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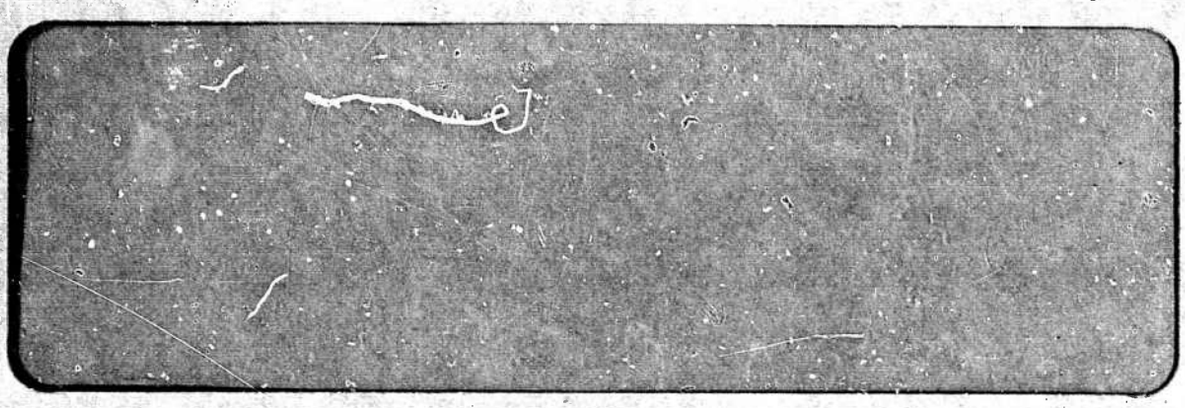

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Ultrafiltration as a Method for Concentrating Rift Valley Fever Virus Grown in Tissue Culture

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Filtration by means of a Diaflo ultrafilter was used to concentrate three 1,000-ml lots and one 3,000-ml lot of tissue culture-grown Rift Valley fever virus. Quantitation of both infectivity and total protein was achieved. Water treatment with continued ultrafiltration of the virus concentrate provided a final virus product approximately 99.25% free of low-molecular-weight materials originally present in the growth medium.

Several methods for concentrating Rift Valley fever (RVF) virus by precipitation with methanol, potassium aluminum sulfate, and ammonium sulfate have been investigated in these laboratories (2). In an attempt to reduce possible alteration or denaturation of the virus, a less drastic concentration and purification procedure involving Diaflo ultrafiltration [Amicon Corporation Publication no. 403 (1970), Lexington, Mass.] was investigated. Information presented in this report indicates that the Diaflo ultrafiltration process yields a virus concentrate with 99.25% of the low-molecular-weight materials removed.

RVF virus (van Wyk strain) was propagated in cells and was processed for use in this study by a method previously described (3). Tissue cells and cellular debris were removed from the virus suspension by low-speed centrifugation (750 × *g*). The supernatant liquid containing the virus was then filtered through an XM-100 membrane in a model 2000 Diaflo ultrafiltration cell (Amicon Corp., Cambridge, Mass.), operated in an ice-water bath (4 C) at an up-stream pressure of 50 psi. The XM series of membranes was chosen because they are "nonionic" and retain particles with a molecular weight of 100,000 or greater and permit passage of materials of low molecular weight, i.e., salts, simple sugars, organic acids, water, and vitamins. The volume of water used in each dialysis experiment is indicated in Table 1. The time required for each ultrafiltration run varied from 24 to 48 hr but never exceeded 48 hr. Flow rates through the membrane started at about 5 ml/min and declined to 0.6 ml/min after 48 hr of operation. Temperature was held at 4 C during the entire operation.

The virus concentration in samples from the ultrafiltration process was determined by the intracerebral inoculation of mice with 10-fold serial dilutions to an LD_{50} end point (4). The method of Matsushita et al. (5) was used to determine total protein. The values for the original virus suspensions and concentrates after ultrafiltration treatment were reduced by the control value for the basic medium. A model 31 conductivity bridge (Fisher Scientific Co.) was used to measure ohms of resistance of RVF virus suspensions. Low-molecular-weight content was read from a plot of ohm resistance against known concentrations of low-molecular-weight material. The known concentrations of low-molecular-weight material were supplied by the medium [Daniels et al. (1), modified Eagle's minimum essential medium] in which the virus was grown, diluted in a series of 10-fold dilutions.

Virus suspensions were concentrated 20 to 24 times; the results (Table 1) indicate that the virus was effectively retained by the Diaflo membrane after a single ultrafiltration or after water additions and continued ultrafiltration and removal of low-molecular-weight materials from the concentrates (Table 1). The effluent contained approximately 1% virus infectivity, indicating little loss of virus through the membrane itself. Quantitation of the concentrates showed 40 to 100% recoveries of infectivity after dialysis and concentration by this process.

