PURIFICATION OF INFLUENZA VIRUS BY PHASE SEPARATION AND SELECTIVE ADSORPTION

Peter v. SENGBUSCH*

The Salk Institute for Biological Studies, San Diego, USA

Received 4 April 1971

1. Introduction

Several methods are available for purification of influenza virus. Those which include adsorption-elution steps are widely used, particularly with red blood cells as adsorbent since influenza virus is selectively adsorbed and subsequently released by action of its own neuraminidase [1]. Comparisons of methods, including other adsorbents (calcium phosphate, aluminium phosphate) and precipitation by ammonium sulphate have been published [2, 3]. None of these methods is completely satisfactory, since unspecific material was always detected in preparations by density gradient centrifugation. The development of the zonal centrifuge [4] gave an alternative procedure for better purification of virus preparations. Since the zonal centrifuge is of limited availability, and requires a prior concentration step [5], a new method for high purification was devised by combining a few simple purification steps. The method is not limited to small scale preparations.

2. Methods

Influenza virus, strain WSE, obtained from Dr. R. Webster (Memphis, Tenn.) and Dr. S. Fazekas de St. Groth (Sydney, Australia), was grown for three days in 11 day old chick embryos. The eggs were subsequently chilled and the infective allantoic fluid was harvested. At this step care was taken to avoid release of blood from the embryo.

The allantoic fluid, filtered through gauze, was mixed with 7 volumes of Freon TF (DuPont de Nemours and Company, Wilmington, Delaware 19898, USA, = Frigen 113, Farbwerke Hoechst AG, Frankfurt, Germany, = Fluorocarbon, $CCl_2F–CClF_2$) to 10 volumes of the aqueous solution and mixed vigourously until a foamy emulsion was obtained: this was essential for improved purification. The emulsion was blended for two to five minutes and the two phases were separated by low-speed centrifugation. A solid bottom phase was formed as a complex between Freon TF, host proteins, lipids, and subcellular structures. The clear supernatant fluid contained all the influenza virus and the haemagglutinating activity was slightly higher (13%) than the untreated allantoic fluid due to removal of non-specific inhibitors. The clear supernatant fluid was adjusted to pH 6.0 by addition of a few drops of 1 N HCl and freshly prepared aluminium phosphate was added to adsorb the virus. The procedure of Miller and Schlesinger [6] was followed closely and the virus was adsorbed quantitatively. After centrifugation virus was eluted completely from the pellet with 0.35 M phosphate buffer, pH 8.0, in an elution volume of about 1/10 the original.

Final high (30,000 *g*, 30 min) and low speed centrifugation yielded the highly purified virus preparation. This centrifugation was essential for removal of low molecular weight substances such as flavines and small proteins.

3. Characterization of the purified preparation

Fluorocarbons (Freon, Frigen) have been used successfully in purification of several viruses [7–9].

* Present address: Max-Planck-Institut für Medizinische Forschung, 69 Heidelberg, Germany.

North-Holland Publishing Company – Amsterdam

The application of this method to some lipid containing viruses was reported to be unsuccessful [9]. This was due to repeated extraction with the fluorocarbon and virus was lost at each step after the first. Virus remained quantitatively in the aqueous phase only in the first cycle of extraction. These results were reproducible and indicate that influenza virus and other host proteins and lipids are denatured by Freon TF and establish an equilibrium after the first extraction cycle. The use of Freon TF in purification of influenza virus is therefore limited to one cycle of extraction.

To characterize the virus sample, a density gradient centrifugation was performed. Only one peak with no low or high density impurities was detected (fig. 1).

