Concentration of Oncogenic Herpesviruses by Methyl Alcohol Precipitation

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Oncogenic herpesviruses, like many other viruses, can be concentrated effectively from large volumes of culture fluids by precipitation with methanol with good recovery of infectivity.

Because ultracentrifugation of large volumes of culture fluids to recover viruses is expensive, time consuming and, in the case of oncogenic viruses, potentially hazardous, we have searched for alternative procedures. In 1947, a procedure for concentrating influenza virus from allantoic fluid was described (2) that used cold methanol to precipitate the virus. We have found that this procedure is effective for oncogenic herpesviruses. Because the procedure currently does not seem to be widely known to many virologists, it is reported here to call attention to it as a useful alternative to certain other procedures for concentrating herpesviruses, such as by polyethylene glycol (1).

The viruses used in this study include Epstein-Barr virus (EBV) from P3HR-1 cells, Herpesvirus saimiri (HVS) grown in Vero, CV-1, or owl monkey kidney cells, herpes simplex virus type 2 (HSV2), strain MS, grown in Vero or in secondary cultures of domestic rabbit kidney cells, and the cottontail rabbit virus (CRV) that causes lymphomas in cottontail rabbits (5), grown in secondary cultures of domestic rabbit kidney cells.

EBV infectivity was assayed by indirect immunofluorescence (3) in Raji cells; the other viruses were assayed by end point dilution or by plaque counts in owl monkey kidney, Vero, or domestic rabbit kidney cells, respectively.

One-half volume of methanol, chilled to −65 C, was mixed slowly into clarified culture fluids containing virus and chilled to +2 C in an ice bath. The temperature of the mixture tended to rise as the methanol dissolved in the aqueous culture fluid; if it rose above +3 C, no further methanol was added until the mixture was again chilled. After the methanol had been added, the mixture was stirred for 3 h. The white precipitate was collected by centrifugation at 2 C in 250-ml bottles in a Sorvall centrifuge (GSA rotor, 10,000 rpm. 15 to 20 min); because of the nature of the precipitate, a low-speed continuous-flow rotor could also have been used, although such a rotor was not tested. The pellets were resuspended in a desired buffer and chilled to 2 C; at this point, the methanol had been diluted out, and the suspension could safely be allowed to warm to room temperature.

Infectivity and complement-fixing (straight-line assay) titers of EBV and HVS concentrated by different methods are compared in Table 1. (The initial titer of EBV was less than 10 antigen-inducing units/ml by immunofluorescence in Raji cells; the initial titer of HVS was 10^4.5 mean tissue culture infective doses/ml.) The standard preparation of EBV concentrated 100-fold by zonal ultracentrifugation had a complement-fixing titer about the same as the complement-fixing titer of EBV concentrated 50-fold by methanol precipitation. The infectivity titer was much greater in the latter, however. “Aging” (4) the P3HR-1 cells for 10 days at 33 C before harvesting the virus increased the complement-fixing titer, but not the infectivity titer. With HVS, the methanol procedure yielded a twofold higher complement-fixing titer than did ultracentrifugation, but the infectivity titer was more than 10-fold higher with the methanol.

The experiments shown in Table 2 were done to test for the percentage recovery of infective virus by using another oncogenic herpesvirus, CRV. Experiments A through D indicate a recovery of about 12 to 30% of the starting infectivity.

To test for the amount of cellular protein that occurs in the precipitate as a contaminant, uninfected cells were labeled by growing them in medium in which 3H-labeled amino acids replaced the usual amino acids. The cells were disrupted by six cycles of freezing and thawing and were mixed with a CRV harvest. As indicated in Table 2, experiments E and F, about
30% of the trichloroacetic acid-precipitable counts in the starting material was recovered in the methanol precipitate. Similar experiments, with similar results, have been done with HSV2 (data not shown).

Methanol precipitation, effective for oncopogenic herpesviruses, is relatively simple, inexpensive, and avoids ultracentrifugation of large volumes of culture fluids. Containment is much more easily accomplished, which is especially important with potentially hazardous viruses. Judging from the experiment with radioactive methanol, the methanol contains almost certainly precipitated virus, which is aggregated, albeit not aggregated, by methanol.

