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Comparison of Techniques for Influenza Virus Purification

C. B. REIMER, R. S. BAKER, T. E. NEWLIN, M. L. HAVENS, R. M. VAN FRANK, W. O. STORVICK, AND R. P. MILLER

Eli Lilly Research and Biological Development Laboratories, Eli Lilly and Co., Indianapolis, Indiana

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Anderson's B-IV zonal-ultracentrifuge rotor (N. G. Anderson et al., Life Sci. 3:667, 1964) has proven useful for purifying relatively large amounts of influenza virus by sucrose density gradient centrifugation (C. B. Reimer et al., Science **152**:1379, 1966). Part of our procedure included a rate-zonal centrifugation step which easily separated influenza virus from soluble micromolecular and macromolecular impurities, insoluble large particulate impurities, and from most of the microsomes. We have used this preparative rate-zonal centrifugation technique for a comparative evaluation of various ancillary procedures for purifying or concentrating influenza virus. As starting material, the same lot of formalin-inactivated PR-8 influenza virus was used which had been concentrated 10-fold and purified 100-fold over allantoic fluid by a differential centrifugation procedure (W. M. Stanley, J. Exptl. Med. **79**:255, 1944). This concentrate contained 1,800 chick cell agglutinating (CCA) units per milligram of protein. Portions of the concentrate were further processed by the various techniques to be described subsequently. As a terminal step, 100 ml of each portion was then subjected to an identical rate-zonal centrifugation in the B-IV rotor.

Figure 1 shows optical density (280 mμ) profiles of sedimenting components in this comparative study. Each profile has been normalized by setting the virus peak equal to 1.0 to permit a qualitative comparison among the various procedures. The relative amplitude of the actual virus peak is graphed in the accompanying ordinate with a number which indicates the actual concentration factor for a particular profile.

The central profile, marked O.V. (original virus), is a typical rate-zonal sedimentation profile for this type of concentrate, without any intervening process. Soluble components are found in the peak on the left. Large particulate impurities are found in the peak on the right. The polydisperse microsomes constitute most of the background adsorbancy between the latter two peaks. The arrow denotes the virus peak, rising out of the microsomal background.

The sedimentation profile in the upper left of Fig. 1, marked $(NH_4)_2SO_4$, was obtained by fractional precipitation with ammonium sulfate as used for plant viruses (W. M. Stanky, p. 447, *in* R. Doerr and C. Hallauer [ed.], Handbuch der Virusforschung, no. 1, Julius Springer, Vienna, 1938). Most of the hemagglutinin was recovered in the fraction which was soluble in 30% and insoluble in 70% ammonium sulfate. This material was resuspended by dialysis against buffer and then was centrifuged in a manner identical to O.V. Significant reduction is seen in the zone of soluble macromolecular impurity, in the zone of large particulate impurity, and in the microsomal background. Evidence for some virus aggregation was noted by the distribution of hemagglutinin, ultraviolet adsorbancy, and by electron microscopy. The $(NH_4)_2SO_4$ procedure diluted virus approximately twofold.

The sedimentation profile in the lower left of Fig. 1, marked $Ca_3(PO_4)_2$, was obtained from a preparation in which the virus had been coprecipitated with calcium phosphate (J. E. Salk, Proc. Soc. Exptl. Biol. Med. **46**:709, 1941) and resuspended in a smaller volume by chelating the calcium with a sodium salt of ethylenediaminetetraacetic acid. Analysis of the data obtained by the zonal ultracentrifuge revealed that this significant virus concentration procedure also purified the original material so that it was free from soluble nonviral components. However, the calcium phosphate coprecipitation procedure had very little effect with respect to removing the other two major classes of impurity, the microsomes and the large heavy particles.

