*J. gen. Virol.* (1968), **2**, 107–114
*With 4 plates*
*Printed in Great Britain*



# Some Observations on the Structure of the Filamentous Particles of Several Plant Viruses

By A. VARMA, A. J. GIBBS*, R. D. WOODS,
*Rothamsted Experimental Station, Harpenden, Hertfordshire*
AND J. T. FINCH
*Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge*

(*Accepted* 28 *July* 1967)

SUMMARY

Several plant viruses with filamentous particles ranging in modal lengths from 0·48 $\mu$ to 1·25 $\mu$ were negatively stained with uranyl formate, examined in the electron microscope, and the electron micrographs analysed in various ways. The particles of all the viruses were helically constructed with a basic pitch of 33 to 37 Å (mean 34 Å), but could be separated into groups by other features of their particles. Various measurements of the particles of five of the viruses suggest that there were 10 to 14 subunits in each turn of the basic helix of their particles.

All plant viruses with elongated particles seem to fall into one of two groups; those with modal lengths of 0·3 $\mu$ or less seem rigid and have a basic helix of pitch 23 to 25 Å, and those with longer particles are filamentous and have a basic helix of pitch 33 to 37 Å.

## INTRODUCTION

The particles of different plant viruses with rod-like or filamentous particles differ greatly in length. Brandes & Wetter (1959) showed that most of the particles of one virus are usually of one characteristic modal or 'normal' length, though some viruses such as tobacco rattle and barley stripe mosaic have particles of several characteristic lengths (Harrison, Nixon & Woods, 1965; Harrison & Woods, 1966).

The most intensive structural investigations have been made on the particles of the shorter rod-shaped viruses, particularly tobacco mosaic virus. Chemical and physical studies on this virus (reviewed by Klug & Caspar, 1960) show it to be built of some 2000 protein subunits arranged helically to form a rigid tube 0·3 $\mu$ in length, with the nucleic acid (RNA) following the basic helix of the protein subunits and enclosed by them at an inner radius. Less detailed studies on tobacco rattle (Nixon & Harrison, 1959; Finch, 1965) barley stripe mosaic and lychnis ringspot (Gibbs *et al.* 1963; Finch, 1965) and pea early browning (Harrison, 1966) viruses indicate that their architecture resembles that of tobacco mosaic virus. In each, the pitch of the basic helix of protein subunits is within the range 23 to 26 Å. (We use the word pitch to describe the axial distance between turns of the basic helix.)

* Present address: John Curtin School of Medical Research, Australian National University, Canberra.

Recently Finch (1964) found that the structure of tobacco mosaic virus particles was more clearly seen in electron micrographs when uranyl formate (Bradley, 1965; Leberman, 1965) was used as negative stain instead of sodium phosphotungstate. We report here the results of examining several filamentous plant viruses in the electron microscope using uranyl formate negative stain. In all the particles examined the pitch was within the range 33 to 37 Å.

## METHODS

The sources of the viruses examined, the plants from which they were obtained, and the acronyms we use for their names are shown in Table 1. Each virus was mounted for electron microscopy on a carbon film, supported on a seven-hole platinum mount. A drop of diluted infective sap or purified virus preparation was put on the grid and excess removed by filter paper, then a drop of 1% aqueous uranyl formate pH 2·5 (Leberman, 1965) was put on the grid and removed in the same way. The grids were examined and photographed in a Siemens Elmiskop I electron microscope at a fixed magnification of 40,000×. The periodicities in the electron micrographs of the particles were analysed using the optical diffraction technique of Klug & Berger (1964). Straight portions (about 0·2 $\mu$ long) of the images of the particles were marked off with opaque tape and used as diffraction subjects in a commercial version (Pullin Ltd) of the Taylor–Lipson diffractometer (Taylor & Lipson, 1964) using a laser light source (Spectra-physics, 130B). Repeating structures and the widths of particles were measured with a double-beam recording microdensitometer (Joyce Loebl and Co. Ltd.). Some particles in PVX preparations were analysed by superimposition and rotation techniques (Markham, Frey & Hills, 1963).

## RESULTS

Plates 1 to 3 show selected portions of some of the clearest micrographs obtained. Individual particles of each virus differed in the clarity and type of substructure they showed, but from an examination of several micrographs, each virus could be put into one of three groups:

(1) *Viruses with flexuous particles clearly showing substructure.* This group contained WCMV, HRSV, PVX, PAMV and SBYV, which has very flexuous particles. Most particles showed clear crossbanding, approximately at right angles to their long axes. They did not often show banding in other directions, corresponding to the higher order helices.

(2) *Viruses with straight particles clearly showing substructure.* This group contained RCVMV and CLV. Most of the particles showed grooves and lines of subunits between them, parallel to their long axes, and showed cross-banding only indistinctly.

