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The Electron Microscopy of Macromolecular Crystals*

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A survey is given of recent applications of electron microscopy to the direct study of the molecular structure of macromolecular solids. The arrangement of molecules in a number of proteins is shown and evidence is obtained on the molecular constitution of the faces of crystals of these materials.

Introduction

Problems of crystal symmetry on two very different levels can be attacked with the help of the electron microscrope. On a coarse level this instrument can be employed as an extension of the optical microscope to investigate how crystals and crystalline aggregates grow, and especially how they are etched. Metallography is primarily concerned with such etching phenomena and the wealth of additional etch detail present in electron micrographs will undoubtedly be of increasing help in solving problems in this and related fields. The electron microscope also opens up an entirely new set of problems on a finer level because of its ability to portray individual macromolecules. Through photography of the arrangement prevailing in macromolecular solids, it is possible to observe and measure directly the molecular structure of para and of true crystals and to examine on this molecular level many of the fundamental questions of crystal growth and dissolution. This paper aims to survey progress made in the two years since the possibility of such measurements was first demonstrated.

The experimental methods employed in the electron microscopy of crystalline preparations have already been described (Williams & Wyckoff, 1944, 1945, 1946; Wyckoff, 1947*a*). Since the preparations must be extremely thin, most single crystals and crystalline aggregates must be investigated through replicas of their surfaces or through surface films prepared by replica techniques. Detail down to macromolecular dimensions can be recorded in collodion replicas subsequently shadowed with either chromium or palladium. Such replicas have been useful for studying the smoothness of individual faces of large crystals, the character of their etch pits, and the influence of twinning on their surface structure. But for studying molecular array, it has been necessary to work with shadowed 'pseudo-replicas'. These have been made by drying the crystals on a clean glass surface and shadowing the resulting deposit by the oblique evaporation on it of gold or palladium. This metallic coating has then been covered with the thinnest possible layer of collodion and floated, together with the metal and the topmost layer of the preparation trapped in it, upon a water surface for the usual final manipulation. Where the molecular order in the deposit was not lost on drying, and where drying has not rendered the bulk of it insoluble and adherent to the film, these 'pseudo-replicas' have shown details of crystalline structure down to the limits of microscopic resolution. Where the procedure has failed, as it has with the bushy stunt and turnip yellow virus protein crystals, this has apparently been due to an actual collapse of the crystal structure during drying.

Protein structures

The simplest experiment to be made on macromolecular arrangement concerns the degree of order in deposits dried from solution (Price, Williams & Wyckoff, 1946). If a solution is sufficiently dilute, its deposit can be prepared on a thin collodion or similar substrate and examined without recourse to the replication described above. All purified virus proteins with spherical or near-spherical molecules have furnished dried deposits that more or less resemble Fig. 1. Where very few particles have been present they have shown a marked tendency to arrange themselves in line, like beads on a string. In regions of greater molecular concentration the molecules have packed together to form a regular net. Over nearly all areas the net has been hexagonal, but most preparations have also contained small areas where the net was square. In still more concentrated regions regular nets of molecules have frequently overlain other molecules in what may be imagined as a first step towards crystallization. Though all the virus proteins have shown some regularity in their particle arrangement, the tendency towards order has not been equally pronounced. It has been noteworthy with the Rothamsted necrosis protein* as shown in Fig. 1. The several independent areas of regularity in this photograph, and the way they meet one another, strongly suggest fields seen in the motion picture of bubbles packed on a water surface (Bragg & Nye, 1947).

Attempts have been made to carry still further this process of crystal formation by desiccation, but thus

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Fig. 1. An electron micrograph of a portion of a microdrop of a water solution of the Rothamsted tobacco necrosis protein dried on collodion. At the bottom the ordered molecules are one layer thick, at the top two layers. Palladium shadowing. Magnification, 50,000.

