MORPHOGENESIS IN MICRASTERIAS

by

THURSTON CASTLE LACALLI

B.Sc., University of Washington, 1968

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in the Department
of
Zoology

We accept this thesis as conforming to the
required standard.

The University of British Columbia

June, 1973

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of ZOOLOGY

The University of British Columbia
Vancouver 8, Canada

Date 23 August, 1973

Abstract

The morphogenetic process responsible for elaboration of cell wall shape in dividing Micrasterias rotata cells is described and the associated ultrastructure discussed. The process is dissected experimentally into its contributing parts; laser microbeam studies reveal the growth sites responsible for morphogenesis, and autoradiography, the associated synthetic patterns. Tip growth similar to that occurring in fungal hypae, root hairs and pollen tubes makes a major contribution to cell shape. Tip growth, together with branching and broadening of the growths produced, accounts for the patterns of morphogenesis exhibited in M. rotata and M. radiata, two species having distinctive shape differences.

The ability of dividing cells to impose a biradiate symmetry on the developing cell wall is discussed and is attributed to a template. Evidence is presented that for both the morphogenetic process and the formation of this template, appropriate spatial information and organization is embodied and maintained in the cell cortex and is not imposed by the cytoplasm or nucleus. Both nucleus and cytoplasm play only an indirect, supportive role; cytoplasmic polarity and specific structures such as microtubules are also not of primary importance. Template formation and tip growth are not reduced to their biochemical mechanisms, but are instead discussed in terms of known cell wall microstructure and physical properties. A discussion of the nature of scientific explanations is included to demonstrate that the explanations employed in

this thesis are scientifically satisfying even though devoid of exact molecular mechanisms.

Table of Contents

List of Tables

List of Figures

Acknowledgement

I wish to thank the members of my thesis committee for their comments and Dr. Acton for his support and encouragement over the past four years. I also thank Mrs. Chas. O. Tolstad for typing the manuscript.

Introduction

The desmids are a rather specialized group of fresh-water green algae distinctive in their methods of reproduction, both sexual (by conjugation) and vegetative (Fritsch,1935). The placoderm desmids, of which Micrasterias is an example, are characterized by a cellulosic cell wall (Klein and Cronquist, 1967) comprising two distinct halves connected by an isthmus (fig. 1). Micrasterias appears deeply constricted at the isthmus as the two cell halves (semicells) are rather large. Each semicell is divided into lobes by a number of further constrictions or notches. There are generally five lobes; a central polar lobe and in each of the lateral wings, two major lobes which branch repeatedly giving the wing perimeter a dentated appearance.

The cell nucleus lies at the very center of the cell, in the isthmus. During vegetative cell division, a daughter nucleus is segregated to each semicell and a septum forms at the isthmus separating the cytoplasm of the two semicells (fig. 2). The septum is distinctly different from the semicell wall in composition and structure, resembling more the primary walls of higher plants. Hence the septum and the wall into which it develops can be considered as the cell's primary wall (Mix,1961). Once formed, the septum bulges out into the shape of a hemisphere (fig. 2a): The hemisphere develops a pattern of lobes and notches as it grows (fig. 2b and c) so as to produce in several hours a new semicell exactly in the image of

the old (Kiermayer and Jarosch, 1962; Lezenweger, 1966). When this morphogenetic process is complete, a thick layer of wall is laid down immediately inside the primary wall and the primary wall is cast off. The new semicell is then left enclosed by a secondary wall only, this being the normal interphase condition (Drawert and Mix, 1962b).

The Micrasterias cells so far figured are rather flat in side view and are examples of cell types one would find in nature (fig. 3a, c and e). The cells are haploid and show a normal or biradiate symmetry. Other symmetry types called "facies" (by Teiling) have appeared spontaneously in cell cultures or have been induced to form (Heimans, 1942; Teiling, 1950; Waris and Kallio, 1964; Kallio and Heikkilä, 1969). Diploid cells frequently become triradiate (fig. 3b) or quadriradiate, by producing one or two extra wings aligned in different planes. Haploid cells occasionally lose one or two wings to become uniradiate (fig. 3d and f) or aradiate. The loss occurs in a single daughter semicell, but the change may be heritable producing from this one semicell a clone having a new symmetry. Symmetry type appears to be inherited through a non-genetic mechanism, at least in the related genus Cosmarium (Starr, 1958; Tews, 1969). That cells with increased nuclear ploidy tend to become tri- or quadriradiate (Kallio 1954, 1960) can be explained as a general rather than a specific effect of the nucleus, a result of nuclear effect on cell turgor (Green, 1969).

The rather elegant process of primary cell wall morphogenesis has been the subject of my thesis work. I will refer to the process as morphogenesis or development and avoid calling it differentiation.

Various workers have studied the morphogenetic process in Micrasterias and tried to explain how the very specific pattern of lobes and notches arises. Teiling (1950) referred to the activity of "meristematic cytoplasm," feeling that the peripheral cytoplasm is regionally differentiated into areas which could support wall growth, hence producing lobes, and those which could not and would become notches. Kiermayer enlarged on these ideas suggesting first that the meristematic regions are distinguished by their ability to deposit cell wall material (Kiermayer and Jarosch, 1962), and second that the meristematic areas are more loosely attached to the cell wall than the areas which become notches. The non-meristematic areas he called fixation zones, as they appeared to exhibit tight fibrillar attachments between cytoplasm and cell wall (Kiermayer, 1964; see fig. 4a). Kiermayer has studied the ultrastructure of growing cells (Kiermayer, 1968, 1970b) and suggested that a directed flow of vesicles containing wall precursors or a regional specialization of the membrane receiving these vesicles could also account for the differentiation of peripheral cytoplasm into distinct meristamatic regions. He has demonstrated that microtubules do not appear to be involved in morphogenesis (Kiermayer, 1968a, 1968b).

Kiermayer (1970b) was the first to try to break

Micrasterias morphogenesis down into its contributing processes. He distinguished five major causal aspects of morphogenesis;

(1) production of cell wall precursors,

(2) transport of precursors to the wall,

(3) maintenance of a cortical template of meristematic regions,

(4) incorporation of precursors into the wall, and

(5) maintenance of a turgor threshold.

Kiermayer considers these five to be harmoniously operating parts of an organized whole. He comments that experimental manipulations will damage the contributing processes directly and not morphogenesis itself. Morphogenesis, like a machine with a damaged part, would then be more likely to grind to a halt than to continue to function but in an altered pattern. This may explain why it is difficult to experimentally interfere with cell morphogenesis in any but very trivial ways. Cells generally grow either normally or not at all; and it is particularly difficult to experimentally alter the symmetry of new semicells in a predictable way (Waris and Kallio, 1964).

