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# Assembly of Flexuous Plant Viruses and Their Proteins

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# Assembly of flexuous plant viruses and their proteins

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[Plates 25–27]

Recent experiments on the disassembly and assembly of some flexuous plant viruses and their proteins are described. The properties of reconstituted potato virus X and those of assembled potato virus Y protein are considered as well as the suitability of other flexuous viruses for reconstitution studies.

## 1. Introduction

The two principal morphological classes of regular anisometric plant viruses are comprised either of rigid tubular particles with an obvious central cavity such as seen in tobacco mosaic virus (TMV) or of relatively long flexuous particles. The latter viruses may be divided into a number of subfamilies composed of particles of various lengths with differences in fine structure. We will deal mainly with members of the potato virus X group (PVX) with particles about 480–580 nm long and the potato virus Y group (PVY) with particles ranging around 720–800 nm in length. Reconstitution studies have been successful with some tubular viruses, particularly TMV, but no indication of success has been reported with the flexuous viruses until recently as reported by Novikov, Kimeav & Atabekov (1972) with PVX.

### *PVX protein*

We have examined PVX and found, unlike Novikov *et al.* (1972), that protein remaining soluble after treatment of the virus with 2 M $CaCl_2$ would not reassemble with RNA under appropriate conditions to form particles whereas protein obtained by freezing viruses in 2 M LiCl was capable of reassembly (Goodman, Horne & Hobart 1975). The protein obtained from the virus contained species sedimenting principally at 2.3S with minor species at 10–15S in 0.1 M NaCl or KCl or in 10 mM sodium 2(*N*-morpholino)ethane sulphonate (MES) pH 6.2. If the 10–15S protein was separated from the 2.3S protein by prolonged ultracentrifugation or by G-200 Sephadex filtration, the 2.3S protein remaining was monodisperse. It represents the chemical subunit which has a molecular mass of 23 000 as determined by amino acid analyses. The protein, either isolated or in the virus, is degraded by unidentified peptidases upon storage.

The 2.3S subunits possess a considerable degree of tertiary structure as determined by tritium exchange studies (Goodman 1975). About 35% of the peptide bond protons are relatively inaccessible for exchange with water (figure 1). The degree of structure was also estimated by circular dichroism (c.d.) measurements which suggested an α-helix content of about 40% (figure 2). The c.d. evidence also suggested that the tertiary structure of PVX coat protein subunits isolated in solution and in the virus was similar although there may be a difference in the tryptophan environment (Homer & Goodman 1975). However, this does not

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invalidate the point that no major difference which may be attributed to a structural constraint imposed upon the subunits upon assembly has been observed. This is probably not surprising when one recalls the flexuous nature of PVX, as well as that of the other elongated viruses, in which the intersubunit interaction must be limited and in which the critical forces which allow stability must be near the inside surface of the helix. This is in contrast to the situation in the relatively rigid TMV in which the helical structure is at least partly stabilized by a reduction in electrostatic repulsion between subunits resulting from hydrogen bonding between two carboxyl-carboxylate pairs near the inner and outer surfaces of the helix (Caspar 1963).

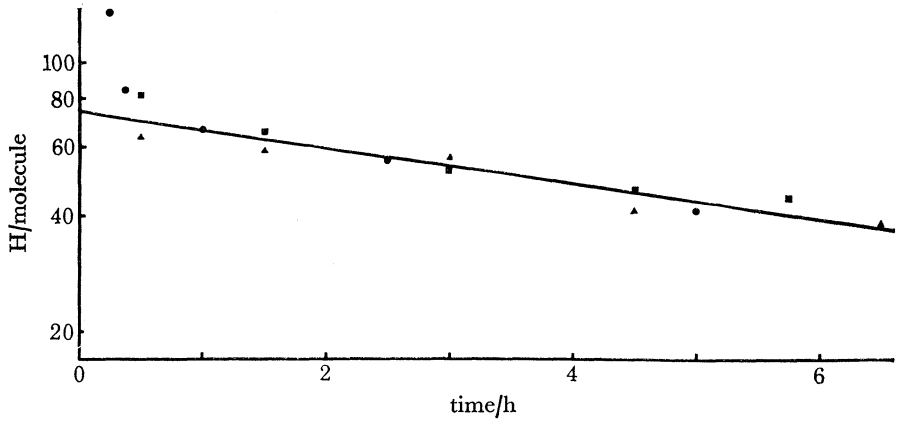


FIGURE 1. Exchange of tritium from PVX coat protein subunits at pH 6.1. Exchange-in was performed at room temperature, pH 8.0, in 10 mM tris HCl containing mCi/ml tritium for at least 7 days. Exchange-out was at 4 °C in 10 mM sodium *N*,*N*-bis(2-hydroxyethyl) glycine. Symbols represent data from three separate experiments. (From Goodman 1975.)

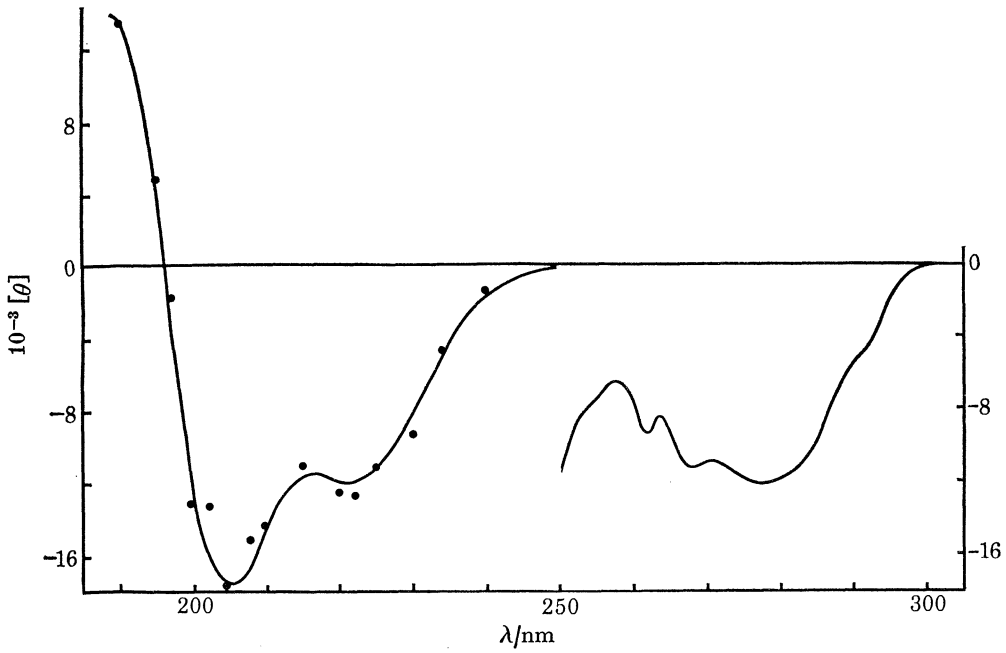


FIGURE 2. Circular dichroism of PVX coat protein subunits (solid line) in the aromatic (right hand scale) and peptide bond (left hand scale) regions. The protein was dissolved in 0.5 mM phosphate, pH 6.45. The spectrum calculated for a protein with 40% α-helix, 5% β-sheet, and 55% random coil is indicated by the dots in the 190–240 nm region. (Homer & Goodman 1975.)


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*Assembly of PVX*

The protein subunits isolated in LiCl would not combine with RNA when both were in 0.1 M NaCl or KCl but would at lower ionic strengths. If the RNA and protein were prepared in 10 or 20 mM sodium *N*,*N*-bis(2-hydroxyethyl)glycine (bicine), 10 mM MES, 10 mM sodium cacodylate, 10 mM sodium acetate, 10 mM tris (hydroxymethyl) methylamine (tris), or 2 mM sodium phosphate around pH 6.2 and mixed at 24 °C so that the RNA to protein ratio was 1:20 (by mass), assembly occurred at levels up to 96 % (figure 3). No reconstitution occurred in 10 mM sodium citrate.

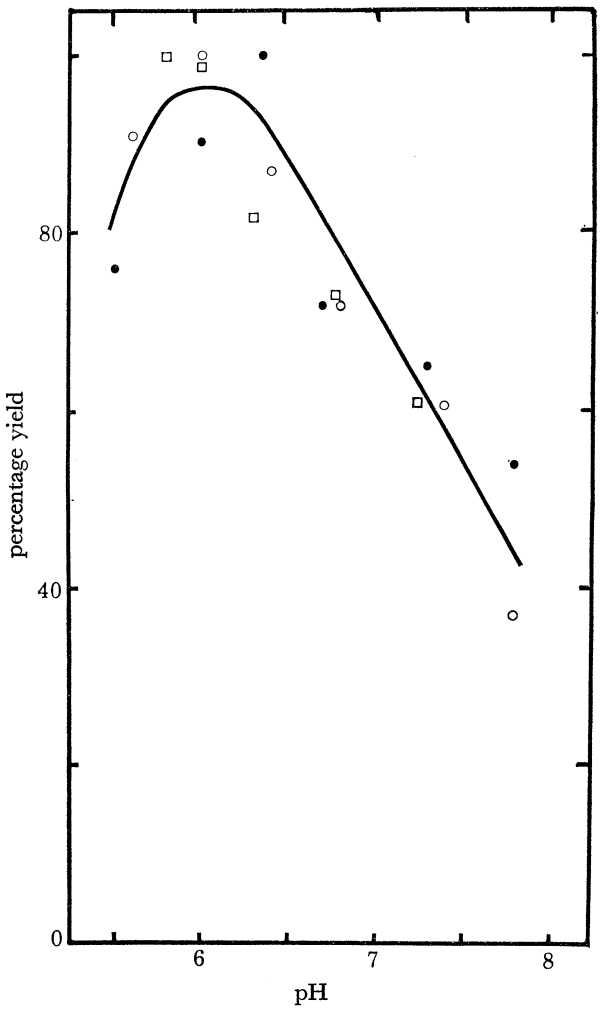



FIGURE 3. Yields of reconstituted PVX nucleoprotein at various pH values. The different symbols represent results from different experiments. (From Goodman *et al.* 1975.)

The morphology of the normal PVX and reassembled particles was studied with the aid of high resolution electron microscopy and optical diffraction following preparation of the suspensions according to the procedures described by Horne & Pasquali-Ronchetti (1974), and are described below.

