THE STRUCTURE OF CRYSTALS OF PURIFIED MAHONEY POLIOVIRUS*

RUSSELL L. STEERE AND FREDERICK L. SCHAFFER
Virus Laboratory, University of California, Berkeley, Calif. (U.S.A.)

The first crystallization of the MEF-1 strain of Type 2 poliovirus was reported by SCHAFFER AND SCHWERDT in 1955[1]. Subsequently the crystallization of Coxsackie virus[2] and Types 1 and 3 poliovirus[3] have been reported. This paper is concerned with the structure of crystals of Type 1 (Mahoney strain) poliovirus.

Crystallization of the virus occurred spontaneously in a highly purified and concentrated suspension. The source and purification of the Mahoney strain poliovirus was identical to that described by SCHWERDT AND SCHAFFER[3]. As mentioned in the description of that purification procedure, considerable virus could be recovered from the "insoluble residue" from the second ultracentrifugation. The crystalline virus described here was derived from such material from four large pools representing 264 liters of original tissue-culture fluid. The residue fraction was extracted several times with pH 9 saline (0.88 *M* NaCl, 0.04 *M* Na_2HPO_4, adjusted to pH 9) and the extracts were combined and clarified at 1000 *g* for 30 min. The virus was then pelleted at 100,000 *g* for 2 h and the pellets were suspended in phosphate-saline (0.01 *M* Na_2HPO_4, 0.11 *M* NaCl, pH *ca.* 7.5) by rocking overnight in the cold. The resulting suspension was clarified at 6000 *g* for 10 min. The clarified product represented about 11 mg of virus material (as estimated by nitrogen[4]) in 2.64 ml of suspension. In purity as estimated by the ultraviolet spectrum, it compared favorably with the best preparations previously reported.

The preparation was set aside in a stoppered Pyrex test tube at 4° for storage. When examined approximately one year later a large number of crystals, many of them nearly perfect in form, were observed on the walls and in the bottom of the tube. The crystals ranged in size from a few microns up to about 0.4 mm in diameter. A group of the larger crystals on the wall of the tube are shown in Fig. 1.

Because of the availability of numerous well-formed crystals several experiments were performed to elucidate their nature and structure.

Bio-assay, pH and ultraviolet absorption

A small number of crystals suspended in mother liquor were withdrawn from the storage tube. They were collected by centrifugation, and the mother liquor was removed and its pH determined to be 7.3. These crystals were dissolved with some difficulty in pH 9 saline. The results of ultraviolet absorption measurements and

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TABLE I

INEFECTIVITY AND ULTRAVIOLET ABSORPTION OF MAHONEY CRYSTALS AND RELATED MATERIALS*

	Infectivity *pfu/ml*	*U.V. absorption ratio* $\frac{E_{260}}{E_{280}}$	*Specific infectivity* $\frac{pfu/ml}{unit\ E_{260}}$
Original preparation (prior to storage)	$5.6 \cdot 10^{10}$	1.67	$1.6 \cdot 10^{9}$
Dissolved crystals	$2.3 \cdot 10^{8}$	1.69	$1.2 \cdot 10^{8}$
Mother liquor	$5.0 \cdot 10^{9}$	1.70	$2.7 \cdot 10^{8}$

* See references 1 and 3 for methods. Infectivities expressed as plaque-forming units (pfu); extinctions (E) are for a 1 cm light path.

bio-assay presented in Table I show a loss of approximately 90 % of the infectivity during the one-year storage period. The low total infectivity of the dissolved crystals is attributable to the small number removed for assay. The difference in specific infectivities of the crystalline and mother liquor fractions may be due to more rapid inactivation in the crystalline form, or to inactivation during solution of the crystals. The difference in total ultraviolet absorption of the original preparation and the mother liquor indicates that about 1/3 to 1/2 the preparation was crystalline.