**Table 1. Comparison of different procedures for concentrating EBV and HVS**

| Virus | Preparation | Infectivity* titer | Complement-fixing titer |
|-------|-------------|-------------------|-------------------------|
| EBV   | Zonal ultracentrifugation* (100×) | <10*<sup>1</sup> | 1:32 (3+) |
| EBV   | Methanol (50×); P3HR-1 cells not "aged" | 10* | 1:8 (4+) |
| EBV   | Methanol (50×); P3HR-1 cells "aged" 10 days at 33 C | 10* | 1:32 (4+) |
| HVS   | Ultrafiltration: 29,000 rpm, 2 h (10×) | 10<sup>4.6</sup> | 1:16 (3+) |
| HVS   | Methanol (10×) | 10<sup>7.2</sup> | 1:32 (4+) |

* Titers for EBV are based on a count of immunofluorescent cells; titers for HVS were obtained by end point dilution assay and are expressed as mean tissue culture infective dose per milliliter.

**Table 2. Recovery of infectivity or of trichloroacetic acid-precipitable radioactive cell protein by methanol concentration of culture fluids containing CRV**

| Preparation | Experiment |
|-------------|------------|
| A           | B          | C           |
| Vol (ml) | PFU/ml | % | Vol (ml) | PFU/ml | % | Vol (ml) | PFU/ml | % |
| Original material Supernatant | 450 | 9.2 × 10<sup>4</sup> | 100 | 550 | 4.0 × 10<sup>4</sup> | 100 | 900 | 3.1 × 10<sup>4</sup> | 100 |
| Resuspended MeOH pellet | 675 | 3.0 × 10<sup>4</sup> | 4.8 | 775 | 5.0 × 10<sup>4</sup> | 176 | 1,350 | 5.0 × 10<sup>4</sup> | 242 |
| | 2.5 | 2.5 × 10<sup>4</sup> | 15 | 10 | 2.5 × 10<sup>4</sup> | 11.4 | 20 | 3.9 × 10<sup>4</sup> | 27.9 |
| D | E | F |
| Vol (ml) | PFU/ml | % | Vol (ml) | Counts/min | % | Vol (ml) | Counts/min | % |
| Original material Supernatant | 800 | 6.8 × 10<sup>4</sup> | 100 | 110 | 197.5 | 100 | 44 | 302 | 100 |
| Resuspended MeOH pellet | 1,200 | 4.2 × 10<sup>4</sup> | 92.6 | 165 | 66.3 | 50.3 | 66 | 207 | 103 |
| | 25 | 2.7 × 10<sup>4</sup> | 12.4 | 5 | 1,326.3 | 30.5 | 2 | 1,916 | 28.8 |

*PFU, Plaque-forming units.
higher infectivity titers by this method (personal communication). M. C. Desgranges and D. V. Ablashi prepared small lots of EBV from P3HR-1 and Ly-38 cell cultures for infectivity and viral deoxyribonucleic acid (DNA) purification. In two separate experiments, higher infectivity titers ($10^{2.0}$ and $10^{2.6}$/ml) were obtained. For preparing viral DNA, concentration by methanol appeared to be as effective as concentration by ultracentrifugation (unpublished data).

Thus, the findings from these two laboratories support the conclusion that this method can be used commercially as well as for further viral purification.

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LITERATURE CITED

1. Adams, A. 1973. Concentration of Epstein-Barr virus from cell culture fluids with polyethylene glycol. J. Gen. Virol. 20:391-394.
2. Aiston, S., E. Bohnel, H. R. Cox., and J. Van Der Scheer. 1947. The purification and concentration of influenza virus by means of alcohol precipitation. J. Immunol. 56:149-163.
3. Henle, G., and W. Henle. 1966. Immunofluorescence in cells derived from Burkitt’s lymphoma. J. Bacteriol. 91:1248-1256.
4. Henle, W., G. Henle, B. A. Zajac, G. Pearson, R. Waubke, and M. Scriba. 1970. Differential reactivity of human sera with early antigens induced by Epstein-Barr virus. Science 169:188-190.
5. Hinze, H. C., and D. L. Wegner. 1973. Oncogenicity of rabbit herpesvirus. Cancer Res. 33:1434-1435.