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Crystalline glycoprotein cell walls of algae: their stucture, composition and assembly

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[Plates 46–53]

Cell walls from various algae within the Chlamydomonaceae display, when negatively stained and examined in the electron microscope, a crystalline lattice component. On the basis of the Fourier transforms of micrographs of the cell wall, the algae have been classified into five classes. Most of the algae examined fall into class II. The two-dimensional repeating morphological unit cell of the wall of each class is described and in each case is a parallelogram whose overall dimensions are all of the same order of magnitude. The implications of the classification for classical taxonomy are discussed. More detailed structural information, to a resolution of about 2.5 nm (based on optical filtering and image enhancement techniques), is presented for two of the cell wall classes. From the evidence of polyacrylamide gel electrophoresis, the cell wall of these algae is constructed primarily from a small number of high molecular mass glycoprotein species. In *Chlamydomonas reinhardi* it has been shown that the hydroxyproline residues in the wall glycoprotein are cross-linked to short oligosaccharides. Some of the conditions necessary for the complete *in vitro* reassembly of the cell wall of *C. reinhardi* are presented and include the presence of a discrete nucleating agent and the presence of a physical surface. Dislocations within the crystalline lattice of the cell walls are described and their role as growth points discussed.

1. Introduction

This paper deals with various aspects and implications of the discovery that numerous primitive single-celled green algae have a crystalline component in their cell walls. It continues a series of papers dealing with the correlated ultrastructural biochemical and genetical analysis of the cell wall of *Chlamydomonas reinhardi* (Roberts, Gurney-Smith & Hills 1972; Hills, Gurney-Smith & Roberts 1973) and extends the approach to various other species in an attempt to throw some light on the distribution, nature, and significance of crystalline cell walls in general and the factors which control their assembly. The prevailing assumption among plant cytologists appears to be that the cell walls of single-celled green algae – for example, *Chlamydomonas* spp. – are much the same as higher plant cell walls, perhaps somewhat simpler but certainly containing a microfibrillar cellulose component. It has emerged, however, during work on the cell wall of *Chlamydomonas* that this picture is now inadequate. Instead, a model is proposed of the cell wall which comprises several distinct layers, some of which form a highly ordered crystalline lattice composed of glycoprotein subunits (Roberts *et al.* 1972). The wall is thin, delicate, held together by non-covalent bonds, and contains no cellulose (Roberts *et al.* 1972; Hills *et al.* 1973; Davies 1972*a*). It would seem unlikely that such a specialized cell-wall type is unique to *Chlamydomonas reinhardi* and this speculation has prompted an examination of numerous related algae to try and discover the extent to which such walls occur, and their possible taxonomic and evolutionary implications. Descriptions of the various crystalline wall classes discovered will be presented and discussed together with thoughts on the nature of the processes involved in assembling such large highly ordered structures.

The available literature on the structure and chemistry of the cell walls of *Chlamydomonas* and related species is both sparse and contradictory. Any indications of regular periodicities or

crystallinity in the walls is even rarer. Sager & Palade (1957), Lewin (1952) and Barnett & Preston (1969) have all stated that the cell wall of *C. reinhardi* comprises a cellulose wall surrounded by a capsule, but do not provide convincing chemical evidence for such a description. Cellulose has also been implicated as the structural wall component in *C. moewusii* by Lewin, Owen & Melnick (1951) and by Brown *et al.* (1968); in *C. eugametos* by Lembi & Lang (1965) and Walne (1966); and in *C. dysosmus* by Lewin *et al.* (1951). Aronson, Klapprott & Lin (1969), however, found no chemical evidence for cellulose in *C. reinhardi*. It was not until more delicate methods of preservation became available that the wall of *C. reinhardi* was revealed to house a highly ordered crystalline lattice within it (Horne, Davies, Norton & Gurney-Smith 1971). Further work on this resulted in the detailed description of this lattice (Hills *et al.* 1973) and some information on its chemical nature (Roberts *et al.* 1972; Davies 1972*a*). A model emerged consisting of a lattice assembled from several discrete high molecular mass glycoproteins, and characterized by a very high concentration of hydroxyproline. Although this would appear to be contradicted by Gotelli & Cleland (1968), who found negligible hydroxyproline in the cell wall, two more recent papers support our findings (Taylor 1969; Miller, Lamport & Miller 1972). Miller *et al.* (1972) extend their work to cover the presence of hydroxyproline hetero-oligosaccharides in *C. gymnogama* and *Volvox carteri*.

Regular periodicities within the cell wall have been reported occasionally for members of the Volvocales. Lembi & Lang (1965), for example, describe regular striations in sections of *Carteria* sp. but find none in *Chlamydomonas* sp. Nakamura, Bray, Costerton & Wagenaar (1973, figure 2), although not describing it as such, show clear freeze-etch evidence for regular periodicities in the cell wall of *C. eugametos*. Using more refined techniques it is possible that further crystalline cell walls will be found in other algae.

The analysis of the *C. reinhardi* cell wall has proceeded along several lines, and has emphasized the suitability of this particular wall for studies on assembly processes. First, there is the highly ordered and defined nature of the wall, and the fact that it is made of a comparatively

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Description of plate 46

Figure 1. Cell wall of wild-type *Chlamydomonas reinhardi*, fixed by Franke *et al.* method (1969) and sectioned. (Magn. × 200 000.)

Figure 2. A similar section, but of the cell-wall mutant CW2. Layers 1 and 7 are missing, but in negative stained preparations the lattice structure is still present (Magn. × 200 000.)

Figure 3. A drawing showing the seven layers of the wild-type cell wall which have been described (Roberts *et al.* 1972).

Figure 4. A cell wall of wild-type *Chlamydomonas reinhardi* negatively stained in ammonium molybdate to reveal the crystalline lattice structure. (Magn. × 225 000.)

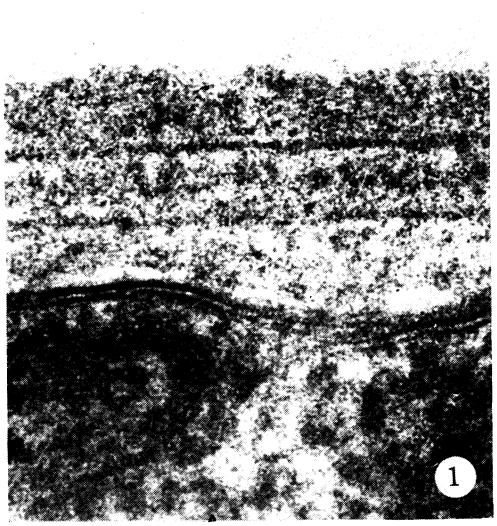
Figure 5. The Fourier transform derived from the micrograph shown in figure 4.

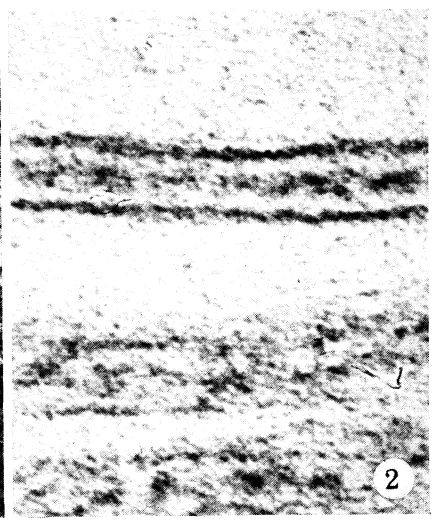
Figure 6. A reconstituted image of the cell wall of *Chlamydomonas reinhardi*, produced on an optical diffractometer, and allowing all the main spots of the Fourier transform (figure 5) to pass through the mask and reform the image. (Magn. 560 000.)

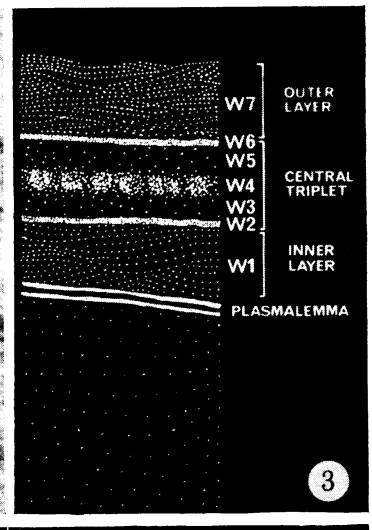
Figure 7. The Fourier transform of the reconstituted image of the cell wall shown in figure 6, showing the diffraction spots used to reconstruct the image.

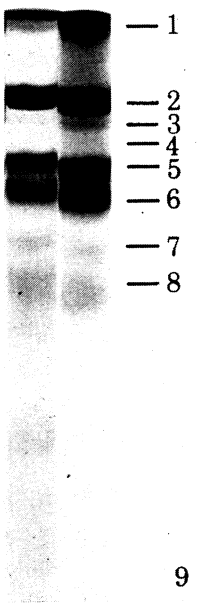
Figure 8. A drawing showing the suggested two-dimensional projection of the ordered lattice within the cell wall of *Chlamydomonas reinhardi*, based on optically filtered images and linearly integrated images.

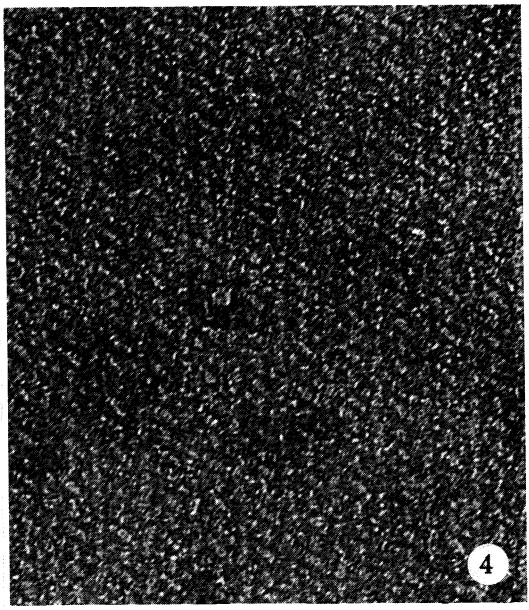
Figure 9. Polyacrylamide/SDS gel electrophoresis of purified wild-type cell walls of *Chlamydomonas reinhardi*. The left-hand gel has been stained for protein with Coomassie Blue and the right-hand gel for carbohydrate using the periodic/Schiff method. The numbering of the gel bands adopted is shown.

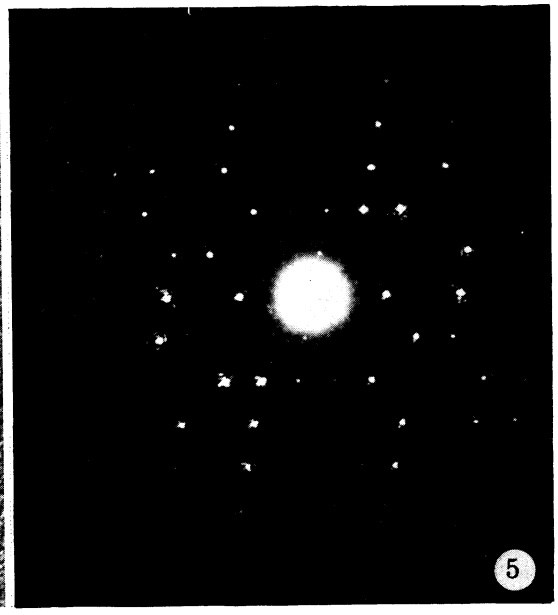


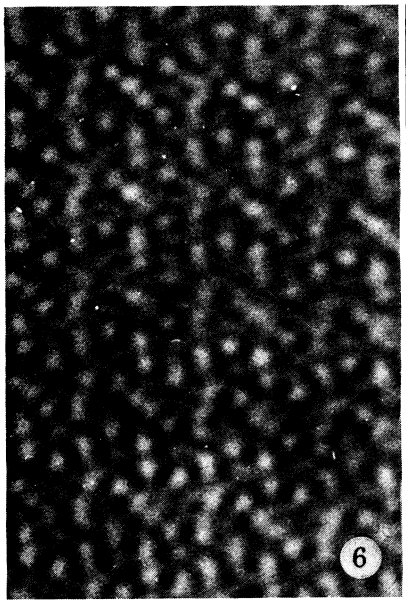


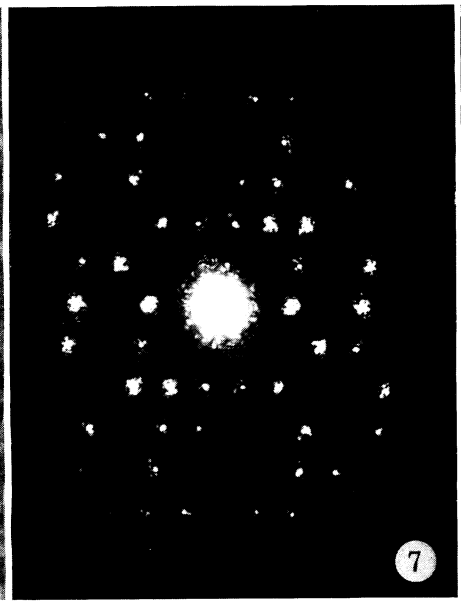


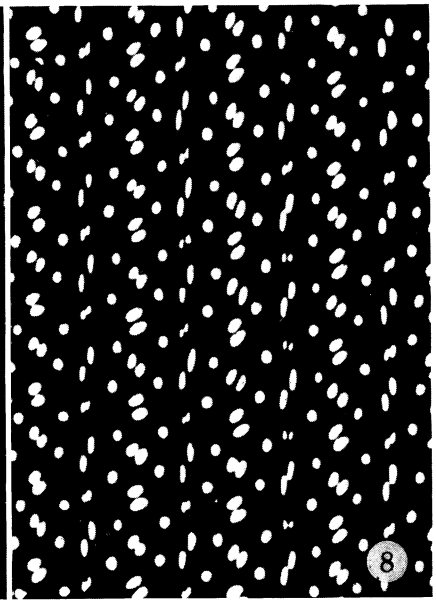












FIGURES 1–9. For description see opposite

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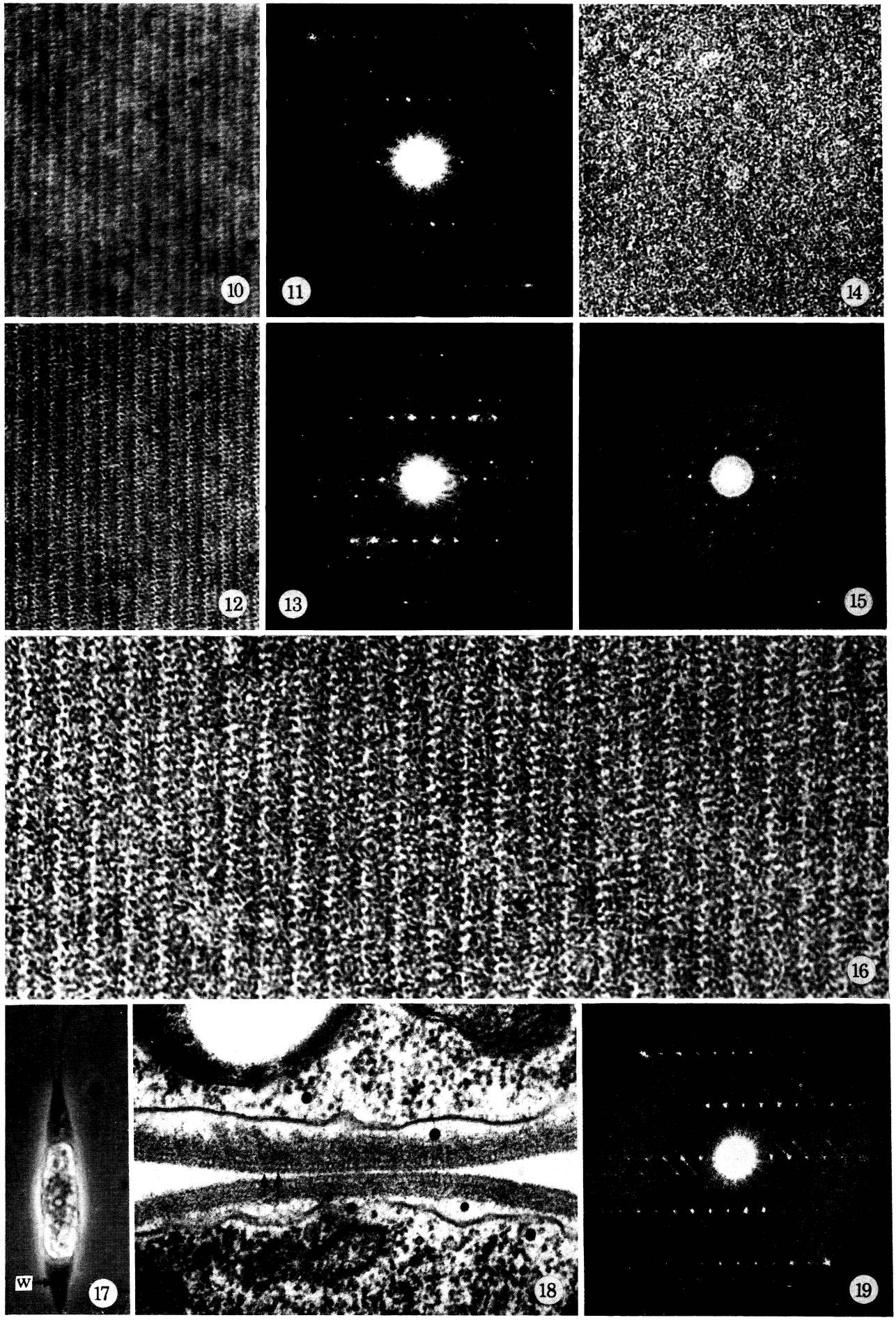

FIGURES 10–19. For description see opposite

small number of macromolecular building blocks (Roberts *et al.* 1972; Hills *et al.* 1973; Davies 1972*a*). Second is the availability of numerous cell-wall-defective mutants, a detailed analysis of which has shown both that single gene differences distinguish most of them from the wild type and that the situation is complicated by the involvement of an extra-nuclear system of control (Davies & Plaskitt 1971; Hyams & Davies 1972; Davies 1972*b*, 1973). Lastly, the facts that the cell walls are easily and quickly purified in a clean state, and that *in vivo* the wall is assembled extracellularly, make the chances of successful *in vitro* reassembly experiments more likely.

2. The cell wall of *Chlamydomonas reinhardi*

The wall lattice

When a cell of *Chlamydomonas reinhardi* undergoes cell division the flagella retract and the protoplast divides to form four new daughter protoplasts within the mother cell wall. Each of these daughters then develops a complete new cell wall around itself, complete with two specialized 'collars' through which the two new flagella will protrude. The old mother-cell wall then splits, releasing the four daughter cells. After several cell generations a liquid culture will contain many of these discarded cell walls, and this fact enables very clean cell-wall preparations to be obtained by differential centrifugation. Two-week-old cell suspensions grown on YAP medium (Davies & Plaskitt 1971) were spun in 250 ml bottles at 8000*g* in a Beckman J. 21 centrifuge for 30 min. At this speed the whole cells form a hard pellet and the residual walls formed a white fluffy layer on the top. This was carefully pipetted off and washed several times. As no cell breakage is involved, negligible contamination from cell debris occurs. Walls prepared by this method have been shown to be identical to those prepared by more elaborate Mickle disintegration methods, by both sectioning and gel electrophoresis.

Preparations of cell walls examined using negative stains have revealed that a complex highly ordered lattice is a constant feature of the wall (figures 4, 5). This lattice can only be

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Description of plate 47

Figure 10. The cell wall of *Chlorogonium elongatum*, negatively stained in ammonium molybdate. The micrograph is taken at the in focus position on the microscope. (Magn. × 160000.)

Figure 11. The Fourier transform of the micrograph shown in figure 10.

Figure 12. The same object as in figure 10, but here the micrograph is taken 2500 nm underfocus. (Magn. × 160000.)

Figure 13. The Fourier transform derived from the micrograph shown in figure 12. Notice the differences in intensity of the diffraction spots, which all lie on the same reciprocal lattice.

Figure 14. The cell wall of *Chlamydomonas ulvaensis* negatively stained in ammonium molydbate. (Magn. × 180000.)

Figure 15. The Fourier transform derived from the micrograph shown in figure 14. The similarities to the basic transform of *C. reinhardi* (figure 5) may be seen.

Figure 16. The cell wall of *Chlorogonium elongatum* negatively stained in ammonium molybdate. The high degree of order within the wall is apparent. This is a class II wall. (Magn. × 310000.)

Figure 17. A phase-contrast light micrograph of a living *Chlorogonium elongatum*. The long pointed cell wall (W) may be seen, projecting at either end beyond the limits of the cell itself.

Figure 18. A section showing the cell wall of *Chlorogonium elongatum*. The wall is thinner than that of *Chlamydomonas reinhardi*. Some indication of periodicity within the wall (→) may be detected. (Magn. × 110000.)

Figure 19. The Fourier transform derived from the micrograph shown in figure 16. This shows the typical reciprocal lattice of class II walls.

preserved by using ammonium molybdate or sodium tungstate as negative stains. Other stains – for example, potassium phosphotungstate – appear to destroy the lattice (Roberts *et al.* 1972). Rotary shadowed carbon replicas of the cell surface have also revealed the presence of the lattice, and the relationship between the images obtained by both methods has been discussed by Hills *et al.* (1973).

Numerous fixatives were tried in order to preserve the lattice and it was found that only the fixative described by Franke, Krien & Brown (1969) enabled the lattice to be subsequently revealed by negative staining. Sections from material fixed in this way showed the wall to have a seven-layered structure (figures 1, 3). Some mutants, e.g. CW 2, although only possessing the central triplet layers of the wall, still show the presence of lattice in negative stain, thus localizing the crystalline wall component to this region (figure 2). A full description of the wall, deduced from sections, has already been published (Roberts *et al.* 1972), and a detailed analysis of the optical transforms of micrographs of negatively stained walls is described by Hills *et al.* (1973). Using the techniques of image reconstruction (Horne & Markham 1972) and linear integration (Hitchborn & Hills 1968) enhanced images of the lattice were obtained (Hills *et al.* 1973) which enabled a detailed two-dimensional model of the unit cell to be derived (figures 6–8), to a resolution of about 2.5 nm. The repeating morphological unit of the model can be described as a parallelogram, composed of several discrete components, with sides of 28.5 nm and 23.6 nm and an angle of 80°. It has been shown by sectioning work that the central triplet, housing the lattice layer, can be solubilized by sodium dodecyl sulphate (SDS), and that the resultant solution can be separated into several distinct components by electrophoresis on polyacrylamide SDS gells (Davies 1972*a*; Roberts *et al.* 1972; Hyams 1972). Wild-type cell walls run on such gels reveal eight glycoprotein components of which four are more distinct (bands 1, 2, 5 and 6 in figure 9) than the others. A more detailed electrophoretic analysis appears in a subsequent section.

Chemical composition

Several samples of purified cell wall have been analysed for amino acid composition in a Beckman Spinco Model 120 amino acid analyser. The percentage of protein in the cell wall is about 30% and hydroxyproline accounts for between about 10 and 14% of the amino acids, depending on the sample. The amino acid composition of a typical sample of cell walls, together with that of wall glycoproteins purified by solubilization in 8 M lithium chloride solution (see section on reconstitution) is given in table 1. Purified cell walls have also been analysed by

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Description of plate 48

Figure 20. A phase-contrast light micrograph of a living cell of *Chlorogonium euchlorum*. The cell itself is fatter than *C. elongatum*, but the wall shows the same sharply pointed ends.

Figure 21. A section of the cell wall of *Chlorogonium euchlorum*. (Magn. × 120000.)

Figure 22. The Fourier transform of the micrograph shown in figure 23 (class II).

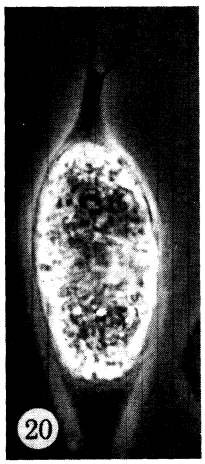
Figure 23. A cell wall of *Chlorogonium euchlorum* negatively stained in ammonium molybdate (class II). (Magn. × 180000.)

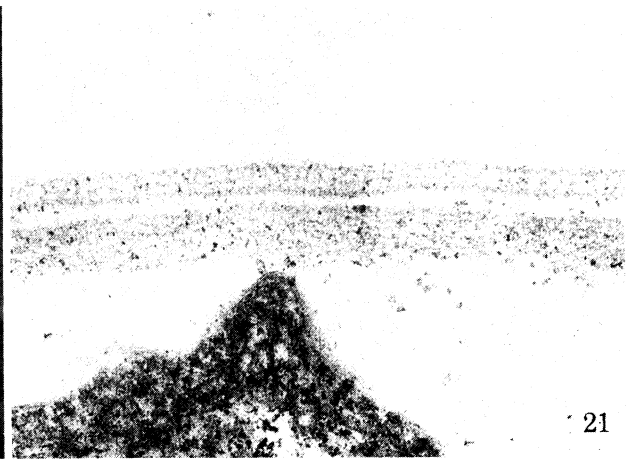
Figure 24. A cell wall of *Polytoma uvella*, negatively stained in ammonium molybdate (class II). (Magn. × 120000.)

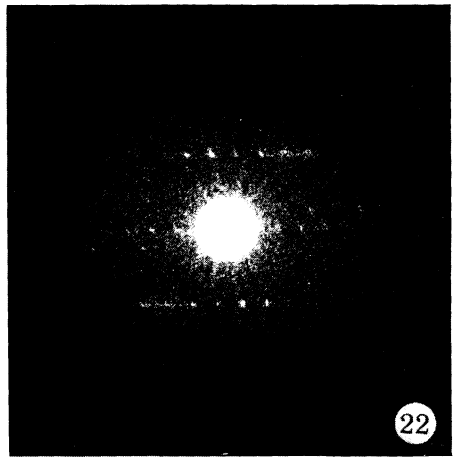
Figure 25. A section of the cell wall of *Polytoma uvella*. (Magn. × 90000.)

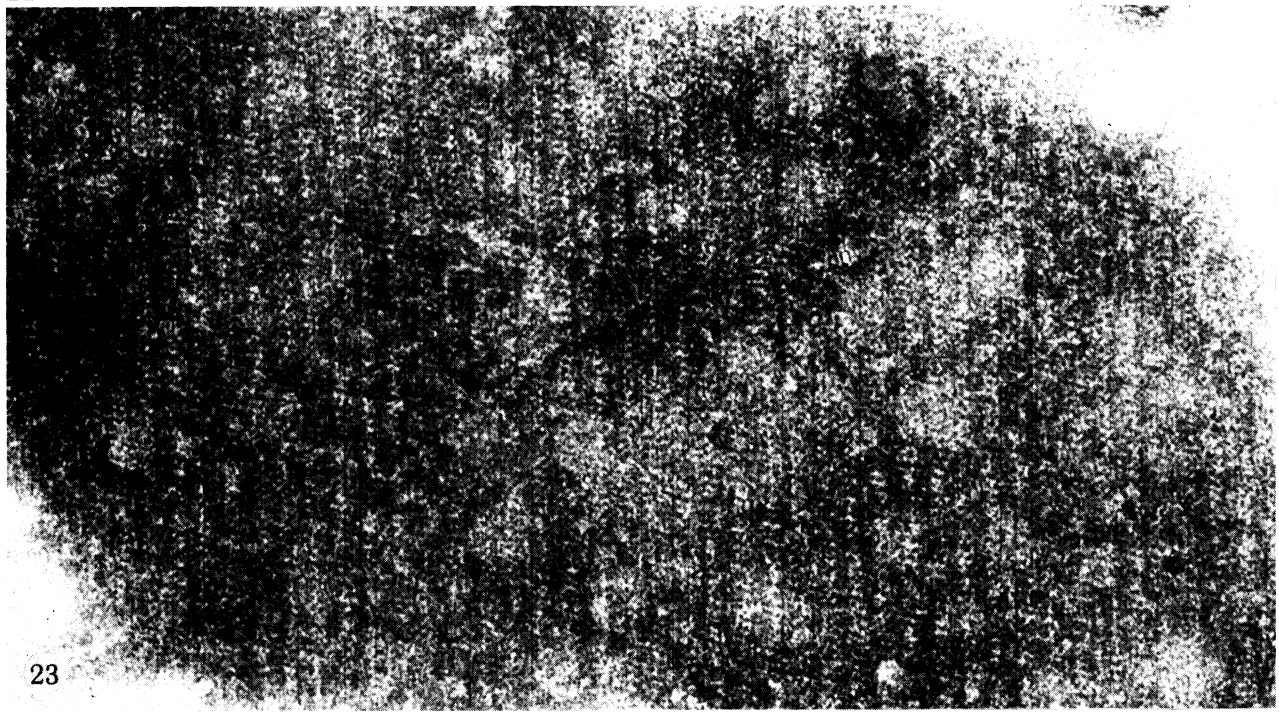
Figure 26. A phase-contrast light micrograph of two living cells of *Polytoma uvella*. As in figures 17 and 20, the gap between the cell wall and the cell is clearly visible.

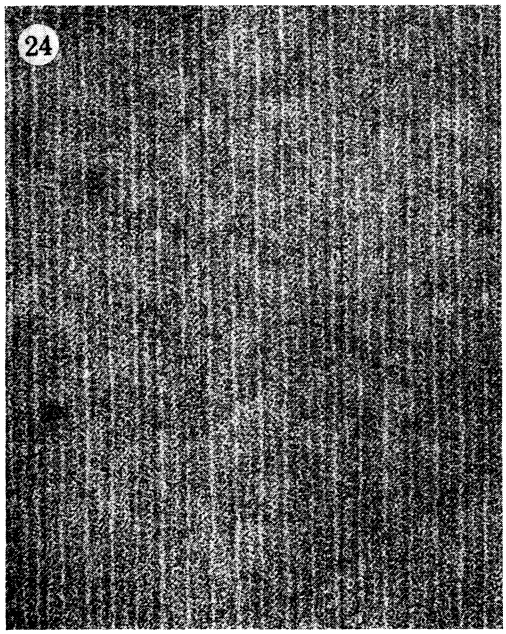
Figure 27. The Fourier transform derived from the micrograph shown in figure 24 (class II).

