ON THE CELL-DIVISION AND MITOSIS IN SOME SOUTH INDIAN DIATOMS*

By R. Subrahmanyan, M.Sc.

Received September 24, 1945

(Communicated by Prof. M. O. P. Iyengar)

(With 88 Text-Figures and 1 Plate)

CONTENTS

Introduction

Lauterborn (1896) was the first to give a detailed account of mitosis in Diatoms. He gave an account of the nuclear division in five Diatoms, *viz.*, *Surirella Capronii* (= *S. calcarata*), *Navicula oblonga* (= *Pinnularia oblonga*), *Pinnularia viridis*, *Nitzschia sigmoidea* and *Gyrosigma attenuatum* (= *Pleurosigma attenuatum*). Other authors following him studied mitotic division in the following forms:—*Eunotia major* (Wisselingh, 1913), *Coscinodiscus subbuliens* (Ikari, 1923), *Achnanthidium brevipes* (Gemeinhardt, 1925), *Synedra ulna*, *S. pulchella* and *S. affinis* (Gemeinhardt, 1926), *Navicula peregrina* (Kolbe, 1927), *Cocconeis placentula* (Geitler, 1927), *Rhoicosphenia curvata* (Cholnoky, 1927 *a*), *Diatoma vulgare* (Cholnoky, 1927 *b*), *Navicula radiosa* and *Eunotia 'formica'* (Geitler, 1929), *Melosira granulata* (Kreiger, 1927), *Coscinodiscus biconicus* (Hofker, 1930), *Meredion circulare* (Geitler, 1932), *Biddulphia sinensis* (Schmidt, 1927, 1933), *Melosira arenaria* (Cholnoky, 1933), *Ditylum Brightwellii* (Gross, 1937/38), *Cyclotella Meneghiniana* (Iyengar and Subrahmanyan, 1944) and *Navicula halophila* (Subrahmanyan unpublished). In most of these cases all the details of the mitosis were not fully followed. Only a few stages were recorded in *Eunotia major*, *Synedra*

* From the Department of Botany, University of Madras.

Part of the Thesis approved for the Degree of Master of Science of the University of Madras.

pulchella, S. affinis, Navicula peregrina, Rhoicosphenia curvata, Meredion circulare and *Ditylum Brightwellii*; and, in *Achnanthidium brevipes, Synedra ulna, Eunotia formica* and *Melosira granulata* a few more stages have been recorded, but all the stages were not fully observed. Only in four cases were all the stages of the mitosis worked out so far, *viz.*, (1) *Surirella Capronii,* (2) *Melosira arenaria,* (3) *Cyclotella Meneghiniana* and (4) *Navicula halophila.*

Practically no work has been done on the cytology of any Diatom in India so far. At the kind suggestion of Professor M. O. P. Iyengar, the writer took up five Diatoms (three belonging to the Centrales and two to the Pennales), which occur commonly in Madras, for a detailed study of their mitosis and cell-division.

1. *Terpsinoë musica* Ehrenberg

This Diatom was found growing as an epiphyte on *Cladophora* and *Pithophora* in a pond inside the Agri-Horticultural Gardens at Madras. The Diatom grows in long zig-zag chains formed by the cells being held together by means of small mucilage pads at the corners (Pl. XXIX, Figs. 2 and 4).

Living material of the *Cladophora* and *Pithophora* with the Diatom growing on them was collected and kept in the laboratory in glass troughs containing the pond water. The Diatom was fixed throughout the twenty-four hours of the day at intervals of one hour each. As it was found difficult to separate the Diatom from the host alga, it was fixed along with the latter. Division generally took place in the evenings between 4 and 6 P.M.

The following fixing fluids were employed:—Chrom-acetic, Flemming's weak solution, Flemming-Benda, Schaudinn's sublimate—acetic alcohol Allen's Bouin (PFA_3), Zenker's fluid, Hofker's fluid, Gilson-Petrum-kewitsch and vom Rath's fluid (without platinic chloride). The Diatom was fairly well fixed in all these fluids, but PFA_3 and hot Schaudinn's fluid proved the most satisfactory. Of these two latter fluids, PFA_3 was slightly better than Schaudinn's.

Since after fixation the Diatom chains break up into the individual cells and also break away from the host alga, bits of the host filaments were frequently shaken in the fixing fluid, and later on in the washing fluids also, to free them of the Diatoms, which then fell to the bottom and got accumulated there. The accumulated Diatom sediment was generally mixed with a lot of dirt. In order to separate the Diatom from the dirt, the sediment was transferred to clear washing fluid in a petri-dish and the dish was gently rocked to and fro and then held somewhat slantingly. After about a couple

of minutes the dirt generally accumulated in the deeper portion of the liquid while the Diatom cells gathered together near the shallow side of the liquid in the dish from where they were carefully removed with the help of a fine pipette and transferred into a tube containing fresh washing fluid. By repeating this process several times, most of the cells could be separated.

The material separated in this manner was washed thoroughly in water or 50% alcohol (according to the fixing fluid employed) by the decantation method and after passing through the necessary grades was finally preserved in 70% alcohol.

The following stains were employed:—Heidenhain's iron-alum hæmatoxylin, safranin, gentian violet, Mayer's hæmalum, Newton's gentian violet, safranin-light green and safranin-gentian violet. Iron-alum hæmatoxylin proved to be the most satisfactory. Excellent preparations were also obtained by staining the material in Feulgen's stain.

Smear preparations of the material were made according to protozoological methods (McClung, 1937, pp. 530–31). The material was also stained *in toto*. The following schedule was followed for smear preparations. A small quantity of the material in 70% alcohol was spread on slides previously smeared with a thin coating of Mayer's albumen, and, before the alcohol dried up completely, the slides were placed in 85% alcohol and left there for about 5 minutes. The slides were then brought down the alcohol grades into water and then washed thoroughly, mordanted in 4% iron-alum solution for 1½ hours, washed in running water for 15 minutes and then stained in ¼% hæmatoxylin for about 4 hours. The staining was differentiated in saturated solution of picric acid. After thorough washing for 3 to 4 hours the slides were passed up through the alcohol grades, cleared in xylol and finally mounted in Canada balsam. Better preparations were obtained when the material was bleached in 10% hydrogen peroxide in 30% alcohol prior to mordanting. Material fixed in osmic fixatives (*e.g.*, Flemming's weak formula and Flemming-Benda) while being bleached was placed in the sun for thorough bleaching.

For staining *in toto*, the material from 70% alcohol was brought down to water and washed well, the changes from one liquid to another being made by decantation. The schedule mentioned above was followed for staining the material. After dehydrating and clearing, the material was transferred to dilute balsam for infiltration and slides were prepared when the balsam reached the desired consistency.

Aceto-carmine preparations also were frequently made for observing division figures. The material stained in aceto-carmine was made permanent as per method given by Lee (1937, p. 687).

Microtome sections of the Diatom were made after embedding the fixed material in paraffin. Sections were cut 20 μ thick. Some excellent views of prophase stages were obtained in these sections, especially valve views, which are otherwise very difficult to obtain owing to the thin and flat nature of the Diatom cell.

Vegetative cell.—The cells of the diatom are rectangular to square with rounded corners in girdle view (Text-Fig. 2) and linear-elliptical with undulations in valve view (Text-Fig. 1). In valve view the poles are capitate. The valves possess transapical septa, usually four in number; but, cells possessing six septa and small cells having only two septa were also frequently met with. The cytoplasm forms a lining layer and a large nucleus is suspended by cytoplasmic strands at the centre of the cell. Numerous disc-shaped or somewhat dumb-bell shaped golden yellow chromatophores are distributed throughout the cell more or less uniformly (Text-Fig. 2).

Mitosis.—The resting nucleus is usually spherical but often appears compressed. The nucleus shows one, occasionally two or three nucleoli and a well-stained reticulum. The chromatin granules stand out very clearly in the reticulum (Text-Fig. 3).

At the beginning of prophase, thin chromosomal threads are seen in the nucleus (Text-Fig. 4). The individual chromosomes next become clearly distinguishable (Text-Figs. 5 and 6). In the meanwhile the cell increases somewhat in volume, the valves loosening a little to allow for this increase. The nucleus still remains at the centre of the slightly enlarged cell. There is no movement of the nucleus in this Diatom towards one side as observed by Cholnoky (1933 *b*) in *Melosira arenaria* and by the writer in *Navicula halophila* (Subrahmanyan, unpublished).

In late prophase a large number of long thread-like chromosomes is seen distributed in the nuclear cavity (Text-Fig. 7; Pl. XXIX, Fig. 9). In the valve view a few chromosomes showed a slightly constricted portion, which was somewhat more lightly stained than the remaining portion of the chromosome. This probably represents the spindle attachment region.

