OBSERVATIONS ON THE FINE STRUCTURE AND DEVELOPMENT OF THE SPINDLE AT MITOSIS AND MEIOSIS IN A MARINE CENTRIC DIATOM (LITHODESMIUM UNDULATUM). II. THE EARLY MEIOTIC STAGES IN MALE GAMETOGENESIS

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SUMMARY
Various cytoplasmic phenomena, including spindle structure and development during prophase of the first meiotic division, are described and illustrated. The living culture is represented by a timed sequence of photographs continuing those previously published with respect to mitotic stages in the same filament. The meiotic preliminaries include the so-called swelling phase, by which the parental frustule is forced open, liberating the contained spermatocytes. This occurs during pachytene on evidence of chromosome structure which is illustrated. A spindle precursor is shown to be present before opening of the frustule; this resembles structurally the mitotic equivalent though the ground plan is oblong instead of square. Growth of the precursor continues until after opening of the frustule, when the spindle itself begins to be laid down. Two stages of developing spindles during the later prophasens are illustrated by sections cut in three planes and by serial sections. Preliminary comparisons are made with metaphase I and with mitosis, both qualitatively and quantitatively, but a full discussion is deferred pending completion of the record for the later meiotic stages.

INTRODUCTION
This paper deals with mainly cytoplasmic events which take place in young spermatocytes of Lithodesmium undulatum during about 4 h after completion of the last spermatogonial mitosis, which was described in a recent paper (Manton, Kowallik & von Stosch, 1969). The material is exactly as used previously except for the times of fixation. Whereas the study of mitosis was limited to one fixation taken at 3.30 p.m. on 9 September 1965, the new observations recorded below are partly derived from material processed at 6.30 p.m. on the same day, supplemented, however, by material fixed in April 1965 at 7 p.m. under comparable conditions. The two sets of material overlap at pachytene as will be shown.

The stages themselves represent little more than the meiotic prophasens up to but not beyond metaphase I. The time taken is nevertheless some indication of the relative importance of the changes involved, since any one of the preceding mitotic divisions
would have been fully completed in less. One of the more interesting attributes of this material is the phenomenal speed with which the whole development from the undivided mother cell to mature male gametes is carried through. In less than 12 h under the experimental conditions used the mother protoplast undergoes 4 successive mitoses and the 2 meiotic divisions. The fact that a third of this total time is taken up by the early meiotic stages with which this paper is concerned is in itself good reason for directing special attention to them.

MATERIAL AND METHODS

Details about the source and handling of the material in culture have already been placed on record in one or other of the previous papers (Manton & von Stosch, 1966; Manton et al. 1969). It is therefore sufficient to say here that sexualization is induced by simple environmental changes involving supply of fresh culture medium and increased temperature and light, after which gamete production occurs daily according to a precisely fixed time-table, reaching a maximum on the third day. Within a culture, synchronization is fairly close although not perfect. The time appropriate for a bulk fixation must therefore be selected after inspection of the culture as a whole and may differ slightly from the actual time taken by any one watched filament. This explains the minor discrepancy between the times quoted above and those recorded in Fig. 1.

The fixative has been post-osmicated glutaraldehyde made up according to the usual formula worked out for the study of marine plankton flagellates (Manton & Parke, 1965). For the April fixation used for the later stages including metaphase, the stock glutaraldehyde had been neutralized with barium carbonate which, on that occasion, gave a precipitate of insoluble barium sulphate crystals when the fixative was mixed with the culture solution. The presence of these crystals injured the very delicate surfaces of the naked spermatocytes to some extent as will be seen in a few of the micrographs but the fixation of the cell interiors was reasonably good. Substitution of calcium carbonate for barium carbonate for the September fixations of the earlier stages avoided some of these difficulties though the overall quality was not improved.

The light microscopy was all carried out in Marburg, mainly by the junior author (K. Kowallik) with the equipment described in Manton & von Stosch (1966). The electron microscopy was all carried out in Leeds, using mainly the Siemens Elmiskop I operating at 60 or 80 kV without thermal regulation, supplemented to a minor extent by the use of the A.E.I. EM6B electron microscope operating at 60 kV with a cold finger charged with liquid nitrogen. Sections cut with a diamond knife from blocks embedded in Epon and mounted on carbon were either stained with lead citrate alone (Reynolds, 1963) or double stained with uranyl acetate (1 % aqueous used for 20 min) preceding lead citrate (7 min).

