X-RAY DIFFRACTION STUDIES OF CUCUMBER VIRUS 4 AND THREE STRAINS OF TOBACCO MOSAIC VIRUS

by

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INTRODUCTION

The various strains of tobacco mosaic virus (TMV) differ from one another in their serological behaviour, in the symptoms which they produce in an infected plant, and in their physico-chemical properties[1]. Chemical analyses have shown that the ribonucleic acid (present to the extent of 6% in the virus) is, within the limits of experimental error, of the same composition in all strains, whereas the amino-acid composition of the virus protein shows considerable variations[1].

There are, however, certain strains which are biologically quite distinct from one another yet show no detectable difference in chemical composition[1]. It was therefore thought to be of interest to attempt to find, by means of X-ray diffraction, in what ways the structure of the virus protein may vary from one strain to another.

In addition to strains of TMV, cucumber virus 4 (CV4) has been investigated. This virus differs from TMV not only in amino-acid composition and in the C-terminal end-group of its protein (alanine in place of the threonine of TMV)[1] but also in the composition of its nucleic acid[1]. Nevertheless it is structurally[2] and serologically[3] closely related to TMV and a comparison of its X-ray diagrams with those of the strains of TMV is therefore of interest.

The purpose of this paper is to show, in a general way, the extent to which the X-ray diagrams of the various TMV strains and of CV4 resemble one another, to enumerate the ways in which they differ, and to give, where possible, some indication of the structural significance of these differences. Certain of the differences are discussed in greater detail in other papers[4,5].

METHODS

Orientated specimens for X-ray examination were prepared by the methods of BERNAL AND FANKUCHEN[2]. That is, dry orientated virus was obtained by evaporating a solution contained between two parallel glass plates, and preparations in the form of gel or concentrated solution were orientated by introducing them into thin-walled glass capillaries of diameter 0.3 to 0.5 mm. It was found that, for solutions containing 5% to 10% by weight of the virus, good orientation generally developed rapidly and spontaneously in the capillary tube. For good X-ray diagrams, however, a concentration of 20% to 35% is required. At these concentrations it is generally found necessary to speed the orientation process by moving the gel specimen repeatedly backwards and forwards over a small length of the tube.

X-ray fibre-diagrams were taken using an Ehrenberg-Spear fine-focus X-ray tube, Ni-filtered Cu radiation, and a Phillips micro-camera modified to give a specimen-film distance of 30 mm and having a lead-glass collimator of bore 50 μ. The camera was hydrogen-filled.

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For calibration of the scale of the X ray diagrams, the (101) reflection of quartz (3.343 A) was used. The capillary tube containing the specimen (or, in the case of dry specimens, the specimen itself) was coated with a film of finely powdered quartz. For each reflection of the quartz, a double ring, corresponding to back and front faces of the specimen, was obtained on the X-ray photograph, and the mean diameter of the ring was used to calibrate the spacings of the 18th and 21st layer-lines of TMV and of CV4.

MATERIALS

Measurements were made on the following three strains of TMV.

Rothamsted strain, prepared by Mr. N. W. PIRIE. The method of purification involved incubation with commercial trypsin.

U1, a "normal" strain, and

U2, a "mild" strain, both prepared by Dr. A. SIEGEL (University College of Los Angeles). The preparation and properties of U1 and U2 have been described in detail[6].

The U1 preparation was pigmented, and the other two were colourless.

The CV4 used in this work was prepared by Dr. C. A. KNIGHT (Virus Laboratory, Berkeley).

RESULTS

X-ray fibre-diagrams of orientated gel preparations of 3 strains of TMV and of CV4 are shown in Figs. 1–4. Fig. 5 shows the diagram of a previously dried specimen of orientated TMV, U2 strain, maintained at a relative humidity of 75% during the exposure. Photographs similar to Fig. 5 were obtained from the same preparation both air-dried and at 92% r.h.

Clearly the strong similarity of all these diagrams shows that the main features of the protein structure are the same in all the materials concerned. Further, calibration with quartz showed that the fibre-axis repeat period (given by the layer-line spacing) was the same for TMV gel, CV4 gel, and dry TMV, being in each case 69 ± 0.5 A.

