fused with those produced by the same sera and a zoster vesicle fluid indicating the identity of the reacting antigens in the two fluids.

With convalescent zoster and concentrated convalescent chickenpox sera, tissue culture fluids produced lines of precipitation only when concentrated 25-fold.

Twenty-two convalescent chickenpox sera failed to produce lines of precipitation with a zoster vesicle fluid. On five-fold concentration, however, 6 sera tested gave lines of precipitation with zoster and chickenpox vesicle fluids which fused with the lines of precipitation given by these fluids and convalescent zoster sera.

The results of this investigation failed to reveal differences in the precipitable antigens of zoster and chickenpox vesicle fluids and the precipitating antibodies in zoster and chickenpox sera, although it appeared that convalescent chickenpox sera had a much lower concentration of antibody than zoster sera. These findings are not unexpected if one accepts the view that zoster is in most cases a clinical expression of the reinvasion of tissues by a latent chickenpox virus.

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