The rate of assembly was rapid as measured turbimetrically (figure 4) but a more direct indication of rapid assembly was obtained by pipetting RNA and protein in the appropriate

stoichiometric mixtures directly onto freshly cleft mica surfaces before stopping reconstitution with 10 mM pH 8.0 sodium ethenylenediaminetetraacetate. Under these conditions the samples could be examined directly in the electron microscope and assembled and partially assembled particles were seen within 30 s of mixing.

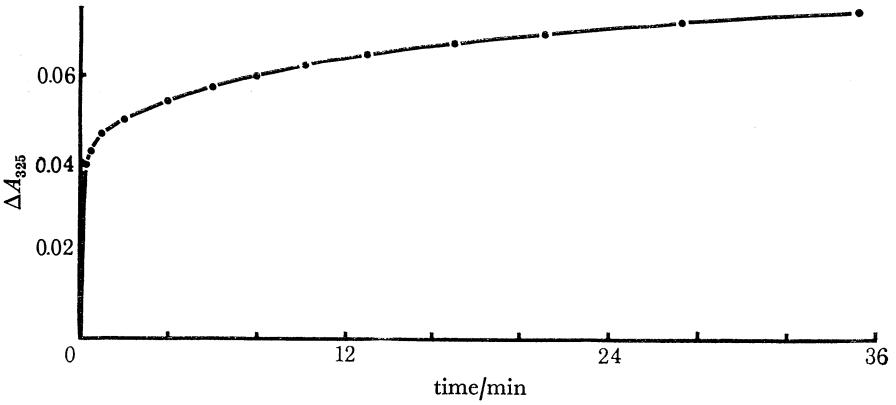



FIGURE 4. Turbididy changes at 325 nm during reconstitution. 1.26 mg protein added to 59 μg RNA in 1 ml at 22 °C, 10 mM sodium bicine, pH 6.3. (From Goodman *et al.* 1975.)

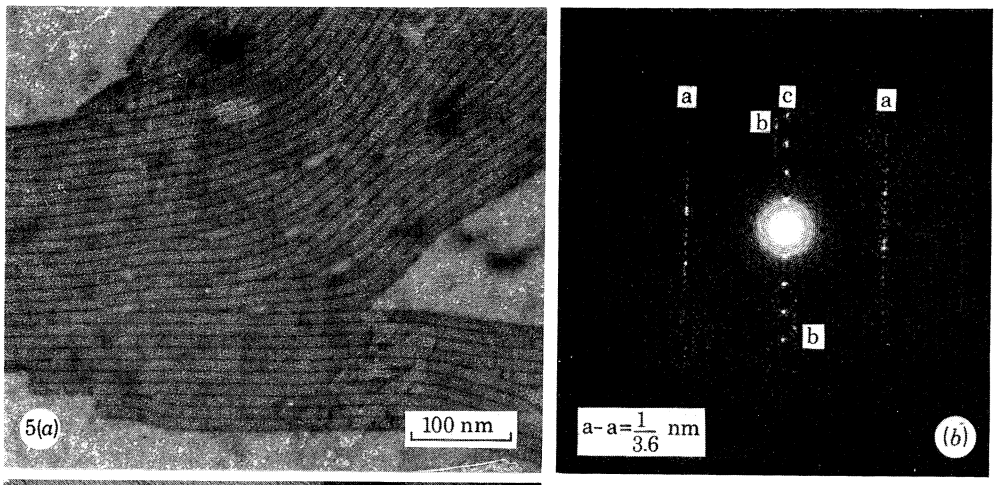
The conditions for assembly are clearly indicative of an entropy-driven reaction since no virus polymerization occurred at 4 °C. This is not necessarily incompatible with the apparent requirement for the rather low ionic strength currently used. A particular charge and/or conformational state is undoubtedly necessary to form the requisite minimum energy structure from what appears to be essentially protein monomers. The *in vitro* assembly conditions outlined are not unreasonable in terms of possible cellular environment. Nevertheless the effect of ionic strength is still under investigation.

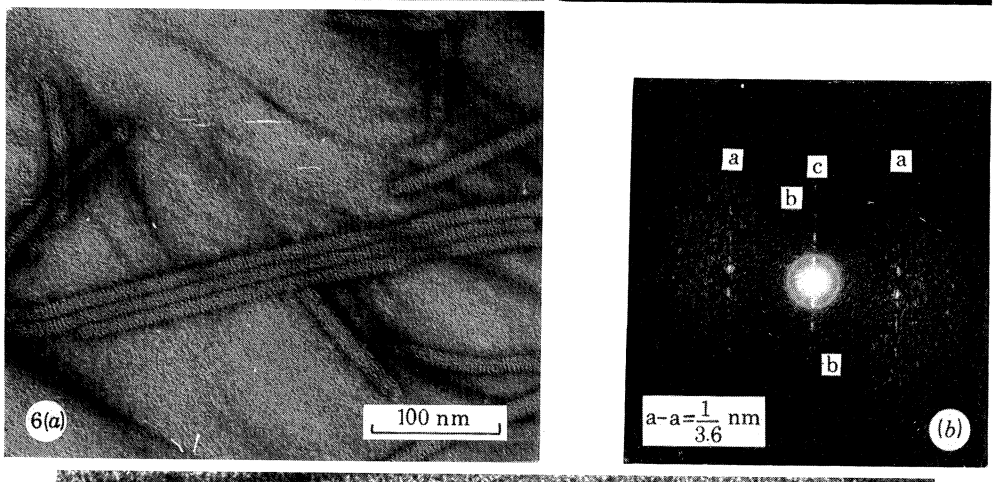
### *Properties of reassembled PVX*

The ribonuclease resistant specific infectivity as measured by local lesions of the reconstituted virus varied from 1 to 14 % of that of the native virus. The rather low infectivity resulted from partial hydrolysis of the RNA after mixing with the coat protein as determined by acrylamide gel electrophoresis of RNA from the mixture. Apparently the coat protein preparations were contaminated with traces of nuclease. This resulted in nucleoproteins with considerable size heterogeneity as determined by electron microscopy and analytical centrifugation. It does not mean that the assembly process was fundamentally incorrect.

Serological assessments do not rely on full length particles. Dissociated coat protein subunits do not cross-react with appropriately prepared antiserum made to the virus. However, the reconstituted virus cross-reacted strongly against such antiserum showing that virus specific determinant groups were generated upon assembly.

The structural features observed in the electron micrographs and their subsequent analysis by optical diffraction indicated basic similarities between native PVX and the reassembled products (figures 5 and 6, plate 25). When negatively stained with uranyl salts a very narrow channel is seen along the central particle axis of at least some strains of PVX (figure 7, plate 25). Microdensitometer tracings across the PVX axis indicate a definite region of density at the centre of the particle corresponding to possible penetration of stain. The mean diameter of



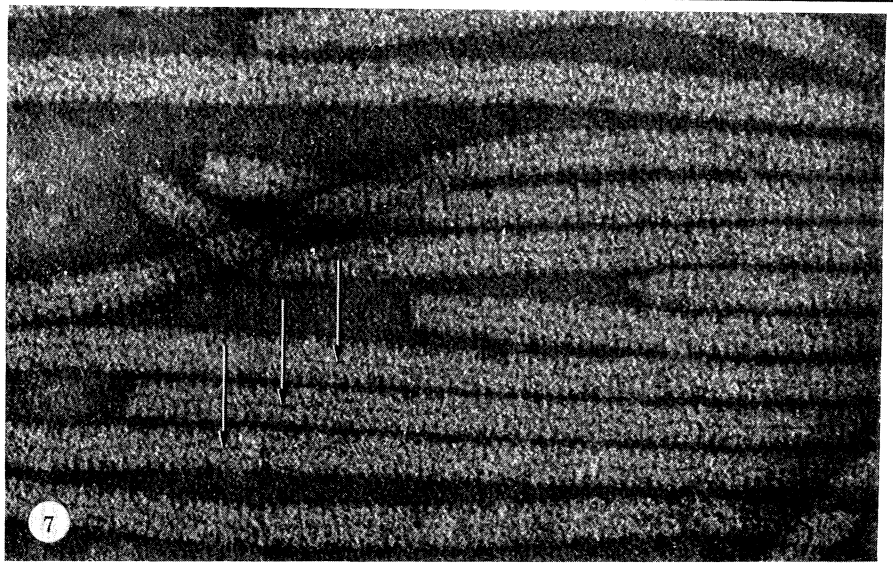




FIGURE 5. (*a*) Part of an area showing native PVX filaments arranged roughly in parallel following preparation by using the negative staining-carbon method. The striations across the filament axis are from the basic pitch of the helix forming the nucleocapsid. (*b*) Optical diffraction pattern from figure 5*a* showing spectra (layer lines) a–a corresponding to the basic pitch of the nucleocapsid helix of about 3.6 nm. The layer lines b–b indicate a secondary helix repeating every 8 turns of the basic helix along the filament axis.

FIGURE 6. (*a*) The filaments are from reconstituted PVX protein and RNA and show the same basic appearance to the particles in figure 5*a*. Some short filaments are also visible in the same area. (*b*) Optical diffraction pattern from figure 6*a*. The layer lines a–a are in the same positions as illustrated in figure 5*b* from native PVX filaments. The spectra at b–b from the secondary helix is very faint, but can be detected on close examination of the diffraction patterns.

FIGURE 7. PVX (papaya strain) nucleocapsids negatively stained with uranyl acetate and mounted directly onto support films. Many of the particles show evidence for a hollow region (arrows) along the nucleocapsid axis. (Reproduced by kind permission of G. J. Hills, John Innes Institute.)

