of the liver of the rats fed mannitol and cacao-butter ranged from 0.62 to 1.80% with an average value of 1.23%, as compared with the average glycogen content of the livers of control rats fed cacao-butter alone of 0.14%.

It is impossible to compare the results of this series with our own experiments since the treatment of the animals was quite different in the 2 series. Under the experimental conditions used by us, it is evident that mannitol did not serve as a ready available source of glycogen. A similar lack of ready utilization of mannitol is shown by the reported failure of mannitol to relieve insulin shock in white rats.[6]

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Concentration of Poliomyelitis Virus by Ultrafiltration.*

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The obvious desirability of finding a means for the more adequate concentration of any of the filterable viruses, led us to attempt to adapt the ultrafiltration method, used by Seibert[1] in the study of tuberculin, to the concentration of poliomyelitis virus. That several of the well studied filterable viruses can be retained by filters or ultrafilters has been known for some time. But the work has been done largely for the purpose of determining the size of the viruses. Krueger and Schultz[2] and Clifton, Schultz and Gebhardt[3,4] for example, have made careful studies of the filterability of poliomyelitis virus through graded collodion membranes. Their results indicate that the size of this virus is certainly less than 50 millimi-

[6] Voegtlin, C., Dunn, E. R., and Thompson, J. W., *J. Pharmacol. and Exp. Therap.*, 1925, **25**, 168.

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[1] Seibert, F. B., *J. Biol. Chem.*, 1928, **78**, 345.

[2] Krueger, A. P., and Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 600.

[3] Clifton, C. E., Schultz, E. W., and Gebhardt, L. R., *J. Bact.*, 1931, **22**, 7.

[4] Poliomyelitis. 1932, p. 49. Milbank International Committee for the Study of Infantile Paralysis.

crons in diameter and probably below 25 millimicrons. We have previously employed distillation *in vacuo* and fractional protein precipitation (Clark, Schindler and Roberts[5] and Clark, Roberts and Preston[6]) for the successful purification and concentration of poliomyelitis virus. Both of these methods, however, necessitate the subsequent elimination of the large amounts of salts by dialysis, before the material can be used for animal inoculation. The method here presented avoids that difficulty and we hope that it may give a more adequate basis for the study of the properties of poliomyelitis and other viruses.

Dried soluble cotton† was dissolved in concentrations of 8, 10, and 12% in glacial acetic acid and allowed to age, as a rule, at least a month, before being used to form the membranes over the alundum shells of medium porosity. Sterile distilled water was employed for washing the membranes, subsequently testing for the presence of acid with Congo Red. At least a liter of water was run through the membrane after the test for acid became negative. The permeability of the membranes was determined by their capacity to hold back Congo Red, a solution of crystallized egg albumin and a solution of hemoglobin. The Congo Red was tested for by the addition of acid to the ultrafiltrate, and the presence or absence of egg albumin was determined by using a specific precipitating serum of high titre. The increasing density of the red color in the "concentrate," when a small amount of laked blood had been added to the material to be filtered, and the colorless character of the ultrafiltrate served as an indicator of value. After our technic had been controlled in this manner a number of times, we discontinued the addition of any of these foreign substances to our filtrates. Careful visual inspection, the rate of flow of the wash water, and the gradual concentration in the ultraresidue of the small amount of hemoglobin normally present in brain cord filtrates proved ordinarily to be sufficient checks of the adequacy of the membranes.

In our earlier experiments, we autoclaved the membranes after they had been washed. This made them less permeable and at the same time more fragile but nevertheless, successful results were obtained by this method. Both the 8% and 10% autoclaved membranes proved adequate for the concentration of poliomyelitis virus; the 12% membranes became too impermeable. Several experiments with this procedure are presented in Table I.

[5] Clark, P. F., Schindler, J., and Roberts, D. J., *J. Bact.*, 1930, **20**, 213.

[6] Clark, P. F., Roberts, D. J., and Preston, W. S., *J. Prev. Med.*, 1932, **6**, 47.

† From Charles Cooper and Company, New York.

TABLE I.*

Experiment 1
Virus concentrated with 8% Autoclaved Membrane.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results	
212	Ultraresidue	2	14-1	Paralyzed in 12 days	Typical Polio.
213	"	0.2	14-1	Remained Well	
214	Ultrafiltrate	2	—	" "	
215	"	0.1	—	" "	
216	Filtrate	0.4	—	" "	

* All injections made under ether anesthesia. Animals held under daily examination for at least a month.
Rockefeller mixed strains of poliomyelitis virus used throughout.
All injections of amounts under 1 cc. made in 1 cc. volumes except Exp. 1 in which 2 cc. volumes were used.
Diagnoses controlled by microscopic examination of sections.