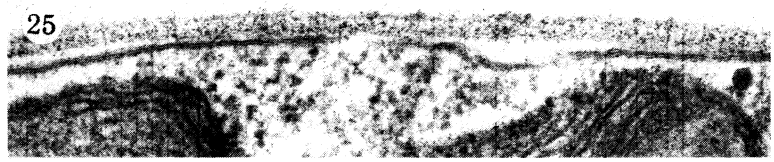


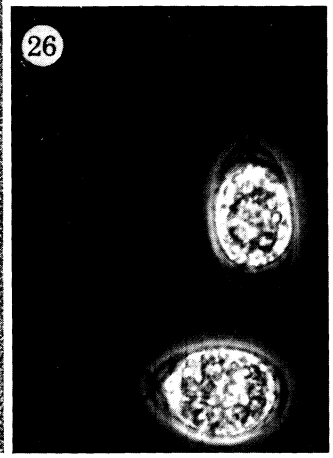


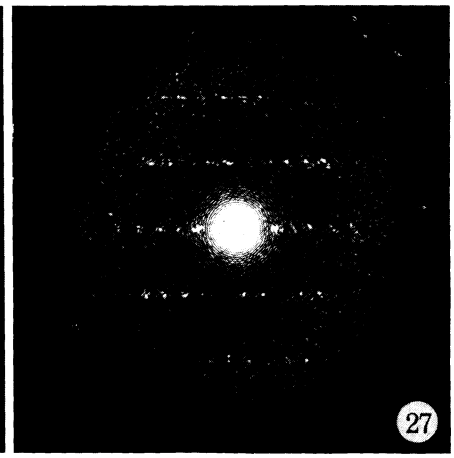












FIGURES 20–27. For description see opposite

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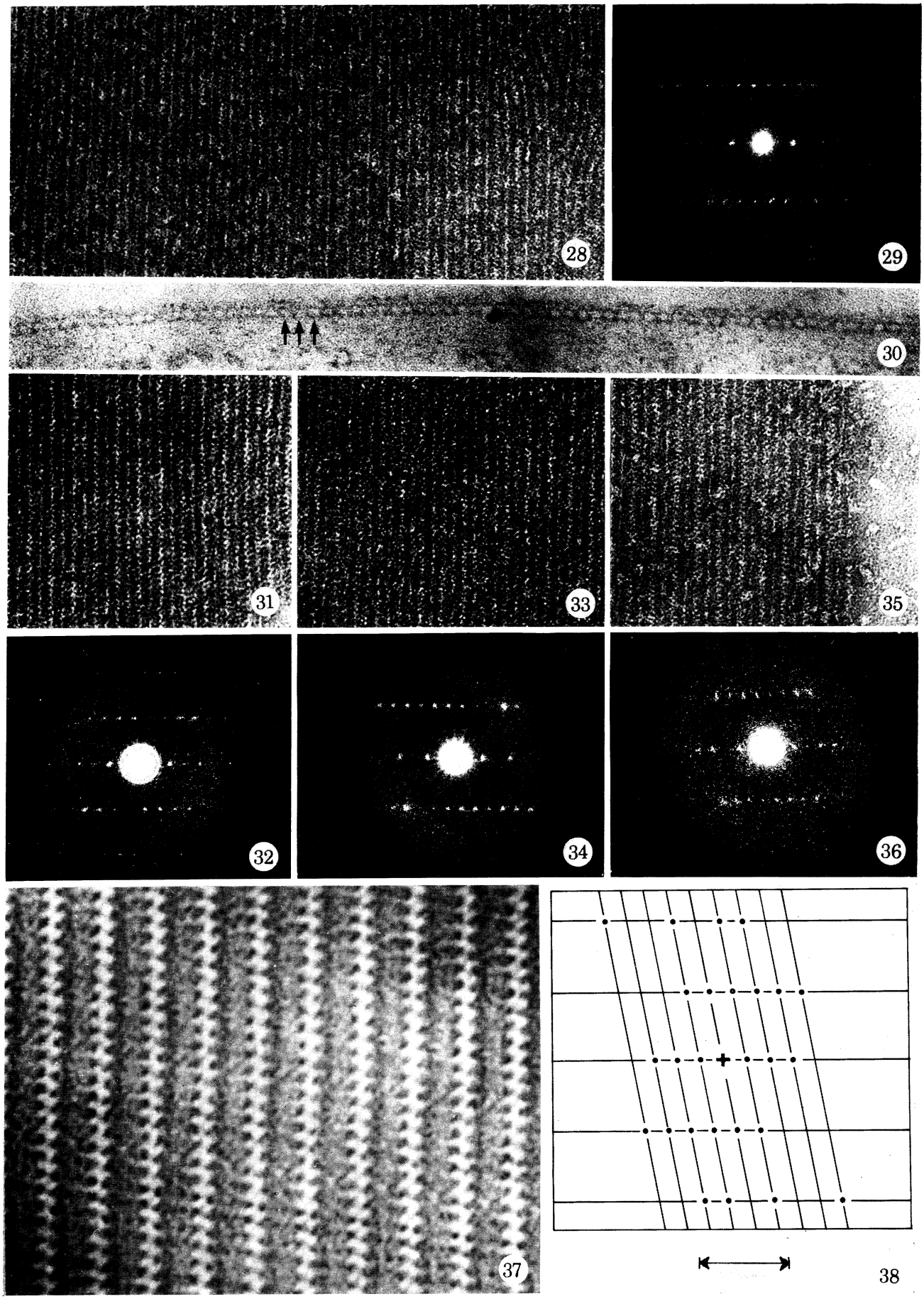

FIGURES 28–38. For description see opposite

paper chromatography for the sugars present in the cell wall (Roberts *et al.* 1972). Qualitatively, it was found that arabinose and galactose were the major sugars, with mannose in smaller quantities, and either xylose or fucose as a constant trace component. The sugar composition has been checked by gas – liquid chromatography of the trimethylsilated derivatives. Arabinose, galactose and mannose, the main constituents, accounted for 47% of the wall by mass.

TABLE 1. AMINO ACID COMPOSITION OF *CHLAMYDOMONAS REINHARDI* CELL WALLS (% BY MASS)

amino acids	8 M LiCl soluble material	clean walls	amino acid	8 M LiCl soluble material	clean walls
Lys	4.43	4.36	Ala	5.77	6.80
His	—	0.74	Val	5.50	5.65
Arg	4.17	5.77	Met	1.17	1.83
Asp	10.00	9.77	Isoleu	3.32	3.02
Thr	6.67	6.17	Leu	6.58	7.52
Ser	6.30	6.91	Tyr	4.61	4.12
Glu	6.83	7.90	Phe	5.16	5.81
Pro	7.32	5.91	Hyp	18.05	13.42
Gly	4.15	4.30			

Details of this are shown in table 2. Using the figures given for the whole cell walls it can be shown that for every mole of hydroxyproline present there are 1.9 mol of mannose. This is sufficiently near 2 to speculate that the molar ratios of hydroxyproline/mannose/arabinose/galactose are 1:2:3:4. As it is known that nearly all the hydroxyproline is covalently bonded to arabinose or galactose (Roberts *et al.* 1972), we can say that the maximum average degree of

TABLE 2. THE SUGAR COMPOSITION OF THE WILD-TYPE CELL WALL OF *CHLAMYDOMONAS REINHARDI*

sugar	molar ratio	% by mass
mannose	2	10.6
arabinose	3.2	14.1
galactose	4.2	22.5

DESCRIPTION OF PLATE 49

FIGURE 28. The negatively stained cell wall of *Chlamydomonas moewusii* (class II) and its Fourier transform (figure 29). (Magn. × 150000.)

FIGURE 30. The cell wall of *Chlamydomonas moewusii* showing the regular periodicities (→) along its length. (Magn. × 110000.)

FIGURE 31. The negatively stained image of the cell wall of *Chlamydomonas dysosmos* (class II) and its Fourier transform (figure 32). (Magn. × 150000.)

FIGURE 33. *Chlamydomonas dorsoventralis*, its negatively stained cell wall and the derived Fourier transform (figure 34). A class II wall. (Magn. × 150000.)

FIGURE 35. The cell wall of *Chlamydomonas eugametos*, negatively stained (class II). (Magn. × 150000.)

FIGURE 36. The Fourier transform derived from figure 35.

FIGURE 37. A micrograph of the negatively stained cell wall of *Chlorogonium elongatum*, that has been linearly integrated along the main line of periodicity. The repeating units that form parallel rows may be clearly seen. (Magn. × 470000.)

FIGURE 38. The reciprocal lattice of the two-dimensional projection of the cell wall of *Chlorogonium elongatum* (class II), showing the main spots that are seen on the selected area electron diffraction pattern from the in focus cell wall. Scale represents 5 nm.

polymerization of the oligosaccharide side-chains of the glycoprotein is 9. (If the mannose is a separate component the figure will be correspondingly lower.) It is of interest that a similar size of oligosaccharide side chain, of arabinose and galactose, is found in the hydroxyproline containing protein of higher plant cell walls (Heath & Northcote 1973).

3. Crystalline cell walls in other green algae

(*a*) *Methods and distribution*

In an effort to see how widely distributed such labile crystalline glycoprotein cell walls are, a large number of algae from the N.E.R.C. Culture Collection of Algae and Protozoa, Cambridge, have been examined. Initially, small samples of cells were disintegrated with glass beads and the resultant material examined in the electron microscope using 5% ammonium molybdate or 5% sodium tungstate (adjusted to pH 6.5 with formic acid) as negative stains. Some of the species which revealed crystalline walls by optical diffraction of the micrographs have been grown up in liquid culture, and wall preparations examined in more detail by both electron microscopy and gel electrophoresis. Electron microscopy was carried out using an A.E.I. EM 6B or a J.E.O.L. JEM 100B electron microscope. Calibration of wall parameters was by the method described in Hills *et al.* (1973) using catalase crystals.

It was found that all the species examined, which showed crystalline cell wall components, fall in the Chlamidomonadineae, a suborder of the Volvocales (Fritsch 1948). On the basis of the Fourier transforms of micrographs of the cell walls it has been possible to classify the crystalline walls into five main classes. These five classes will be described in detail in the next section. Table 3 shows the algae which have been examined and the class to which they have been assigned. Table 4 shows those algae examined in which an ordered cell wall could not be found. It should be pointed out that it is possible that more work, using other stains for example, may reveal lattices within the walls of even some of these algae. *Haematococcus lacustris*, for example, is a likely candidate, as another species of *Haematococcus* has already been shown to contain a lattice (table 3).

The rationale on which the classification of the crystalline wall types discovered is based requires a few words of explanation. In all cases the Fourier transform of the electron micrograph is used. It is possible to arrange the diffraction spots of the Fourier transform on a regular grid and it is the dimensions and angles of this basic grid that have been used to determine

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Description of plate 50

Figure 39. The negatively stained cell walls of *Chlamydomonas asymmetrica*. That this image is of two cell walls placed or folded back to back in register may be seen from its Fourier transform (figure 41). (Magn. × 150000.)

Figure 40. A single piece of cell wall of *C. asymmetrica*, negatively stained. This is a class III cell wall. (Magn. × 150000.)

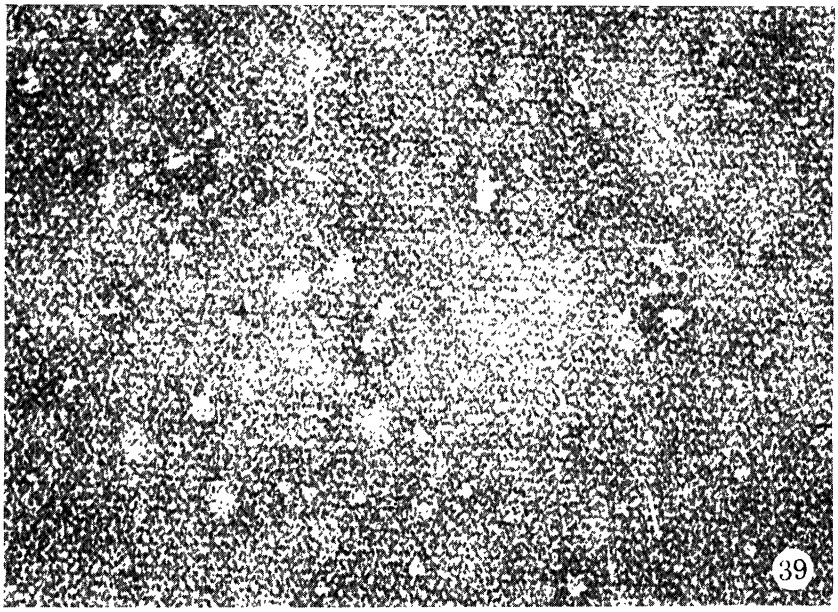
Figure 42. The Fourier transform of a class III wall, derived from the micrograph shown in figure 40.

Figure 43. A key to the reciprocal lattice of the two-dimensional projection of the cell wall of *C. asymmetrica* (class III), showing some of the major diffraction spots usually found on it. Scale represents 5 nm.

Figure 44. The negatively stained cell wall of *Chlamydomonas angulosa*, a class IV wall. (Magn. × 150000.)

Figure 45. The Fourier transform derived from the micrograph shown in figure 44.

Figure 46. A key to the reciprocal lattice of the two-dimensional projection of the cell wall of *Chlamydomonas angulosa* (class IV), showing the main diffraction spots encountered on it. Scale represents 5 mn.



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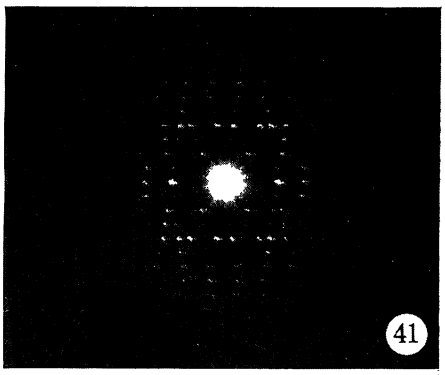

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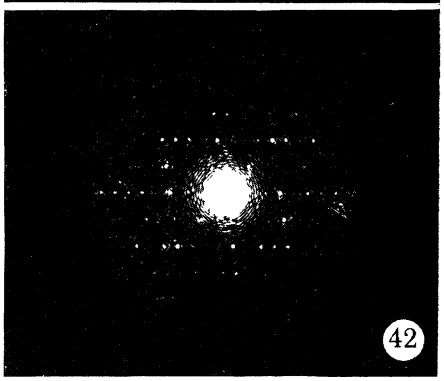

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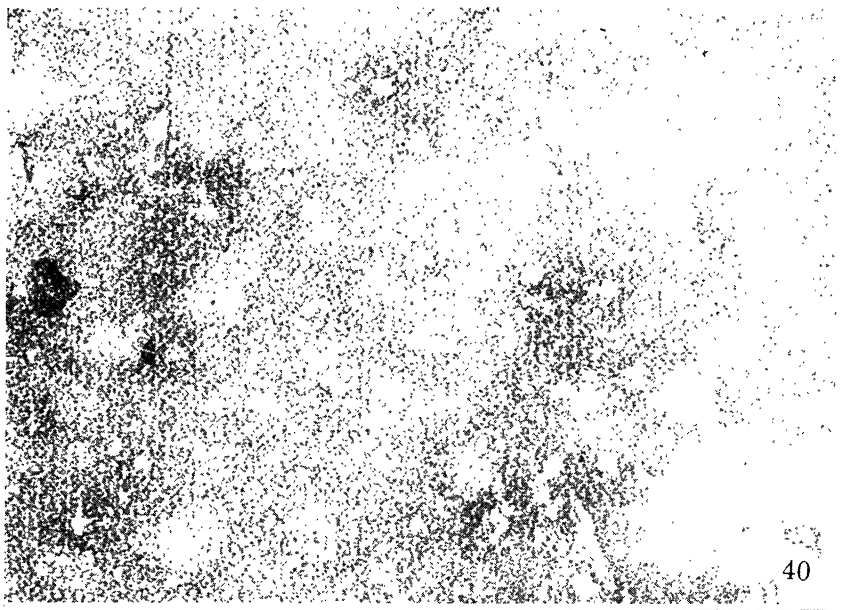

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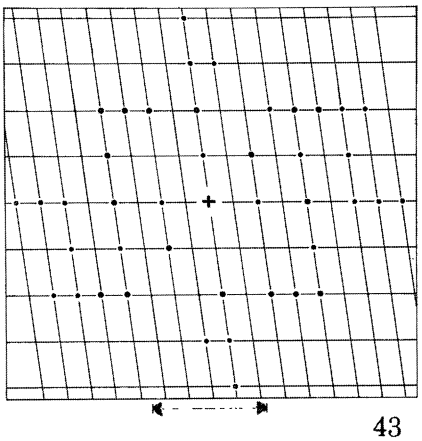

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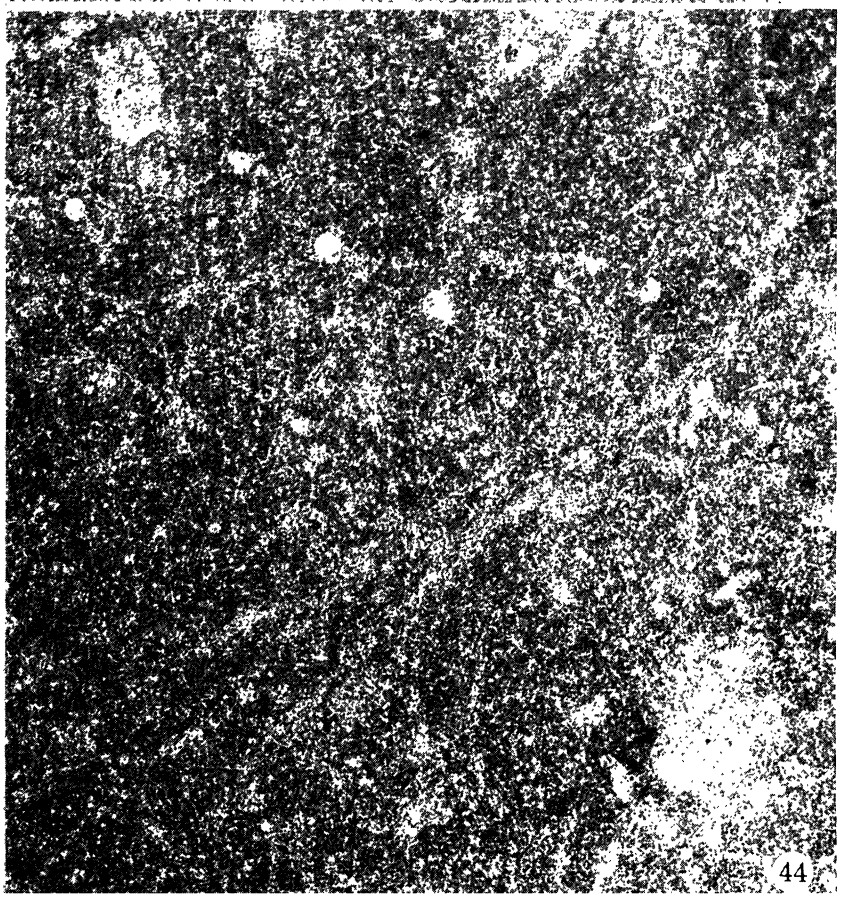

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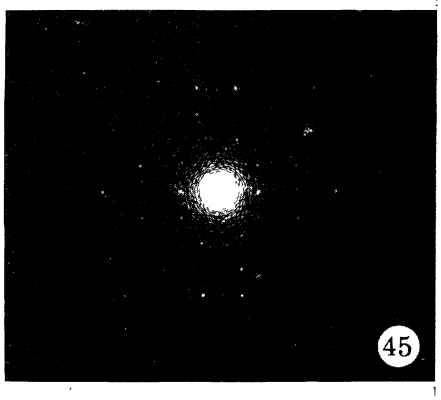

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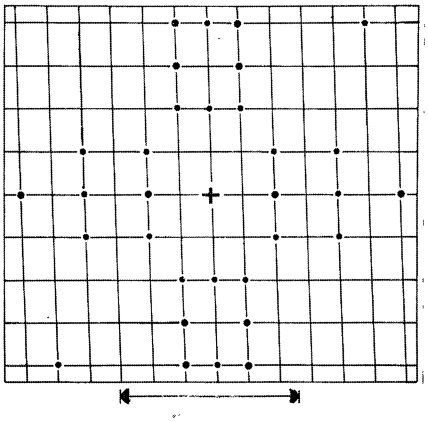

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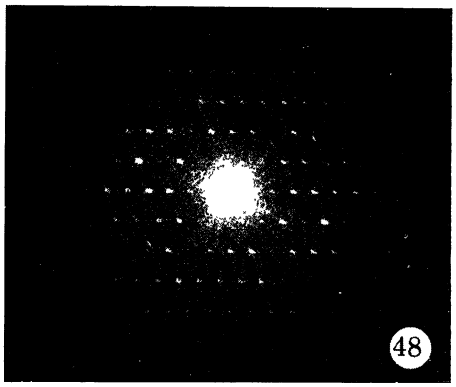

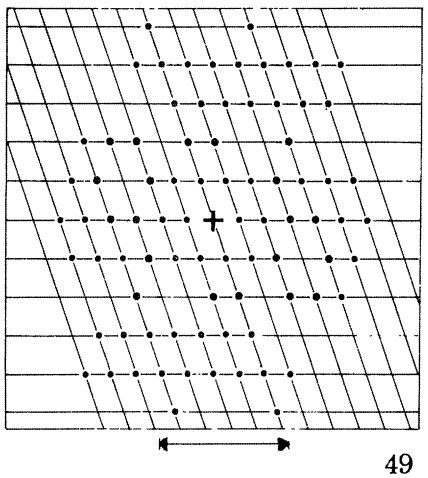

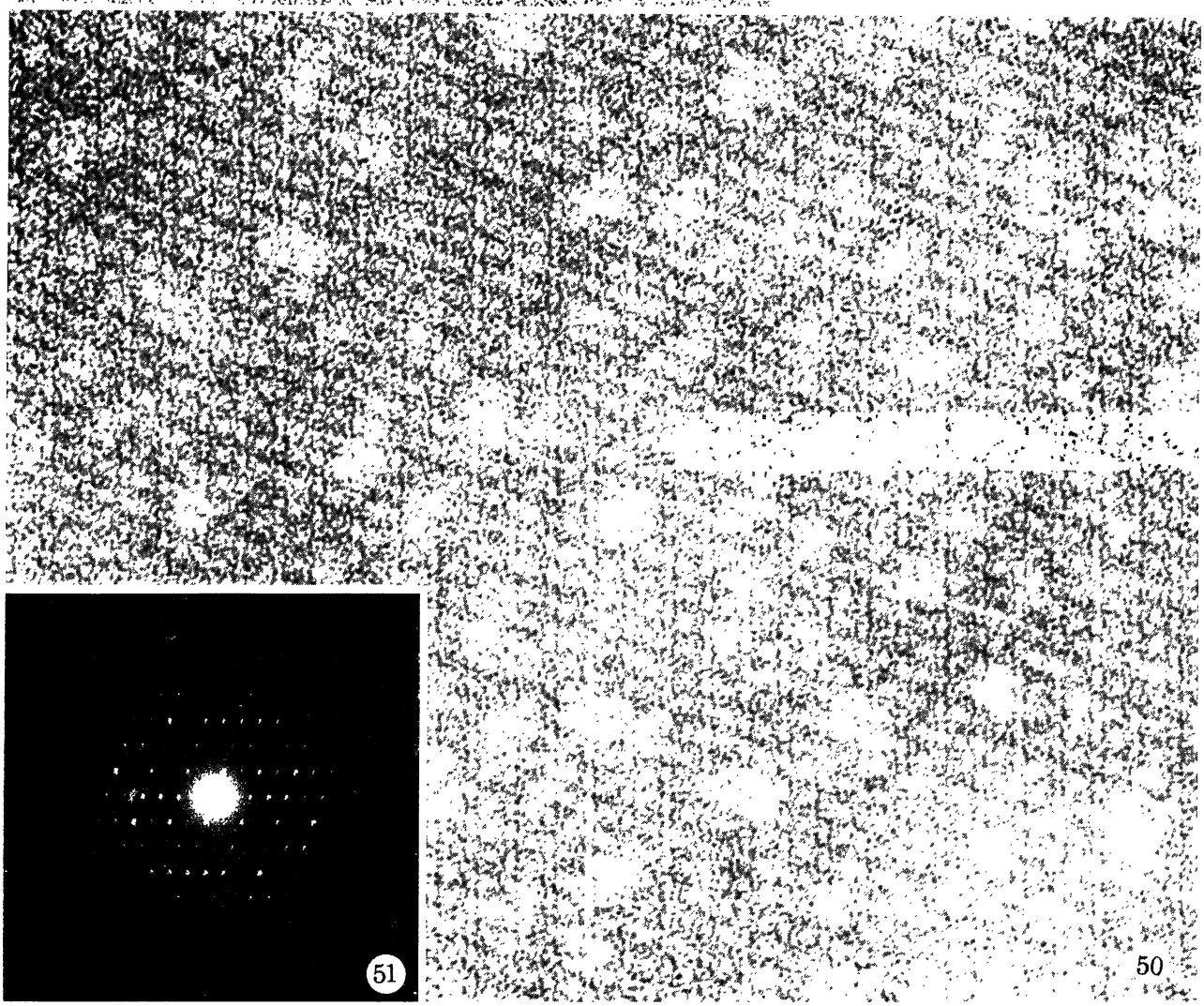

FIGURES 47–51. For description see opposite

whether two walls are identical or not. Great care is needed, as has already been demonstrated for *Chlamydomonas* (Hills *et al.* 1973), that a single area of cell wall is chosen for diffraction as mistakes can easily arise by multiple layers of wall, in particular those showing preferential stacking. It is possible that individual diffraction spots, lying on the intersections of the basic

TABLE 3. THE FIVE CLASSES OF THE ALGAE HAVING CRYSTALLINE CELL-WALL COMPONENTS

wall class	alga	Cambridge culture collection no.
I	*Chlamydomonas reinhardi WT*	
		11/32c
		11/32a
		11/45
	Volvox aureus	LB 88/16
Ia	*Chlamydomonas ulvaensis*	11/58
II	*Chlorogonium elongatum*	
	C. euchlorum	12/3
	Polytoma uvella	
	Carteria crucifera	8/7a
	C. eugametos	8/3
	C. incisa	LB 8/4
	Chlamydomonas moewusi	11/16f
	C. dorsoventralis	11/4
	C. eugametos	11/5c
	C. applanata	11/2
	C. shaerella	11/27
	C. rosae	11/66
	C. dysosmus	11/36a
	C. chlamydogama	11/48a
	Haematococcus capensis	LB/344b
	Brachiomonas submarina	7/1a
III	*Chlamydomonas asymmetrica*	11/41
IV	*Chlamydomonas angulosa*	11/59
V	*Chlamydomonas incisa* (= *Sphaerellopsis*)	11/10
	Lobomonas piriformis	45/1

grid, may show a very wide range of intensities from one picture to another. This may arise for two main reasons, one being the type and amount of negative stain present, the second being the precise degree of underfocus used in taking the original micrograph (Erickson & Klug 1971). This emphasizes that it is the basic grid on which the spots fall, and not which spots are present and at what intensity, that is really of value in clarifying wall types. Figures 10 and 12, for example, show the in-focus image of a negatively stained cell wall of *Chlorogonium elongatum*

DESCRIPTION OF PLATE 51

FIGURE 47. The negatively stained cell wall of *Chlamydomonas incisa* showing the appearance of a class V cell wall. (Magn. × 150 000.)

FIGURE 48. The Fourier transform obtained from figure 47.

FIGURE 49. A key to the reciprocal lattice of the two-dimensional projection of the cell wall of *Chlamydomonas incisa*, a class V wall, and the main diffraction spots usually found on it. Scale represents 5 nm.

FIGURES 50, 51. The negatively stained cell wall (Magn. × 210 000) of *Lobomonas piriformis* (class V) and the Fourier transform obtained from it (figure 51). Compare this with figures 47–49.

and the same object taken approximately 2500 nm under-focus (the sort of degree of underfocus at which one might take micrographs). Figures 11 and 13 show their respective optical transforms. These clearly show the variation in spot distribution that may be encountered over the same basic grid. The electron diffraction pattern of the in-focus object obtained using the selected area diffraction mode of the JEM 100B microscope has also been used to compare wall

TABLE 4. THOSE ALGAE EXAMINED IN WHICH A CRYSTALLINE WALL COMPONENT HAS NOT BEEN FOUND

alga	Cambridge culture collection no.
Polytomella caeca	LB/63/2a
Gymnodinium sp.	LB 1117/2
Pedinomonas tuberculata	LB 1965/2
Ochromonas sociabilis	933/3
Haematococcus lacustris	10/7
Gonium sociale	24/2
Euglena gracilis	1224/52
Dunaliella primolecta	11/34

types. Further, this electron diffraction pattern may assist in choosing the diffraction spots of the optical transform (e.g. of *Chlorogonium elongatum*) that should be allowed to reform the filtered image on the optical diffractometer. A more detailed discussion of this topic will be presented elsewhere (Roberts & Hills, in preparation). For convenience, all diffraction patterns are shown to the same scale in the figures.

(*b*) *The five provisional wall classes*

Class I

Chlamydomonas reinhardi has already been described and summarized in the previous section *Volvox aureus*, a multicellular chlamydomonad, is the only other species found so far to have a similar wall type. *Chlamydomonas ulvaensis* has a wall which, although having a Fourier transform displaying the same basic underlying grid, shows a consistent difference in the distribution of spots on it (figures 14 and 15). It has been temporarily assigned to a class I*a* until further work has clarified the nature of its relation with class I walls.