(3) *Viruses with flexuous particles with indistinct substructure.* This group contained PVY, CYVV, BYMV and HMV. Although the surface of the particles was verrucous no pattern was obvious.

Particles of all the viruses seemed very variable in structure, and only in the clearest micrographs could the lines of subunits or grooves between them be occasionally traced the full width of a particle or for 200 Å or more along its length. Presumably this variability was caused not only by variations in the thickness and penetration of

Table 1.

| Virus group* | Virus | Modal length* ($\mu$) | Host plant | Nature of preparation† | Source |
|---|---|---|---|---|---|
| Potato virus X-group | White clover mosaic (WCMV) | 0·459 | *Phaseolus vulgaris* L. ('Prince') | P | Rothamsted |
| | Hydrangea ringspot (HRSV) | 0·500 | *Hydrangea hortenensis* Smith | S | Littlehampton (M. Hollings) |
| | Potato virus X (PVX) | 0·515 | *Nicotiana tabcum* L. ('White Burley') | P | Rothamsted |
| | Potato aucuba mosaic (PAMV) | 0·580 | *Solanum tuberosum* L. ('Ninety-fold') | P | Cambridge (D. E. Richardson) |
| | Centrosema mosaic (CMV) | 0·580 | *Crotalaria spectabilis* Roth. | P | New Guinea (R. J. van Velsen) |
| Potato virus S-group | Red clover vein mosaic (RCVMV) | 0·642 | *Pisum sativum* L. ('Onward') | P | Rothamsted |
| | Carnation latent (CLV) | 0·650 | *Dianthus barbatus* L. | P | Littlehampton (M. Hollings) |
| | Ryegrass mosaic (RGMV) | 0·700 | *Lolium perenne* L. | P | Rothamsted |
| Potato virus Y-group | Potato virus-Y (PVY) | 0·730 | *Nicotiana tabacum* L. ('White Burley') | P | Edinburgh (D. A. Govier) |
| | Henbane mosaic (HMV) | 0·730 | *Nicotiana tabacum* L. ('White Burley') | P | Rothamsted |
| | Bean yellow mosaic (BYMV) | 0·750 | *Phaseolus vulgaris* L. ('Prince') | P | Rothamsted |
| | Clover yellow vein (CYVV) | 0·767 | *Nicotiana clevelandii* Gray | P | Rothamsted |
| | Sugar beet yellows (SBYV) | 1·250 | *Beta vulgaris* L. ('Klein E') | P | Rothamsted |

* Virus groups and modal lengths from Brandes & Wetter (1959) and Gibbs, Varma & Woods (1966).
† S = sap; P = purified preparation.

the stain, and by the superposition of the images of both sides of the particles (Klug & de Rosier, 1966), but also by flexibility of the particles indicated by their flexuous outlines. Despite differences in their appearance the optical transforms of the particles of all three groups were similar. The helical nature of the particles was shown by the appearance in the optical diffraction patterns of near-meridional diffraction maxima at spacings corresponding to a basic helix which for all particles was of pitch 33 to 37 Å (Table 2). The maxima on opposite sides of the meridian were usually about the same intensity with a clear minimum on the meridian between them, indicating that detail from both near and far sides of the particle was superposed in the images, the particles being enclosed in negative stain. Near-equatorial reflexions were found on some patterns of RCVMV and WCMV, corresponding to very steep (near-longitudinal)

Table 2.

| Group | Virus | No. of optical transforms examined | Mean pitch basic helix (Å) |
|---|---|---|---|
| Potato virus X | PVX | 15 | 34±1* |
| | HRSV | 2 | 37 |
| | WCMV | 14 | 34±1* |
| | PAMV | 2 | 35 |
| | SBYV | 2 | 34 |
| Potato virus S | RCVMV | 26 | 34±1 |
| | CLV | 2 | 33 |
| Potato virus Y | PVY | 2 | 33 |
| | CYVV | 2 | 35 |
| | BYMV | 2 | 34 |

* Occasional patterns of PVX and WCMV showed weak layer lines respectively about halfway and $\frac{2}{7}$ of the distance from the equatorial to the meridional reflexions.

helices on the particles. The reflexions with RCVMV were consistent with a spacing of about 33 Å but with WCMV the reflexions were broad and ranged in spacing from 30 to 40 Å. Without information about which side of the particles was associated with which diffraction maxima, we could not determine whether the various helices and hence the surface lattice were left- or right-handed. A small proportion of the diffraction patterns showed traces of diffraction along layer lines between the equator and the near-meridional maxima as noted in Table 2. The apparent variability of the particles did not depend on pH; separate suspensions of WCMV, PVX and RCVMV were adjusted to either pH 4, 7 or 10 for 4 hr before mounting for electron microscopy, but all gave similar optical transforms, and particles fixed with formaldehyde before staining resembled untreated ones.