Castle, in his work on growth of Phycomyces sporangiophores, comments on morphogenesis as follows (Castle, 1953; pg. 369):

> "Growth is very close to being life itself, with everything included and implied thereby, so that multiple causation of any phenomenon of growth is generally to be anticipated. The writer is convinced, nevertheless, that problems of orientation are exceptional, and that the bulk of physical and chemical processes contributing to growth do not confer upon it directional properties. Ultimately these are undeniably

> contributed by interaction between the given genetic make-up and the environment, but in a more immediate sense definite facts of structure - molecular or gross - must be involved."

Kiermayer feels he has pinpointed the directional contribution to Micrasterias morphogenesis in his third causal aspect, the cortical template. His template is formed when the septum is formed (Kiermayer, 1967). Its information, perhaps embodied in the adjacent cytoplasm, directs the formation of meristematic areas. The septum could contain instructions for building the whole of the elaborate cell wall or for doing only the first few steps.

In a long series of experiments, Waris and Kallio (1964) demonstrated that the nucleus is not directly required for cell morphogenesis. Cells enucleated by centrifugation, UV irradiation or drug treatment continue to grow and develop for several hours. No matter how early the enucleation is done, dividing cells always produce at least three lobes, one corresponding to the polar lobe and one to each of the two lateral wings (fig. 4b). Waris and Kallio, therefore, proposed that the morphogenetic template depends upon some aspect of the cytoplasm or of cytoplasmic structure. The idea that cytoplasm may have an oriented structure, that it is thixotropic, is a very attractive one. For example, Picken (1960; pg. 163) has quoted Frey-Wyssling;

> "'...the structure of plasm must be of a wonderful co-ordination. The framework cannot represent an unordered pile, but must possess an organized and well-defined structure.'"

but on consideration of the experimental evidence (Moore, 1935; Crick and Hughes, 1950) Picken added;

> "We can agree whole-heartedly that the structure of plasm must be of wonderful coordination, if the statement refers simply to the fact that processes in the cytoplasm are coordinated. But that there is an organized and well-defined structure, in the sense of a firmly bonded framework, is denied by the evidence in those cells that have so far proved accessible to examination."

In Micrasterias the cytoplasm streams actively throughout morphogenesis (Kiermayer, 1964) and so it is particularly difficult to imagine that a firmly bonded cytoplasmic structure is present. Waddington (1962) suggested that a structure might still exist but be dynamic in nature. It is clear that Stentor, for example, shows dynamic organization. Minced cells are able to reorganize their jumbled bits of cell cortex into a normal whole (Tarter, 1960).

Whether dynamic or static, cytoplasmic structure does offer itself as a possible template for Micrasterias morphogenesis. Selman (1966) has shown that such a template does not depend directly on the old semicell as cell damage is not heritable (fig. 4c) except in cases of considerable general cell damage (Selman, 1966; figs. E to H, plate 1). Kallio (1972b) has demonstrated heritable damage in M.torreyi resulting from UV irradiation of the cytoplasm, but interpretation of these experiments is difficult as the damage produced is very non-specific. M.torreyi is, in any case, a very mutable species (Kallio, 1968). Kallio (1972b) has most recently suggested that the template may depend on a particular spatial ordering of cytoplasmic organelles. This has also been

proposed as an explanation for zygospore morphogenesis in Micrasterias (Kies, 1970), but no appropriate order has yet been found in the vegetative cell (Kiermayer, 1970a). Waddington's ideas about dynamic structure are highly speculative. He is at a loss when asked to give more concrete explanations of the template.

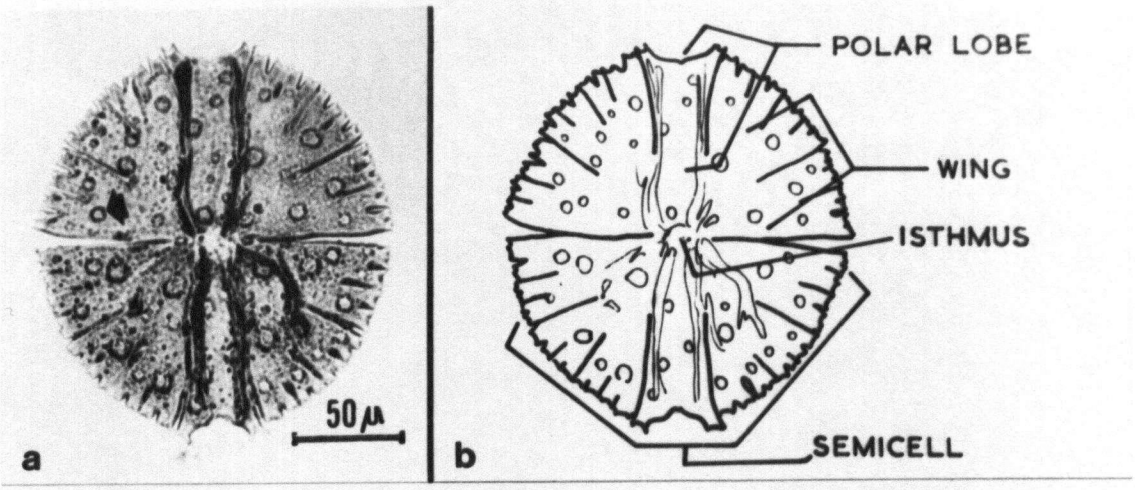
> "It seems to me extremely difficult to envisage a mechanism by which spatial arrangements of material on this scale of magnitude could be brought about. The conventional deus ex machina to be invoked is 'local specialization of the cell surface'; but the pattern of the new half-cell is hardly affected by the pattern of the old surface."

Hence morphogenesis in Micrasterias remains an unsolved problem. It is a very striking process but still a mysterious one. I took a rather broad approach to the problem, wanting to understand the growth process itself but also wanting to explore existing hypotheses, particularly those of Waddington concerning dynamic structure; wanting also to find out about the template, its position and the nature of its substance.

Figure 1 Normal interphase cell of M.rotata
(a) Cell viewed face on to show its shape. The cell is packed with chloroplast and pyrenoids (arrow) except at the isthmus where a clear area indicates presence of the nucleus.
(b) Diagram of the cell from (a) showing its parts. The cell consists of two semicells joined at an isthmus, each semicell having a central polar lobe with a wing on each side.

