

## CONCENTRATION OF INFLUENZA VIRUS USING POLYETHYLENE GLYCOL

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*Summary.* — Maximum yield of influenza virus was obtained by treating infected allantoic fluid with 8 per cent PEG for 2 hours at 4 °C. Precipitated virus was deposited at 6000×g for 20 min, resuspended carefully and finally purified on a sucrose density gradient.

*Key words:* influenza virus; virus purification; polyethylene glycol

### Introduction

Polyethylene glycol (PEG) has been used to concentrate many viruses (Vajda 1978). Different authors (Kanarek and Tribe 1967); Polson *et al.* 1972; Polson 1974; Hayward *et al.* 1977) suggest different conditions for optimum recovery of influenza virus from infected fluids. Therefore the precise conditions for recovery of influenza virus from allantoic fluids using PEG have been determined and this method compared with adsorption on to and elution from fowl red blood cells (RBC) according to Laver (1969).

### Materials and Methods

Influenza strains A/Japan/305/57 and A/Hong Kong/1/68 were grown in embryonated eggs and infected allantoic fluids harvested after 48 hr at 37 °C and clarified by low speed centrifugation. Haemagglutinin was titrated by the microtitre method using 0.5 per cent fowl RBC Neuraminidase was assayed as described by Aymard-Henry *et al.* (1973) using 0.6 per cent (w/v) fetuin. After concentration virus samples were layered onto 28 ml linear gradients of 10-40 per cent (w/v) sucrose which were spun at 40,000×g for 40 min. A 40 per cent w/v stock solution of PEG 6000 (BDH Reagents Ltd) in phosphate buffered saline (PBS) pH 7.2 (sterilised by autoclaving) was added to allantoic fluids to the desired concentration and the mixture held at 4 °C for the desired time. The precipitate was pelleted by centrifugation for 20 minutes and resuspended in PBS. Excess PEG was deposited at 3000×g for 5 minutes and the supernatant recovered. The optimum conditions for each stage were assessed.

### Results

PEG concentrations from 1-10 per cent were tried. Complete virus recovery was achieved only using 8-10 per cent. Maximum virus recovery required precipitation to proceed for 2 hr with 8 per cent PEG. At 1 hr haemag-

glutinin was still detectable in the supernatant. After 24 hr haemagglutinin began to reappear in the supernatant. Centrifugation at different speeds showed that over 99 per cent of virus was deposited at 4,500–5,000 rev/min ( $6000\times g$ ). This is easily achieved using a bench centrifuge. The deposited virus – PEG precipitate was resuspended in PBS and rotamixed for various times to disaggregate complexes. Unexpectedly at all times tested there appeared to be a virus concentration factor of  $10^6$  (haemagglutinin measurement) when the volume had only been decreased by a factor of 20. The presence of PEG was necessary to show this apparent increase in virus titre and this effect of PEG disappeared with ten fold dilution. Rotamixing infected allantoic fluid in the absence of PEG has no effect on haemagglutinin titre, and only whole virus particles were seen by electron microscopy after PEG concentration. Rotamixing (optimum time 30s) was therefore deemed to have no deleterious effect. After rotamixing residual PEG was deposited at  $3000\times g$  for 5 min and the supernatant collected. The necessity for sonicating this supernatant before sucrose density gradient centrifugation (Heyward *et al.* 1977) was investigated although not using PEG-concentrated material because of the interference with haemagglutination. The haemagglutinin titre of fresh infected allantoic fluid was unaffected by sonication, but there was an increase in titre if such fluid was left overnight sonicated and assayed again. This suggested aggregation of virus particles overnight and their disaggregation by sonication. Following observations support further this assumption: (1) sucrose gradient centrifugation of freshly concentrated virus gives a clear band midway down the tube with very little sediment; (2) sucrose gradient centrifugation of stored virus gives a broader band together with a sediment showing considerable haemagglutinin activity; (3) if fresh or old concentrated virus was sonicated for 1 minute for sucrose gradient centrifugation, the virus band was distinct and narrow with very little sediment.

Sucrose gradient centrifugation of an 8 per cent PEG solution gave no bands but a surupy solution remained on top of the gradient. Sucrose gradient centrifugation of virus recovered from allantoic fluid by adsorption to and elution from fowl RBC (Laver 1969) resulted in a clear fairly broad virus band midway down the tube. After centrifugation through sucrose gradient PEG-concentrated virus was found as a broad opaque band  $\frac{1}{2}$ – $\frac{2}{3}$  rds down the gradient, but there was also a second clearly defined narrow yellow brown band close to the interface between the PEG layer and sucrose gradient. This second band had some complement fixing activity but little or no haemagglutinin or neuraminidase activity.

Of the two concentration methods, the PEG method gave a higher titre (haemagglutinin) than the red cell adsorption/elution method, and the latter was slightly higher than the theoretical yield. An apparent decrease in neuraminidase titres was probably due to interference by PEG in the enzyme assay because after density gradient centrifugation, which removes residual PEG (Alberts 1967), high enzyme titres returned. Electron microscopic observation of PEG-concentrated virus after sucrose gradient centrifugation

showed both isolated and aggregated particles. The aggregated particles were not tightly adherent to one another and did not have interlocking spikes. Very little contaminating material was seen on the grid.

### Discussion

Adsorption to and elution from fowl red cells is fairly specific for influenza virus and thus gives a measure of purification in addition to concentration. The PEG method is non-specific and appears to result in precipitation of haemagglutinating material that would probably be unable to elute from red cells and other material such as that giving the yellow second band on the gradient. When using allantoic fluid after several days storage PEG precipitated both whole and fragmented virus whilst the red cells selected out biologically active virus particles. Both methods take about the same time to perform and after sucrose gradient centrifugation which removes most impurities give similar products. PEG reagents can be kept on a laboratory shelf and is probably the simpler and cheaper technique.

The PEG method was successful with A/PR/8/34 as well as A/Jap and A/HK, and is probably applicable to most influenza strains. AO/NWS has no neuraminidase activity under standard conditions so *V. cholerae* neuraminidase would have to be added to red cells to elute the virus. AO/BEL can elute from fowl but not human RBC.

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