COUNTER-CURRENT DISTRIBUTION OF POLIO VIRUS[1]

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It has been shown recently that large protein molecules (Albertsson, 1960; Albertsson and Nyns, 1959), nucleic acids (Lif et al., 1961; Albertsson, in press) and cells (Baird et al., 1961; Albertsson and Baird, in press) can be fractionated by counter-current distribution using aqueous mixtures of two water soluble polymers such as dextran and polyethylene glycol as phase systems (Albertsson, 1960). By this method particles are separated according to their physico-chemical properties, mainly those of the particle surface. In the case of bacteria this method seems to be highly specific; even fairly closely related strains may thus be separated from each other (Baird et al., 1961; Albertsson and Baird, in press). It has also been shown that a number of viruses, including enteroviruses (Wesslén et al., 1959; Philipson et al., 1960; Norrby and Albertsson, 1960) can be distributed in aqueous polymer two-phase systems in a reproducible manner without significant loss in activity. This paper presents results obtained from counter-current distribution of poliovirus in a dextran sulfate-polyethylene glycol phase system.

Virus. Poliovirus type 2 strain MEF 1 and poliovirus type 1 strain 1423/53 (a Swedish strain) were used. The viruses had been passaged several times in primary monkey kidney cultures and were not plaque purified.

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Virus assays. Techniques for growth and assay of virus have been described elsewhere, (Choppin and Philipson, 1961).

Counter-current distribution. The experiments were carried out in a hand-driven battery with 20 tubes (E.C. Apparatus Co., 538 Walnut Lane, Swarthmore, Pa., U.S.A.). The phase system consisted of 7 % (w/w) sodium dextran sulfate (Dextran sulfate 500' from AB Pharmacia, Uppsala, Sweden), 1.2 % (w/w) polyethylene glycol ('Carbowax 6000' from Carbide and Carbon Chemicals Company, New York, U.S.A.)) in 0.6 M NaCl with 0.01 sodium phosphate buffer pH 7.0 and was prepared by dissolving the polymers in the buffered salt solution. This system forms no phases at room temperature; at 4°C phases of approximately equal volume are formed. The system was purified, as described elsewhere (Bengtsson and Philipson, 1962). The battery was filled by mixing equal parts of the top and bottom phase and dispensing 20 ml of phase system mixture containing the virus to be tested. The battery was placed in the cold and left for about half an hour when phase systems had formed. The phases were mixed by turning the battery at least fifty times and the phases were then left to separate for 30 minutes. After this time the top phases were transferred to the adjoining tubes and the phases again mixed and left to separate. Usually 19, but in some cases 20, transfers were carried out.

After completion of the transfers the phases were emptied into tubes and assayed for infectivity.

Results and discussion. Experiments were first carried out to find a phase system where the distribution of the virus was about equal between the top and bottom phase, i.e. the partition coefficient is about 1, which is essential for a successful counter-current distribution. Virus adsorbtion at the interface was considered to be of disadvantage. It has previously been shown (Philipson et al., 1960) that in the dextran sulfate-polyethylene glycol system viruses distribute in favour of the top phase at high NaCl concentrations but in favour of the bottom phase at lower

NaCl concentrations. On the basis of these findings we tested the distribution of poliovirus type 2 strain MEF 1 in a number of systems with varying NaCl content and also polyethylene glycol concentration. The results are given in Table 1.

Table 1. Distribution of poliovirus type 2 strain MEF 1 in phase systems with different composition.

System No.	Final concentration of polymers in per cent w/w		Final concentration of NaCl in M	Partition coefficient (K)	Per cent recovery of virus	Interface adsorption
	Dextran sulfate (DxS)	Polyethylene glycol (PEG)				
1	7.0	1.0	0.70	> 12	19	0
2	7.0	1.2	0.60	0.57	69	0
3	7.0	1.3	0.50	< 0.05	60	+
4	7.0	1.3	0.55	0.05	100	+
5	7.0	1.3	0.60	0.2	62	0
6	7.0	1.5	0.50	0.05	110	+
7	7.0	1.5	0.55	0.05	62	+
8	7.0	1.5	0.60	1.25	72	+
9	7.0	1.5	0.70	1.5	52	+

It is clearly seen that for a given polymer composition the partition coefficient tends to increase as the NaCl concentration is raised. Only systems Nos. 2, 8 and 9 gave partition coefficients around 1 and since the two latter showed interface adsorption, system No. 2 was selected for counter-current distribution. Poliovirus type 1 strain 1423/53 behaved similarly in preliminary experiments.