Class II

This class contains by far the greatest number of algae examined so far. The one chosen as the type organism is *Chlorogonium elongatum* (figures 16–19). This alga has a highly elongated, spindle-shaped cell wall, containing a more rounded protoplast within it (figure 17). Two

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DESCRIPTION OF PLATE 52

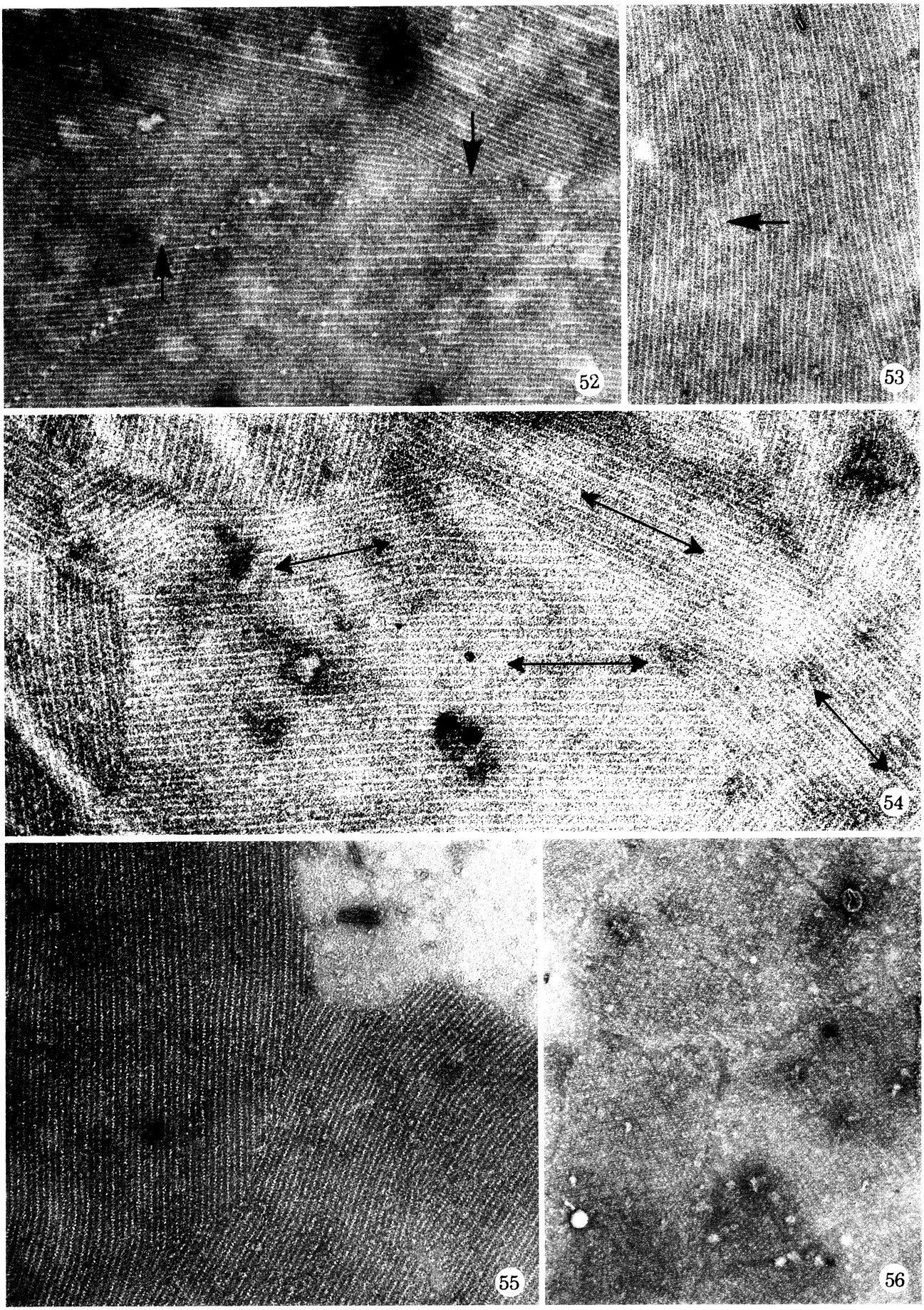
FIGURE 52. Local faults in the lattice structure within the cell wall of *Polytoma uvella* (→) (Magn. ×75000.)

FIGURE 53. As figure 52 (Magn. ×90000).

FIGURE 54. The cell wall of *Polytoma uvella* (class II) showing the abutment of numerous 'plate' like areas of lattice showing different orientations (↔). (Magn. ×90000.)

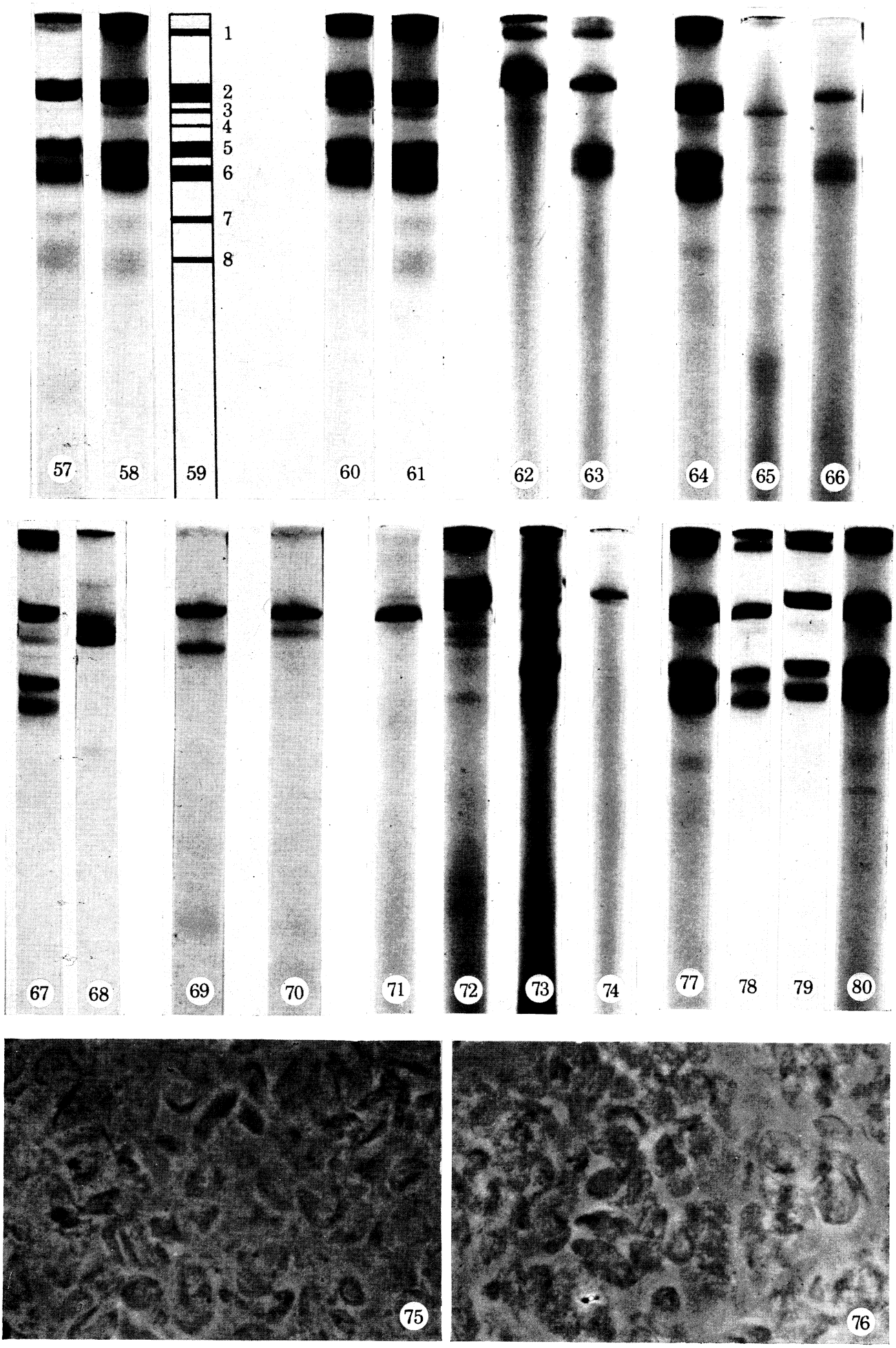
FIGURE 55. Dislocation within the cell wall of *Chlamydomonas dorsoventralis*. (Magn. ×90000.)

FIGURE 56. The cell wall of *Chlamydomonas incisa* shows numerous 'plate' like areas with 'open boundaries' between them (unlike figure 54). These may represent a growth phase of the cell wall, or it is possible that the wall may fracture on drying down in the negative stain. (Magn. ×51000.)



FIGURES 52–56. For description see opposite

(*Facing p.* 136)



FIGURES 57–80. For description see opposite

specialized regions, or collars, appear at one end of the wall, providing an exit for the two flagella. Other algae, although having the same crystalline wall lattice, show a completely different overall cell morphology (*Chlorogonium euchlorum* (figure 20) and *Polytoma uvella* (figure 26), for example, show two other cell types, both having class II cell walls). In sections of cells fixed using the method of Franke *et al.* (1969) the cell wall can be seen (figure 18) to have an outer component, occasionally showing periodicities, composed of at least two electron-dense layers separated by a less electron-dense layer. This component is about 35 nm in thickness, and carries on its inside face a more amorphous cell wall layer, again about 35 nm in thickness but more variable. Other algae having class II wall components do not necessarily show the same appearance in thin section. *Chlorogonium euchlorum* (figure 21) shows a highly defined outer 'triplet' layer, more comparable with the sectioned appearance of *Chlamydomonas reinhardi* and its 'central triplet' layer. *Polytoma uvella* seems to have a much thinner ($\sim$ 30 nm) and simpler

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Description of plates 53

FIGURES 57–74. Polyacrylamide/SDS gels prepared of different cell walls under various conditions. PAS indicates that the gel is stained with the periodic–Schiff method for carbohydrate and CB indicates that it is stained with Coomassie blue for protein. For running conditions see text.

FIGIRE 57. Wild-type *Chlamydomonas reinhardi* walls (PAS).

FIGURE 58. Wild-type *C. reinhardi* walls (CB).

FIGURE 59. Drawing to show the main bands obtained from *C. reinhardi* walls.

FIGURE 60. *C. reinhardi* walls digested at 37 °C for 1 min in 1% SDS before gels were run. Note multiple bands at position 2. (PAS.)

FIGURE 61. *C. reinhardi* walls digested at 100 °C for 1 min in 1% SDS before gels were run (PAS).

FIGURE 62. *C. reinhardi* walls, dissolved in 8 m LiCl solution, run without digestion in SDS (PAS)

FIGURE 63. As figure 62 but digested in 1 % SDS at 100 °C for 1 min before running. Note reappearance of bands 5 and 6. (PAS.)

FIGURE 64. *C. reinhardi* WT walls (PAS).

FIGURE 65. *C. reinhardi* 11/45 (PAS) cell wall.

FIGURE 66. *C. reinhardi* 11/32C (PAS) cell wall.

FIGURE 67. *C. reinhardi* walls (PAS).

FIGURE 68. *Chlamydomonas moewusii* cell walls (PAS).

FIGURE 69. *Chlorogonium elongatum* cell walls (PAS).

FIGURE 70. *Chlorogonium euchlorum* cell walls (PAS).

FIGURE 71. *Chlamydomonas dysosmos* cell walls (PAS).

FIGURE 72. *Chlamydomonas chlamydogama* cell walls (PAS).

FIGURE 73. *Lobomonas piriformis* cell walls (PAS).

FIGURE 74. *Carteria eugametos* cell walls (PAS).

FIGURE 75. Phase-contrast light micrograph of purified cell walls of wild-type *Chlamydomonas reinhardi*. They have been frozen and thawed and have lost their original purely spherical shape. (Magn. × 1000.)

FIGURE 76. The same batch of walls as shown in figure 75, dissolved in 8 M LiCl solution, may be recovered again by dialysis against water. The reassembled cell walls are shown here. (Magn. × 1000.)

FIGURES 77–80. Polyacrylamide gel electrophoresis of *C. reinhardi* cell walls. For conditions, see text.

FIGURE 77. Wild-type cell walls (PAS).

FIGURE 78. Wild-type walls dissolved in 8 M LiCl solution, spun at 15000 *g* for 6 min and supernatant run on gel (PAS).

FIGURE 79. Purified reassembled cell walls from an 8 M LiCl solution.

FIGURE 80. Wall fragments assembled by dialysis of 8 M LiCl digest of cell walls in the absence of any nucleating agent.

wall when seen in section (figure 25). *Chlamydomonas moewusii* is more like *Chlorogonium elongatum* but does show, when sectioned at the correct angle (figure 30), very marked evidence of the main periodicities seen in negative stained preparations. *C. moewusii* is unusual compared with the other members of class II in that the crystalline component of the wall is stable when negatively stained with uranyl acetate. Figure 16 shows the appearance of a fragment of cell wall of *Chlorogonium elongatum* negatively stained in 5% ammonium molybdate and figure 19 shows the derived Fourier transform. The diffraction spots fall at points on two layer lines forming an underlying grid having as its main component a parallelogram with an angle of 80° (figure 38). Using the methods of optical reconstruction and linear integration (figure 37) it was possible to derive a two-dimensional structural model of the arrangement of subunits within the lattice to a resolution of about 2.5 nm (Roberts & Hills, in preparation). The main repeating structural unit is a parallelogram of sides 21.5 and 7.0 nm and an angle of 80°. This model has been confirmed by work on computer phase-contrast image reconstruction (Gerchberg 1972) based on the electron diffraction pattern of the cell wall.† The essential feature of the wall lattice is the presence of stacked subunits forming parallel rows 21.5 nm apart.

All the other algae that have been placed in class II have cell walls which give Fourier transforms on the same basic grid. In other words, they have similar-shaped subunits aggregated in a similar manner. Selected examples of these other algae have been chosen for illustration. *Chlorogonium euchlorum* is shown in figures 20–23 and *Polytoma uvella* in figures 24–27. *Chlamydomonas moewusii*, *dysosmos*, *dorsoventralis* and *eugametos* are shown in figures 28–36. Although at higher magnification these walls all appear similar, at lower powers the walls display differences in their appearance on the grid. Two main differences are apparent. One is the size of the wall fragments encountered and the second is their method of folding down on the grid surface. The size of wall fragments varies enormously from the pieces found in *Chlorogonium elongatum*, which are sometimes as much as 10 μm across, to those obtained from *Chlamydomonas applanata* or *Chlamydomonas rosae*, which are rarely more than 500 nm across. Walls may either lie down flat during negative staining on the grid surface, or they may roll or fold to varying degrees, and this seems to be a function of the wall examined rather than the method of examination. Thus, *Chlorogonium elongatum* tends to show large flat single sheets of wall, while *Carteria crucifera* or *Chlamydomonas chlamydogama*, for example, exhibit a large degree of folding, making clear single areas of wall hard to locate. The degree to which the wall breaks up and/or folds may well be a function of the number and types of dislocations present in the wall lattice (wall dislocations are discussed in a subsequent section). Some of the algae which have been placed in class II show a variation in the *a* and *b* dimensions (table 5) which may reflect small variations in the glycoprotein monomers which form the lattice. For evidence on this point see the section on gel electrophoresis of wall components.

Class III

This class contains one alga; *Chlamydomonas asymmetrica*. The wall appears as small fragments with a readily visible crystalline structure. Fourier transforms of micrographs show, however, that the majority of fragments are in fact double structures, composed of two overlapping walls, This phenomenon is described for *Chlamydomonas reinhardi* by Hills *et al.* (1973). As in that case double walls tend to show a preferential orientation with respect to each other. Walls, back to

† This work, done in conjunction with Dr R. Gerchberg of the Cavendish Laboratory, Cambridge, will be published separately.

back and exactly in register, are very common (figures 39, 41). Only very rarely is an area found which displays the single Fourier transform (figures 40, 42). The diffraction spots again fall on a simple grid, or reciprocal lattice, the size of which shows that the repeating two-dimensional morphological subunit in the cell wall is a parallelogram with sides of 38.0 nm and 20.0 nm and an angle of 82° (table 5). It should be mentioned that in all walls examined the angle of the lattice may vary by about ± 2°.

TABLE 5. THE AVERAGE PARAMETERS OF THE TWO-DIMENSIONAL REPEATING MORPHOLOGICAL UNIT OF THE VARIOUS CRYSTALLINE WALL CLASSES

wall class	*a*/nm	*b*/nm	angle/deg
I	28.1	23.6	80
II	21.5	7.0	80
III	38	20	82
IV	28	21	88
V	25	16	71

Classes I and II have been calibrated from catalase crystals (Hills *et al.* 1973) and the other classes have been derived from these. The error in classes III–IV will therefore be correspondingly larger.

Class IV

This class again only has one algal species in it; *Chlamydomonas angulosa*. When negatively stained, smallish fragments of wall were seen which at first sight displayed no organized structure. Fourier transforms of the micrographs, however, revealed a faint but definite crystalline lattice. It did not appear to be present continuously over all the area of each wall fragment. Doubled over or stacked walls were commonly revealed upon diffraction analysis. The diffraction spots could again be placed on a simple grid, the reciprocal lattice, which revealed that the repeating structural morphological unit of the cell wall is a parallelogram of sides 28 and 21 nm (the variation among individual walls may be as much as 2 nm) and an angle of 88° (see table 5).

Class V

This class contains two algae: *Chlamydomonas incisa* and *Lobomonas piriformis*. Walls from *C. incisa*, when negatively stained, appear as a series of small plates or plaques, clearly showing lattice-like structure, interdigitating with one another. Very rarely are double areas or folded walls seen. The Fourier transform of the micrograph is very clear (figure 48) with spots falling on a reciprocal lattice which indicates that the repeating two-dimensional morphological subunit of the wall is a parallelogram, 25 × 16 nm, with an average angle of 71° (figure 49). The position with *Lobomonas* is more complicated as there is some doubt about its direct affinity with *Chlamydomonas incisa*. Negatively stained preparations show very clear evidence of the high degree of order present within the cell wall (figure 50) and the Fourier transform (figure 51) shows basically the same reciprocal lattice. However, slight variation of the *b* parameter (table 5) has been noticed and the distribution of spots on the lattice shows small but consistent variations. Also the wall itself does indeed look different, but this could be due to possible variations in other amorphous wall components obscuring the lattice. The decision whether or not these two algae have identical, or different but closely related, wall structures will await more detailed work involving focal series and optical reconstructions. At present they show such close affinities that they are classed together.

4. Dislocations in the cell wall

The cell walls of the algae discussed in the previous section are examples of biological surface crystals showing translational periodicity in two dimensions. Such spherical closed surfaces must of necessity show dislocations (Harris & Scriven 1970) and this has been demonstrated clearly in this case. The type and degree of dislocation varies from species to species. *Chlorogonium elongatum* is a very long thin cell, and has very few apparent dislocations. *Polytoma uvella*, on the other hand, has a wall showing numerous local defects (figures 52 and 53) as well as interdigitated plate like areas of wall lattice (figure 54). Other algae show similar faults in their walls. *Chlamydomonas dorsoventralis* (figure 55) and *Chlamydomonas incisa* (figure 56) are examples illustrated.

It is of considerable interest that walls from the same crystalline class can display specific differences in the pattern of dislocations found in their surface crystal. Harris & Scriven (1970) have suggested that the dislocations in closed surface crystals may act as the sites for the addition of new subunits. In other words, the essential growth of the cell wall by intussusception of new subunits may take place at the dislocations. They present a model for growth by the 'non-conservative climb' of dislocations. Local wedge disclinations are regarded as the source of and sink for, the dislocations required. This model would fit admirably as an explanation of the growth of the closed surface crystal that is the algal cell wall. It would seem likely, however, that the pattern of dislocations, although to some extent species specific and hence inherited, is a dynamic one, possibly varying greatly during the life of the cell. During the period of rapid growth, after the daughter cells leave the mother cell wall, one would expect a great number of dislocations acting as growth centres. In the stationary phase, when the wall expands no more, the number of dislocations could become minimal. Further work is planned to test this idea using *Chlorogonium* or *Polytoma*.

5. Cell wall analysis by polyacrylamide gel electrophoresis

Electrophoretic analysis of the cell wall of *Chlamydomonas reinhardi* has revealed the presence of at least eight SDS soluble glycoprotein components (Davies 1972*a*; Hyams 1972). These have been labelled 1–8, starting with the least mobile component on the gel (figure 57–59). It has been shown from work carried out on cell-wall mutants (Davies 1972*a*) that the glycoproteins are all derived from the central triplet (layers 2–6) of the wall which contains the crystalline lattice (figure 3). The work is extended in this paper to gain some more detailed information about the bands obtained from *Chlamydomonas reinhardi*, and to extend this to cover some of the other algae whose walls have been described. The methods used for polyacrylamide gel electrophoresis are identical to those described by Davies (1972*a*).

The first experiments performed were to clarify the relationship between the various bands seen on gels of *Chlamydomonas reinhardi*. Samples of cell walls were incubated for 1 min in buffered 1% SDS + 1% mercaptoethanol at 37, 60 and 100 °C to solubilize the wall components and were then run on gels. Those incubated at 60 and 100 °C show consistent bands as described previously, but those at 37 °C show that band 2 appears as three separate bands each of which stain for both carbohydrate and protein. This result (figures 60, 61) is hard to interpret. If one assumes that the 100 °C digestion is complete then one would expect more, not fewer, components. Alternatively, if the three bands are polymers then one would expect much slower

migration rates at 37 °C. All subsequent digestions were carried out at 100 °C. The amount of SDS present in the digestions is adequate for the concentration of walls used as ½% SDS and 2% SDS gave exactly the same results in these experiments. Experiments in which a wild-type wall preparation was incubated with and without SDS present, before running on the gels, gave patterns as shown in figures 62 and 63. Bands 5 and 6 only appear on the gel from material incubated with SDS. Here it can reasonably be assumed that the component in band 2 is far more readily released from the wall than the components in bands 5 and 6. It would seem unlikely that bands 5 and 6 could derive from the breakdown of a component in band 2 as their molecular masses would be too high. Assuming that the logarithm of the molecular mass of these glycoproteins is proportional to the distance migrated on the gel (see, for example, Weber & Osborne 1969), a rough estimate of the molecular masses of the wall components was made. Walls, digested at 100 °C for 1 min in 1% SDS, were run together with samples of bovine serum albumin (fraction V). This gave rough molecular masses for the main bands as follows: band 2, 295000; band 5, 215000; band 6, 185000. Further work is obviously required to get a more reliable estimate, but the general point is that we are dealing here with very large glycoprotein molecules. Gels were run on walls digested with and without 1% mercaptoethanol present. There was no difference between the number and amount of the bands, suggesting disulphide bonds to be unimportant in cross-linking. It has already been shown (Davies 1973) from experiments with the various cell-wall mutants that the presence of the cell-wall lattice is almost always accompanied by the presence of prominent bands 1, 2, 5 and 6 on gels. Two other cultures of *Chlamydomonas reinhardi* from different sources were obtained from the Culture Collection of Algae and Protozoa, Cambridge. These both proved interfertile with our wild-type strain. Neither produced walls, shed off in the medium during cell division, in very large quantities, suggesting the presence in these cultures of degradative enzymes. However, enough walls were obtained to show that they both had identical walls to our standard Class I *C. reinhardi*. These walls when run on polyacrylamide gels show a similar pattern, with, however, small but distinct differences (figures 64–66). Strains 11/32*a* and 11/32*c* show identical bands to each other, there being no difference between + and − mating type walls, but they show slower mobility than in our wild type. Strain 11/45 shows almost identical bands to our wild type. The exact number of distinct components that are required to build the lattice in the wall is still open to doubt, but we can say that the number is likely to be small. Bands 1, 2, 5 and 6 are very prominent and are probably the major components. The exact relation between these components and the two homogeneous species found in the analytical ultracentrifuge at 6.8 *S* and 9.3 *S* after dissolving the cell wall in 8 M lithium chloride (Hills 1973) remains to be found.

Cell-wall preparations from several other algae have also been run on SDS polyacrylamide gels, following digestion with 1% SDS at 100 °C for 1 min. The results are shown in figures 67–74. Two main facts emerge from these initial results. One is that all the walls examined are built up from a few components all staining on the gels for both carbohydrate and protein, and all having molecular masses of the same order as those in *Chlamydomonas reinhardi*. The other point emphasizes the differences between the gels, for it is clear that walls of the same crystalline type may yet show considerable differences in the pattern of bands revealed on the gels. For example, *Chlamydomonas chlamydogama* (figure 72), *Chlamydomonas dysosmos* (figure 71) and *Chlamydomonas moewusii* (figure 68), although all having the same crystalline surface lattice within their walls (class II), show variations in the glycoprotein components that are used to

build it. Similarly, *Chlorogonium elongatum* (figure 69) and *Chlorogonium euchlorum* (figure 70), although similar morphologically and having the same wall structure, show distinct differences when their walls are analysed electrophoretically. More detailed analysis is required to obtain answers to the problems of how identical crystalline lattices can be composed of different component subunits, and of the exact relation between the different bands found on the gels. Electrophoretic evidence of another type supports our conclusion that *Chlamydomonas moewusii* and *Chlamydomonas eugametos* are very closely related (i.e. both class II walls) and are very different from *C. reinhardi* (class I). This evidence comes from the isoenzyme patterns of acetone extracted proteins from the three algae described by Thomas & Delcarpio (1971).

6. The *in vitro* assembly of the cell wall

One of the main problems that is of general interest concerning the cell wall of *Chlamydomonas reinhardi* (the cell wall about which we have the most information) is how it is assembled in the cell from its subunits. Is the process one of self-assembly, or is it one of directed assembly necessitating a seed or template? Evidence pertaining to such a problem may come from three main directions. First is the data obtained from work on cell wall mutants in an attempt to try and formulate a morphogenetic pathway of wall formation (Davies 1973) in much the same way as has been done for phage morphogenesis. Second is the careful analysis of the *in vivo* situation, in which the daughter protoplasts reform a complete cell wall very swiftly within the mother cell wall. Third, and perhaps immediately the most feasible, is the attempt to mimic the *in vivo* situation by experiments *in vitro* on the disassembly and reassembly of purified cell walls. In this case, conditions may be precisely monitored and the steps involved in reassembly investigated. The initial successful *in vitro* reassembly of the cell wall has been achieved by Hills (1973), and one or two remarks about it are in order. Briefly, he has shown that the purified cell walls are soluble in aqueous 8 M lithium chloride and are recoverable as spherical intact walls (having identical structure and composition to the original walls) by back dialysis of the solution against water.

The appearance of the original cell walls and those that have been reconstituted in this manner are shown in figures 75 and 76. Hills has further shown that a nucleating agent, insoluble in aqueous 8 M lithium chloride, is essential for the recovery of wall-shaped products upon dialysis of the wall solution. Using gel electrophoresis, some more information may be gained about the process of wall disassembly and reassembly. Cell walls, dissolved in 8 M LiCl solution, were centrifuged at 15000 *g* for 5 min and the supernatant, containing the soluble wall components, was run on gels as described earlier. It can be seen from figures 77 and 78 that all the components that go to form the normal wild-typed cell wall are soluble in 8 M LiCl. Further evidence that all the main wall components go into solution is seen in the fact that the amino acid composition of the LiCl soluble material is close to that of the intact cell wall (table 1). In addition, the walls recovered by reassembly following dialysis, when spun down, washed, and run on gels show that all the components are used in the reassembly process (figure 79). That the nucleating agent is essential only for the production of cell walls of the right size and shape may be demonstrated as follows. If a LiCl digest of cell walls is spun to remove the nucleating agents (Hills 1973) the remaining supernatent will not produce wall-shaped reassembly products. However, in the light microscope a large amount of 'fluffy' material may be seen to be produced in the dialysis sac. This assembly product when spun,

washed and run on gels shows that it too contains all the components of the normal cell wall in the correct proportions (figure 80). The nucleating centre, which is not an existing piece of cell wall, in some way therefore determines the precise size and shape of the reassembly product. In its absence, reassembly merely produces heterogeneous wall fragments. It is of interest that the *in vivo* situation suggests that pre-existent wall lattice is not essential for correct wall assembly to occur, for after meiosis a complete new wall is formed within the zygote wall, a structure completely different from the usual mother cell wall. Not all the soluble components are re-used in the reassembly process, as gels run of the supernatant from a dialysis sac which had run to equilibrium against water showed that walls were still present in the solution and still showed the correct proportions of each component.

Investigation of the conditions of dialysis has also thrown some light on the reassembly process. Although walls themselves are insoluble in 2 M LiCl solution, an 8 M LiCl solution of walls diluted to 2 M does not give any reassembly products, which suggests that the effect of dialysis for reassembly is not just one of dilution. Davies (1973) has investigated some of the factors which control cell-wall assembly in some of the cell-wall mutants, and concludes that a particular kind of physical structure (e.g. dialysis sac, agar or Millipore filter) is essential for wall formation in some of those mutants which do not usually produce walls. Following from this fact, 8 M LiCl solutions of cell walls were placed on Millipore filters floating on water, and also as drops trapped in 2% agar blocks. In both cases reassembly to form intact spherical cell walls occurred. However, it can be shown that it is not the surface alone which is essential for reassembly, as pieces of agar block, or dialysis sac, if added to 8 M LiCl wall digests do not initiate reassembly, even upon dilution. In all the cases where reassembly occurs to give intact cell walls, three conditions appear to be necessary: a physical surface of the right kind, a gradient of salt (LiCl) concentration across the surface, and the presence of a nucleating agent. Further work on the nature of the nucleating agent and the conditions of dialysis will be reported elsewhere.

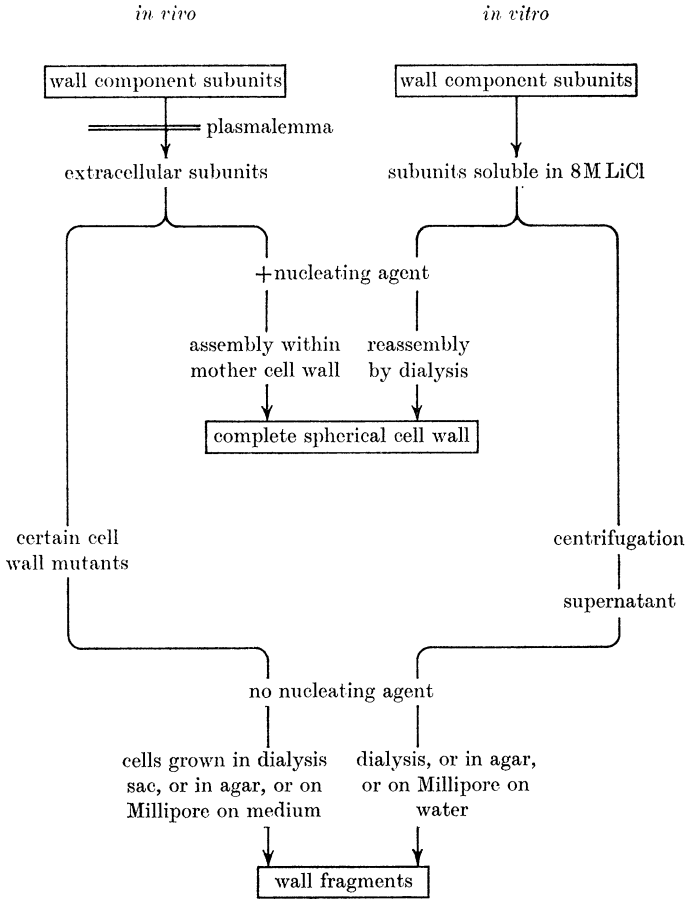
A suggested scheme of comparison for the methods of cell-wall assembly *in vivo* and *in vitro* is shown in table 6 in a diagrammatic form. The relation of layers 1 and 7 of the wall (figure 3) to the crystalline lattice and how they fit into the scheme of wall assembly is not known.

