In general, the results indicate that the method is useful with synthetic solutions under controlled conditions. Its accurate application to blood sera would require in any case corrections for effects of varying concentrations of sodium chloride, glucose and urea upon both the specific gravity and relative viscosity values and of possible abnormal serum proteins upon the relative viscosity in various pathological conditions.

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Ultrafiltration of the Virus of Equine Encephalomyelitis.

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Meyer, Haring, and Howitt[1] were the first to show that the virus of equine encephalomyelitis passes readily through Berkefeld V and N filters. Olitsky, Cox and Syverton[2] reported that the virus passed through Seitz filters in a relatively high concentration. Krueger, Howitt and Zeilor[3] filtered the virus through acetic acid collodion membranes and found that it passed through 3%, but was retained by 3.5% membranes. From these results they estimated the particle size of the virus to be approximately 500 mμ. It was considered of interest to determine the size of this virus more accurately by filtration through finely graded collodion membranes of relatively uniform porosity.

The collodion membranes used in our experiments were prepared according to the method of Elford[4] with certain modifications adopted by Bauer and Hughes.[5] The filtration technique described by Bauer and Hughes[6] in their study of yellow fever virus was closely followed. All filtrations were conducted under positive pressure of nitrogen of 100 cm. Hg. The effective filtration area of the membranes comprised about 5 cm.^{2} and the amount of filtrate collected through such an area varied from 8 to 10 cc.

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1 Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227.

2 Olitsky, P. K., Cox, H. R., and Syverton, J. T., *J. Exp. Med.*, 1934, **59**, 159.

3 Krueger, A. P., Howitt, B., and Zeilor, V., *Science*, 1933, **77**, 288.

4 Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

5 Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

6 Bauer, J. H., and Hughes, T. P., *Am. J. Hyg.*, 1935, **21**, 101.

The Eastern and Western strains of the virus were studied. These were the same strains that had previously been employed by Cox and Olitsky[7] in their immunization experiments with the virus adsorbed on aluminum hydroxide. Brains of mice that succumbed to experimental infection were used as source of virus, and in one experiment the virus grown in tissue cultures was employed. In preparing material for filtration, 5 to 8 brains were finely ground in a sterile mortar and suspended in a diluent made up of equal parts of hormone broth, ascitic fluid and distilled water. The suspension was centrifuged in the angle centrifuge for 30 minutes at a speed of 3,000 r.p.m., and the supernatant fluid was passed through a Seitz filter. Portions of this stock filtrate were passed through a series of collodion membranes of varying porosities. With a view to reducing the extent of adsorption of the virus by the membranes, 4 cc. of broth were passed through each membrane prior to the filtration of the virus. When tissue culture material was used as a source of virus, there were added to 60 cc. of tissue culture 20 cc. each of hormone broth, ascitic fluid, and distilled water. The mixture was then centrifuged and the supernatant fluid passed through a collodion membrane with an average pore diameter of 600 mμ to serve as stock filtrate. The presence of the virus in the filtrates was determined by injection of the filtrates intracerebrally into mice; 4 to 6 mice were used for each filtrate and each mouse was given 0.03 cc. In each experiment the virus content of the stock filtrate was titrated in mice using 4 to 6 mice for each dilution.

TABLE I.
Filtration Experiment with Mouse-Brain Virus, Western Strain.

No. of Membrane	Aver. pore diameter mμ	Amt. of filtrate collected cc.	Results of inoculation of filtrate in mice*	Titration of stock filtrate	
				Dilution	Results*
207	75	9	5/6	10^{-2}	6/6
222	70	9	4/6	10^{-3}	6/6
139	66	9	2/6	10^{-4}	4/6
208	60	8	0/6	10^{-5}	0/6
176	55	8	0/6		

*In all the tables the numerator represents the number of mice that succumbed to infection; the denominator, the number of mice used in the test.

In Table I are summarized the results of one of the 4 identical filtration experiments carried out with the Western strain of the mouse-brain virus. The virus passed through membranes with an average pore diameter of 75, 70, and 66mμ, but was held back by

[7] Cox, H. R., and Olitsky, P. K., *Science*, 1934, **79**, 459.

those of 60 and 55 mμ. The results of the experiments carried out with the Eastern strain, using both mouse-brain and tissue culture virus are shown in Tables II and III. The results were exactly the same as those obtained with the Western strain.

TABLE II.
Filtration Experiment with Mouse-Brain Virus, Eastern Strain.

