Pelleting Viruses and Virus-Infected Cells for Thin-Section Electron Microscopy (33731)

KENDALL O. SMITH AND WARREN D. GEHLE

Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014

Large numbers of purified virus particles are ordinarily required to produce centrifuged pellets sufficiently large to manipulate. For example, about 10^{13} particles of a medium sized (68 mμ diam.) virus are required to produce a pellet 1 mm^3 in size. Even pellets this large are sometimes difficult to process for thin sectioning and electron microscopy because of the many manipulations required for embedding. Centrifugation in round-bottomed tubes, a common practice, produces virus pellets whose dimensions are unfavorable for ultrathin sectioning. The thin, disc-shaped pellets thus produced sometimes disintegrate during manipulation. Because simple mechanical problems associated with pelleting and plastic embedding frequently result in loss of precious materials, large amounts of material must be available to insure satisfactory results.

Our laboratory is concerned with recognizing *small* quantities of viruses by electron microscopy, for example, adventitious viruses occurring in cell cultures. We have designed a routine, simple method for pelleting viruses or virus-infected cells into the tips of small, pointed, commercially available plastic tubes. Virus material, which is deposited at the tip of the tubes, remains in a fixed position throughout fixation, dehydration, and plastic embedding. No mechanical manipulation of the pellet is required and virtually the entire pellet is available for sectioning. Good results have been obtained with quantities of virus so small that no pellet could be visualized with the unaided eye.

Materials and Methods. Figure 1 shows the components used for pelleting virus. Part numbers 1 and 3 were constructed of Lucite by the Fabrication Section, N.I.H. Instrument Shop. Part number 2 is the size 00 BEEM[1] polyethylene capsule (this capsule is cylindrical, tapering to a flat point approximately 1 mm^2). Part number 4 is a Lusteroid Spinco centrifuge tube designed for the SW-39 swinging bucket rotor. Parts 1, 2 and 3 are assembled as suggestd in Fig. 1 and placed into the Lusteroid tube, part 4 (Lucite parts were machined so as to give a close fit within the tube; tolerance between parts 1–3 and the Lusteroid tube was about 0.002 in.). About 3.0 ml of virus suspension was loaded into the assembly, placed in the Spinco SW-39 rotor, centrifuged at about 100,000*g* for 1 hr and the supernatant fluid was removed. The parts were disassembled and the BEEM capsule, which contained the virus pellet, was removed for further processing.

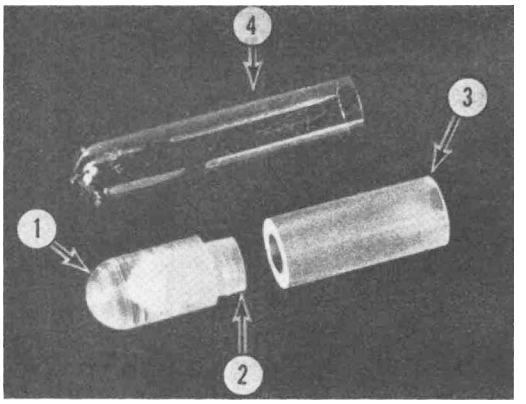

FIG. 1. Lucite assembly (Parts 1, 3) used for pelleting virus into a polyethylene tube (Part 2); Parts 1, 2, and 3 are placed in a Spinco Lusteroid tube and centrifuged in the Spinco SW-39 swinging bucket rotor.

The pellet at the tip of the capsule was fixed for 1 hr in 3% gluteraldehyde at room temperature (Sorensen's phosphate buffer without $CaCl_2$ was used as diluent throughout), rinsed three times in 0.2 *M* sucrose–phosphate solution during a 2-hr period or left overnight at 4°. Postfixation was for 1 hr in 1% osmium tetroxide at 4°. This was followed by three phosphate buffer rinses

[1] Obtained from Better Equipment for Electron Microscopy, Inc., P. O. Box 132, Jerome Avenue Station, Bronx, New York 10468.

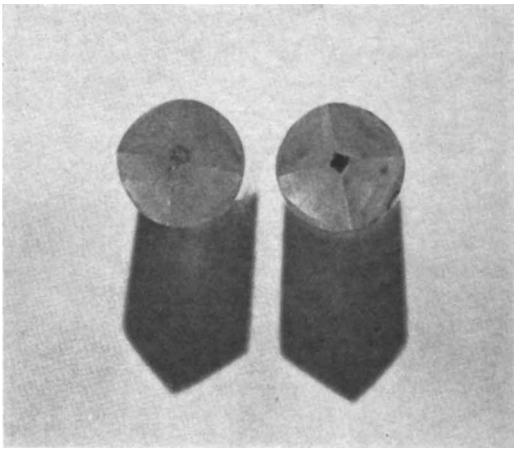

FIG. 2. Top view of plastic-embedded virus pellets produced by centrifugation into polyethylene tubes.

and dehydration in graded ethyl alcohol solutions. The pellet was placed in two successive changes of propylene oxide, followed by a mixture of equal volumes propylene oxide and the final Epon 812 resin mixture. The resin was poured into the BEEM capsules (containing pellets), incubated overnight at 45°, then at 60° for 2 days. After allowing to cool for 1 hr at room temperature, the polyethylene capsule was cut away, leaving the plastic embedded virus pellets similar to those seen in Fig. 2. Ultrathin sections were cut on a Porter-Blum microtome model MT-2. Sections were stained using a saturated solution of uranyl acetate in 50% ethyl alcohol, followed by lead citrate.

