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ARENAVIRUS CONCENTRATION BY MOLECULAR FILTRATION.(U)
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Arenavirus Concentration by Molecular Filtration

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ABSTRACT

Liter volumes of a human arenavirus pathogen (Machupo) and a nonpathogen (Tacaribe) were concentrated 30 to 100 times in less than 90 min without significant loss of particle infectivity.

Various molecular weight polyethylene glycols have been used in the concentration of several arenaviruses (2, 7), and other animal and bacterial viruses (1, 5, 8, 11). Under ordinary laboratory conditions this method is quite satisfactory; however, under the rigorous containment conditions required for virulent viruses this method is cumbersome, time consuming, and therefore, less desirable. In addition, even small amounts of polyethylene glycol are undesirable in virus concentrates prepared for human vaccine studies. To circumvent these problems, we have utilized a molecular filtration unit manufactured by the Millipore Corporation (Pellicon Cassette System, Bedford Mass.). Virus concentration by molecular filtration has been previously described (3, 4, 9). While numerous system designs are available, the two commonly used basically consist of multiple or single membranes through which fluid flows in a recirculating or nonrecirculating mode. We have utilized both designs in our attempts to concentrate liter volumes of several arenaviruses and have found the recirculating multiple membrane Pellicon Cassette System superior, due to the speed of concentration and efficiency of virus recovery. Because of these features and the ease with which this system can be handled in a P-4 containment facility, much of the work involved in the concentration of human arenavirus pathogens can be eliminated. Figure 1 illustrates the Pellicon Cassette unit and the concentration design used in these studies.

Cell culture fluids containing 10^7 - 10^8 $\log_{10}$ plaque forming units (PFU) and 10^8 - 10^9 physical particles per milliliter of Tacaribe or Machupo virus were prepared by infecting baby hamster kidney cells (BHK-21) grown in 1/2-gallon roller bottles with the Malale or Carvallo strain of Machupo virus and strain TRVL 11573 of Tacaribe virus. The

multiplicity of inoculation for Machupo virus was 1.0 while that for Tacaribe virus was 0.1 PFU/cell. Supernatant fluids containing the virus suspended in maintenance medum E-199 (Grand Island Biologics) and 5% fetal calf serum were harvested 48-72 h postinoculation and stored at -70°C. When sufficient volumes (3 to 6 liters) of these supernatants were obtained, they were thawed and pooled prior to concentration. The supernatants were then poured into holding tank A of our concentration unit, clarified of extraneous cellular debris by filtration through two Millipore pre-filter pads contained in B and collected in holding tank C (Fig. 1A). The clarified fluid was pumped through the intake port of the molecular filtration unit D which contained five 225 cm^2 Pellicon membrane filters with 10^6 retention capacity, layered on top of one another and sandwiched between two leucite blocks (Fig. 1B). Fluid entering the unit passed over these filters (from right to left) in a tangential flow. Components with a molecular weight larger than 10^6 were presumably retained while those with a molecular weight less than 10^6 passed through the filters. These retentates and filtrates were collected separately by a manifold system contained within the filtering apparatus. The retentate was recirculated into holding tank C while the filtrate was collected separately in tank E. Flow rates were determined by the viscosity of the sample, the number and retention capacity of filters used, and the pressure applied to the recirculating fluid. Flow rates with viscous concentrates and five filters at 10 lb/in^2 averaged 60 ml/min. Table 1 lists the results from several different concentration runs with both Tacaribe and Machupo viruses.

The results illustrate the ease and efficiency of arenavirus concentration afforded by the Pellicon Cassette System. Of significance

was the fact that, even under minimal pressures, 30 to 100-fold concentrations were obtained in less-than 2 h without significant loss of virus infectivity. With its high flow rate and gentle action, this system is well suited for the concentration of fragile viruses and also for the concentration of viral antigens to be used as vaccines in humans.

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TABLE 1. Concentration of Machupo and Tacaribe viruses in a Pellicon Cassette System

Processing conditions and results	Machupo virus		Tacaribe virus, lot		
	Malale	Carvallo	1[a]	2	3
Time (min)[b]	60	45	90	60	90
Pressure (lb/in^2)[c]	10	10	10	25	25
Results					
Volume (ml)					
Starting	3600	2300	6000	4500	6000
Final	115	75	60	125	60
Concentration factor	31	30	100	36	100
Infectivity (PFU/ml)					
Starting	6.0×10^7	3.9×10^7	-	2.0×10^6	2.0×10^6
Final	1.7×10^9	9.0×10^8	-	9.0×10^7	1.0×10^8
Virus particles/ml[d]					
Starting	6.0×10^8	-	2.5×10^8	-	-
Final	1.7×10^{10}	-	2.0×10^{10}	-	-
% Recovery[e]					
PFU	90	75	-	100	50
Vp	91	-	80	-	-

[a] Formalin-inactivated

[b] Total time required for indicated concentration.

