Method	Virus	Volume (ml) Start	Volume (ml) Final[a]	PN (mg/ml) Start	PN (mg/ml) Final	PN eliminated (%)	HA/mg PN Start	HA/mg PN Final	Purification factor y	Virus recovery[b] (%)
MF	B×1	4100	695	0.776	0.024	96.9	827	25725	31.1	100
MF	A-MRC 12	30000	3000	0.644	0.027	95.8	3975	94815	23.8	100
MF	B×1	30000	3000	0.721	0.029	96.0	718	17655	24.6	100
MF + chromatography	B×1[d]	50	83	0.11	0.026	76.4	72727	280827	3.9	93.3
MF + chromatography	B×1[e]	50	265	0.938	0.600	36	68230	88274	1.3	82.8
MF + zonal ultracentrifuge	A-MRC 12[d]	3000	100	0.27	0.032	88.1	94815	686185	7.2	86.7

[a] PN recalculated according to the initial volume; [b] Virus recovery expressed as HA value. HA tests performed at room temperature in tubes using 2fold dilutions of virus in 0.5 ml of PBS + 0.5 ml RBC. HA was read as the highest dilution causing complete RBC agglutination; [c] Sepharose 2B chromatography performed on 3,7×54 cm column; [d] Virus concentrated 10×; [e] Virus concentrated 100×.

and which could therefore be separated from virus particles by membrane molecular filtration. The process has been performed with the Pellicon® Cassette System, using PSVP membranes with a cut-off limit of 1×10^6 daltons (Millipore, Bedford, Mass., USA), and a piston pump generating 4 at working pressure.

For these experiments, membranes with a total surface of 4600 cm^2 were chosen. Lots of MRC 12 type A and B×1 type B inactivated influenza virus strains were used in amounts of 4–30 l. The starting suspensions had a protein nitrogen content (PN) of 0.6–0.8 mg/ml; hemoagglutinin value (HA/mg PN) 1100–4000 for type A and 700–2000 for type B. Positive polyacrylamide gel electrophoresis (PAGE) and immunoelectrophoresis (IEP) against hens' egg proteins were found in all lots.

The virus suspension was first concentrated (from 10:1 to a maximum of 100:1) by forced tangential flow through PSVP membranes. The retentate was then washed with 0.1 M phosphate buffer pH 7.3, until constant PN was obtained.

A typical analytical follow-up of the complete cycle is shown in the figure. The whole cycle was carried out in 9 h. Analysis of the retentate obtained after washing showed 96% elimination of PN and a purification factor of 23–40, expressed as the ratio between the final and starting HA/mg PN value. IEP and PAGE control for hens' egg proteins were negative in all lots prepared by this method. The recovery of influenza viruses calculated as HA activity was 100%. In order to find out to what extent it is possible to eliminate residual impurities from membrane-purified vaccine, the retentates were either chromatographed through a Sepharose 2B column (Pharmacia AB Uppsala, Sweden) or ultracentrifuged with a zonal centrifuge (Beckman, type L2-65B equipped with a rotor type CF-32 Ti). The results are shown in the table. With this simple, 1-step concentration and purification method, more than 95% of PN were eliminated, as shown in the 3 lots presented in the table.

It has also been shown that further processing of MF membrane-purified retentate, either with zonal ultracentrifuge or by Sepharose chromatography, results in reduction of PN to less than 1%, in cases where extremely high purity is required. In comparison with other methods used for influenza vaccine concentration and purification, MF membranes with an exclusion limit of 1×10^6 daltons have the advantages of higher yield, more rapid working cycles, and extremely simple management of molecular filtration.

A newly designed decantation for purified synaptic vesicle

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Summary. A new decanting device of fine stainless steel needles has been developed, which can remove a layer from a gradient with little contamination from the adjacent upper or lower layers. This new apparatus can be used for removal of a very narrow band from a density gradient and has been successfully used in the fractionation of synaptic vesicles from brain homogenates.

There are many instruments available for marking the density gradients used in ultra-centrifugal fractionation[1,2]. However, little attention has been paid to decantation and to the problems of contamination between adjacent layers which are encountered with the routine method[3–5]. A new decanting device of fine stainless steel needles is shown in figure 1.

Methods and results. Fine needles of outer diameter 0.4 mm ⌀ were used in the device as shown in figure 1. We have called the device a 'multi-fine needle' decanter. 25 stainless steel needles were gently inserted into the solution in the centrifugation tube (diameter 14 mm) and a layer of the solution was pushed out by applied pressure. The outflow rate could be controlled by altering the pressure by using an O-ring. The contamination made by turbulence around each of the needle tips was reduced to a minimum compared to the conventional methods shown in figure 2. Quantities as little as 0.5 ml could be removed from the tube without contamination. For a layer

1 R. J. Britten and B. B. Roberts, Science *113*, 32 (1958).
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4 R. J. Heckly, Analyt. Biochem. *7*, 97 (1960).
5 V. P. Whittaker, Meth. Neurochem. *2*, 1 (1972).

centrifugation tube, the only modification required was to increase the number of stainless steel needles. Brain homogenate was fractioned as shown in Figure 3[6], 0.5 ml fractioned were collected from the density gradient using the multi-fine needle decanter and were then examined in the electron microscope (figure 4).

