Purification of Infectious Feline Leukemia Virus from Large Volumes of Tissue Culture Fluids

L. E. MATHES,* D. S. YOHN, AND R. G. OLSEN

Department of Veterinary Pathobiology and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210*

Received for publication 4 December 1976

Feline leukemia virus from tissue culture fluids was concentrated 100 times by continuous-flow molecular filtration. Virus concentrates retained 100% of their original infectivity. Further purification was accomplished by a single sucrose density gradient centrifugation.

Assembly of C-type ribonucleic acid viruses occurs at the cytoplasmic membrane by a budding process that releases mature virions into tissue culture fluids. The initial step in recovering virus is usually a concentration procedure that reduces the volume of these fluids to a size that can be conveniently accommodated by conventional density gradient centrifugation. Some of the methods used to reduce the volume include $(\text{NH}_4)_2\text{SO}_4$ precipitation (1, 7, 9), polyethylene glycol concentration (2, 3, 9, 11), concanavalin A precipitation (10), and pelleting by centrifugation (6, 9). All of these techniques are effective means of concentrating virus, but they may have a deleterious effect on viral infectivity. Loss of infectivity may be due to disruption or alteration of the surface membrane structures characteristic of C-type ribonucleic acid viruses.

The objective of this study was to evaluate continuous-flow molecular filtration as an alternate procedure for concentrating tissue culture fluids and to specifically test for recovery of viral infectivity.

With continuous-flow molecular filtration, fluids are continuously passed between two membrane filters. Molecules with molecular weights smaller than the exclusion size of the membrane can pass across the membrane (filtrate), whereas larger molecules are retained (retentate) and cycled back into the starting reservoir. As the fluids recycle through the filtration unit, the retentate volume continuously decreases.

For this study, the molecular filtration system (Millipore Corp., Bedford, Mass.) was equipped with one 5-square-foot (0.46-m^2) membrane cassette having an exclusion size of approximately 10^6 daltons. A 20-liter closed flask with input and output connections was attached to the unit, and a high-volume peristaltic pump (Cole-Parmer Instrument Co., Chi-

cago, Ill.) was placed in line on the output side of the flask. The discard filtrate was collected in a second flask. The filtration unit was decontaminated by pumping 2% formalin in normal saline into the system and allowing it to stand for 1 h, or continuously during the period when the unit was not in use. Just prior to use, the formalin was flushed from the unit by pumping 3 liters of sterile distilled water through the system. During operation the molecular filtration system was housed in a 4°C constant-temperature room.

The F422 cell line, provided by F. De Noronha, Cornell University, Ithaca, N.Y., was grown in suspension culture and used as the source of the Rickard strain of feline leukemia virus. Cells were seeded at a concentration of 1.6×10^6 to 1.8×10^6 cells per ml in 2-liter roller bottles containing 200 ml of McCoy 5-A suspension medium (GIBCO, Grand Island, N.Y.) with 20% fetal bovine serum (Reheis Chemical Co., Phoenix, Ariz.). At 3 days, the cultures were fed with 200 ml of fresh McCoy 5-A medium containing 15% fetal bovine serum, and at 5 days the tissue culture fluids were separated from the cell by centrifugation at $400 \times g$ for 10 min. Usually, 24 roller bottles, containing a total of 9,600 ml, were processed each time.

Tissue culture fluids were concentrated by recycling fluids through the system until the volume was reduced 10- to 100-fold. The entire concentration step was accomplished in approximately 3 h. Twenty milliliters of the concentrate was then applied to the top of each 0 to 50% preformed sucrose gradient (68 ml) in TNE [0.01 M tris(hydroxymethyl)aminomethane, 0.1 M NaCl, 0.001 M ethylenediaminetetraacetate] buffer, pH 7.6. The preformed gradients were produced in 1.5- by 4-inch (3.8 by 10.2-cm) cellulose nitrate centrifuge tubes (Beckman Instruments, Inc., Palo Alto, Calif.) using the ISCO model 570 gradient former (ISCO, Lincoln,

FIG. 1. Feline leukemia virus tissue culture fluids, concentrated 10 times, were purified on 0 to 50% sucrose gradients. Twenty milliliters of viral concentrate was layered onto the 68-ml gradient in 1.5- by 4-inch cellulose nitrate centrifuge tubes. The gradients were centrifuged for 2 h at $45,000 \times g$ in a 21 rotor. The virus band formed between 32 and 36% sucrose (density, 1.14 to 1.16). Symbols: ●, optical density; ○, percent sucrose.