The widths of some of the particle images were measured with a microdensitometer. The values found (Table 3) were variable but were less than those reported by other workers who measured shadowcast preparations (Brandes & Wetter, 1959). In addition, near-longitudinal lines of subunits were visible on some images of WCMV, PVX, RCVMV, CLV and SBYV. The distance between these lines, measured with the microdensitometer, was within the range 26 to 35 Å (Table 4). If the edges of the particles and the longitudinal lines of subunits are all assumed to lie on the same cylindrical surface, a simple calculation shows that there are 10 to 14 such lines on the surface of the whole particle. Four of the viruses seemed to have 10 to 12 lines

(i.e. 10 to 12 subunits in each turn of the basic helix), whereas SBYV seemed to have about 14. This difference may be real but it may have been caused by flattening of the more flexible particles of SBYV when they were mounted for electron microscopy.

Some electron micrographs of purified PVX preparations contained several nearly circular particles (Pl. 1, 4) with a diameter similar to that of the intact PVX particles. These particles seemed to be short lengths of the PVX particles (perhaps only one turn of the basic helix, or a disc of similar size), which were lying on the electron microscope grid with their axes at right angles to the surface of the grid. They showed a

Table 3. *Widths of particles* (Å)*

| | | | | | |
|---|---|---|---|---|---|
| Potato virus X group | PVX | 115±3 | Potato virus S group | RCVMV | 100±3 |
| | HRSV | 133±4 | | CLV | 118±3 |
| | WCMV | 118±1 | Potato virus Y group | PVY | 105±3 |
| | PAMV | 132±4 | | CYVV | 123±2 |
| | SBYV | 114±4 | | BYMV | 133±2 |

* Means of 15 to 20 measurements taken from a total of 10 particles of each virus.

Table 4. *Longitudinal bands*

| | | Distance between bands at centre of image (Å)* | No. of bands per particle (i.e. subunits/turn). |
|---|---|---|---|
| Potato virus X group | PVX | 35±1 | 10·4 |
| | WCMV | 34±1 | 11·0 |
| | SBYV | 26±1 | 13·9 |
| Potato virus S group | RCVMV | 32±1 | 10·0 |
| | CLV | 31±1 | 12·0 |

* Means of 15 to 20 measurements taken from a total of 10 particles of each virus.

central hole of variable diameter surrounded by about ten radially arranged subunits with a circular dark zone at a radius of about 35 Å. A composite photograph (Pl. 4, fig. 22) was made of the images of five of the particles (Pl. 4, fig. 17 to 21) and showed ten equally spaced peripheral subunits; the images were aligned using the centre of each particle and its clearest peripheral subunit as reference points.

The composite photograph was also examined by rotational superposition (Markham *et al.* 1963), and showed subunits most clearly when rotated to give five- and ten-fold superposition( Pl. 4, fig. 23), when the dark zone at radius 35 Å was also clear; similar results were obtained when original electron micrographs were also rotated. The dark zone may indicate the position of the nucleic acid; since it is at the radius of a helix of pitch 34 Å it may be the same length as the nucleic acid molecule in a particle of tobacco mosaic virus.

## DISCUSSION

Plant viruses with elongated particles seem to be of two types: those with short rod-shaped particles (0·5 $\mu$ or less in length) with a basic helix of pitch 23 to 25 Å and those with longer filamentous particles with a basic helix of pitch 33 to 37 Å. Brandes & Wetter (1959) showed that plant viruses with elongated particles could be grouped by the 'normal' length of their particles. We have found that groups of viruses showing

similar structure when negatively stained in uranyl formate include one or more of the groups recognized by Brandes & Wetter. Thus viruses can be more easily and certainly identified or grouped not only by measuring the lengths of their particles, but also by examining the particles in uranyl formate negative stain. For example, we also examined particles of *Centrosema* mosaic virus (CMV) and ryegrass mosaic (RGMV). The modal length of CMV 0·58 $\mu$c (Crowley & Francki, 1963) suggests that it is one of the PVX-group, and the apparent structure of its particles, which show clear crossbanding in uranyl formate (Pl. 1, fig. 5) agrees with this. Similarly, RGMV, a mite-transmitted virus, has particles (modal length, 0·7 $\mu$), resembling particles of viruses of the PVY-group (modal length 0·73 to 0·76 $\mu$) more closely than those of viruses of the PVS-group (modal length 0·65 $\mu$) (Pl. 3, fig. 13).