7. Problems for taxonomy

The taxonomy of the primitive green algae is both confusing and artificial, based for the most part on gross overall morphology (e.g. by Bourelly 1966). Fritsch (1948) divides the Volvocales into three suborders of which one, the Chlamydomonadinae, contains four families, of which two contain all the algae so far examined and found to have crystalline cell walls. These are the Chlamydomonaceae and the Sphaerellaceae. He admits, however, that the latter are better regarded as 'representing a specialized type derived from the Chlamydomonads'. Such a view is represented also by an artificial algal key (Bourelly 1966) in which these two subfamilies are joined. However, it must be admitted straight away that, allowing for the fact that all those algae that possess crystalline walls fall within this group, their further classification within that group on the basis of crystalline types as outlined here bears no resemblance to the subdivisions of the group that have been proposed elsewhere. This is obviously not to suggest that such artificial algal keys should be dropped in favour of a classification based on wall structure, even though this may be valid on a somewhat larger scale (e.g. Dawes 1966). What is

proposed is that such crystalline wall classes should be further investigated and taken into account in the classification of the Chlamydomonaceae. It is surely of significance, for example, that the majority of the algae examined fall into class II. Such conservation of ultrastructure must have evolutionary significance. Similarly it would seem that the multicellular forms (e.g. Volvox) have evolved from a class I precursor such as *Chlamydomonas reinhardi*. It is tentatively

TABLE 6. COMPARATIVE SCHEME FOR CELL WALL ASSEMBLY *IN VIVO* AND *IN VITRO* FOR *CHLAMYDOMONAS REINHARDI*



proposed that class II walls are the most widely spread and probably the most primitive and that the others arose from this by modifications of the glycoprotein subunits until new crystal packings became energetically more favourable. It is also possible that such crystalline cell walls have been conserved, evolutionary speaking, and are present in some form in more advanced plant cell walls, masked by the enormous depositions of pectin, hemicellulose and cellulose, but still containing hydroxyproline.

7. GENERAL CONCLUSION

It has been demonstrated, as a result of detailed electron-microscopical investigation, that numerous members of the algal family Chlamydomonaceae possess within their cell walls a highly ordered, crystalline lattice component. This has been confirmed by negative staining

methods, shadowed replicas and thin sections. On the basis of the Fourier transforms of the negatively stained cell walls, the algae examined have been classified into five main classes. The two-dimensional unit cell of each class is a parallelogram whose sides and angles have been calculated. It is of interest that the size of the parallelogram is of the same general order in all the classes with sides between about 10 and 40 nm and an angle between 70 and 90°. More detailed structural information has so far been obtained only for class I and II walls. This has been obtained by the combined techniques of optical reconstruction, linear integration and electron diffraction, and has enabled models of the cell wall to be proposed to a resolution of 2.5 nm. The nature of the components that assemble these crystalline walls is being investigated by two approaches, biochemical analysis and polyacrylamide gel electrophoresis, and it has emerged that the walls are constructed from a small number of different high molecular mass glycoproteins. For *Chlamydomonas reinhardi* it has been shown that these glycoproteins contain large amounts of hydroxyproline which is cross-linked to short oligosaccharides of neutral sugars. These crystalline cell walls are of very great value as experimental material for studying the processes of biological assembly systems. As a result of *in vitro* reassembly experiments it has been shown that certain conditions are essential for the complete *in vitro* reconstitution of whole cell walls. Among these is a specific nucleating agent which may well correspond to the heritable extranuclear informational system proposed by Davies (1973) to be essential for wall formation. It is proposed that during wall formation the requisite subunits are accumulated within the mother cell wall, and that in the presence of the nucleating agent these may aggregate within minutes to form a new intact cell wall. In the absence of either a nucleating agent or the mother cell wall, only fragments of wall or no wall at all will be formed. To be incorporated into this model are two more features. One is the presence in the wall of the two flagellar collars (Roberts *et al.* 1972), whose structure and role will be discussed in another paper. The second is the presence in the crystalline walls of numerous faults and dislocations. These have been discussed in terms of the wall as a dynamic organelle, within which dislocations may act as mobile growth centres. In some unknown way the nucleating agent determines both the pattern of dislocations and the final overall cell-wall shape, which in some cases may be very different from the shape of the cell within, for example, *Chlorogonium elongatum* (figure 17). So far, reassembly experiments on class II walls have been unsuccessful and the exact conditions remain to be found. The implications of the various classes of crystalline algal cell walls that have been discovered, to algal evolution and taxonomy, are also briefly discussed. The directions of future research in this field will be along two lines: a more detailed chemical and structural analysis of the five wall classes, and an elucidation of the factors which control the *in vitro* and *in vivo* assembly of the cell wall.

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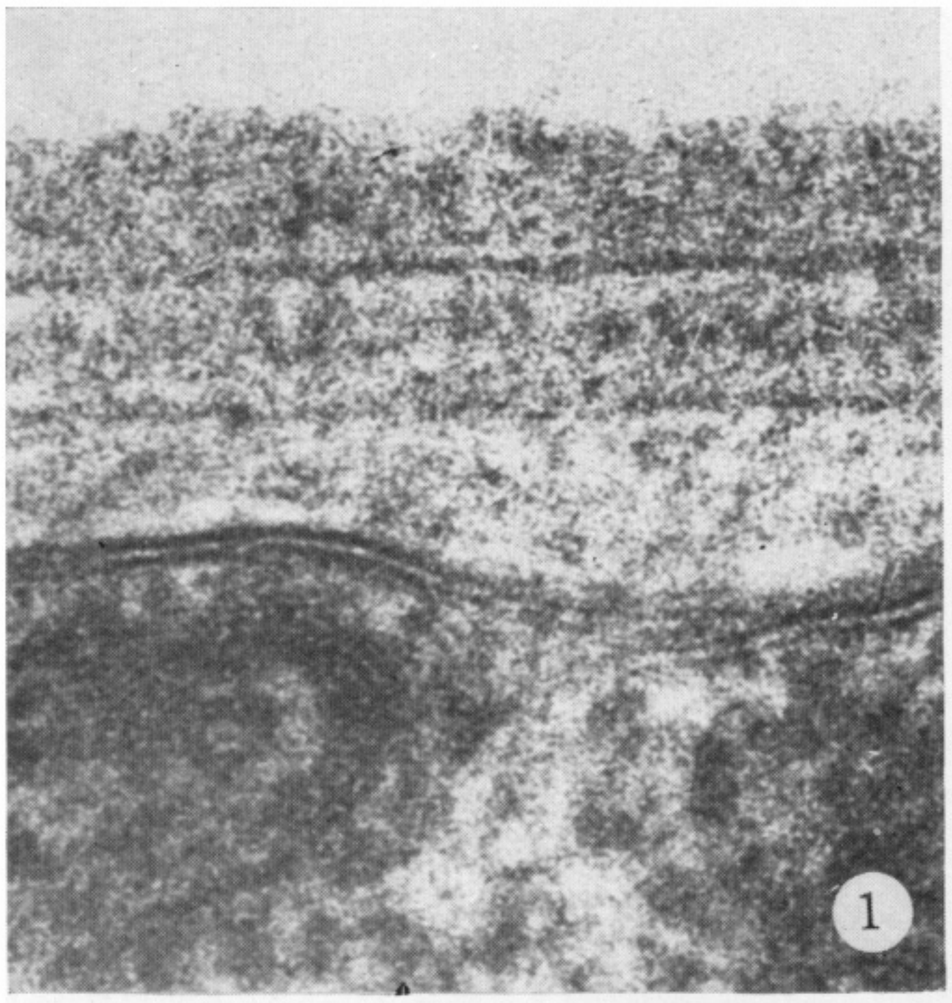

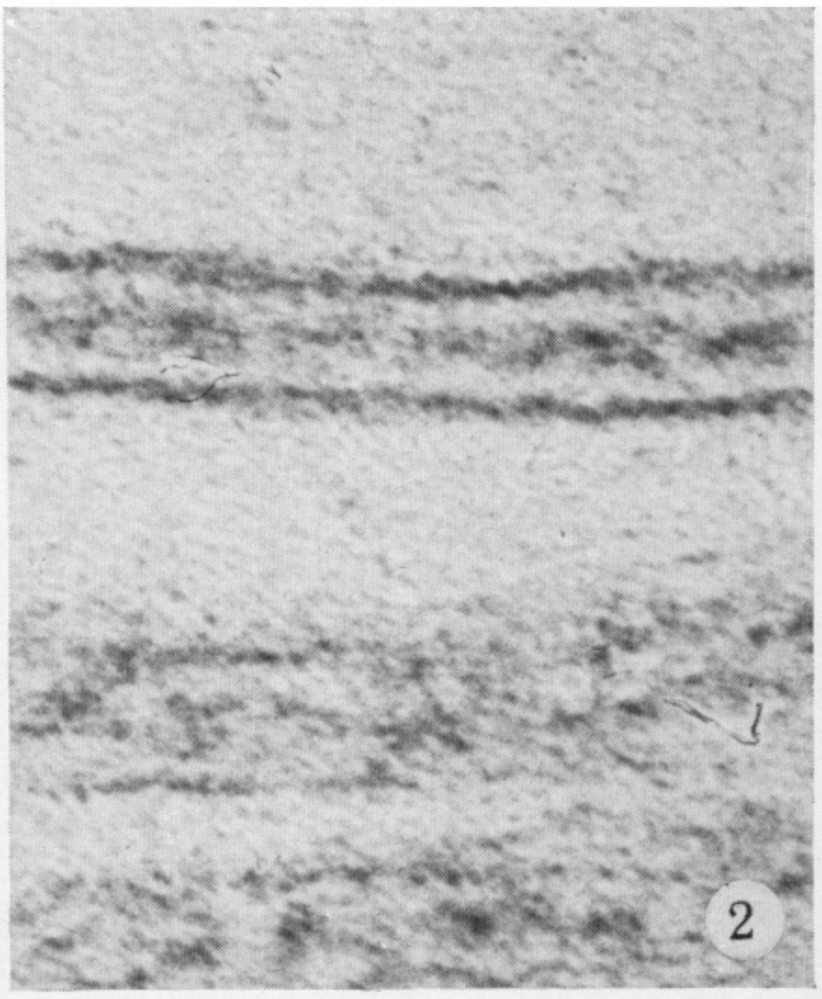

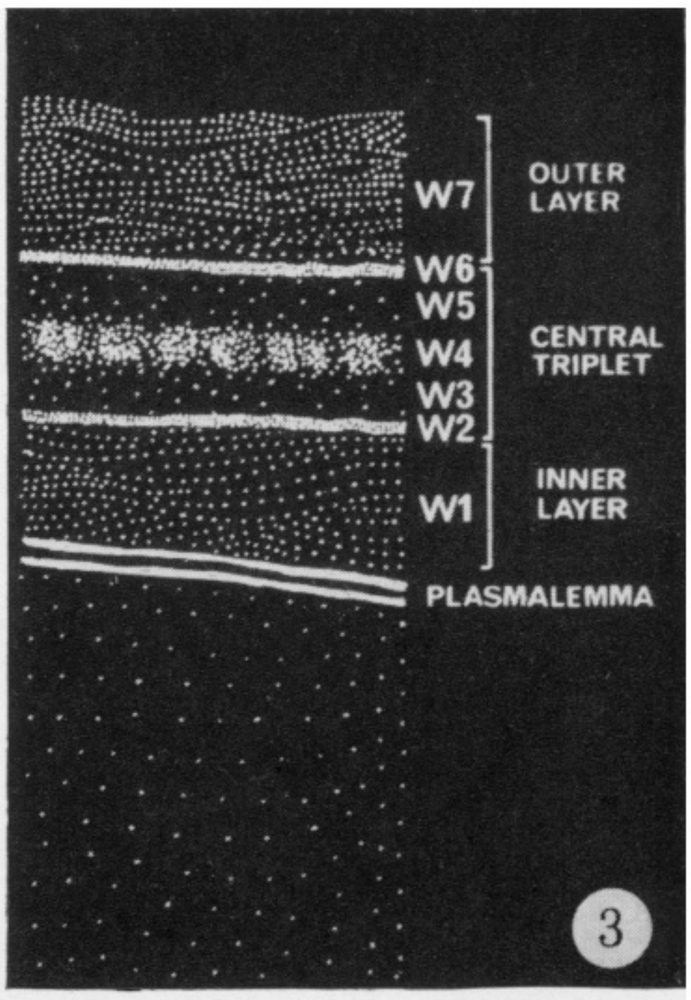

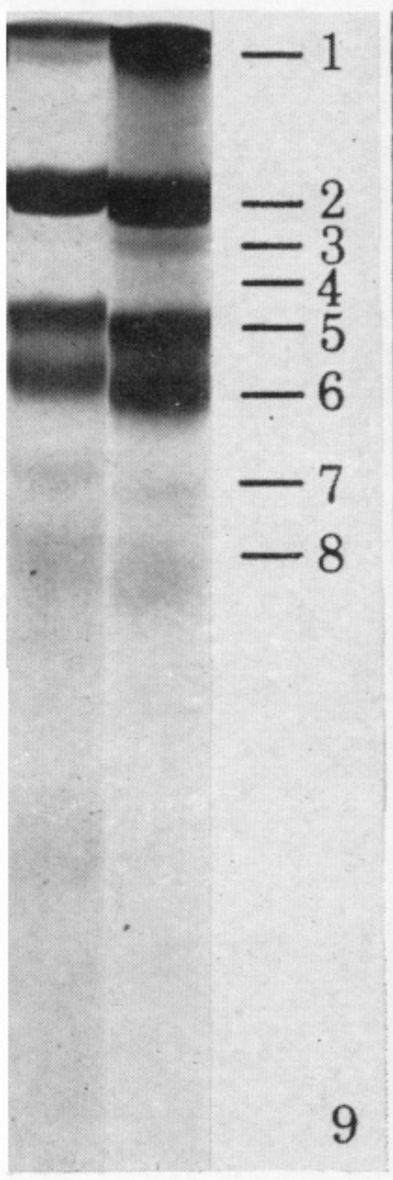

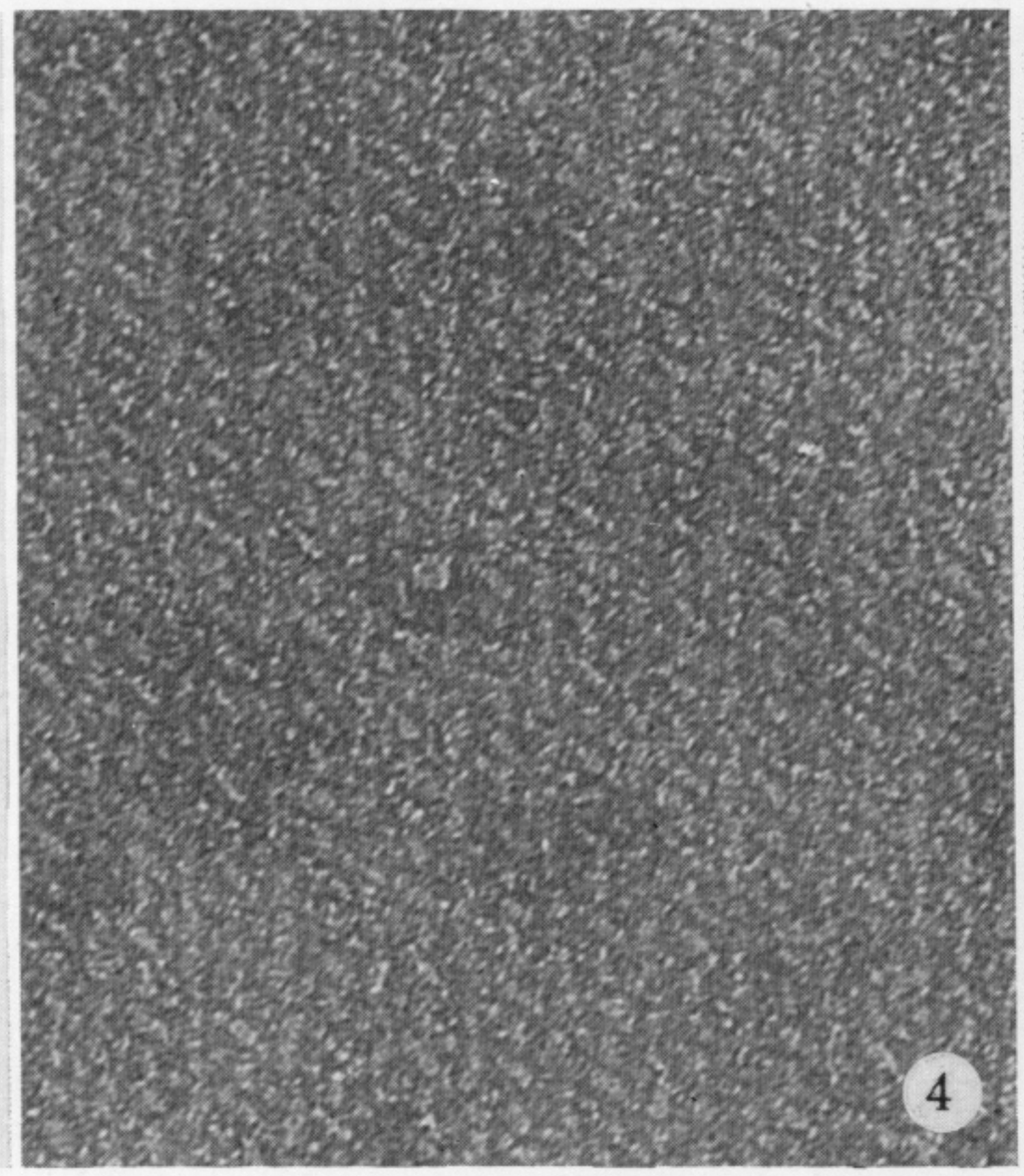

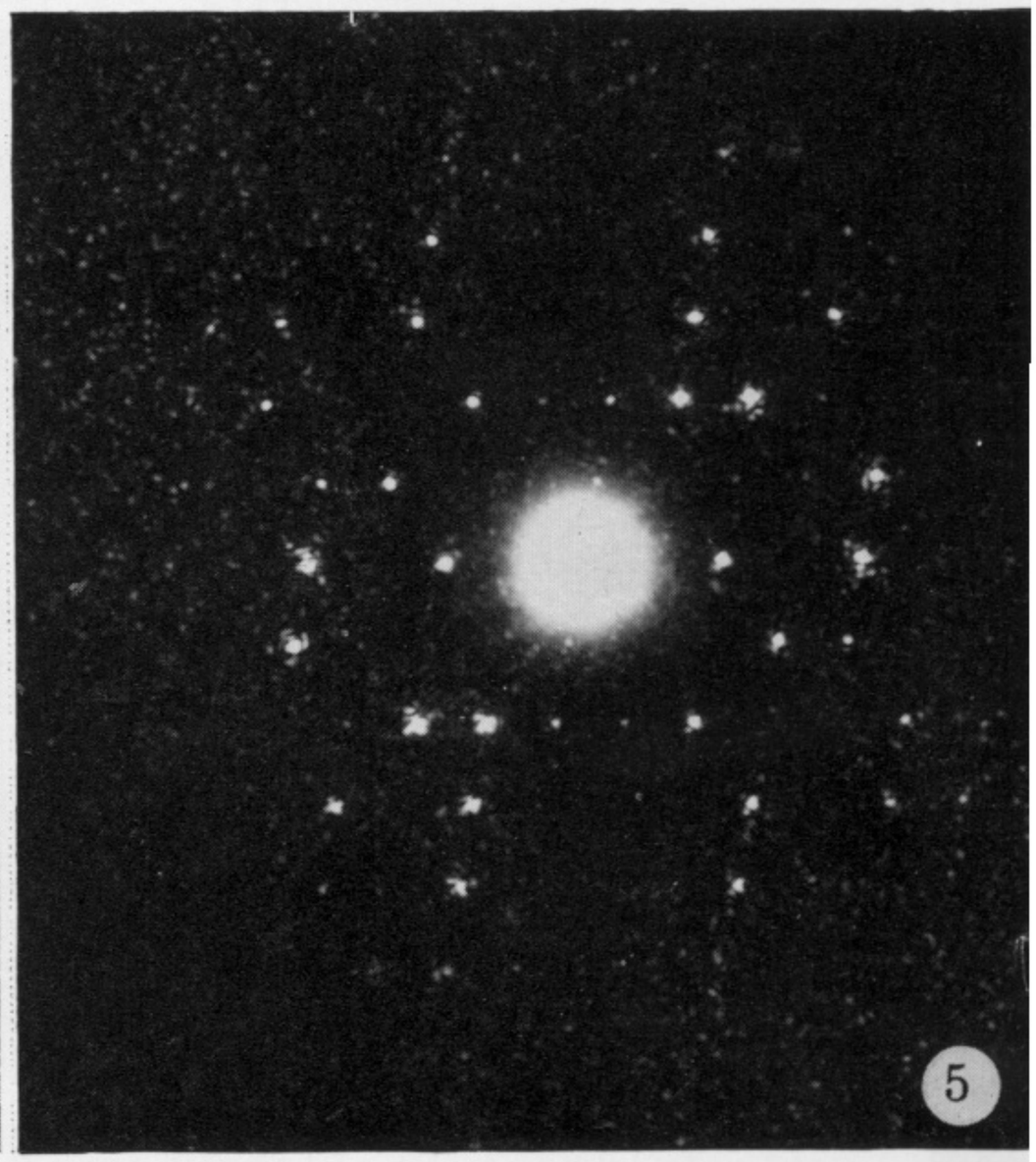

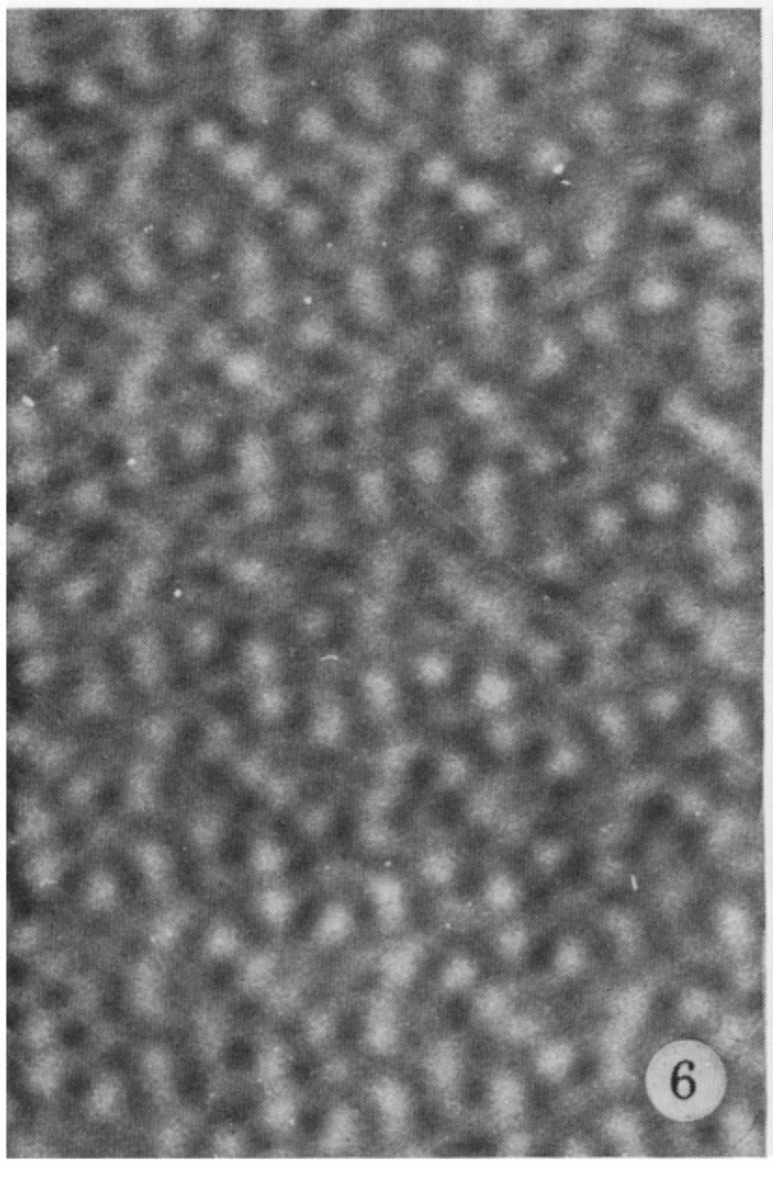

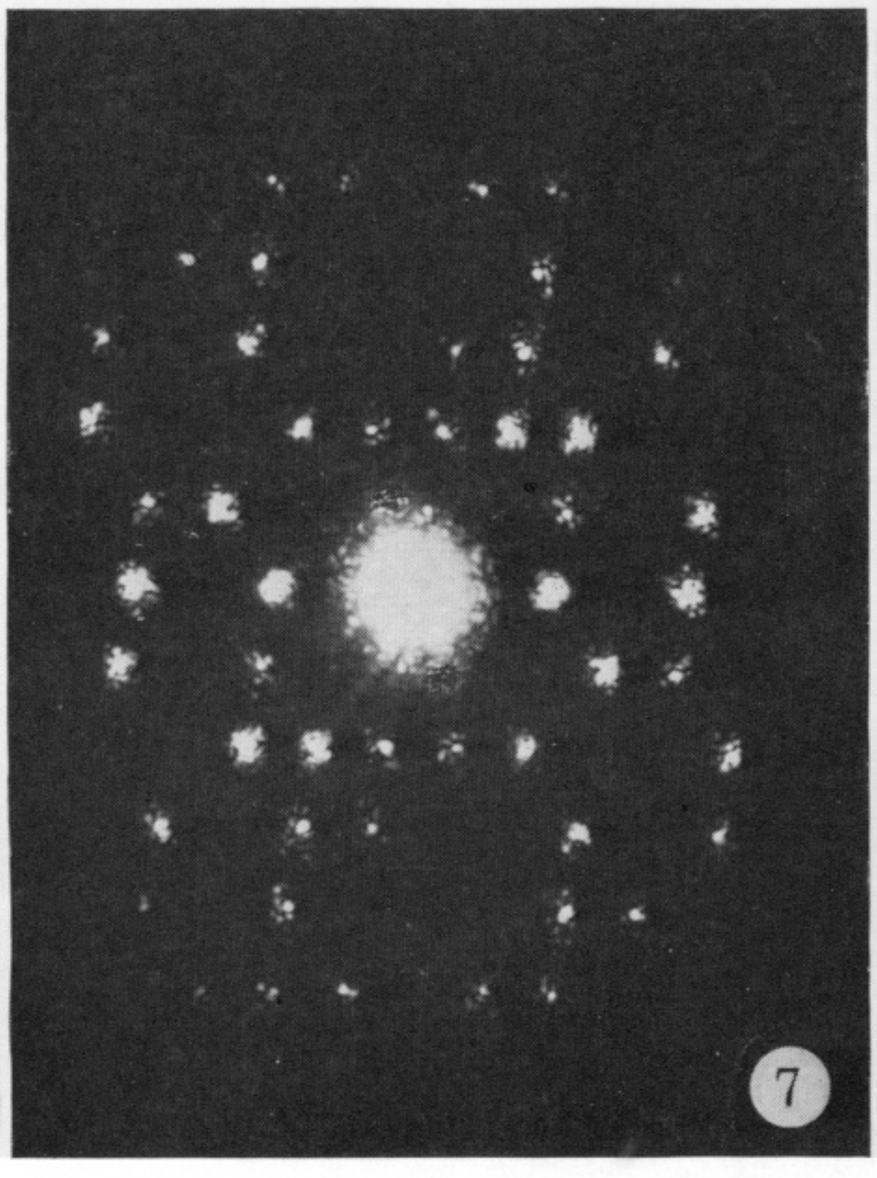

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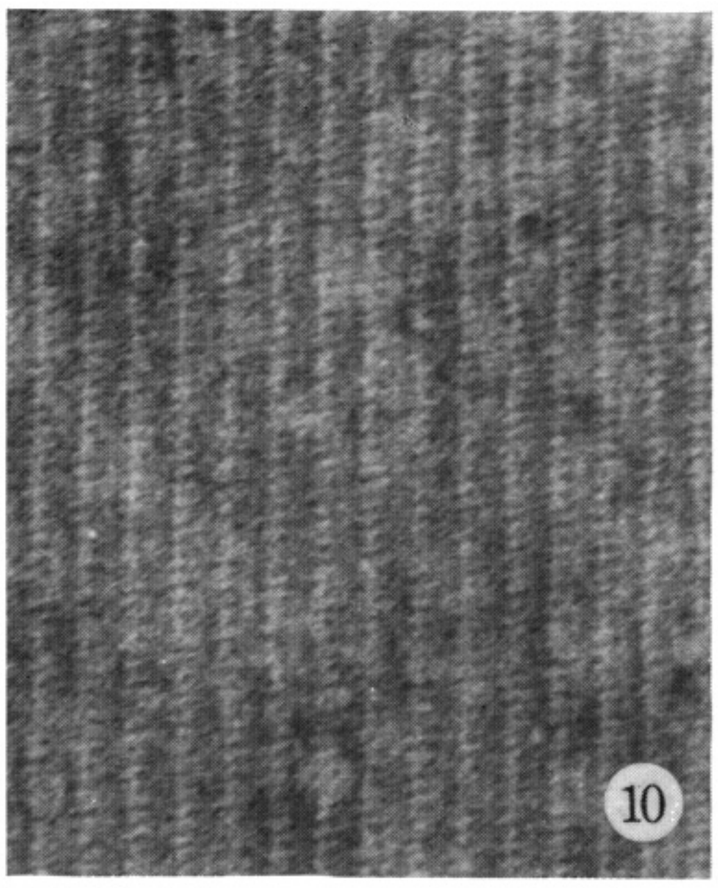