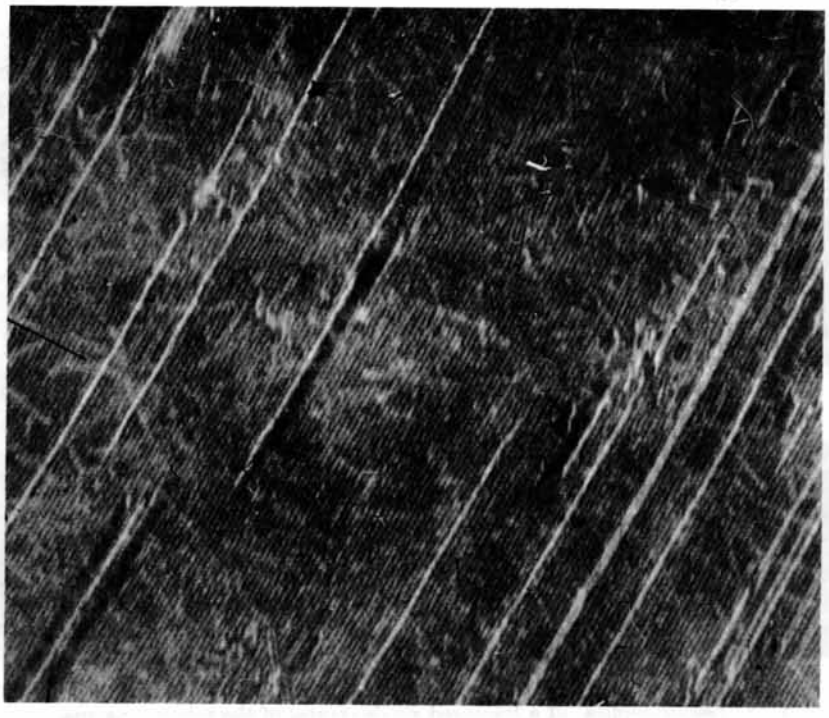

Fig. 2. A 'pseudo-replica' of the top surface of a dried tobacco mosaic virus protein gel showing the molecules in a strictly parallel array. The molecular rods have diameters of 150 A. Magnification, 50,000.

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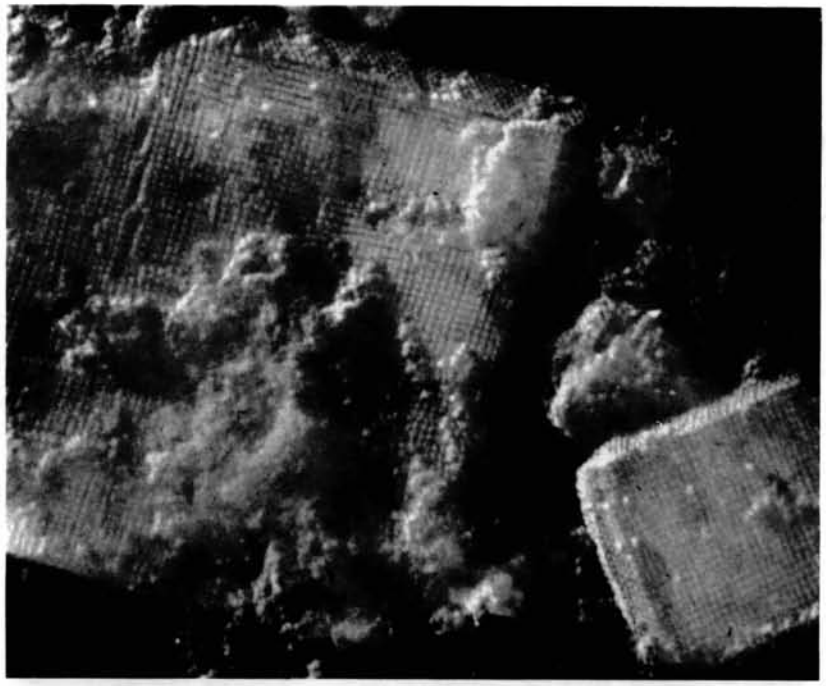

Fig. 3. Several cube octahedra of a necrosis virus protein showing their molecular arrangement. The disordered mass of molecules covering much of the (100) face of the largest crystal were probably in solution in the mother liquor from which the crystals were taken. Shadowed twice from directions at right angles to one another to bring out molecular arrangement on side faces. Palladium shadowing. Magnification, 55,000.