Our results agree with most of those already published on the structure of filamentous virus particles. Bernal & Fankuchen (1941) found by X-ray diffraction studies that PVX had a basic repeating structure every 33 Å along its axis. This was confirmed by Tollin *et al.* (1967), who found that narcissus mosaic, whose particles are slightly longer (0·55 $\mu$) than PVX, also has a basic axial repeat every 33 Å in dry particles and 36 Å, in wet ones; the variability in pitch in the particles we examined may have been caused by a similar change on drying. Lister, Bancroft & Nadakavukaren (1965) published electron micrographs of a 'latent' virus of *Malus* species with particles resembling those of SBYV but only 0·62 $\mu$ long, and showing clear crossbanding every 32 to 34 Å. Cadman & Cathro (1964) reported that the particles of citrus tristeza virus, which also resemble those of SBYV but are about 2 $\mu$ long, showed crossbanding at about 40 Å intervals. Francki (1966) reported that the particles of *Cymbidium* mosaic virus (modal length 0·475 $\mu$) negatively stained with uranyl acetate showed crossbanding at 28 Å intervals, which is less than we would have predicted, but his method of estimating this distance was very indirect.

Our observations on the structure of SBYV do not agree with those of Russell & Bell (1963), who reported that the repeating structures on SBYV particles were 26 to 30 Å apart, and suggested that the primary helix of the particle repeated every 3 turns and 19 subunits (i.e. $6\frac{1}{3}$ subunits/turn), whereas we found the pitch of the basic helix to be about 34 Å with perhaps 12 to 14 subunits in each turn. Russell & Bell used neutral phosphotungstate as negative stain, so we also prepared electron micrographs of SBYV using this stain and found (by microdensitometer) repeating units 36 to 40 Å apart. Measurements of the micrograph published by Russell & Bell suggest at least 10 subunits per turn of the helix.

## REFERENCES


Bradley, D. E. (1965). The structure of the head, collar and base plate of 'T-even' type bacteriophages. *J. gen. Microbiol.* **38**, 395.

Brandes, J. & Wetter, C. (1959). Classification of elongated plant viruses on the basis of particle morphology. *Virology* **8**, 99.

Bernal, J. D. & Fankuchen, I. (1941). X-ray and crystallographic studies of plant virus preparations III. *J. gen. Physiol.* **25**, 147.

Cadman, C. H. & Cathro, J. (1964). *Scot. Hort. Res. Inst. Ann. Rept* 1963–64, p. 70.

Crowley, N. C. & Francki, R. I. B. (1963). Purification and some properties of *Centrosema* mosaic virus. *Aust. J. biol. Sci.* **16**, 468.

Finch, J. T. (1964). Resolution of the substructure of tobacco mosaic virus in the electron microscope. *J. molec. Biol.* **8**, 872.

FINCH, J. T. (1965). Preliminary X-ray diffraction studies on tobacco rattle and barley stripe mosaic viruses. *J. molec. Biol.* **12**, 612.

FRANCKI, R. I. B. (1966). Isolation, purification and some properties of two viruses from cultivated Cymbidium orchids. *Aust. J. biol. Sci.* **19**, 555.

GIBBS, A. J., VARMA, A. & WOODS, R. D. (1966). Viruses occurring in white clover (*Trifolium repens* L.) from permanent pastures in Britain. *Ann. appl. Biol.* **58**, 231.

GIBBS, A. J., KASSANIS, B., NIXON, H. L. & WOODS, R. D. (1963). The relationship between barley stripe mosaic and lychnis ringspot viruses. *Virology* **20**, 194.

HARRISON, B. D. (1966). Further studies on a British form of pea early browning virus. *Ann. appl. Biol.* **57**, 121.

HARRISON, B. D. & WOODS, R. D. (1966). Serotypes and particle dimensions of tobacco rattle viruses from Europe and America. *Virology* **28**, 610.

HARRISON, B. D., NIXON, H. L. & WOODS, R. D. (1965). Lengths and structure of particles of barley stripe mosaic virus. *Virology* **26**, 284.

KLUG, A. & BERGER, J. E. (1964). An optical method for the analysis of periodicities in electron micrographs, and some observations on the mechanism of negative staining. *J. molec. Biol.* **10**, 565.

KLUG, A. & CASPAR, D. L. D. (1960). Structure of small viruses. *Adv. Virus Res.* **7**, 225.

KLUG, A. & DE ROSIER, D. J. (1966). Optical filtering of electron micrographs, reconstruction of one-sided images. *Nature, Lond.* **212**, 29.

LEBERMAN, R. (1965). Use of uranyl formate as a negative stain. *J. molec. Biol.* **13**, 606.

LISTER, R. M., BANCROFT, J. B. & NADAKAVUKAREN, M. J. (1965). Some sap-transmissible viruses from apple. *Phytopathology* **55**, 859.