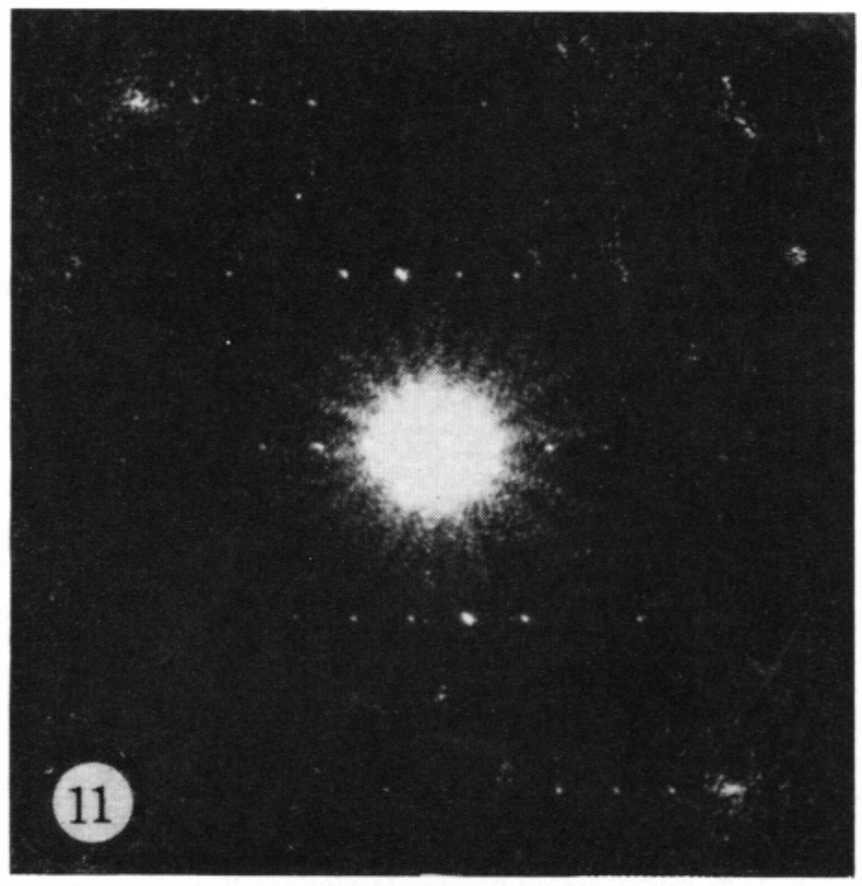

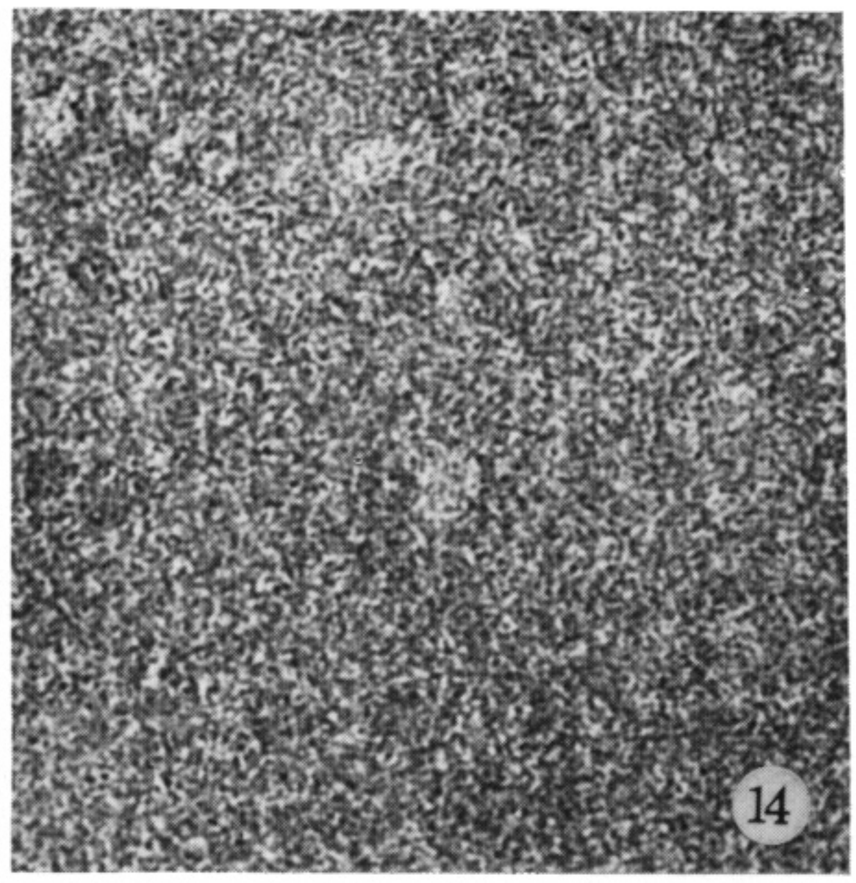

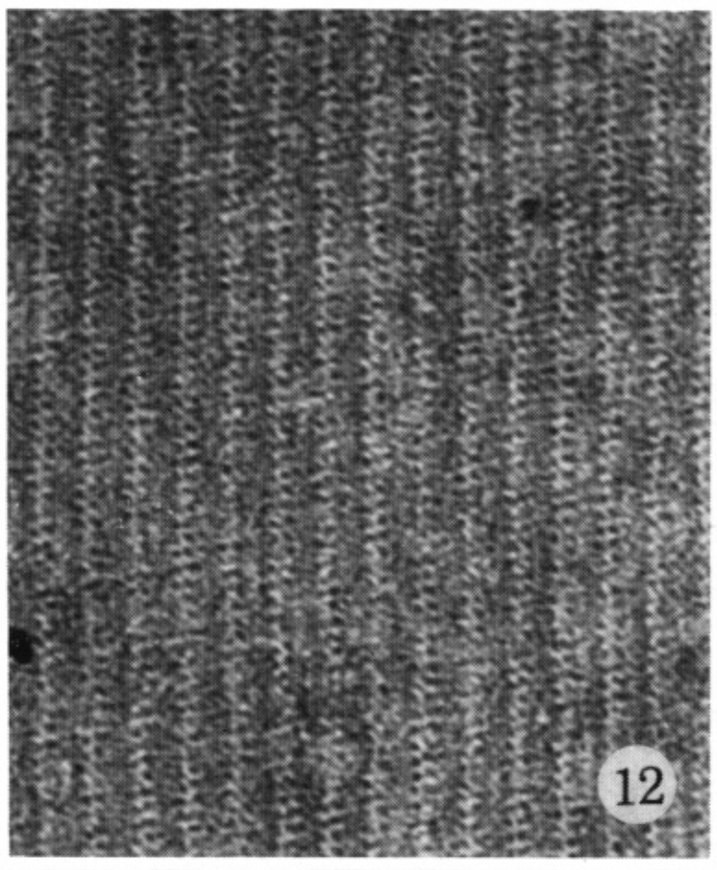

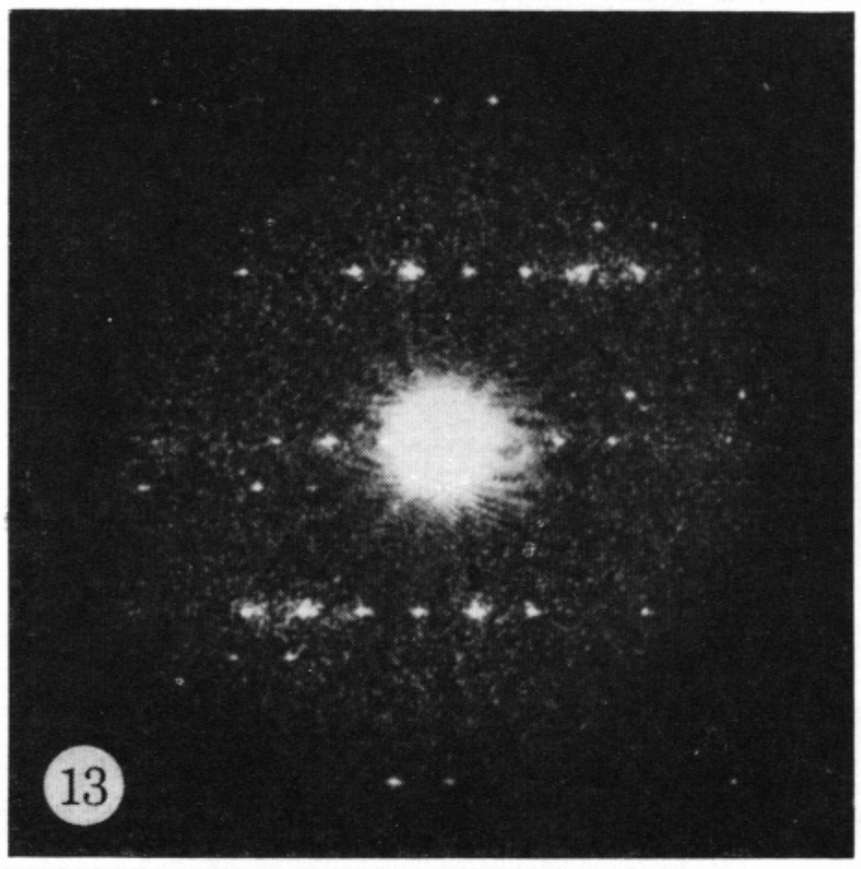

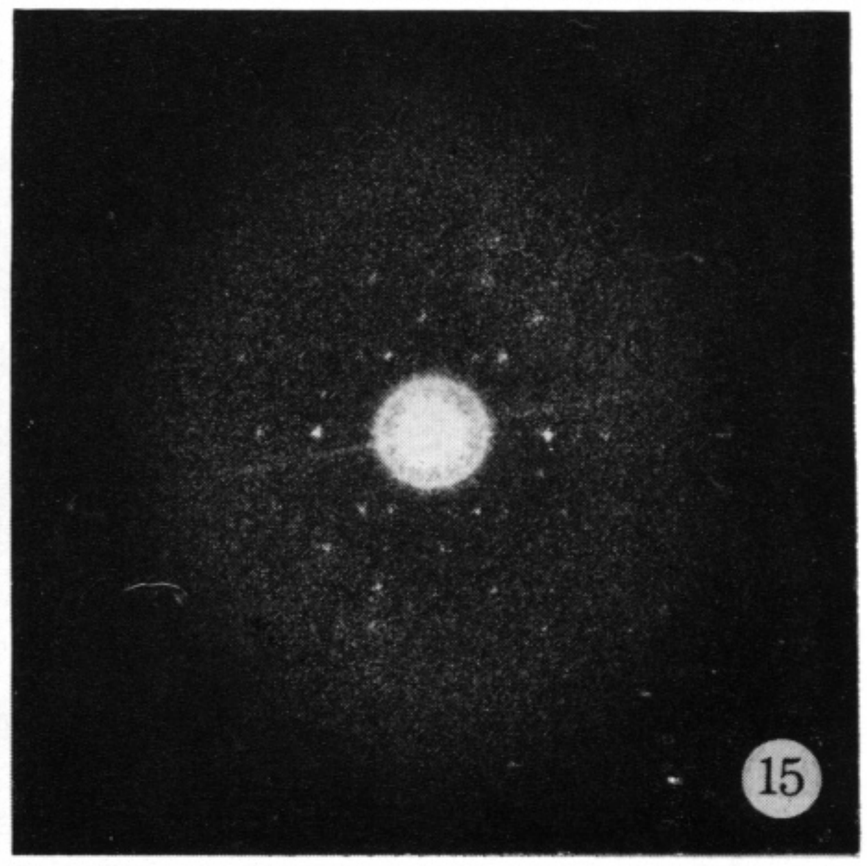

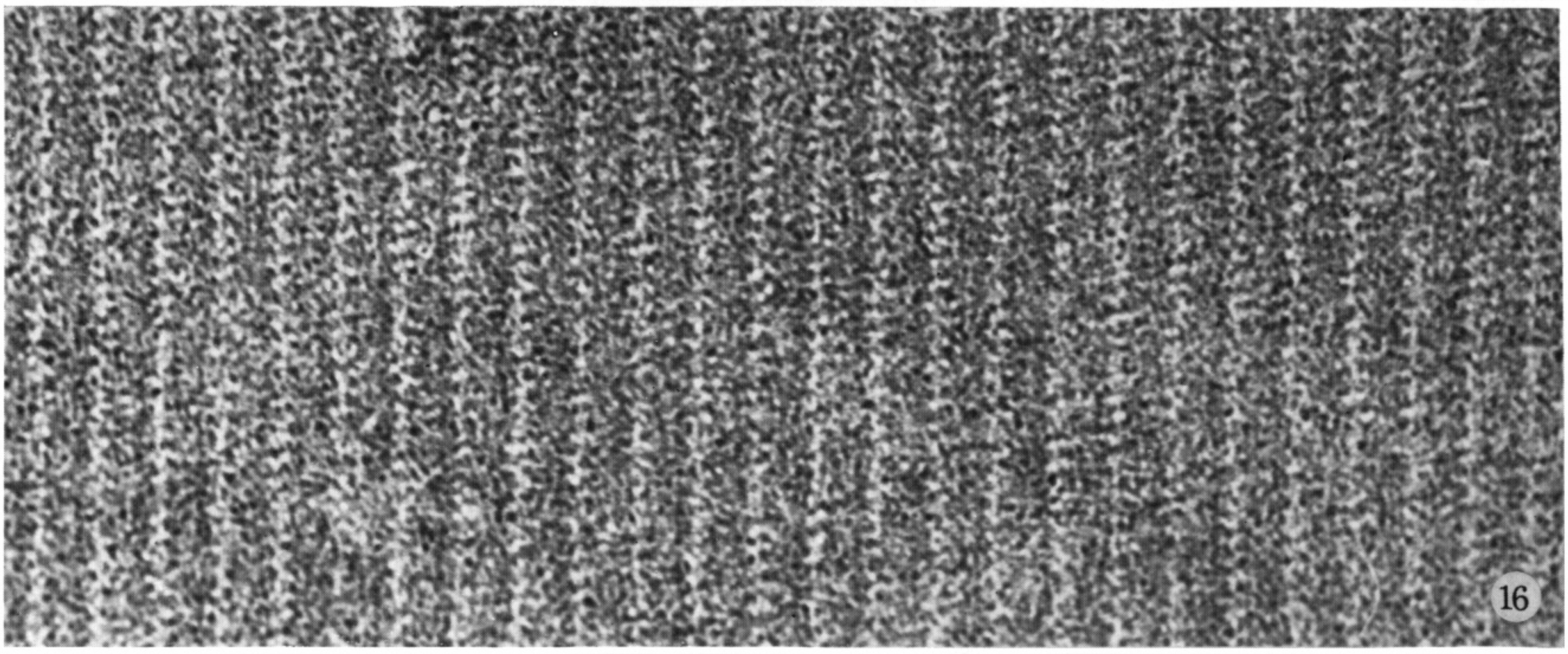

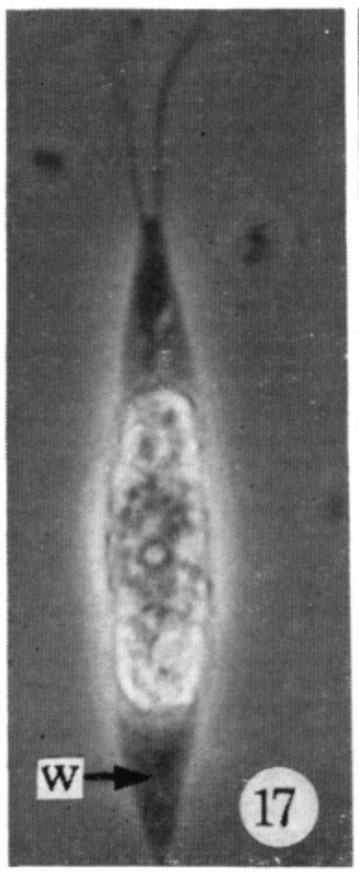

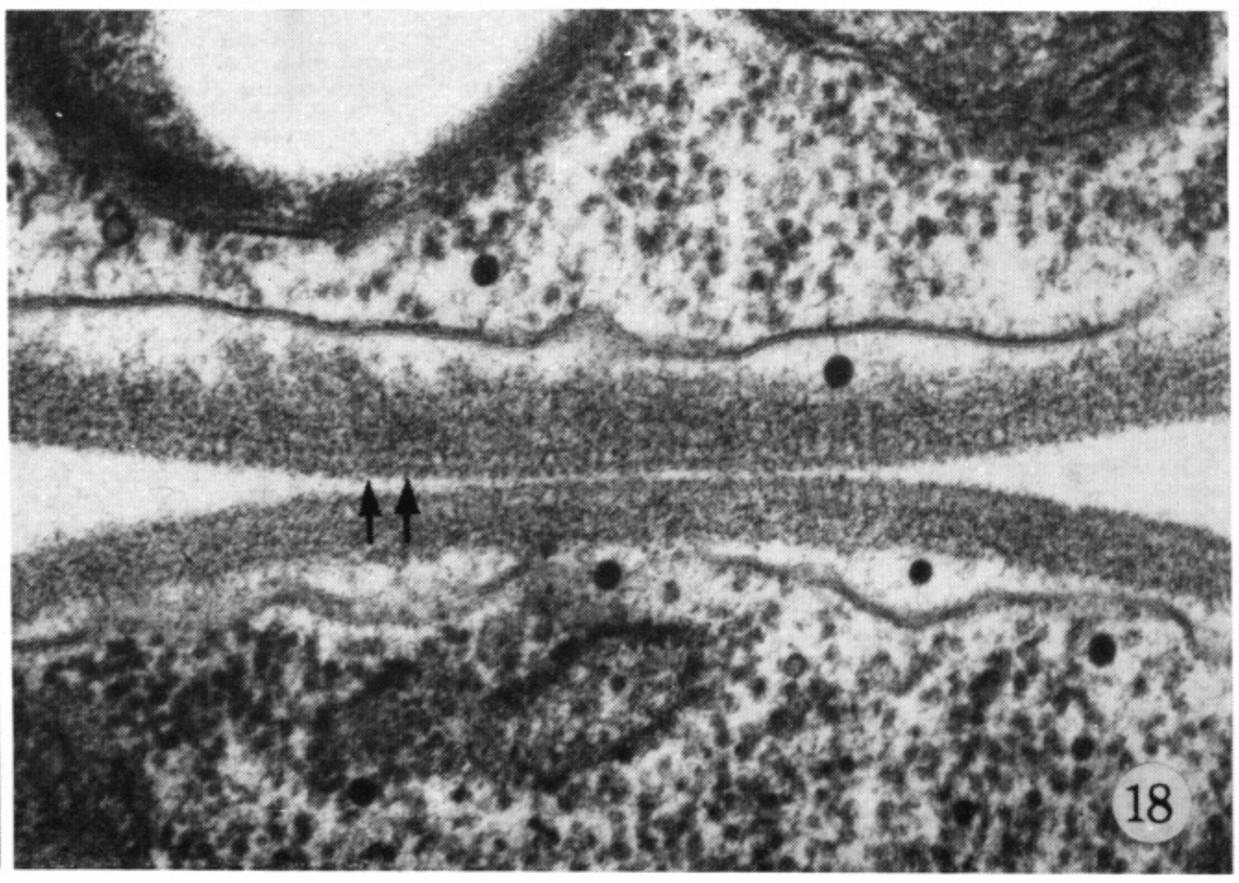

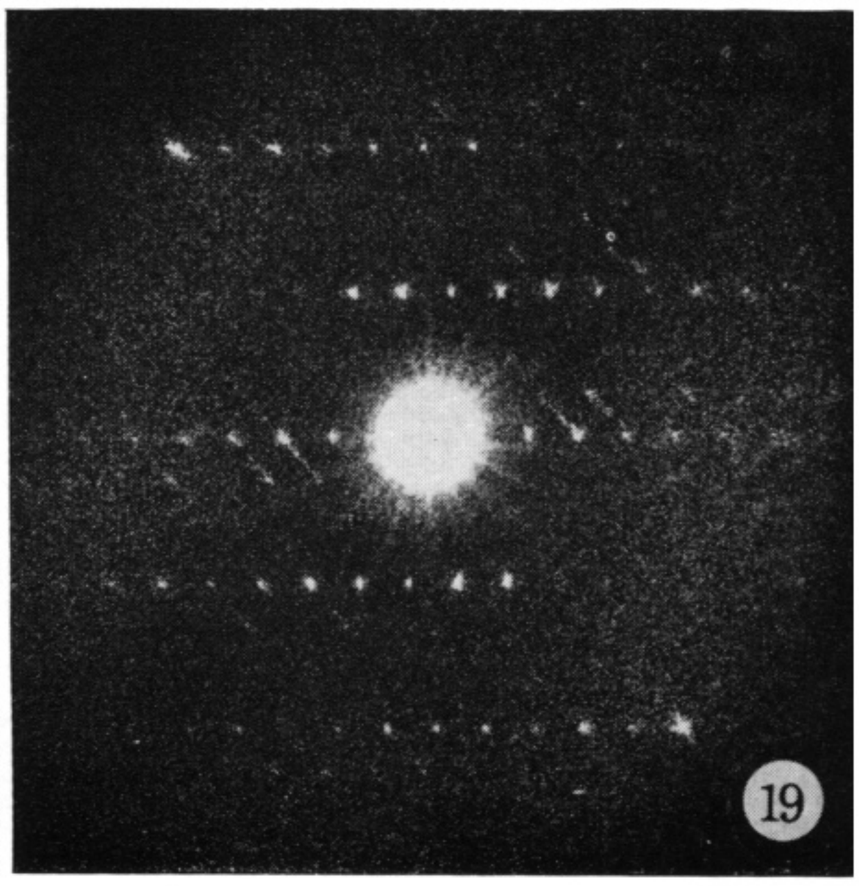

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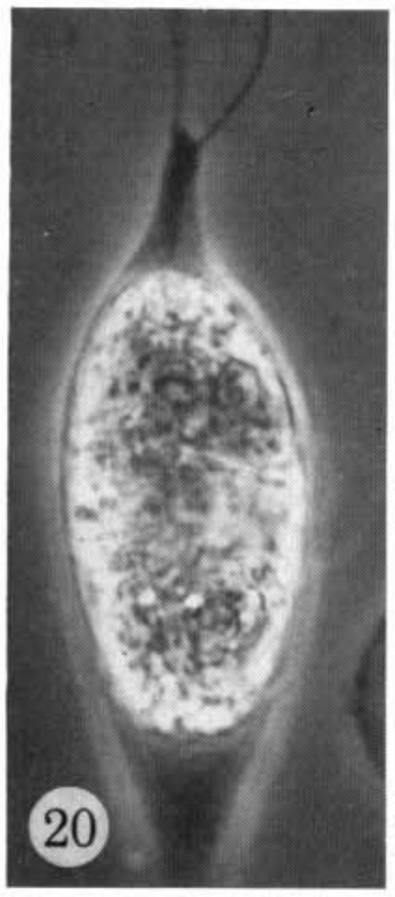