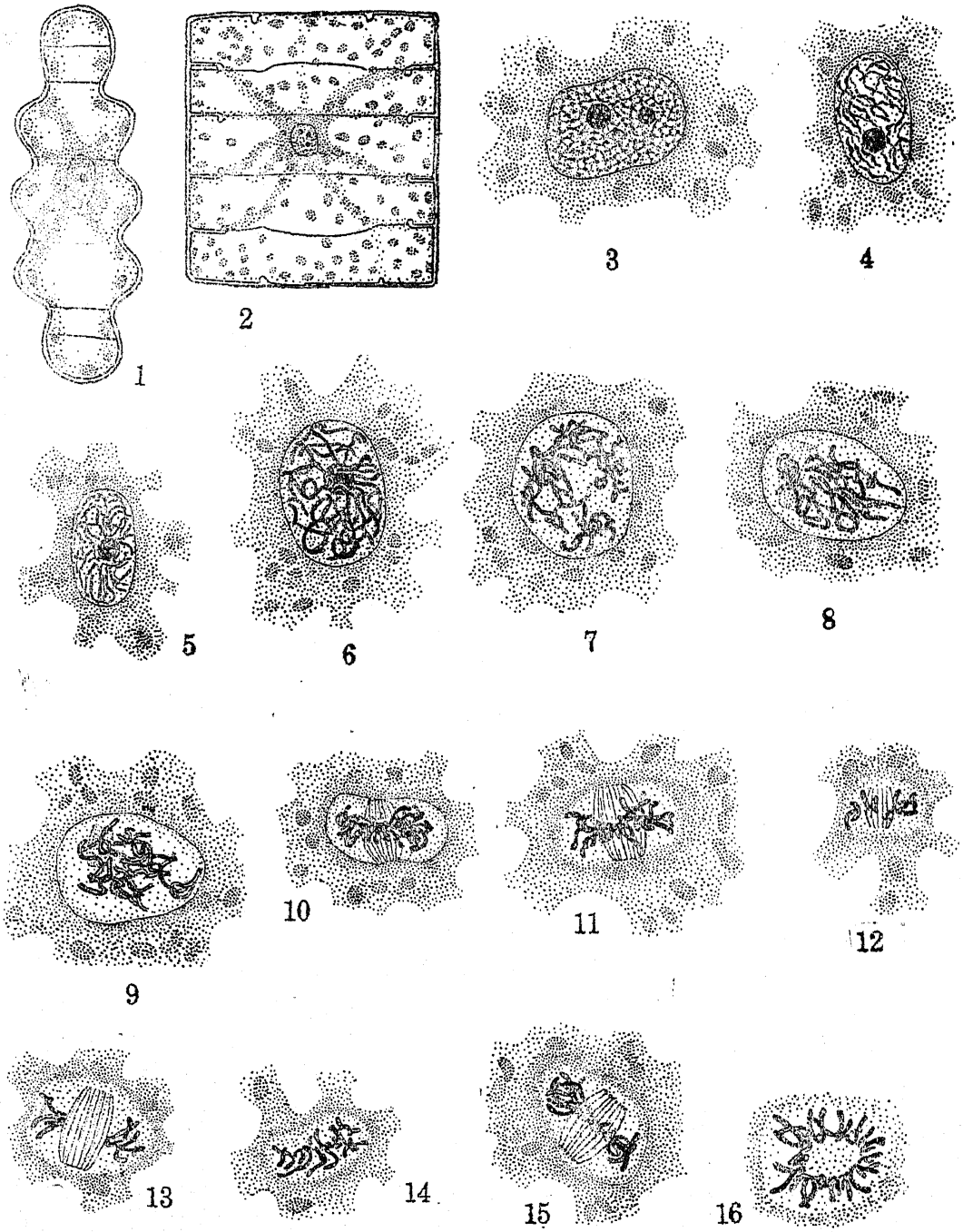
In late prohase there is no trace of the nucleolus (Text-Fig. 7). The nuclear membrane persists for a long time; in some instances it was seen even during metaphase (Text-Fig. 10). And even after the disappearance of the nuclear membrane the nuclear space is seen standing out clearly (Text-Fig. 11).

In early metaphase the chromosomes are seen directed towards one side of the nuclear cavity all around the spindle which becomes first evident

about this time (Text-Figs. 8 and 9). At late metaphase the chromosomes are seen arranged more or less in a ring around the spindle (Text-Fig. 16). The distribution of the chromosomes in the equatorial plane is not uniform. There are more chromosomes in one half of the ring than in the other, and a small gap could be seen in that half where the chromosomes are fewer (Text-Figs. 12–14). The spindle is barrel-shaped (Pl. XXIX, Fig. 7). No centrosome could be seen, though in a few preparations a few granules were observedat the poles of the spindle (Text-Fig. 15), as was found by Cholnoky (1933 *b*, p. 708) in *Melosira arenaria*.

The chromosome number could not be determined with certainty as no clear polar view of metaphase was obtained. Further, as the chromosomes are long and differently shaped and somewhat compactly arranged, individual chromosomes could not be recognised clearly. However, in an aceto-carmine preparation, a slighly oblique polar view of metaphase was obtained (Text-Fig. 16) in which the chromosome number could be approximately estimated as about 28 ($2n$). This number is supported by the counts in prophase stages.

The chromosomes appear longitudinally divided even in early metaphase (Text-Fig. 9). During anaphase the daughter chromosomes separate and move towards the poles of the spindle. The anaphasic figures (Text-Figs. 17, 18 and 20; Pl. XXIX, Fig. 8) are characterised by long trailing chromosomes which lie parallel to the axis of the spindle and in some cases the sister chromosomes could still be distinguished for quite a long time owing to their incomplete separation. Very early anaphase stages were not found in any of the preparations in spite of careful search of numerous preparations. Therefore, an attempt was made by the writer to follow with the help of water immersion objectives (N.A. ·75 and 1·18) the several stages of nuclear division in the living specimens to find out if by any chance any of the earlier stages of anaphase could be observed. Dividing cells could be easily recognised by the aggregation of the chromatophores around the nuclear cavity and also by the orientation of the chromatophores along the plane of division. At this stage, the nucleus, when carefully examined, showed a prophasic condition with the long thread-like chromosomes clearly distinguishable. The nucleus then passed into metaphase. In this stage the spindle is barrel-shaped and the chromosomes are seen arranged in a ring around the equator of the spindle. After late metaphase, the two sets of daughter chromosomes were observed to move away from each other very rapidly as if pulled apart by some force and finally come to lie at the poles of the spindle. This anaphasic movement was observed in dozens of individuals and in every case was found to be extremely rapid, the movement



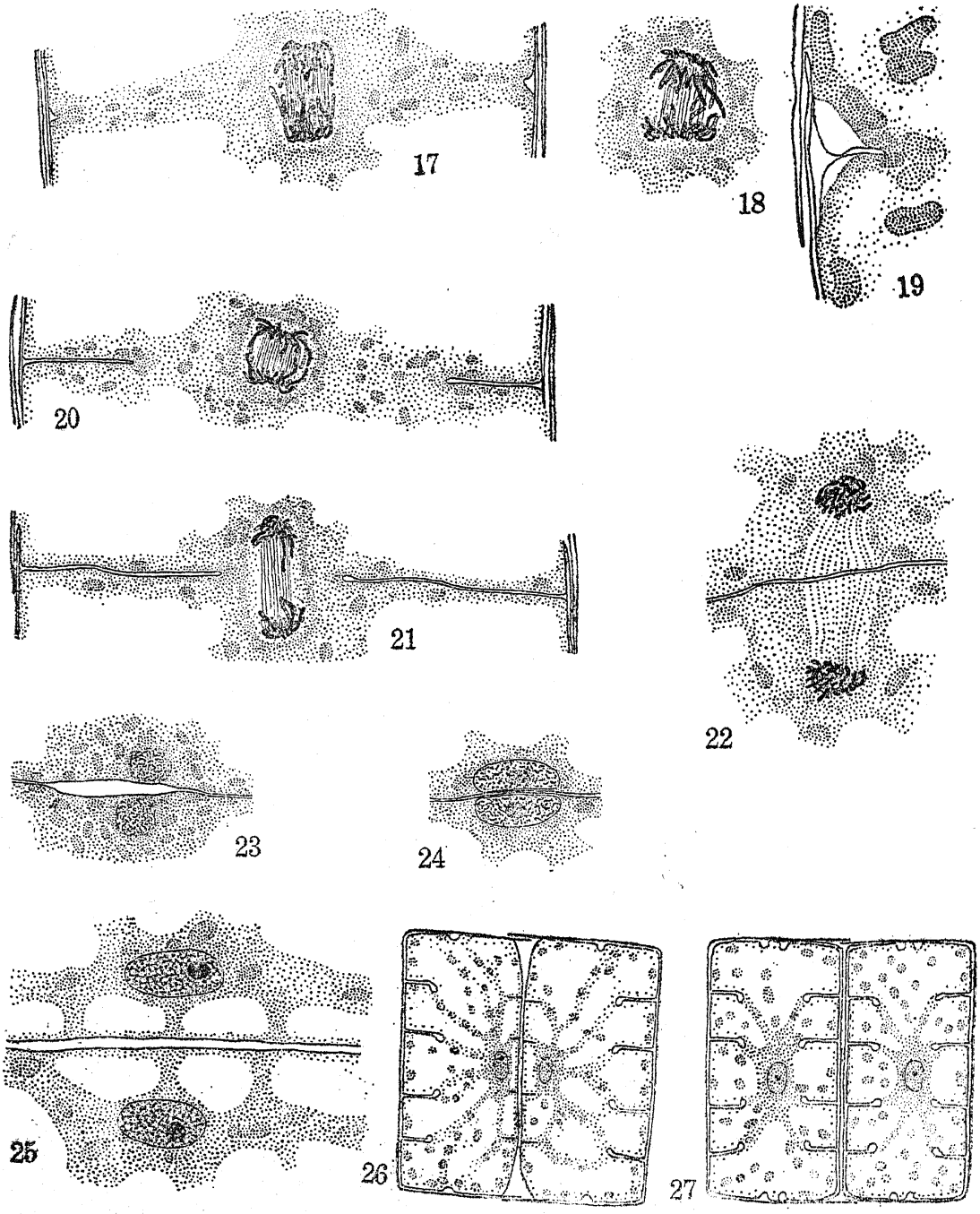
Text-Figs. 1–16. *Terpsinoë musica*.—Fig. 1. Cell showing nucleus and chromatophores (valve view). Fig. 2. Do. (girdle view). Fig. 3. Resting nucleus. Note vacuolate nucleoli. Figs. 4–5. Early prophase. Fig. 6. Prophase. Nucleolus still present. Fig. 7. Late prophase, seen from valve side; nucleolous disappeared. Figs. 8–9. Early metaphase. Fig. 10. Metaphase; nuclear membrane still intact. Fig. 11. Late metaphase; nuclear membrane disappeared. Figs. 12–14. Metaphase in three different foci, showing the arrangement of the chromosomes around the spindle. Fig. 12, upper focus; Fig. 13, median focus; Fig. 14, lower focus. Fig. 15. Metaphase, median focus showing tiny granules at the poles of

