Ultracentrifugal examination of dissociated polio virus

It is generally known that poliomyelitis virus cannot be lyophilized without serious loss of infectivity. The mechanism involved was explained by HAMPTON[1], who showed that when dried droplet preparations of purified suckling-mouse-adapted MEF_1 poliomyelitis virus, intended for electron microscopy, were re-wetted, the virus particles dissociated into smaller units. In the present work the dissociation products were examined in the Spinco analytical ultracentrifuge as well as in the electron microscope.

The virus used in this study was the MEF_1 strain of Type II polio and was prepared from 20 l of infected tissue-culture fluid. The purification method was that used by POLSON AND HAMPTON[2]. It involved the initial concentration of the infective agent by pervaporation followed by the removal of undesired tissue protein by chloroform treatment and differential ultracentrifugation. Before lyophilization the homogeneity of the virus suspension was confirmed by electron microscopy and analytical ultracentrifugation. The virus suspension recovered from the ultracentrifuge cell was freeze-dried, reconstituted in the same volume of distilled water and again ultracentrifuged. Electron micrographs were also made of the reconstituted materials.

In Fig. 1 (a) and (b) are shown sedimentation diagrams of the virus before and after freeze-drying respectively. It will be noticed that the virus suspension prior to lyophilization contained one major component with an indication of two slower sedimenting components. The sedimentation coefficients of the three components were 156 for the fast component and approximately 80 and 20 Svedberg units (S) for the slower ones. In the centrifugation run with the lyophilized virus suspension a component of sedimentation coefficient 66 S was found together with material which did not sediment far enough to enable the sedimentation velocity to be estimated. In a second experiment using a different preparation of undissociated MEF_1 virus the material was ultracentrifuged at a higher rotor velocity with the object of resolving the fraction which failed to sediment in the first experiment. The undissociated preparation (Fig. 2a) again contained, in addition to the main component, small amounts of two slower sedimenting fractions, the three sedimentation coefficients being 156, 73 and 25 S. In the lyophilized preparation (Fig. 2b) two components were resolved; these had sedimentation coefficients of 69 and 15 S units respectively. No trace of the component having a sedimentation coefficient of 156 S could be detected in either of the lyophilized preparations. Details of the ultracentrifugal analyses are given in Table I.

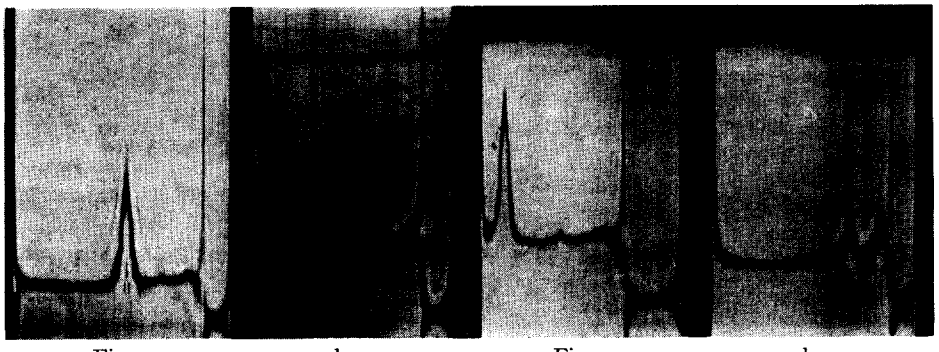

Fig. 1a b Fig. 2a b

Fig. 1. Sedimentation diagrams of purified MEF_1 virus, first preparation, (a) before and (b) after lyophilization. Phaseplate angle, 20° in both cases. (a) was taken after 35 min at 14,290 r.p.m. and (b) after 27 min at 14,290 r.p.m. + 7 min at 29,500 r.p.m.

Fig. 2. Sedimentation diagrams of purified MEF_1 virus, second preparation (a) before and (b) after lyophilization. Phaseplate angle, 15°, and rotor velocity, 20,410 r.p.m., in both cases, (a) was taken after 25 min and (b) after 27 min at the indicated rotor velocity.