RESULTS

The living culture

The more obvious changes manifested by a living culture undergoing some of the earlier stages of sexualization were illustrated photographically in Manton et al. (1969) as they took place on the morning of 25 August 1965. Between 9 a.m. and 1:30 p.m. (13:30 h) the protoplast of a mother cell giving rise to spermatogonia divided 4 times, the products of each division becoming successively smaller as their number increased from 2 to 4, 8 and eventually 16. Since the object of the previous study was to add details of fine structure during the last of these divisions the photographic series was discontinued at this point except for one supplementary photograph of the same filament taken late in the evening of the same day to show that gamete formation was proceeding normally to completion.
Figure 1 continues the series from the point reached at the end of the mitotic phase, the details being taken from 3 cells introduced into the previous communication as exposures A and B. For ease of cross-reference the first exposure included here in Fig. 1 is labelled K to show that it follows fairly closely after that reached by exposure H in Manton et al. (1969). The serial order of subsequent exposures and the time intervals between them are as indicated in Fig. 1.

The morphological event dominating the sequence of changes shown by exposures K to O is the opening of the frustule brought about by the so-called swelling phase. Before this begins the group of small spherical spermatocytes resulting from completion of the preceding mitotic phase occupy without completely filling the cavity of the parental frustule (exposure K). Some 3 h later (exposure L) the swelling phase begins to be detectable, reaching a maximum within 30 min (exposure N). As water is taken in the cells become more translucent and their diameter increases until they become pressed so tightly together that their contact faces become flattened (exposure M). When the frustule halves begin to separate (exposure N) this pressure is relieved and as the filament breaks up the newly exposed protoplasts rapidly return to approximately their former size (exposures O–Q). Each then individually undergoes meiosis. The series of photographs ends (exposure R) with one protoplast in advance of the others and in the elongated condition characteristic of late anaphase of the first meiotic division.

Preliminary observations on the fine structure of the swelling phase

Full understanding of the mechanism of swelling will require a more detailed study than we have at present been able to make. It is nevertheless impossible to trace the early stages of spindle development without including some observations on spermatocytes before liberation from the frustule. Figures 2–4 assemble in serial order 3 conditions appropriate for comparison from this point of view, selected details being illustrated at higher magnifications in Figs. 5–16.

Figure 2 illustrates the general appearance of spermatogonia within a mother cell towards the end of the mitotic phase. The material is from the 3.30 p.m. fixing used for the previous investigation (Manton et al. 1969) and was selected for cutting because it could be seen in the intact block to contain more than 8 though less than 16 protoplasts. The search for mitotic stages was successful in the sense that prophase spindles were found in certain protoplasts, notably one at bottom left, although other smaller protoplasts such as those present at top right in Fig. 2 seemed likely to have completed their last mitosis. Other examples of these two conditions are illustrated in Figs. 5 and 6 at a higher magnification. The cell of Fig. 5 also contained a young prophase spindle near the point of the arrow. A spindle was not encountered in the cell of Fig. 6 but the interphase condition of the nucleus as well as the general appearance of the cytoplasm suggests interkinesis after completion of the last mitotic division.

Figures 3 and 4 are very different. Both come from the 6.30 p.m. fixing but they are not duplicates. The specimen of Fig. 3 is younger than that of Fig. 4 on evidence presented by both cytoplasm and nuclei. If the latter are considered first there is...
clearly a marked difference of nuclear size between the two stages, and the details of nuclear contents are also dissimilar. The nuclei of both stages differ substantially from any mitotic equivalent so far encountered, including those illustrated in Figs. 5 and 6. It is not possible to establish the precise stage of Fig. 3, though the uniformly mottled appearance of the nuclei together with the inconspicuous, superficially placed, nucleoli suggest a very early meiotic prophase rather than interkinesis. Fortunately the larger nuclei of Fig. 4 contain evidence of chromosome structure permitting a far more precise recognition of the stage involved. Further details are illustrated in Figs. 10 and 12. In the latter, which relates to a field from another part of the specimen of Figs. 4 and 10, the well-known axial structure characteristic of paired chromosomes at pachytene is displayed (see Fawcett, 1956; Moses, 1958; Coleman & Moses, 1964; Roth, 1966; Sotelo & Wettstein, 1966; Aldrich, 1967). A better-preserved specimen from the April fixing is reproduced in Fig. 13 to show the tufts of contorted filaments attached to the edges of the ribbon-shaped synaptinemal complex. Since the specimen of Fig. 13 was outside the frustule while that of Fig. 12 was still enclosed it is probable that the relatively long prophase stage of pachytene is that in which the swelling phase reaches a maximum and opens the frustule.

