Some X-ray Measurements on Single Crystals of Tomato Bushy-Stunt Virus

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X-ray measurements on single crystals of tomato bushy-stunt virus have confirmed that the crystal is cubic body-centred with a cell side of 386 A. for the wet and 314 A. for the dry state. Owing to the large unit cell of the crystal there are many crystallographic planes in a stationary crystal in position to reflect monochromatic X-rays, thus giving rise to a photograph showing concentric rings of discrete reflexions when the X-ray beam is travelling parallel to a crystallographic axis. From such photographs it can be shown that both the Bravais lattice and the cell side can be simultaneously determined for the wet crystal without the need of resorting to oscillation or rotation photographs. It is found that the 'ring' effect has completely disappeared in the dry state, and instead the appearance of reflexions near the centre of the photograph suggests that a slight disorientation of internal crystallite regions has possibly taken place.

The experiments indicate the necessity for the use of high-intensity sources of X-rays for work on weakly scattering biological materials.

Crowfoot & Schmidt (1945) have discussed and explained the type of X-ray photograph obtained from a stationary crystal, that of tobacco necrosis virus derivative, containing a large unit cell. In this case the crystal is triclinic, and their use of 'stills' enabled them to define the reciprocal cell dimensions before taking the oscillation photographs necessary to give them the degree of resolution required for a further detailed interpretation. We have obtained similar 'stills' with the cubic body-centred wet crystalline tomato bushy-stunt virus of cell side 390 A., which is considerably larger than the cell dimensions of tobacco necrosis virus derivative, and have shown that it is possible from a series of such stills to determine both the type of lattice and the cell dimensions uniquely for a cubic crystal without necessarily resorting to oscillation photographs. This is one particular application of 'stills' to the general procedure suggested by Crowfoot & Schmidt.

We show in Fig. 1, photographs of a stationary wet crystal of tomato bushy-stunt virus which crystallizes in beautiful isotropic rhombic dodecahedra. These specimens were supplied to us by Drs Bawden and Pirie, of Rothamsted, and the crystal sizes varied in dimensions from 0·05 to 0·50 mm. side. In order to obtain good photographs from such weakly scattering crystals it was found necessary to use a hydrogen medium and a high-intensity source of X-rays. The former eliminated the X-ray scattering due to air; the latter was obtained from the 50 kW. copper target tube of the Davy-Faraday Laboratory, put at our disposal through the kindness of Sir Henry Dale. Exposures on this tube, with a specimen–film distance of 10 cm., were made in 20–30 min., whereas about 3 days were required to obtain a comparable photograph with the

less powerful, commercially produced, sealed 1 kW. X-ray tubes. In photographing the wet crystals we have followed the procedure, now accepted, of enclosing the specimen in a thin-walled borosilicate capillary. Most of the liquid is withdrawn from the capillary, leaving just a trace with the crystal sticking to the wall of the tube. The capillary is sealed at both ends, leaving the crystal in a wet atmosphere.

From X-ray photographs, like those in Fig. 1, it has been possible to deduce that the wet crystals of tomato bushy-stunt virus are cubic body-centred with a cell side of 386 ± 11 A., in agreement with the earlier findings of Bernal, Fankuchen & Riley (1938) based on morphological examinations and X-ray powder photographs. The very fine rings indicate just how close together are the reciprocal-lattice points, yet individual reflexions on the rings are sufficiently discrete to be observed and point to a high degree of internal regularity within the molecules, a regularity which extends to spacings of about 7·5 A.