Figure 2 Stages of morphogenesis in M.rotata. The stages shown are separated from one another by about one hour. Figure 18 gives a more precise representation of stages.
(a) Stage 3: Mitosis is complete and the two daughter cells have been separated by a septum of primary wall. The septum has begun to bulge out.
(b) Stage 7: Five lobes have appeared on each new semicell. The chloroplast has advanced into the new semicell and vacuoles populate the cytoplasm.
(c) Stage 10: An almost complete complement of lobes is present on each semicell. The chloroplast continues its invasion of the new semicells.

Figure 1



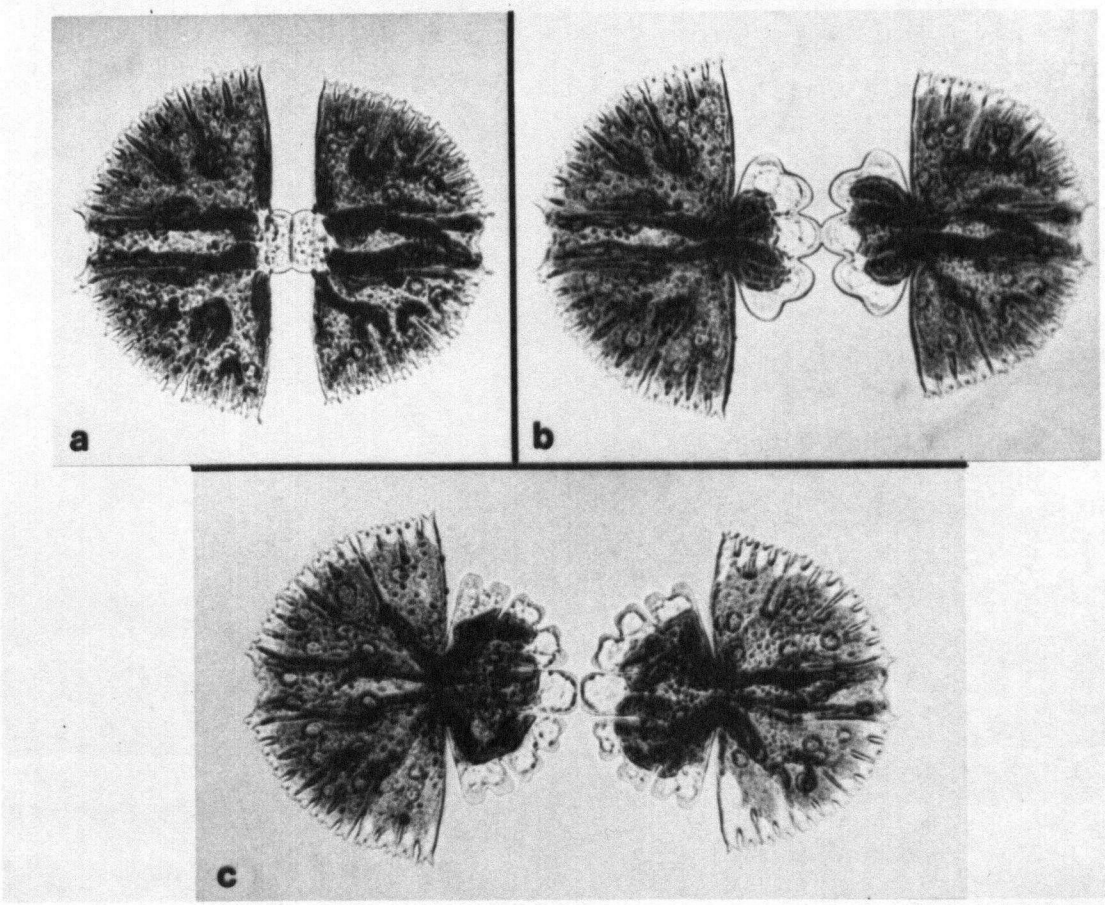


Figure 2

Figure 3 Scanning and light micrographs of normal and abnormal facies of three Micrasterias species

(a) M.rotata biradiate
(b) M.rotata triradiate
(c) M.torreyi biradiate
(d) M.torreyi uniradiate
(e) M.thomasiana biradiate
(f) M.thomasiana uniradiate