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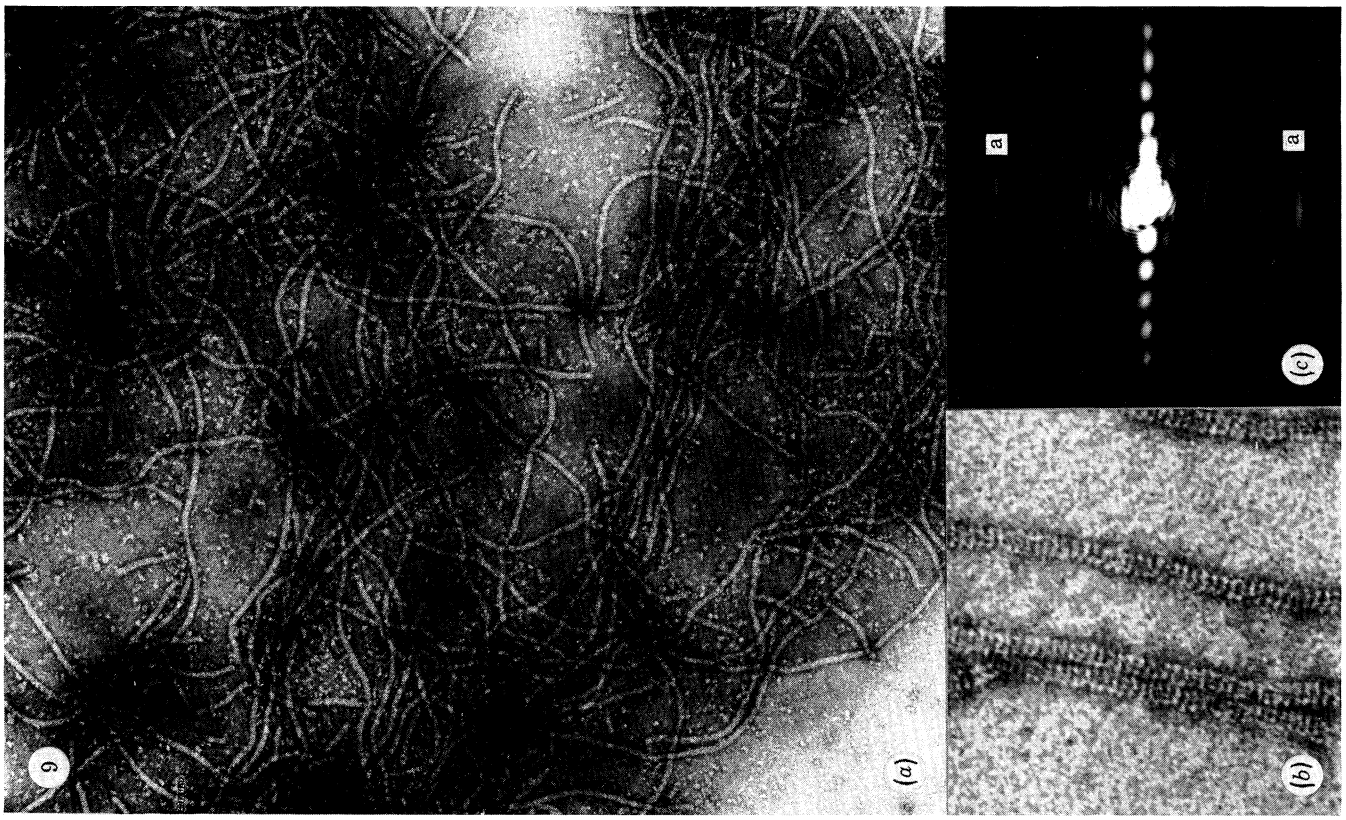


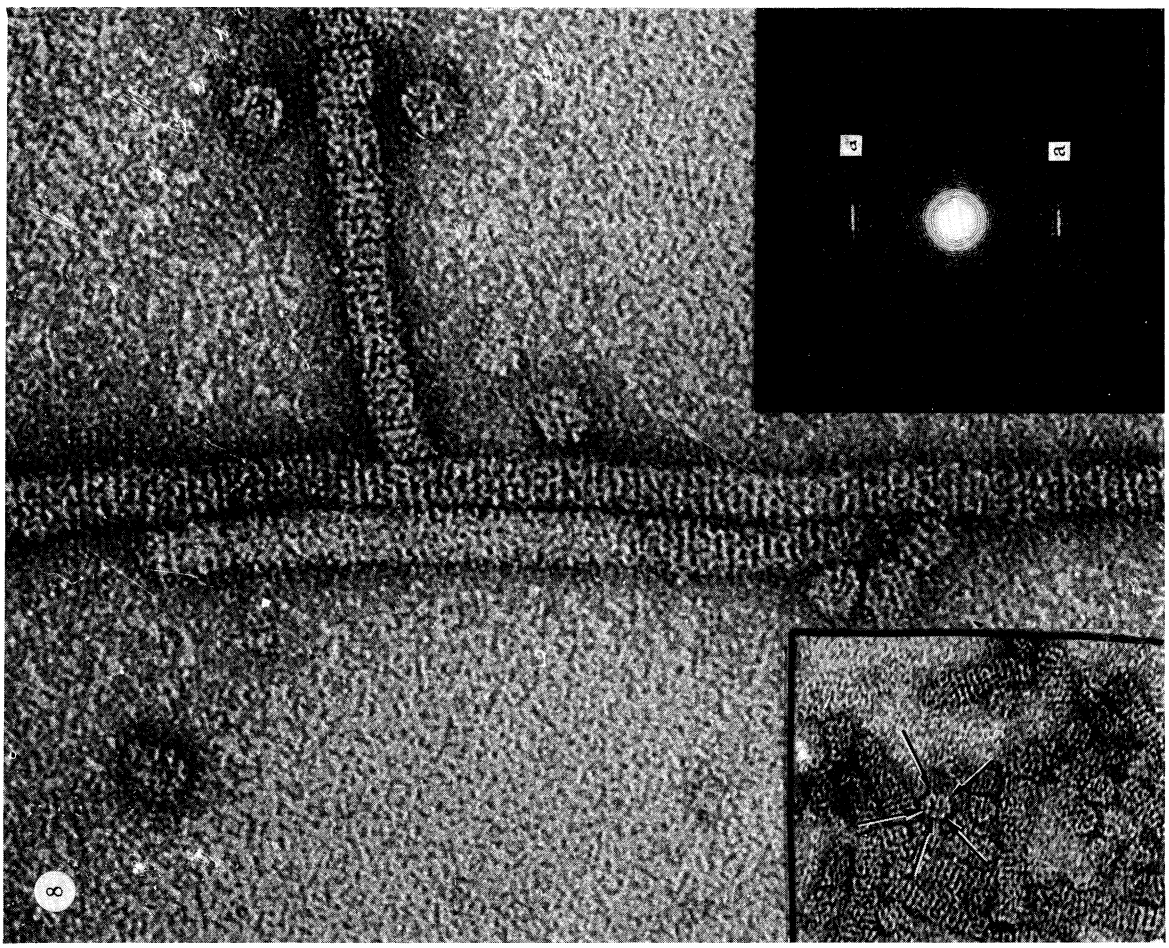


FIGURES 8 AND 9. For description see opposite.



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PVX was estimated to be about 12.6 nm and the near-meridinal spectra (figure 5*b*), show a basic pitch of the helix of 3.6 nm, which is similar to the value reported by Varma, Gibbs, Woods & Finch (1968). A repeat of every 8 turns of the helix is indicated by the first layer line shown in figure 5*b*. The reconstituted particles (figure 6), look the same as natural virus and show essentially the same structure (figure 6*b*) although the first layer line is not as strong as with the natural virus.

The reconstituted virus resembles natural PVX in a number of important ways but the details of assembly have not yet been examined. The reaction may not be specific to PVX–RNA since reconstitution of flexuous particles also occurs with TMV–RNA. The fractionated protein used for the assembly experiments did not appear to contain rings of subunits or stacked discs but this does not necessarily mean that such aggregates are not required for particle initiation and/or for elongation. Particles sedimenting at 10–15S were observed in the unfractionated protein. They may correspond to rings composed of 9 subunits, a value not inconsistent with estimates based on the volume of the subunits in relation to the diameter of the virus and its pitch. Such structures can be seen in figure 8, plate 26, along with loosely-packed particles which are aggregates of protein with a pitch of 4.0 nm.

### *Assembly of PVY protein*

The assembly of PVY protein has been examined more closely than that of PVX. The virus can be dissociated in 2 M LiCl, or 2 M $MgCl_2$, $CaCl_2$, $SrCl_2$ or $BaCl_2$ at pH 5–8 to form a species which mainly sediments at 3S. Such a preparation is probably composed of protein monomers which, like those of PVX, are easily degraded on storage. The molecular mass for the PVY monomer seems to be about 34 000 in the best preparations. Minor peaks sedimenting at 10S have also been observed. If the protein is dialysed against 1–100 mM phosphate at pH 6–9, long particles composed of rings or stacked disks (figure 9, plate 26) with a pitch of 4.0 nm (figure 9*b*, *c*) are formed in abundance (McDonald, Beveridge & Bancroft 1975). The particles appear to lack fine structure if phosphate ions are absent. The basic pitch of the virus is 3.3 nm and no secondary helices have been observed (figure 10, plate 27).

If the assembled protein particles are dialysed to pH 10.5, they disassemble to form 3S proteins which can be reassembled into the flexuous protein particles again by lowering the pH. Thus, the system is clearly pH dependent and lysyl residues are probably involved. No long particles were formed at pH 5, but rather an aggregate sedimenting at about 15S which is probably constructed of rings containing 7 subunits assuming their estimated molecular mass is correct. These assemble into long particles at pH 6 to 9. If particles are made at pH 8 and are dialysed to pH 5, they retain their integrity. Therefore, no helical structures could be induced to form with protein alone under conditions where TMV protein and cowpea chlorotic

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### Description of plate 26

Figure 8. Reaggregated PVX protein showing elongated structures interpreted as being a stacked-disk assembly of annuli. Isolated annuli viewed end on (insert) show individual subunits (arrows) and there are about 9 subunits in each annulus. The optical transform shows a repeat of about 4.0 nm (a–a) along the particle axis and the positions of the spectra indicate a stacked-disk array.

Figure 9. (*a*) Particles of PVY protein formed by dialysing against 1–100 mM phosphate at pH 6–9. (*b*) High magnification area of PVY protein forming stacked disk-like aggregates. (*c*) Optical transform from PVY particle of the type shown in figure 9*b*.




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mottle virus (CCMV) protein form capsids identical to those of the virus and which are under the control of carboxyl-carboxylate pairs (Butler, Durham & Klug 1972; Bancroft 1970). That the protein subunits used with PVY are intrinsically capable of forming helical structures similar to the virus has been shown in preliminary reconstitution studies. Consequently, the control of assembly may be new for PVY.