TABLE 1. Infectivity of feline leukemia virus preparations taken at various steps during purification

Material tested	Vol (ml)	Titer (FFU ^a /ml)	Concn factor	Total infectivity ^b
Unconcentrated tissue culture fluids	9,600	1.32×10^4	0	1.27×10^8
100-fold concentrated tissue culture fluids	100	1.20×10^6	96	1.20×10^8
Once-banded virus	80	1.82×10^5	120	1.46×10^7
Filtrate	9,500	<5	0	$<4.75 \times 10^4$

^a FFU, Focus-forming units.

^b Infectivity assays were performed by the method of Schaller and Olsen (8).

Neb.). The gradients were centrifuged at 4°C for 2 h in a 21 rotor at $45,000 \times g$ using a model L2 65B ultracentrifuge (Beckman). The opaque virus band was harvested from the gradients by puncturing the bottom of the tube and collecting 2.5-ml fractions (Fig. 1). The purified virus suspension was dialyzed overnight against 50 volumes of TNE buffer to remove the sucrose. The virus infectivity assay was described elsewhere (8).

Viral infectivity assays (Table 1) revealed no loss of infectivity during concentration. However, after one banding, the infectivity titer was reduced approximately one $\log_{10}$. No infectious virus was detected in the filtrate.

Loss of infectivity of oncornaviruses during purification has been a serious problem. The delicate membrane structures are known to be easily denatured by changes in osmotic pressure, the shearing forces of centrifugation, and freeze-thawing (4, 5). By using the molecular filtration system, it was possible to concentrate virus from large volumes of tissue culture fluid as much as 100-fold without measurable loss of

infectivity. After density gradient centrifugation, however, viral infectivity was reduced by approximately one $\log_{10}$.

We have routinely processed 10-liter batches of virus by the molecular filtration procedure and find the method superior to other techniques. The facts that the entire procedure can be accomplished in a single day and that the system is relatively inexpensive to operate make it ideal for routine virus purification. Because the molecular filtration system is closed during operation, the concentrate remains sterile. The closed system also prevents aerosolized infectious virus from escaping. This aspect is of particular importance when moderate- or high-risk agents are being purified.

This study was supported in part by Public Health Service contract N01-CP-5-3571 within the Virus Cancer Program of The National Cancer Institute and by Public Health Service grant P01-CA-15147-01 from the National Cancer Institute.

LITERATURE CITED

1. Duesberg, P. H., H. L. Robinson, W. S. Robinson, R. J. Huebner, and H. C. Turner. 1968. Proteins of Rous

- sarcoma virus. *Virology* 36:73-86.
2. Leberman, R. 1966. The isolation of plant viruses by means of simple coacervates. *Virology* 30:341-347.
 3. McSharry, J., and R. Benzinger. 1970. Concentration and purification of vesicular stomatitis virus by polyethylene glycol precipitation. *Virology* 40:745-779.
 4. Moenning, V., H. Frank, G. Hunsmann, I. Schneider, and W. Schafer. 1974. Properties of mouse leukemia viruses. VII. The major viral glycoprotein of Friend leukemia virus. Isolation and physiochemical properties. *Virology* 61:110-111.
 5. Moenning, V., G. Hunsmann, and W. Schafer. 1973. Partial purification and biological-serological characterization of carbohydrate containing components from preparations of Friend-leukemia-virus. *Z. Naturforsch. Teil C* 28:785-788.
 6. Moloney, J. B. 1969. Biological studies on a lymphoid-leukemia virus extracted from sarcoma 37. I. Origin and introductory investigations. *J. Natl. Cancer Inst.* 24:933-951.
 7. Robinson, W. S., A. Pitkanen, and H. Rubin. 1965. The nucleic acid of the Rous sarcoma virus: purification of the virus and isolation of nucleic acid. *Proc. Natl. Acad. Sci. U.S.A.* 54:137-144.
 8. Schaller, J. P., and R. G. Olsen. 1975. Determination of subgroup-specific feline oncornavirus-neutralizing antibody. *Infect. Immun.* 12:1405-1410.
 9. Smith, R. E., and E. H. Bernstein. 1973. Production and purification of large amounts of Rous sarcoma virus. *Appl. Microbiol.* 25:346-353.
 10. Stewart, M. L., D. F. Summers, R. Soeiro, B. N. Fields, and J. V. Maizel, Jr. 1973. Purification of oncornavirus by agglutination with concanavalin in A. *Proc. Natl. Acad. Sci. U.S.A.* 70:1308-1312.
 11. Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. *Virology* 40:734-744.