molecule only to provide a certain number of important but insufficient relations among the values which may be ascribed to all these interesting quantities. Joint consideration of series of related molecules should prove more productive.

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A Comparison of X-ray Measurements on Air-Dried Tobacco Necrosis Protein Crystals with Electron-Microscope Data

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From X-ray and goniometric measurements on large air-dried crystals of the Rothamsted tobacco necrosis protein, lattice constants for the triclinic unit cell have been determined and compared with observations made by Wyckoff using the electron microscope.

In examining crystals of the Rothamsted and other strains of tobacco necrosis protein, Wyckoff (1948) showed that it was possible to observe in the electron microscope the arrangement of the molecules in the different crystal faces, and so to make deductions about the probable crystal symmetry. The electron micrograms show that the particles are roughly spherical, although more space-filling than true spheres, and Wyckoff suggested that the faces shown were the cube and octahedral faces of a crystal having an essentially cubic close-packed structure. We have now carried out X-ray measurements on large air-dried crystals of the Rothamsted strain of tobacco necrosis protein and these provide an interesting comparison with Wyckoff's deductions. While the X-ray data give little exact evidence of the molecular shape, the shorter wave-lengths used do enable a more precise picture of the orientation and inter-particle distances to be obtained. These are found to be essentially similar to those suggested by Wyckoff, although the crystal symmetry is actually triclinic and not cubic.

The crystals of the tobacco necrosis protein were prepared by Dr N. W. Pirie at Rothamsted (Bawden & Pirie, 1945). They had separated from aqueous solution and had been allowed to dry very slowly over a period of months. In form they were weakly birefringent triclinic blocks, bounded by {100}, {010} and {001}, 0·15–0·5 mm. across. Direct observation under the microscope of the interfacial angles gave $\alpha = 99$–$102°$, $\beta = 108$–$112°$, $\gamma = 118$–$121°$. The faces developed were sufficiently clear for goniometric measurements to be attempted, though they showed some distortion and gave blurred signal images. The angles measured by Miss M. W. Porter are

	Observed	Calculated from α, β, γ below
$(0\bar{1}0):(001)$	64° 47′–66° 1′	64° 58′ α^*
$(100):(001)$	59° 4′	59° 49′ β^*
$(0\bar{1}0):(100)$	52° 38′	52° 49′ γ^*

A series of X-ray photographs was taken of single crystals using chromium radiation and plate-to-crystal distances of 10 and 20 cm. These were all 2° or 4° oscillation photographs taken with each axis in turn as rotation axis, the beam direction being initially parallel to a crystal face. The photographs showed at most only two orders of the main-face reflexions, and on the *c*-axis photographs the layer lines were practically invisible. No reflexions were observed with spacings smaller than 58 A. From the photographs, in conjunction with the microscope measurements, the following lattice constants were derived:

$$a = 157, \quad b = 154, \quad c = 147 \text{ A.},$$
$$d_{100} = 116, \quad d_{010} = 119, \quad d_{001} = 123 \text{ A.},$$
$$\alpha = 100, \quad \beta = 110, \quad \gamma = 120°.$$

The unit-cell volume calculated from these figures is 2,572,000 A.^{3}, and the crystal density measured by flotation in toluene/*o*-dichlorobenzene mixture was 1·317 g.cm.$^{-3}$. On drying in vacuo at 100° C. Weiler & Strauss found that the loss of solvent was 21·4% by weight of the air-dried crystals. The molecular weight of the protein present, calculated from these figures, is 1,600,000, in good agreement with earlier estimates derived from the wet crystals (Crowfoot & Schmidt,

1945). The accuracy of this value, however, is low, of the order ± 100,000, on account of the limited X-ray data available. The error may be even greater if there is an error in the value for the solvent content, which is possible owing to the very small amount of crystals used in this determination. The figure does, however, agree reasonably well with the ultracentrifuge measurements made by Ogston (see Bawden & Pirie, 1945); which, on the assumption of a spherical molecule, correspond to 16 mμ diameter and a minimum molecular weight of $1{\cdot}85 \times 10^6$.

Although the crystal lattice now found is not as nearly cubic as suggested by Wyckoff, the actual correspondence between the electron-microscope photographs and our observations is very close. On one face, the *c* face of the crystal, the packing of the molecules is in a hexagonal close-packed array within the limits of experimental error. Microscopically, just as in the electron microscope, the crystal can be seen to be built up by packing together these hexagonal layers (see Wyckoff, 1948, Fig. 7). The actual relative positions of the layers adopted is, however, some distance from that required for octahedral planes in cubic close packing; the angle between the *b* and *c* axes in the pseudo-cube face, our (100) face, is not 90° but nearly 100°, and in the second 'octahedral' face, our (010) face, the angle between the *a* and *c* axes is not 120° but 110°. With this distortion the electron-microscope pictures appear to be reasonably in agreement, judged by the appearance of an extended model of the crystal lattice. Moreover, the distances between the particles, corresponding to the *a* and *b* lattice constants, 157 and 154 A., have been estimated on different photographs given to us by Wyckoff as between 130 and 165 A. Wyckoff considers that the best value for this distance is 140 A. and that under the conditions of electron microscopy a small further shrinkage of the crystal from the air-dried state may occur (Wyckoff, 1950, p. 192). The electron microscope also shows that the dried crystals are far from regular; their disorder limits the X-ray diffraction effects visible to those that depend only on the approximate close packing of roughly similar spherical units.

The earlier measurements carried out on wet tobacco necrosis protein crystals indicated a more complex type of triclinic lattice than that found now, with two non-equivalent molecules in the unit cell. If, however, we take the approximate positions of the molecular centres in the wet crystals, as shown by the X-ray spectra, we obtain a pseudo cell closely similar to that now described:

$$a' = 179, \quad b' = 176, \quad c' = 179 \text{ A.},$$
$$\alpha = 83°, \quad \beta = 109°, \quad \gamma = 119°.$$

The difference between the cell dimensions of the wet and dry 'unit cells' is only of the order of 20–30 A., as in many smaller protein crystals; again there is one plane in which the packing is nearly hexagonal close packed. When the crystals shrink, however, the close-packed planes slide into rather different positions in relation to one another, the angle α changing from the acute to the obtuse angle on passing from the wet to the dry crystal. At the same time the disorder introduced makes it impossible to detect differences in the relative orientation of the neighbouring molecules in the dry-crystal lattice. Only the precise triclinic character of the lattice remains as evidence that we are dealing with units which have a specific internal structure and a shape which is not exactly spherical.

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