the spindle. Fig. 16. Metaphase, polar view. Note arrangement of the chromosomes in a ring; the spindle fibres seen as dots in the centre of the ring. Fig. 1, ×400; Fig. 2, ×275; rest, ×880.

taking hardly 4 to 5 seconds. This rapid separation of the daughter chromosomes during anaphase evidently explains the absence of early anaphase stages in the preparations. The writer was, however, able to fix some of the earlier stages of anaphase by keeping some cells on a slide under observation and quickly fixing it by running acetic-alcohol under the cover galss as the daughter chromosomes began to move apart. Aceto-carmine preparations were made of these stages. The stage shown in Fig. 17 was obtained by this method. It may be mentioned in this connection that, while investigating the life-history of *Ditylum Brightwellii*, Gross (1937/38, p. 7) was not able to observe even a single case of mid-anaphase. He presumed that the anaphasic movement in the Diatom took place with great rapidity. The writer's observations on the present Diatom (*Terpsinoë musica*) would appear to fully justify Gross' presumption that the anaphasic movement takes place very rapidly in his Diatom.

The spindle increases in length progessively as the daughter chromosomes move farther apart. This was observed in the living material. The chromosomes having moved as far apart as possible, begin to contract and show a clumped appearance (Text-Figs. 21 and 22) and soon the outline of the individual chromosomes becomes very difficult to distinguish. In telophase, the two groups of chromosomes are at first far apart (Text-Fig. 22), but after cytokinesis is complete the two nuclei come to lie very close to each other (Text-Figs. 23 and 24). Later on, as the new valves are being formed the daughter nuclei move to the centre of the respective daughter cells (Text-Figs. 25–27).

Cytokinesis.—Though several stages of cytokinesis were found in the fixed material, the actual details of cytokinesis as observed in the living specimens were most interesting. In the first place the process is extremely rapid and is completed within 3–4 minutes. Simultaneously with the rapid anaphasic movement of the daughter chromosomes, a cleavage furrow starts at the periphery of the cell (Pl. XXIX, Fig. 3) and gradually advances towards the centre of the cell. In optical section (in girdle view) it is seen as a small cleavage furrow starting just at the middle where the two valves overlap each other (Text-Figs. 17 and 19; Pl. XXIX, Fig. 6). The cleavage further advances inwards very rapidly until it reaches the spindle region of the division figure (Text-Fig. 21). But, after reaching the spindle region, the cleevage process becomes very much slowed up as if some kind of resistance is being offered by the spindle. Finally



Text-Figs. 17–27. *Terpsinoë musica.*—Fig. 17. Early anaphase. Note beginning of cytokinesis from two girdle sides (optical section); aceto-carmine preparation. Fig. 18. Anaphase. Fig. 19. Beginning of cytokinesis; from a specimen just killed with osmic acid. Fig. 20. Anaphase; cytokinesis advanced half way to the centre. Fig. 21. Late anaphase; cytokinesis nearing completion; aceto-carmine preparation. Fig. 22. Telophase; cytokinesis just completed; aceto-carmine preparation. Fig. 23. The two nuclei which were very much apart during late telophase (Fig. 22) have come very close to each other at this stage. Fig. 24. Daughter nuclei fully formed. Nucleolus lightly stained. Fig. 25. Daughter nuclei moving

towards the centre of their respective cells. Fig. 26. The two daughter cells. New transverse septa being formed at the centre. Note cytoplasmic strands connecting them with the nucleus. Fig. 27. The two daughter cells after formation of valves and transverse septa. Figs. 17, 20 and 21, ×575; Figs. 18, 22–25, ×890; Fig. 19, ×1170; Figs. 26 and 27, ×250.

the cleavage cuts through the spindle and the cytokinesis becomes complete. The whole process from the commencement of the cleavage furrow at the periphery of the cell up to the completion of the cytokinesis takes about three minutes and a half. The time taken for the cleavage to reach the spindle is about one minute and the time taken for the cleavage to cut through the spindle is about two minutes and a half. Such an extreme rapidity of the process as observed here does not appear to have been recorded previously among Diatoms. The only previous record appears to be by Gross (1937-38, p. 5) on *Ditylum Brightwellii*, where the process according to him takes 30 minutes.

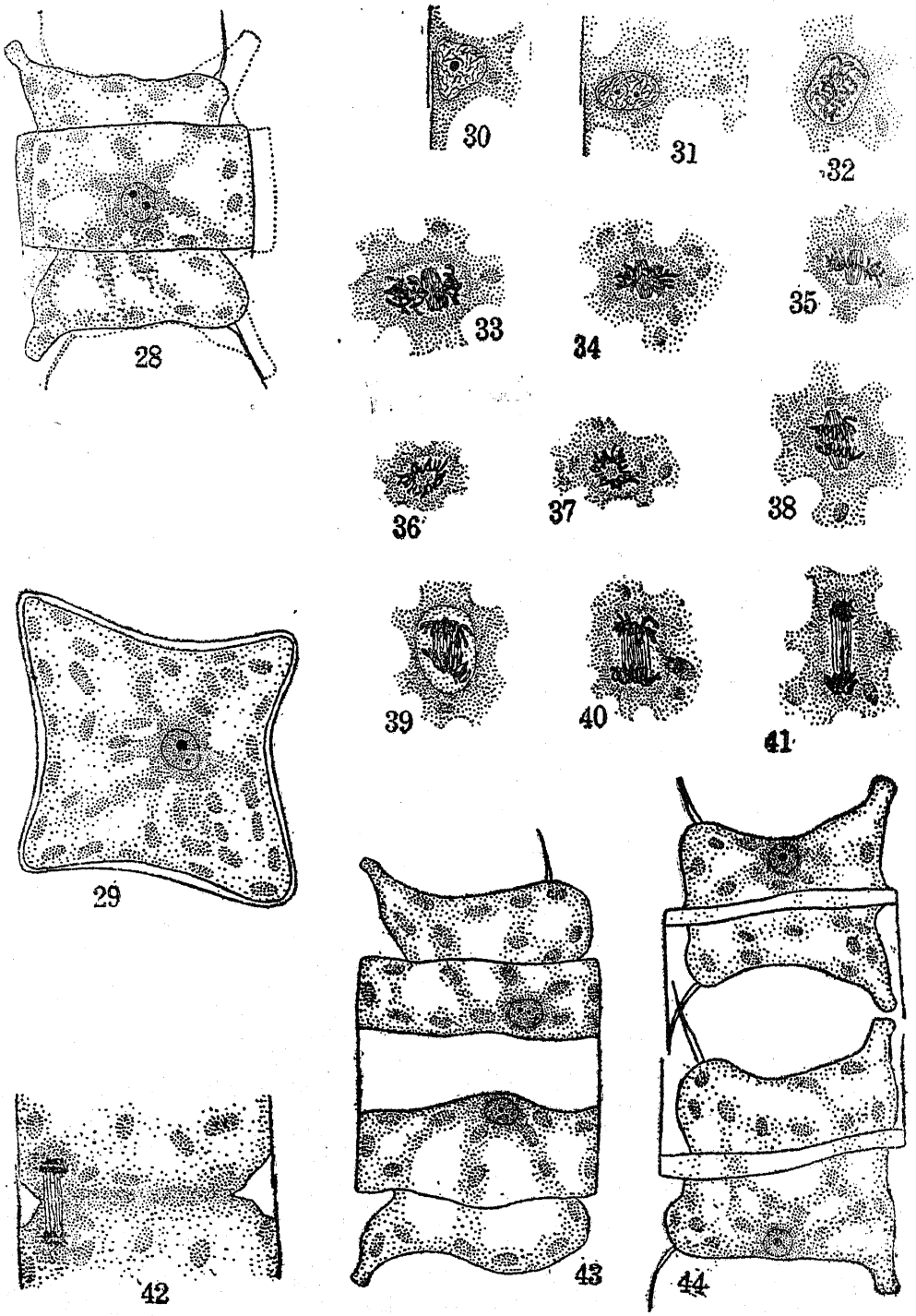
Soon after the completion of cytokinesis, each daughter protoplast secretes a silicified valve. After the new vavles are secreted by the protoplast the transverse septa start as small protuberances from the periphery of the new valves and grow inwards (Text-Figs. 26 and 27). In the early stages of their formation the growing septa are connected with the nucleus by definite cytoplasmic strands. The time taken after completion of cytokinesis to form new valves and transverse septa is about 3 to 4 hours.