### *Klosterviruses*

The thread-like klosterviruses (Bar-Joseph & Hull 1974) illustrated in figures 11 to 14, plate 27 can be disassembled after overnight dialysis in 0.01 M pH 7 phosphate or tris (Lister & Hadidi 1971). The subunits will not reassemble into helices at pH 5, but sediment at about 17S perhaps as rings probably containing 9 subunits similar to PVX. Also, like PVX, there seems to be a nucleotide to protein ratio of 4:1 which differs from the 6:1 ratio estimated for PVY. Interestingly, the viruses tested are stable at neutrality in the presence of $Mg^{2+}$, but do not require it around pH 5. The situation is reminiscent of that for cowpea chlorotic mottle virus which can be stabilized at pH 7 only in the presence of divalent cations (Bancroft 1970). The klosterviruses are susceptible to ribonuclease under any conditions, however, which is a reflection of limited subunit interaction. It is clear that reassembly should take place *in vitro* considering the ease of disassembly and these viruses, notwithstanding their very low yields, are worth attention.

## Conclusions

The helical plant viruses are easy to take apart under reasonably mild conditions which do not destroy the capability of the protein subunits to interact. No polymerization forms have yet been found that might not be expected. However, the details of assembly require attention, as indeed do the details of the composition and structure of many of the native particles. Nevertheless, it is clear that these viruses are going to serve as useful systems for the study of assembly processes which may have relevance to other systems including animal viruses containing helical nucleocapsids.

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## Description of plate 27

Figure 10. (*a*) PVY virus particles prepared by the negative staining-carbon method. (*b*) Optical transform from PVY particles showing a basic pitch of the nucleocapsid helix of about 3.3 nm.

Figure 11. Sugar beet yellows virus showing similar structural features to the images in figures 5 and 6.

Figure 12. Optical diffraction pattern from an area in figure 11. The pitch of the helix at a–a is about 3.9 nm.

Figure 13. Chlorotic leafspot mottle virus formed into a two-dimensional aggregate at the surface of mica. Many areas show the filaments in roughly parallel arrays together with regular striations across the particle axis.

Figure 14. Optical transform from part of figure 13 showing spectra at a–a corresponding to a basic pitch of the nucleocapsid of about 4.0 nm. A secondary helical repeat of about 12.0 nm is indicated at b–b. (From Horne *et al.* 1975.)



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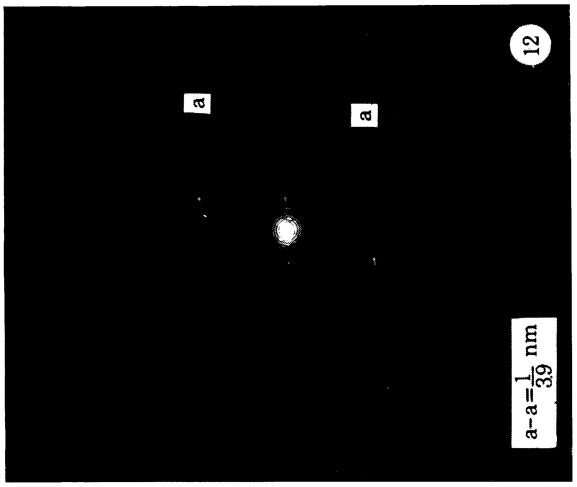


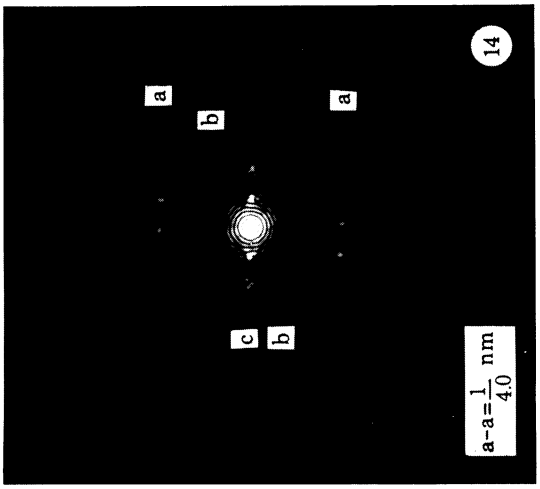



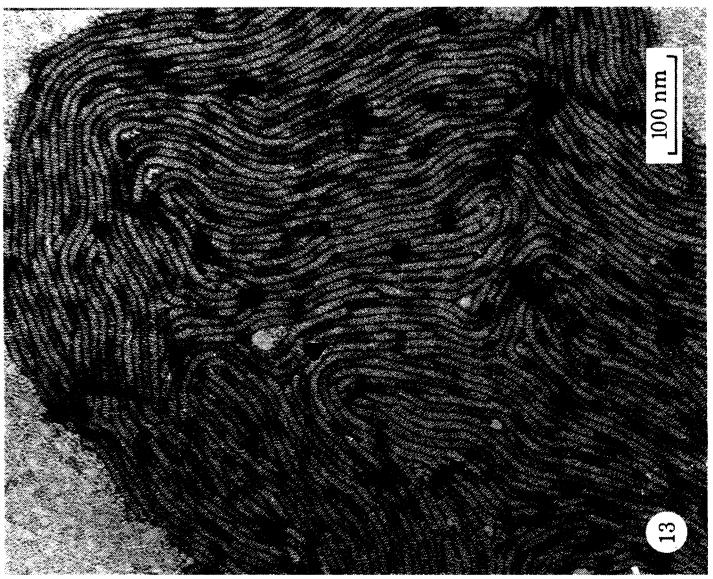


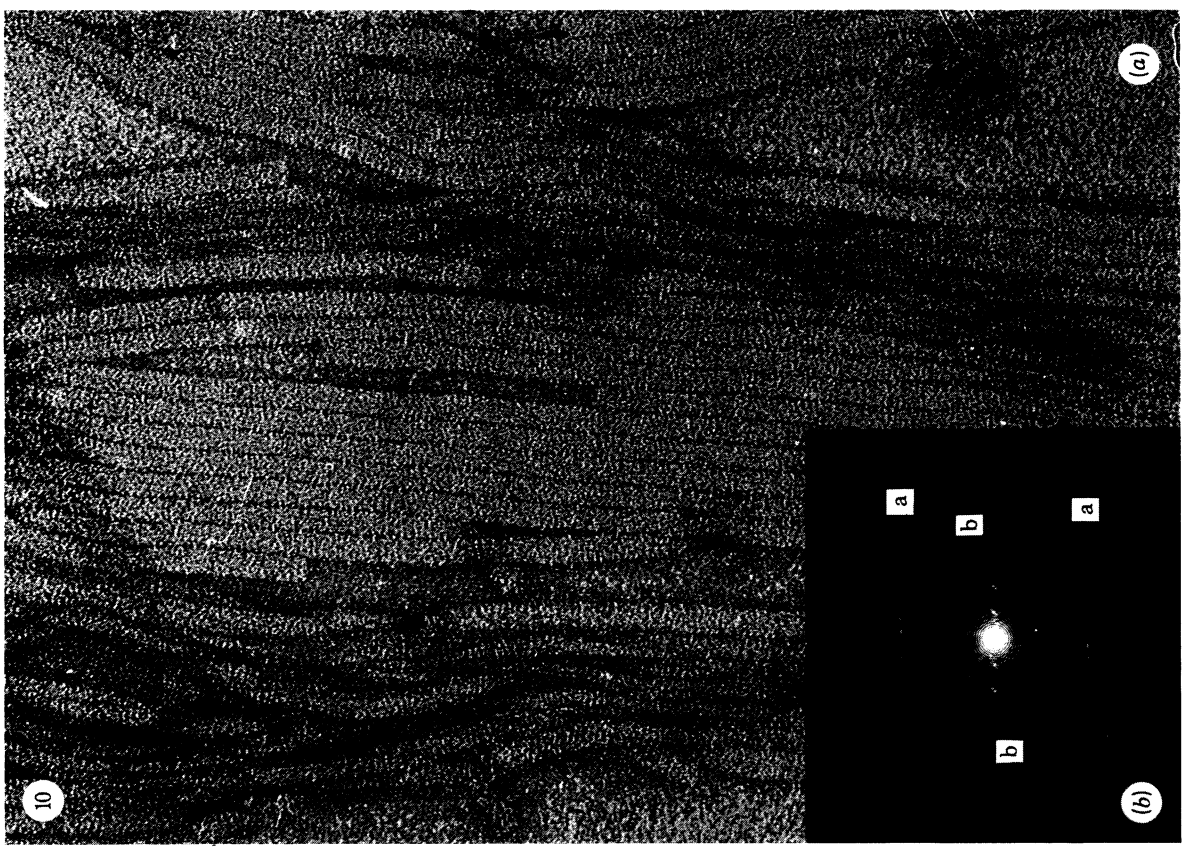


FIGURES 10–14. For description see opposite. (*Facing p.* 178)