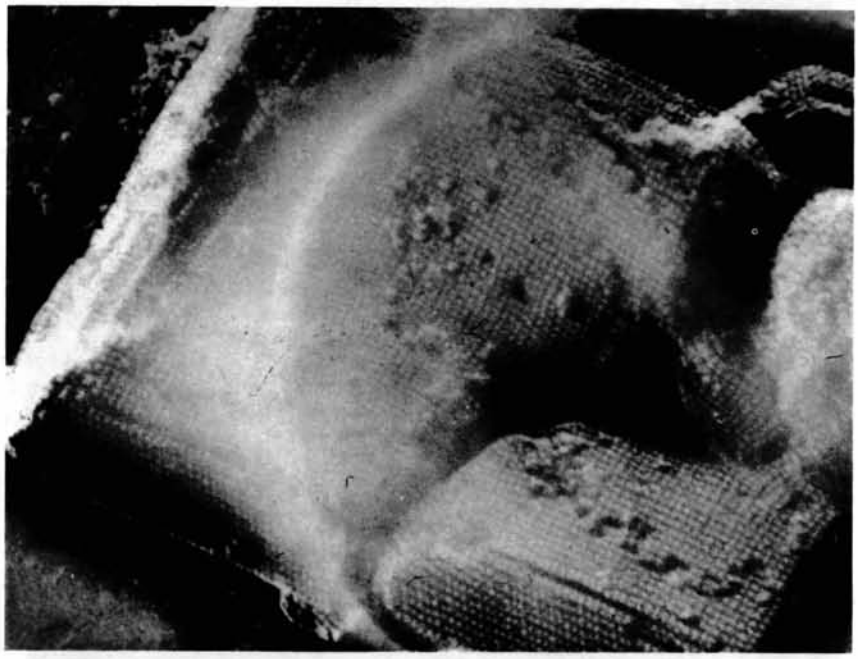

Fig. 4. A 'pseudo-replica' of a fractured single crystal of the tobacco necrosis virus protein. Molecular arrangement on a (111) face is better shown than in Fig. 3. Magnification, 50,000.

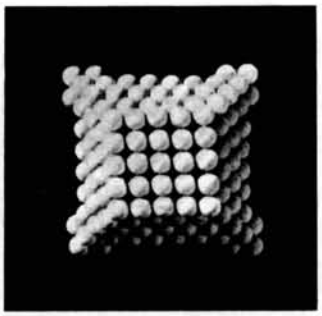

Fig. 5. A cork-ball model of a cubic close packing showing the same face development as the crystals of Figs. 3 and 4.

Fig. 6. A 'pseudo-replica' of the top face of an orthorhombic crystal of the southern bean mosaic virus protein. The molecules in the crystal are not spherical and the net is rectangular but not square. Gold shadowing. Magnification, 40,000.

Fig. 7. A 'pseudo-replica' of part of a single crystal of the Rothamsted necrosis protein showing its terraced faces. This is one of the few untwinned examples observed. The (100) face in the upper right, like the top (111) face, is reasonably flat, though terraced; the side faces on the left are nothing but shelving series of steps. Palladium shadowing. Magnification, 50,000.

