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# PURIFICATION AND CONCENTRATION OF PARAINFLUENZA VIRUS

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*Summary.* — Concentration and partial purification of type 3 parainfluenza virus was achieved by adsorption onto barium sulphate or precipitation by zinc acetate or ammonium sulphate.

The yields of parainfluenza viruses obtained from infected cell culture fluids are low. This fact prevented to a certain extent the studies on the chemical composition of parainfluenza viruses. Their concentration by adsorption onto formalin-treated guinea pig erythrocytes has been reported (Zakstelskaya *et al.*, 1963), but the resulting virus eluates may contain products of erythrocyte disintegration. We found previously (Smirnova *et al.*, 1968) that type 2 and 3 parainfluenza viruses can be concentrated by adsorption onto barium sulphate.

In the present work we studied the concentration of type 3 parainfluenza virus, in addition to adsorption onto barium sulphate, also by precipitation with either 0.02 M zinc acetate according to the method described by Neurath *et al.* (1966) or ammonium sulphate at 50% saturation. The VOK stock strain of type 3 parainfluenza virus was propagated in stable human amnion cell cultures. Virus was concentrated from cell-free culture fluids harvested 5 days after inoculation. The precipitates obtained with ammonium sulphate or zinc acetate were dissolved and dialyzed against frequently changed physiological saline at 4° C for 2—3 days. Elution of virus from barium sulphate was done with 0.25 M sodium citrate solution.

Infectivity of the preparations was estimated by inoculating cell cultures with various dilutions of the virus-containing fluids; presence of virus was checked by haemadsorption and the titres were calculated by the formula of Reed and Muench. The complement fixation (CF) reaction was carried out for 18 hours at 4° C, using 2 units of complement and a 3% suspension of sheep erythrocytes. The haemagglutinating (HA) activities were determined by mixing serial 2-fold dilutions of the test fluids with a 0.6% suspension of guinea pig erythrocytes (the erythrocyte concentration was adjusted on a photoelectric colorimeter). Proteins were estimated by the method of Lowry *et al.* (1951).

The supernatants obtained after adsorption or precipitation were practically free of infectious virus or CF and HA antigens.

The results presented in Table 1 indicate that the titres of infectious virus and CF and HA antigens increased with all 3 concentration procedures employed. On adsorption of virus onto barium sulphate, the increase in HA activity was considerable, which could have been due to some disintegration of the virions. Virus purification resulted in all cases in the removal of low molecular weight substances of the culture medium and of a part of high molecular weight cellular substances which were not precipitated under the conditions of the experiment. The data presented suggest virus purification with respect to protein contents of the preparations. The total protein content of the preparations was lower after concentration than

**Table 1. Concentration of type 3 parainfluenza virus by adsorption onto barium sulphate or precipitation with zinc acetate or ammonium sulphate**

| Exp. No. | Treatment | Infectivity (log units) | | HA titres* | | CF titres* | | Protein ($\mu$g/ml) | | Concentration factor with respect to volume |
|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | I | II | I | II | I | II | |
| 1 | Ba-sulphate | 1 | 2 | 20 | 160 | 2 | 16 | 36 | 140 | $\times 10$ |
| 2 | | 2.7 | 3.4 | 4 | 64 | 4 | 64 | 50 | 400 | $\times 10$ |
| 3 | | 1 | 2 | 4 | 160 | 8 | 64 | 68 | 480 | $\times 10$ |
| 4 | Zn-acetate | 2 | 3.3 | 10 | 160 | 1 | 8 | 64 | 760 | $\times 20$ |
| 5 | | 1 | 2.3 | 20 | 320 | 2 | 16 | 36 | 580 | $\times 20$ |
| 6 | | 1 | 2.3 | 4 | 80 | 4 | 64 | 45 | 630 | $\times 20$ |
| 7 | $(NH_4)_2SO_4$ | 2 | 3.7 | 10 | 320 | 1 | 16 | 64 | 710 | $\times 50$ |
| 8 | | 1 | 2.3 | 20 | 320 | 2 | 16 | 36 | 365 | $\times 20$ |
| 9 | | 2 | 3.3 | 2 | 40 | 2 | 32 | 34 | 160 | $\times 20$ |

* Dilution reciprocals.
I and II — Values before and after concentration, respectively.

in the starting material. The best purification was obtained with ammonium sulphate — the total protein contents decreased 2—4-fold at the same amount of infectious virus and viral antigens. Thus the biological activity per protein unit increased.

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