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Ultrafiltration of the Virus of Vesicular Stomatitis.

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Galloway and Elford[1] recently filtered the virus of vesicular stomatitis through graded collodion membranes and found that the virus passed through membranes which had an average pore diameter of 160 mμ or greater, but was completely held back by those which had an average pore diameter of 130 mμ or less. From the analysis of their results they estimated the size of the virus particles to be 70-100mμ. They used the vesicular fluid from the pads of the feet of guinea pigs as a source of virus, and tested their filtrates for the presence of virus by intradermal inoculation into the pads of guinea pigs. Two different strains of the virus, serologically distinct, were studied, but no difference in the particle size was found.

* C represents a complete, P a preliminary manuscript.

[1] Galloway, I. A., and Elford, W. J., *Brit. J. Exp. Path.*, 1933, **14**, 400.

Recently, Olitsky, Cox, and Syverton[2, 3, 4] have shown that mice are highly susceptible to this virus when inoculated intracerebrally and also that the virus can be maintained in tissue cultures. It was considered of interest to find out whether the particle size of the virus, derived either from the brain of infected mice or from the tissue cultures, is of the order of magnitude found by Galloway and Elford with virus from the vesicular fluid of guinea pig pads. To decide this point, the experiments here presented were carried out.

The collodion membranes used in these experiments were prepared according to the method of Elford,[5] with certain minor modifications adopted by Bauer and Hughes.[6] Infected mouse brains or tissue cultures were used as source of virus. The brains were ground in a mortar and suspended in a diluent consisting of equal parts of hormone broth, ascitic fluid, and distilled water. The concentration of the brain tissue in the suspension varied from one to 2% by weight. The suspension was centrifuged, and the supernatant fluid was passed first through a Seitz filter and then through a series of membranes of varying porosity. When the virus grown in tissue culture was used, 20 cc. of hormone broth and a similar amount of ascitic fluid were added to 60 cc. of the tissue culture. The mixture was centrifuged, and portions of the supernatant fluid were passed through a series of graded collodion membranes without preliminary filtration through a Seitz filter. All filtrations were carried out under positive pressure of nitrogen. In most of the filtrations the pressure was 100 cm. Hg., and in only a few instances were 2 atmospheres applied. The effective filtration area of each membrane was about 5 sq. cm., and the amount of filtrate collected from each membrane varied from 5 to 11 cc. The presence of virus in filtrates was tested in mice by intracerebral inoculation. A group of 6 mice was used for testing each filtrate, and the amount injected into each mouse was 0.03 cc. The virus content of the Seitz filtrate, or when tissue culture was used the unfiltered portion of the virus-containing material, was determined by titration in mice, using 4 or 6 mice for each dilution.

Two strains of the virus, the "Indiana" and the "New Jersey"

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

[3] Cox, H. R., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 653, 654.

[4] Cox, H. R., Syverton, J. T., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 896.

[5] Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

[6] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

strains, were studied. These were the same strains as those which had been used by Galloway and Elford in their studies, and they were sent us through the kindness of Dr. W. E. Cotton of the U. S. Bureau of Animal Industry. A total of 8 filtration experiments were carried out. In 5 of these the mouse-brain virus of the "Indiana" strain was used, in 2 the mouse-brain virus of the "New

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

No. Membrane	Ave. pore diameter mμ	Amt. of filtrate collected cc.	Results of inoculation of filtrate in mice.*	Titration of stock filtrate	
				Dilution	Results*
205	180	10	6/6	10^{-2}	4/4
97	170	10	6/6	10^{-3}	3/4
173	160	10	6/6	10^{-4}	1/4
203	150	10	6/6	10^{-5}	0/4
181	150	10	6/6		
174	140	10	6/6		
206	130	10	0/6		

* The numerator represents the number of mice that succumbed to infection; the denominator, the number of mice employed in the test.

TABLE II.
Filtration Experiment with Mouse-Brain Virus, "New Jersey" Strain.

No. Membrane	Ave. pore diameter mμ	Amt. of filtrate collected cc.	Results of inoculation of filtrate in mice.*	Titration of stock filtrate	
				Dilution	Results*
137	160	10	6/6	Undiluted	6/6
203	150	10	2/6	10^{-1}	6/6
174	140	10	4/6	10^{-2}	5/6
215	140	10	0/6	10^{-3}	3/6
206	130	10	0/6	10^{-4}	1/6
259	130	10	0/6		
221	120	10	0/6		
219	120	10	0/6		

* The numerator represents the number of mice that succumbed to infection; the denominator, the number of mice employed in the test.

TABLE III.
Filtration Experiment with Tissue Culture Virus, "New Jersey" Strain.

No. Membrane	Ave. pore diameter mμ	Amt. of filtrate collected cc.	Results of inoculation of filtrate in mice.*	Titration of unfiltered suspension	
				Dilution	Results*
137	160	8	6/6	10^{-1}	4/4
173	160	6	6/6	10^{-2}	4/4
181	150	8	6/6	10^{-3}	3/4
174	140	5	0/6	10^{-4}	2/4
206	130	8	0/6	10^{-5}	0/4
138	120	6	0/6		

* The numerator represents the number of mice that succumbed to infection; the denominator, the number of mice employed in the test.

Jersey" strain, and in one the tissue culture virus of the latter strain. Three typical experiments are shown in Tables I, II, and III. It will be seen from these tables that the virus passed through all membranes which had an average pore diameter of 150 mμ or greater. The passage through the 140 mμ membranes was irregular, as in some of the experiments the filtrates proved infective, while in others they failed to produce infection. The filtrates of membranes which had an average pore diameter of 130 or less gave uniformly negative results. The filtration end-point, therefore, is considered to be approximately 140 mμ. These results are in close agreement with those obtained by Galloway and Elford.

Summary. The filtration end-point of the virus of vesicular stomatitis, or the average pore diameter of the finest membrane passing the virus, was found to be approximately 140 mμ. Two immunologically distinct strains of the virus, the "Indiana" and the "New Jersey" maintained either in tissue culture or in mouse brain, were studied, and the filtration end-point was found to be the same irrespective of the source or serological type of the virus.

Our results confirm those of Galloway and Elford, who found that the virus passes through collodion membranes which have an average pore diameter of 160 mμ but is held back by those of 130 mμ. From their results they estimated the particle size of the virus to be between 70 and 100 mμ.

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Early Diagnosis of Rabies by Mouse Inoculation. Measurement of Humoral Immunity to Rabies by Mouse Protection Test.

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A method for early and reliable diagnosis of rabies by animal inoculation and a protection test for measuring humoral immunity to the virus may become feasible by the use of highly susceptible strains of mice. Ordinarily the injection of brain tissue from rabid animals into rabbits or guinea pigs incites the disease irregularly and after incubation periods of 2 to 8 weeks. Mice as test animals have proved in the past even less satisfactory.[1] Mice especially bred for

[1] Koch, J., *Kolle-Kraus-Uhlenhuth. Handb. der Path. Mikroorg.*, 1930, **8**, 547.