A number of points of difference between the X-ray diagrams of the various strains of TMV are, however, clearly visible, while between CV4 and TMV the differences observed are greater. (In comparing the diagrams it is, of course, necessary to ignore such differences in appearance as are due only to different degrees of orientation in the specimens, this being of no structural significance.)

TMV Strains

i. *Splitting of the layer-lines.* The layer-lines of the X-ray diagrams of TMV are not exactly equally spaced. This is most clearly seen in Fig. 3 (U2 gel). The separation between the inner regions of layerlines 1 and 2 is less than that between layer-lines 0 and 1 and layer-lines 2 and 3. Similarly, the separation between the inner regions of layer-lines 4 and 5 is small, while that between 3 and 4 and between 5 and 6 is large. Further from the centre of the diagram this effect is reversed.

The same effect occurs, to a lesser extent, in the Rothamsted and U1 strains (Figs. 1 and 2).

ii. *The 3rd layer-line.* The positions of the intensity maxima, in all regions of the X-ray diagram are closely similar for all 3 strains. However the *relative intensities* of the maxima on the 3rd layer-line differ considerably from one strain to another.

iii. *Higher layer-lines.* Small differences in the strength of intensity maxima can be detected in various parts of the diagram. Parts of the 8th layer-line of U2 are markedly different from the other strains.

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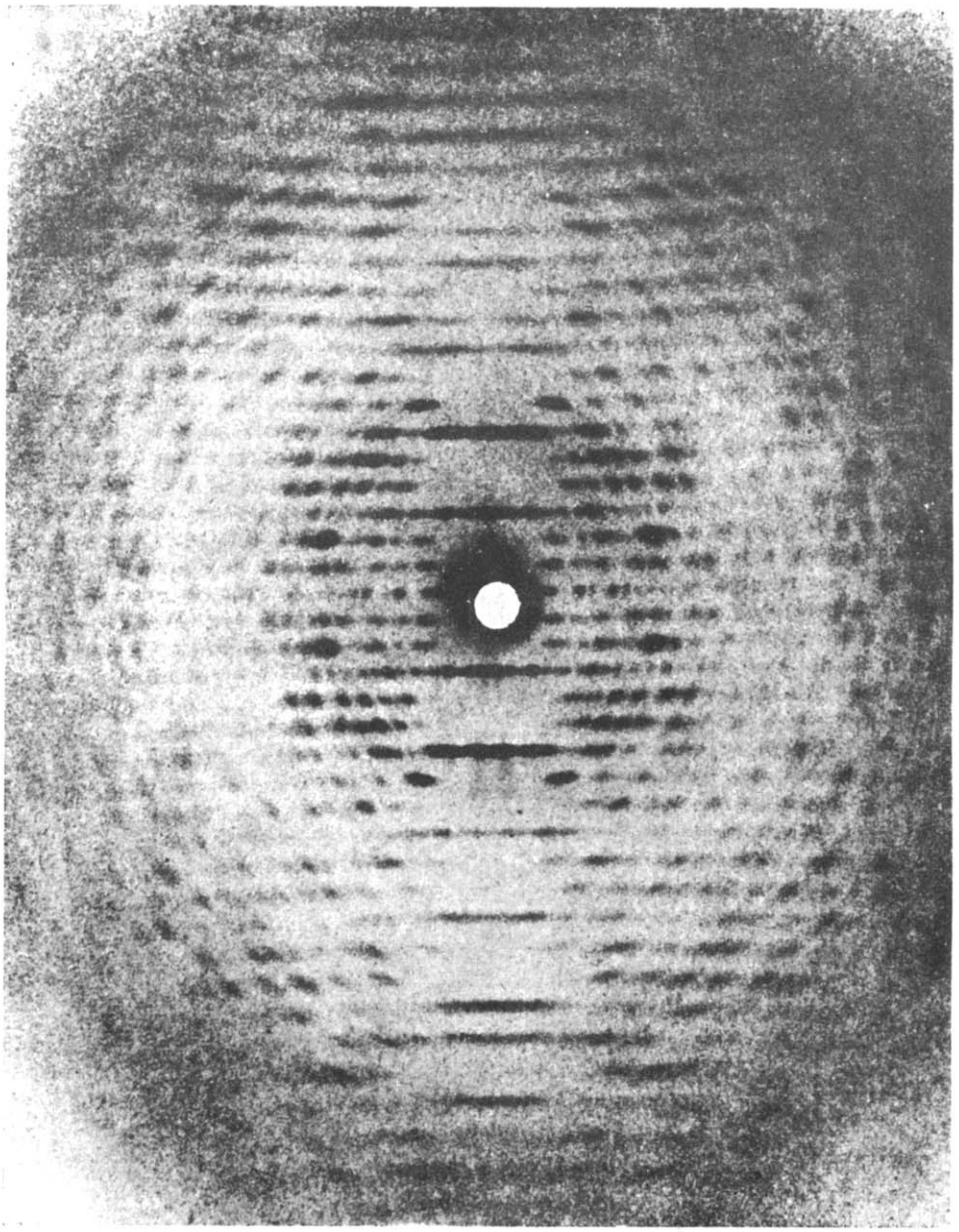

