To correlate the appearance of virus in blood and brain of nasally infected mice with the appearance of lesions in the central nervous system, groups of white-face mice were inoculated intranasally and 4 killed each day for 8 days. The brains and cords were sectioned and stained for study. Definite lesions were not observed before the 5th day, at which time they were not extensive. On the 6th and 7th days, however, the lesions were fully developed.

It appears, therefore, that louping-ill virus introduced into the noses of susceptible mice gains access to blood and brain and is demonstrable in both by the 2nd day. It persists in the blood until cortical lesions develop and the animal becomes ill, at which time it tends to disappear. Virus is present in the brain at least 4 days before the animal appears sick and persists and multiplies there until the animal succumbs to encephalitis.

TABLE I.

Relation of Presence of Louping-Ill Virus in Blood and Brain of Intranasally Inoculated Mice to Appearance of Central Nervous System Lesions and Course of Disease.

Remarks	Days after Intranasal Inoculation									
	1	2	3	4	5	6	7	8	9	10
Virus in blood	0	+	+	+	+	±	±	±	±	±
" " brain	±	+	+	++	++	++	++	++	++	++
Lesions in central nervous system	0	0	0	0	±	+	++	++	++	++
Signs of disease	0	0	0	0	±	+	++	++	++	++

7281 P

Ultra-Filtration Experiments with the Encephalitis Virus from the St. Louis Epidemic.

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Webster and Fite[1] showed recently that the virus isolated from cases of encephalitis in the St. Louis and Kansas City epidemics of 1933, passes through Berkefeld N candles in a relatively high concentration. While these findings indicate that the virus particles are exceedingly small, it was considered of interest to determine

[1] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

their size more accurately by filtration through graded collodion membranes of known porosity.

The membranes used in these experiments were prepared according to the method of Elford[2] with certain minor modifications adopted by Bauer and Hughes.[3] They were sterilized by steaming for one hour at 100°C. The average pore values were calculated according to Poiseulle's law from data obtained by measurement of the thickness of the membrane, its water content, the rate of flow of water through a known area, and the pressure producing the flow. These measurements were made after the sterilization.

The virus-containing material used consisted of fresh brain tissue from mice which had succumbed to the experimental disease. In most of the experiments 4 brains were ground in a sterile mortar, suspended in 100 cc. of a diluent of equal parts of hormone broth (pH 8.0), ascitic fluid, and distilled water. The suspension was centrifuged for 30 minutes at about 3000 r.p.m. The supernatant fluid was passed through a Seitz filter, and then through a collodion membrane with an average pore diameter of about 0.25μ. This stock filtrate was then passed through a series of membranes of different pore sizes. From 6 to 8 different membranes were used in each experiment. The effective filtration area for each membrane was about 5 sq. cm., and the amount of the virus suspension passed through such area was usually 10 cc. To reduce the amount of adsorption of proteins from the virus suspension by the membranes, 3 cc. of sterile broth was passed through each membrane before filtration of the virus suspension. All filtrations were carried out under applied positive pressure of nitrogen. The amount of pressure used in most of the experiments was 100 cm. mercury, and only in one experiment, in which membranes of the finer grade were used, were three atmospheres applied. In no case was there observed any stretching or damage to the membranes.

Each filtrate was tested in a group of 6 mice, and the amount injected into each mouse was 0.03 cc. intracerebrally. The stock filtrate in each experiment was titrated in mice to a dilution of 1:100,000, and in most of the tests was found to be infective in this dilution.

Nine filtration experiments were carried out, using a total of 25 membranes of different porosities. These experiments are summarized in Table 1.

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

[3] Bauer, J. H., and Hughes, T. P., to be published shortly.

TABLE I.
Summary of ultra-filtration experiments carried out with the encephalitis virus.

No. of Membrane	Aver. pore diam. m. μ	Thickness membrane mm.	No. filtration exper.	No. times filtrate infective
165	257.7	.15	1	1
157	218.8	.13	1	1
137	162.3	.15	1	1
99	121.3	.18	1	1
107	100.0	.22	1	1
87	90.8	.17	4	4
86	82.6	.17	2	2
189	72.3	.16	2	2
89	71.2	.18	2	2
213	69.3	.17	2	2
160	67.6	.14	4	1
77	67.0	.20	4	4
186	66.9	.16	2	2
139	66.1	.11	6	3
184	62.5	.16	2	None
208	59.7	.14	1	"
91	57.0	.19	4	"
149	55.7	.17	2	"
151	54.8	.17	1	"
202	51.8	.13	2	"
79	51.8	.17	2	"
131	49.3	.14	4	"
132	43.0	.14	2	"
114	38.8	.16	1	"
153	28.0	.13	2	"

As seen from this table, the virus passed consistently through all membranes with an average pore diameter greater than 66 millimicrons, while membranes with pore diameter smaller than this gave uniformly negative results. However, the amount of the virus passing through membranes between 72 and 66mμ, appeared to be greatly diminished. The strain of mice used in these experiments is highly susceptible to the virus, and the titrations of the stock filtrates almost invariably produced 100% mortality in the mice inoculated with dilutions up to 1:10,000. On the other hand, the mortality rate among the mice inoculated with the filtrates of the membranes between 72 and 66mμ, was usually 4 or 5 in a group of 6. Of the 6 experiments carried out with membrane No. 139, which has a calculated average pore diameter of 66.1mμ, three gave entirely negative results, in one experiment only 2 of the 6 mice died, and in the remaining 2 experiments all mice died. The filtration end point, in these experiments, appears to be about 66 millimicrons.

All collodion membranes have a strong adsorptive affinity for proteins, and in filtration experiments usually little protein passes through a membrane before this affinity is satisfied. This adsorption naturally reduces the diameter of the pores, the extent being depend-

ent on the amount of cohesion of protein molecules taking place in the capillary channels of the membrane. In view of this phenomenon, Elford[4] has estimated that in filtration experiments with membranes of greater porosity than 10mμ, a particle must have a diameter only from one-half to one-third of that of the pore in order to traverse it. The application of this formula to the above experiments would indicate that the probable size of the encephalitis virus lies somewhere between 22 and 33 millimicrons.

7282 P

Observations on Induction of Anaphylactic Shock by the Specific Carbohydrates of Type-I Pneumococcus.

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Further comparative studies of the soluble specific substance of Avery and Heidelberger and of the specific "cellular carbohydrate"[1] by means of the experimental induction of anaphylactic shock have given additional information regarding the antigenic activities of these preparations. Avery and Tillett[2] induced fatal anaphylactic shock in passively sensitized guinea pigs with the homologous soluble specific substance of the pneumococcus, and Enders[3] reported that antiserum, after precipitation with soluble specific substance, still sensitized guinea pigs to his "A" substance but not to the soluble specific substance. This report records experiments with the type-I specific substances injected intracardially into guinea pigs which had been passively sensitized with type-I antipneumococcus rabbit serum 24 or 48 hours previously.

The minimum amount of immune serum necessary to sensitize guinea pigs so that fatal shock developed when they were injected intracardially with soluble specific substance was always greater than that required in the case of the cellular carbohydrate. On the other hand, the minimum amount of each of the specific substances which induced fatal shock in the guinea pig previously sensitized by

[4] Elford, W. J., *Proc. Roy. Soc. B.*, 1933, **112**, 384.

[1] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1933, **24**, 349.

[2] Avery, O. T., and Tillett, W. S., *J. Exp. Med.*, 1929, **49**, 251.

[3] Enders, J. F., *J. Exp. Med.*, 1930, **52**, 235.