It is not possible at present to ascertain exactly the stage in the nuclear cycle at which the swelling starts, and interpretation of the swelling mechanism will need to be worked out by means of a special study which we have not yet been able to undertake. Two observations can nevertheless be made on the fine structure of the cytoplasm of the stages illustrated in Figs. 3 and 4, one of which concerns the spindle precursor which will be dealt with in the next section but the other can appropriately be mentioned briefly here. Details of the cytoplasm from marked fields will be found in Fig. 7 with respect to the stage of Fig. 3, and Figs. 14 and 15 with respect to the stage of Fig. 4. Both contain various inclusions some of which, such as mitochondria, plastids and Golgi bodies, can be recognized as such, while others, notably the numerous vesicles with contents, present prominently in Fig. 7, cannot be so easily identified. Some vesicles in the latter are undoubtedly of Golgi origin since in at least 2 places continuity with the Golgi system is evident. In this specimen there are, however, few if any cisternae which can be recognized as belonging unequivocally to the endoplasmic reticulum (ER) except the nuclear envelope and the layer surrounding each plastid, both of which are in an apparently quiescent condition.

The contrast between this and the later condition illustrated in Figs. 14 and 15 is striking and perhaps important. These two fields refer to the pachytene cells of Fig. 4, the actual sites being indicated by the arrows in Fig. 10. Both show cytoplasm penetrated by distended cisternae which can be recognized as ER without ambiguity by means of the ribosomes attached in many places to the outer sides of the bounding membranes. There is moreover continuity between such inflated cisternae and other sites of ER. Thus in Fig. 14 they are clearly related to the layer surrounding a chloroplast, while those in Fig. 15 seem to be proliferating from a dilation of the nuclear envelope which elsewhere is undistended. Continuity between all the profiles of ER present in either of these fields was shown by means of serial sections. There is no doubt, therefore, that pachytene is a stage at which greatly increased activity of ER
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is taking place and this naturally includes the nuclear envelope as part of the endo-
plasmic reticular system.

Figure 16 adds a final example of an extreme dilation of nuclear envelope which, if
seen with the light microscope, might have been interpreted as a vacuole. The stage
of this specimen is less fully documented than that of the pachytene cells already
discussed but it is introduced to show the sort of activity which will need to be taken
into account before the details of the swelling stage itself can be properly understood.

The spindle precursor

Elucidation of the relative order of the stages discussed above permits attention to
be effectively directed to the spindle and spindle precursor. Only the latter is repre-
sented up to and including pachytene, but since the structural details agree closely
with those of the mitotic equivalents (Manton et al. 1969) it will be sufficient here to
comment mainly on the differences.

Figures 7–9 illustrate spindle precursors from the specimen of Fig. 3 in two planes
of section. Figure 9, cut tangentially to the nuclear surface, is the most informative.
It shows the series of parallel plates of different density exactly as previously de-
scribed, though there is one conspicuous difference in relative dimensions. Whereas
the spindle precursor at a mitotic interphase (measured in many examples) was
arranged on a square ground plan, that of Fig. 9 is oblong, the longer dimension being
already 50% greater than the shorter. This difference is of course not detectable in
sections cut perpendicularly to the nuclear envelope as in Figs. 7 and 8 but it suggests
that growth of the spindle precursor accompanies onset of the meiotic prophase in a
manner not encountered at interphase or a mitotic prophase.

This growth continues during and indeed after pachytene. Figure 11 illustrates a
tangential view of a slightly deformed spindle precursor from the specimen of Figs. 4
and 10; if the deformity is ignored the long dimension is approximately twice the
short one. Another detail visible in Fig. 11 which should be noted in passing is the
patch of discontinuous dense material present between the spindle precursor and an
adjacent plastid. This may perhaps be the meiotic equivalent of the mass of ancillary
material, of different appearance but perhaps similar function, to which attention was
directed at a mitotic prophase (Manton et al. 1969).