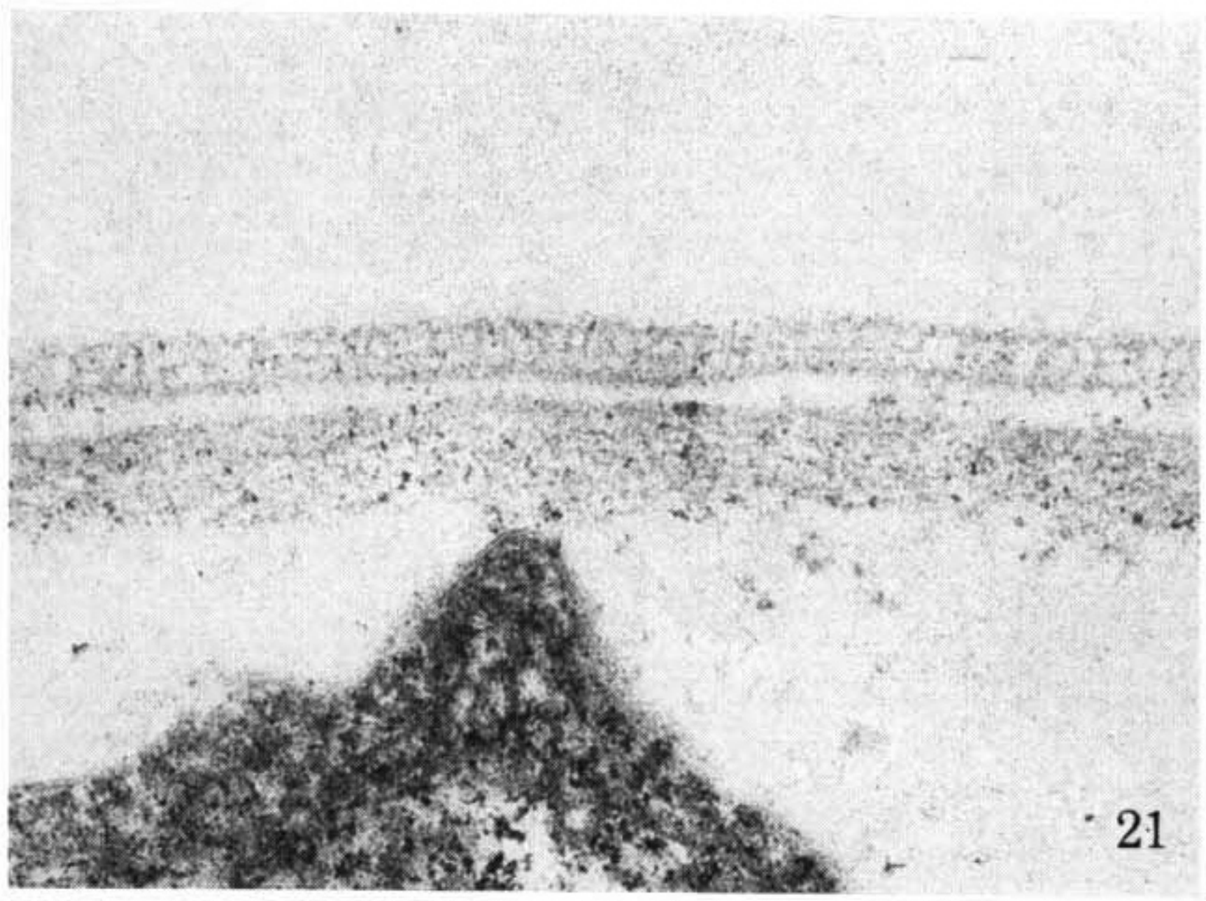

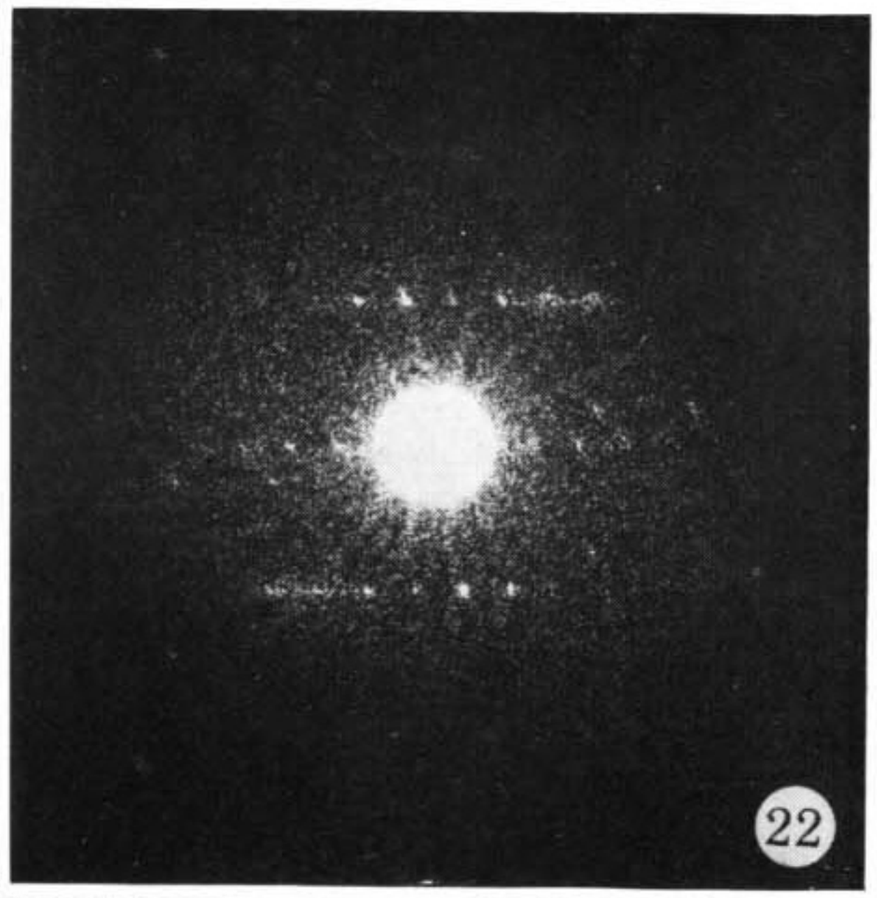

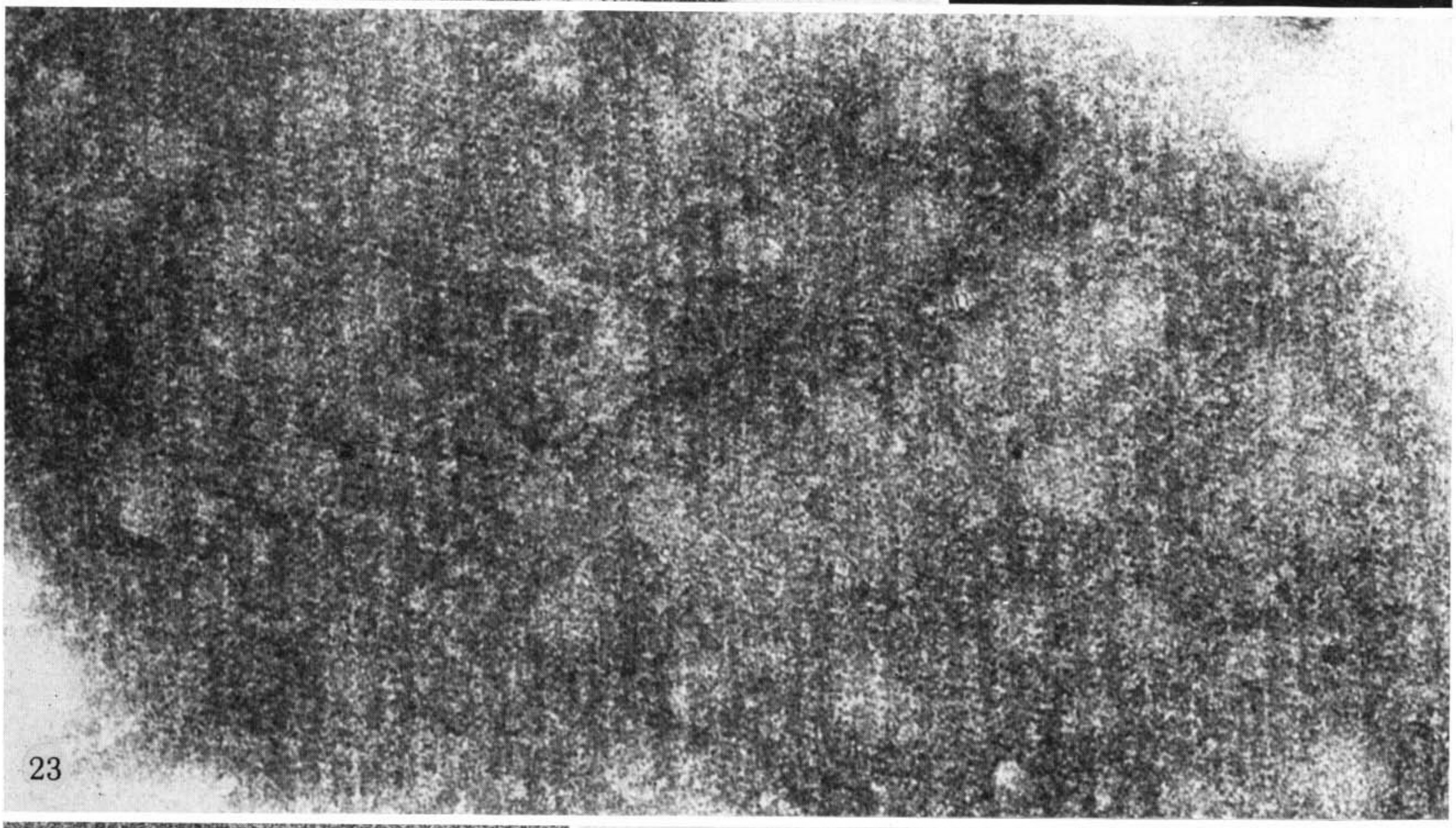

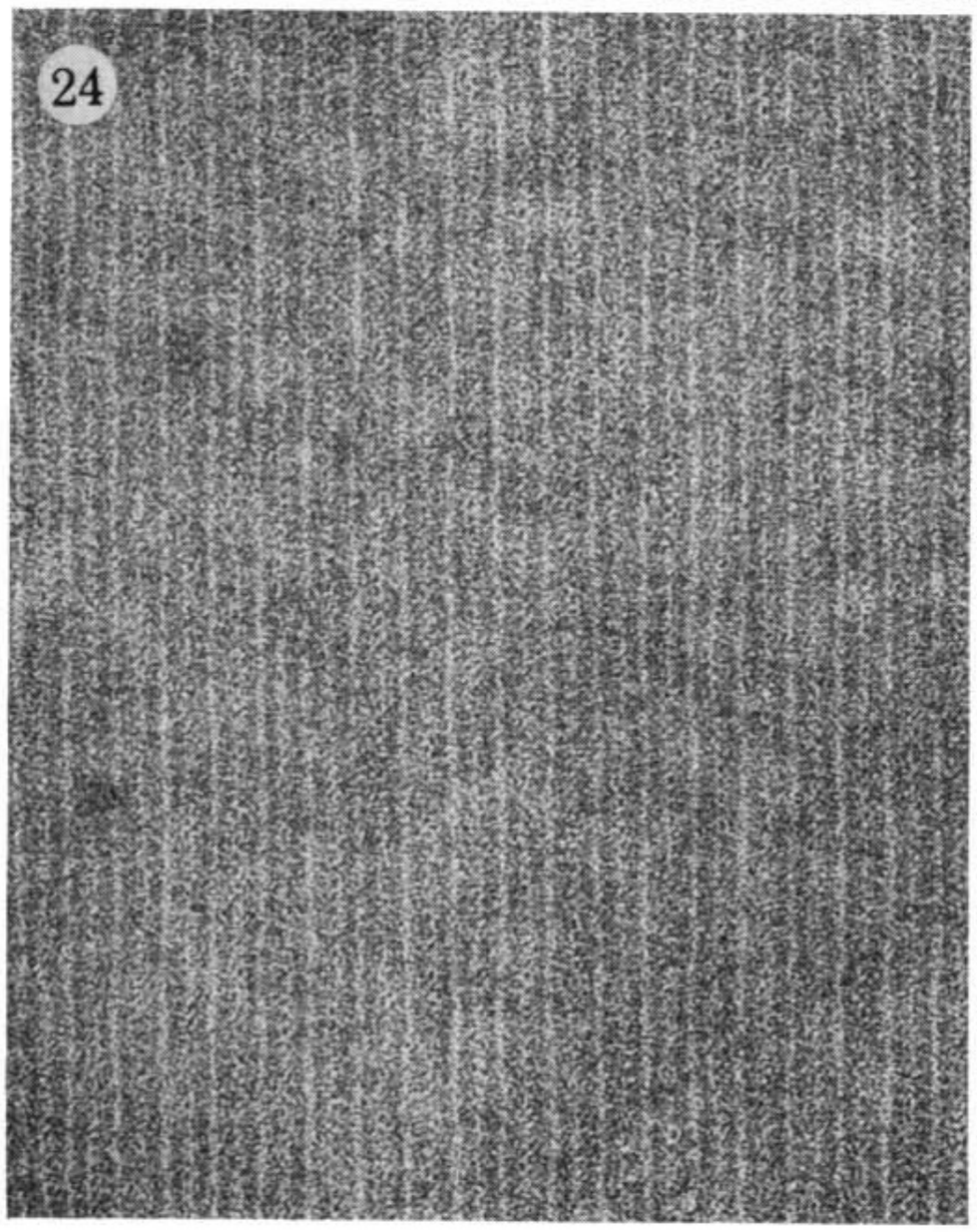

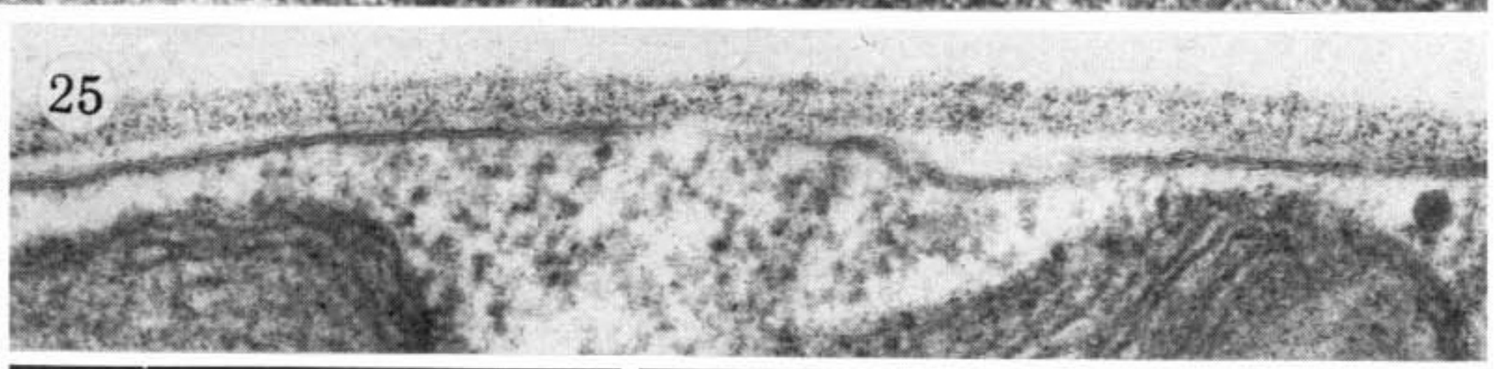

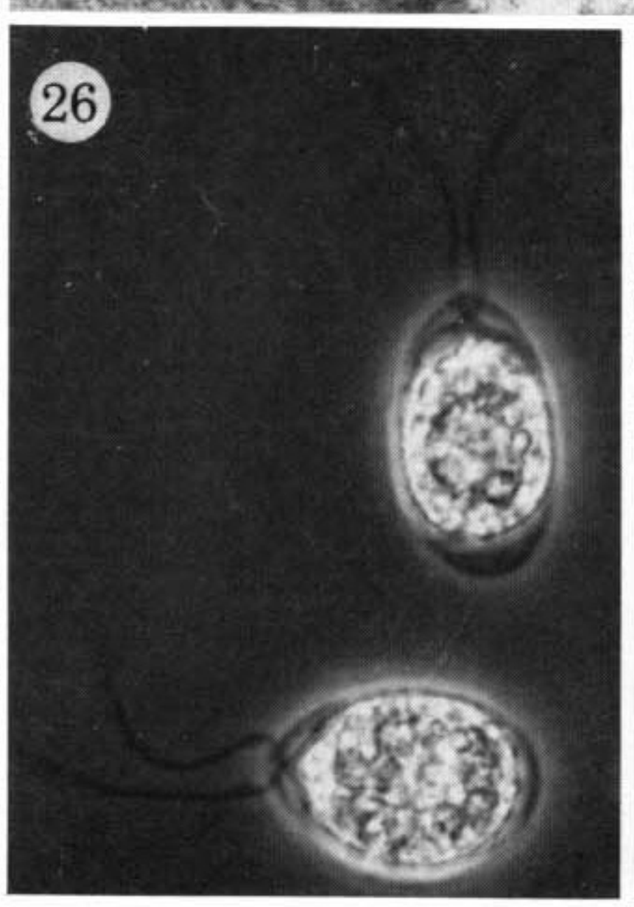

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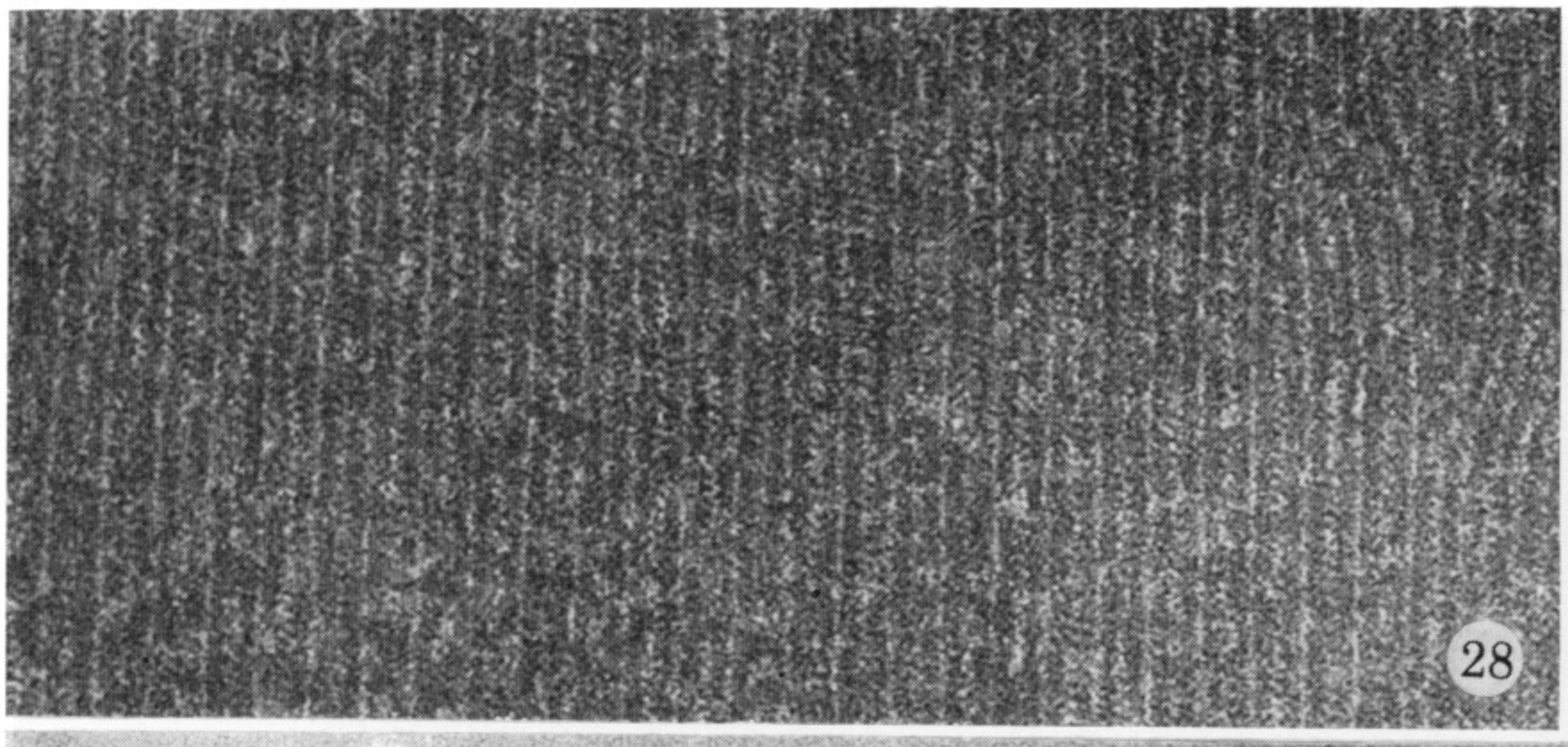
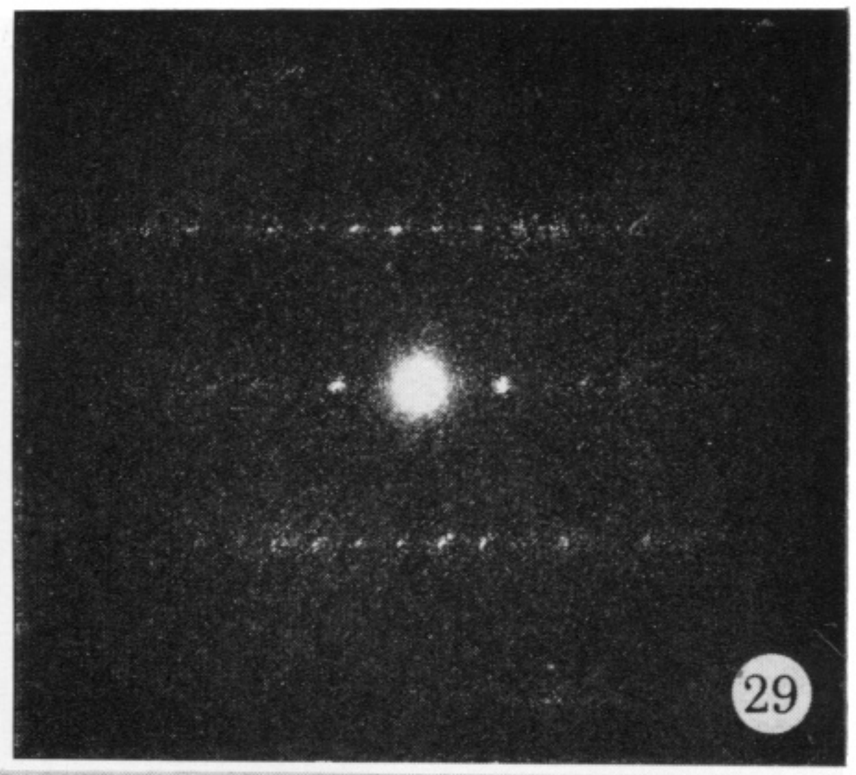
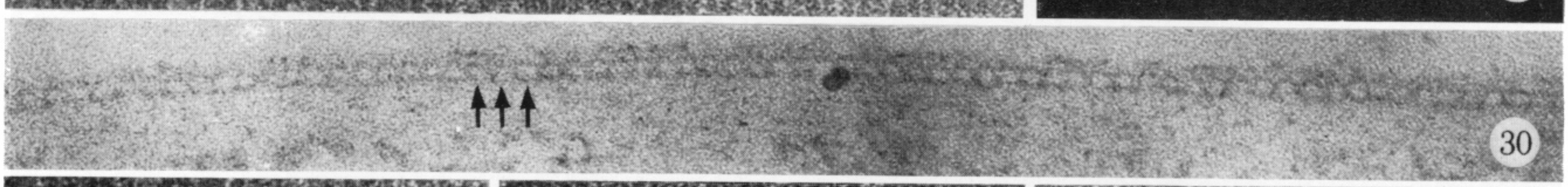
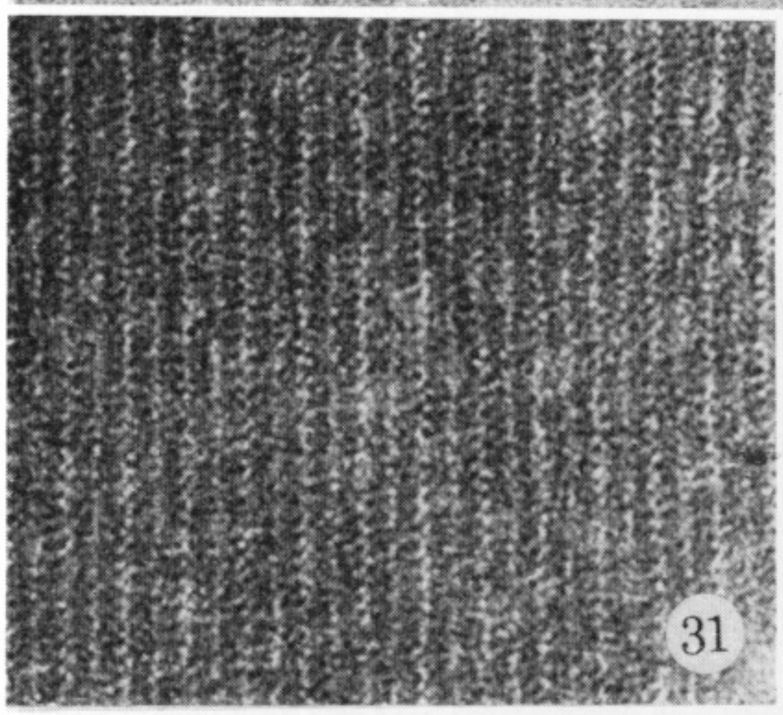
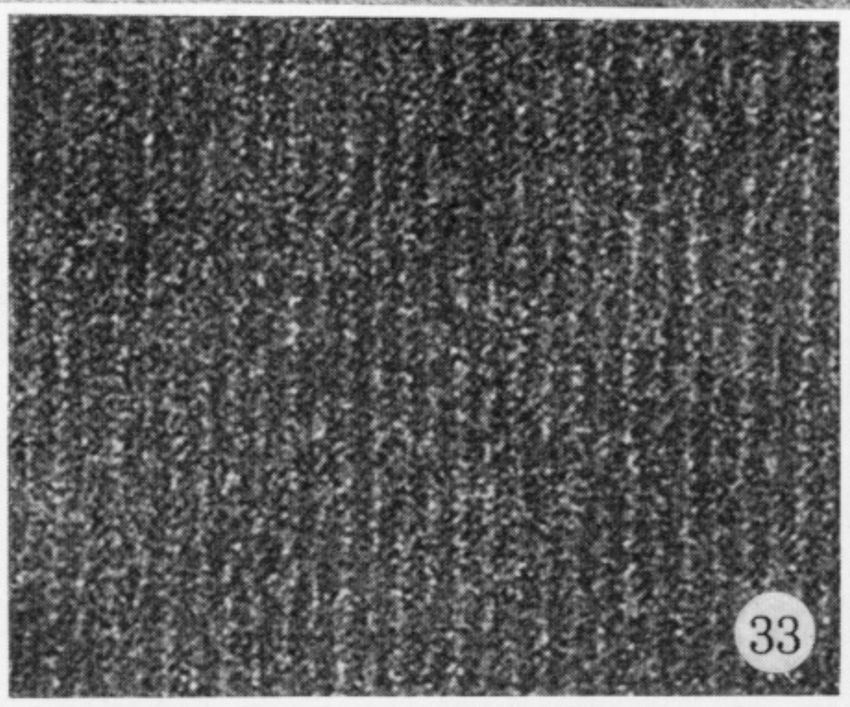
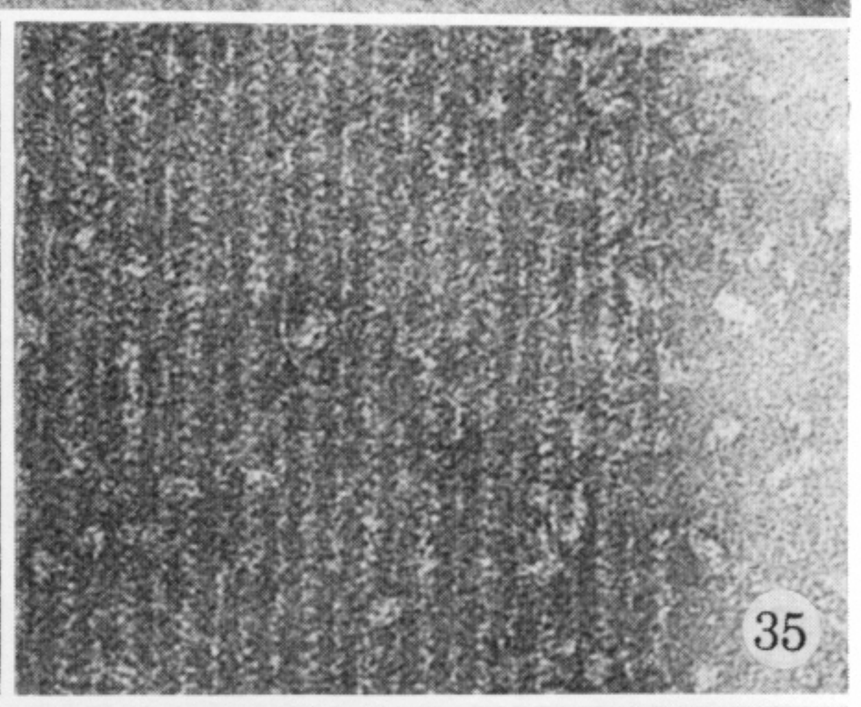