Photographs were also taken of air-dried crystals which could be mounted on fibres. The same specimen–film distance of 10 cm. was maintained, and it was noticeable that the ring effect had now completely disappeared; in its place reflexions were observed near the centre of each photograph, indicating that the molecules had not collapsed quite randomly but that a lattice still persisted. It is quite possible that in the process of drying and shrinking crystallite regions persist within the main crystal, and that the molecules maintain a body-centred packing, as suggested by Bernal *et al.*, but that the regions themselves are slightly disordered with respect to their previous parallel orientations. An attempt was made to measure the cell dimensions of the dried crystal from photo-
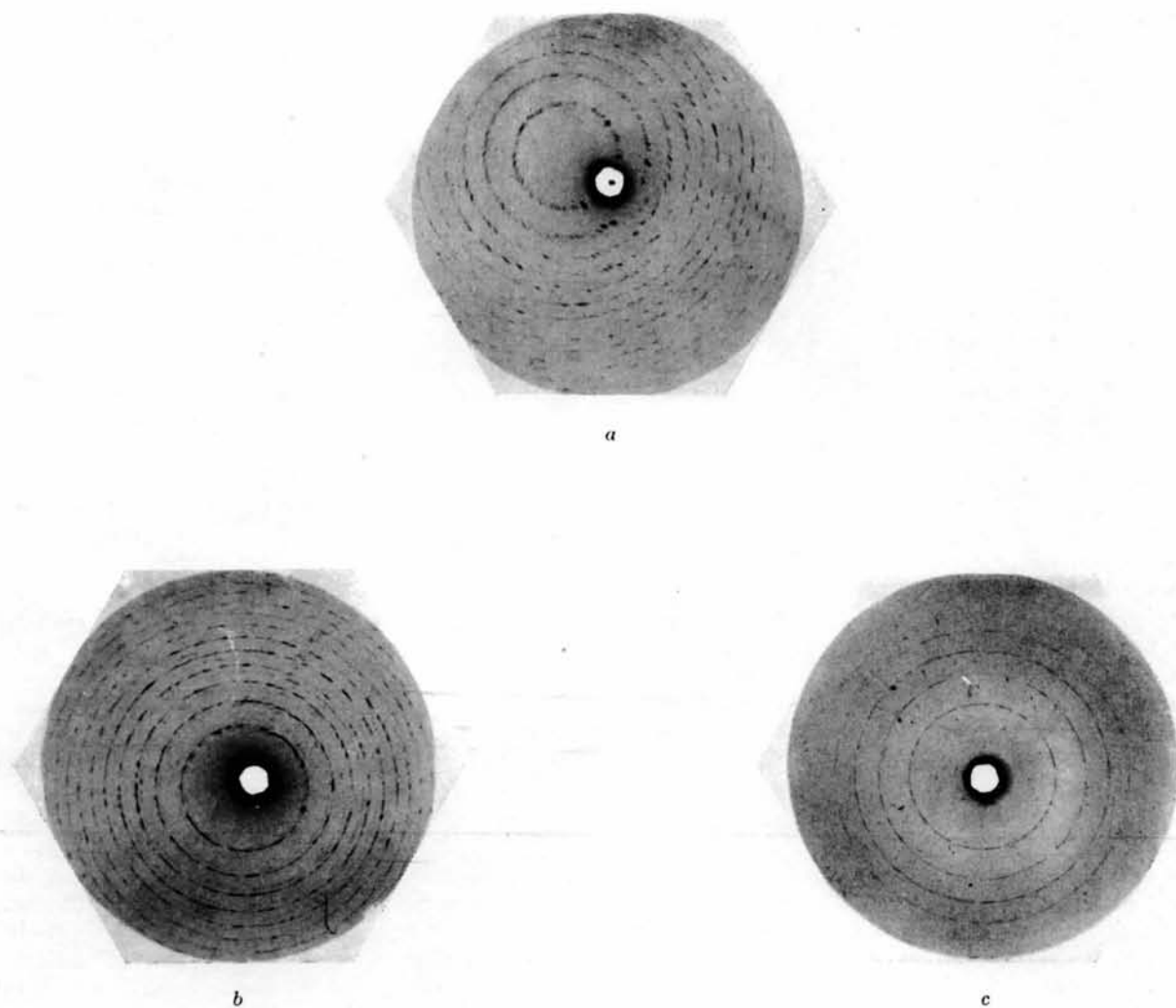
Fig. 1. In *a* and *b* the X-ray beam is nearly parallel to the twofold axis. The crystal is still not correctly set in *b*. In *c* the beam is travelling down a fourfold axis. Specimen–film distance, 10–11 cm.

graphs taken with the X-ray beam travelling parallel to the 2-, 3- and 4-fold axes in turn, but owing to the very small number of reflexions observed reliable values could hardly be expected and a larger specimen-film distance is required to obtain the necessary accuracy in measurements. In spite of this limitation the measurements yielded a cell side of 314 ± 16 A., which is in reasonable agreement with that found by Bernal et al. The single-crystal X-ray data on the dried specimens, however, are too meagre at present to state whether the crystal retains its original system on drying; and experiments are in progress to test this point.

