LETTERS TO THE EDITORS

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Molecular Arrangement in Crystals of the Southern Bean Mosaic Virus Protein

ABOUT ten years ago, it was shown[1] that electron microscopic examination of 'psuedo-replicas' could reveal the ordered particle arrangement on the surface of a macromolecular crystal. The necessary preparations were made by metal-shadowing the dried crystals lying on a glass surface, coating with collodion, freeing this composite layer and dissolving the crystalline deposit from the replica thus produced. The molecular order on several kinds of crystal was seen in this way; but a broader application of the method was limited by factors such as the fragility of the collodion film and the need to work with proteins not denatured by vacuum desiccation and the evaporation of metal.

The possibilities inherent in this approach to problems of crystal growth, as well as structure, have been so great that continued efforts have been made to improve this initial technique. Thus 'silicon monoxide' films have been employed[2] to reproduce molecular order in crystals of certain proteins. We have now found that evaporated films of carbon[3] permit a very significant advance in both the quality of replication and the kinds of macromolecular crystals that can be successfully studied.

Molecular order was first demonstrated using crystals of the southern bean mosaic virus protein[1]. In this case, however, as in practically all others, the initial method did not replicate enough different faces to permit a conclusive deduction of the molecular arrangement within the crystal. The numerous crystal faces that can be seen when carbon films are used for preparing specimens of this substance are illustrated in Figs. 1 and 2. These better results are to be attributed in part to the advantageous mechanical and electron optical properties of the carbon membranes, and in part to their chemical inertness and the possibilities this offers of various purifying procedures.

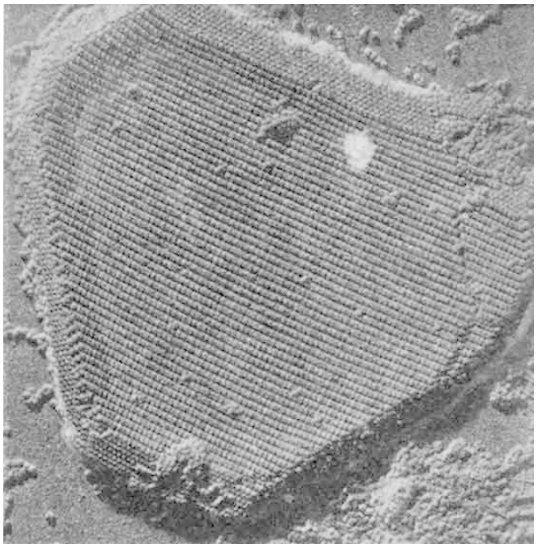

Fig. 1. An electron micrograph of a complete southern bean mosaic virus crystal showing the molecular arrangement on several of its faces. The large face is 110. Magnification × 30,000

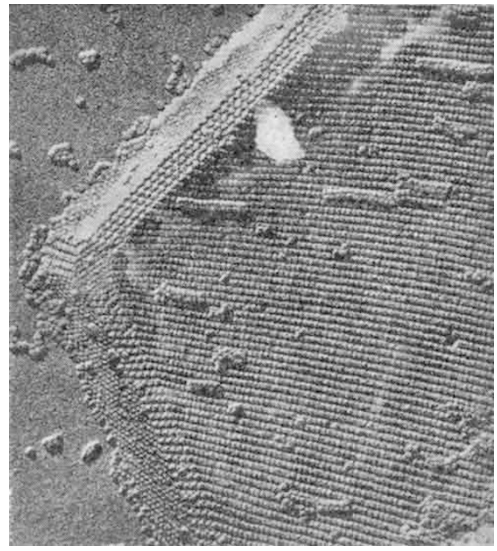

Fig. 2. An electron micrograph of the molecular distribution on several faces developing on one corner of a southern bean mosaic virus crystal. Two [110], [120], [111] and one [100] faces are shown here. Magnification × 30,000

The accompanying electron micrographs are typical of approximately a hundred thus far made of crystals of this virus protein. The crystal symmetry, as determined from measurements of inter-particle spacings and of angles between the edges of crystal faces, proves to be very nearly, if not exactly, cubic. The principal face has invariably been that of a dodecahedron. Faces of the several forms that have been seen have been identified by their relative positions on the crystal and by the particle configurations and spacings they exhibit. This identification has been confirmed by comparisons between the electron micrographs and models built of cork balls which, using the chosen molecular arrangement, reproduce the observed faces. The crystals shown in the figures exhibit faces of the forms [100], [111], [120] and [113], in addition to the dominant [110].

The molecular arrangement in this case is a cubic close packing of essentially spherical molecular particles of diameter about 230 A. The edge-length of the unit cube, containing four of these molecules, is about 325 A.

We have been using this improved method of specimen preparation for studying crystallographic problems connected with the growth of these crystals and for determining the molecular arrangement in a number of other crystalline proteins. These investigations are continuing and will be described in detail elsewhere.

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[2] Hall, C. E., *J. Biol. Chem.*, **185**, 45 (1951).
[3] Bradley, D. E., *Brit. J. App. Phys.*, 5, 65, 96 (1954).