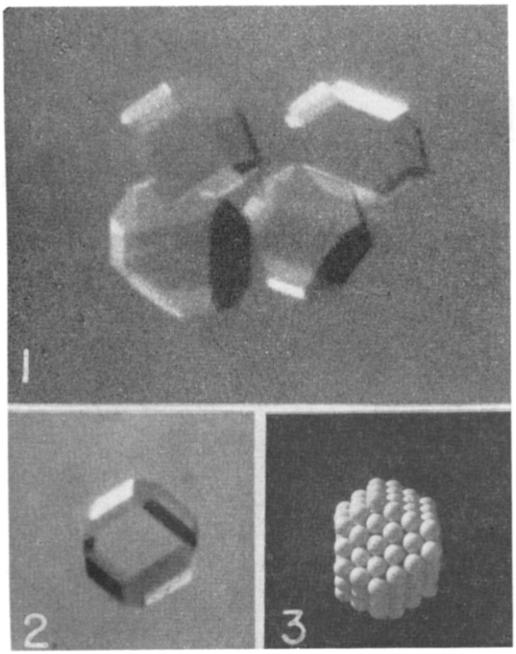

Fig. 1. Poliovirus crystals on inside wall of test tube. × 100.

Fig. 2. Poliovirus crystal removed from test tube and mounted on glass microscope slide. × 80.

Fig. 3. Model of crystal built of plastic balls glued together in packing of a face-centered cube.

Visual observations and manipulations

The crystals were found to be quite fragile upon handling, tending to fracture into extremely small crystallites. With care, however, a few were teased loose from the wall of the test tube below the liquid surface and were transferred along with a small volume of mother liquor to microscope slides with no observable damage to their structure. They were mounted inside a small ring of stopcock grease and a cover slip was pressed down carefully from above to make good contact with the grease and the liquid inside the grease ring. Care was taken to prevent crushing of the crystals. A crystal isolated and mounted in this manner is shown in Fig. 2. Most of the manipulations and observations were made in the cold room at 4°. Mounted crystals lost the

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sharpness of crystalline edges within one hour at 20° to 25°, and regained it upon chilling. This temperature effect became irreversible, however, after a few cycles, the crystals failing to redevelop.

Measurement of interfacial angles

Several crystals were isolated for measurement of the angles between the numerous faces in order to allow a description of the crystals to be made. These crystals were placed on glass slides, the mother liquor removed, a drop of DuPont "Duco waterproof clear cement" placed over each crystal, and a cover slip pressed down on top. The cement was allowed to harden overnight and interfacial angles of three crystals were measured* within 24 h before appreciable disintegration of the crystals had occurred.

For the crystal which had the most suitable faces for measurement, six faces were measured and it was presumed that the crystal was resting on a seventh face parallel to the slide.

The faces fall into the following zones (each zone including faces parallel to a common direction):

1, 3, 7, 6
2, 3, 4, 5
1, 2, 5, 6

Disposition of these zones closely approximates (within 2°) requirements for: Face 1, 2, 4, 5, 6, 7 = Octahedron, (111) of isometric system. Face 3 = Cube, (100) of isometric system. Interfacial angles measured: six octahedron-to-cube angles of 55° (± 2) [theoretical angle = 54 3/4°]. These are the angles between cube face 3 and the six octahedron faces. Six octahedron-to-octahedron angles average 70 1/2° (± 3) [theoretical angle is 70 1/2°].

In a second crystal two octahedron-to-cube angles were measured and found to be 54° and 55°. For the third crystal two octahedron-to-octahedron angles of 70° and three octahedron-to-cube angles of 51° to 55° averaging 54° were measured.

Most of the crystals failed to exhibit any birefringence whatsoever when placed between crossed nicol prisms. One large crystal (0.2 × 0.3 mm and 0.09 mm thick), however, did exhibit a very low birefringence, 0.001 or less.

The above measurements and observations are in as good agreement as can be expected for the following:

The crystals are isometric or nearly so. The habit is octahedral (111) with minor development of the cube faces (100). Crystals are distorted in shape by overdevelopment of some octahedron and suppression of some cube faces.

*Index of refraction***

A single well-developed crystal was placed on a gelatin-coated glass slide and the

* We are much indebted to Professor F. J. TURNER of the Geology Department on this campus for making these measurements and interpreting the results. Measurements were made on a Lietz universal stage attached to a petrographic microscope. This permits measuring interfacial angles and observing zonal relations on very small crystals; but precision is relatively low, being ± 2°–3° for all interfacial angles.