Fig. 1. X-ray fibre-diagram of orientated gel of TMV, Rothamsted strain.

CV4

i. *The positions of the intensity maxima.* Although the diffraction pattern of CV4 closely resembles those of the TMV strains in its general features, measurement of the positions of corresponding maxima reveals that, in CV4, they *all* lie slightly further from the meridian. The discrepancy is approximately 3%.

ii. *Relative intensities.* Differences in relative intensities, as compared with TMV,

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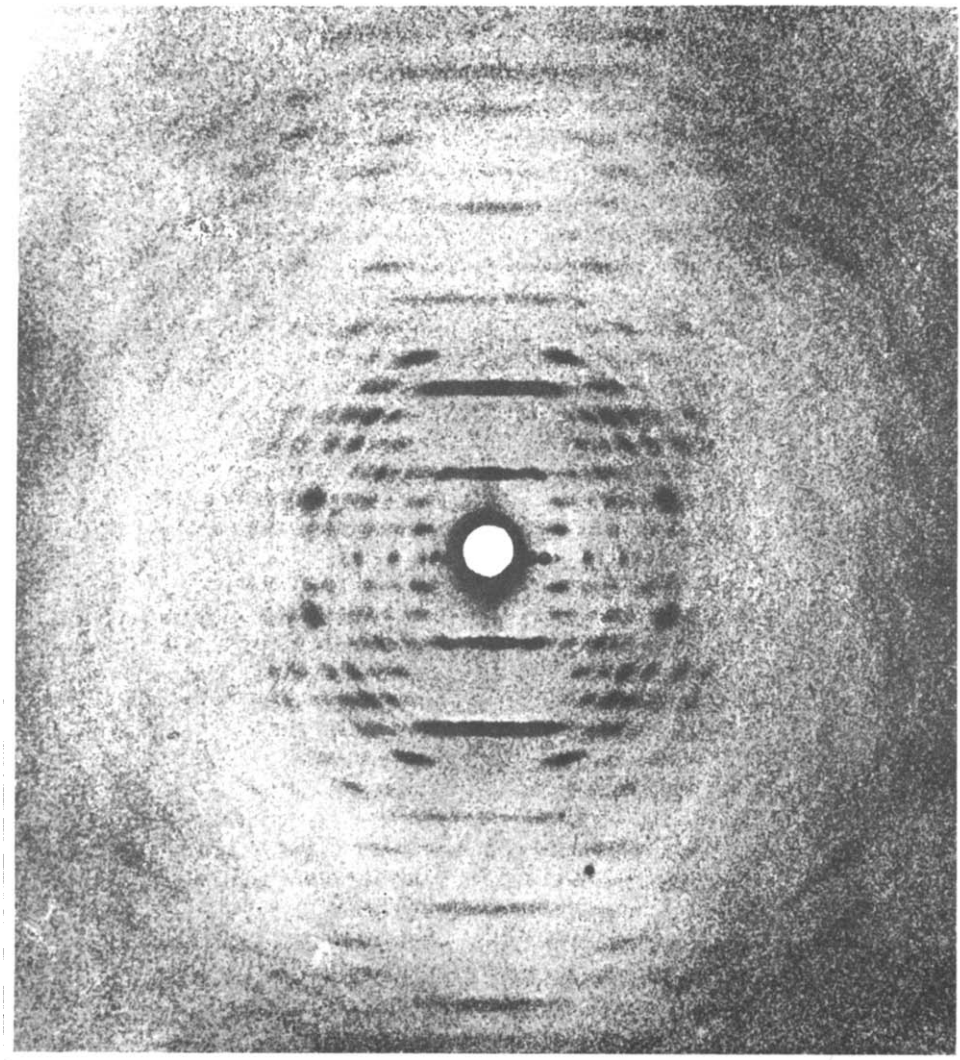