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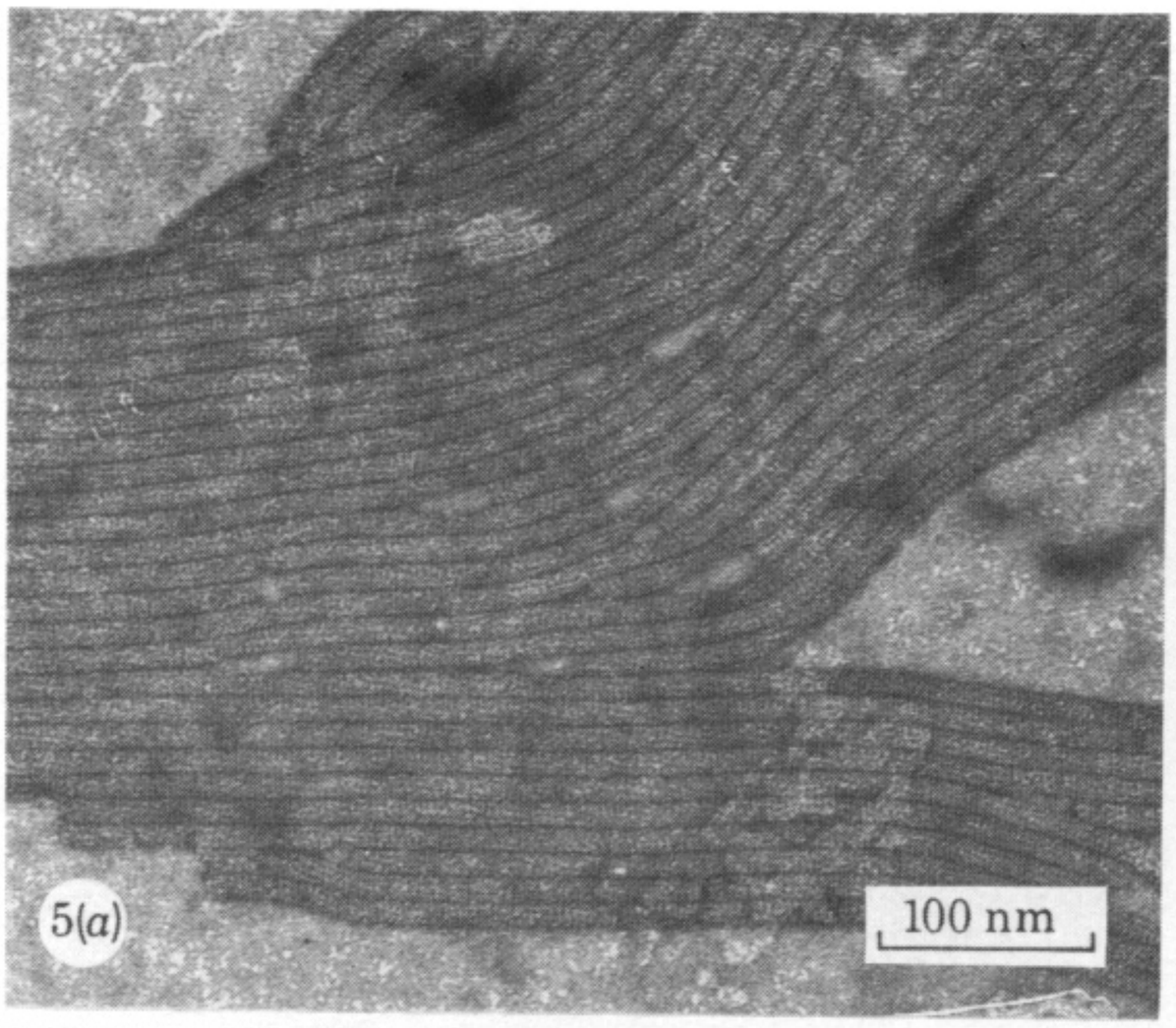