2. *Triceratium dubium* Brightwell
(= *Biddulphia dubia* Cleve)

This Diatom occurs commonly in the marine plankton of the Madras coast. The Diatom was isolated from the plankton catch and grown in cultures in the laboratory. The methods employed for isolating and growing the Diatom in cultures were the same as those used for *Cyclotella Meneghiniana* (Iyengar and Subrahmanyan, 1944, pp. 127–28), only, instead of pond water, candle-filtered sea-water was used for making the culture solutions. Both agar and liquid cultures were tried. The Diatom did not thrive well in agar cultures, but grew very well in liquid cultures. The Diatom multiplied fairly rapidly in the cultures and formed very long chains (Pl. XXIX, Fig. 1).

For fixing and staining the Diatom the methods* employed were the same as those used for *Terpsinoë musica* Ehrenberg, only sea-water was used for making up the fixing solution. Of the several fluids employed only Flemming's weak solution and PFA_3 were found most suitable. The Diatom

* The methods are given in detail under *Terpsinoë musica*, only the deviations are indicated here and in the following forms.



Text-Figs. 28-44. *Triceratium dubium*.—Fig. 28. Cell in girdle view; (the dotted line shows cell outline in the lower focus). Nucleus in resting condition. Fig. 29. Cell in valve view. Figs. 30-31. Early prophase. Fig. 32. Late prophase. Figs. 33-34. Early metaphase with the spindle and the chromosomes arranged round it at the equatorial region. Fig. 35. Metaphase. Figs. 36-37. Metaphase in valve view. Note ring-like arrangement of the chromosomes.

Fig. 38. Late anaphase. Fig. 39. Early anaphase; note nuclear membrane still persisting. Figs. 40–42. Late anaphase; note beginning of cytokinesis in Fig. 42. Fig. 43. The two daughter protoplasts just separated after cytokinesis. Fig. 44. The two daughter cells after formation of new valves, horns and processes. All figs. ×1200.

chains were pipetted out from the cultures and transferred to the fixing fluid. In all the cases the chains broke up into the individual cells in the fixing fluid. Staining in iron-alum hæmatoxylin gave the best results. Bleaching for 15 minutes, mordanting for one hour and staining for three hours were found quite sufficient for this Diatom.

Vegetative cell.—The cells of *Triceratium dubium* are box-shaped. In girdle view, two angles show a stout horn-like prolongation and the other two angles show each a short, blunt process (Text-Fig. 28). In valve view the Diatom is usually four-sided (Text-Fig. 29) but three-sided and five-sided valves also were observed (*cf.* Hustedt, 1930, p. 807, Fig. 469). The valves and the girdles are strongly sculptured. The cytoplasm forms a lining layer in which are imbedded numerous yellowish brown chromatophores. The nucleus usually lies in the centre of the cell suspended by cytoplasmic strands (Text-Figs. 28 and 29).

Mitosis.—Material fixed during the early hours of the morning (4–6) A.M. showed plenty of division figures. The resting nucleus is spherical and shows one, rarely two or three nucleoli and a lightly stained reticulum. (Text-Figs. 28 and 29).

Just prior to division the nucleus, which usually lies at the centre of the cell in the resting condition, moves towards one side of the cell and remains in the lining layer of cytoplasm where all the stages of division take place. A similar behaviour of the nucleus was observed by Cholnoky (1933) in *Melosira arenaria* and by the writer in *Navicula halophila* (unpublished). The cell about this time increases somewhat in volume, the valves loosening a little to allow for this increase in size.

During prophase the reticulum is stained deeper and thin chromosomal threads appear in the nuclear cavity (Text-Figs. 30 and 31). In late prophase the chromosomes become slightly thicker and shorter and are distributed more or less evenly in the nuclear cavity (Text-Fig. 32).

During metaphase, the spindle, which is barrel-shaped, becomes evident (Text-Figs. 33 and 34); and the chromosomes, which are long and variously shaped, are seen arranged in a compact ring round the spindle at its equatorial region (Text-Figs. 35–37). The distribution of the chromosomes is more or less even in the ring unlike in *Terpsinoë musica* where they are slightly unequally distributed. The nucleolus disappears at about

early metaphase. The nuclear membrane also disappears about this time. But occasionally it was seen persisting till anaphase (Text-Fig. 39).

The exact chromosome number could not be accurately determined since the chromosomes are many and very compactly arranged. As far as the writer was able to count, the number of chromosomes appeared to be 26–30 ($2n$).

In anaphase the daughter chromosomes separate and move towards the poles of the spindle (Text-Figs. 38, 39 and 40). The chromosomes in anaphase lie somewhat parallel to the axis of the spindle. After reaching the poles of the spindle the chromosomes contract; at this stage it is very difficult to make out the outline of the individual chromosomes (Text-Figs. 41 and 42). During telophase the daughter nuclei are organised. The daughter nuclei which are at first close to each other (Text-Fig. 43) finally move to the centre of the respective cells. Occasionally they move to the opposite ends of the cells and remain in the parietal layer of the cytoplasm (Text-Fig, 44).

Cytokinesis.—During anaphase cytokinesis commences. In optical section it is seen to commence as a small cleavage furrow near the periphery of the cell and the furrow progresses centripetally (Text-Fig. 42) and finally the protoplast is divided into two. As soon as the fission is complete, the daughter protoplasts appear to contract a little leaving a fairly wide space in between (Text-Fig. 43). A similar phenomenon was observed by Gross (1937/38) in *Ditylum Brightwellii.* After this the new valves with their characteristic markings and processes are soon formed.

The daughter cells generally do not separate immediately after division but remain attached to each other by means of a mucilage pad formed at one of the angles. In this way long zig-zag chains are formed, the mucilage pads generally being formed in diagonally opposite angles in alternate cells.

3. *Biddulphia mobiliensis* Bail.

The only previous account of this Diatom appears to be by Peragallo in 1907. Unfortunately the writer could not get Peragallo's original paper. But a brief summary of Peragallo's observations together with his figures of cell division is given by Oltmanns (1922, Bd. I, p. 128). Peragallo's observations regarding nuclear division as far as could be judged from the account given by Oltmanns appear to be meagre and not quite satisfactory. Oltmanns states finally that further observations should be awaited on this Diatom.

This Diatom was found in the marine plankton of the Madras coast. It was isolated from the other forms and grown in cultures in the laboratory.

The same procedure was followed as for the previous Diatom (*Triceratium dubium*) for isolating the Diatom and growing it in cultures in the laboratory. For fixing and satining, the same methods as those used for the previous forms were employed. Fixing in PFA_3 and staining in iron-alum hæmatoxylin gave the best results.

Vegetative cell.—The cells of *Biddulphia mobiliensis* Bail. are box-shaped in girdle view and elliptical in valve view. In girdle view the corners are prolonged into horns and between the horns two long spines are situated on a slightly raised portion. The cells at times form long or short chains. The cytoplasm forms a lining layer and the nucleus is suspended by cytoplasmic strands at the centre of the cell. Numerous disc-shaped and somewhat slightly lobed brownish-yellow chromatophores are seen distributed at the periphery of the cell (Text-Fig. 45).

Mitosis.—Material fixed between 4 and 6 A.M. showed plenty of division figures, but dividing cells were also seen frequently at other times of the day in the cultures. The resting nucleus is spherical and shows one, rarely two nucleoli and a well-stained reticulum. Prior to division the cell increases in volume, the valves loosening slightly to allow for this increase. The nucleus during division is seen situated in the parietal layer of cytoplasm as in the previous form.