Figure 3

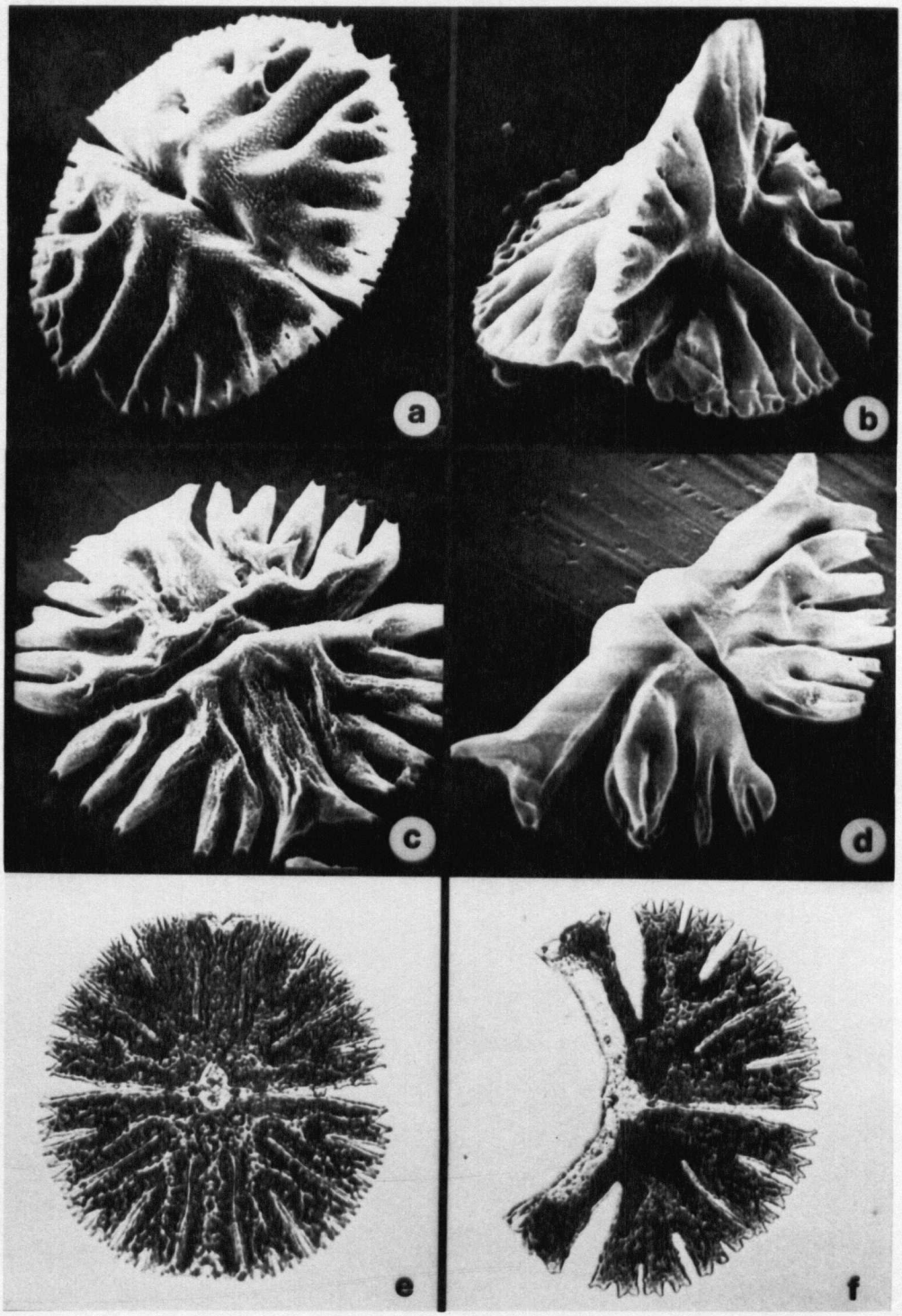
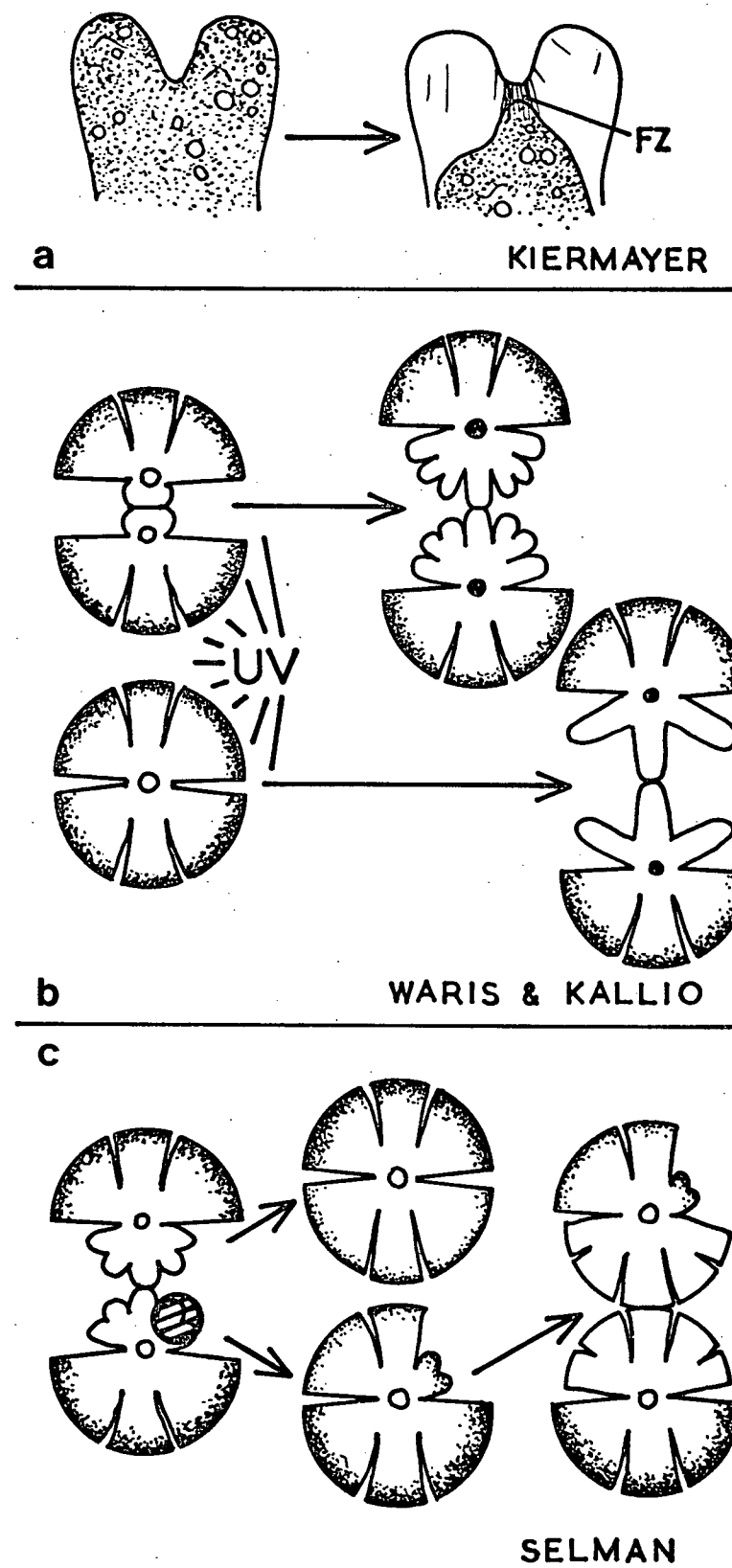


Figure 4 A diagrammatic summary of published experiments concerning Micrasterias morphogenesis
(a) Experiments by Kiermayer (1964) showing by plasmolysis that the protoplast is most tightly attached to the cell wall at lobe notches. Under appropriate conditions, fibrillar connections between cytoplasm and cell wall can be demonstrated at these fixation zones (FZ).
(b) Experiments by Waris and Kallio (1964) in which dividing cells were enucleated with UV radiation. Cells already developing can develop further in the absence of a nucleus. Cells just preparing to divide also develop when enucleated and always produce at least three lobes representing the three major axes; polar lobe and two wings.
(c) Experiments by Selman (1966) in which developing semicell lobes were irradiated with a UV dose sufficient to arrest lobe growth. The abnormal cell thus produced showed normal semicell morphogenesis after its next division and hence the UV induced abnormality is shown not to be heritable.