In Fig. 3 is shown an electron micrograph of the second preparation before dissociation by lyophilization. The sample appears to be reasonably homogeneous and the virus particles are similar in shape and size as those earlier observed by, for instance, HAMPTON[1] or TAYLOR AND MCCORMICK[3]. After freeze-drying no such particles could be detected (Fig. 4). The electron micrograph revealed less well defined bodies of varying size and opacity to electrons and with great tendency to aggregate into clumps.

Tests on the second preparation by the tissue-culture method showed that the infectivity dropped from $10^{8.5}$ to $10^{2.5}$ T.C.I.D.$_{50}$ per ml during freeze-drying. Whether the remaining activity was due to undissociated virus cannot be stated with certainty.

TABLE I

SEDIMENTATION COEFFICIENTS AND RELATIVE CONCENTRATIONS OF THE DIFFERENT COMPONENTS OF THE UNDISSOCIATED AND DISSOCIATED MEF_1 POLIO-VIRUS PREPARATIONS

Preparation	*Total concentration of sedimented fractions*	*S^0_{20} in Svedberg units (relative concentration in %)*		
	mg/ml	*I*	*II*	*III*
1	0.87	156 (100%)	80 (trace)	20 (trace)
1 (freeze-dried)	0.24	—	60 (100%)	not sedimented
2	1.16	156 (80%)	73 (8%)	25 (12%)
2 (freeze-dried)	0.72	—	69 (40%)	15 (60%)

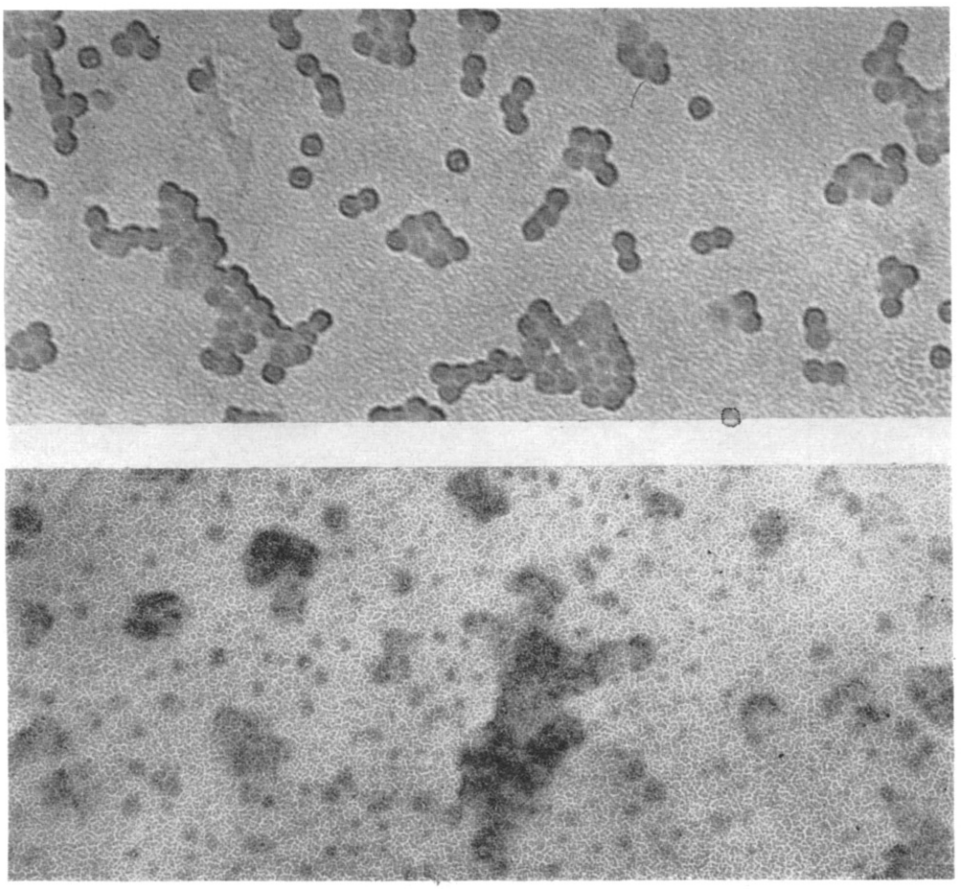

Fig. 3. Electron micrograph of undissociated MEF_1 virus, second preparation. × 88,000. The material was dialyzed against distilled water before being subjected to electron microscopy.