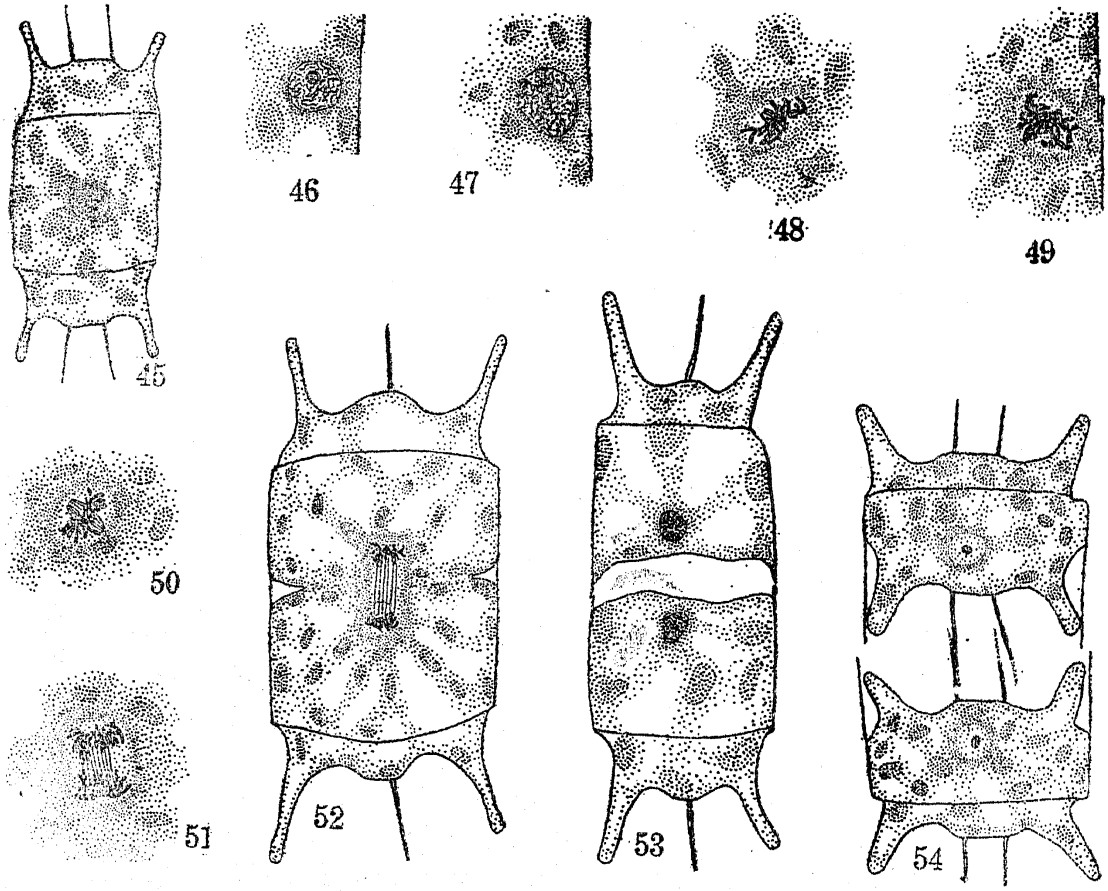
In early prophase thin chromosomal threads are seen in the nuclear cavity (Text-Figs. 46 and 47). In late prophase the chromosomes become shorter and thicker and are seen distributed in the nuclear cavity (Text-Fig. 49). The nucleolus and nuclear membrane disappear about late prophase, sometimes early metaphase.

During early metaphase the chromosomes move to the equator of the spindle which becomes evident at about this stage (Text-Fig. 48). A little later the chromosomes are seen arranged in a ring at the equator of the spindle (Text-Fig. 50). Good polar views of metaphase were not observed but the ring-like arrangement could be clearly made out by proper focussing.

The number of chromosomes could not be determined with certainty owing to the compact arrangement of the chromosomes. Their number appeared to be about 20 ($2n$).

In anaphase the daughter chromosomes move to the poles of the spindle (Text-Figs. 51 and 52) and in telophase the daughter nuclei are organised.

Cytokinesis.—Cytokinesis begins during anaphase as in the previous cases. It is seen in optical section as a clevage furrow, which advances in a centripetal manner and cuts the protoplast into two (Text-Figs. 52 and 53). Cytokinesis took place even more rapidly than in *Terpsinoë musica.* The time taken from the commencement of the furrow at the sides of the cell to the completion of the division of the protoplast was less than 30 seconds. The division was so rapid that it was impossible to make any camera lucida drawings of the stages.



Text-Figs. 45–54. *Biddulphia mobiliensis.*—Fig. 45. Vegetative cell in girdle view. Figs. 46–47. Early prophase. Fig. 48. Early metaphase. Note the spindle. Fig. 49. Late prophase. Fig. 50. Late metaphase. Fig. 51. Anaphase. Fig. 52. Anaphase; note beginning of cytokinesis. Fig. 53. Cytokinesis completed. Fig. 54. Daughter cells after formation of valves and spines and horns. Figs. 45 and 54, ×760; rest, ×1170.

As soon as the cleavage is completed the daughter protoplasts are separated as in the previous form (*Triceratium dubium*) by a wide empty space between them. The exact significance of this space is not clear. Gross (1937/38, p. 5) states in the case of *Ditylum Brightwellii* that " the fact that the two daughter cells together fill a space far smaller than the original

parental one suggests the existence of a strong bipolar tension prior to and during the division."

The development of the characteristic horns and spines of this Diatom (Text-Fig. 54) has been described in detail by Iyengar and Subrahmanyan (1944 *a*).

4. *Achnanthes inflata* (Kutz.) Grun.

This Diatom occurs as an epiphyte on *Cladophora* and *Pithophora* along with *Terpsionoë musica* in a pond in the Agri-Horticultural Gardens at Madras. The Diatom often forms short chains. The basal cell is attached to the algal filament by a mucilage pad at one corner (Text-Fig. 55).

The Diatom was fixed both in the field and in the laboratory. Division figures were found in plenty in material fixed between 11 P.M. and 2 A.M. The material was fixed in the several fluids used for *Terpsinoë*, but only Schaudinn's sublimate-acetic-alcohol and PFA_3 gave good results. The material was washed and stained in iron-alum hæmatoxylin in the same manner as the previous forms.

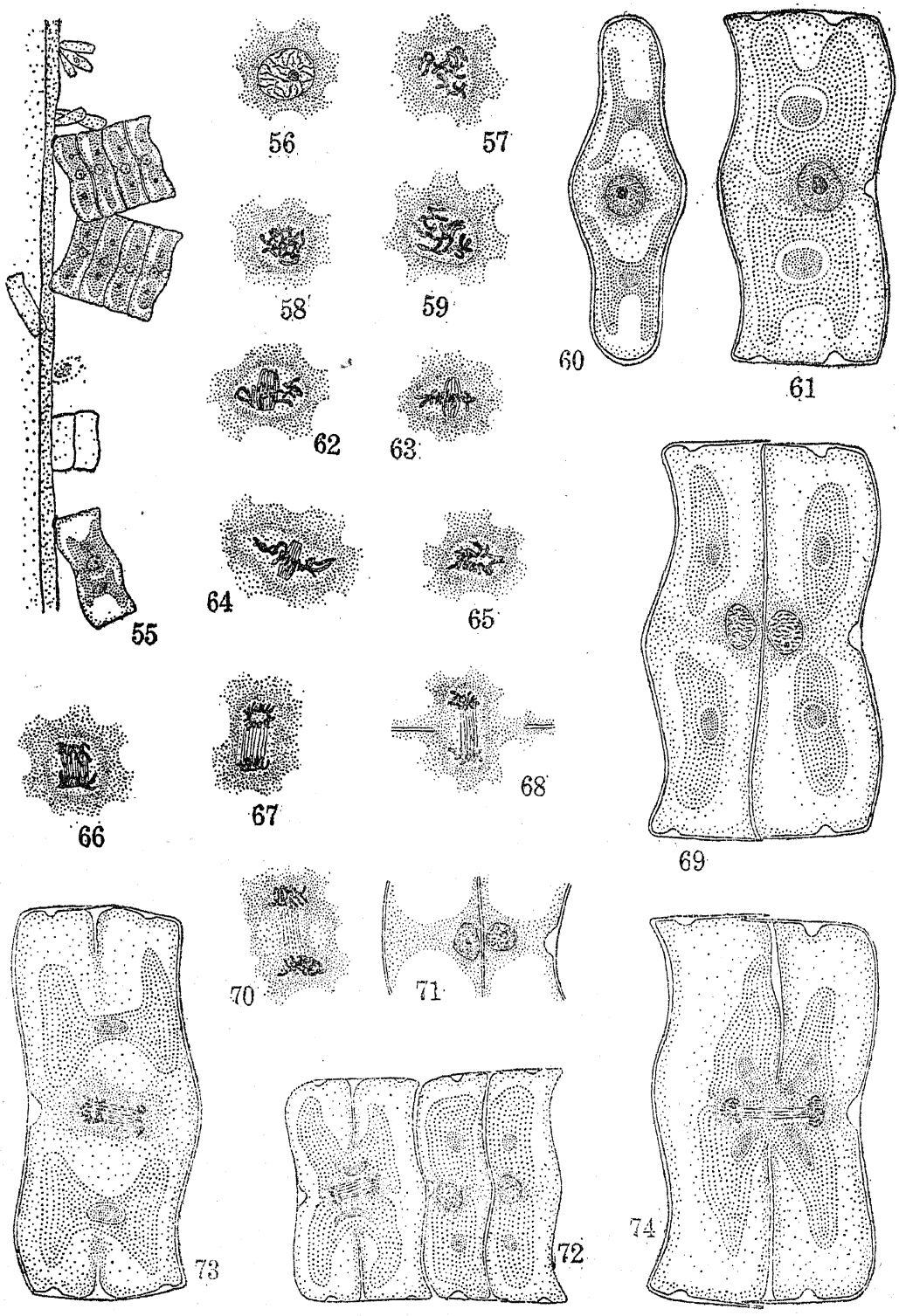
Vegetative cell.—The cells of *Achnanthes inflata* are rectangular with the two long sides curved (Text-Fig. 61) and in valve view are linear and bulged out in the middle with broadly rounded poles (Text-Fig. 60). Two plate-like chromatophores lie on either side of the nucleus. Each chromatophore possesses a pyrenoid (Text-Fig. 60). In valve view the chromatophore is H-shaped (Text-Fig. 60). The nucleus is situated in a dense mass of cytoplasm at the centre of the cell (Text-Figs. 60 and 61).