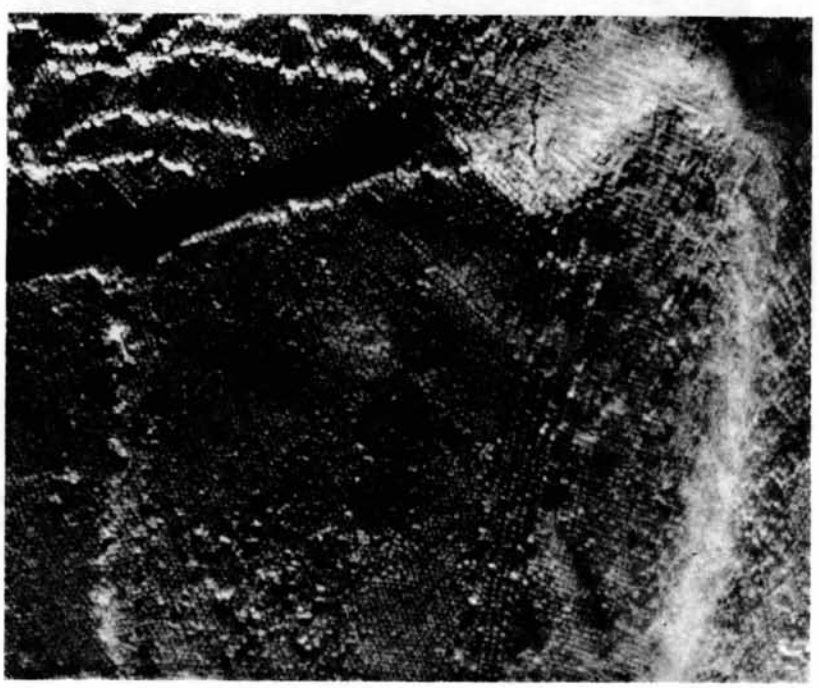

Fig. 8. A 'pseudo-replica' of a part of a highly twinned crystal of the Rothamsted necrosis protein. The (100) side face is at the right. The top (111) face shows clearly the composite nature of the crystal by the frequent changes in direction of the axes of the hexagonal net. Palladium shadowing. Magnification, 55,000.

far there has been no success in producing better-ordered aggregates, even in the presence of ammonium sulfate and other agents for 'salting-out'. There is, however, need for much more experimentation in this direction. Particles and molecules ordered into regular nets on drying from solution have been obtained from many substances besides virus proteins. Certain of the hemocyanins show this ordering and it has even been found with the thin-tailed bacteriophages once they have been obtained sufficiently pure.

The tobacco mosaic virus protein, with its greatly elongated molecular particles, forms deposits which exhibit many of the characteristic properties of para-crystalline materials (Wyckoff, 1947*b*). Its solutions, when sufficiently concentrated, separate into two layers, the bottom one of which is optically anistropic. This anisotropy must of course result from some sort of order in molecular arrangement. Electron micrographs of frozen-dried preparations from such solutions have shown extensive membranous associations of tobacco-mosaic particles. Careful examination shows that several of these sheets frequently overlie one another and consequently they cannot be explained as a surface film formed at some stage of the preparation of the sample. It is more reasonable to suppose that they exist as such in the liquid and are responsible for its anisotropy.

Solutions still more concentrated are gels whose stiffness increases with the content of tobacco-mosaic protein. Such gels of various water-contents can be prepared for subsequent electron microscopy by the wedge method of Bernal & Fankuchen (1941). They show areas in which the molecules are in remarkably perfect parallel arrangement (Fig. 2).

The arrangement in these well-oriented gels of tobacco mosaic is far superior to that in the so-called crystals of this protein obtained by iso-electric or ammonium-suphate precipitation. As numerous electron micrographs have indicated, such 'crystals' are in fact only sheaves of poorly oriented molecules. The lack of order they present is in striking contrast to the perfection of molecular arrangement in a true crystal. This is apparent from Figs. 3 and 4 which show cubic crystals of one of the necrosis virus proteins (Markham, Smith & Wyckoff, 1947, 1948). The faces of these crystals are strictly flat and have their molecules in close-packed regular nets which are square on the top and hexagonal on the pyramidal faces. Comparison with the cube-octahedral model of Fig. 5 shows that this is to be expected if the molecules are in a cubic close packing and if the indices of the top and pyramidal faces are (100) and (111) respectively. Axes of the unit cube, diagonal to the square edges of the top face, have a length, $a_0 = 340$ A., equal to the distance between adjacent molecules in this diagonal direction.

The dimensions measured on electron micrographs apply to completely desiccated crystals, and for the moment there is no way to determine how different they may be from those of the hydrated crystals. The overall excellence of the molecular arrangement as photographed and its continued cubic symmetry suggest, however, that this crystal loses its water without serious alterations in its structure.