Experiment 2
Virus concentrated with 10% Autoclaved Membrane.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results	
201	Ultraresidue	1	32-1	Paralyzed in 15 days	Typical Polio.
241	"	.05	32-1	" " 11 "	" "
230	Ultrafiltrate	1	—	Remained Well	
231	"	.05	—	" "	

Experiment 4.
Virus concentrated with 8% Autoclaved Membrane.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results	
250	5% Paper Filt.	2	—	Remained Well	
257	" "	.1	—	" "	
254	Ultraresidue	2	54-1	Paralyzed in 7 days	Typical Polio.
255	"	.1	54-1	Remained Well	
259	Ultrafiltrate	2	—	" "	

The great difficulty in handling the brittle autoclaved membranes led, however, to a change in the method. Beginning with the sterile glacial acetic acid solutions of cotton, aseptic procedures were employed throughout. Cylindrical suction flasks were specially blown, each barely large enough to admit a single shell covered with its membrane and so small as to leave a residue of only 5 to 15 cc. at the end of the filtration process. As reservoirs for sterile distilled water for washing the membranes, large suction flasks were provided with two hole rubber stoppers, one hole for an outlet glass tube and the other for a receiving brass funnel which had a screw top which could be made air tight with sterile vaseline. A battery of such small ultrafiltration flasks can be set up each with its own reservoir

and attached to a common suction pump. In practice the whole apparatus is assembled and autoclaved with all its connecting tubes and clamps before coating the alundum shells with the cellulose solution. The shells are then removed from the covered vessel in which they have been autoclaved, using sterile cotton gloves in the handling, since rubber gloves are difficult to sterilize and were a source of contamination earlier in the process. The shells are hermetically sealed into the suction flasks with a hot sealing mixture consisting of 3 parts resin and 2 parts beeswax. Care must be used to have the flasks full of fluid so that the membranes shall be kept wet; otherwise, bubbles will be formed with so high a surface tension as to make filtration impossible with an ordinary water pump. After the washing has been completed, the virus filtrate is introduced through the brass funnel into the same reservoir and the ultrafiltration actually begun. The ultrafiltrate comes over with increasing slowness as the membranes become coated with the somewhat viscous residue. When large amounts have been in process of filtration requiring several days for completion, we have usually protected the virus by immersing the suction flasks in a refrigerating system of cold running water.

This method has been fairly satisfactory. Many details must be

TABLE II.*

Experiment 9.

Virus concentrated with 10% unautoclaved membranes.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results	
341	Filtrate	1	—	Paralyzed in 11 days	Typical Polio.
342	"	.1	—	Remained Well	
343	"	.01	—	" "	
345	Ultraresidue	.1	20-1	Paralyzed in 8 days	" "
346	"	.01	20-1	Remained Well	

Experiment 11.

Virus concentrated with 10% unautoclaved membranes.

Monkey No.	Inoculum	Amt. Injected cc.	Degree of Conc.	Results	
342	Ultraresidue	.0001	165-1	Remained Well	
344	"	.001	165-1	Paralyzed in 6 days	Typical Polio.
356	"	.01	165-1	" " 6 "	" "
353	"	.1	165-1	" " 6 "	" "
357	Filtrate	.001	—	Remained Well	
343	"	.01	—	Paralyzed in 15 days	" "
347	"	.1	—	" " 8 "	" "
352	Ultrafiltrate	2	—	" " 11 "	" "

*Details of injections, the same as in Table 1.

controlled, but, with care, sterile concentrates can be obtained. Not only are hemoglobin and egg albumin held back by 8% and 10% membranes, but also the virus of poliomyelitis may be concentrated by this method as indicated by representative experiments in Table II. In only 2 out of many series did the virus pass the membranes of 8% concentration or higher. One of these was an experiment in which the membranes were sterilized in 70% alcohol in order to avoid the difficulties of the autoclaved membranes. It is well known that alcohol increases the permeability of such membranes. The other instance of virus passage (Table II. Experiment 11) was probably due to some unobserved break in the membranes, since we have repeatedly controlled the ultrafiltrate, even to the extent of concentrating it by distillation *in vacuo* and injecting this concentrated ultrafiltrate into monkeys without obtaining an infective dose of the virus. In testing these "concentrates" by the only known method, injection into susceptible monkeys, we find that although increase in potency is demonstrable, the infectivity does not increase proportionately to the actual decrease in the volume. In Table II, Experiment 9, for example, the virus filtrate was concentrated 20 times by volume, but the ultraresidue was only 10 times as effective as the original filtrate in producing complete paralysis in monkeys. In Experiment 11, the virus filtrate was concentrated 165 times by volume, but was still only 10 times as potent as the original virus. Doubtless considerable virus is adsorbed on the membrane in the process of filtration, but it would appear probable that we also have in these experiments indication of the concentration of an inhibiting factor in poliomyelitis brain cord suspensions, as suggested by Leiner and v. Wiesner[7] and studied more completely by Jungeblut.[8]

Conclusions. A method for the ultrafiltration and concentration of poliomyelitis virus is described. This should be applicable to the further study of the properties of poliomyelitis and other filterable viruses. The method does not result in a direct proportional volumetric increase in the potency of the ultraresidue. A concentration of an inhibiting agent is suggested.

[7] Leiner and v. Wiesner, *Wien. Kl. Woch.*, 1910, **23**, 817.

[8] Jungeblut, C. W., *J. Immun.*, 1932, **22**, 99.