Mitosis.—The resting nucleus shows a prominent nucleolus and a well stained reticulum. In some cases the nucleolus appears vacuolated (Text-Fig. 60).

During early prophase thin chromosomal threads are seen in the nuclear cavity (Text-Fig. 56). These later contract and in late prophase 12–16 somewhat long chromosomes are seen lying in the nuclear cavity (Text-Figs. 57–59). By about this stage both the nucleolus and the nuclear membrane disappear completely.

While the prophasic changes are taking place the cell increases in volume by the valves loosening a little so that when division is completed each daughter cell is only slightly less broad than the mother cell.

In early metaphase the chromosomes move to the equator of the spindle (Text-Fig. 62) and form a compact ring at metaphase (Text-Figs. 63–65). The spindle is barrel-shaped as in the previous cases and is first noticeable in early metaphase (Text-Fig. 62).



Text-Figs. 55–73. *Achnanthes inflata*.—Fig. 55. Habit. Note the cells forming chains and the attachment mucilage pad. Fig. 60. Valve view of cell. Note the H-shaped chromatophores with pyrenoid. Nucleus in resting condition. Fig. 61. Girdle view of cell; nucleus

in resting condition. Fig. 56. Early prophase. Figs. 57–59. Late prophase. Note disappearance of both nucleolus and nuclear membrane. Figs. 62–64. Metaphase. Fig. 65. Metaphase, polar view (slightly oblique). Figs. 66–68, 70. Anaphase. Cytokinesis well advanced in Fig. 68. Fig. 73. Anaphase. Note beginning of cytokinesis. Fig. 71. Daughter nuclei organised. Fig. 69. Cell after completion of cytokinesis. Note one nucleus and two chromatophores in each cell. Fig. 72. One cell (left) showing cytokinesis and division of chromatophores and elongated pyrenoids; cell on the right completely divided. Fig. 74. Cell undergoing division; chromatophores and pyrenoid just divided; cytokinesis not complete; nuclei in telophase. Fig. 55, ×270; Fig. 72, ×750; rest, ×1150.

In anaphase the daughter chromosomes move to the poles of the spindle (Text-Figs. 66, 67, 68 and 70) and then contract. During telophase the daughter nuclei are organised (Text-Figs. 74.)

Cytokinesis.—Cytokinesis begins during anaphase as a small cleavage furrow which progresses in a centripetal manner and finally cuts the cell into two (Text-Figs. 72, 73, 74 and 69). During division, the chromatophores and the pyrenoids elongate and divide by constriction (Text-Figs. 72–74). After the division of the cytoplasm and the chromatophores is completed, new valves are formed (Text-Fig. 72, right cell).

The observations agree in a general way with those of Gemeinhardt (1925) on *Achnanthidium brevipes* (Ag.) Cl. But Gemeinhardt did not observe the several stages of prophase and the spindle clearly. Again the chromosomes in his form are short and dot-like whereas in the present form they are long and thread-like.

5. *Pleurosigma angulatum* (Quekett) W. Smith

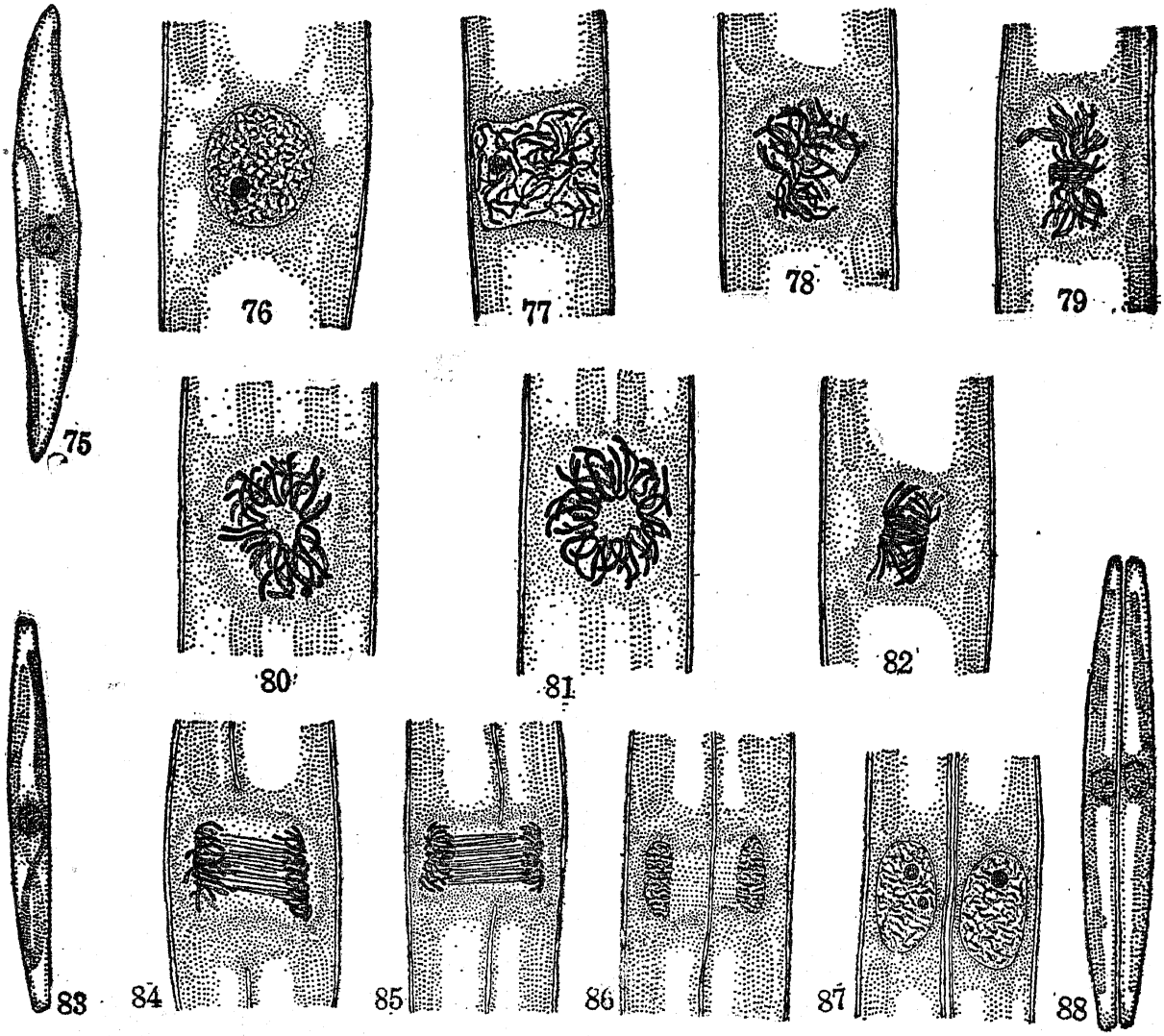
This pennate Diatom is a brackish-water form and occurs in plenty as a brownish scum on the moist sand at the edge of the water near the mouth of the river Adyar at Madras. It occurred more or less in a pure formation except for a few smaller pennate Diatoms.

Fixing in PFA_3 made up with the river water and staining in iron-alum hæmatoxylin proved quite satisfactory. Nuclear figures were found in plenty in the material fixed between 5 and 7 P.M.

Vegetative cell.—The cells of *Pleurosigma angulatum* appear like a drawn out ' S ' in valve view and rectangular in girdle view (Text-Figs. 75 and 83). The cells are several times longer than broad. The chromatophores are ribbon-shaped and lie more towards the girdle side in the resting cell. The large nucleus is situated at the centre of the cell in a dense mass of cytoplasm (Text-Figs. 75 and 83).

Mitosis.—The resting nucleus shows a single nucleolus and a deeply stained reticulum (Text-Fig. 76). The reticulum in this Diatom is more strongly stained than in all the previous forms studied by the writer.