The photographs of the dried crystals were taken with the 5 kW. X-ray tube of the Davy-Faraday Laboratory through the kindness of Prof. E. K. Rideal. It is not possible to compare the times of exposures between the wet- and dry-crystal photographs taken with different X-ray tubes of such widely differing power, but it may be of interest to note that a dried crystal of size 0·2 mm. gave an X-ray photograph in 5–6 hr. with Cu K radiation at 30 kV. and 150 mA. The same size of wet crystal gave an X-ray photograph in about 20 min. on the 50 kW. tube working at 35–40 kV. and 1000–1200 mA. These figures alone are sufficient argument for the use of high-intensity sources of X-radiation when investigating the weakly scattering macromolecules of biological importance.

We give here the method employed to measure the cell dimensions directly from the 'still' photographs of the wet virus crystals. In general, we can state that for each photograph the diameter of the rings can be related to the spacing d^* of planes of reciprocal-lattice points perpendicular to the n-fold axis down which the X-ray beam is travelling. Making no assumption therefore about the type of Bravais lattice present, each photograph can give a set of interplanar spacings d relative to the three lattice types, primitive (P), face-centred (F) and body-centred (I). From such photographs, taken with the X-ray beam travelling parallel to known symmetry axes in turn, we can correlate the results and so determine both the Bravais lattice and the cell dimensions simultaneously. In the case of a cubic crystal the obvious axes to set parallel to the X-ray beam are the 2-, 3- and 4-fold axes. This technique is highly reminiscent of that employed by the Braggs in the early days of crystal-structure determination when establishing the cell dimensions and lattice types of simple cubic crystals.

Fig. 2 recalls briefly what Crowfoot & Schmidt have already pointed out. This shows a diagrammatic representation of planes of reciprocal points AA', BB', CC', etc., with spacing d^*, and the sphere of reflexion. XY is the photographic plate and R is the distance of the plate from the crystal specimen centred at O. The crystal axis OP is offset from the direction of the X-ray beam OR_0 by an angle α. Projections from O to the points where AA', BB', CC', ... cut the sphere of

reflexion will give ellipses on XY whose major axes are $R_0 R_0'$, $R_1 R_1'$, $R_2 R_2'$, ... (with decreasing α the ellipses degenerate into circles). Thus the rings of reflexions can be regarded as arising from the intersection of the sphere of reflexion with equidistant planes of reciprocal-lattice points normal to a crystallographic axis, which is itself parallel or nearly parallel to the X-ray beam.

Now if OP is, say, parallel to [100], reflexions falling on $R_0 R_0'$ will be of the type $0kl$, those falling on $R_1 R_1'$ are $1kl$, and so on. If n_k and m_k are distances of reflexions on the kth ellipse measured along the major axis from R_0, and $2\phi_k$ is the angle of the kth cone, then, from a treatment of the reciprocal lattice, according to Bernal (1927), it is possible to show that the following relations hold;

$$n_k/R = \tan(\phi_k + \alpha) \quad \text{and} \quad m_k/R = \tan(\phi_k - \alpha); \quad (1)$$

$$kd^* = \cos\alpha - \cos\phi_k. \quad (2)$$

Fig. 2.

By a simple trigonometrical expansion of (2),

$$kd^* = 2\sin\tfrac{1}{2}(\phi_k + \alpha)\sin\tfrac{1}{2}(\phi_k - \alpha)$$

$$= 2\sin(\tfrac{1}{2}\tan^{-1}n_k/R)\sin(\tfrac{1}{2}\tan^{-1}m_k/R). \quad (3)$$

If R is sufficiently large (say about 10 cm. or more), (3) can be reduced to the simple expression

$$kd^* = n_k m_k / 2R^2. \quad (4)$$

It is not always possible to locate the exact point of impact of the main beam on an X-ray photograph, and accordingly it is more convenient to measure $n_k + m_k$, the major axis of the kth ellipse. If we call this distance D_k, and if $0 < 2\alpha^2 < d^*$, i.e. if α is small, then

$$d^* = (D_{k+1}^2 - D_k^2)/8R^2. \quad (5)$$

d^* should be calculated from (3), but for approximate purposes it is sufficient to use (5) for cell dimensions above 300 A. or more, and with necessary correction terms this relation can be made to yield fairly reliable values. In the case of tomato bushy-stunt virus the

use of (5) did not lead to errors greater than 3 % for small angles of missetting.