Fig. 2. X-ray fibre-diagram of orientated gel of TMV, U1 strain.

are noticeable in various parts of the diagram. One on the 6th layer-line is indicated by an arrow in Fig. 4.

iii. *Splitting of the layer-line.* A *slight* splitting of the layer-line is observed, but in the opposite sense to that in TMV. That is, the distance between the central regions of layer-lines 1 and 2 *etc.* is slightly greater than the mean layer-line spacing.

Change in the diagram on drying

The principal differences between the diagrams of the gel and of dried virus preparations subsequently maintained at about 75% r.h. lie in the equator and the central region of the 3rd layer-line. The changes observed are similar for all three strains of TMV and for CV4.

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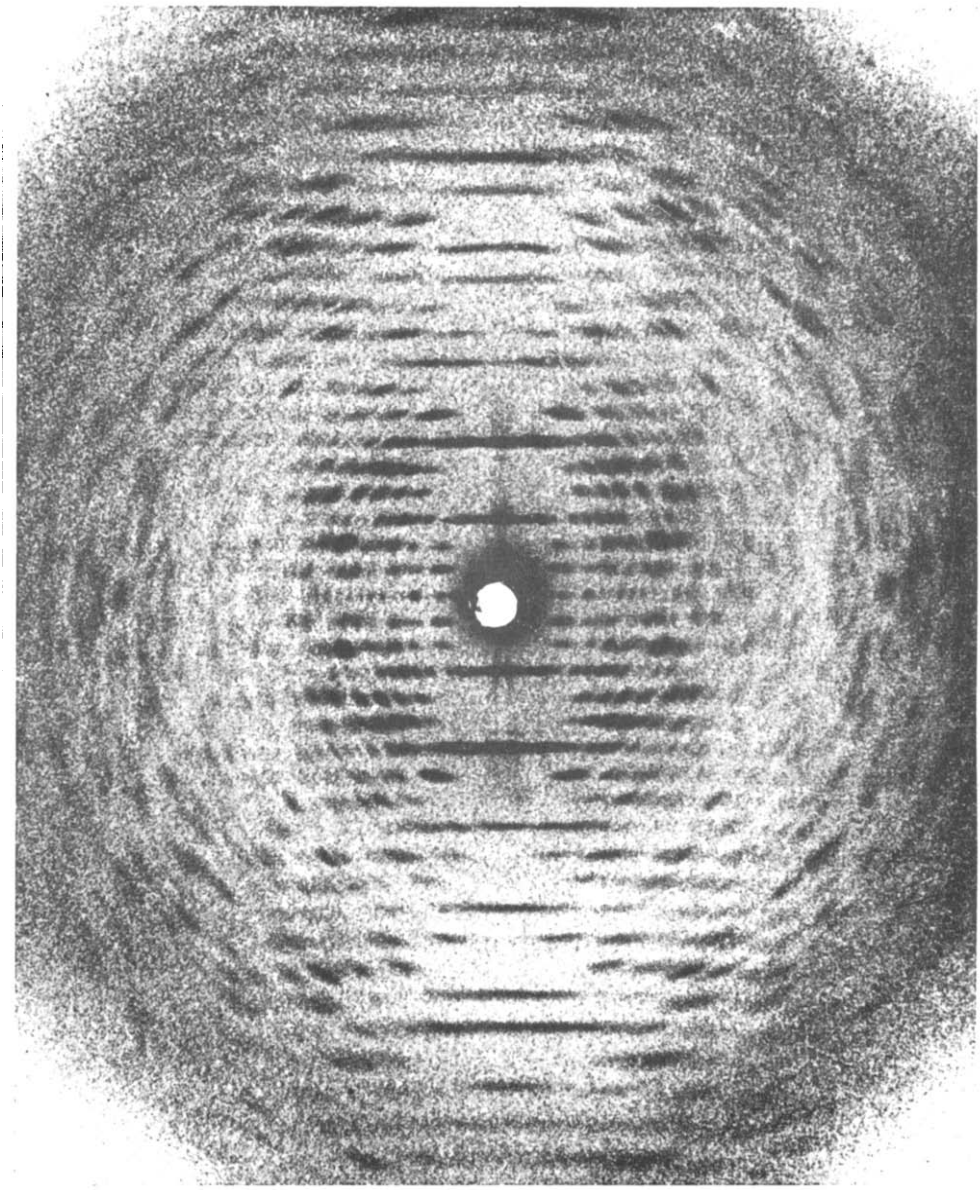