Discussion. The conventional methods for decanting density gradients[3,4] use a single needle; such methods are shown in figure 2. In figure 2, solution is sucked out of the tube, and in the other, the solution is allowed to drip from a small hole in the bottom of the tube. The turbulence around the tip of the needle and the quantity of solution removed depend upon the diameter of the needle and/or the decanting speed. If all the solution in the centrifugation tube is of the same viscosity, and we assume that the solution moves horizontally towards the needle from the periphery of the tube, then when a volume, V, of the solution is sucked upwards into the single needle as shown in figure 2, it is to be expected that a volume, ΔV, moves from the side of the tube towards the tip. As it is difficult to make ΔV move slowly, this leads to turbulence and mixing; that is, it moves broad part of the solution between centre and periphery of the centrifugation tube.

In the multi-fine needle method (figure 1), there is only a small distance between the needles. Therefore, the solution surrounding a needle has to move far less than in the conventional method, thereby preventing contamination from the upper or lower layers. When the instrument was used to the 0.1 M sucrose band of a density gradient designed for separation of synaptic vesicles (figure 3), 0.5 ml layers were obtained[6]. When these layers were examined under the electron microscope, the synaptic vesicles were found mainly in the upper A (figure 4) and B fractions, and membrane contamination was found in the lower fractions, C and D. In conclution, we have developed a device which can be used to remove narrow, defined bands from density gradient with little contamination from adjacent layers.

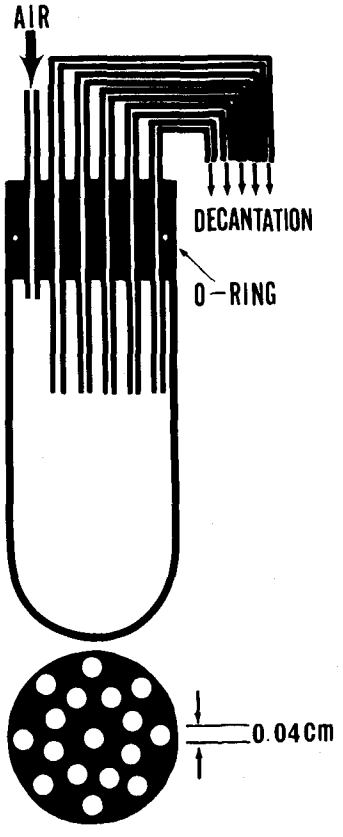

Fig. 1

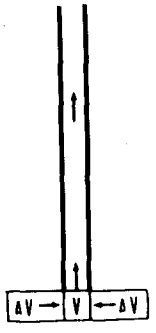

Fig. 2

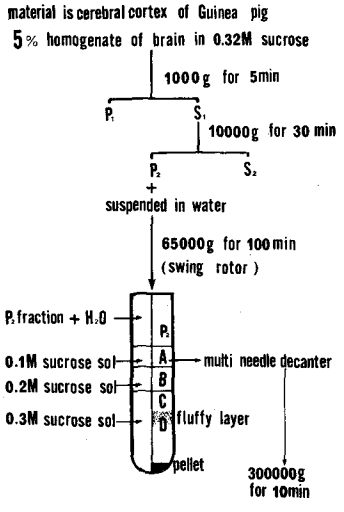

Fig. 3

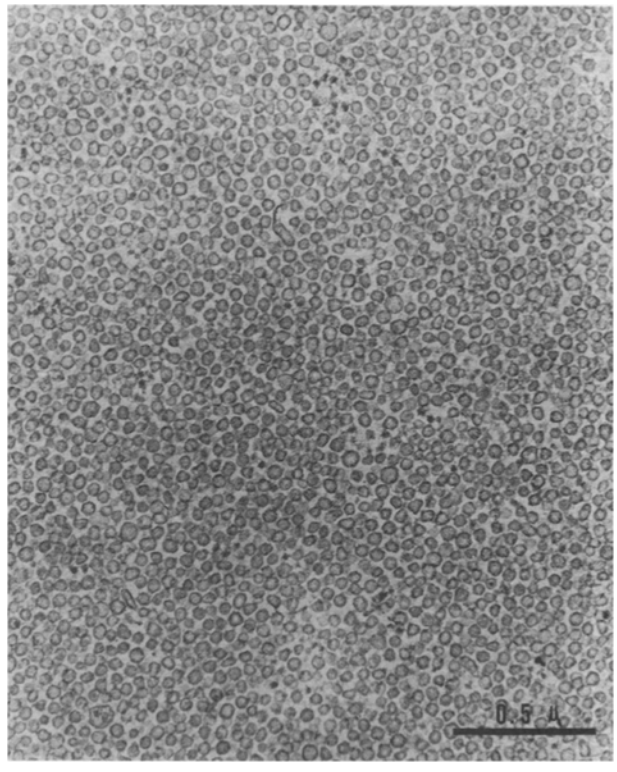

Fig. 4

6 K. Ohsawa and K. Uchizono, Proc. Jap. Acad. Sci. *51*, 202 (1975).