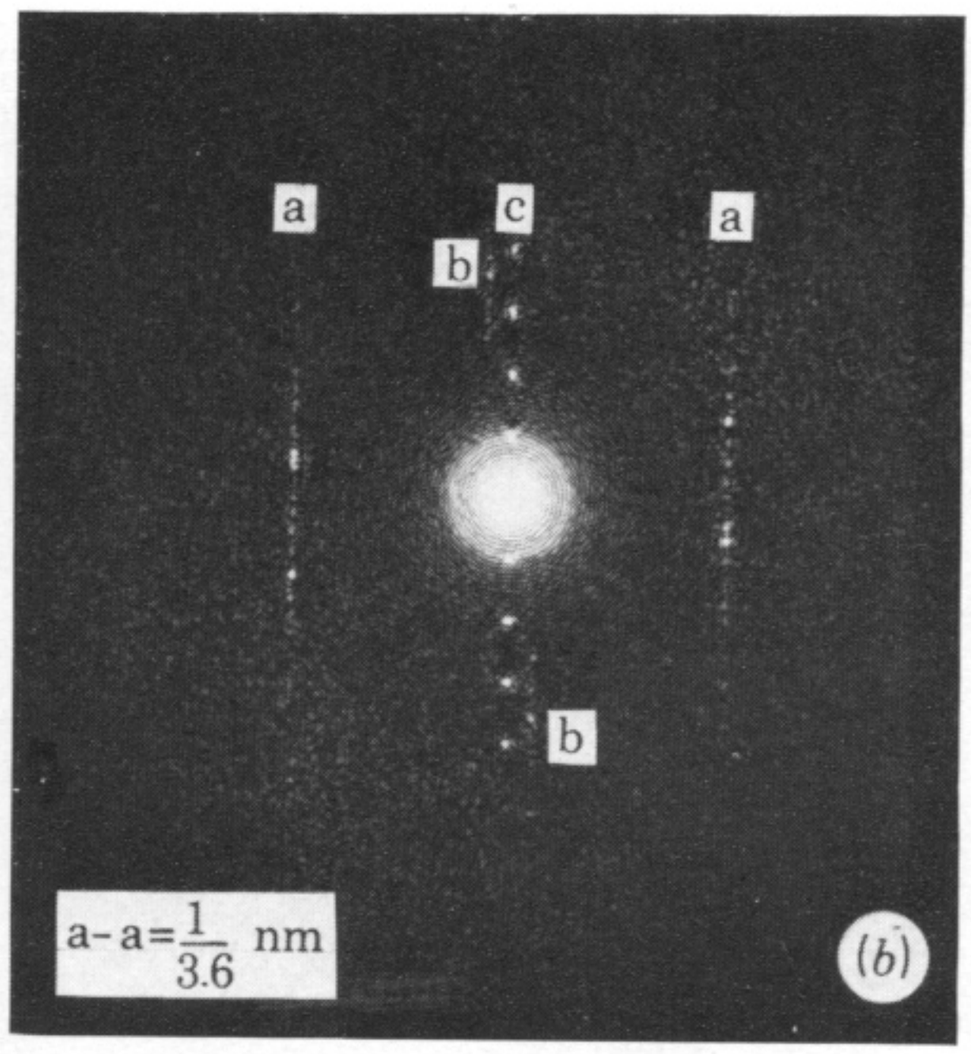


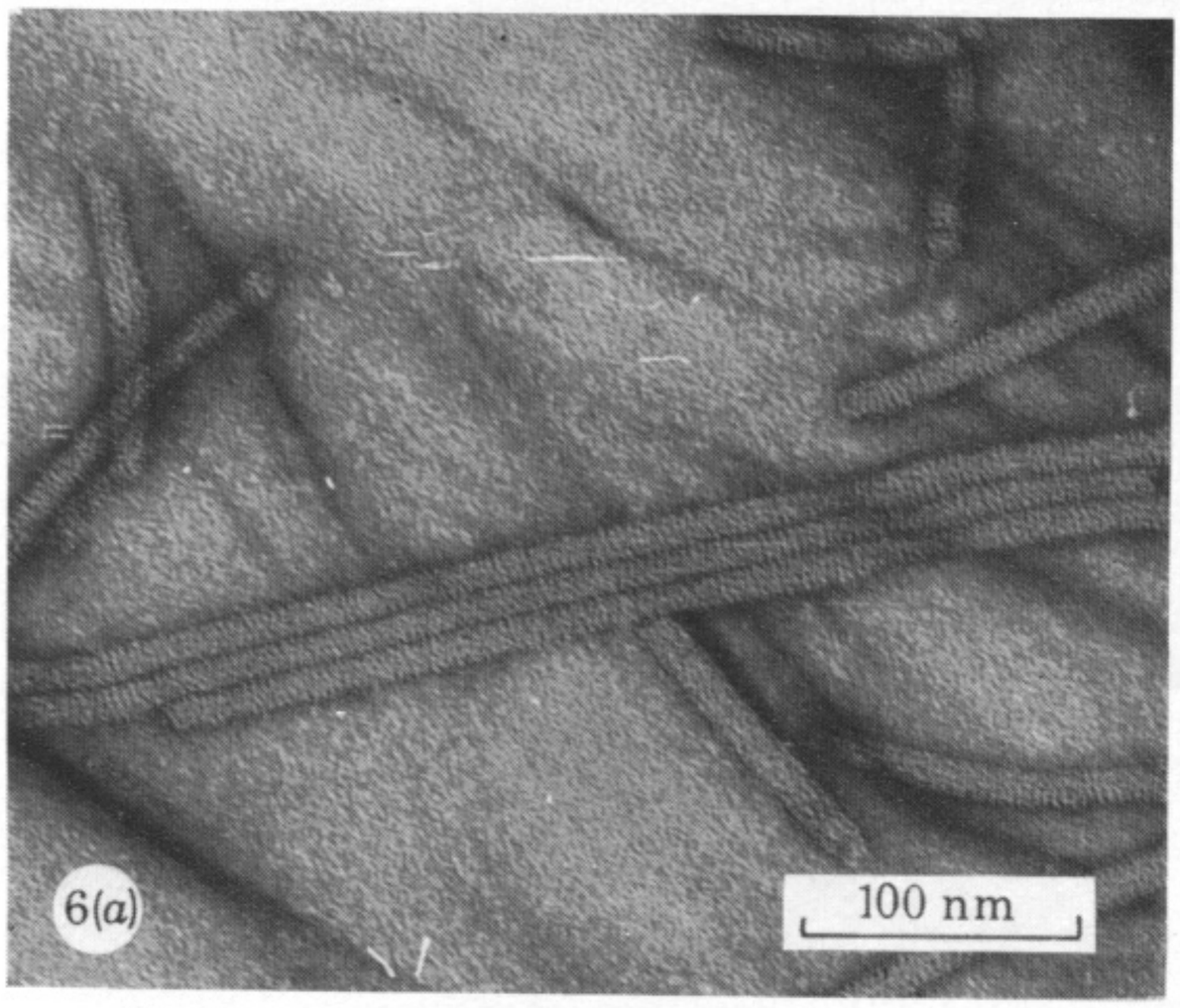


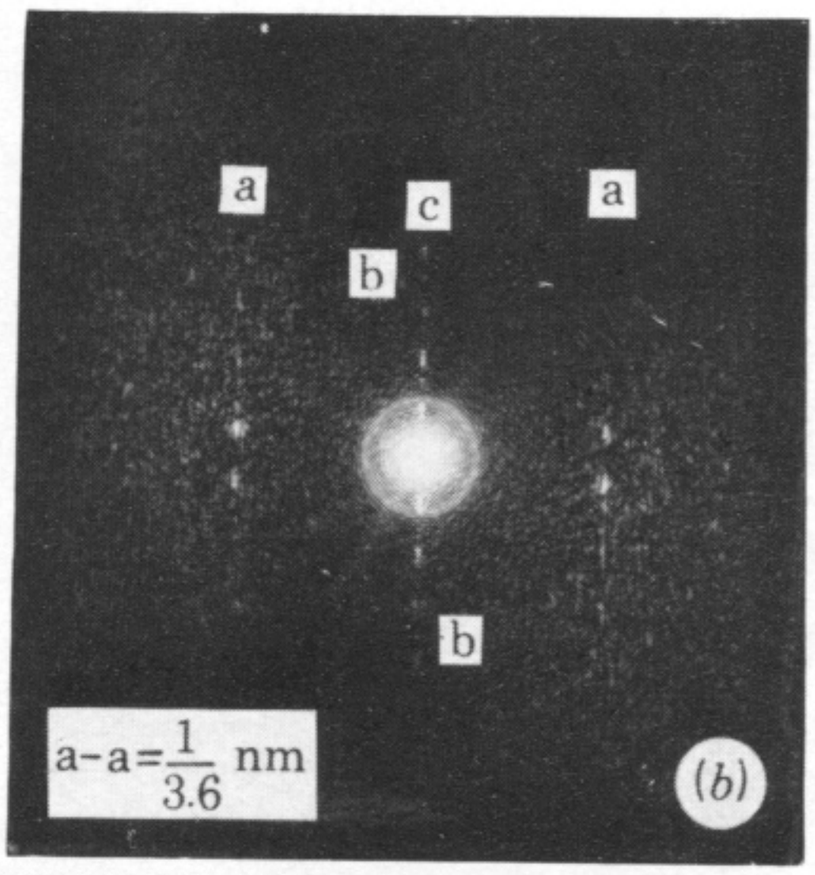


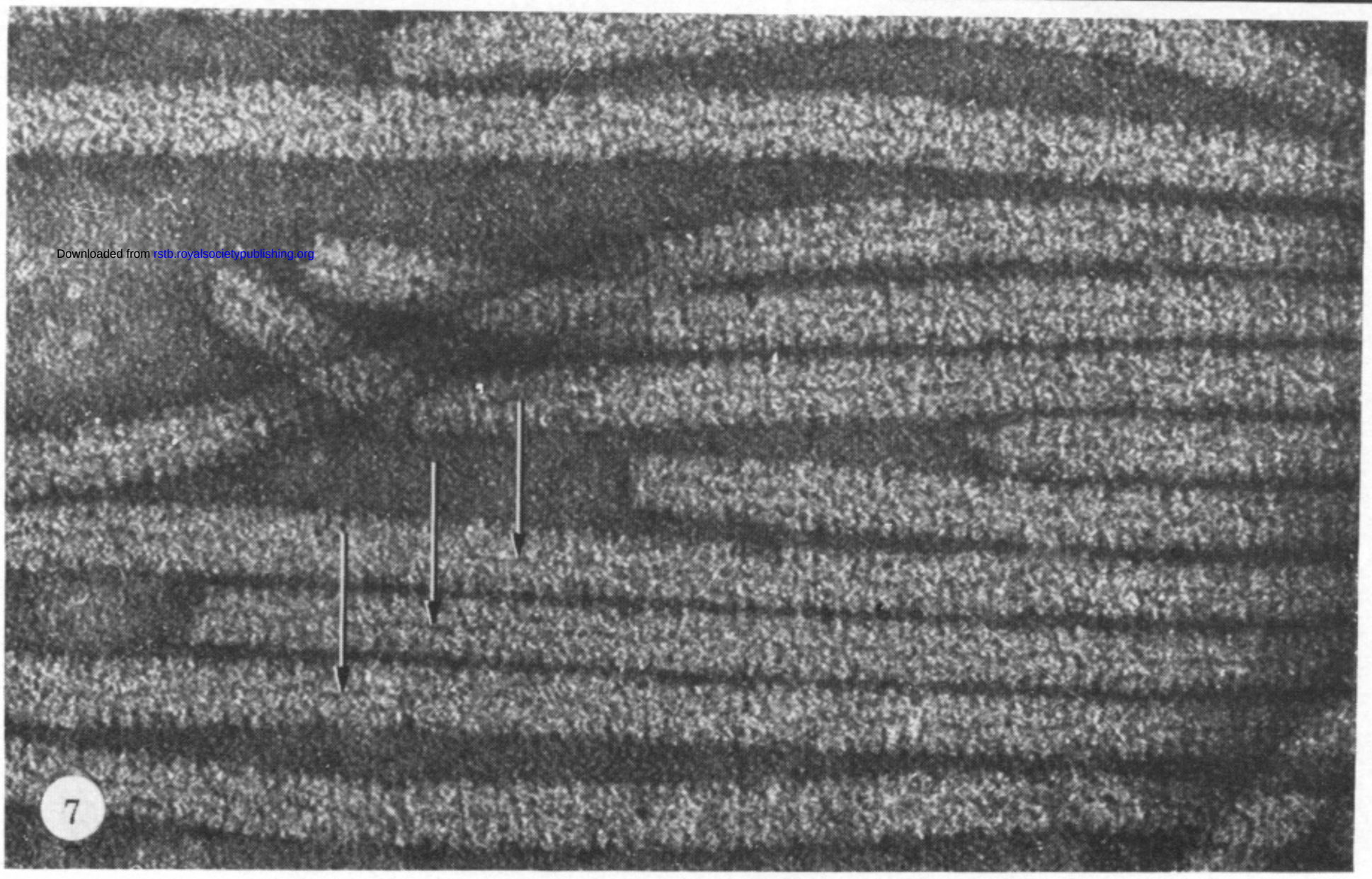




IGURE 5. (*a*) Part of an area showing native PVX filaments arranged roughly in parallel following preparation by using the negative staining-carbon method. The striations across the filament axis are from the basic pitch of the helix forming the nucleocapsid. (*b*) Optical diffraction pattern from figure 5*a* showing spectra (layer lines) a–a corresponding to the basic pitch of the nucleocapsid helix of about 3.6 nm. The layer lines b–b indicate a secondary helix repeating every 8 turns of the basic helix along the filament axis.

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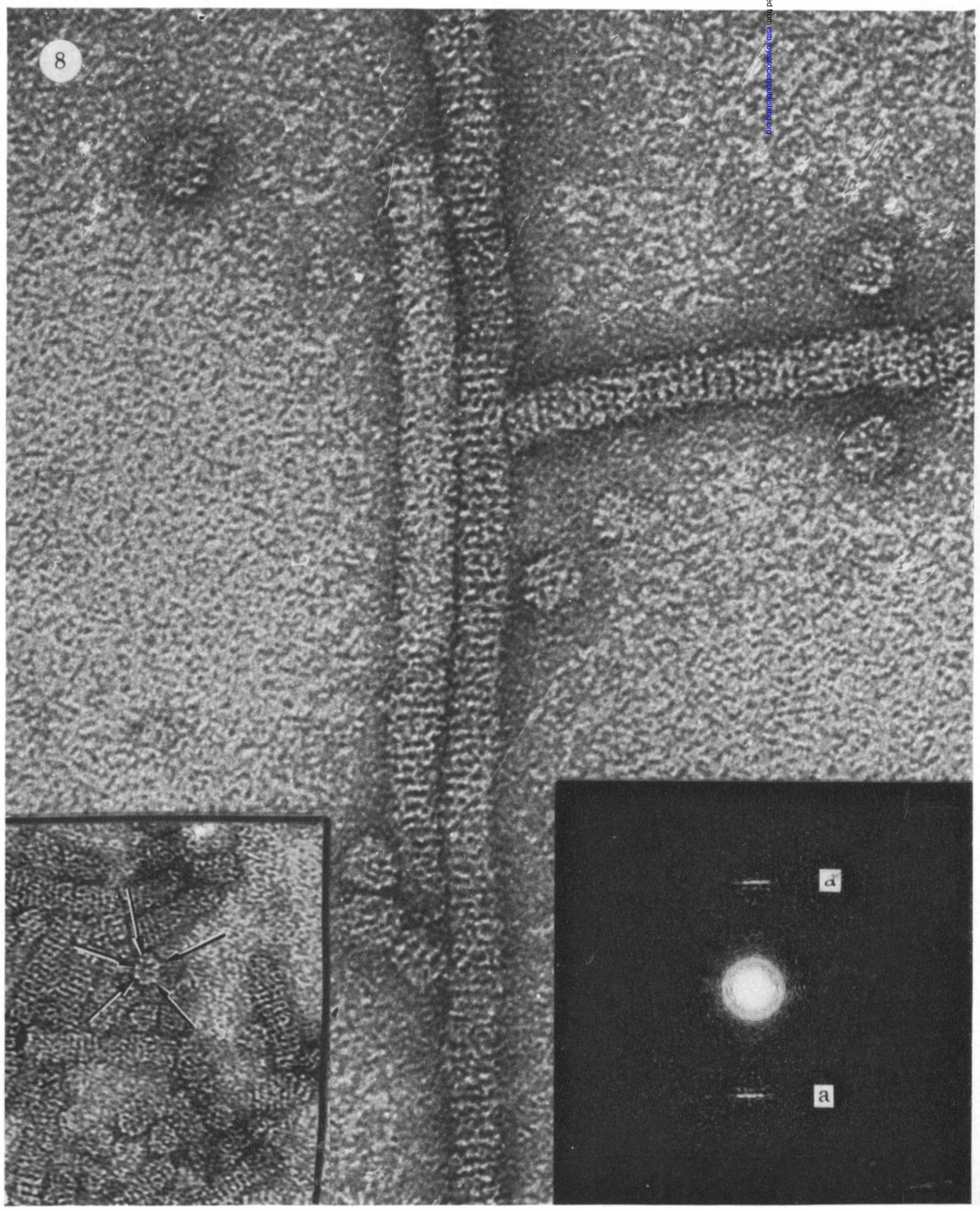


