Electron Microscopy of Poliovirus Antigen Antibody Precipitates Extracted from Gel Diffusion Plates

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Two antigens detected in poliovirus preparations are called either N (native) or H (heated) or D and C from their position in a density gradient. Most of the infectivity and RNA of poliovirus preparations is in the D zone on gradient centrifugation and virus inactivation by heat or mercuric ions changes the antigenic reactivity from N or D to H or C with release of viral RNA (Le Bouvier, 1959; Dimmock, 1967). Hummeler, Anderson & Brown (1962) showed by electron microscopy that empty virus particles (penetrated by phosphotungstate) were aggregated by antiserum to heated virus and full virus particles (not penetrated by phosphotungstate) by antiserum to native virus. Watson et al. (1966) described a method of examining virus precipitin lines under the electron microscope, and we have examined the D and C antigen precipitates of type 1 poliovirus by this technique.

Plate 1 shows a gel diffusion test prepared as described by Beale & Mason (1962). The antigen in the peripheral wells was concentrated type 1 poliovirus with a titre of $10^9$ TCD 50/ml. Alternate wells were filled with unheated virus and virus heated at 50°C for 15 min. The antiserum in the centre well reacted with both unheated (D) and heated (C) preparations.

After 48 hr incubation at room temperature the precipitin lines were cut out with a scalpel and placed on a slide. The fragments of agar were then frozen and cut up into tiny pieces with the scalpel. After thawing, a drop of 3% phosphotungstate (pH 6-1) was added and the mixture taken up into a Pasteur pipette. A drop was expelled on to a carbon-coated Formvar-covered grid and dried after removing excess fluid with filter paper. Samples were examined in a Siemens Elmiskop 1A electron microscope at a magnification of $\times 40,000$. Plates were exposed at a magnification of $\times 60,000$. Plate 2, fig. 2, shows the appearance of the material extracted from the D antigen line. There are full virus particles surrounded by antibody, whereas the C line seen in Pl. 2, fig. 3, shows only empty virus particles surrounded by antibody. We have used the gel diffusion technique (Beale & Mason, 1962) to measure the amount of D antigen and the electron microscopy of the gel diffusion lines gives visual confirmation that the D and C antigen lines are associated with complete and empty virus particles respectively.

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REFERENCES


Short communications


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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Gel diffusion plates with alternate D and C antigenic peripheral wells and anti D+C serum in the centre. The more prominent lines are produced by D antigen.

PLATE 2

Fig. 2. Electron micrograph of D antigen line.

Fig. 3. Electron micrograph of C antigen line.