Figure 4



Materials and Methods

Certain of the species used in this work (details in Table I) were purchased from the Indiana Culture Collection (Starr, 1964). Others were isolated locally and unialgal cultures established from single individuals with identification made by reference to West and West (1905). On occasion abnormally shaped cells were observed in the cultures. These were isolated and cultured and in a few cases gave clones of an abnormal but stable cell type. Cells were grown in a salt solution, the MS medium of Waris (1953). A simple light box served for routine work, supplying fluorescent light of 50 to 120 ft. candles on a cycle of 12 hrs. light, 12 hrs. dark. On this light regime the cells became roughly synchronized, about 20% dividing each day. Those dividing on any given day were synchronized to within about an hour of one another and began division from one to two hours before the light period. Temperature could not be accurately controlled in the light box but generally kept between 20 and 23° C. Stock cultures were kept in an environment room at 20° C under similar light conditions.

About 30 cells at a time were prepared for electron microscopy following the method of Kiermayer (1968) with some adjustments. Initial fixation was in 1% glutaraldehyde for 8 hrs. at 4° C followed by a 2 hr. wash and postfixation in 2% OsO_4 overnight. Dehydrating solutions were made up by mixing methanol with 0.25% aqueous NaCl; dehydration was begun at 15%

Table I

Cell Cultures

Culture	Source
M. rotata	Isolated February, 1970 Beaver Lake, Vancouver, B. C.
M. radiata	Isolated March, 1971 by Marion McCauley Loon Lake near Haney, B. C.
M. denticulata	Isolated February, 1970 Beaver Lake, Vancouver, B. C.
M. thomasiana biradiate	Indiana Collection LB 543
M. thomasiana uniradiate	Indiana Collection LB 548
M. torreyi biradiate	Indiana Collection LB 794
M. sol	Indiana Collection LB 649
M. radiata pygmy	K & S Biological Supply Vancouver, B. C.
M. torreyi uniradiate	Isolated from biradiate cultures June and August 1972
M. rotata triradiate	Isolated from biradiate cultures June 1972

with changes of 10% every 5 minutes. Cells were not centrifuged into a pellet but were processed individually through the solutions using a pipette. Cells were taken through propylene oxide into Spurr's embedding medium (Spurr, 1969) and sectioned on an LKB microtome. Thick sections (1 μ) were cut for light microscopy, mounted on glass slides and stained with toluidine blue. Thin sections were supported on grids coated with paraloidin and carbon and stained with uranyl acetate and lead citrate or with uranyl acetate alone. Staining times of up to two hours were required; lead staining was done according to Reynolds (1963). Stain made from commercial lead citrate was not satisfactory and left a peppery residue. Micrographs were taken using the Hitashi HS- 7S and AEI 801S microscopes. Electron microscopy was done only on M.rotata.

Dividing cells treated in various vital dyes were irradiated with a helium-neon gas laser microbeam, the laser (Metrologic Instruments) producing an 0.5 mWatt continuous beam in the red region at 633 mμ. The microbeam apparatus has been previously described (Lacalli and Acton, 1972). The beam was focussed using the condenser (Leitz #77) of a Leitz Ortholux microscope with the condenser front lens in place (fig. 5). Lasings were observed and photographed by means of standard microscope optics with a green interference filter interposed so as to reduce the beam intensity. Direct viewing of the

beam is probably not dangerous under these conditions (Bloom, 1968). Photographic records of lasings were taken as double exposures, the beam being photographed with the condenser front lens in place and the object photographed with normal illumination and the lens removed. All the laser experiments reported here were done with M.rotata, though experiments with M.radiata gave similar results.

In a typical laser experiment, 5 to 10 dividing cells were placed in a dish containing a known concentration of a dye in culture medium (details in Results, Part IV). After 30 minutes, a cell was removed and placed in a drop of medium between a sandwich of coverslips (fig. 6), and this put on the microscope stage. The cell would be lased for from 1 to 60 seconds with spots of diameter 2 μ or greater, though the cell itself really experiences a cone of laser light (fig. 7). Finally, a photograph was taken to record the exact stage of the lased cell and the point of lasing, and the cell removed to a spot plate depression filled with culture medium so that development and later cell divisions could be observed. In this fashion, one dividing cell could be lased about every 5 minutes. After 15 minutes, the dye solution would be freshened by adding sufficient dye from a stock solution to double the dye concentration, as many of the dyes tend to precipitate from solution. Generally only 5 or 6 cells were lased each day so that the dye solution was freshened only once.

Patterns of cell wall synthesis were studied in M.rotata by exposing dividing cells to tritiated compounds,

isolating the cell walls as ghosts and preparing autoradiograms from them. Usually 30 to 50 dividing cells were placed in a small dish containing 0.3 to 0.4 ml of culture medium made up to a label concentration of 200 μC/ml (glucose, methionine or proline) or 50 mC/ml (water). Cells were left in the solution for a measured length of time and then removed to a dish of distilled water, washed in several changes of water, centrifuged to the bottom of a test tube containing distilled water to which grains of sand had been added and the tube immersed in an ultrasonic cleaner for 20 to 30 seconds. The ultrasonic cleaner served to break cells apart, releasing the protoplasm. Several standard commercial models were tried and all worked. The time from first washing to rupture by ultrasonics was standardized at 5 minutes.

Isolated walls were stored in 5 to 10 ml distilled water to which a few drops of commercial formalin had been added. For mounting, a few drops of 0.1% aqueous crystal violet were also added to the solution. This colored the primary walls so that they could be picked out under a dissecting microscope and dried onto albuminized slides, about 25 cell walls to a slide. The slides were dipped in acetone to remove the dye and in Carnoy's fixative for 10 minutes to wash out small molecules. For autoradiography, slides were dipped once in Ilford L-4 nuclear emulsion made up in hot water as 2 gm/10 ml. Slides were left in light-tight boxes with dessicant for one month at 4° C, developed for 4 minutes in D-19, stained in 0.1% crystal violet and mounted.

A few simple staining tests were done on the primary wall to determine its chemical properties. Cell wall ghosts were mounted as described above for autoradiograms. Various extraction methods were employed and these, with controls, were stained with PAS, alcian blue or crystal violet (Table II). In the case of enzyme extractions, controls were done with enzyme first dissolved in a small volume of distilled water and immersed in a boiling water bath for an hour. Sets of slides which were to be directly compared were all stained together.