During prophase a number of long thread-like chromosomes is seen lying in the nuclear cavity (Text-Fig. 77). The spindle is barrel-shaped and becomes evident during early metaphase (Text-Figs. 78 and 79). The chromosomes move towards the equator of the spindle and are seen divided longitudinally (Text-Fig. 79). They are then arranged uniformly in a ring around the spindle at metaphase. Only polar views of the spindle were



Text-Figs. 74–88. *Pleurosigma angulatum*.—Fig. 75. Cell in valve view. Fig. 83. Cell in girdle view. Fig. 76. Resting nucleus. Fig. 77. Prophase. Note long thread-like chromosomes. Fig. 78. Early metaphase; nuclear membrane and nucleolus disappeared. Fig. 79. Metaphase. Note the spindle; the chromosomes already divided. Figs. 80–81. Metaphase, in polar view. Note arrangement of chromosomes in a ring and the spindle fibres seen as dots inside the chromosome ring. Fig. 82. Early anaphase. Fig. 84. Anaphase. Cytokinesis well advanced. Fig. 85. Late anaphase. Cytokinesis advanced still further and very near the spindle. Fig. 86. Telophase. Cytokinesis completed. Fig. 87. Daughter nuclei organised and new valves formed. Fig. 88. Completed daughter cells. Figs. 75, 83, 88, ×420; rest, ×1200.

seen. Side view of metaphase was not observed. Inside the chromosome ring could be seen a number of dots representing the spindle fibres in polar view (Text-Figs. 80 and 81; Pl. XXIX, Fig. 5).

An exact chromosome count could not be made either at metaphase or the earlier stages as the chromosomes were long and many and very compactly arranged. The number of chromosomes appears to be about 40 (2 *n*).

In anaphase the daughter chromosomes move to the poles of the spindle, the spindle lengthning as the daughter chromosomes move farther apart (Text-Figs. 82 and 84). The chromosomes then contract and the outline of the individual chromosomes becomes very difficult to make out (Text-Fig. 85). In telophase the daughter nuclei are organised (Text-Fig. 86).

Cytokinesis.—Cytokinesis commences during anaphase as a tiny cleavage furrow advancing to the centre and finally cutting the cytoplasm into two in the valvar plane (Text-Figs. 84–86). Later two new valves are secreted (Text-Figs. 87 and 88).

Discussion

Nucleolus.—Schmidt (1927) while investigating nuclear division in *Biddulphia sinensis* came to the conclusion that in the resting nucleus all the chromatic substance was situated in the nucleolus and that the outer nucleus (reticulum area) contained no chromatin material. The chromosomes according to him were derived solely from the nucleolar substance.

Cholnoky (1927 *b*) held in the case of *Diatoma vulgare* that a wandering of chromatin substance takes place from the nucleolus to the chromosomes.

Geitler (1928, p. 500) after severely criticising Schmidt's (*l.c.*) view, holds that the nucleolus does not represent so to say a superfluous reserve substance for the formation of the chromosomes, but that the nucleoli of Diatoms are quite comparable to those of the Metaphyta.

Later on, Cholnoky (1933) after investigating the nuclear division in *Melosira arenaria,* states that the chromosomes are well developed long before the nucleolus disappears and that the nucleolus is not chromatic in nature. He also states that his former view (*l.c.* 1927 *b*) regarding the wandering of chromatin from the nucleolus to the chromosomes was based on the then generally accepted ideas.

Schmidt reinvestigated *Biddulphia sinensis* in 1933 and as a result of his observations withdrew his former opinion that the chromosomes were derived from the nucleolus.

The writer's observations on the nuclear division of the five Diatoms investigated clearly show that the chromosomes never take their origin from the nucleolus. They always take their origin from the reticulum. In all the five Diatoms he found that the chromosomes were already well formed in the nuclear cavity (outer nucleus) while the nucleolus is still in tact. Again, Feulgen's reaction was tried by the writer for all the five forms several times. The nucleolus in all the five forms always gave a negative reaction, while the reticulum (in the resting nucleus) and the chromosomes (in the division figures) gave a positive reaction. The result of the Feulgen's reaction clearly showed that the nucleolus is not chromatic in nature.

Spindle.—There is a certain amount of controversy regarding the origin of the spindle. According to Lauterborn (1896), the spindle in *Surirella Capronii* is formed outside the nucleus by a round body derived from the centrosome. This body becomes a cylindrical spindle and penetrates into the nuclear cavity during the prophase of mitosis. But Belar (1926, p. 285) considers such an origin of the spindle as not probable when compared with what takes place in other forms with a central-spindle mitosis.

Ikari (1923) also thought that the spindle is extra-nuclear in origin. He observed in *Coscinodiscus subbuliensis* the spindle lying in close contact with the nucleus but outside it. Cholnoky (1933 *b*, p. 704) states that Ikari probably saw the 'normal' centrosomes with radiation, but interpreted them according to Lauterborn.

Karsten (1900, 1912) found during reduction division in *Surirella splendida* (*S. Saxonica*) that the single centrosome present elongates and forms the spindle.

Geitler (1927) found in *Cocconeis placentula* var. *klinoraphis* the centrosome penetrating into the nuclear cavity during prophase and forming the spindle.

It may be mentioned in this connection that centrosomes have been recorded by Cholnoky (1933 *a*) in *Cymbella cistula, Gomphonema capitatum* and *Gyrosigma accuminatum.* In the first form the centrosomes were observed at the poles of the spindle during reduction division.

Cholnoky (1933 *b*) states in the case of *Melosira arenaria* that he was not able to establish the exact origin of the spindle. He could see the spindle only in late prophase stages.

In the five forms dealt with in the present paper the writer was not able to find a definite centrosome. The spindle in every case was first seen in early metaphase and became more definite in metaphase. Its exact origin,

however, could not be traced. Again, no evidence for an extra-nuclear origin of the spindle was found though numerous preparations were very carefully examined.

It may be mentioned in this connection, however, that in *Navicula halophila* (Subrahmanyan, unpublished), a darkly stained body was seen close to the nucleus during prophase. This body could not be seen during early metaphase and later stages. Nor could it be seen in the resting nucleus though numerous preparations were carefully searched. The only stages during which it could be seen are mid- and late prophase. This dark body showed a close resemblance to the centrosome in *Cocconeis placentula* var. *klinoraphis* as figured by Geitler (1927, Taf. 13, Figs. 4 and 5). According to Geitler, the centrosome in this Diatom penetrates into the nuclear cavity and then forms the spindle. In *Navicula halophila* the writer could not observe the penetration of the dark body into the nuclear cavity. The fact that the disappearance of the dark body is soon followed by the appearance of the spindle inside the nuclear cavity in early metaphase would appear to suggest that here also the spindle is formed by the centrosome as in *Cocconeis placentula* var. *klinoraphis* as recorded by Geitler (1927). But until the actual entry of this dark body into the nuclear cavity and its modification into the spindle inside the nucleus are observed, nothing further could be definitely stated regarding this matter.

Cytokinesis.—Almost all the previous observations on the process of cytokinesis in Diatoms are based on fixed material. Gross (1937-38) appears to be the only worker so far to have followed cytokinesis in a living Diatom (*Ditylum Brightwellii*). He found that the process took 30 minutes to complete. In the present investigation the writer followed the cytokinesis in the living specimens of two Diatoms, *viz.*, *Terpsinoë musica* and *Biddulphia mobiliensis*. In both these Diatoms the process was surprisingly rapid. In *Terpsinoë musica* the process took only three and a half minutes to complete, while in *Biddulphia mobiliensis* the process took hardly 30 seconds to complete. It may be mentioned, however, that the cytokinesis in *Terpsinoë* was not uniformly rapid throughout. It was very rapid until the spindle region was reached. But after reaching the spindle, the cytokinesis was slowed up very much as if some resistance was being offered by the spindle. It took just a minute to reach the spindle region but took nearly two and a half minutes to cut through the spindle. In the case of *Biddulphia*, however, this slowing up of the process when the spindle region was reached was not noticeable. The process appeared to be fairly uniformly rapid throughout. It would be interesting if more Diatoms are studied in their living condition as regards their cytokinesis.

SUMMARY

Nuclear and cell division was followed in detail in five Diatoms, three belonging to the Centrales (*Terpsinoë musica, Triceratium dubium* and *Biddulphia mobiliensis*) and two to the Pennales (*Pleurosigma angulatum* and *Achnanthes inflata*).

In the resting nucleus are seen a well-developed reticulum and one or occasionally two or three nucleoli. The chromosomes become well differentiated during prophase and are distributed evenly inside the nucleus.

The nucleolus in all the five forms invariably disappears in late prophase and reappears when the daughter nuclei are organised.

The nuclear membrane generally disappears about metaphase in *Terpsinoë musica,* and about late prophase or about early metaphase in the other forms. The nuclear membrane was observed to persist till anaphase only in one case in *Triceratium dubium.*

The spindle makes its appearance during early metaphase. The chromosomes at metaphase are arranged in a ring round the spindle.

After anaphase and telophase the daughter nuclei are organised.

The chromosomes do not originate from the nucleolus, but are derived from the reticulum. The nucleolus gives a negative reaction with Feulgen stain, while the reticulum and the chromosomes give a positive reaction.