Fig. 3. X-ray fibre-diagram of orientated gel of TMV, U2 strain.

Comparison of Figs. 3 and 5 shows clearly the difference in intensity distribution on the equator for gel and solid preparations of U2 strain.

The change in the central region of the 3rd layer-line is shown separately in Fig. 6, since this part of the diagram is over-exposed in the photographs shown in Figs. 1–5. Fig. 6a, from a weak exposure of orientated gel of the Rothamsted strain of TMV, shows a series of maxima lying close to the axis on the 3rd layer-line. The distance of the first of these from the axis corresponds to a spacing of about 270 A.

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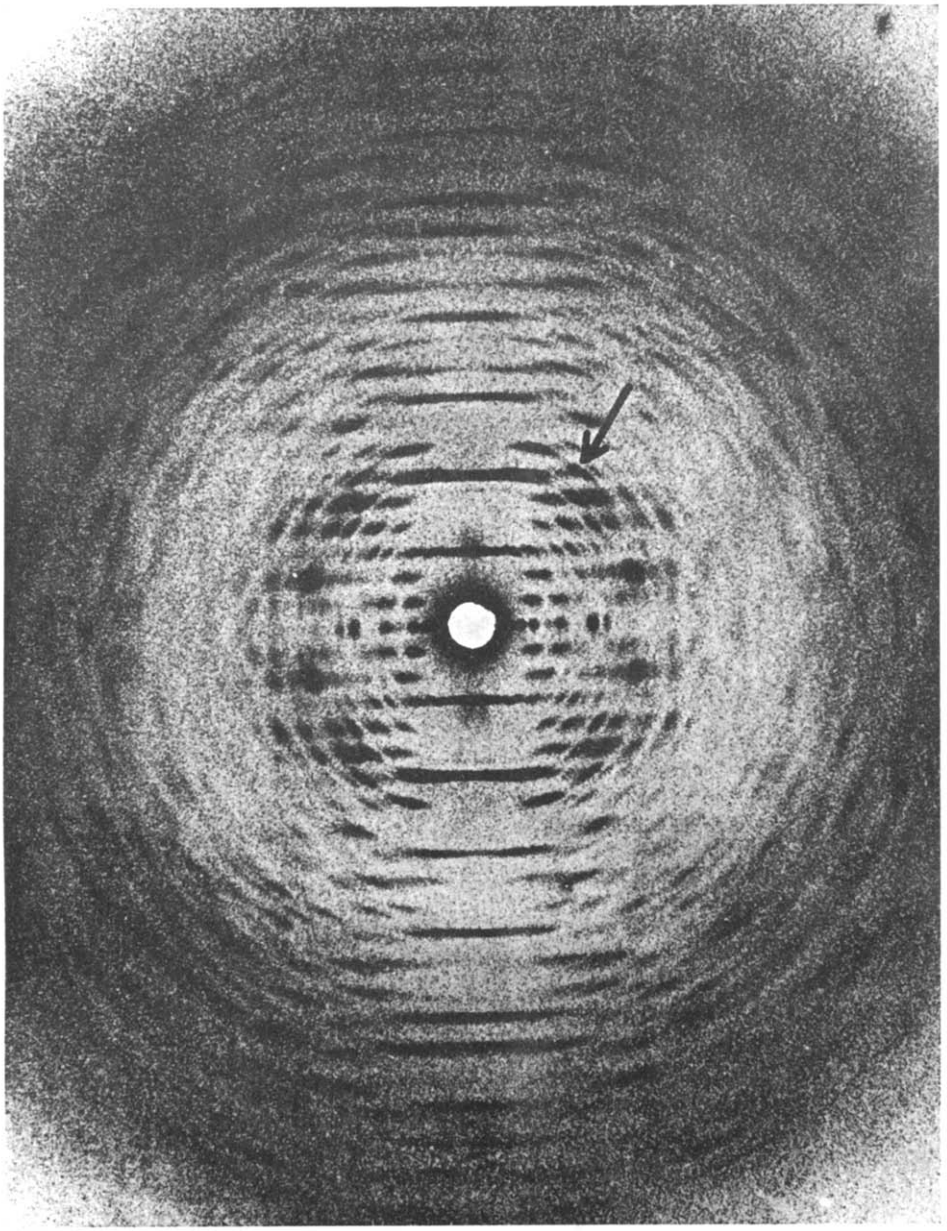