Various chemicals and enzymes were tested in dividing cells. These were dissolved in culture medium, and several dozen dividing cells at various early stages were then introduced. Results were discarded if they did not consistently point to one effect, as a chemical may produce diverse effects, killing some cells while causing others to burst, swell or arrest their morphogenesis. Effective concentration ranges were determined for each chemical thought to be of interest, though no attempt was made to determine to what extent the various chemicals could penetrate cells. These experiments and those on cell wall analysis were done with M.rotata.

The shapes of interesting cells were recorded in photographs, as the outline of cells is rather complex and a hand sketch was not thought precise enough.

Table II

Treatments for Cell Wall Preparations

Treatment	Procedure	Reference
Wash for autoradiograms	Carnoy's fixative, 30 min. at 20° C	
Mild alkali extraction	0.5% ammonium oxalate, 24 hrs. at 60° C	Jensen (1962)
Alkali extraction	4% NaOH, 24 hrs. at 20° C	Jensen (1962)
Strong alkali extraction	17.5% NaOH, 24 hrs. 20° C	Jensen (1962)
Mild methylation	2% $SOCl_2$ in abs. methanol, 6 hrs. at 20° C	Pearse (1968)
Strong methylation	0.5% HCl in abs. methanol, 12 hrs. at 50° C	Pearse (1968)
Saponification	1% HaOH in 80% ethanol, 1 hrs. at 20° C	Pearse (1968)
Chromic acid	10% chromic acid, 4 hrs. at 20° C	Mix (1961)
Pectinase	0.5% enzyme in 0.2 M sodium acetate buffer pH 4.0, 24 hrs. at 27° C	Kertesz (1951)
Cellulase	0.5% enzyme in 0.2 M sodium acetate buffer pH 4.5, 24 hrs at 37° C	Pigman (1951)
Hemicellulase	0.5% enzyme in 0.2 M sodium acetate buffer pH 5.5, 24 hrs. at 37° C	Pigman (1951)
Trypsin	0.1% enzyme in 0.05 M Tris with 0.02 M $CaCl_2$ pH 8.1, 16 hrs. at 27° C	Walsh (1970)
Pectin esterase	0.1% enzyme in 0.1 M phosphate buffer pH 7, 24 hrs. at 27° C	Kertesz (1951)
PAS	1% periodate for 20 min., Schiff reagent for 30 min., 2 fresh sulfite washes	Jensen (1962)
Alcian blue pH 2.5	0.3% alcian blue in 3% acetic acid for 20 min., washed in 3% acetic acid	Parker and Diboll (1966)
Alcian blue pH 0.5	0.3% alcian blue adjusted to pH 0.5 for 20 min., washed in water adjusted to pH 0.5	Parker and Diboll (1966)
Crystal violet	0.1% crystal violet, 10 min. at 20° C	Drawert and Metzner-Kuster (1961)

Cells were prepared for scanning electron microscopy by freeze-drying cells onto aluminum stubs and coating with gold. Stubs were observed using a Cambridge Stereoscan microscope.

Sources for some of the chemicals used in this work are listed in Table III.

Table III

Chemicals Used

Chemical	Source
L- methionine (Methyl-^{3}H)	ICN
D- glucose - 1 - ^{3}H	ICN
Water - ^{3}H	ICN
L- proline - ^{3}H (generally labeled)	ICN
D-glucose-6 - ^{3}H	ICN
Pectin methyl esterase (tomatoe)	Sigma
Cellulase, practical type 1 (Aspergillus)	Sigma
Pectinase, purified (fungal)	Sigma
Trypsin	Difco
Hemicellulase, crude grade II (Rhizopus)	Sigma
Pronase, B-grade	Cal Biochem
Alcian blue	Gurr (ESBE)

Figure 5 Laser microbeam apparatus utilizing a standard microscope of which only the condenser, stage and objective are shown. A condenser front lens of high numerical aperture and an additional short focal-length lens are shown introduced into the light path to further reduce beam diameter. The mirrors shown are front-surfaced to reduce reflection of multiple images.

Figure 6 Method of mounting cells for laser irradiation. The chamber is constructed of cover glasses and cover glass fragments and serves to sandwich a drop of culture medium containing the cell to be lased.

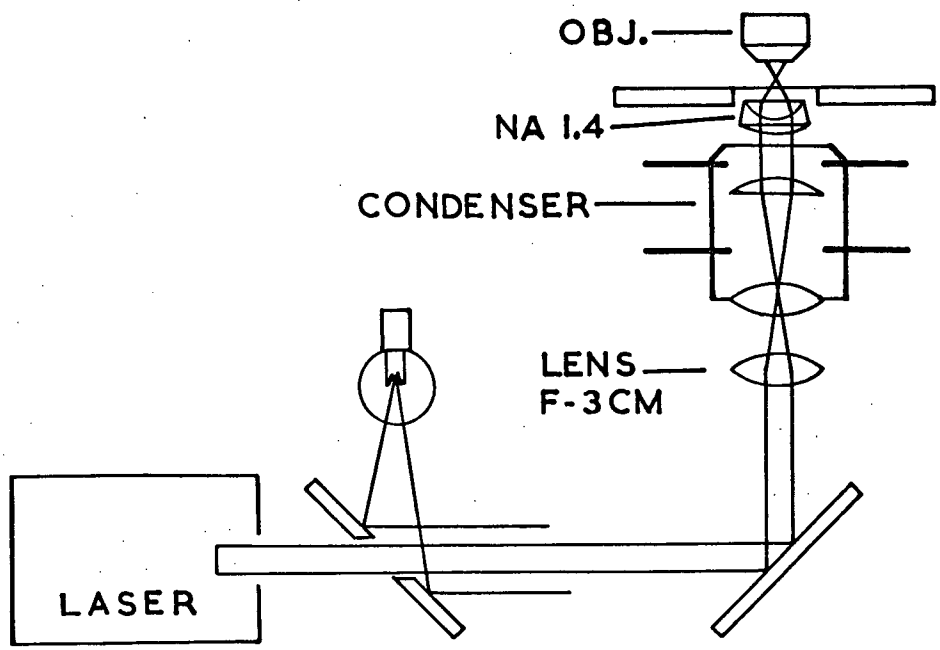
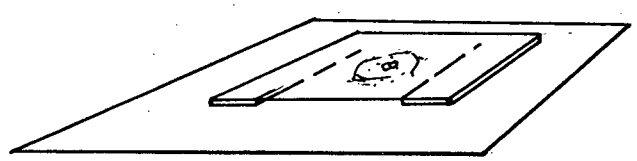