MARKHAM, R., FREY, S. & HILLS, G. J. (1963). Methods for the enhancement of image detail and accentuation of structure in electron microscopy. *Virology* **20**, 88.

NIXON, H. L. & HARRISON, B. D. (1959). Electron microscopic evidence on the structure of the particles of tobacco rattle virus. *J. gen. Microbiol.* **21**, 582.

RUSSELL, G. E. & BELL, J. (1963). The structure of beet yellows virus filaments. *Virology* **21**, 283.

TAYLOR, C. A. & LIPSON, H. (1964). *Optical Transforms*. London: Bell.

TOLLIN, P., WILSON, H. R., YOUNG, D. W., CATHRO, J. & MOWAT, W. P. (1967). X-ray diffraction and electron microscope studies of narcissus mosaic virus. *J. molec. Biol.* **26** (In the Press).

(*Recieved* 29 *June* 1967)

## EXPLANATION OF PLATES

### PLATE 1

Figs. 1 to 6. Electron micrographs of particles of viruses mounted in uranyl formate. Fig. 1. White clover mosaic virus. Fig. 2. Hydrangea ringspot virus. Fig. 3. Potato virus X with two 'discs' (arrowed). Fig. 4. Potato aucuba mosaic virus. Fig. 5. Centrosema mosaic virus. Fig. 6. Sugarbeet yellows virus.

Fig. 7. Microdensitometer trace along the image of a particle of sugarbeet yellows virus stained with uranyl formate. Slit at right angles to the long axis of the particle (slit width, 70 $\mu$). Vertical axis, density of image.

Fig. 8. Microdensitometer trace across the image of the particle of sugarbeet yellows virus used for obtaining the trace shown in fig. 7.

### PLATE 2

Figs. 9 to 11. Electron micrographs of particles of viruses mounted in uranyl formate. Figs. 9, 11. Red clover vein mosaic virus. Fig. 10. Carnation latent virus.

Fig. 12. Optical diffraction pattern of particles shown in fig. 3.

### PLATE 3

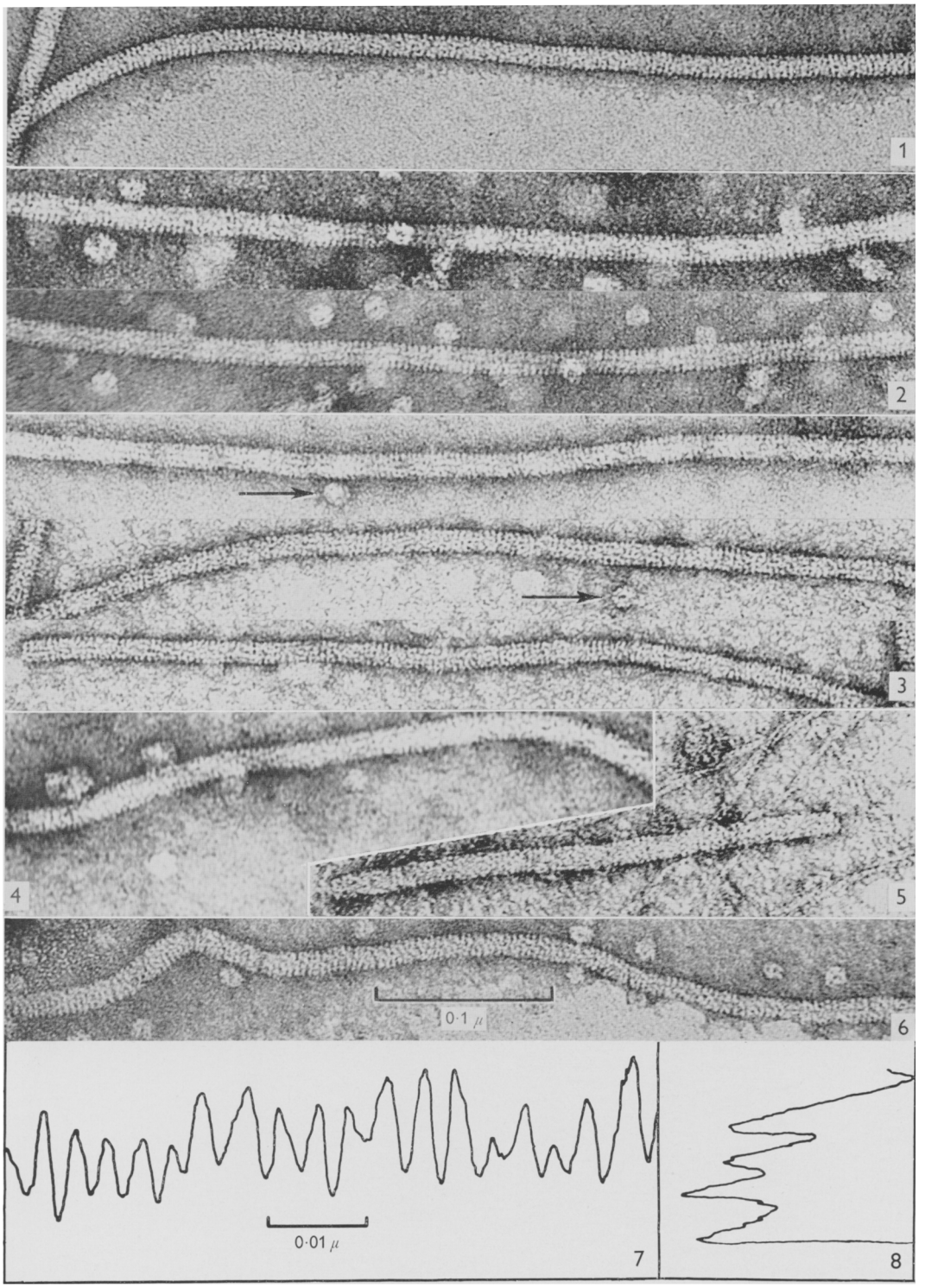
Figs. 13–16. Electron micrographs of particles of viruses mounted in uranyl formate. Fig. 13. Ryegrass mosaic virus. Fig. 14. Potato virus Y. Fig. 15. Bean yellow mosaic virus. Fig. 16. Clover yellow vein virus.