** We are indebted to Dr. CHARLES M. GILBERT of the Geology Department of this campus for his assistance in the determination of this index of refraction by the standard petrographic technique of grain immersion using immersion oils of various refractive indices.

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mother liquor removed leaving the crystal adhering firmly to the slide. This crystal was allowed partially to dry by standing exposed for 1/2 h in a 4° cold room. Various immersion oils were applied until the index of refraction was determined. Slide and crystal were thoroughly washed with CCl_4 between applications of the different oils. As measured at 4° in this manner the index of refraction of the poliovirus crystal was 1.535 ± 0.003. One must remember, however, that the crystal was only partially dried in the cold and that the amount of water present in the crystal is unknown. These measurements, consequently, are of doubtful significance.

Internal structure by electron microscopy

In order that the detailed internal structure of the crystal might be determined, several crystals were placed in a drop of mother liquor on a small brass block, rapidly frozen and prepared for electron microscopy following slight modifications of the procedure of STEERE[5].

Electron micrographs of replicas of fractured crystals show the arrangement of the virus particles within the crystal lattice (Fig. 4). The predominant plane in this micrograph shows the square packing to be associated with a cube face (100) (A-Fig. 4). Visible in the same micrograph are planes tilted with respect to the (100) plane and showing the close packing of the octahedron face (111) (B-Fig. 4). In a few places this crystal was fractured also along the (110) plane (C-Fig. 4). Of the five crystals for which replicas were obtained four were fractured along a (111) plane (Fig. 5) and only one was fractured in such a manner that both the (100) and the (111) faces were visible.

In order to obtain a measurement for particle diameter within the crystalline array, a suspension of Dow polystyrene latex lot 580G with a diameter of 259 mμ[6] was sprayed onto the surface of a replica of one of the crystals fractured along the (111) plane. Electron micrographs were taken in which the latex particles were superimposed on the replicated crystal areas. In this manner the length of a row of 10 or more virus particles could be compared with the diameter of the latex particles. The average diameter of the Mahoney strain of poliovirus as determined in this manner is 27.3 ± 1.4 mμ, a value which is in good agreement with other values obtained for this virus[7].

Across the width of the micrograph (Fig. 4) approximately 30 levels within the crystal are represented. In the original micrograph from which this portion was selected, a total of 55 levels were counted.

DISCUSSION

Since the individual particles of the Mahoney strain of poliovirus are spherical or nearly so[7], it is not surprising that crystals which develop in suspensions of the purified virus appear to be of the isometric system, as deduced from the lack of detactable birefringence in most crystals and the closeness of measured interfacial angles to the theoretical values. The small amount of birefringence observed in one crystal could result from a slight strain or distortion or from discontinuities such as that observed at D-Fig. 4. From the measurement of interfacial angles and from visual observation of numerous crystals, the crystal habit was determined to be octahedral with minor development of cube faces. Additional faces were observed on some crystals.

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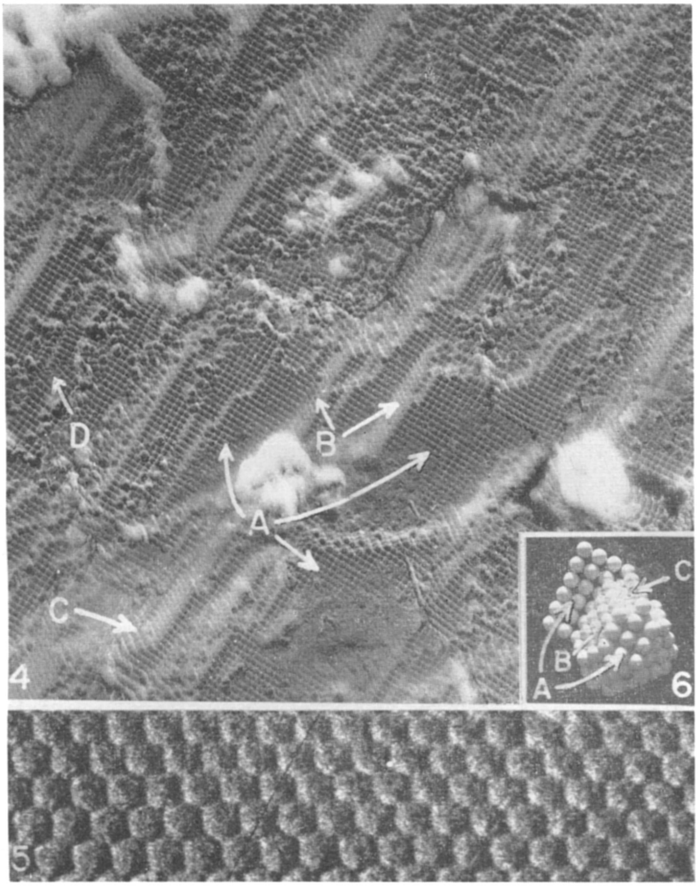