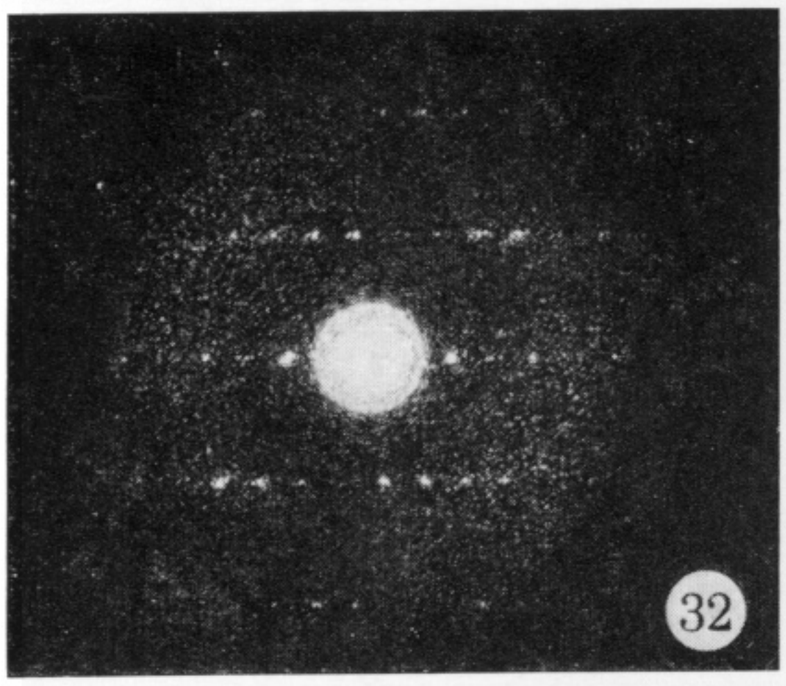


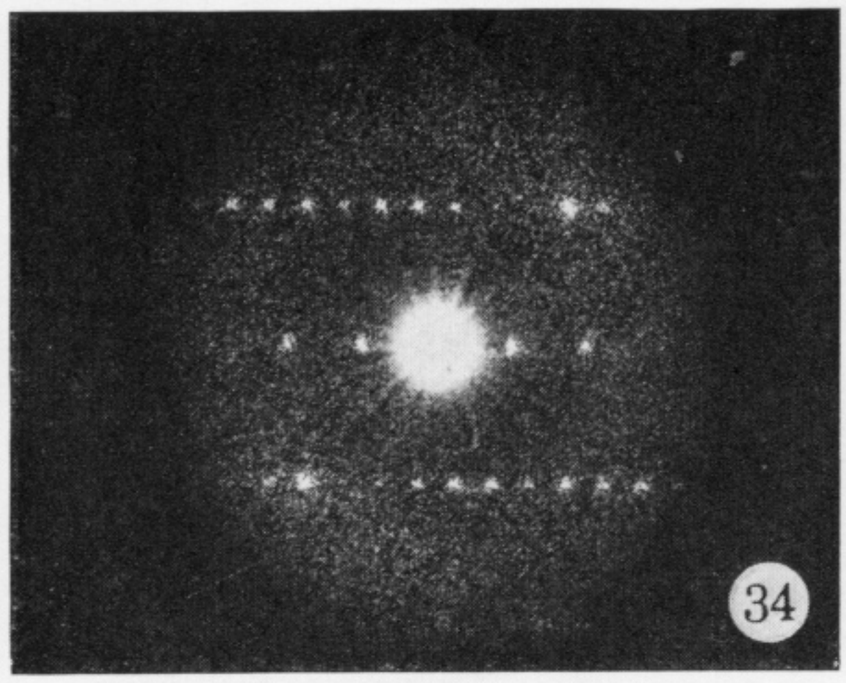


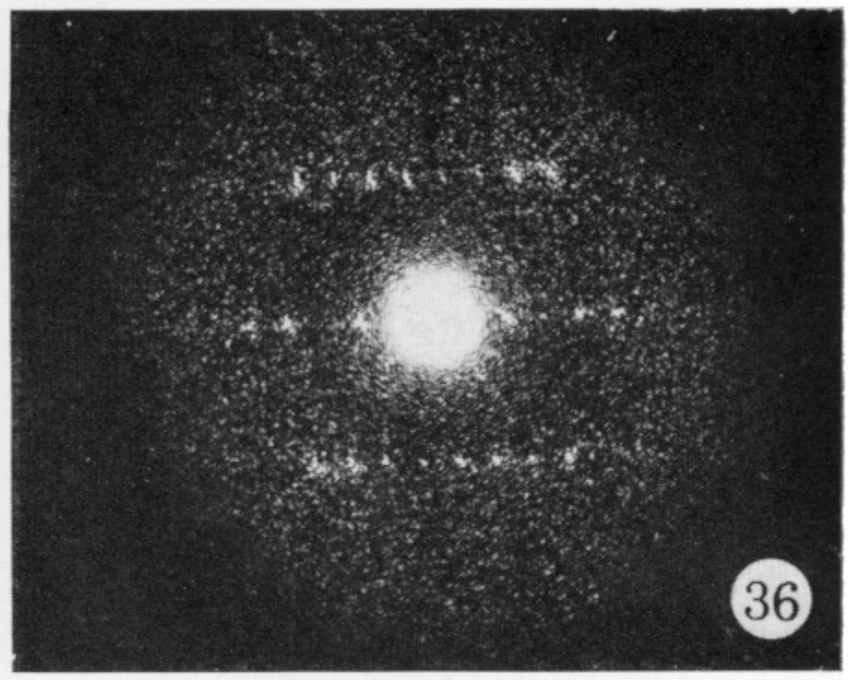


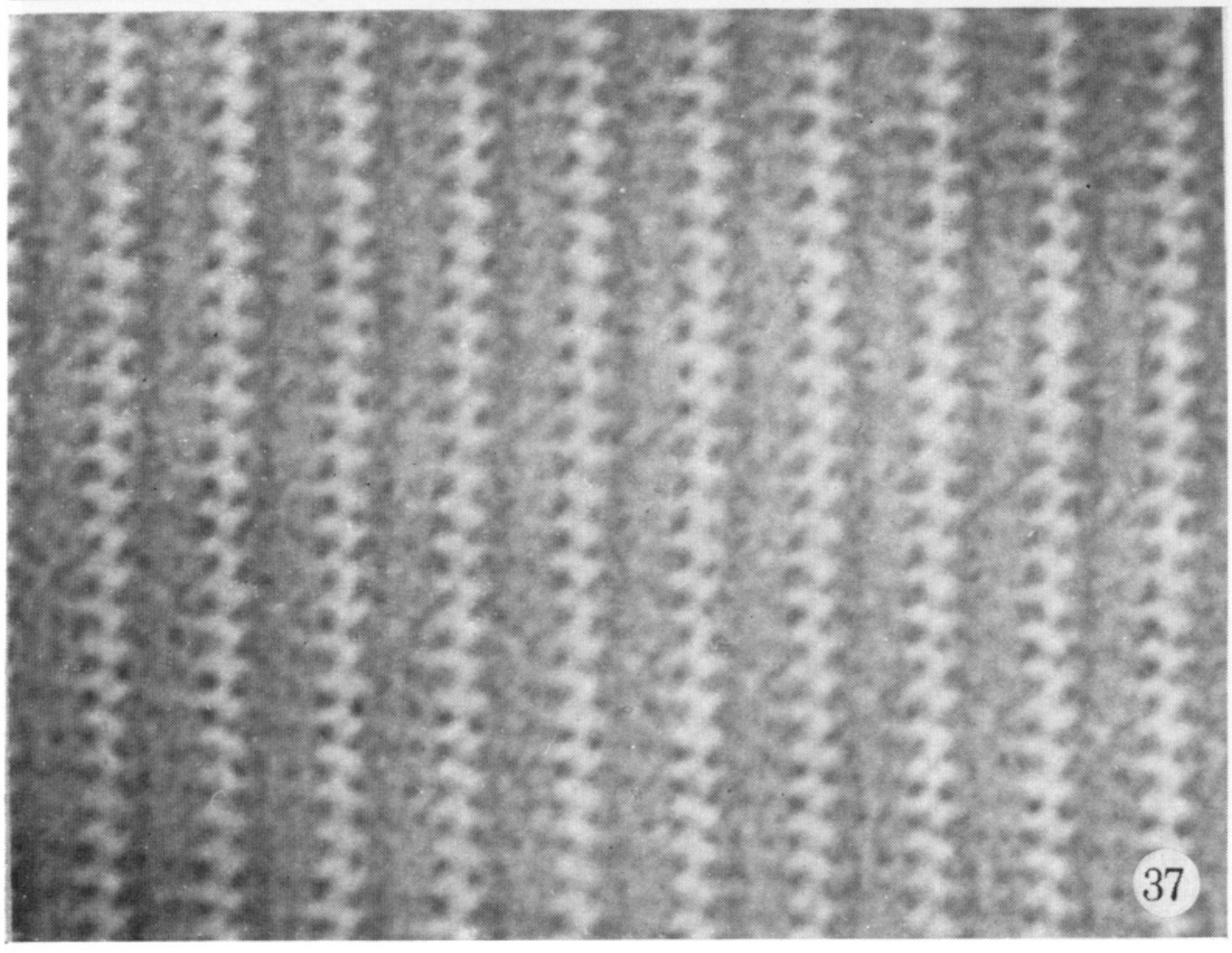


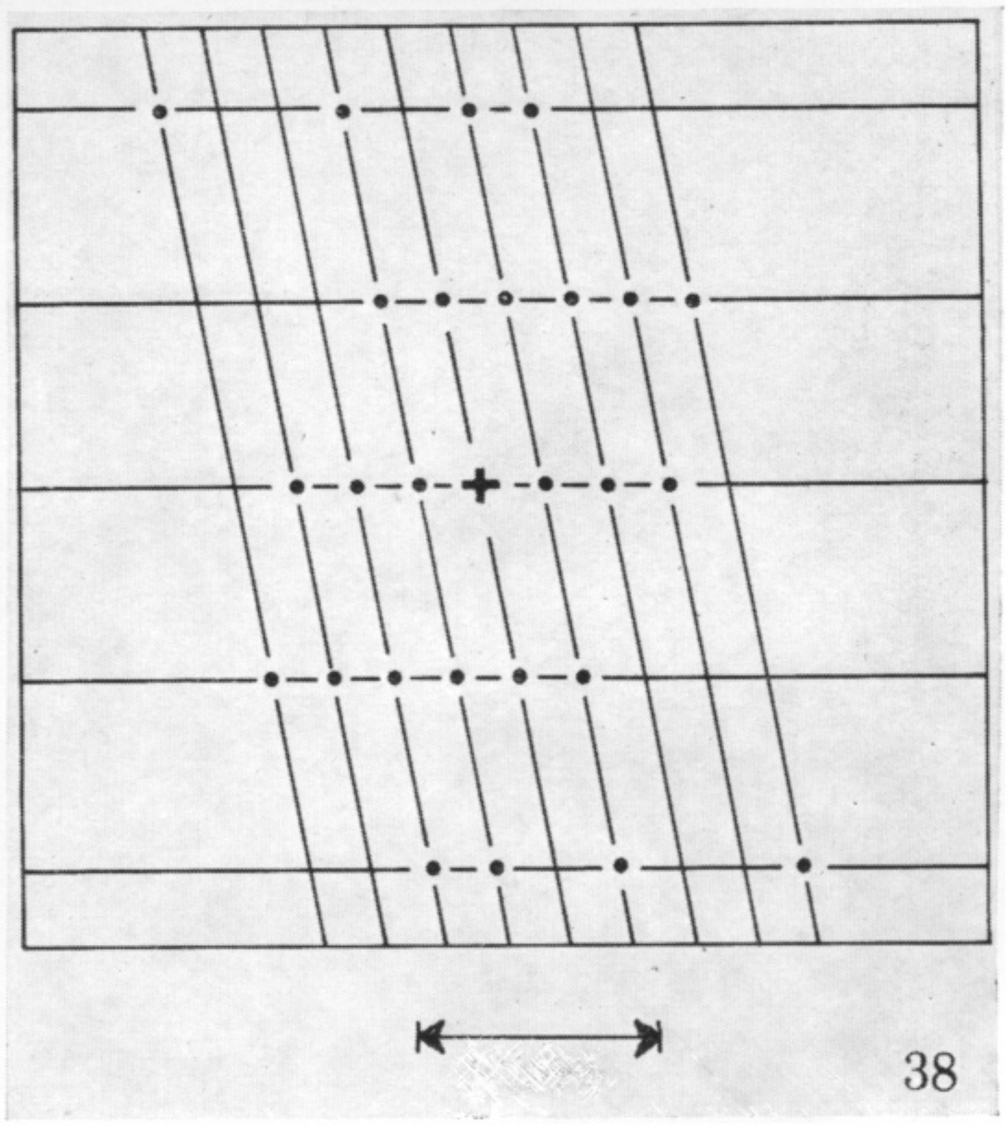




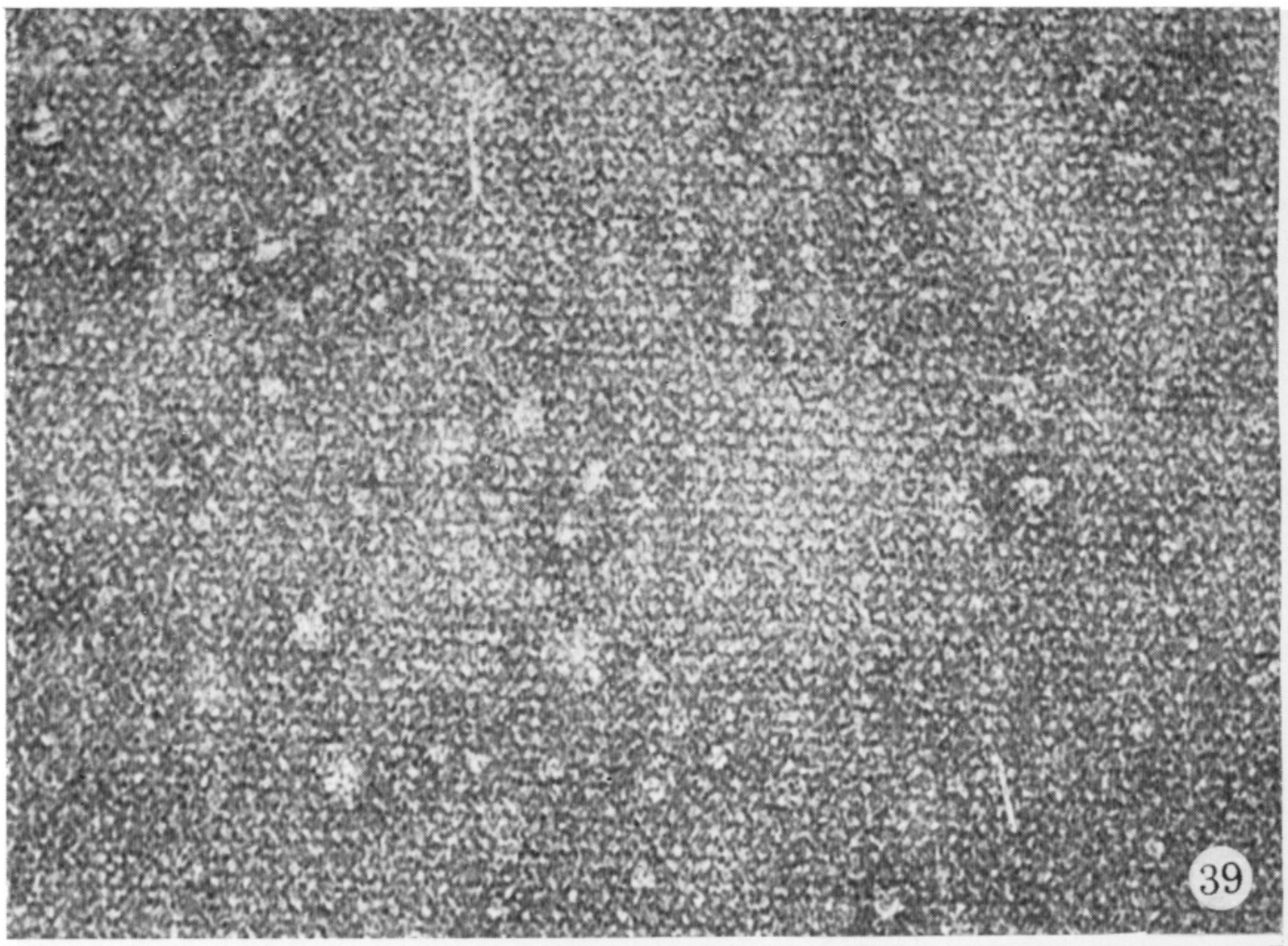


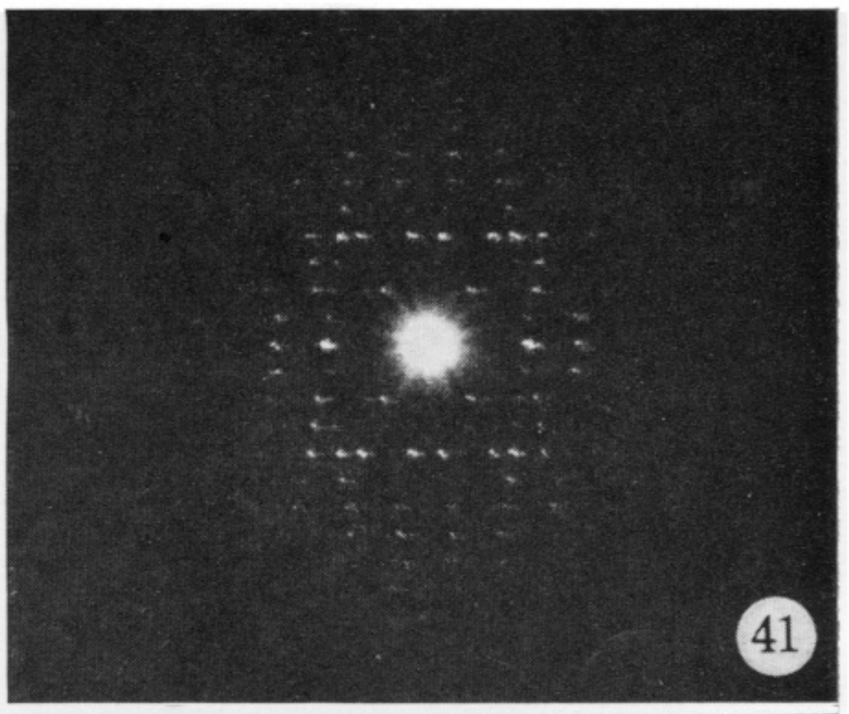


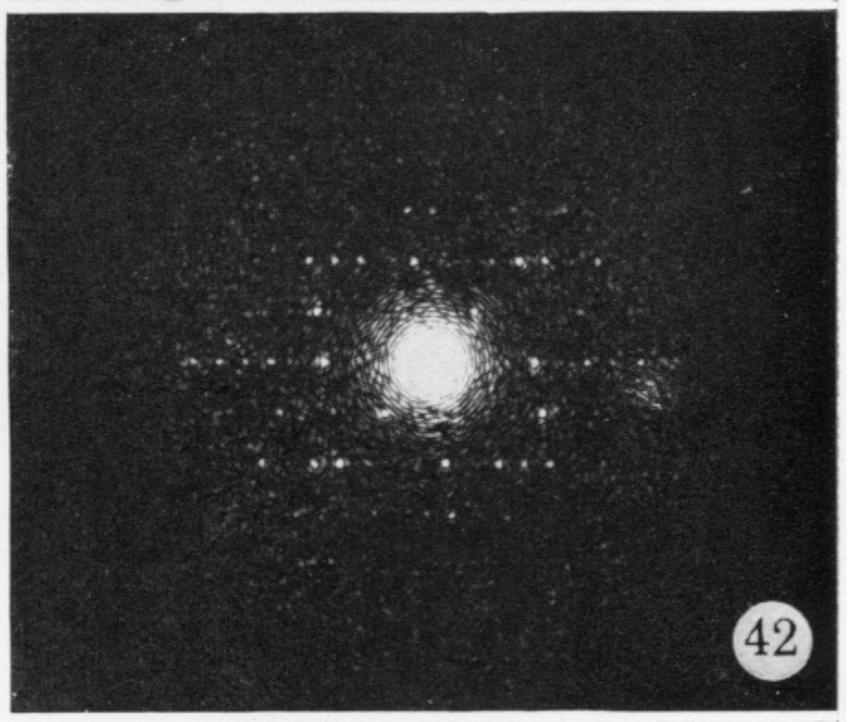


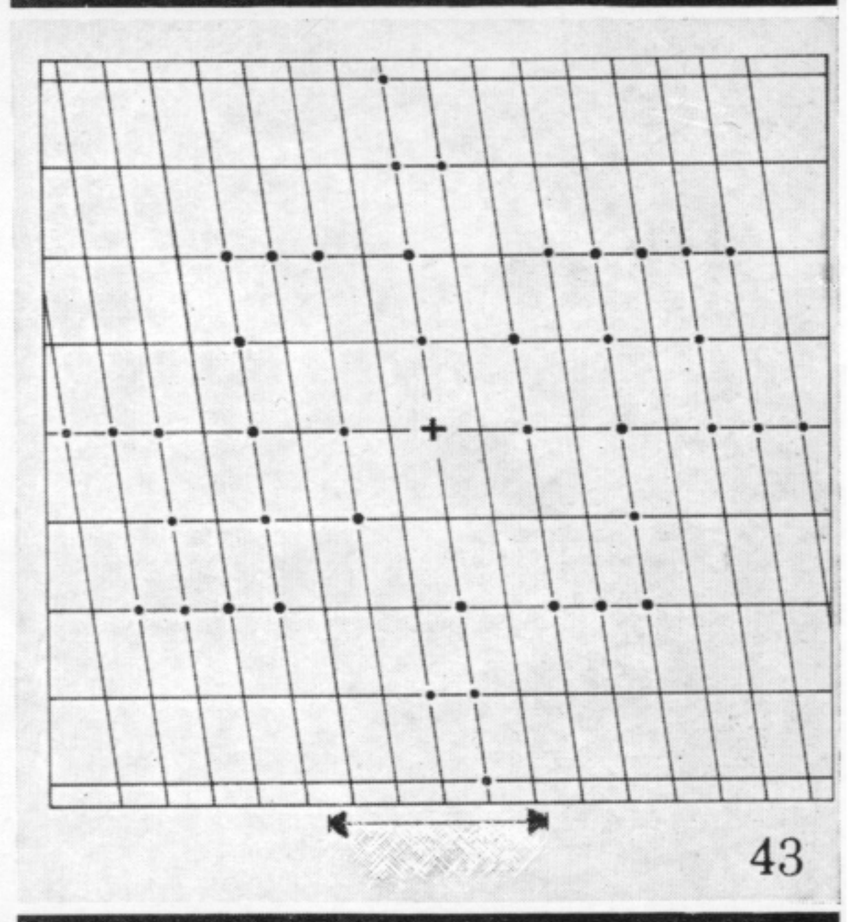


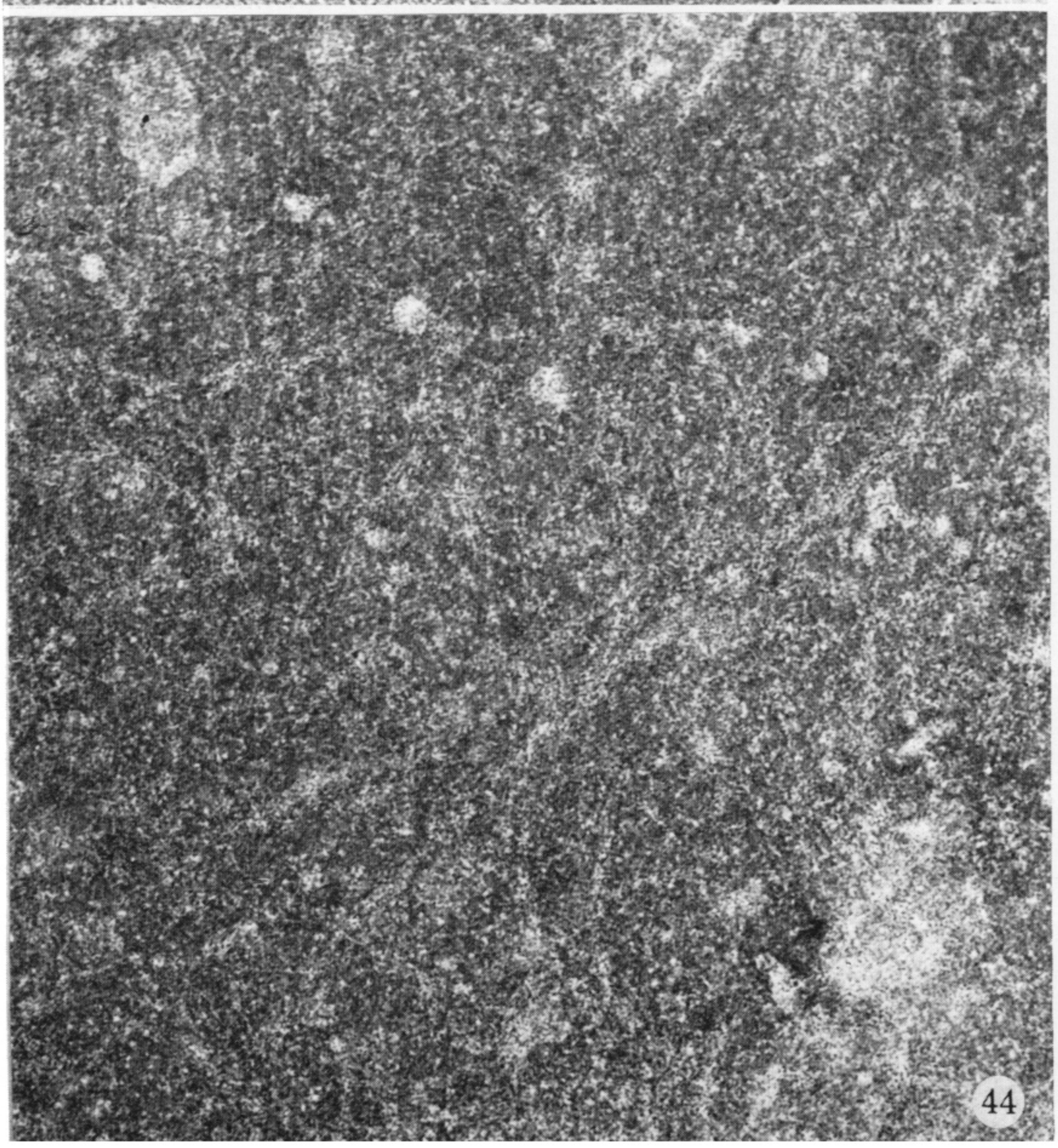
FIGURES 28–38. For description see opposite