The upper diagram in Fig. 1 shows the result of a 19 transfer counter-current distribution of strain 1423/53 of polio type 1. Two, and possibly three, peaks are obtained in the diagram indicating a considerable heterogeneity. The fractions numbered 3 and 4 were pooled and sub-

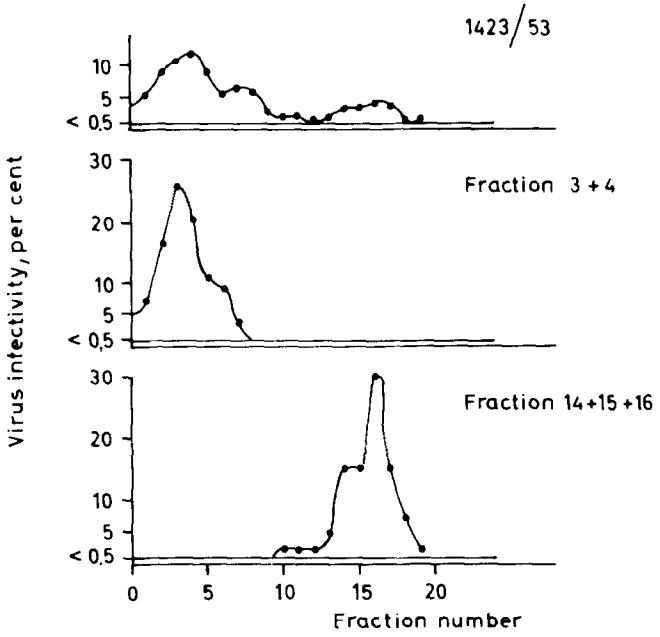

Fig. 1. Counter-current distribution of poliovirus type 1 strain 1423/53 in a phase system $DxS_{7}^{0.48}$ - $PEG_{1.2}^{6000}$ (0.6 M NaCl).
Top: Original distribution of strain 1423/53.
Intermediate: Redistribution of a pool of fractions 3+4 from the original experiment.
Bottom: Redistribution of a pool of fractions 14-16 from the original experiment. Virus infectivity in respective fractions given in per cent of virus recovered.

jected to a new counter-current distribution. The diagram thus obtained is also given in Fig. 1. A peak is obtained at the same position as the left peak in the upper diagram. Similarly the fractions 14, 15 and 16 were pooled and upon redistribution gave rise to a peak at the same position as the peak to the right in the upper diagram of Fig. 1. This would indicate then that the heterogeneity of the strain 1423/53 of polio is genuine and not an artifact produced by the counter-current distribution. This is further supported by marker tests which indicate that the two virus fractions are genetically different. The results of marker tests and also counter-current distribution of other strains of polio virus will be reported elsewhere (Bengtsson and Philipson, in preparation).

It should be possible to apply counter-current distribution to other viruses by using phase systems of the type described here. However, it would be necessary to carry out preliminary experiments of the kind described in Table 1 in order to find a suitable polymer composition and salt concentration. Although we have used counter-current distribution primarily as an analytical tool, it should also be possible to apply it for preparative purposes such as virus purification. It has been demonstrated elsewhere (Philipson et al., 1960; Norrby and Albertsson, 1960) that the dextran sulfate may be removed from the virus by precipitation with $BaCl_2$ or KCl.

REFERENCES

Albertsson, P.Å., Partition of Cell Particles and Macromolecules, Almqvist & Wiksells, Stockholm; John Wiley and Sons, Inc., N.Y., 1960.
Albertsson, P.Å., and Nyns, Ed.J., Nature 184, 1465 (1959).
Albertsson, P.Å., Arch. Biochem. Biophys., in press.
Albertsson, P.Å., and Baird, G.D., Exp. Cell Res., in press.
Baird, G.D., Albertsson, P.A., and Hofsten, B., Nature 192, 236 (1961).
Bengtsson, S., and Philipson, L., in preparation.
Choppin, P.W., and Philipson, L., J. Exp. Med., 113, 713 (1961).
Eagle, H., Science 130, 432 (1959).
Lif, T., Frick, G., and Albertsson, P.Å., J. Mol. Biol. 3, 727 (1961).
Norrby, E.C.J., and Albertsson, P.Å., Nature 188, 1047 (1960).
Philipson, L., Albertsson, P.Å., and Frick, G., Virology 11, 553 (1960).
Wesslén, T., Albertsson, P.Å., and Philipson, L., Arch. Ges. Virusforsch. 9, 510 (1959).
Youngner, J.S., Proc. Soc. Exp. Biol. Med. 85, 202 (1954).