Fig. 4. X-ray fibre-diagram of orientated gel of CV4. The arrow indicates a region of the 6th layer-line which is markedly different from the TMV diagrams.

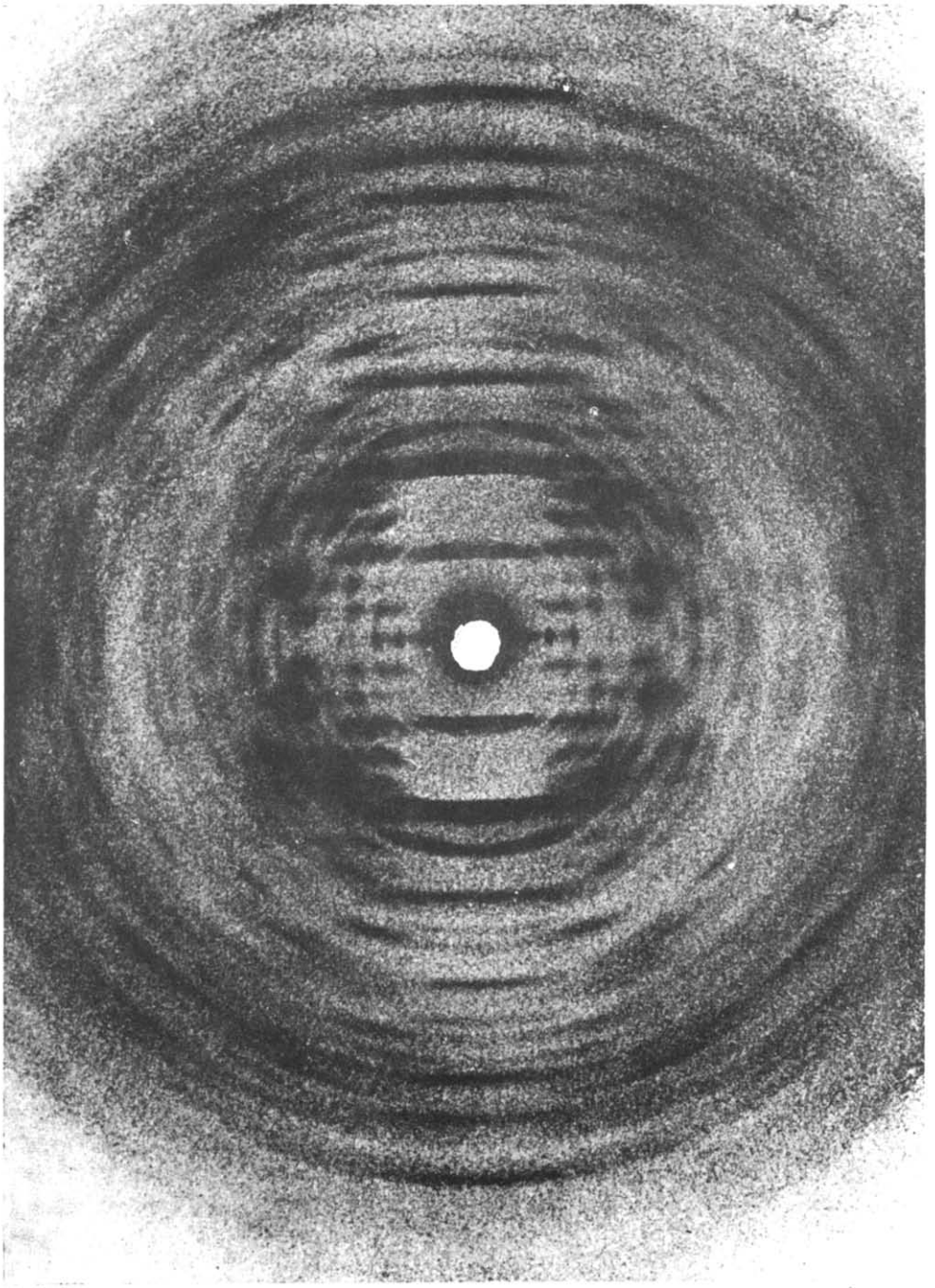

Fig. 5. X-ray fibre-diagram of solid orientated TMV, U2 strain, at relative humidity 75 %.

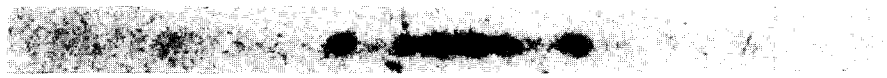

Fig. 6a

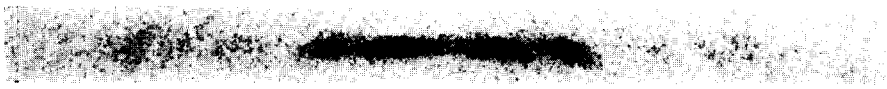

Fig. 6b

Comparison of central region of 3rd layer-line of TMV (Rothamsted strain) in the wet and dy states. Fig. 6a. Orientated gel. Fig. 6b. Air-dried orientated solid.

Fig. 6b shows the same part of the diagram for an orientated air-dried specimen of the same strain. In this the first maximum is entirely absent, while the second is both displaced and reduced in intensity.

When solid preparations of orientated virus are *strongly* dried, further changes in the X-ray diagram occur. These will be described and discussed elsewhere.

DISCUSSION

It has been shown that TMV protein consists of structurally equivalent sub-units of molecular weight about 30,000 lying in helical array about the long axis of the particle, and that there are $3n + 1$ such sub-units in 3 turns of the helix, with n probably 12[7,8]. The nucleic acid forms a central core[7,9,10]. The strong general similarity of the X-ray diagrams (Figs. 1–4) shows that this basic structural arrangement exists not only in the various strains of TMV but also in CV4. There are, however, some important points of difference.

The slight irregularity of layer-line spacing—the splitting of the layer-lines—indicates that there is not *exactly* a whole number of sub-units in 3 turns of the helix. This effect is discussed in detail elsewhere[4]; it is shown that, if $n = 12$, there are for the U2 strain of TMV 37.05 and for the U1 and Rothamsted strains 37.02 sub-units in the axial period of 69 A. In CV4 the direction of the effect is reversed and there are approximately 36.98 units in 3 turns of the helix.

