Chromatography of Vaccinia Virus

In a previous paper (Hanafusa, 1960), it was demonstrated that multiple infection of UV- or heat-inactivated vaccinia caused the death of host cells. From studies of this agent by means of sedimentation and immunological reactions it was concluded that the inactivated virus particles may be responsible for this killing action. The IHD strain of vaccinia virus used for this work had been passaged ten times in Ehrlich's ascites cells and then more than 20 times in L cells. It did not produce haemagglutinin either in L cells or in the chorioallantoic membrane of eggs, as reported by Cassel and Fater (1958). Therefore the killing phenomenon is independent of the haemagglutinin. With adenovirus, the cytotoxic agent obtained from a crude virus preparation has been separated from the virus particles by ion exchange chromatography (Klemperer and Pereira, 1959; Philipson, 1960).

To obtain further information on the relation of the cell killing capacity to the virus particle, a vaccinia virus preparation was chromatographed on DEAE-cellulose according to the method of McCrea and O' Loughlin (1959), and the infectivity and cell killing activity in each fraction of the effluent measured.

Vaccinia-IHD virus was obtained by sonic disruption of infected L cells and dialyzed overnight against 0.01 M Tris-succinate buffer. The dialyzed virus material (1.5×10^8 PFU/1.5 ml) was put onto a column (0.9×15 cm) of DEAE-cellulose (Eastman Kodak) which had been equilibrated with 0.01 M Tris-succinate buffer, pH 7.4. The adsorbed virus was eluted by gradient elution; a continuously increasing concentration of NaCl in buffer was run through the column and the eluate collected in 4 ml fractions at a flow rate of about 15 ml per hour. The infectious units and cell killing action of aliquots of each tube were measured, and the remainder of each fraction was used for measurement of the extinction at 260 mμ and 280 mμ. The infectivity of the virus was assayed by plaque counting on L cell monolayers. Fig. 1 illustrates a representative chromatogram. Both the infectivity and the early cytotoxic effect were found mainly at tubes 15 to 25 and no other tubes had a cytotoxic effect. Most proteins and nucleic acids other than those of the virus was eluted more rapidly. The recovery of infectivity was about 90 per cent. The virus in tubes 20 and 21 was concentrated by high speed centrifugation and inactivated by heat or UV. Samples inactivated in both ways caused death of L cells in their respective manners. These results show clearly that inactivated virus particles are responsible for the killing of L cells.

A small peak was always found at tubes 31 to 33. However a similar chromatogram to that shown in Fig. 1 was obtained by chromatography of a virus clone isolated from this peak. The peak may have been due to some heterogeneity in structure of the virus.

As a similar chromatographic pattern was obtained with ectromelia virus, viruses

belonging to the pox group seem to have similar surface structures.

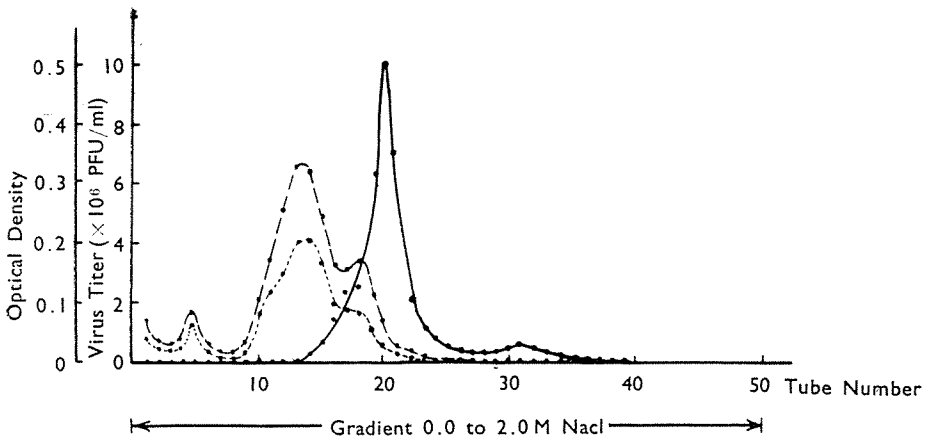

Fig. 1. Chromatography of vaccinia IHD on DEAE-cellulose by gradient elution with increasing concentrations of NaCl obtained by running 3.2 M NaCl in 0.01 M Tris-succinate buffer into a mixing flask containing 200 ml of 0.01 M buffer.
Solid line : infectivity of eluates.
Broken line : absorbance at 260 mμ.
Dotted line : absorbance at 280 mμ.

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Received on November 30, 1960