Fig. 4. Electron micrograph of a replica of a fractured, frozen crystal of poliovirus showing various planes within the crystal and a discontinuity. A, the cube (100) plane; B, the octahedron (111) plane; C, the (110) plane; and D, the discontinuity. × 30,000.

Fig. 5. Electron micrograph of a replica of a frozen crystal of poliovirus fractured along the octahedron (111) plane only. × 180,000.

Fig. 6. Model constructed of plastic balls with the packing of a face-centered cube. Planes marked A, B, and C correspond with those in Fig. 4.

Electron micrographs were obtained of fractured crystals in which cube (100) and octahedral (111) planes were clearly demonstrated. Using a print of a micrograph showing good reproduction of a (111) plane, similar to that of Fig. 5, three intersecting lines were drawn through a single particle in such a manner that they continued through the center of lines of particles on either side of the original. The angles between adjacent lines all measured 60°. Furthermore the lengths of lines to the far edge of the 30th particle in each of the six directions were equal. From these measurements, it was determined that the particle packing within the crystal has the space-lattice of a face-centered cube. Such measurements would be incompatible with a body-centered cube.

Models of the crystal have been constructed using small plastic balls packed in the form of a face-centered cube. It was possible in this manner to build up an octa-

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hedron with cube faces (Fig. 3) corresponding favorably with those of the poliovirus crystal (Fig. 2). Furthermore, by continuing the development of certain rows of particles the three faces as seen in A, B and C of Fig. 4 were able to be shown in their same respective positions (A, B and C of Fig. 6).

It is of interest that when poliovirus crystals frozen in mother liquor are fractured they tend to fracture preferentially along an octahedron (111) plane or along a cube (100) plane, there being only small portions (C-Fig. 4) where clean fractures along a (110) plane are observable. The major external faces of the poliovirus crystals should have particles arranged in the (111) and the (100) planes. This contrasts with the observations of LABAW AND WYCKOFF[8] in their recent studies of the external surfaces of southern bean mosaic virus crystals. They found that the largest flat planes of their crystals were the (110) planes. The particle packing of both types of crystals appears to be the same (face-centered cubic), the closest possible 3-dimensional packing for spherical objects.

X-ray crystallography should extend our knowledge of the structure of poliovirus crystals and may yield information concerning the structure of the virus particles themselves. Such studies might prove particularly interesting in view of the recent work of KLUG, FINCH AND FRANKLIN[9] with the crystals of turnip yellow mosaic virus, the particles of which are of comparable size with poliovirus but were found to be packed in such a manner that they lie with their centers at the lattice points of a body-centered cubic unit cell.

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SUMMARY

Crystals of Mahoney strain poliovirus have been examined to determine their crystal structure. It was concluded from these studies that the crystals were isometric or nearly so with habit being octahedral with minor development of the cube faces; and with unit packing, within the crystals, being that of a face-centered cube.

Electron micrographs of fractured crystals showed virus particles in cube (100) and octahedron (111) faces and also a few surfaces fractured along the (110) plane. The size of the particles within the crystal, as measured in a (111) plane, was found to be 27.3 ± 1.4 mμ.

A value of 1.535 ± 0.003 was determined for the index of refraction, but this is of doubtful worth because the degree of hydration of the crystal was unknown.

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