### PLATE 4

Figs. 17 to 21. Electron micrographs of pieces of particles of potato virus X, stained with uranyl formate.

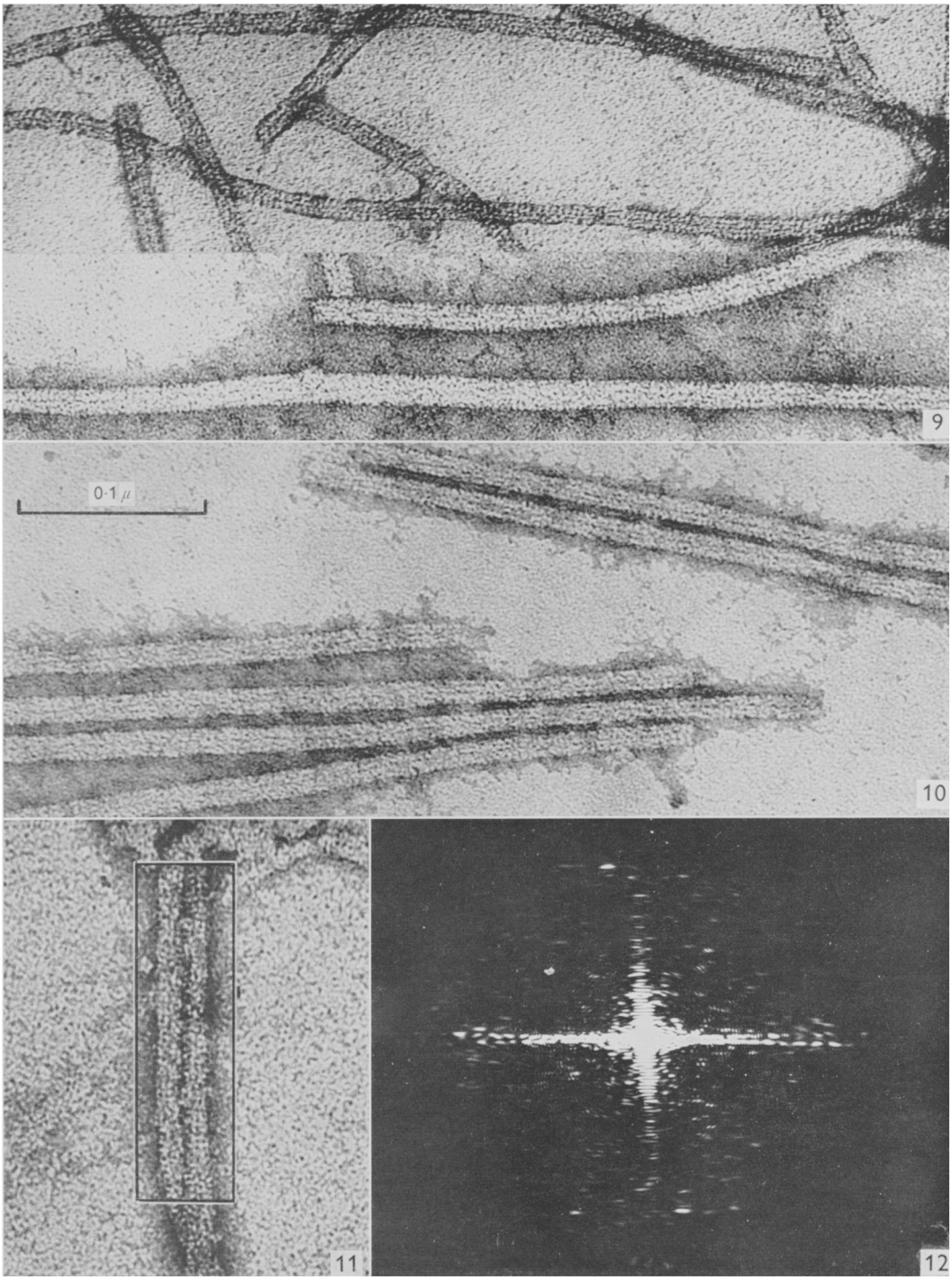
Fig. 22. Composite picture of five end pieces of potato virus X, obtained by superimposing electron micrographs shown in Figs. 17 to 21.