A larger assembly was designed to accomodate about 16 ml of fluid (this fits into the Spinco SW-25.2 rotor). The inside cavity in the top part (equivalent to part 3, Fig. 1) was tapered from a relatively large mouth to a base whose inner diameter was slightly less than the inner diameter of the BEEM capsule. Virus sedimenting within the assembly thus drops without obstruction into the BEEM capsule and is deposited at its lower tip. Multiple filling and pelleting in the same BEEM capsule can significantly increase the size of pellets when virus concentrations are low.

The virus used in the study reported here was SV-15, a simian adenovirus. Virus was grown in BSC-1 cells, purified by CsCl density gradient centrifugation (1), then dialyzed free of CsCl.

Virus-infected cells were handled in a manner almost identical to that described above for cell-free virus, except that centrifugation at 2500*g* for 5–10 min was sufficient to pellet unfixed cells. Better results were obtained by fixing cells *after* pelleting rather than before. *Cell* pelleting could be done in an ordinary swinging-bucket, low-speed table centrifuge.

Results. Figure 3 illustrates the appearance of SV-15 virus which had been processed in this manner. Either thick or extremely thin sections could be cut without difficulty. The first material sectioned at the tip of the block was uniformly packed virus; pellets appeared to be well fixed and penetrated with plastic. Virus-infected cells which were prepared in this manner were well-preserved and easily handled.

Discussion. Centrifuging viruses or virus-infected cells into pointed polyethylene capsules produces a small, compact pellet which need not be transferred to another container or manipulated in any way during the fixation-embedding procedures required for thin-sectioning. Consequently, less virus material is required and satisfactory results are more uniformly obtained.

The standard BEEM capsule has the following disadvantage for this work: virus or virus-infected cells tend to cling to the creases in the faceted lower portion of the

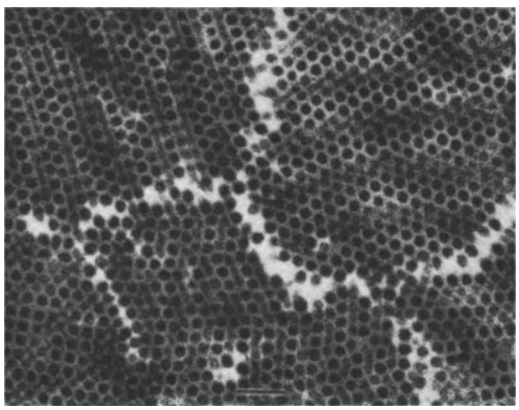

FIG. 3. An ultrathin section through a pellet of SV-15 virus (bar equals 200 mμ).

tube, so that all the material is not deposited at the tip. The BEEM Company has modified the design of the tube, however, to eliminate this problem. They have produced experimental lots of tubes which have no facets, and which "neck-down" to either a pointed or a flat tip. We have found that the pointed tip is more desirable for pelleting small amounts of cell-free virus, whereas the flat tip is more desirable for cells. All the particulate material appears to be deposited at the tips of these tubes.

Summary. A method is described which permits cell-free virus or virus-infected cells to be pelleted into the tips of polyethylene tubes in such a way that the pellets can be fixed, plastic-embedded and sectioned *in* place. This permits successful sectioning of pellets so small that they can not be visualized with the unaided eye. Thus thin-section electron microscopy is possible on specimens containing small concentrations of virus or virus-infected cells.

1. Smith, K. O., J. Immunol. 94, 976 (1965).

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Platelet Alkaline Phosphatase: Normal Values and Variations in Myeloproliferative Syndromes* (33732)

BURNESS F. ANSELL, JR. AND ROBERT B. SCOTT
(Introduced by G. Watson James, III)
Division of Hematology, Department of Medicine, Medical College of Virginia, Richmond, Virginia 23219

Leukocyte alkaline phosphatase (LAP) activity is well established as an aid in the diagnosis of the various myeloproliferative disorders. Measurements of leukocyte alkaline phosphatase have shown elevations in polycythemia vera and infectious leukocytosis, low values in chronic granulocytic leukemia, and variable value in agnogenic myeloid metaplasia and essential thrombocythemia (1, 2). Alkaline phosphatase activity has been measured not only in leukocytes but also in platelets (3, 4). This fact, coupled with the observations of both qualitative and quantitative platelet abnormalities in the various diseases making up the "Myeloproliferative Syndrome," led to our research. The purpose of the present work was to determine whether or not other myeloid cells responded to the same control mechanism which determines the alkaline phosphatase activity of leukocytes. This was tested by measuring alkaline phosphatase activity of leukocytes and platelets simultaneously in conditions in which abnormalities of leukocyte alkaline phosphatase are known to occur. If a single mechanism affected the whole myeloid series one would expect to see similar changes in the activity of alkaline phosphatase in platelets as in white blood cells.

Methods. Whole blood (50–100 ml) was collected from 15 normal controls and from 11 diseased patients using 0.1 vol of 2% disodium ethylenediaminetetraacetate as an anticoagulant. All procedures were carried out at 4°. The plasma was separated by centrifugation at 105*g* for 15 min. The plasma was removed and cell counts were performed. Of the remaining white blood cells, 90–95% were lymphocytes, and a few were eosinophiles. If, however, the white blood cell count was above 5000/mm^3, or more than 10% granulocytes were found, the plasma was respun for an additional 10 min. Platelet counts were done by a phase method (5). The number of platelets per sample varied from 155,000 to 1,795,000 (mean 498,000)/mm^3.

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