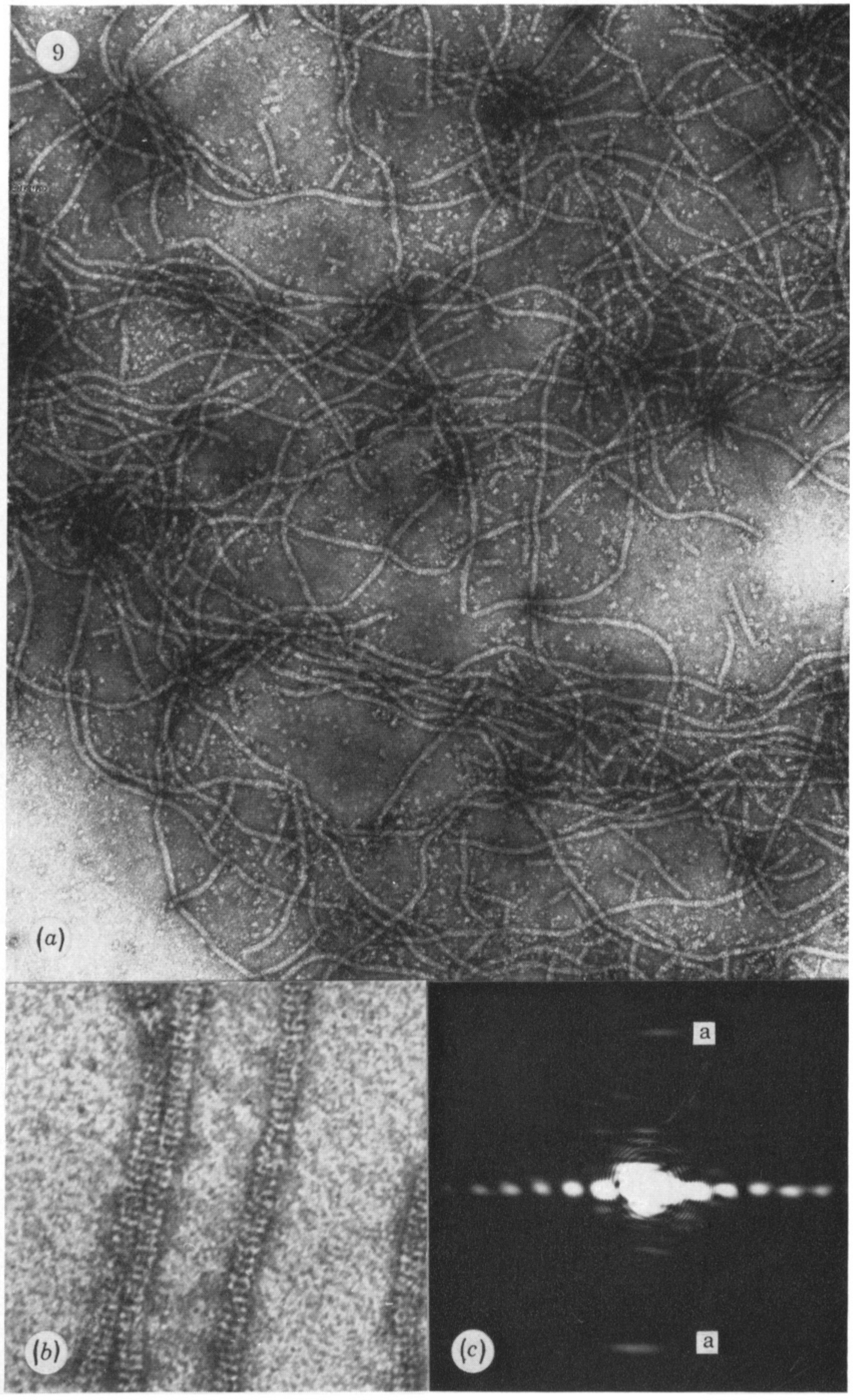


FIGURES 8 AND 9. For description see opposite.

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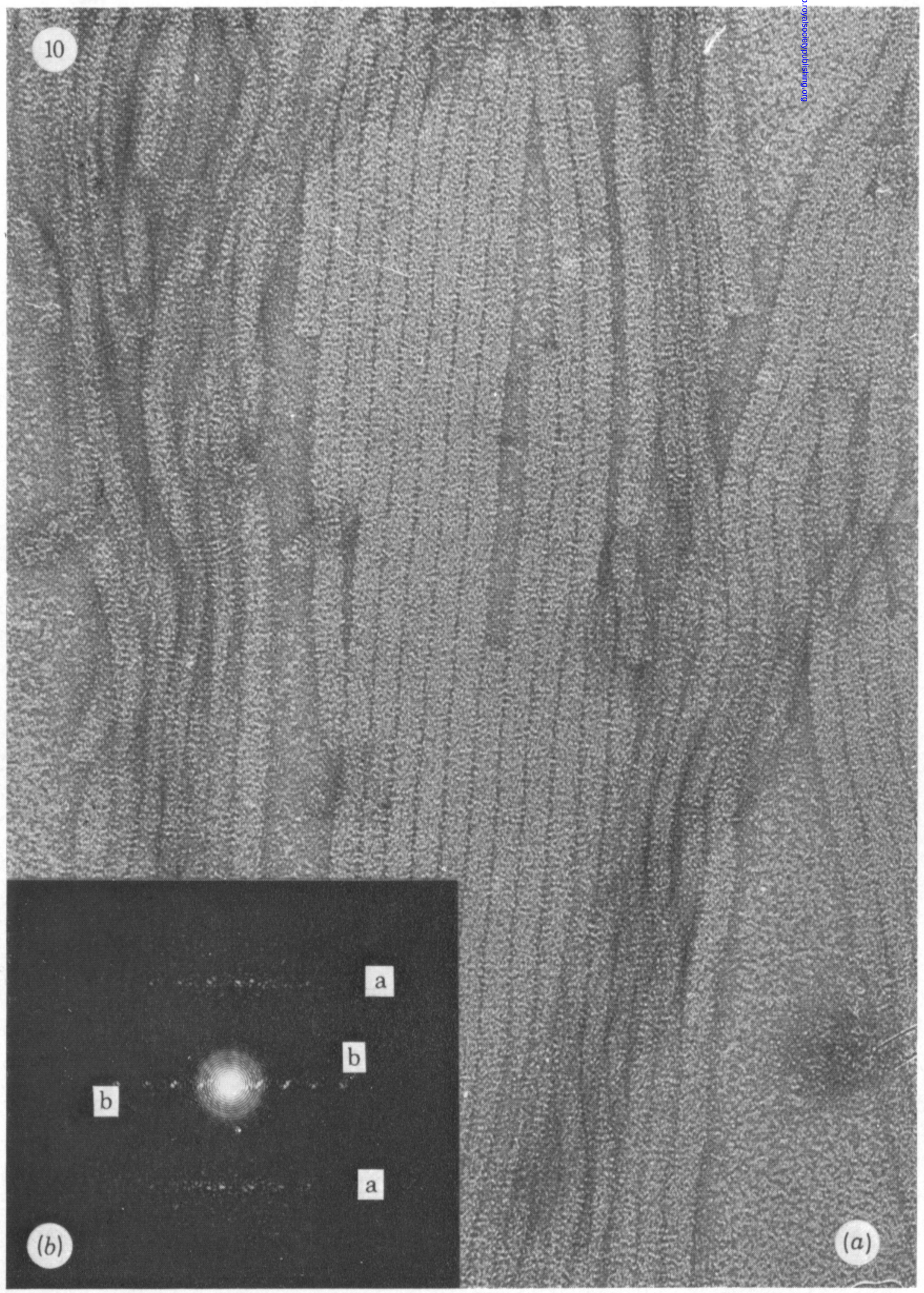


