ULTRAFILTRATION OF THE VIRUS OF POLIOMYELITIS

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That the inciting agent of human poliomyelitis should be classified with the filtrable viruses has been recognized since the pioneer work of Landsteiner and Levaditi (1) and Flexner and Lewis (2), who showed that this virus passes through Berkefeld filters. Efforts to determine more nearly the size of the virus were made by Amoss (3), who showed that it passes easily through a Berkefeld V filter, less easily through an N filter, and is diminished in quantity after passage through a W filter. More accurate methods for determining the size of virus particles have been made available by the important work of Elford, who has described a method for preparing collodion membranes of graded porosities, and has shown that it is reliable (4). Using his methods, we have obtained the additional evidence here presented regarding the size of the poliomyelitis virus particle.

EXPERIMENTAL

The graded collodion membranes used in these experiments were prepared according to the method of Elford, with certain minor modifications. The technic of preparing the membranes and the apparatus used have been described by Bauer and Hughes (5).

Six filtration experiments were carried out. In each experiment five to eight membranes were used. These varied in porosity from 30 mμ to 265 mμ average pore diameter, and in thickness from 0.12 mm. to 0.22 mm. The filtration area was approximately 5 sq. cm.

The strain of poliomyelitis virus used throughout these experiments was the M.V., for which we are indebted to Dr. Simon Flexner. This strain was chosen on account of its high and constant virulence.

Preparation of Virus Suspensions.—Infective spinal cords to be used for the preparation of virus suspension for filtration purposes were removed from monkeys

shortly after the onset of paralysis. The incubation period of these monkeys, *i.e.* the number of days elapsing between inoculation and onset of paralysis, varied from 7 to 11 days. On account of the low concentration of virus in the brain, only the cord was used. In the first four experiments the whole cord was used, but in the last two only the cervical and lumbar enlargements were used. The infective tissue was ground thoroughly in a mortar with quartz sand and a suitable diluent. The concentration of tissue in these suspensions varied from 4.1 to 10 per cent by weight. The virus suspensions were then centrifuged for from 30 to 90 minutes at 3,000 R.P.M. In the last four experiments an angle centrifuge was used. The supernatant fluid was then passed through a Seitz filter. This filtrate was distinctly turbid because of the presence of finely divided fat. It is essential that the material to be filtered through the finer collodion membranes should be absolutely clear. Consequently, attempts were made to clarify the Seitz filtrate further by passing it through coarser membranes prior to filtration through membranes of fine porosity. It was found, however, that the fat globules passed through membranes with an average pore diameter of 500 mμ or greater, and the filtrate of those membranes had practically the same turbidity as the original Seitz filtrate. On the other hand, when membranes with an average pore size of 300 mμ were used, the filtrates were clear but the membranes became clogged completely after the passage of a few cubic centimeters. Attempts were also made to use the sand and paper pulp filters described by Galloway and Elford (6) for the clarification of the monkey cord emulsion, but these attempts were unsuccessful in that the filters invariably became clogged after the passage of a few cubic centimeters of the suspension. It was therefore decided to use straight Seitz filtrates as stock filtrates for passage through the finer graded membranes for the determination of the filtration end-point of the virus.

The composition of the diluent used for the suspension of finely ground monkey cord varied in each experiment, as shown in Table I. As indicated in this table, hormone broth and phosphate buffer solutions in varied proportions were incorporated into the diluent used in all experiments. The importance of the broth in ultrafiltration has been pointed out by Galloway and Elford (6) and Bauer and Hughes (7). In one experiment 5 cc. of broth were passed through the membranes prior to the filtration of the virus suspension.

All filtrations were carried out under a positive pressure of nitrogen. The amount of pressure applied was 100 cm. of mercury, except in one experiment in which three atmospheres were employed. The amount of filtrate collected varied from 5 to 10 cc. The protein content of the filtrates was determined in every instance. The infectivity of the filtrates was tested by the intracerebral injection of monkeys. The amount inoculated was 1 cc. in each experiment, except in Experiment 4 when 2 cc. were injected. Monkeys were kept under observation for at least 5 weeks. Diagnosis of poliomyelitis was made on clinical grounds; but most of the monkeys inoculated with the filtrates were killed after the development of paralysis, and the diagnosis was confirmed by histopathological

TABLE I

Details of Methods Followed in Filtration of Poliomyelitis Virus

	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
Source of virus	1 whole cord	2 whole cords	1 whole cord	1 whole cord	Cervical and lumbar enlargements of 3 cords	Cervical and lumbar enlargements of 5 cords
Percentage of infective tissue in stock suspension	4.1	10.0	5.0	5.0	5.0	6.8
Composition of diluent	Hormone broth pH 8.0, 25 cc. Phosphate buffer solution M/15, pH 8.0, 50 cc. Distilled water 20 cc. Normal monkey serum 5 cc.	Hormone broth pH 8.0, 50 cc. Phosphate buffer solution M/15, pH 8.4, 25 cc. Distilled water 25 cc.	Hormone broth pH 8.0, 25 cc. Phosphate buffer pH 8.4, 25 cc. Ascitic fluid 25 cc. Distilled water 25 cc.	Hormone broth pH 8.0, 50 cc. Phosphate buffer M/15, pH 8.0, 50 cc. Normal monkey serum 5 cc.	Hormone broth pH 8.0, 50 cc. Phosphate buffer pH 8.0, 50 cc. Normal monkey serum 5 cc.	Hormone broth pH 8.0, 40 cc. Phosphate buffer M/15, pH 8.2, 40 cc. Normal monkey serum 5 cc.
Filtration pressure	100 cm. Hg	228 cm. Hg	100 cm. Hg	100 cm. Hg	100 cm. Hg	100 cm. Hg
Amount of filtrate injected into monkeys	1 cc.	1 cc.	1 cc.	2 cc.	1 cc.	1 cc.
Amount of filtrate collected	6–10 cc.	6–10 cc.	8 cc.	5 cc.	5–6 cc.	5.5–6.5 cc.

