CRYSTALLIZATION OF PURIFIED MEF-1 POLIOMYELITIS VIRUS PARTICLES

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Communicated by W. M. Stanley, October 20, 1955 and read before the Academy, November 3, 1955

Highly purified preparations of poliomyelitis virus, strain MEF-1, have been shown by analytical ultracentrifugation to contain only one homogeneous component.1 Electron microscopic and other studies have revealed this component to consist of spherical particles approximately 27 μ in diameter which are readily aligned in two-dimensional, close-packed arrays and with which infectivity is associated.1, 2 The present report is concerned with the preparation and the description of properties of three-dimensional crystals, readily visible in the light microscope, which were obtained from a highly purified and concentrated preparation of such virus particles. Although several plant viruses have been crystallized3 since the initial crystallization of tobacco mosaic virus by Stanley in 1935,4 this is believed to be the first report of the crystallization of a virus affecting man or animals.

Crystallization.—The source of virus was the fluid harvested from monkey kidney tissue cultures infected with strain MEF-1 poliomyelitis virus and provided by the Connaught Laboratories, Toronto. The initial purification procedure, which has been previously outlined,1 included methanol precipitation, butanol extraction, ultracentrifugation, nuclease digestion, and electrophoretic fractionation. Further purification of the preparation was achieved by sedimentation through a sucrose density gradient in a swinging cup rotor (Spinco SW-39), as described by Brakke for plant viruses.5 In this way approximately 1.2 mg. of nucleoprotein material was obtained as a single sedimenting band in 0.73 ml. of 0.14 M NaCl containing approximately 30 per cent sucrose. This represented the virus concentrated from 15 liters of infected monkey kidney tissue culture fluid. The preparation was diluted to 2.0 ml. with 0.14 M NaCl and was sedimented by ultracentrifugation in an average centrifugal field 100,000 × g for 2.5 hours. The clear, gel-like pellet was covered with 0.3 ml. of unbuffered 0.14 M NaCl at pH 5.9 and gently rocked over- night at 4° C. After this treatment the pellet appeared white and opaque, and upon suspension discrete crystals were seen.

After removing a small fraction of the suspended crystals for further observation, the mother-liquor was removed from the remaining crystals. The latter were washed twice with isotonic saline and the washings pooled with the mother-liquor. The crystals were readily dissolved by warming to room temperature in 0.14 M NaCl adjusted to pH 8.5 with dilute NaOH.

The virus was recrystallized as follows: An aliquot of the dissolved crystals was diluted with a small volume of 0.14 M NaCl buffered with 0.001 M phosphate at pH 7.6, and the virus was then sedimented in a sealed capsule6 in the swinging cup rotor of the ultracentrifuge. After removal of the supernatant fluid, the pellet was sus- pended in a small volume of isotonic saline buffered with 0.01 M phosphate at pH 5.9 and allowed to stand at 4° C. Small crystals were seen by microscopic examination. After low-speed centrifugation, the mother-liquor was removed from the re-
crystallized material, which was washed with pH 5.9 saline. The washings were pooled with the mother-liquor. These crystals again were easily dissolved in isotonic saline buffered at pH 7.8 with 0.01 M phosphate.

Properties of the Crystals.—A series of photomicrographs of the crystals taken in ultraviolet light at various wave lengths is shown in Figure 1. The rapid decrease in transmission between 295 and 280 m\(\mu\) is consistent with the nucleoprotein nature of the virus particles and their ultraviolet absorption spectrum in suspension.\(^1\) The photomicrograph in visible light shown in Figure 2 gives some idea of the shape of the crystals. They appear to be tetragonal prisms with pyramids at both ends. The larger specimens measure approximately 30 \(\mu\) in length.

Evidence that the crystals contained the virus particles was provided by the essentially constant specific infectivity measurements of solutions of washed crystals, of mother-liquors, and of the two fractions of purified and concentrated virus from which the crystals were derived. Infectivity measurements were made by plaque assay on monkey kidney cultures by a modification\(^7\) of the technique of Dulbecco and Vogt.\(^8\) Specific infectivities were expressed as the number of plaque-forming units (PFU) per unit mass of nucleoprotein. The latter was measured by optical density at 260 m\(\mu\) in a Beckman spectrophotometer and in some instances by direct
chemical analysis for nitrogen. By means of analytical electron microscopy, an estimate was also made of the ratio of countable physical particles to PFU. Since there was no electron microscopic evidence of material other than the 27-mu particles in the dissolved crystals, it was possible to estimate the mass of a single physical particle from the experimental data. The results of various experiments are summarized in Table 1.