[c] Average pressure for run.

[d] Determined by quantitative electron microscopy (6, 10).

[e] (Postconcentrate/preconcentrate) x 100.

Figure Legends

Figure 1a. Concentration apparatus assembled in a P-4 containment hood line. (A) crude sample holding tank, (B) tripod filter holder containing two Millipore pre-filter pads, (C) clarified fluid holding tank, (D) Millipore Pellicon Cassette Molecular Filtration unit and peristattic pump with rate controller (not labeled). (E) filtrate holding tank.

Figure 1b. Diagramatic sketch of molecular filtration unit.

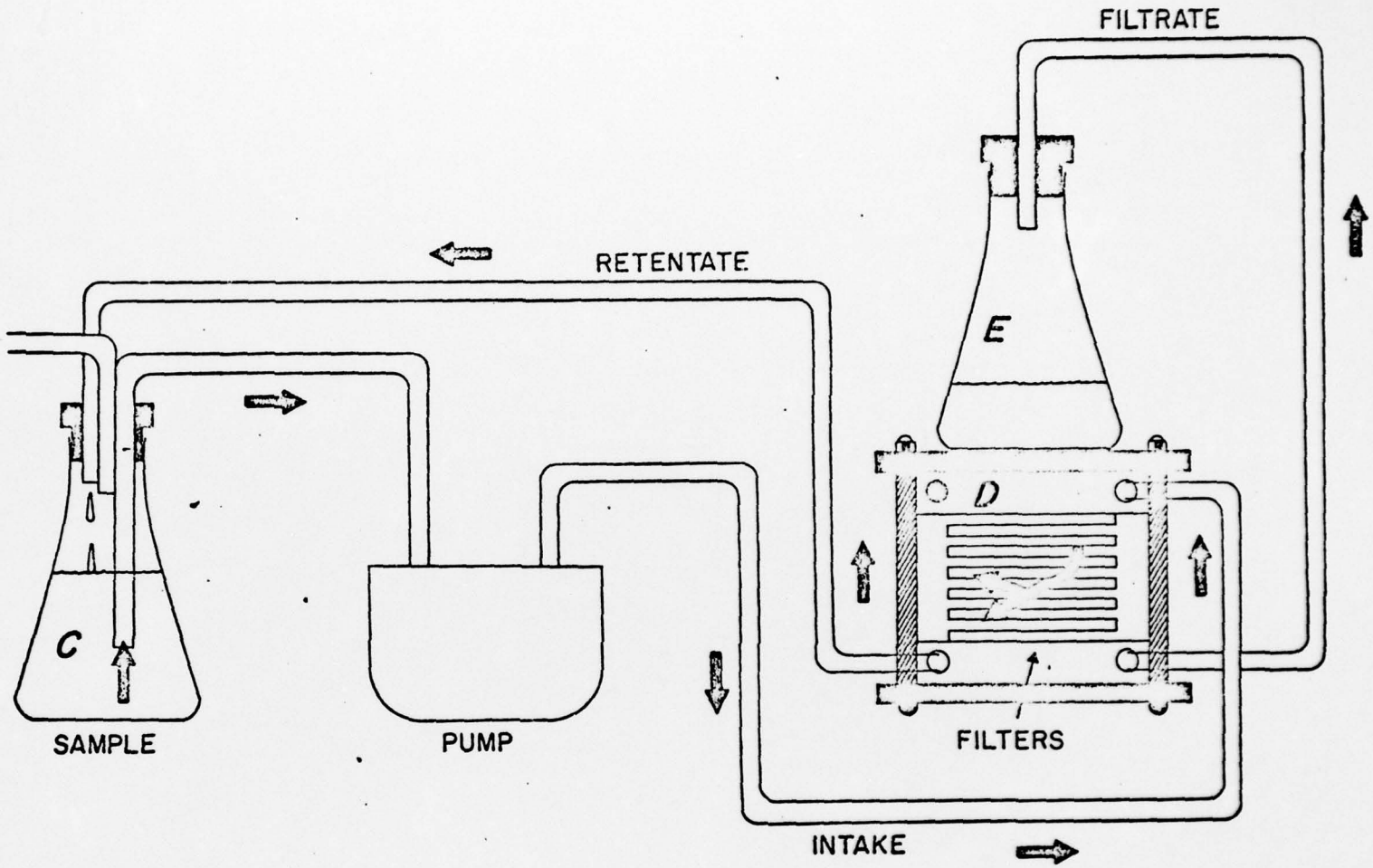

FIG 3B

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