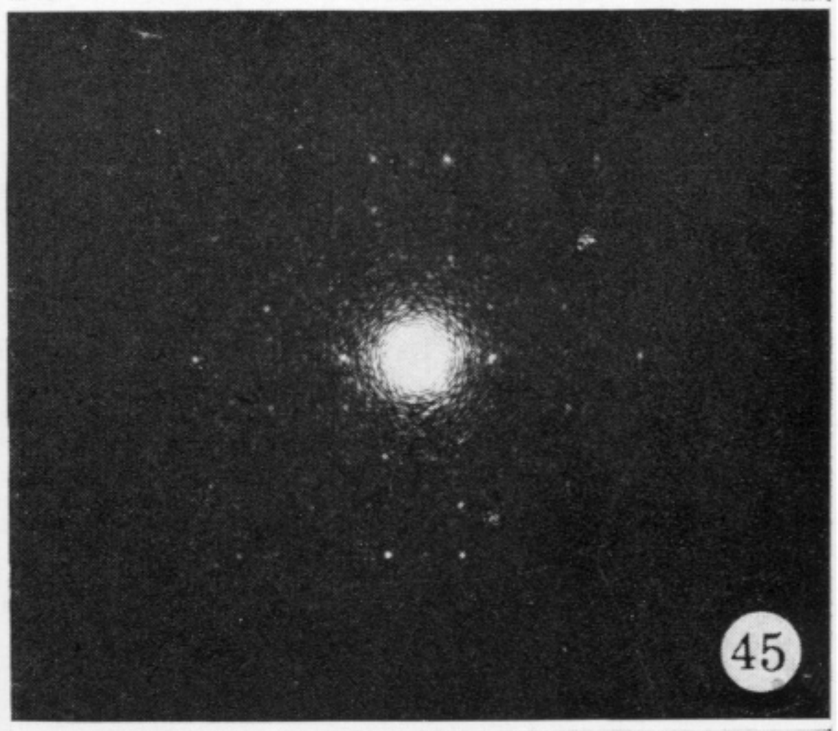


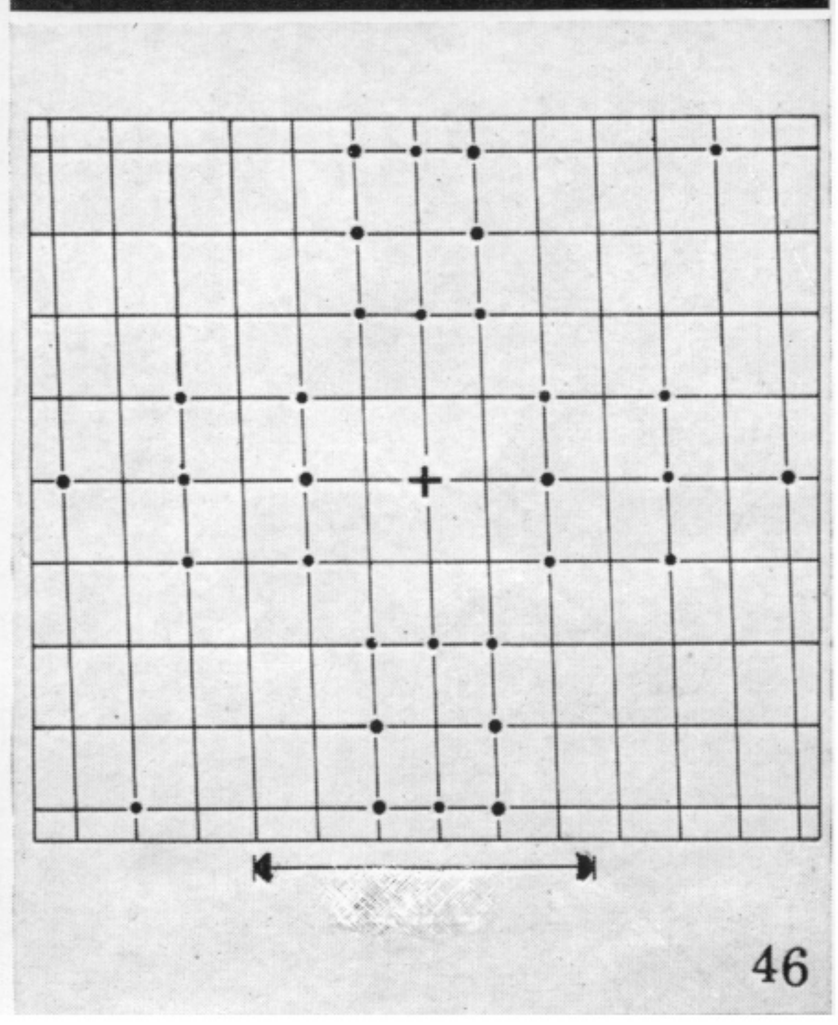




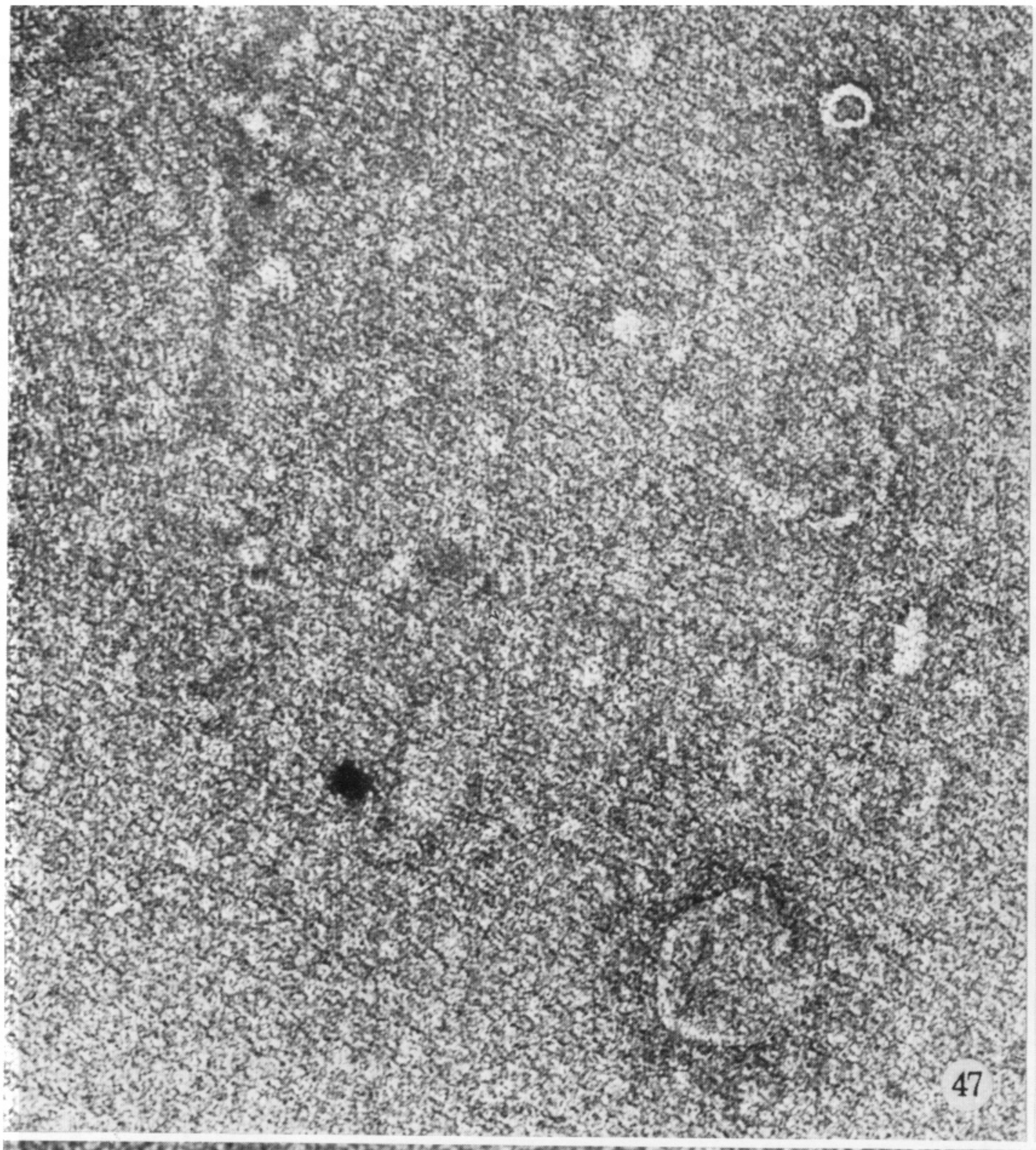


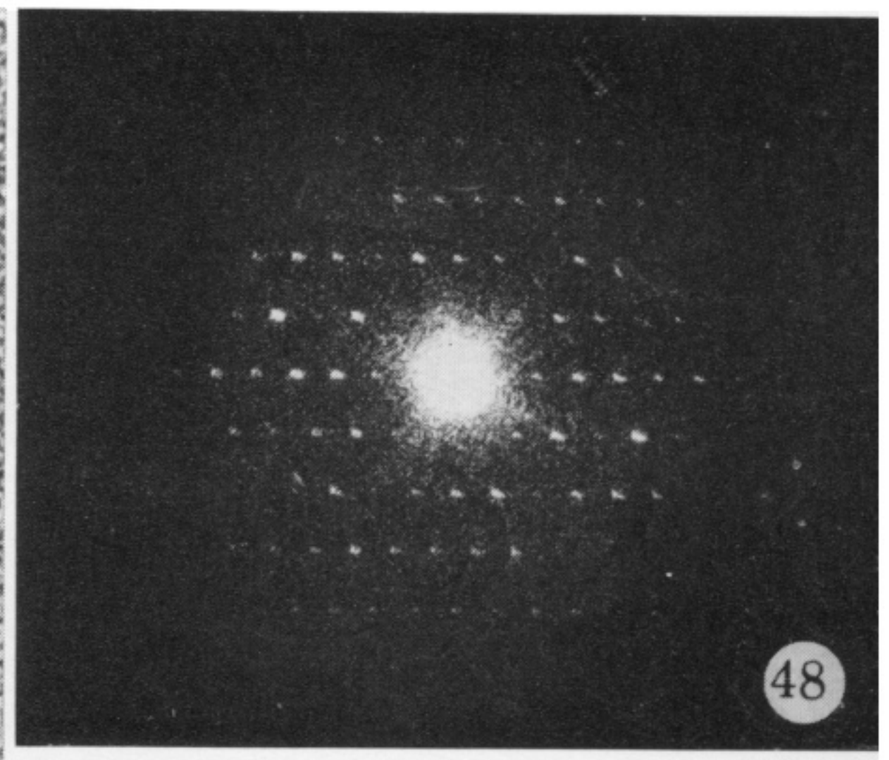


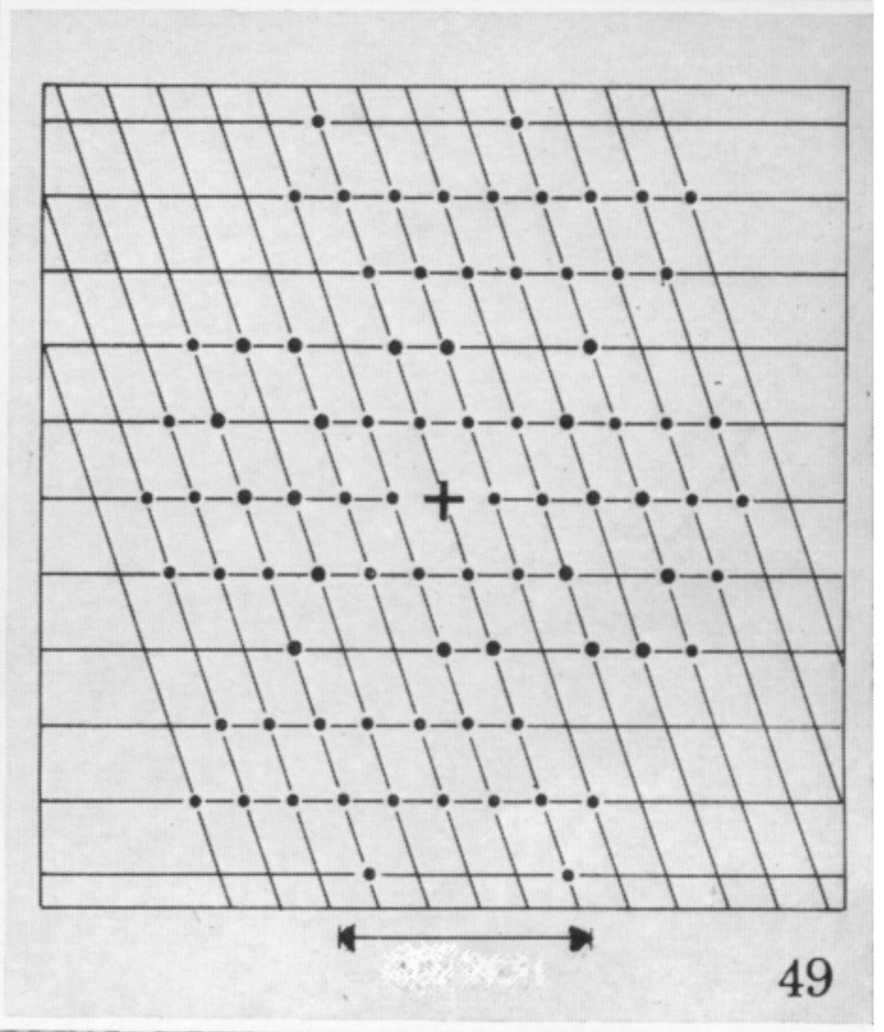


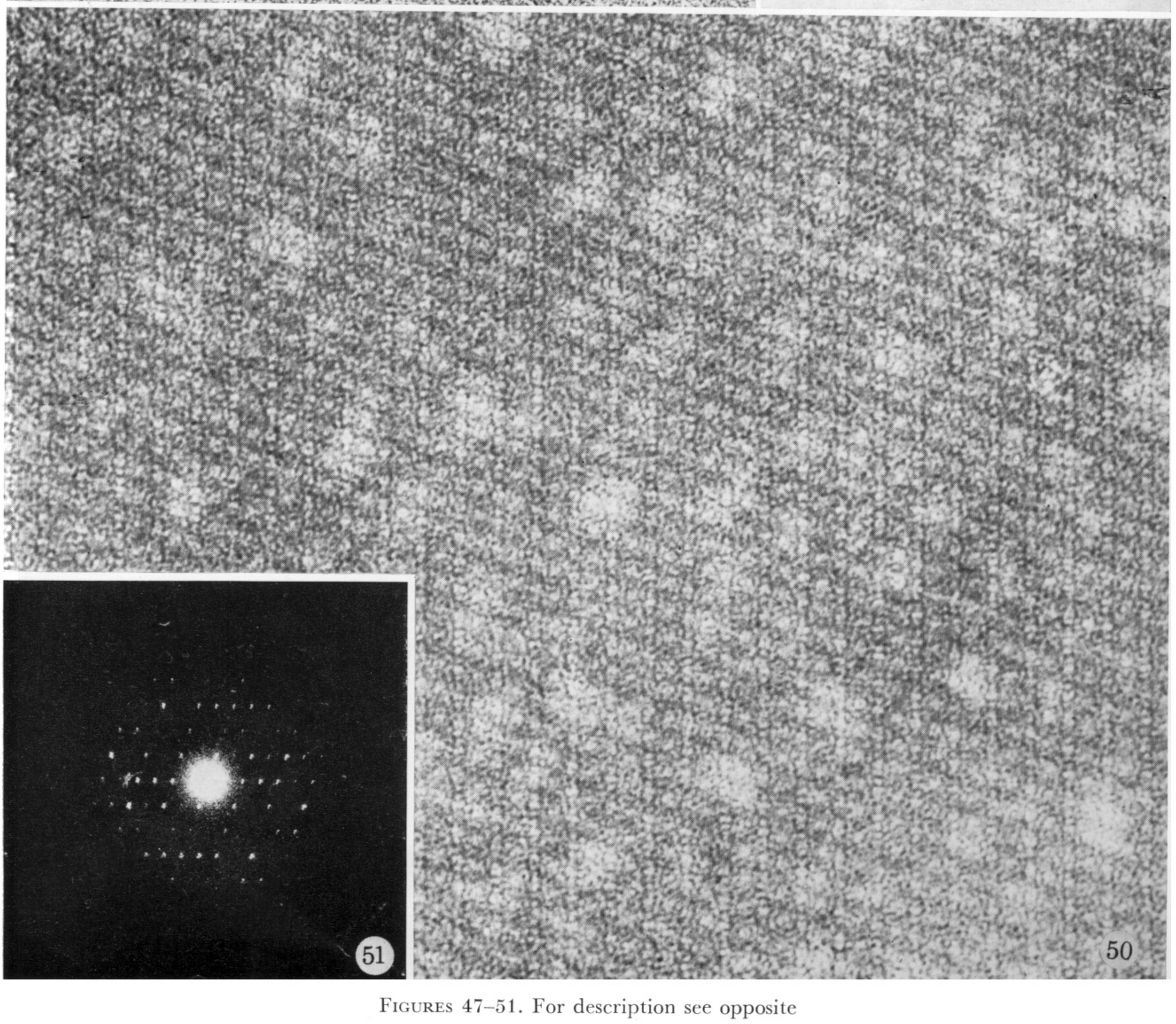


FIGURES 39–46. For description see opposite

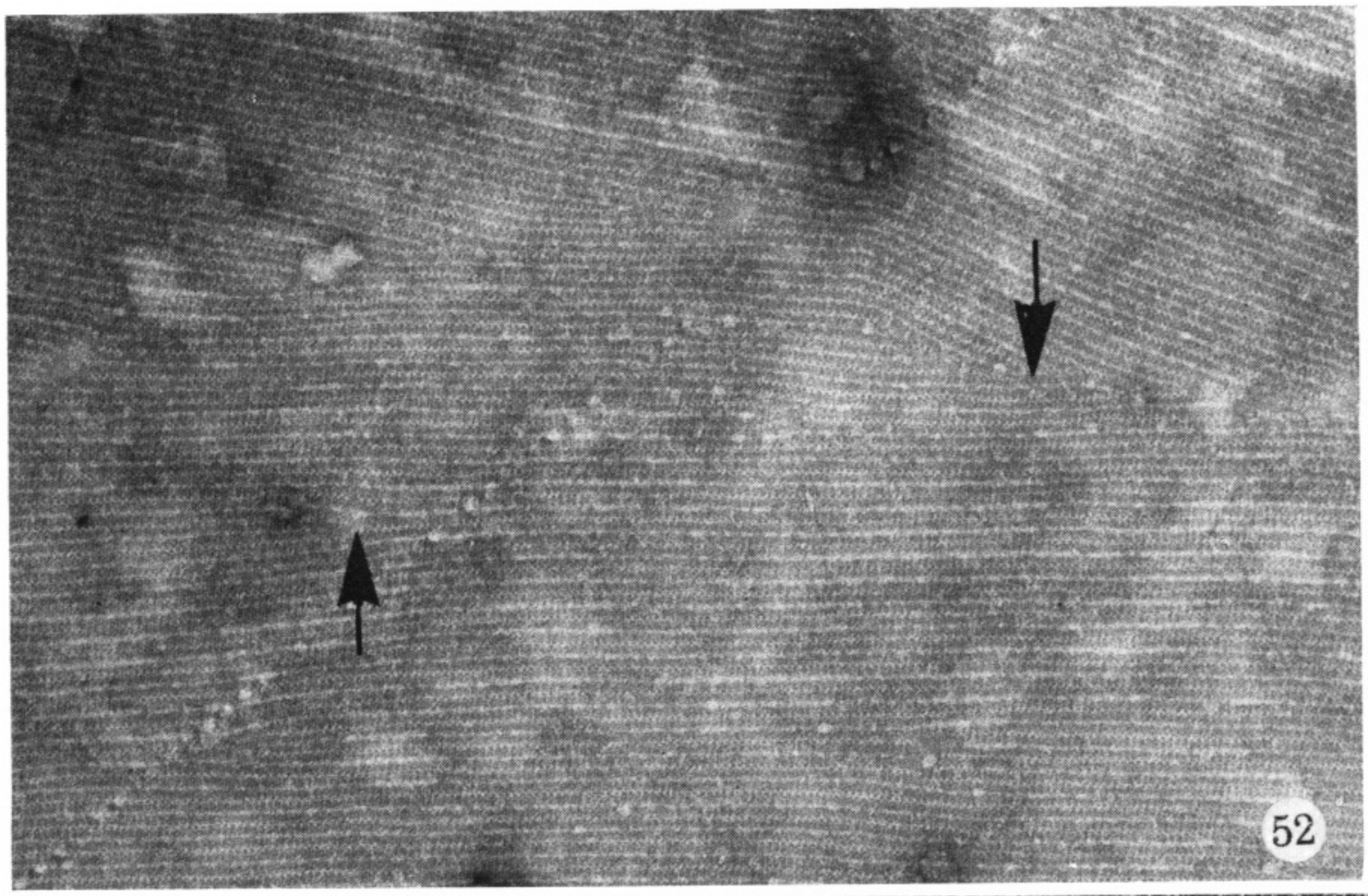


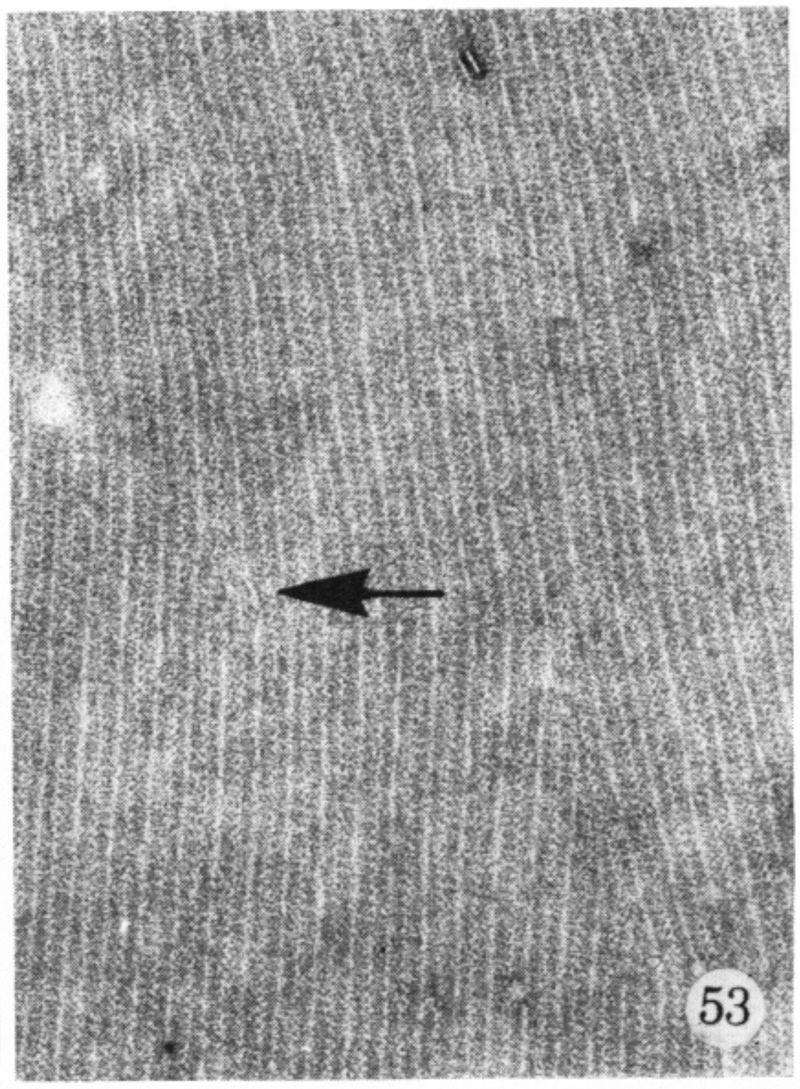


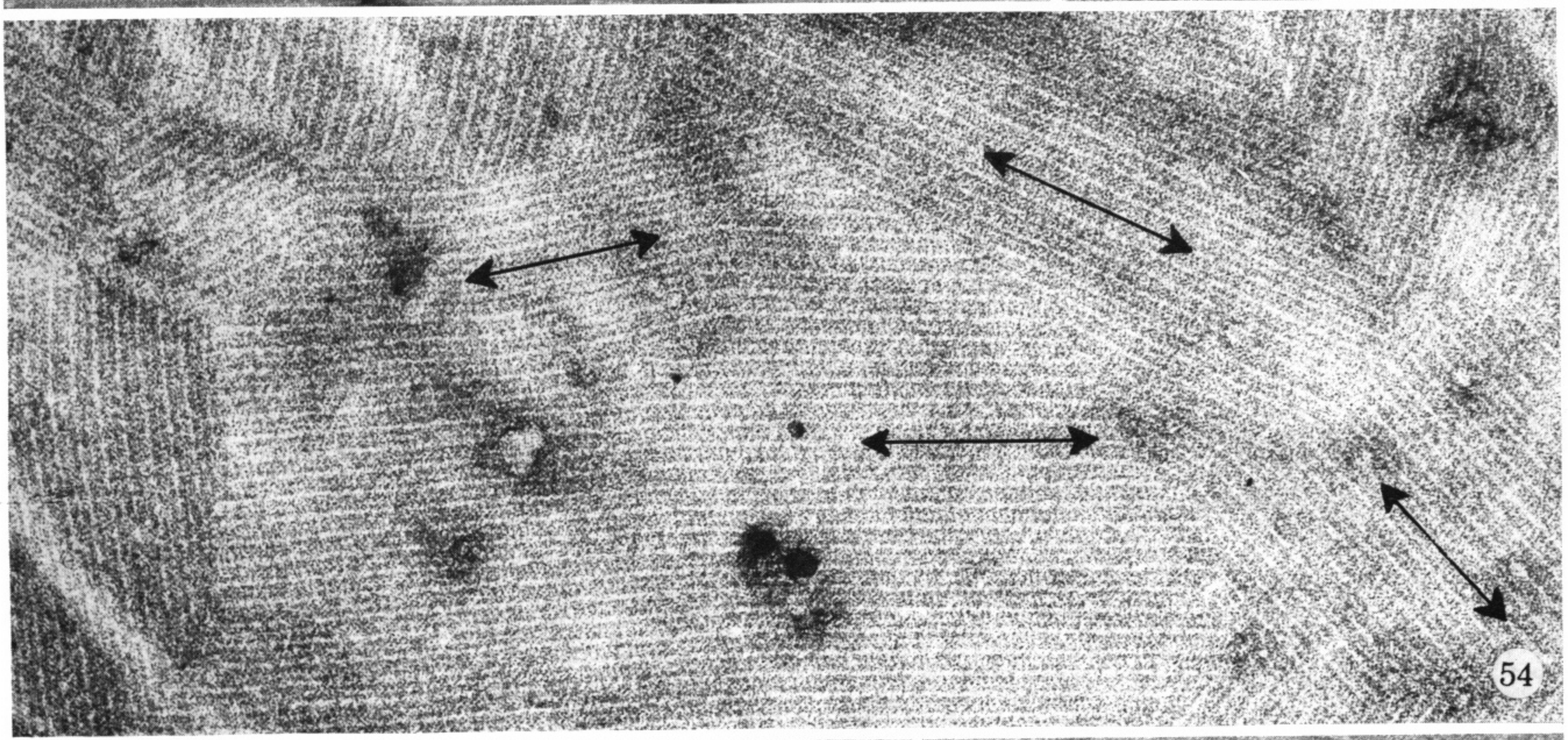


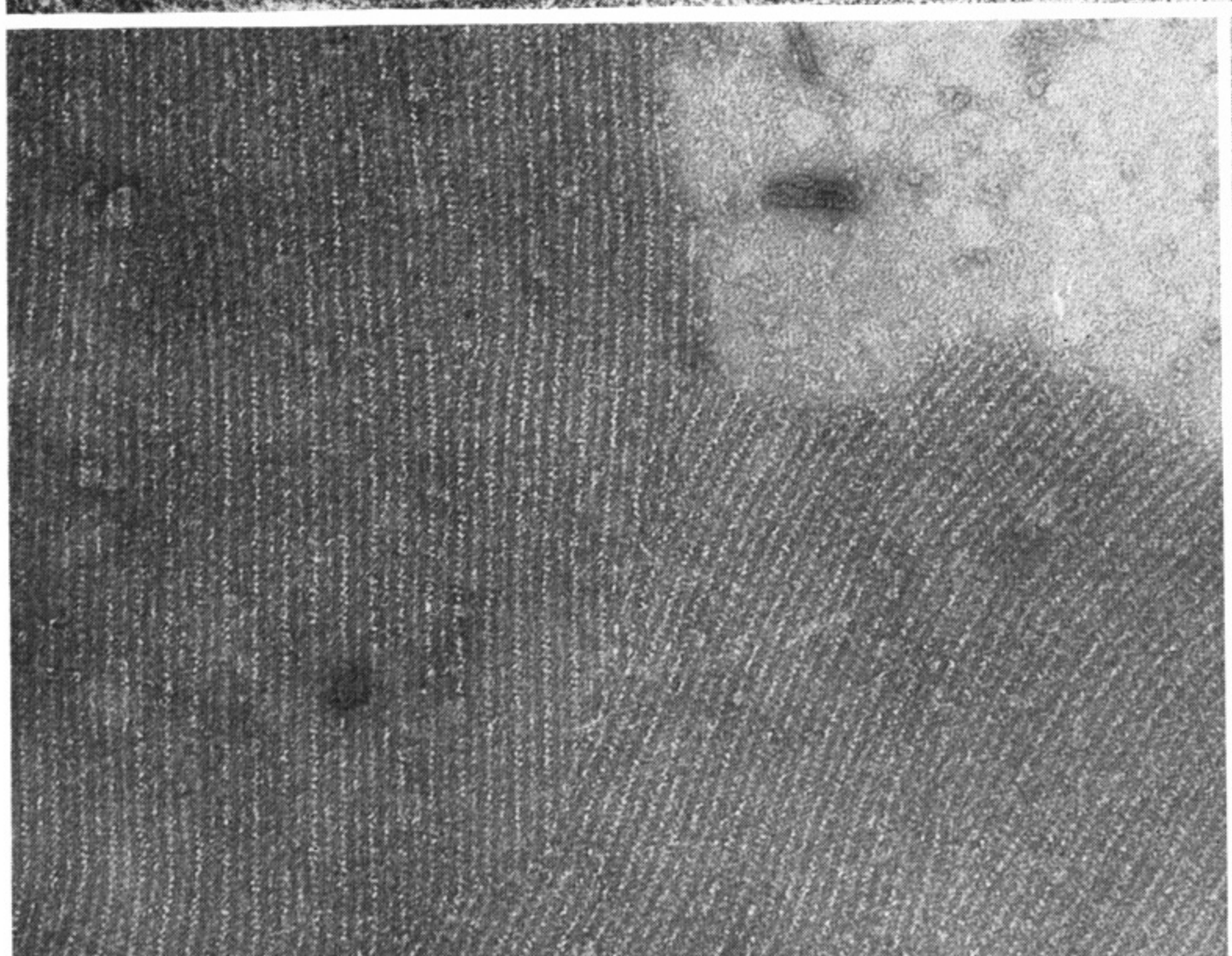


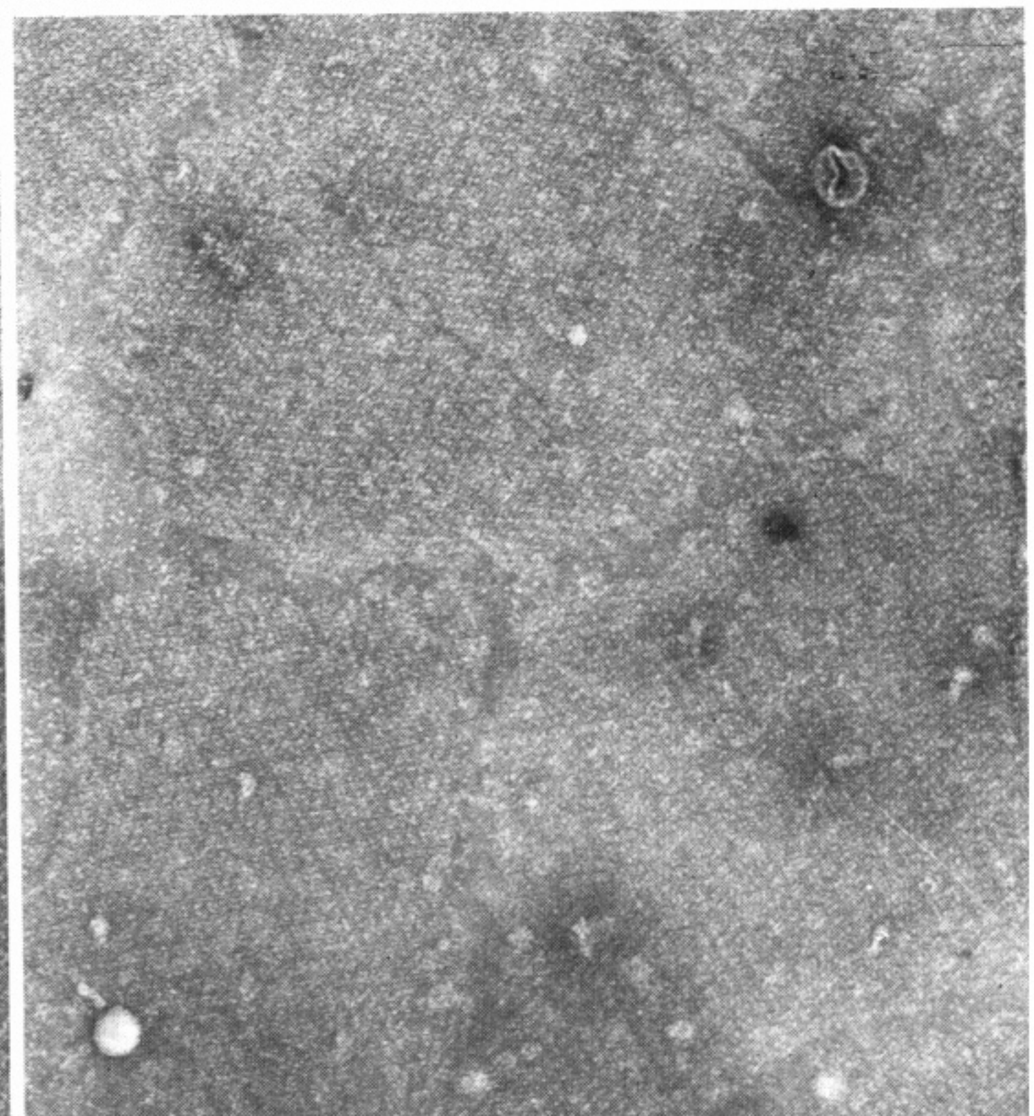
FIGURES 47–51. For description see opposite



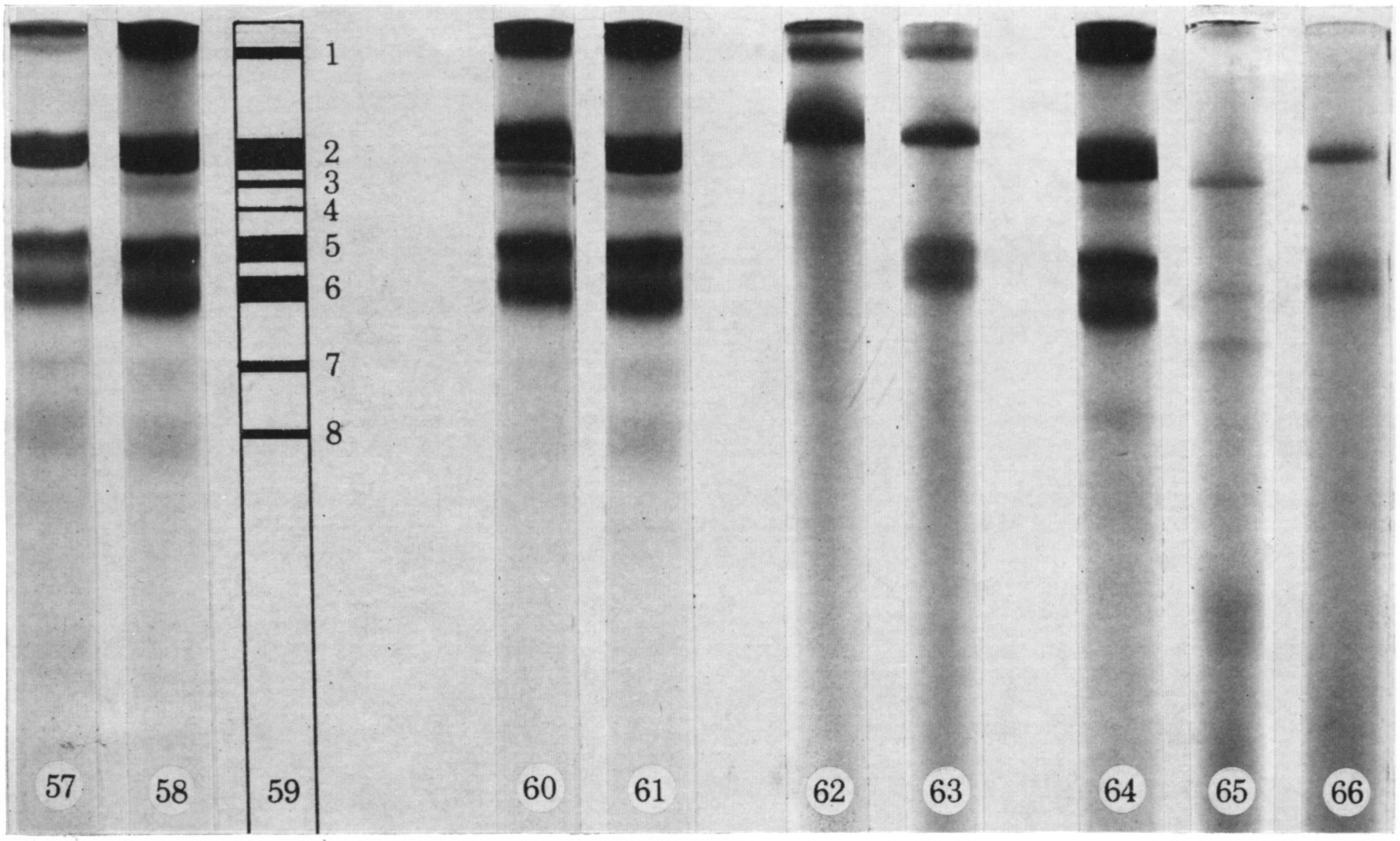


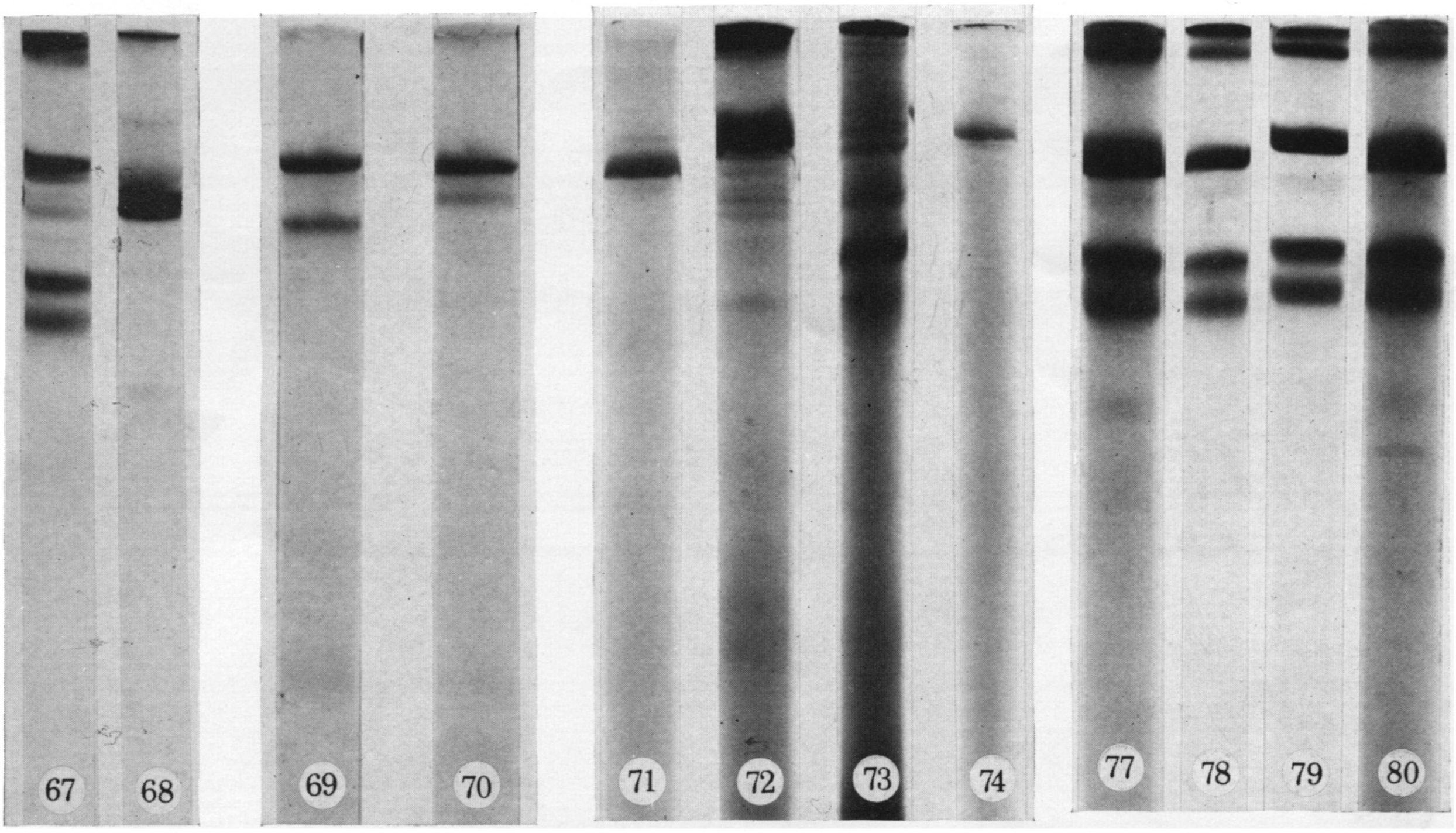


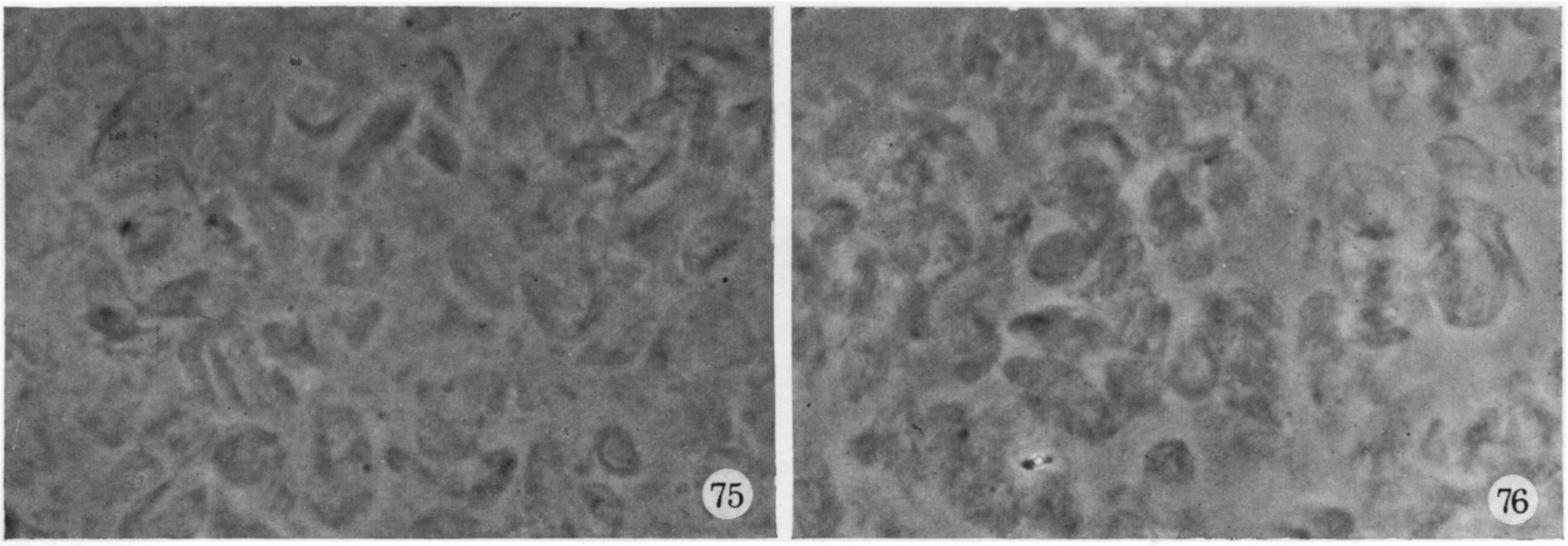




FIGURES 52–56. For description see opposite







FIGURES 57–80. For description see opposite