The magnitude of this variation in the number of protein sub-units per turn of the helix is small; the difference between the two extremes represented by U2 and CV4 corresponds only to a shift of 0.7 A at the outermost shell of the virus particle. Nevertheless, the existence of such a variation in the position of any one protein sub-unit with respect to its neighbours above and below probably means that there is little specific chemical bonding between one turn of the helix and the next.

In CV4, all important intensity maxima occur about 3% further from the meridian than in the TMV strains, indicating that the structure of the virus protein, though similar, is somewhat more compact, the diameter of the particle being about 5 A less than that of TMV, while the axial spacings are the same. This agrees well with the observation of BERNAL AND FANKUCHEN[2] that the inter-particle distances in their dry preparation were 146 A for CV and 152 A for TMV.

The greatest variation in intensity distribution between one strain and another occurs on the 3rd layer-line. In all preparations of TMV and of CV4, however, the

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3rd layer-line shows the same general features; an oscillating intensity function of short period is modulated by one of much longer period. It is shown in the following paper[5] that this feature of the X-ray diagram is related to the detailed external form of the virus particle, and indicates the presence of some kind of helical groove following the line of the main protein helix. The existence of the groove is confirmed by the disappearance of the first maximum on the 3rd layer-line on passing from gel to dry preparations. The variation in the detail of the intensity distribution on the 3rd layer-line (Figs. 1–4), however, indicates that the detailed form of the groove may vary from one strain to another.

ACKNOWLEDGEMNTS

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SUMMARY

X-ray diagrams of 3 strains of tobacco mosaic virus, and of cucumber virus 4 are closely similar in their main features but differ significantly from one another in points of detail.

The conclusion is drawn that the helical arrangement of the virus protein is essentially the same in all four substances, but that there is a *slight* variation in the number of protein sub-units in one turn of the helix, and also a slight variation in the surface structure of the virus particles.

RÉSUMÉ

Les diagrammes aux rayons X de trois souches du virus de la mosaïque du tabac et du virus 4 du concombre possèdent des caractéristiques très semblables mais diffèrent de façon significative les uns des autres par des points de détail.

Les auteurs concluent que la structure hélicoïdale de la protéine du virus est essentiellement la même pour les quatre substances, mais qu'il y a une légère variation dans le nombre des sub-unités protéiques par tour d'hélice, et également une légère variation dans la structure de la surface des particules de virus.

ZUSAMMENFASSUNG

Röntgendiagramme von drei Tabakmosaikvirusstämmen und von Gurkenvirus 4 weisen im Wesentlichen grosse Ähnlichkeit, im Einzelnen jedoch bedeutende Unterschiede auf.

Es wird daraus gefolgert, dass die Schraubenkonfiguration des Virusproteins in allen vier Substanzen im Wesentlichen dieselbe ist, während in der Anzahl der in einer Schraubenwendung befindlichen Proteinsubeinheiten, sowie in der Oberflächenstruktur der Viruspartikeln eine geringe Variation besteht.

REFERENCES

1 C. A. KNIGHT, *Advances in Virus Research*, 2 (1954) 153.
2 J. D. BERNAL AND I. FANKUCHEN, *J. Gen. Physiol.*, 25 (1941) 111.
3 S. E. LURIA, *General Virology*, 1953, p. 121.
4 R. E. FRANKLIN AND A. KLUG, *Acta Crystallographia*, 8 (1955) 777.
5 R. E. FRANKLIN AND A. KLUG, *Biochim. Biophys. Acta* (in press).
6 A. SIEGEL AND S. G. WILDMAN, *Phytopathology*, 44 (1954) 277.
7 J. D. WATSON, *Biochim. Biophys. Acta*, 13 (1954) 10.
8 R. E. FRANKLIN, *Nature*, 175 (1955) 379.
9 G. SCHRAMM, G. SCHUMACHER AND W. ZILLIG, *Nature*, 175 (1955) 549.
10 R. G. HART, *Proc. Natl. Acad. Sci. U.S.*, 41 (1955) 261.

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