Approximately 70 to 75% of the protein was recovered in the dialysates (Table 1); the remaining 25 to 30% was recovered in the effluent. Thus, 100% of the protein was accounted for in all three Diaflo runs.

The retention of low-molecular-weight materials in the dialysates (concentrates) varied directly with the amount of water used for

TABLE 1. *Rift Valley fever (RVF) virus recovery and product characteristics before and after Diaflo ultrafiltration*

Virus lots (Diaflo concd)	Test measurements		Total protein		Infectivity			Low-molecular-weight materials	
	Vol of RVF virus (ml)	Vol of water for dialysis (ml)	Amt[a] (μg/ml)	Per cent recovery	Log_{10} $MICLD_{50}$ per ml	Log_{10} $MICLD_{50}$[b]	Per cent recovery	Resistance (ohm-cm)[c]	Content (%)
1									
Unconcn	1,000		108,000		5.52	8.52		100	100
Concn	62	450	75,640	74	6.52	8.28	57	4,000	4.25
2									
Unconcn	1,000		102,000		7.19	10.19		90	100
Concn	41	1,880	67,035	74	8.85	10.46	100+	18,000	0.75
3									
Unconcn	1,000		103,000		6.69	9.69		90	100
Concn	50	1,800	51,250	69	7.60	9.30	41	10,000	0.90
4									
Unconcn	1,000		ND[d]		7.19	10.67		86	100
Concn	155		ND		8.29	10.48	64	1,300	9.00

[a] Volume × μg/ml = total protein.
[b] Volume × log_{10} $MICLD_{50}$ per ml = log_{10} total $MICLD_{50}$.
[c] Low-molecular-weight content was read from a plot of ohm-cm resistance against known concentrations of low-molecular-weight material. The known concentration of low-molecular-weight material was the growth medium diluted 10-fold.
[d] ND, not done.

TABLE 2. *Conductivity of certain medium combinations*[a]

Solution	Description of medium combinations	Ingredient added	Resistance (ohms-cm)	Comments on changes in conductivity
1	Distilled water		500,000	
2	Solution 1 + phenol red	0.002% Phenol red	10,000	Di-sodium salt present
3	Solution 2 + dextrose	1.0% Dextrose	10,000	
4	Solution 3 + vitamins	2.0% Vitamins (stock solution)	10,000	
5	Solution 4 + nonessential amino acids	2.0% Nonessential amino acids (stock solution)	10,000	
6	Solution 5 + L-glutamine	2.0% L-glutamine (stock solution)	2,300	Formation of aminoglutaramic acid
7	Solution 6 + lactalbumin	0.25% Lactalbumin hydrolysate	1,400	Hydrolyzed with enzyme and neutralized with salt formation
8	Solution 7 + reducing agents L-cysteine and ascorbic acid	0.026% L-Cysteine 0.005% Ascorbic acid	1,200	Sodium salts of cysteine and ascorbic acid
9	Solution 8 + bovine serum	10% Bovine serum	430	Normal blood salts
10	Solution 9 + salts (complete medium)	0.82% Salts	70	Highly ionized salts

[a] Daniels et al. (1) modified Eagle's minimum essential medium.

dialysis through a ratio of about 1:3 (virus material:water); thereafter, no appreciable change in conductivity occurred (Table 1). As measured by the change in conductivity from 80 to 100 to about 20,000 ohms, the low-molecular-weight material was reduced from 100 to 0.75% low-molecular-weight content in the final virus product. As expected, the conductivity was essentially all attributed to the ionized salts present in the infecting medium (Table 2) and not due to high-molecular-weight materials.

The concentration technique just described

demonstrates its usefulness in both concentrating and partially purifying RVF virus after growth in a tissue culture system. The method is simple and yet capable of processing large amounts of tissue culture-grown RVF virus. Additional studies with other viruses are warranted on the basis of these findings. Other advantages are (i) that equipment is cheaper and therefore more available to the average laboratory and (ii) there is less danger of inactivating virus than by chemical or solvent precipitation methods. There are certain disadvantages to the system. First, it is difficult to keep the system sterile for long periods of time, thereby reducing the amount of material that can be dialyzed or processed at any given time. However, continued addition of antibiotics to the system may be helpful in this respect because most antibiotics are quite soluble and would pass into the effluent and not build up in the concentration. Second, difficulty was experienced in achieving a high concentration of the virus because of buildup of residues and apparent plugging of the filter membrane itself.

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