A New Method for Purification of Myxoviruses by Zonal Centrifugation with Two Different Sucrose Density Gradients (36434)

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A number of problems are encountered during the purification of lipid-containing viruses. Host components, *e.g.*, occur as contaminants in the virus preparations and can only be separated with difficulties from the virions. Furthermore, enveloped viruses are not uniform particles of the same size.

These problems can be overcome if both methods of density gradient centrifugation, rate zonal and isopycnic centrifugation, are applied sequentially, particularly in a zonal rotor where large quantities can be handled (1). In contrast to the conventional swing-out rotors, the zonal centrifugation has the advantage of allowing the particles to migrate relatively freely in sector-shaped rotor chambers to form sharp bands for better resolution.

In a newly developed method two different density gradients are applied to the rotor, thereby allowing rate zonal and isopycnic centrifugation simultaneously in the same run.

Materials and Methods. Virus and assay of viral activities. Fowl plague (FPV) and

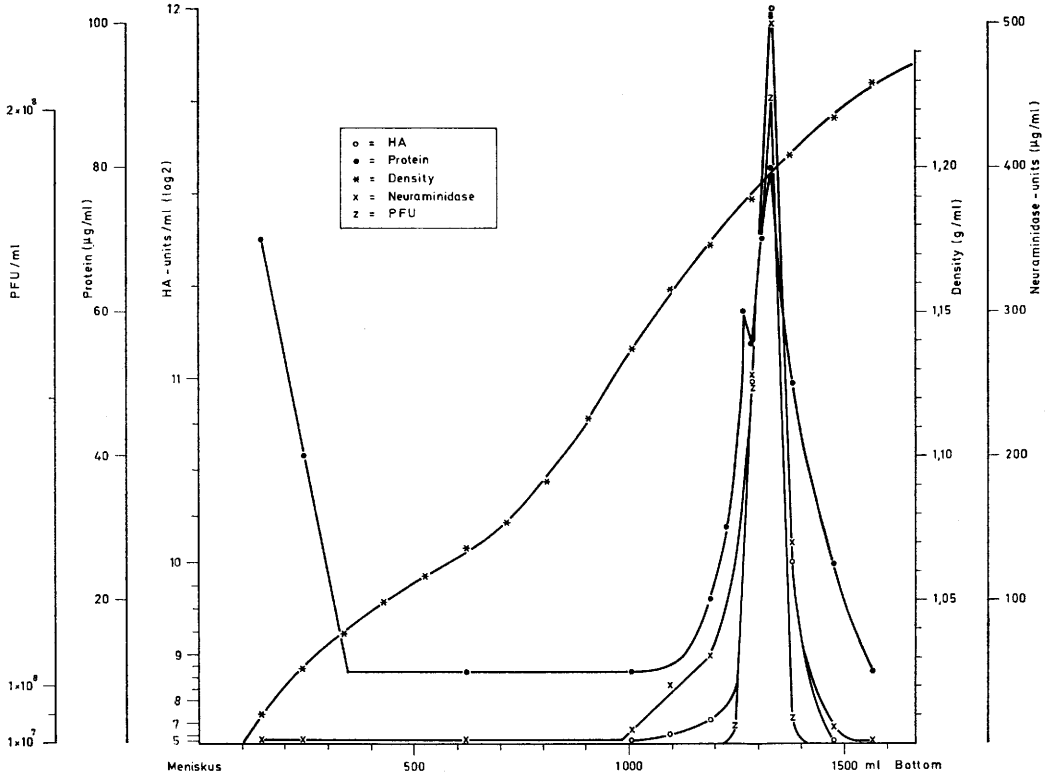

FIG. 1. Distribution of protein and FPV specific activities after centrifugation with two sucrose density gradients. The density was determined by refractometer.