study. For controls one or more monkeys were injected with the stock Seitz filtrate in every instance. In several experiments the infectivity of the Seitz filtrate dilution 1/10 and 1/100 was also tested. The immunity of the monkeys that remained well after injection with filtrates was tested in later experiments

TABLE II

Results of Filtration Experiments with Poliomyelitis Virus

Membrane			No. of experiments	No. of monkeys inoculated with filtrates	No. of monkeys developing poliomyelitis	Incubation period
No.	Average pore diameter	Thickness				
	mμ	*mm.*				*days*
135	265	0.12	1	1	1	17
85	215	0.19	1	1	1	16
83	100	0.17	1	1	1	14
107	100	0.22	2	2	2	10, 19
87	90	0.17	1	1	1	8
160	67	0.14	1	1	0	
77	67	0.20	1	1	0	
139	65	0.12	2	2	0	
208	60	0.14	2	2	1	8
78	60	0.20	1	1	1	12
91	57	0.19	2	2	1	31
149	55	0.15	2	2	0	
176	55	0.12	1	1	1	9
131	50	0.14	2	2	0	
202	50	0.13	3	4	4	12, 16, 8, 15
150	45	0.17	3	6	1	27
132	43	0.14	1	1	0	
152	40	0.16	3	6	2	8, 13
114	39	0.16	1	1	0	
154	35	0.13	3	6	1	27
113	35	0.16	1	1	0	
115	30	0.15	6	6	0	
Seitz filtrate.....			6	17	14	7, 8, 9, 10, 10, 11, 11, 11, 12, 12, 12, 12, 13, 17
Seitz filtrate 1/10.....			3	3	2	8, 13
Seitz filtrate 1/100.....			4	4	1	11

by intracerebral inoculation of stock Seitz filtrates. These monkeys served as additional control animals for the infectivity of the stock virus filtrate.

Results of Experiments.—The results of the filtrations are shown in Table II. The irregularity of the results obtained is probably due to

the well known wide range in susceptibility of *rhesus* monkeys to the virus of poliomyelitis. This is accentuated when filtrates are used which certainly have a much smaller concentration of virus than is present in the suspensions of virus commonly used in work with poliomyelitis.

On two occasions the virus was demonstrated in the filtrates of the membranes with an average pore diameter of 40 mμ, and on one occasion in the filtrate of a 35 mμ membrane. No monkeys became infected with the filtrates obtained from a membrane of 30 mμ average pore diameter. The filtration end-point of the virus of poliomyelitis in our experiments is consequently 35 mμ. In view of the fact that in filtration experiments a considerable amount of protein is adsorbed in the membranes, which necessarily reduces the diameter of the pore, Elford (8) has estimated that with membranes with an average pore diameter greater than 10 mμ, a particle must have a diameter not greater than from one-third to one-half of that of the pore in order to traverse it. Applying this formula to our results, it would seem that the particle size of the poliomyelitis virus lies somewhere between 12 and 17 mμ.

DISCUSSION

Our results show that the virus of poliomyelitis is extremely small, approximating that of foot-and-mouth disease (8–12 mμ, Galloway and Elford (6)), and it would seem probable that it is even smaller than our results would indicate. In evaluating ultrafiltration experiments, all that can legitimately be concluded is that under the conditions of the experiments the virus passed through membranes of a certain pore size. The observations are of only minor value unless the filtrations are carried out under optimum conditions. Such conditions were by no means present in our experiments. Minute particles of fat, which passed through the Seitz filter and through the coarser membranes, interfered considerably in the filtration through membranes of smaller porosities. Furthermore, Galloway and Elford (6) have shown that in ultrafiltration experiments the concentration of virus is of great importance and that only a high concentration of virus in the stock filtrate gives a sharp filtration end-point. Poliomyelitis virus is present in infective cords in relatively small amounts.

In our experiments on three occasions the infectivity of a 1/100 dilution of the Seitz filtrate was tested by the intracerebral injection into monkeys. Only once was virus demonstrated. Another factor of importance which renders the interpretation of the results difficult is the wide variation in the susceptibility of the monkeys to minute doses of the virus.

CONCLUSIONS

From the ultrafiltration analysis the size of the virus of human poliomyelitis has been estimated to be somewhere between 12 and 17 mμ. Technical difficulties encountered and the low concentration of the virus make it seem possible that the virus is even smaller.

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