Fig. 23. Pictures obtained by rotational superposition of Fig. 22; 3 to 11 superpositions.

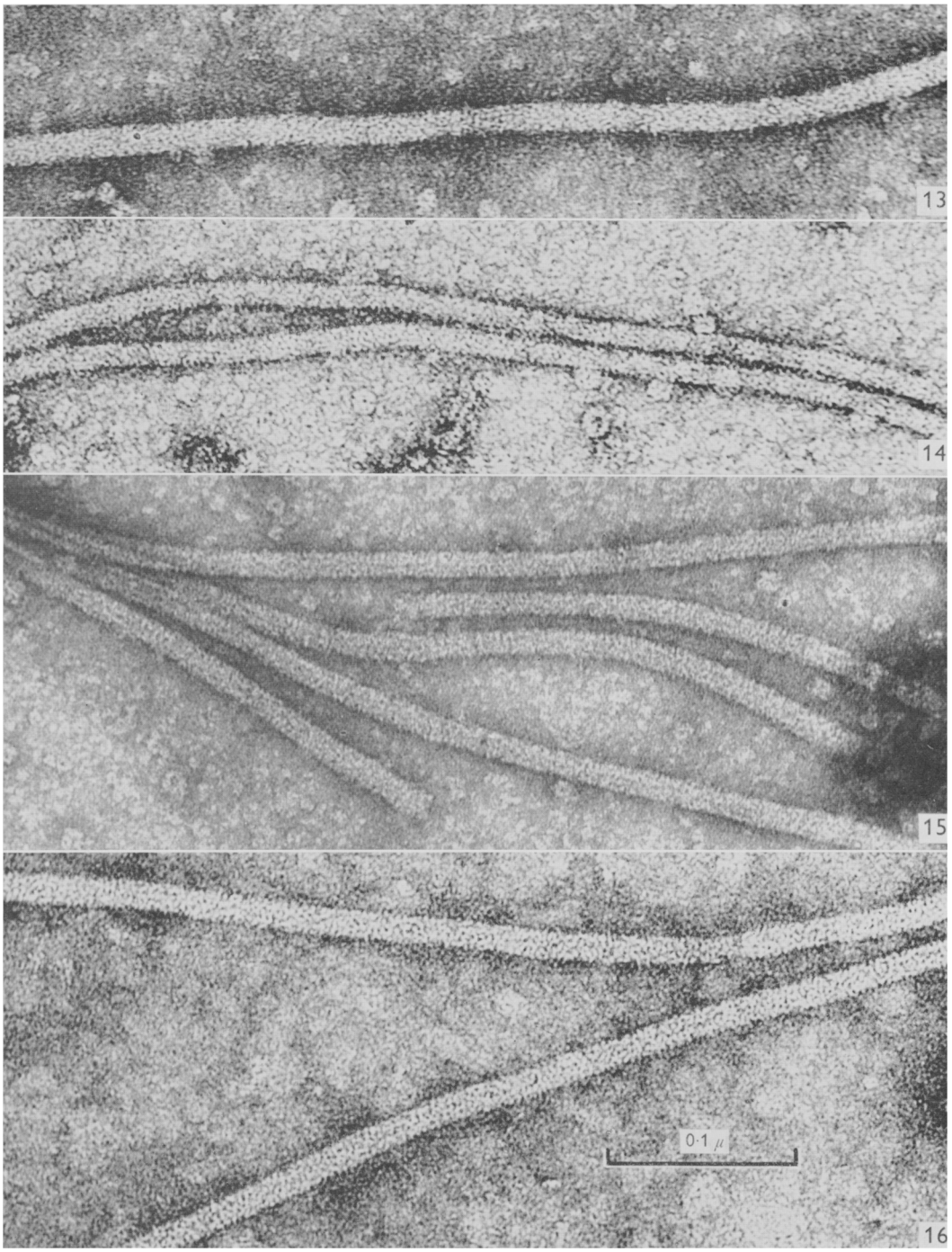


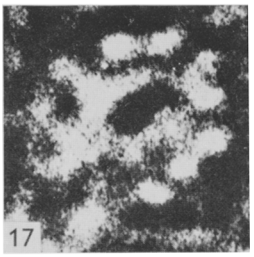

A. VARMA, A. J. GIBBS, R. D. WOODS AND J. T. FINCH

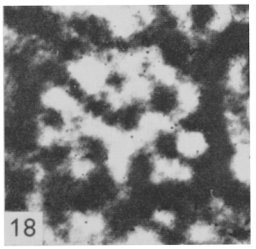
(*Facing p.* 114)