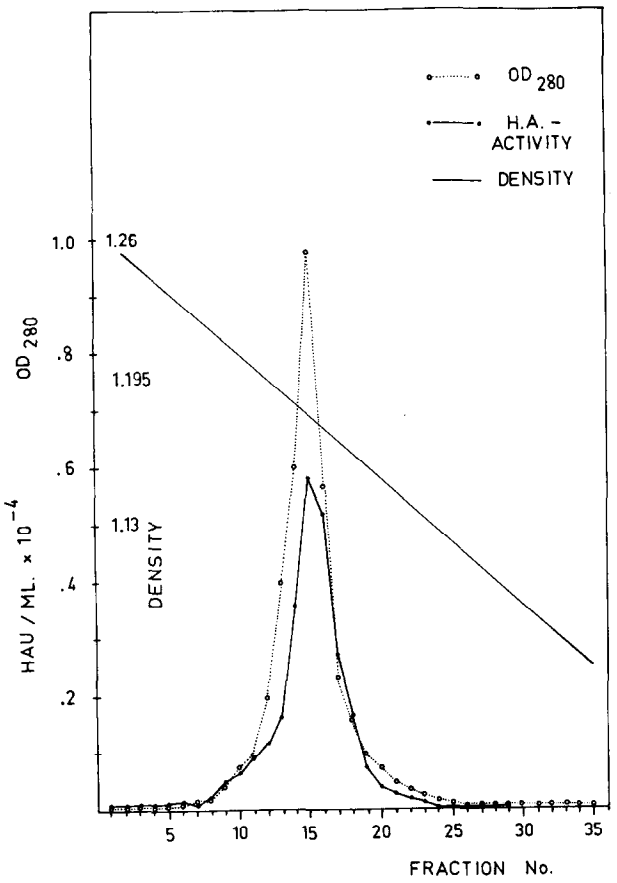

Fig. 1. Density gradient centrifugation of an influenza virus sample (WSE), purified by the Freon method. The virus was banded in a linear sucrose gradient (2.0–0.5 M) in a Spinco L centrifuge (SW 25.1 rotor, 22,000 rpm for 16 hr).

Table 1
Antigenicity of influenza virus (WSE) in an inbred mouse strain (F/St).

Preparation	No. of mice	Activity in haemagglutination inhibition		
		log 2 units /ml	standard deviation	standard error
Freon method (fresh)	38	14.44	1.70	0.28
Red blood cell adsorption-elution				
16 month old	32	14.15	0.62	0.10
Fresh	35	14.25	0.82	0.14

The virus (2500 HA units/0.1 ml) was injected intravenously and antibody activity in inhibition of haemagglutination was assayed 3 weeks later.

The calculated density of the virus was 1.18 g/ml, in agreement with Reimer et al. [5]. The haemagglutinating activity at comparable protein concentrations (absorbancy, A_{280}) was 101×10^3 HA units [10] for the Freon prepared virus and 98×10^3 HA units for a commercial preparation (Zonomune, Ely Lilly and Company, Indianapolis: V-1305, control 1YU978). The observed difference, if significant, may be due to different specific activities of the virus types compared. The haemagglutinating activity of the virus preparation remained constant for five months at 4° in physiological saline with 0.08% sodium azide as preservative. Electron microscopy revealed a uniform population of virus particles. Spherical, eliptical and kidney shaped forms were characteristic of the influenza strain WSE. Applied to Biogel A 50 (Bio-Rad Laboratories, Richmond, Calif.) column, the virus eluted in the void volume and no material was retarded. The antigenicity of the preparation, as measured by the injection of a saturating dose (2500 HA units/0.1 ml) of antigen in inbred mice (table 1) was comparable to that for samples purified by adsorption–elution with red blood cells.

4. Conclusion

These results indicate that the Freon method provides active virus preparations which are comparable in purity with those obtained by zonal centrifugation. The method provides large amounts of purified influenza virus as antigen and should be useful for inexpensive production of vaccines.

Acknowledgements

This work was supported by a Dernham Fellowship (J 129) of the American Cancer Society, California Division and an NIH grant (AI 06544) to Dr. E.S. Lennox (The Salk Institute, San Diego). I wish to thank Mr. R. Austin for his excellent technical assistance during the course of the work.

References

[1] K.G.Hirst, J. Exptl. Med. 76 (1942) 195.
[2] F.M.Davenport, J. Am. Med. Assoc. 182 (1962) 11.
[3] C.B.Reimer, R.S.Baker, T.E.Newlin, M.L.Havens, R.M. van Frank, W.P.Storcick and R.P.Miller, J. Bacteriol. 92 (1966) 1271.
[4] N.G.Anderson and C.L.Burger, Science 136 (1962) 646.
[5] C.B.Reimer, R.S.Baker, T.E.Newlin and M.E.Havens, Science 152 (1966) 1379.
[6] H.K.Miller and R.W.Schlesinger, J. Immunol. 75 (1955) 155.
[7] H.G.Wittmann, Z. Vererbungsl. 95 (1964) 333.
[8] L.Philipson, in: Methods in Virology, Vol. 2, eds. K. Maramorosch and H.Koprowski (New York, 1967) p. 235.
[9] V.V.Hamparian, F.Müller and K.Hummeler, J. Immunol. 80 (1958) 468.
[10] S.Fazekas de St. Groth and R.G.Webster, J. Exptl. Med. 124 (1966) 331.