Fig. 4. Electron micrograph of MEF_1 virus dissociated by lyophilization. × 88,000.

The work described above confirms HAMPTON's observation that polio virus is dissociated by lyophilization. Our observations, however, differ from HAMPTON's in that we have found that the virus particles do not break up into units of uniform size, but that a variety of particles of different dimensions were formed. From results obtained in the ultracentrifuge it would appear that two main components are formed as products of the dissociation and have sedimentation coefficients of 66 to 69 S and approximately 15 S, respectively. It cannot be decided at the moment whether the faster of these components consists of the intact nucleic acid nucleus or the protein shell or some bigger fragments of either part of the virus. The slower component undoubtedly consists of smaller dissociation products with molecular weights of probably less than a million. As only a trace of the lyophilized preparations was insoluble and only 40–60 % was recovered as sedimented material, much must have been converted to even smaller particles of sedimentation coefficients less than 15 S.

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The sialic acids of hog pancreas

The amino group of sialic acids can be substituted with an acetyl group (N-acetylsialic acid) or a glycolyl group (N-glycolylsialic acid). BLIX *et al.*[1] isolated the glycolyl-containing sialic acid (P-sialic acid) from hog-submaxillary mucin. When we tried to isolate N-glycolylsialic acid from other tissues of hog, *e.g.* kidney and serum, the crystals were found to be composed of about 15 % N-glycolylsialic acid and 85 % N-acetylsialic acid[2,3]. On the other hand, the sialic acids isolated by us from the submaxillary gland and mucin of hog contained 80 % and 90 % N-glycolylsialic acid, respectively. Therefore, we assumed that the mucoids in the secretions from the hog glands contained practically pure N-glycolylsialic acid. To test the hypothesis we have isolated the sialic acids from hog pancreas and investigated their composition.

2 kg of hog pancreas freed from most of the fat and connective tissue were homogenized in a Turmix blendor. The homogenate was poured under stirring into 8 l ethanol and boiled for 30 min. The ethanol was filtered off and the residue was reboiled with the same volume of ethanol. The extracted material was collected on a Büchner funnel and washed several times with ethanol and finally suctioned dry. The washing of the material and the isolation of sialic acids were carried out as described for human liver[4]. The lyophilized crude fraction contained 315 mg sialic acid determined by the resorcinol method[5]. As the amount of N-acetylsialic acid in the original material was found to be 725 mg, the yield was 43 %.

Lyophilized material, corresponding to 275 mg sialic acids, was dissolved in 2 ml water and diluted with 20 ml methanol. 50 ml diethyl ether were added under continuous agitation. A large amorphous precipitate was filtered off and an additional 10 ml of ether added. Crystallization started immediately. The crystals were redissolved in water and methanol, and ether was added as before. The yield of recrystallized sialic acids was 137 mg (Fracion A).

The amorphous precipitate and the mother liquors were rechromatographed on Dowex-2. A second fraction of sialic acids could be crystallized (Fraction B = 61 mg).

Table I shows the glycolic acid content of the lyophilized material and of Fractions A and B.

Only two spots were found, with the same R_F values as N-acetylsialic acid and N-glycolylsialic acid, when the material was subjected to paper chromatography with *n*-butanol–*n*-propanol–0.1 NHCl (1 : 2 : 1, v/v/v) as solvent[7].

The X-ray powder diagram was identical with that of P-sialic acid[1,2] (N-glycolylsialic acid). The infrared spectrum was of the same type as that of a mixture of the two sialic acids[7].