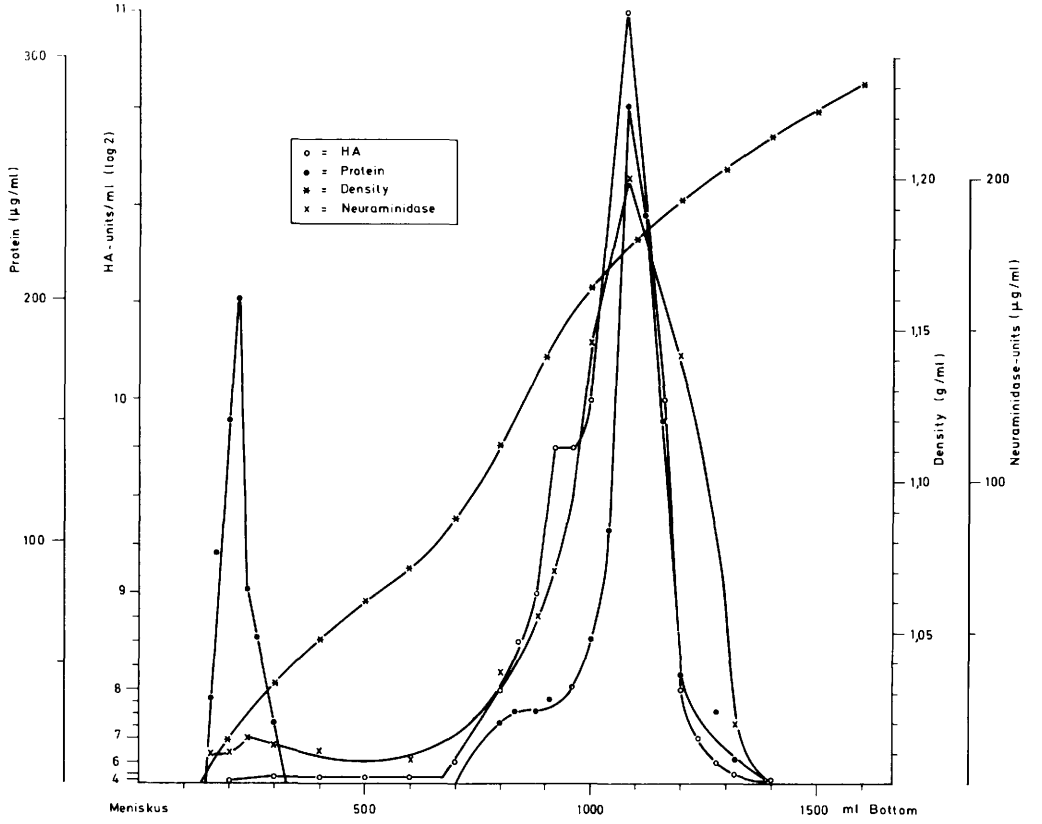

FIG. 2. Distribution of protein and NDV specific activities after centrifugation with two sucrose density gradients.

Newcastle disease viruses (NDV) were used as representatives of ortho- and paramyxoviruses. The viruses were grown in the allantoic cavity of chick embryos. Allantoic fluid with a hemagglutinating titer of 2^{-8} was used as starting material.

Plaque-forming units (PFU), hemagglutination (HA), and neuraminidase activity were assayed as described elsewhere (2). The Folin reaction was used for determining the protein content (3).

Centrifugation. Crude debris was removed by centrifugation at 3000 rpm for 20 min and the virus was sedimented at 35,000*g* for 120 min. Virus pellets obtained from about 4 liters of allantoic fluid were suspended in 150 ml of phosphate buffer saline (PBS), pH 7.2, and centrifuged again for 20 min at 3000 rpm. The supernatant was subjected to zonal centrifugation (Spinco, L2-65B, Beckman Instruments). A 5–20% sucrose gradient (600 ml) was pumped into the rotor chamber. This gradient was underlayed with 800 ml of a 30–50% sucrose gradient which was followed by 265 ml of a 50% sucrose solution to fill the rotor. The virus preparation (150 ml) was then applied to the rotor content. Subsequently the virus sample was displaced from the central axis with 100 ml of PBS. The filling procedure was carried out at 15° and 3000 rpm. The rotor was run at 20000 rpm at 15°. After 50 min the speed was increased to 35000 rpm, and centrifugation was continued for 6 hr at 4°. Finally, fractions of 20 ml were collected at 3000 rpm (15°) by pumping a 55% sucrose solution through the inlet.

Results. The results of representative centrifugation runs (Figs. 1 and 2) demonstrate that both FPV and NDV band in sharp peaks which are clearly separated from con-

taminating proteins. The virus-specific activities, PFU, HA, and neuraminidase, coincide in the same density, which amounts to 1.2 g/ml for FPV and 1.8 g/ml for NDV. The specific activities of FPV and NDV reached efficiency values of 2×10^{-8} g protein/HA-unit (FPV) and 1.4×10^{-8} g protein/HA-unit (NDV). The second minor peak of protein (Fig. 1) and the shoulder of HA-activity (Fig. 2) were regularly found. At present, we have no interpretation for these observations. After this type of purification the material can be concentrated in a Rotor No. 30 (Spinco centrifuge) and the pellet can be resuspended in the desired volume of PBS.

Discussion. The proposed technique of sucrose density centrifugation in a zonal rotor described above resulted in a sharp peak of virus material. The relatively broad band of virus which is obtained by the conventional rate zonal centrifugation in a sucrose gradient is narrowed and condensed at the transition zone of higher sucrose concentration. The virus migrates from there as a sharpened band to its position of isodensity. By applying this principle it is possible to obtain high yields of purified virus in a relatively short time. The specific activities of such viral preparations are in the same range as those found in material produced by adsorption/elution to erythrocytes, by chromatography on calcium phosphate, or by density gradient centrifugations in separate runs (4–6).

The method described leaves space for a variety of modifications. The characteristics of the gradients, *e.g.*, can be adapted to the specific properties of the material to be purified which ensures optimal separations.

Summary. A method was developed that permits rate zonal and isopycnic centrifugation in a zonal rotor in a single run. This procedure effected a high degree of purification of enveloped viruses in a relatively short time.

The authors thank Mr. G. Göttlicher for his assistance. The work was supported by the Sonderforschungsbereich 47, Virologie.

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Received Sept. 10, 1971. P.S.E.B.M., 1972, Vol. 140.