The values of d^* as calculated from (5) gave, in turn, interplanar spacings of 542, 390 and 330 A. for directions perpendicular to (110), (100) and (111) respectively. These spacings are in the ratios $1/\sqrt{2} : 1 : 2/\sqrt{3}$, indicating a cubic body-centred lattice with a cell side of 386 A.

In general, the procedure outlined above gives directly the interplanar spacings of planes of reciprocal points perpendicular to any chosen crystallographic axes set parallel to the X-ray beam. Bravais lattices and cell dimensions are accordingly not difficult to determine for crystals of large unit cells, especially if the crystals are well formed.

We would like to express our appreciation to Mr H. Smith of the Davy-Faraday Laboratory for the invaluable help he gave us while using the 5 and 50 kW. X-ray tubes of that laboratory.

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The Crystal Structure of Sodium Selenochromite, NaCrSe₂, and Preliminary Investigations on Related Compounds

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Attempts have been made to prepare new model-substances for magneto-chemical investigations. Sodium selenochromite, NaCrSe₂, rubidium selenochromite, RbCrSe₂, and rubidium thiochromite, RbCrS₂, of high purity have been prepared by reaction of the pure elements, and a potassium selenochromite, K₀.₅CrSe₂ or KCrSe₂.CrSe₂, by fusion of KCN with Cr₂O₃ and selenium.

The crystal structures of the alkali selenochromites and rubidium thiochromite have been investigated with the following results:

NaCrSe₂: hexagonal rhombohedric; D_{3d}^5–$R\bar{3}m$; $I_a = 3\cdot708$ A., $I_c = 20\cdot29$ A. $\pm 0\cdot05$ A.; parameter z of Se: $95\cdot3° \pm 0\cdot3°$; density obs. $= 4\cdot49_7$ g.cm.$^{-3}$; density calc. $= 4\cdot77$ g.cm.$^{-3}$; the hexagonal unit cell contains three, the rhombohedral cell one molecule of NaCrSe₂.

K₀.₅CrSe₂: hexagonal rhombohedric; C_{3v}^5–$R\bar{3}m$; $I_a = 3\cdot44$ A., $I_c = 24\cdot2$ A.; parameter z of Se: $141\cdot5°$; parameter z of K: 60–$62°$.

RbCrSe₂: hexagonal rhombohedric; space group not yet determined; $I_a = 3\cdot34$ A., $I_c = 26\cdot9$ A.; density obs. $= 5\cdot02$ g.cm.$^{-3}$, density calc. $= 5\cdot26$ g.cm.$^{-3}$

RbCrS₂: hexagonal rhombohedric; space group not yet determined; $I_a = 3\cdot39$ A., $I_c = 16\cdot20$ A.

The radius of the Se²⁻ ions decreases from $1\cdot85$ A. in NaCrSe₂ to $1\cdot71_5$ A. in RbCrSe₂. Preliminary magnetic measurements on NaCrSe₂ show the compound to obey the Curie-Weiss law with a θ value of about $+70°$ C.

Introduction

The compounds of the transition-group elements with the non-metals of the sixth group differ considerably in their magnetic behaviour from the pure ionic compounds, i.e. the salts and the oxygen-containing acids. Commonly they show only moderate paramagnetism or ferromagnetism. To explain this behaviour Haraldsen & Klemm (1934) assumed that covalent bonds are developed between the cations. To correlate the formation of such covalent bonds with the distances between the cations in the crystal structure it seemed useful to investigate model substances, in which for structural reasons the development of covalent bonds occurs in one direction only.

Rüdorff & Stegemann (1943) realized this condition in the alkali thiochromites. These compounds have the