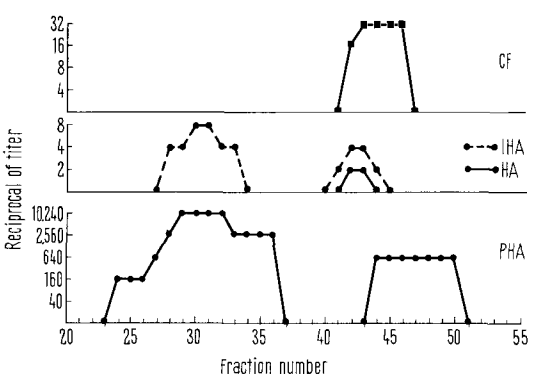
Separation and Identification of Adenovirus Type 7A Components

Various studies have demonstrated the structural complexity of the adenoviruses. Two reviews summarizing many of their characteristics have recently been published[1,2]. The purpose of this study was to separate and identify serologically the components of adenovirus type 7A (strain S-1058).

Materials and methods. Soluble antigen preparations were made from concentrates of infected HeLa cell lysates after intact virions were removed by ultracentrifugation. Viral antigens used for antisera production were prepared from concentrates of intact virions which had been purified by equilibrium ultracentrifugation in CsCl. Methods used for the separation of the viral components were similar to that of NORRBY[3], and consisted of DEAE-Sephadex column chromatography and sucrose density gradient centrifugation. Fractions obtained by these methods were examined by using micro-volume modifications of the following techniques: complete hemagglutination (HA), incomplete hemagglutination (IHA), passive hemagglutination (PHA), hemagglutination inhibition antibody consumption (HIC), and complement fixation (CF). Details concerning these procedures have been reported previously[3,4].

Results and discussion. Satisfactory resolution of antigenic components was not obtained by DEAE-Sephadex chromatography in the presence of the complete HA. When desired, the complete HA was removed by 3 adsorptions with packed Rhesus erythrocytes. The Figure illustrates a profile of viral fractions obtained by elution of an absorbed preparation from DEAE-Sephadex with a 0.0 to 0.35 *M* NaCl gradient. Several peaks of activity were found when fractions were reacted with immune rabbit sera. The first component to elute exhibited IHA and PHA activities but not HA activity. Its properties were suggestive of isolated pentons. Since PHA activity was also found in these fractions, a determinant possessing PHA activity must be located on the penton. A second component having HA and IHA activity eluted at a slightly higher molarity of NaCl. This activity probably represents residual dodecon (HA) structures not removed by erythrocyte adsorption. Group specific CF activity (representing hexon components) was determined using an adenovirus type 2 antiserum. Complement fixing antigen was usually found in a peak which overlapped the complete HA although some heterogeneity was encountered in its elution pattern. In several experiments 2 peaks of CF activity were found; one eluted with penton activity and the other eluted with the complete HA. This lack of homogeneity has been reported previously[5]. The last antigenic component was a second peak of PHA activity which eluted at approximately the same position as the HA but could also be demonstrated in viral preparations after removal of HA activity. The hemagglutination patterns associated with this peak were somewhat less complete than those associated with the first PHA peak. The viral component responsible for this activity is unknown.



Fractionation of adenovirus type 7A components on DEAE-Sephadex. CF, complement fixation; IHA, incomplete hemagglutination; HA, complete hemagglutination; PHA, passive hemagglutination.

Centrifugation of 5% to 20% sucrose gradients was used to further separate viral components. All HA activity, representing dodecon structures, was found near the bottom of the tube. These fractions exhibited neither PHA nor group specific CF activities. The lack of CF activity associated with dodecon structures was not surprising, since the hexon component is not present in the architecture of the dodecon. The lack of PHA activity in dodecon fractions was unexpected, since pentons which comprise dodecon structures were active by PHA methods. The spacial configuration of the dodecon may be such that firm attachment between erythrocytes and 'PHA active sites' on penton units of the dodecon cannot be achieved.

Slower sedimenting components, represented by CF and PHA activities, required longer periods of centrifugation for separation. Activity associated with penton components was found in fractions near the bottom of the centrifuge tube. This finding was similar to that of NEURATH et al.[6]. Heterogeneity of CF activity demonstrable in fractions eluting from DEAE-Sephadex was also found using sucrose gradient centrifugation techniques. An isolated peak of HIC activity which probably represented free fiber components was found near the center of the gradient. Fractions of this peak were not active using either IHA or PHA methods indicating that the active sites involved with these reactions were probably not located on the fiber.

The results reported here are similar to those reported for other adenovirus types. The PHA represents another useful tool for the characterization of these components. The antigenic site associated with the IHA and the principal determinant of PHA activity may be located on or near the vertex capsomer. The second peak of PHA activity may represent free vertex capsomers.

Zusammenfassung. Adenovirus Typ 7 A wurde durch DEAE-Chromatographie und Dichtegradientzentrifugation in seine Komponenten zerlegt und diese mittels verschiedener serologischer Methoden charakterisiert.

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