The number of chromosomes appeared to be about 28 ($2n$) in *Terpsinoë musica,* about 26–30 ($2n$) in *Triceratium dubium,* about 20 ($2n$) in *Biddulphia mobiliensis,* about 12–16 ($2n$) in *Achnanthes inflata* and about 40 ($2n$) in *Pleurosigma angulatum.*

Cytokinesis was followed in living specimens of *Terpsinoë musica* and *Biddulphia mobiliensis.* The process in these two Diatoms is extremely rapid, the time taken for the whole process being three and a half minutes in *Terpsinoë* and about thirty seconds in *Biddulphia.*

In conclusion, the writer wishes to express his great indebtedness to Prof. M. O. P. Iyengar, M.A., Ph.D. (Lond.), F.L.S., for his constant guidance and valuable help throughout the course of the present investigation. The writer's sincere thanks are also due to the authorities of the University of Madras for the award of a studentship during the tenure of which this investigation was carried out.

LITERATURE CITED

Cholnoky, B. von .. "Über die Auxosporenbildung von *Rhoicosphenia curvata* (Kg.) Grun.," *Arch. Protistenk.*, 1927*a*, **60**, 8–33.

———— .. "Über Kern- und Zellteilung des *Diatoma vulgare* Bory," *Mag. Bot. Lapok*, 1927*b*, **26**, 69–94 (cited from *Bot. Centralbl.*, N.F., 1928-29, **13**, 99–100).

———— .. "Die Kernteilung von *Melosira arenaria* nebst einigen Bemerkungen über ihre Auxosporenbildung," *Zeitschr. Zellforsch. u. Mikr. Anat.*, 1933, **19**, 698–719.

Fritsch, F. E. .. *The Structure and Reproduction of the Algæ*, 1935, **1**, Cambridge.

Geitler, L. .. "Somatische Teilung, Reductionsteilung, Copulation und Parthenogenese bei *Cocconeis placentula*," *Arch. Protistenk.*, 1927, **59**, 506–49.

———— .. "Neue Cytologische Arbeiten über Diatomeen," *ibid.*, 1928, **64**, 495–506 (*Ein Sammel referat*).

———— .. "Über den Bau der Kerne zweier Diatomeen," *ibid.*, 1929, **68**, 623–36.

———— .. "Der Formwechsel der pennaten Diatomeen (Kieselalgen)," *ibid.*, 1932, **78**, 1–226.

Gemeinhardt, K. .. "Zur Cytologie der Gattung *Achnanthidium*," *Ber. d. Deutsch. Bot. Ges.*, 1925, **43**, 544–50.

———— .. "Die Gattung *Synedra* in systematischer, zytologischer und okologischer Beziehung," *Pflanzenforschung*, 1926, Heft 6.

Gross, F. .. "Life-history of some Marine Plankton Diatoms," *Phil. Trans. Roy. Soc., London*, 1937-38, ser. B, **228**, 1–48.

Hofker, J. .. "Über die Fortpflanzung einiger Diatomeen der Zuidersee," *Zeitschr. Zellforsch. u. Mikr. Anat.*, 1930, **10**, 769–82.

Ikari, J. .. "On the nuclear and cell-division of a Plankton diatom *Coscinodiscus subbuliens* Jorgensen. (Prel. note)", *Bot. Mag., Tokyo*, 1923, **37**, 95–108 (cited from Cholnoky, 1933).

Iyengar, M. O. P., and Subrahmanyan, R. "On the Structure and Development of the Spines or Setæ of some Centric Diatoms," *Proc. Nat. Acad. Sci., India*, 1944*a*, **14**, pt. 3, 114–24.

———— .. "On Reduction Division and Auxospore-formation in *Cyclotella Meneghiniana* Kutz.," *Journ. Ind. Bot. Soc.*, 1944*b*, **23**, (4), 125–52.

Kolbe, R. W. .. "Zur Okologie, Morphologie und Systematik der Brackwasser Diatomeen. Die Kieselalgen des Sperenberger Salz-gebiets," *Pflanzenforschung*, 1927, Heft 7.

Krieger, W. .. "Zur Biologie des Flussplanktons," *ibid.*, 1927, Heft 10.

Lauterborn, R. .. *Untersuchungen uber Bau, Kernteilung und Bewegung der Diatomeen*, Leipzig, 1896 (Cited from Fritsch, 1935 and Wisselingh, 1913).

McClung, C. E. .. *Handbook of Microscopial Technique*. 2nd Edit., New York, 1937.

Lee, Bolles .. *The Microtomist's Vade-Mecum*, London, 1937. Edited by Gatenby, J. B., and Painter, T. S.

Oltmanns, F. .. *Morphologie und Biologie der Algen*, **1**, Jena, 1922.

Peragallo, H. .. "Sur la division cellulaire du *Biddulphia mobiliensis*," *Trav. Stat. Biol. Soc. Scient. d'Arcachon*, 1907, **10**, 1–26 (cited from Oltmanns, 1922).

Schmidt, P. .. "Weiteres über die Fortpflanzung der Diatomee *Biddulphia sinensis*," *Internat. Rev. Ges. Hydrobiol. u. Hydrograph.*, 1927, **11**, 114–47 (cited from Geitler, 1928).

——— .. "Neue Ergebnisse zur Biologie der *Biddulphia sinensis* Grev.," *Flora*, N.F., 1933, **28**, 235–68.

Subrahmanyan, R. .. "Somatic Division, Reduction Division, Auxospore-formation and Sex Differentiation in *Navicula halophila* (Grunow) Cleve" (unpublished).

Wisselingh, C. van .. "Die Kernteilung bei *Eunotia major* Rabenh.," *Flora*, 1913, **105**, 265–74.

Explanation of Plate XXIX

Fig. 1. *Triceratium dubium.*—Portion of a chain of the Diatom (living material). ×500.

Fig. 2. *Terpsinoë musica.*—Shows habit. Diatom forming zig-zag chains attached to *Cladophora*. ×34.

Fig. 3. *Terpsinoë musica.*—One girdle side of a living cell just before beginning of cytokinesis. Arrow points to the region where furrowing will take place. ×1200.

Fig. 4. *Terpsinoë musica.*—A portion of the Diatom chain ×62.

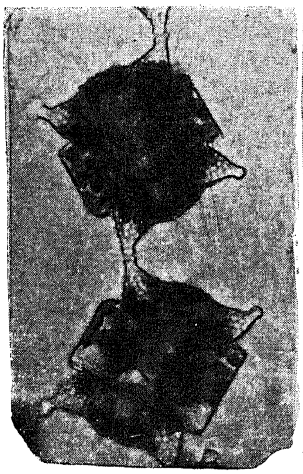
Fig. 5. *Pleurosigma angulatum.*—Metaphase, polar view. Note chromosomes arranged round the spindle. ×1500.

Fig. 6. *Terpsinoë musica.*—The same specimen shown in Fig. 3 killed in osmic acid when cytokinesis just started. Arrow points to the furrow. ×1200.

Fig. 7. *Terpsinoë musica.*—Metaphase. Note barrel-shaped spindle. ×1500.

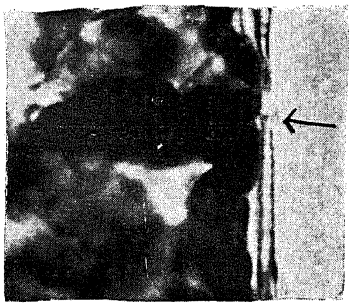
Fig. 8. *Terpsinoë musica.*—Anaphase. ×1500.

Fig. 9. *Terpsinoë musica.*—Late prophase seen from valve view. Note long thread-like chromosomes. ×1500.

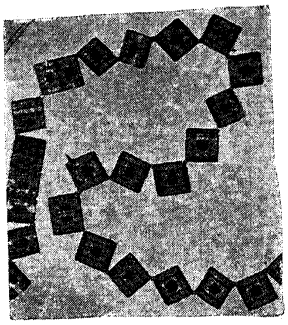


1

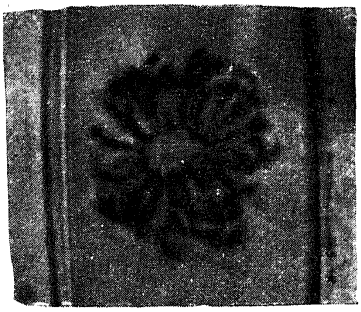
2



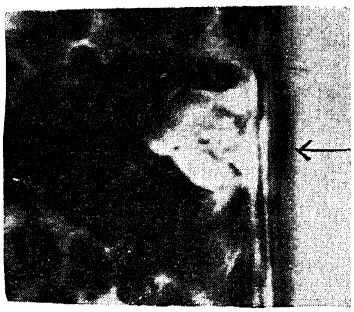
3



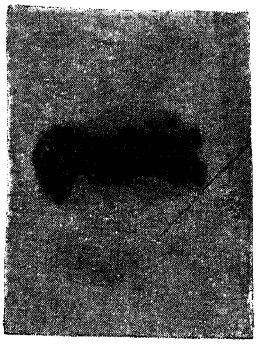
4



5



6



7

8

9