The southern bean mosaic virus protein has given excellent records of its crystal structure (Price & Wyckoff, 1946). Its symmetry is probably orthorhombic, however, and this, along with the difficulty of getting crystals of sufficiently diverse habit, has interfered with a complete deduction of molecular arrangement. The available crystals have been tabular with a top face that was molecularly flat. Their side faces have usually not been well replicated but some at least appear flat. As can be seen from Fig. 6, the net on the top face is rectangular with one axis about 1·4 times longer than the other. This is evidently due to the flattened shape of the molecules; but the reason for their departure from sphericity is unknown. The separate particles of this protein, as seen in dried deposits, have seemed spherical; it is important to determine if this is true and if the forces involved in crystal formation have been sufficient to bring about this molecular compression. There is good reason, based on the symmetry of the wet crystals, for believing that the distortion is not a consequence of desiccation. In the few instances where it has adequately been reproduced, the net on side faces has been hexagonal. This naturally suggests that the structure of this crystal may be an orthorhombic distortion of the same cubic close packing found for the necrosis virus, but better photographs of side faces will be required to establish this fact.

Recent experiments with a crystalline product of the Rothamsted necrosis virus have introduced new questions of crystallographic importance. This protein, unlike others thus far derived from virus-diseased material, crystallizes spontaneously from sufficiently concentrated water solutions (Bawden & Pirie, 1942, 1945). As a consequence, the pellet formed by ultra-centrifugation is largely crystalline. All the crystals thus far photographed have been obtained in this way. Those resulting from slower crystallization apparently are of more diverse habit, and their photography will inevitably add much to the present results.

The constitution of crystal faces

Two types of crystal have been seen in preparations made by smearing the crystalline pellets on a glass surface for 'replication'. One consists of well-formed polyhedrons, the other of flat lozenge-shaped objects whose faces are not molecularly flat but consist of series of steps. In the typical example of Fig. 7 the top face is nearly flat, but some sides of the tablet are bounded by terraces, the breadth of whose steps in different directions statistically determines the inclination of the bounding 'face'.

Such photographs introduce the general question of the molecular constitution of crystal faces. Most of

those occurring in nature cannot be planar nets of particles. Evidently there must be two sorts of planes and faces on crystals: (1) those like the preceding whose particles in close contact form a planar net; and (2) those more thinly populated faces whose particles in a plane could make at best very incomplete contact with one another. It is to be expected that only faces of the first sort will be molecularly flat. The character of the roughness of the others will become apparent when faces of less simple forms are examined, but the terraced faces of Fig. 7 suggest one form this roughness may take.

Photographs of any of the Rothamsted crystals at a sufficiently high magnification (Fig. 8) demonstrate that the molecules on the top faces form a hexagonal net, like that prevailing on (111) faces of the virus crystals of Figs. 3 and 4. On every polyhedron examined and on most of the other crystals, however, this particle distribution has not been continuous over the entire face. Instead these faces have consisted of a number of areas with particle arrangements turned 30° with respect to those in adjacent areas. That this is not a superficial phenomenon can be seen by tracing the lines of contact between different areas as they pass beyond one face of a crystal. Evidently these polyhydra demonstrate the kind of intimate shifts in molecular arrangement that are responsible for at least one type of penetration twinning. This twinning in crystals prepared by ultracentrifugation is perhaps not so surprising in view of the rapidity with which they have been formed. When more slowly grown crystals can be examined they may be found to be simple, untwinned individuals.

In spite of the twinning shown by these crystals, a study of the molecular distribution over their side faces provides a good idea of their molecular arrangement. The side face to the right of Fig. 7 and the corresponding face of Fig. 8 have rectangular, apparently square, nets. Study indicates that they correspond closely to a cubic close-packed arrangement bounded on the top by an octahedral and on the four sides by cubic and octahedral faces. In fact, taking into account the twinning that is present, available electron micrographs show no detectable departure from such an arrangement. Several years ago X-ray reflections were obtained from crystals that may have been from the same necrosis protein (Crowfoot & Schmidt, 1945). These crystals had a definite low symmetry and the diffraction data pointed to a unit that was at most monoclinic. It is possible that the molecular arrangement in the crystals examined here is a minor distortion of the cubic close packing, but this will only become apparent when untwinned individuals are photographed.

These experiments leading to direct visual determinations of the molecular arrangement in crystals are obviously of a fragmentary character, but gradual improvements in methods of specimen preparation are extending their range of application. At the same time, the intimacy and diversity of the information already obtained suggest that this type of electron microscopy can be the source of information about fine structure that applies beyond the restricted realm of macromolecular aggregates.

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