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Separation of Reticuloendotheliosis Virus from Avian Tumor Viruses

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Velocity sedimentation and isopycnic density gradient centrifugation indicate that reticuloendotheliosis virus has a different mass and buoyant density than members of the avian tumor virus group. The group-specific antigen of the avian tumor virus group was not detected in concentrated and purified reticuloendotheliosis virus preparations.

The reticuloendotheliosis virus (REV) was isolated from a turkey poult with leukosis-like lesions in 1958 by Twiehaus (*unpublished data*). The virus is unique in its extreme virulence for experimental animals (5). The relationship of REV, if any, to the avian tumor virus complex is not clear. Complement fixation tests for the group-specific antigen of the avian tumor virus complex (COFAL) have been negative (7). Similarly, host range, immunological, and viral interference studies comparing this virus to the A and B subgroups of the avian tumor virus complex suggest that REV is not related to the complex (1). However, the morphology of REV is very similar to that of the avian tumor viruses (9; K. L. Baxter-Gabbord, Bacteriol. Proc., p. 222, 1971), and REV also contains the ribonucleic acid-dependent deoxyribonucleic acid polymerase activity which is characteristic of this group of viruses (D. A. Peterson et al., Bacteriol. Proc., p. 221, 1971). We have found that REV can be separated from viruses of the avian tumor group by sucrose and cesium chloride gradient centrifugation.

The buoyant density of REV in sucrose gradients is similar (K. L. Baxter-Gabbard, Bacteriol. Proc., p. 222, 1971) to the buoyant density reported for avian tumor viruses (2, 3). We were able to show sedimentation differences between REV and avian tumor viruses by mixing experiments.

As representatives of the avian tumor virus complex, we used Rous sarcoma viruses with the envelopes from avian leukosis viruses RAV-2 and RAV-3. These viruses were kindly provided by P. K. Vogt, University of Washington School of Medicine, Seattle. REV was a generous gift of A. S. Levine, Indiana University Medical School, Indianapolis. The viruses were cultured separately in secondary chicken embryo cell cultures with Eagle's medium containing 6% dialyzed newborn calf serum. The monolayers were treated with diethylaminoethyl dextran (25 μg/ml) for 2 hr before infection to enhance virus attachment as described by Vogt (8). Two days after infection, ^{3}H-uridine (10 μCi/ml) was added to the REV-infected cultures, and $^{32}PO_4^{3-}$ (2 μCi/ml) was added to the RSV-infected cultures. After a 48-hr labeling period, the culture fluids were removed and the virus particles were concentrated by differential centrifugation. The suspended virus preparations were mixed in equal volumes, layered on a 25 to 42% (w/w) sucrose gradient, and centrifuged at 110,000 $\times$ *g* for 4 hr in an SW41 rotor. As indicated in Fig. 1, REV was seperated from RSV(RAV-2)-RSV(RAV-3) by velocity sedimentation in sucrose. Labeled REV also cosedimented with virus purified from liver preparations obtained from REV moribund chicks. Both of these REV preparations were infectious for newborn chicks. The ^{32}P-labeled RSV(RAV-2)-RSV(RAV-3) peak was COFAL-positive.

To determine whether REV has a different buoyant density than members of the avian tumor virus complex, isopycnic centrifugation in a CsCl gradient was performed. REV and RSV(RAV-2)-RSV(RAV-3) preparations were fixed with 3% formaldehyde for 24 hr as described by Sydiskis (6) to prevent disruption of the virus particles during the cesium chloride gradient centrifugation. Equal volumes (0.2 ml) of REV (^{3}H-uridine) and RSV(RAV-2)-RSV(RAV-3) ($^{32}PO_4^{3-}$) were mixed in a cesium chloride solution of an approximate density of 1.20 g/cm^{3}. Centrifugation was for 48 hr at 105,000 $\times$ *g* in an SW50.1 rotor. Fractions were collected through the bottom of the tube, and the density of the fractions was determined. Each fraction was precipitated on membrane filters (Millipore Corp.) with 10% cold trichloroacetic acid. The profile obtained in this mixing experiment is illustrated in Fig. 2.

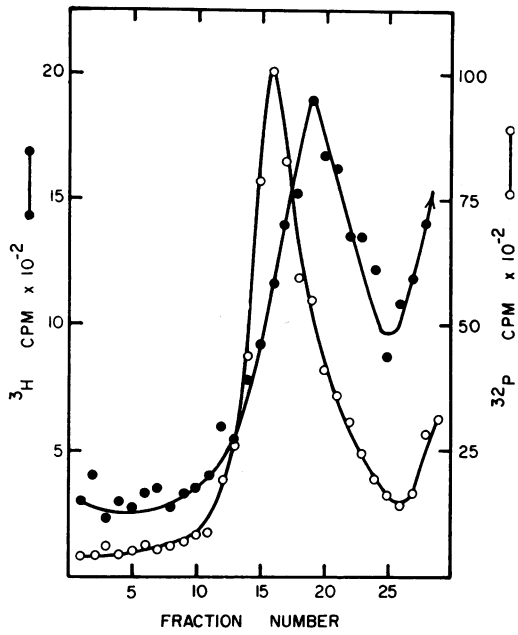

FIG. 1. *Centrifugation of ^{3}H-uridine-labeled REV, ●, and ^{32}P-labeled RSV(RAV-2)-RSV(RAV-3), ○, in a 25 to 42% linear sucrose gradient for 4 hr at 110,000 × g in an SW41 rotor.*