At a substantially later stage, after opening of the frustule, the long dimension of
the spindle precursor has grown to 3 times the short dimension as seen in a tangential
section such as that of Fig. 19. By this time, however, the spindle itself is beginning
to be laid down and this stage is therefore more properly considered in the next
section.

The meiotic prophase spindle

At meiosis, as at mitosis, it is necessary to distinguish clearly between the spindle
precursor and the spindle. The latter begins to be formed only after the frustule has
opened and an early condition of it is illustrated in 3 planes of section in Figs. 17–20.
It consists of a layer or layers of close-packed aligned tubules, each just long enough
to span the distance between the end plates of the precursor (Fig. 18). Since the tubules
are laid down between the precursor and the underlying nuclear envelope, the precursor itself is pushed away from the nuclear surface by this intrusion. This movement is, however, accompanied by corresponding growth of the end plates which, when cut in a plane at right angles to that of Figs. 18 and 19, give the very striking appearance seen in Figs. 17 and 21. Comparison with the earlier condition seen in Fig. 8 will at once indicate in a general way what has happened. The end plates have grown mainly towards the nuclear surface but the pronounced inward curvature of their distal edges shows that they are extending their area at this edge also, though doubtless under different local conditions. This flexure is temporary, but while it lasts a section of a meiotic spindle is so distinctive that it can be recognized at a glance.

In both the specimens represented by Figs. 17 and 21 patches of dense ancillary material surrounding the spindle apparatus resemble the mitotic equivalent in a general way, though with differences of detail. This material also resembles that already noted in a different position in Fig. 11.

Finally the transverse section illustrated in Fig. 20 shows both the position and number of the spindle tubules at this early stage. The area occupied by tubules is more extended laterally than in the mitotic equivalent owing to the prophase growth of the meiotic spindle precursor, to which attention has already been directed (Figs. 18, 19). In other respects the resemblance to mitosis is close.

A substantially later meiotic prophase condition which is approaching the stage at which the nuclear envelope will break down is illustrated in Figs. 22–24. A transverse section of such a spindle (Fig. 24) will at once show the considerable increase in tubule numbers compared with the earlier condition of Fig. 20, but the effect of the disappearance of the spindle precursor, which takes place at this time, is better seen in a side view (Figs. 22, 23). As long as the spindle precursor is intact the end plates cannot separate and the spindle tubules remain short (Fig. 18). Sooner or later, however, the centre of the precursor breaks down and the enlarged end plates move apart as the spindle tubules lengthen. As this happens the differentiation between the equator and the spindle ends begins to become evident (Fig. 23) and this differentiation, with minor modification, persists until metaphase.

Further information about the structural basis of the equatorial differentiation visible in Fig. 23 is obtainable from Fig. 25. This illustrates a series of 8 transverse sections through a spindle at approximately this stage, the series including the two ends and the equator. For ease of reference the spindle ends are placed side by side at the top of the page but the serial order of the various levels is indicated alphabetically. Section A is at one end and section H at the other, the equator being represented by sections C–E. Numerical information at a high level of accuracy is obtainable from this series in spite of slight obliquity of the tubules at one end of the series. In the most densely crowded equatorial level (section C) the number of tubules present is approximately 147. At one end (section G which has had to be used to avoid the dirt on section F) the number is 85. At the other end (section A) the count is less exact owing to obliquity of the tubules but the number is of the same order, i.e. not less than 80. These numerical facts confirm the general resemblance between the two ends of one spindle while exposing a discrepancy between these and the equator.
This discrepancy is of exactly the same type as that previously encountered at a mitotic metaphase, though the actual numbers involved are quite different. In both it has been shown that the number of tubules present at the equator is nearly but not quite twice the number encountered at either end. The discrepancy (i.e. the difference between the equatorial count and the sum of the end counts) is of the order of 10% and the explanation put forward for the mitotic metaphase seems equally applicable to a meiotic prophase. This is that most of the spindle tubules attached to any one pole terminate shortly after passing the equator but that a few go the whole way. These few—that is the discrepant 10%—being attached to both poles, are therefore counted twice in an estimate of tubule numbers based on counts at the two ends.