Figure 5

Figure 6



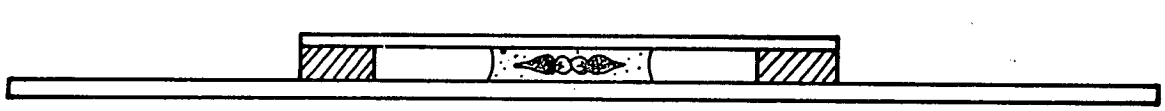
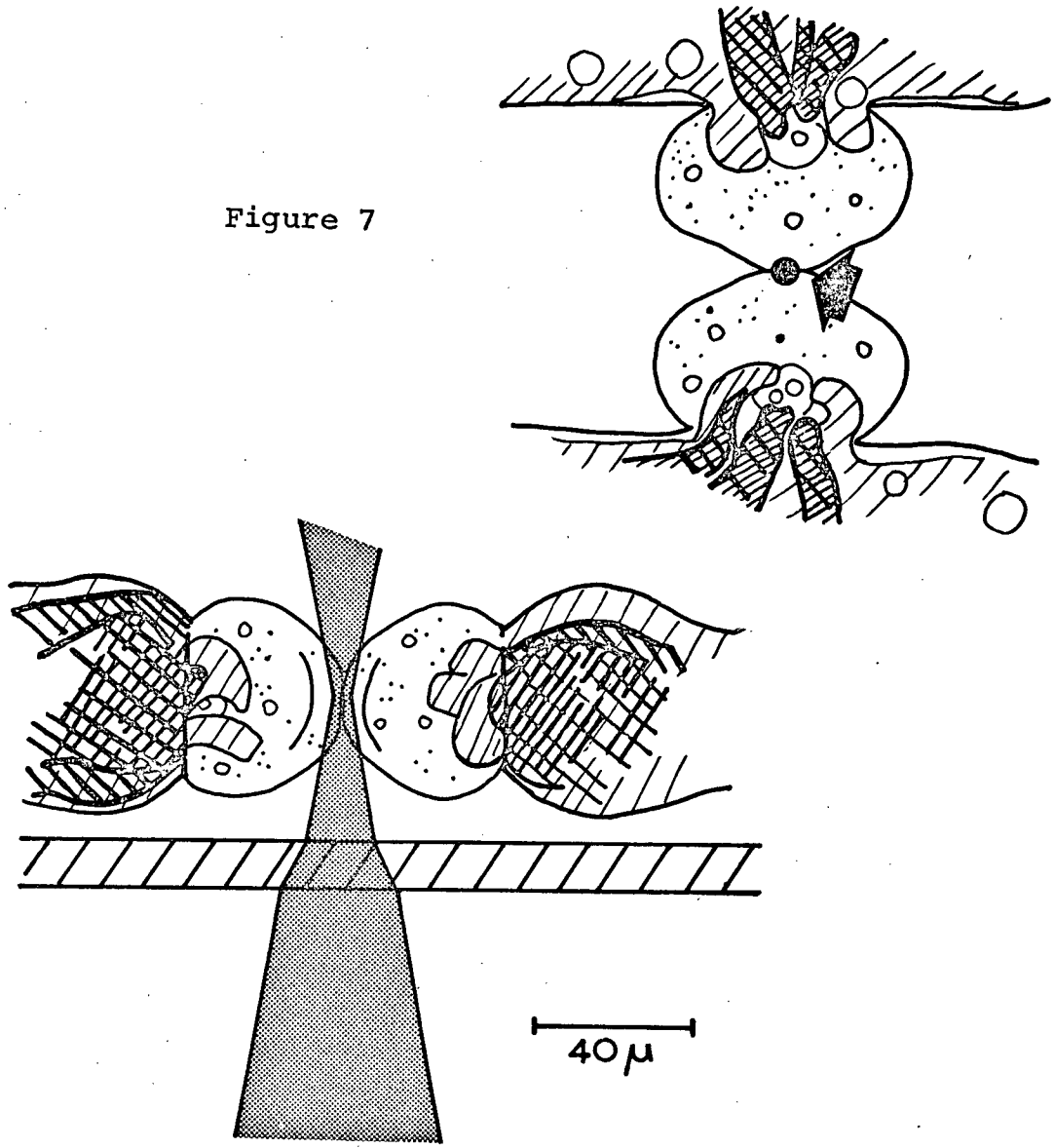


Figure 7 Laser microbeam dimensions. An experimental lasing viewed through the microscope is seen only as a spot of light (arrow). In side view the microbeam is a double cone of light which may irradiate a much larger area of wall than is immediately apparent.

Figure 7



Results and Discussion

I. Observations on Sectioned Material

Reports have been published on the fine structure of several Micrasterias species describing general characteristics of the cells and their organelles; nucleus and nucleolus (Drawert and Mix, 1961b, 1961c), chloroplast and pyrenoids (Drawert and Mix, 1961d, 1962a), cell wall (Drawert and Mix, 1961a; Kiermayer and Staehelin, 1972), golgi (Drawert and Mix, 1961e; Staehelin and Kiermayer, 1970), mitochondria (Drawert and Mix, 1961f) and the microtubular system (Kiermayer, 1968b). Kiermayer (1970a) has studied the structure of dividing cells and Kies (1970) of the developing zygospore. My own EM studies have been brief, but directed toward the solution of specific questions concerning morphogenesis. My survey of general structure in M.rotata confirms the published reports, but I also looked at the ultrastructure of septum formation for which there is no published description. Very early stages of septum formation cannot be distinguished by light microscopy.

For this study, about 30 cells were selected from a synchronized culture in which a number of cells were beginning to divide. When sectioned, 15 of these could be seen to be in interphase and the others in various early division stages; 4 in post-telophase, 4 in telophase, 2 in metaphase and 5 in prophase. One cell was at a particularly early stage of cell division and showed only the barest suggestion of a forming primary wall.

Problems with fixation and infiltration were encountered. I solved the latter by using Spurr's embedding medium rather than Epon, as Spurr's is much less viscous. The fixation problem was not so easily solved. The cytoplasm of both dividing and interphase cells was found to be filled with large empty spaces, probably caused by the bursting of slime-filled vesicles during fixation. Kiermayer (1968b) found similar damage in the cytoplasm of M.denticulata, but eliminated the spaces and maintained a population of large, membrane-bound vesicles by using glutaraldehyde very sparingly, fixing in 1% glutaraldehyde for 10 minutes. I was unable to improve the appearance of M.rotata cytoplasm using Kiermayer's fixation schedule and hence relied routinely on long glutaraldehyde fixation so as to minimize general shrinkage of the protoplast. This was important as I was interested in the cytoplasm-cell wall interface.