The sedimentation profile in the lower right of Fig. 1, marked agar, was obtained from a preparation which had been passed through a "molecular sieve" column containing 4% agar chips (R. L. Steere and G. K. Ackers, Nature **194**:114, 1962). The hemagglutinin band from this column was submitted to an identical rate-zonal ultracentrifugation. When compared at equal virus concentration, this agar procedure is qualitatively very similar to the calcium phosphate procedure in its ability to purify PR-8 influenza virus. The soluble macromolecular impurities are largely

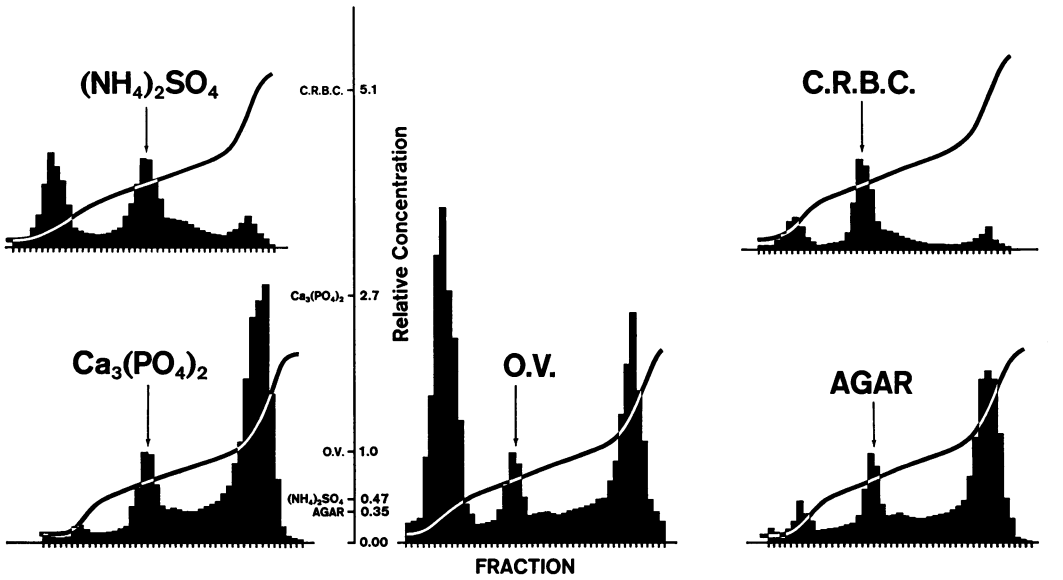

FIG. 1. *Comparison of rate-zonal sedimentation profiles obtained with PR-8 influenza virus which was prepurified by various ancillary procedures. The arrow indicates the virus peak found by hemagglutination and by electron microscopy. The curved line shows the sucrose concentration gradient, on the left (centripetal) side increasing as a sigmoid to 10% sucrose, then increasing linearly to 30% sucrose, and finally rising sharply to 60% sucrose on the right (centrifugal) side.*

removed, but the microsomal and large particulate components are unaffected. For an intact virus as large as influenza, the calcium phosphate procedure is preferred over the molecular sieve procedure with 4% agar, because the former leads to significant virus concentration but the latter leads to a dilution of virus.

The sedimentation profile in the upper right of Fig. 1, marked C.R.B.C., was obtained from a preparation in which virus was adsorbed to chicken red blood cells (C. A. Knight, p. 12, *in* Protoplasmatologia, vol. 4, Chemistry of Viruses, Springer Verlag, Vienna, 1963), washed, eluted into a small volume with the aid of receptor-destroying enzyme, and separated from the erythrocytes by low-speed centrifugation. The rate-zonal profile of this material indicated that a significant reduction had occurred in all three major classes of impurity. The contaminating receptor-destroying enzyme, hemoglobin, red cells, and red cell ghosts were easily separated from the virus by the subsequent rate-zonal centrifugation.

This limited comparative study clearly demonstrated the ease and superiority of zonal ultracentrifugation procedures for effectively purifying influenza virus. Some of the other procedures studied could be employed efficiently in conjunction with the zonal ultracentrifuge.