A final structural detail should be noted before leaving the late prophase spindle. In transverse sections such as those in Figs. 24 and 25 the arrangement of spindle tubules in a stack of slightly curved layers is sufficiently uniform to correspond approximately to ‘square’ close-packing. There are a few imperfections, some layers being shorter or less completely filled than others, but on the whole the layer lines and row lines are well marked and nearly at right angles to each other, though both may be tilted with respect to the nuclear surface. Square close-packing of this kind was also recognizable at a mitotic prophase, though the evidence was less satisfactory. Since the condition at metaphase is different it is perhaps important to draw attention to this feature here, since Figs. 24 and 25 strengthen the evidence considerably for this particular detail.

The spindle at metaphase

The nuclear membrane is still present in all the stages illustrated up to and including that in Fig. 25. Shortly thereafter it breaks down and, while continuing to enlarge, the spindle sinks into the mass of chromosomal material. The central position with respect to the chromosomal mass is then equally evident in longitudinal (Fig. 26) and transverse view (Fig. 28). Nevertheless, both the dimensions and structure of the spindle have changed in several respects.

Some of these changes parallel exactly the condition at a somatic metaphase which has already been explored (Manton et al. 1969). Some further details about the structure of the poles are, however, illustrated in Fig. 27. The dense material of the polar plate is here partially covered externally by vesicles and flattened cisternae, some of which are probably derived from the disrupted nuclear envelope which has settled on the outer face of the polar plate itself (see also Fig. 26). Towards the interior of the nucleus a massive bundle of approximately parallel tubules passes inwards from the polar plate, with a few individual tubules diverging laterally. At the equator, however, the spindle tubules become united laterally into discrete bundles (Fig. 26) which, as at a mitotic metaphase, show hexagonal close-packing internally (Fig. 28). Some dense vesicles of unknown nature and origin but closely resembling those seen at mitosis are also invariably present among the tubules in the spindle area.

The number of bundles present in the specimen of Fig. 28 cannot be exactly determined, though it is of the order of 18. This compares closely with the number of
tubule bundles present at a mitotic metaphase which was similarly estimated (Manton et al. 1969) as approximately 16. The total number of tubules in the meiotic spindle is nevertheless greater, the count for Fig. 28, which is less than median, being 287 as opposed to slightly less than 200 for median levels at the preceding mitosis. The meaning, if any, of these data will be further discussed when information is available for both meiotic divisions. Enough, perhaps, has been presented to show that the increased size of the spindle precursor and spindle, encountered at a meiotic prophase compared with a spermatogonial prophase, is sustained up to and including metaphase and is therefore a characteristic of meiosis I in this material.

**DISCUSSION**

To anyone familiar with what has gone before, more especially Manton et al. (1969), the observations recorded here will seem to some extent like a more elaborate version of a familiar story. The special character of the diatom spindle, notably its flat poles, central position and differentiated equator are exactly as previously described for the premeiotic mitosis in this organism and, indeed, also as foreshadowed by the very skilful light microscopy of Lauterborn (1896), working with pennate diatoms. This degree of familiarity may at first obscure the very unusual character of the diatom mitotic apparatus among plants and protista generally. When prophase is also remembered, in which most though not quite all of the structural characteristics of the mature spindle are developed outside the nucleus before breakdown of the nuclear envelope, we have a type of mitotic apparatus which is virtually unique.