No. of Membrane	Aver. pore diameter mμ	Amt. of filtrate collected cc.	Results of inoculation of filtrate in mice	Titration of stock filtrate	
				Dilution	Results
207	75	10.0	6/6	10^{-2}	4/4
139	66	10.0	6/6	10^{-3}	4/4
208	60	8.5	0/6	10^{-4}	4/4
176	55	8.0	0/6	10^{-5}	0/4
202	50	7.5	0/6		
150	45	7.0	0/6		

TABLE III.
Filtration Experiment with Tissue Culture Virus, Eastern Strain

No. of Membrane	Aver. pore diameter mμ	Amt. of filtrate collected cc.	Results of inoculation of filtrate in mice	Titration of stock filtrate	
				Dilution	Results
207	75	10	6/6	10^{-2}	6/6
222	70	10	5/6	10^{-3}	6/6
139	66	10	5/6	10^{-4}	6/6
208	60	10	0/6	10^{-5}	4/6
176	55	10	0/6		

Bauer and Hughes[6] have shown that yellow fever virus passes through collodion membranes with pore diameters close to the filtration end-point in a relatively high concentration, indicating a remarkable uniformity in the size of both the virus particles and the pores of membranes. A similar experiment was carried out with the Eastern strain of mouse-brain virus, in which the virus content of the filtrates of different membranes was titrated in mice. All dilutions were made in a diluent consisting of equal parts of ascitic fluid, broth, and distilled water, as used in the preparation of the stock filtrate. The results are shown in Table IV. It was rather surprising to find that although the filtration end-point was sharp and clear-cut, the virus appeared in the filtrates in a fairly low concentration, proving infective only in 1-100 dilution. It will be noticed also that 75% of the protein present in the stock filtrate had been adsorbed or held back by the membranes. As to whether the low concentration of the virus in the filtrates was caused by the lack of uniformity in the particle size of the virus, or the pore size of the membranes, or by the reduction of the size of the pores in the

TABLE IV.
Filtration Experiment with Mouse-Brain Virus, Eastern Strain. Titration of filtrates.

No. of Membrane	Aver. pore diameter mμ	Amt. of filtrate collected cc.	Protein content of filtrate %	Results in mice inoculated with dilutions of the filtrates					
				Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Seitz			0.2	6/6	6/6	6/6	6/6	6/6	5/6
207	75	8.0	0.05	6/6	5/6	6/6	0/6	0/6	1/6*
222	70	8.0	0.05	6/6	6/6	2/6	0/6	0/6	0/6
139	66	8.5	0.05	6/6	6/6	3/6	0/6	0/6	0/6
208	60	8.0	0.05	0/6	0/6	0/6	0/6	0/6	0/6
176	55	8.0	0.05	0/6	0/6	0/6	0/6	0/6	0/6

*The cause of death uncertain; probably not encephalitis.

membranes through the adsorption of a large amount of protein, is impossible to determine.

Summary. The virus of equine encephalomyelitis, both the Eastern and the Western strains, was found to pass collodion membranes with an average pore diameter of 66 mμ, but was completely held back by those of 60 mμ. The results indicate that this virus has the same filtration end-point, and consequently the same particle size, as that of St. Louis encephalitis, which was shown by Bauer, Fite, and Webster,[8] and Elford and Perdrau[9] to be 20 to 30mμ.

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Staphylococcal Antihemolysin in Osteomyelitis and Other Staphylococcal Infections.

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The possible clinical application of staphylococcal toxoid in staphylococcal infections makes it necessary to know the amount of circulating antitoxin in the blood of normal individuals, and of persons with staphylococcal infections before treatment with toxoid.

Several recent reports[1-6] have recorded antihemolysin (antitoxin?) values for normal healthy individuals. The earlier reports are not readily comparable because of differences in standards employed in various laboratories. The general conclusion, however, is that sera from normal persons exhibit a wide individual variation of titers. With the establishment of a provisional International Standard for staphylococcus antitoxin,[7] it is possible to render reports in entirely comparable terms. Dolman[3] has reported that the normal value for healthy individuals is from ⅓ to 1 International Unit. Murray[5]

[8] Bauer, J. H., Fite, G. L., and Webster, L. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 696.

[9] Elford, W. J., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 143.

[1] Bryce, L. M., and Burnet, F. M., *J. Path. and Bact.*, 1932, **35**, 183.

[2] Dolman, C. E., *J. Am. Med. Assn.*, 1933, **100**, 1007.

[3] Dolman, C. E., *Lancet*, 1935, **1**, 306.

[4] Parish, H. J., O'Meara, R. A. Q., and Clark, W. H. M., *Lancet*, 1934, **1**, 1054.

[5] Murray, D. S., *Lancet*, 1935, **1**, 303.

[6] Gross, H., *Klin. Wchnschr.*, 1933, **12**, 907.

[7] Hartley, P., and Llewellyn Smith, M., *Quart. Bull. Health Organis., League of Nations*, 1935, Jan., 68.