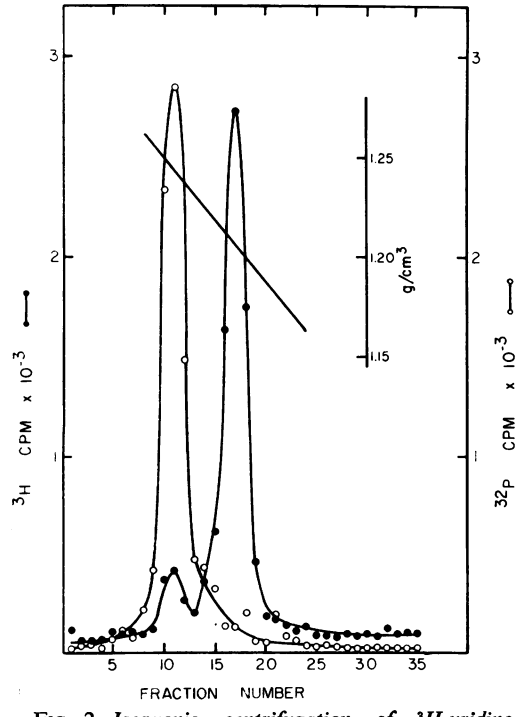

FIG. 2. *Isopycnic centrifugation of ^{3}H-uridine-labeled REV, ●, and ^{32}P-labeled RSV(RAV-2)-RSV(RAV-3), ○, in a cesium chloride gradient for 48 hr at 105,000 × g in an SW50.1 rotor. The viruses were fixed with formaldehyde before centrifugation.*

REV had a bouyant density of 1.203 g/cm^3, whereas the RSV particles banded at a density of 1.242 g/cm^3. The small ^{3}H-uridine peak which cosedimented with the $^{32}PO_4^{3-}$ RSV particles is probably avian leukosis virus present in our cell cultures.

REV does not contain the group-specific antigen of the avian tumor virus complex (7). However, previous tests for the antigen were done with unconcentrated culture fluids obtained from REV-infected cells. To obtain concentrated preparations of REV, livers from infected birds were homogenized with a Ten Broeck tissue grinder. The virus was recovered by differential centrifugation, the high-speed pelleting being through a 40% glycerol cushion. The virus was then centrifuged in a 25 to 40% (w/w) linear sucrose gradient at 110,000 × *g* for 4 hr in an SW41 rotor. Fractions (0.5 ml) were collected and diluted to 1 ml for optical density measurements. Each fraction was then centrifuged, and the pellets were suspended in saline and subjected to three freeze-thaw cycles. COFAL tests (4) were performed with antisera obtained from Microbiological Associates. RSV(RAV-2)-RSV(RAV-3) from culture fluid concentrated fourfold by centrifugation had a titer of 16. Figure 3 demonstrates that

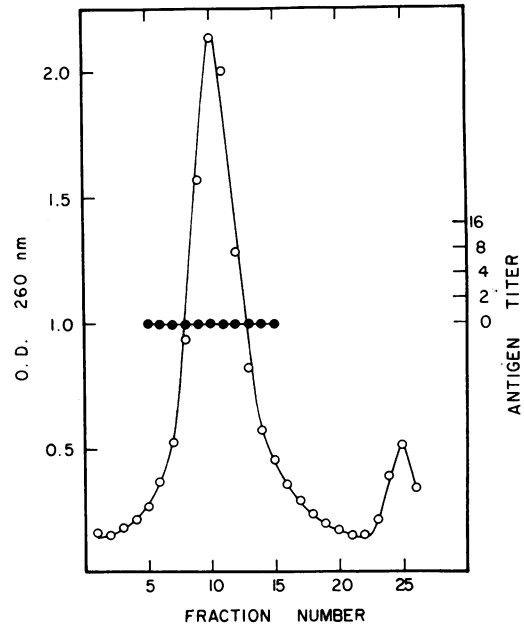

FIG. 3. *Absence of avian leukosis virus group-specific antigen in concentrated REV preparations. Micro complement fixation tests were performed with 2 units of guinea pig complement per well. The results are expressed as the reciprocal of the highest antigen dilution showing 50% hemolysis. Symbols: ○, optical density; ●, antigen titer.*

REV does not have the group-specific antigen of the avian leukosis complex.

These studies indicate that REV has a mass and buoyant density different from members of the avian tumor virus complex. Concentrated REV preparations disrupted by repeated freeze-thaw cycles do not contain the group-specific antigen of the avian tumor viruses. These results are consistent with previous studies (1, 7) that indicate that REV is not a member of the avian tumor virus complex.

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