Further comment on these lines must, however, be deferred until the descriptive facts have been more fully assembled to include not only meiosis II but also some observations on anaphase which cannot be effectively examined at any stage other than anaphase I, which is not yet before us. Limiting present comment to the comparison between mitosis and meiosis as recorded, it is clear that the more massive character of the young meiotic spindle permits observations to be made upon it which would have been difficult or impossible at the mitotic equivalent. The long series of sections through a complete young spindle as assembled in Fig. 25 are therefore in a sense complementary to equivalent observations already introduced on the basis of full mitotic metaphase in an abnormal (giant) cell. In both cases direct counts of tubules at the two ends of a spindle were approximately equal, though the actual numbers in the two spindles were different and, in both, the number of tubules transected at the equator was nearly but not quite equal to the sum of those seen at the ends. This discrepancy was explained in both cases in terms of a minority of tubules traversing the whole length of the spindle as opposed to a majority which end shortly after passing the equator. The actual counts in both cases limit the minority to not more than 10% of the total. Since the spindle used for Fig. 25 is still in the cytoplasm and separated from the nucleus by an intact nuclear envelope it shows clearly that the basic character of the spindle does not require for its initiation any direct contact with the chromosomes. The break-up into bundles which distinguishes metaphase from prophase is nevertheless a later development for which chromosome contact may be needed.
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One of the more unexpected details revealed more clearly at the first meiotic division than at any other is the difference in the pattern of close-packing exhibited by tubules at two ages of spindle. In the prophase spindle of Fig. 24 quadrangular ("square") close-packing is unmistakable, that previously demonstrated for the mitotic equivalent (Manton et al. 1969, fig. 12) being similar though less complete. At metaphase, on the other hand, the larger bundles in all 3 examples, including the two mitotic metaphases previously illustrated, as well as that from meiosis I recorded in Fig. 28, show hexagonal close-packing of individual tubules within bundles. This suggests that in the latter condition, where a bundle is complete, the component tubules must be attached laterally and are therefore not able to move with respect to one another as freely as may be the case at prophase. Further, it is probable that the first tubules to be laid down in the very young spindle may all span the whole distance between the facing polar plates as suggested by Figs. 17 and 18, but subsequent elongation (Fig. 23) may involve detachment of individual tubules from one pole or the other, with subsequent growth only at the end retaining contact with a pole. Search has been made at all stages for signs of isolated tubules free at both ends but without success. It therefore seems probable that the polar plates are the main if not the sole source of tubule growth during the stages so far examined.

REFERENCES


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Fig. 1, k-r. *Lithodesmium* meiosis I. Part of a living filament showing the early stages of meiosis at the times indicated; an undivided vegetative cell entering the field at top and bottom; two spermatogonial mother cells between these, the upper one normal and containing 16 spermatocytes of uniform size, the lower one abnormal with fewer cells and mixed sizes. × 320.
Fig. 2. Spermatogonia within a mother cell at about the stage of the last premeiotic mitosis, from the 3.30 p.m. fixing. The two lower cells (left) containing young mitotic spindles and therefore not yet divided; the two upper cells (right) have probably just completed division. Micrograph D 2209, × 2000.

Fig. 3. An early stage in the 6.30 p.m. fixing. Sixteen spermatocytes within a closed frustule, the nuclei probably in very early meiotic prophase. For further details see Figs. 7–9. Micrograph D 3416, × 2000.

Fig. 4. A later stage in the 6.30 p.m. fixing. Nuclei greatly enlarged and in pachytene stage, though the frustule is still closed. For further details see Figs. 10–15. Micrograph D 3355, × 2000.
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Fig. 5. Spermatogonium in prophase of the last mitotic division from the 3.30 p.m. fixing; the position of a prophase spindle indicated by the arrow though the spindle itself (previously illustrated) is too small to see at this low magnification. Micrograph D 2090, × 5000.

Fig. 6. Interphase spermatogonium or spermatocyte from the 3.30 p.m. fixing; the nucleus in the resting stage and the cytoplasm with the usual display of organelles and vesicles. Micrograph Z 1945, × 5000.

Fig. 7. Part of a spermatocyte from the 6.30 p.m. fixing from a site marked by arrows in Fig. 3; the nucleus (n), a spindle precursor (arrow) closely pressed against the nuclear envelope; elsewhere in the cytoplasm the usual organelles together with vesicles of many sizes. Endoplasmic reticulum inconspicuous but present round the plastids in an apparently quiescent condition. Micrograph D 3412, × 15000.

Fig. 8. Spindle precursor more highly magnified from a specimen similar to that in Fig. 7. Micrograph D 2453, × 30000.

Fig. 9. Tangential section of a spindle precursor otherwise similar to that of Fig. 8, showing the series of parallel bands; for further description see text. Micrograph D 2094, × 30000.
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Fig. 10. Part of two spermatocytes from the specimen of Fig. 4. The frustule is still closed but the greatly enlarged nuclei are now unmistakably in pachytene. Arrows indicate the fields shown more highly magnified in Figs. 14 and 15; for other details from this specimen see Figs. 11-13. Micrograph D 3351, × 5000.