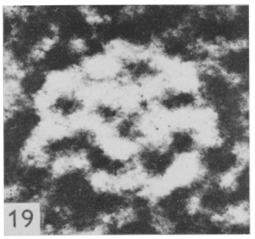


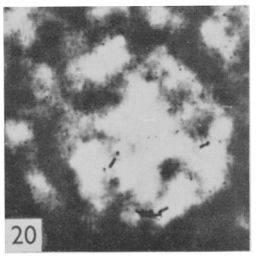

A. VARMA, A. J. GIBBS, R. D. WOODS AND J. T. FINCH

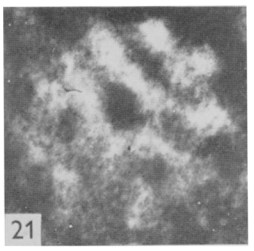

13
14
15
0·1 μ
16

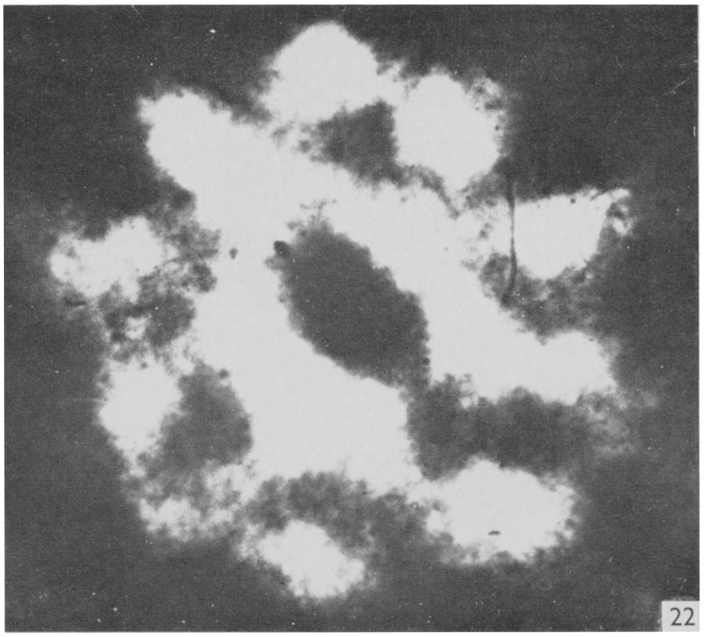

17

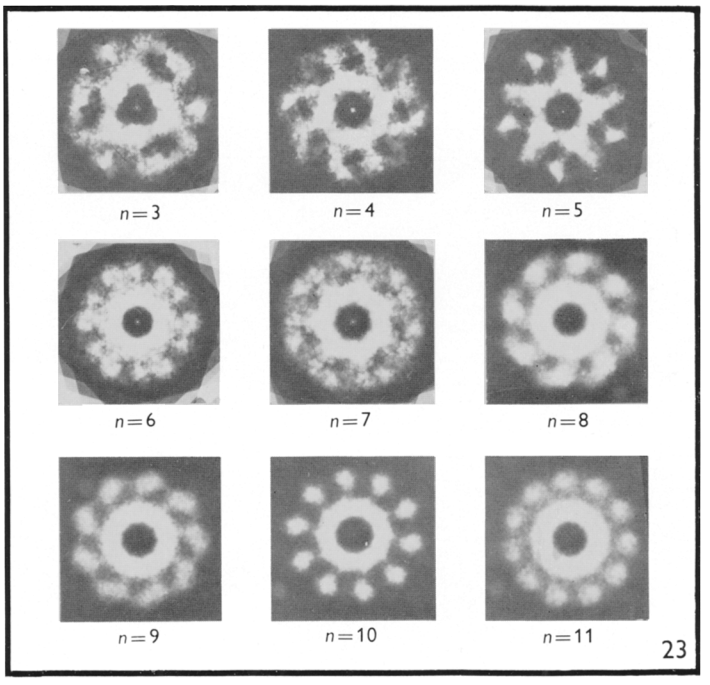

18


19


20


21


22



23