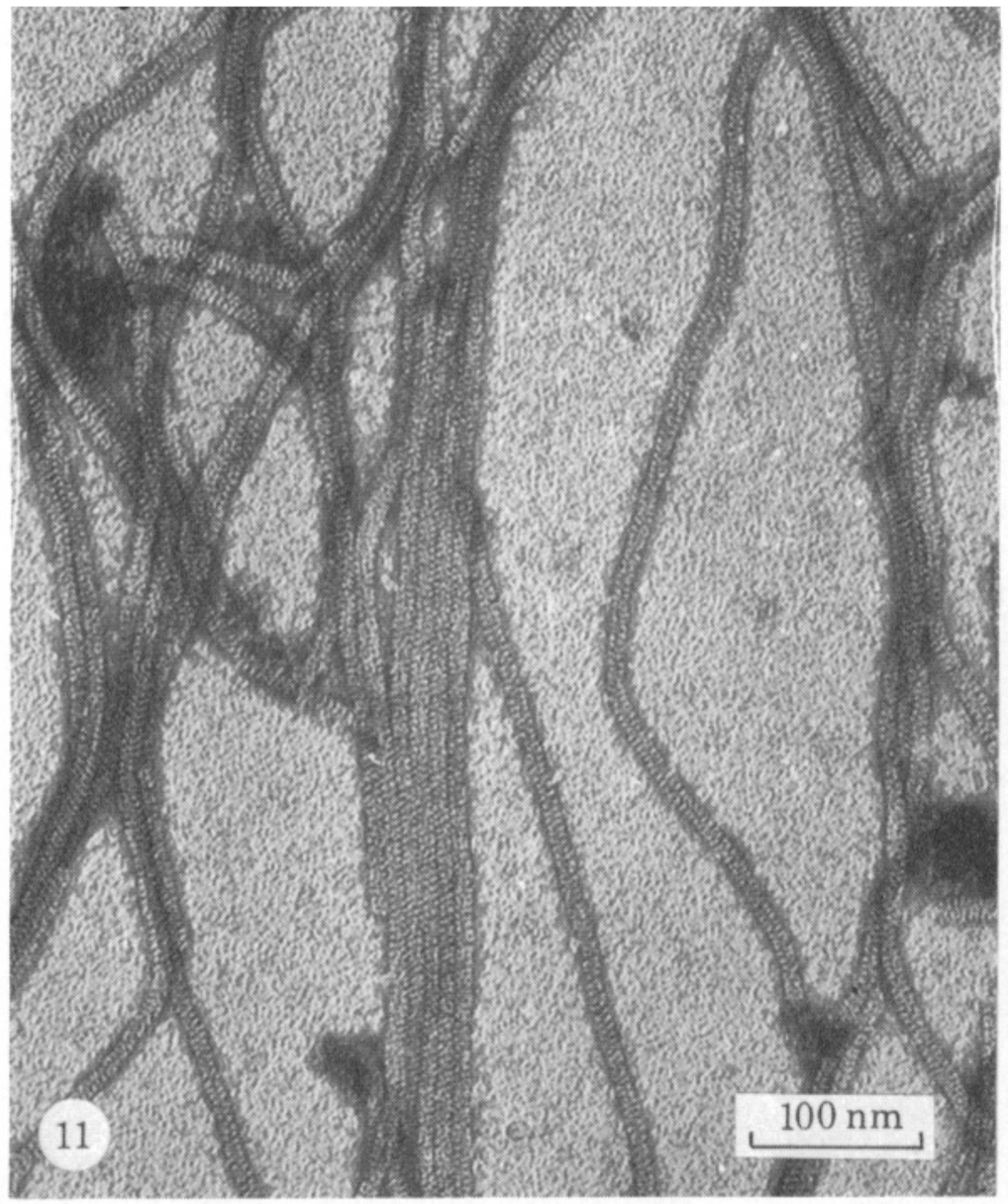


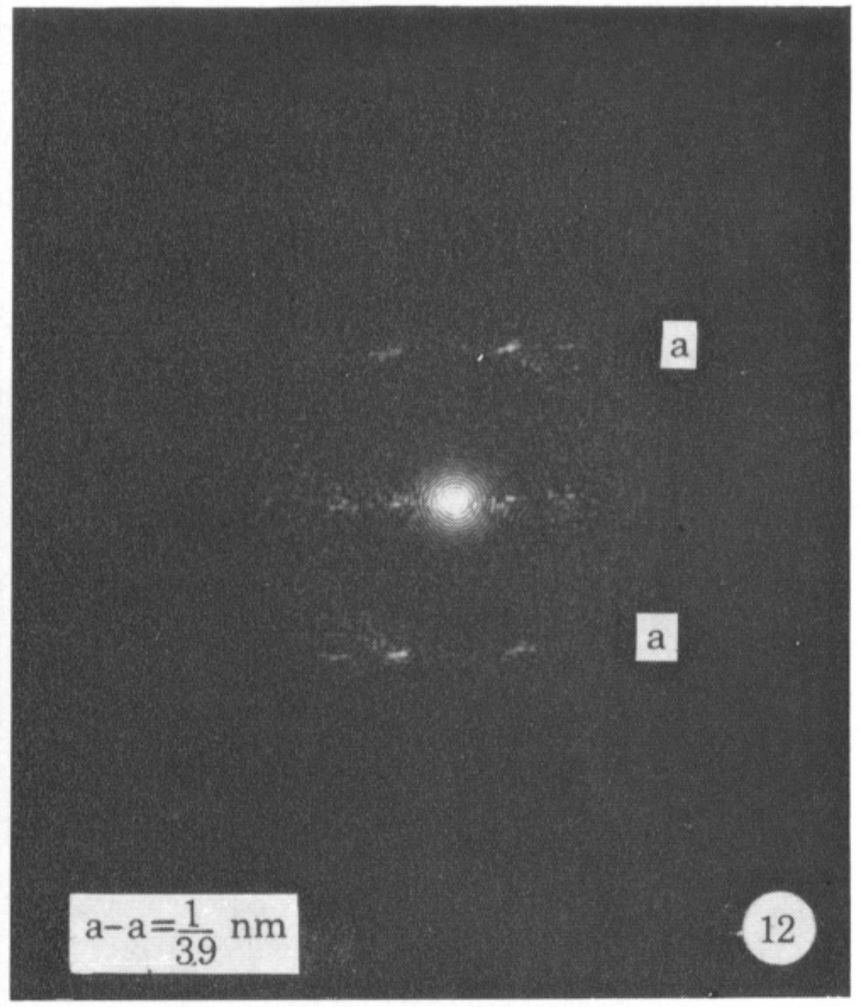


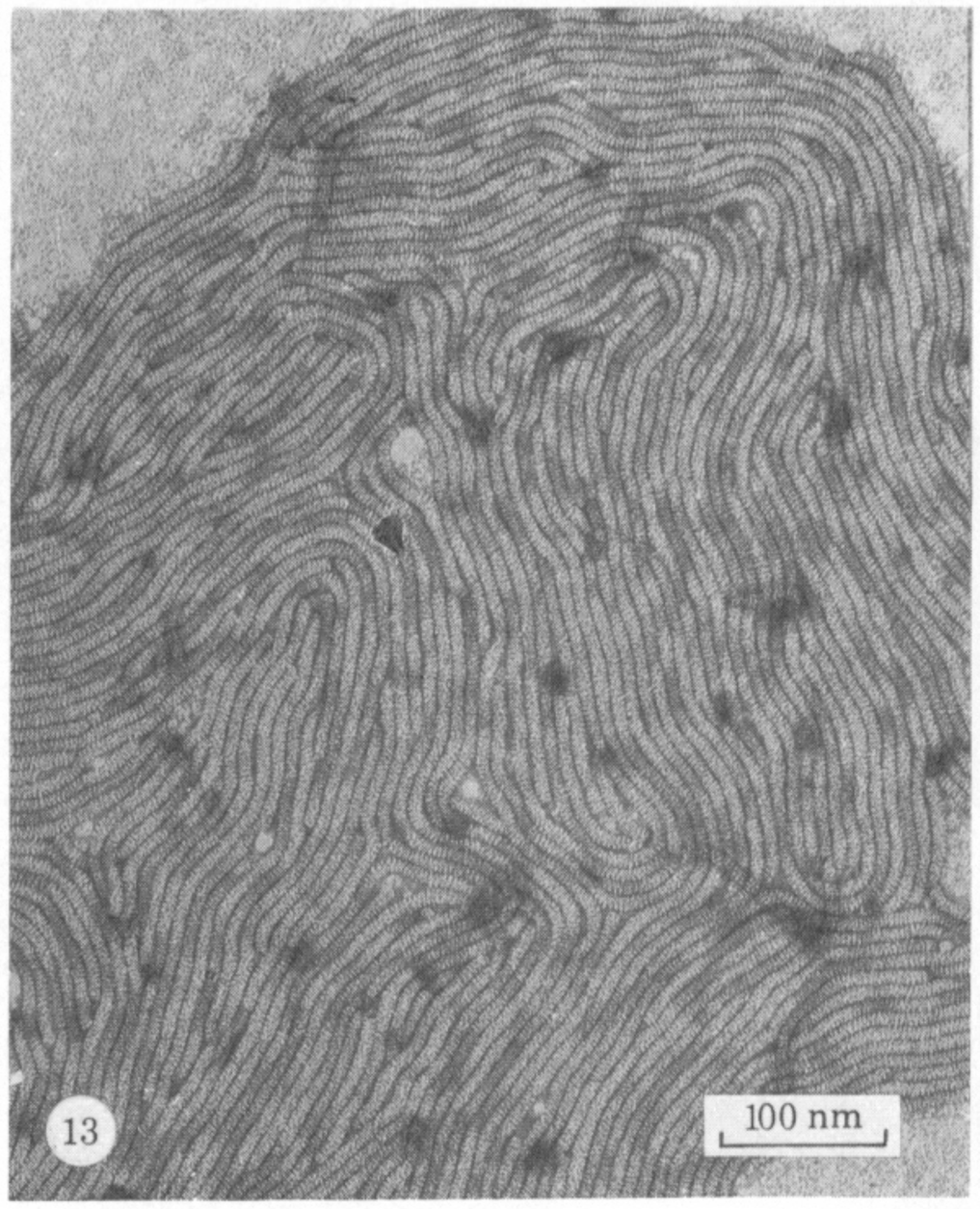


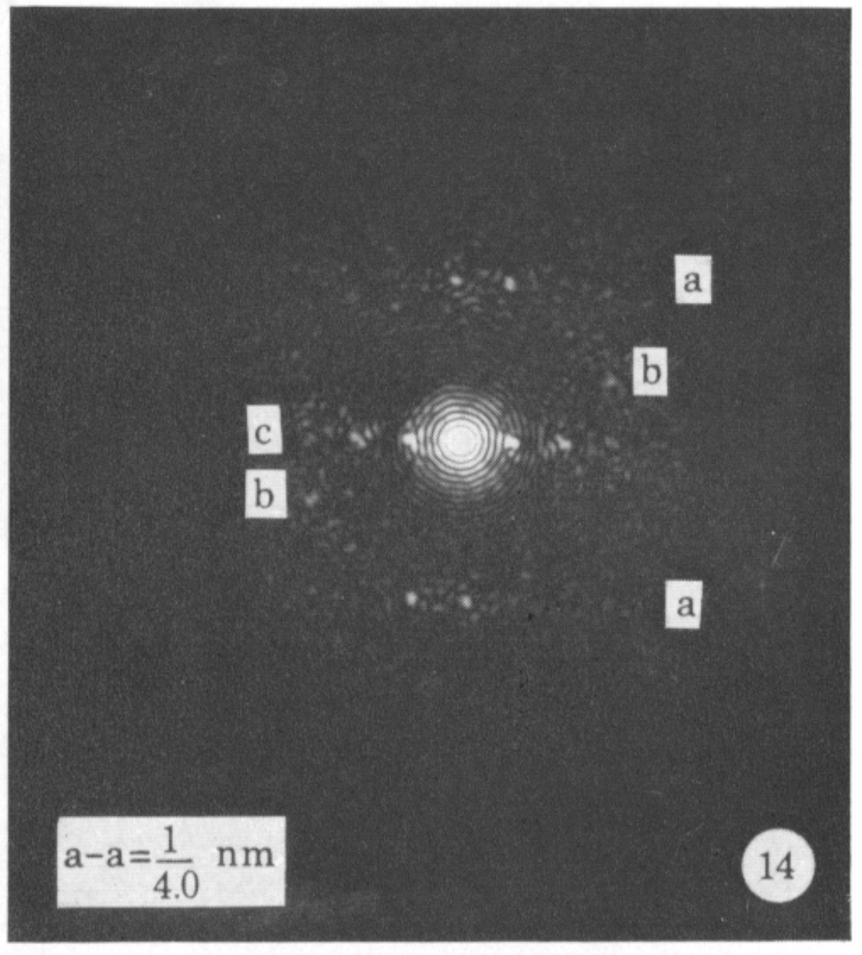


FIGURES 10–14. For description see opposite.