Fig. 11. Tangential view of a spindle precursor, slightly deformed but appreciably longer than that in Fig. 9, from a pachytene cell in the specimen of Figs. 4 and 10. Part of a plastid entering the field at the top and a fragmented patch of dense material between this and the spindle precursor (arrows). Micrograph D 3375, × 30000.

Fig. 12. Part of a nucleus (n) from the specimen of Figs. 4 and 10, showing the axial complex of a pachytene chromosome near the nuclear envelope; from the September fixing (compare with Fig. 13). Micrograph D 3360, × 30000.

Fig. 13. Part of a thick section through a pachytene cell after liberation from the parental frustule; from the April fixing. The axial complex characteristic of pachytene is similar to that in Fig. 12 but the tufts of contorted filaments are better preserved. Micrograph D 92, × 30000.
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Fig. 14. Part of the upper cell in Fig. 10, showing distended endoplasmic reticulum (er) in continuity on both sides of the chloroplast. (n, nucleus.) Micrograph D 3358, x 25000.

Fig. 15. Part of the surface of the lower cell in Fig. 10 (arrow), showing proliferation of endoplasmic reticulum (er) from the nuclear envelope into the cytoplasm (n, nucleus.) Micrograph D 3353, x 20000.

Fig. 16. Part of a cell from another specimen, showing a greatly distended dilation of the nuclear envelope (arrows). Micrograph D 2823, x 15000.
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Fig. 17. Surface of a nucleus (n) at a later stage of prophase, showing a Golgi body and a spindle precursor beginning to generate a spindle (contrast with Fig. 8). Micrograph C 9930, × 30000.

Figs. 18, 19. Two successive sections through a spindle and spindle precursor at about the stage of that of Fig. 17 cut tangentially, showing the greatly elongated pole-plates (contrast with Figs. 9 and 11). The parallel bands of the precursor are still present (Fig. 19) with tubules beneath (Fig. 18). Micrographs D 1367 and 1369, × 30000.

Fig. 20. Section through a young spindle cut in a plane at right angles to that of Figs. 19 and 20 showing spindle tubules arranged against the nuclear envelope. (n, nucleus.) Micrograph D 99, × 60000.
Fig. 21. Spindle precursor and young spindle cut in a plane perpendicular to the nuclear envelope (as in Fig. 17) from another specimen, more highly magnified. Micrograph D 1377, × 50000.

Fig. 22. A spermatocyte towards the end of prophase of the first meiotic division reduced to its normal size after completion of the swelling phase and with the spindle beginning to elongate after breakdown of the centre of the spindle precursor. Micrograph D 998, × 5000.

Fig. 23. The spindle from the cell of Fig. 22, showing the centrosomal plates enlarged and moving apart (contrast with Figs. 17 and 21). The differentiation characteristic of the equator versus the spindle ends is already clearly visible; the nuclear envelope is still intact. Micrograph D 1000, × 30000.

Fig. 24. Transverse section through the equator of an exceptionally large specimen at approximately the stage of Fig. 23, showing the spindle tubules in slightly curved rows with a few gaps; apart from these the arrangement approximates to 'square' close-packing. Micrograph D 1633, × 60000.
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Fig. 25. Series of 8 sections through a spindle at approximately the stage of Fig. 23 showing the differences in numbers of tubules between the equator and the spindle ends. The order of the sections is indicated alphabetically. For further description see text. Micrographs D 631, D 632, D 634, D 636, D 639, D 606, D 601 and D 645, x 50000.
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Fig. 26. Slightly oblique longitudinal section through a spermatocyte at metaphase of the first meiotic division, showing the central spindle. Micrograph D 2609, x 15000.

Fig. 27. Spindle pole at metaphase of the first meiotic division, showing a layer of dense material covered externally by flattened vesicles probably derived from the disrupted nuclear envelope. Spindle tubules more or less parallel centrally but a few individual tubules diverge widely. Micrograph D 557, x 40000.
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Fig. 28. Nearly median transverse section through the central spindle of a spermatoocyte in metaphase I, showing tubules united into bundles with hexagonal close-packing; the bundles at the top of the field are not complete owing to obliquity of the equator. For further description see text. Micrograph D 325, x 80000.