<table>
<thead>
<tr>
<th>Relative Sequence of Fractionation</th>
<th>PFU/MI per Unit OD†</th>
<th>PFU per Gram†</th>
<th>27-mu Particles per PFU</th>
<th>Estimated Grams per Particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoretic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.2 × 10⁹</td>
<td>1.6 × 10¹⁴</td>
<td>3,000</td>
<td>2.1 × 10⁻¹⁷</td>
</tr>
<tr>
<td>2</td>
<td>3.1 × 10⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.3 × 10⁹</td>
<td>2.2 × 10¹³</td>
<td>2,250</td>
<td>2.0 × 10⁻¹⁷</td>
</tr>
<tr>
<td>Mother-liquor from 1st crystallisation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.2 × 10⁹</td>
<td>2.3 × 10¹³</td>
<td>1,300</td>
<td>3.3 × 10⁻¹⁷</td>
</tr>
<tr>
<td>Recrystallized virus</td>
<td>3.5 × 10⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>850</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother-liquor from recrystallisation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.4 × 10⁹</td>
<td></td>
<td></td>
<td>1,200</td>
</tr>
</tbody>
</table>

† OD 260mu = optical density measured at 260-mu wavelength for a solution 1 cm. thick.
‡ Nitrogen × 6.25.

Discussion.—The constant specific infectivity values listed in the third and fourth columns of Table 1 for the density gradient, crystalline, and mother-liquor fractions attest to the comparable degrees of purity of these fractions. In the fifth column, the reciprocal specific infectivities expressed as physical particles per PFU are more variable. This probably can be accounted for by a variable loss of physical particles by adsorption and disintegration experienced in the preparation, dilution, and spraying of extremely small samples of highly purified virus for quantitative electron microscopy.

Physical and chemical data obtained earlier on highly purified though not crystalline MEF-1 virus provided sufficient information to estimate by Stokes's low a virus-particle mass of 1–1.5 × 10⁻¹⁷ gm. The mass of the particles is also calculable from the nucleoprotein and physical-particle concentrations of the purified virus preparations determined by direct chemical analysis and analytical electron microscopy, respectively. If the physical-particle count is low, owing to losses discussed above, the value of the mass per particle (sixth column) will be high. The difference between the particle mass estimated from Stokes's law and the 2.0 × 10⁻¹⁷ gm. obtained for the particles from crystalline virus can probably be attributed, therefore, to low electron microscope counts. The above data, supported by ultracentrifugal and electron microscopic evidence for homogeneity, lead to the conclusion that the crystals were composed of the 27-mu particles.

It is probable that a large percentage of the particles in the crystalline state are inactive due to prolonged storage of the virus. That fraction which is inactive is difficult to determine because of our uncertain knowledge of the efficiency of the plaque-assay technique. It is possible, however, that essentially all the physical particles were at one time mature, viable virus and that biological inactivation resulted in no physical and chemical alteration detectable by presently available tests.
This idea is compatible with the ability of the particles to crystallize, since it has been generally observed that no denatured protein will crystallize. 10

Preliminary experiments in our laboratory with purified Saukett poliomyelitis virus indicate that it can also be crystallized under conditions successfully applied to the MEF-1 virus. It does not seem unreasonable, therefore, to expect all three types of human poliomyelitis as well as other animal viruses of small size to be capable of crystallization under proper conditions. This crystallization of a virus affecting man and animal emphasizes anew certain basic similarities between animal and plant viruses.

Summary.—The crystallization of human poliomyelitis virus particles from a highly purified and concentrated preparation has been reported for the first time. The appearance of the crystals and the evidence that the crystals are composed of characteristic virus particles, 27 mp in diameter, are presented and discussed.

The authors are indebted to Drs. Russell L. Steere and Robert C. Backus for the visible and ultraviolet photomicrographs, respectively, of the virus crystals, and to Miss Joan Sprecher and Miss Helen Fisher for their excellent technical assistance.

* Aided by a grant from the National Foundation for Infantile Paralysis. This paper was presented before a meeting of the National Academy of Sciences at Pasadena, California, on November 3, 1955 (see Science, 122, 879, 1955).
5 M. K. Brakke, Arch. Biochem. and Biophys., 45, 275, 1953.
6 R. C. Backus and R. C. Williams, Science, 117, 221, 1953.

INFLUENCE OF AUXIN ON CELL-WALL METABOLISM*

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Communicated September 4, 1955

INTRODUCTION

The plant hormone auxin (indole-3-acetic acid, IAA) controls rate of increase in size of the cells of many plant tissues. Thus, excised sections of the oat coleoptile respond to addition of the hormone by increasing more rapidly in length. It is known that the auxin-induced increase in cell volume of the oat section is immediately due to the uptake of water by the cell.1 Auxiliary induced uptake of water by the coleoptile is in turn an osmotic phenomenon.2 Net water uptake by the coleop-