Figure 8 summarizes the ultrastructure of a typical interphase cell. Mitochondria are long and threadlike but generally are seen in cross-section. A row of microtubules lies adjacent to the cell wall at the isthmus, forming rings around the nucleus. Dictyosomes show large and regularly stacked cisternae and line the chloroplast membrane in all parts of the cell. Small vesicles with dark centers appear throughout the cytoplasm but particularly around the dictyosomes. They correspond to Kiermayer's dark vesicles (DV) and appear to be associated with the dictyosome tubules. The dictyosomes are clearly not producing vesicles from their cisternae.

Large vesicles appear throughout the cytoplasm; their contents are contracted to the center of each vesicular space and resemble in appearance the slime contained in the pores of secondary wall. From thick sections the chloroplast can be seen to be a thin wafer, coated, as is the cell wall, with a thin layer of cytoplasm. Cell volume is largely taken up with vacuole or packed with large vesicles.

Figure 9 illustrates cells probably in prophase. A girdle of wall material encloses the isthmus and is of different thickness in different cells. This girdle appears to be primary wall and closely resembles in structure the bits of primary wall left from previous cell divisions. Dictyosomes throughout the cell appear active. They are not so closely associated with the chloroplast as in interphase, and their outer cisternae are greatly enlarged. Stacks of ER are also present and lie near the chloroplast. Microtubules do not appear to be as abundant at the isthmus as in interphase cells, but I have only a few micrographs on which to base this conclusion. There is nothing distinctive about the nuclei in these cells to indicate that the cells are in prophase of mitosis. Prophase as seen in the light microscope may last several hours and precedes septum formation (Waris, 1950a). These cells appear to be at a stage of division approaching septum formation and so I will refer to this stage of wall development as a prophase stage.

Figures 10 and 11 illustrate a telophase cell with the septum about half formed. Nuclear envelope encloses the many small chromosomes, and spindle microtubules are still visible.

Microtubules appear to be entirely absent from the septum and isthmus area. The cytoplasm is particularly dense around the inner edge of the septum, perhaps because large vesicles appear to be excluded from this area. A few membrane-bound vesicles lie along the primary wall external to the plasma membrane.

Figures 12 and 13 illustrate a cell of about stage 7 of cell morphogenesis. Chloroplast lobes enter the new semicell on either side of the nucleus. Dictyosomes cluster about the ends of the chloroplast in an area which in live cells does not stream as actively as cytoplasm in the semicell lobes is observed to do (Jarosch, 1962). Lobes themselves are filled with vesicles and ER. By stage 7, microtubules should again be present at the surface of secondary wall at the isthmus (Kiermayer, 1968b), but I have not observed them. Microtubules are present adjacent to the nucleus. Vesicles are occasionally seen between the plasma membrane and cell wall. Peculiar lamellar structures also occur in these spaces and in the cytoplasm, but their distribution in the cell follows no conspicuous pattern. The primary wall appears to be of similar structure throughout and is not noticeably different in structure from the primary wall present at earlier stages.

Figure 14 illustrates sections of a single cell which appears to be at a very early stage of primary wall formation. I will call this stage an early prophase stage, though again, EM does not properly reveal the state of nuclear organization. In the early prophase cell, a thin, uniform layer of primary wall-like material girdles the isthmus. Deposits of similar

material occur also in interphase cells and are found most often along the inside of secondary wall at notches in old semicells. The early prophase cell represents the only case in which such a deposit was found at the isthmus. In addition, dictyosomes appeared more active in this cell than in interphase cells. They appear in the cytoplasm at some distance from the chloroplast and have outer cisternae which are slightly enlarged. Microtubules are present at the isthmus as is a band of vesicles lying external to the plasma membrane and encircling the isthmus.

Figure 15 shows semidiagrammatically the course of septum development as suggested by the EM evidence presented here. Of particular interest is the movement of microtubules. Bands of wall microtubules similar to the isthmus band are seen in other desmids, in Closterium (Pickett-Heaps and Fowke, 1970a) and in Penium (Gerrath, 1968). The isthmus band is reminiscent of the preprophase band of microtubules found to encircle the nucleus in some higher plants. These bands may supply microtubules to the mitotic spindle either in subunit form or as whole tubules able to migrate from wall to spindle during prophase (Pickett-Heaps and Northcote, 1966). It is not surprising then that microtubules are absent from the isthmus wall during mitosis in M.rotata. Microtubules also lie along side the post-telophase nucleus. Kiermayer (1968a) suggests that these may anchor the nucleus and return it to the isthmus following chloroplast migration in a fashion similar to that observed in Closterium. When Micrasterias is treated with colchicine (Kiermayer, 1968a), both nucleus

and chloroplast take up abnormal positions in the new semi-cell. Post-telophase microtubules may act through their ability to stabilize cytoplasmic gels (Porter, 1966), strengthening the cytoplasm around the invading chloroplast and nucleus.

There is considerable argument as to whether microtubules act to direct deposition of cell wall materials. There is good evidence that they do direct secondary wall deposition (Pickett-Heaps, 1967), but far less as regards primary walls (Newcomb, 1969). Only with Nitella has colchicine been shown to alter synthetic patterns (Green, 1962) and here the observations were rather cursory. Microtubules are present along many growing primary walls but absent from many others, particularly from the growing regions of cells showing tip growth (Newcomb, 1969). Only a very few microtubules are found along the growing primary wall of M.denticulata, and colchicine does not alter morphogenesis of this species (Kiermayer, 1968a, 1968b) or of M.rotata. Microtubules may orient septa or direct the initiation of cross-walls in some cells. Microtubules are present in some cells which divide by furrowing (Johnson and Porter, 1968) and are important in the construction of cell plates, in which they may lie longitudinally as in phragomoplasts or transversely as in phycoplasts (Pickett-Heaps, 1972). The argument that preprophase bands of microtubules act to orient cell division or cell plate formation has been discredited (Pickett-Heaps, 1969a). Colchicine will interfere with cell plate formation (Whaley et al., 1966) but does not affect septum formation in Micrasterias (Kiermayer, 1968a).

It is interesting to speculate as to why the septum in Micrasterias initiates at the isthmus and not somewhere else. Double cells exist having two isthmuses but only one nucleus. These cells can form a complete septum at both isthmuses (Kallio, 1963) and hence the position of the nucleus is not an important factor, rather there is something peculiar about the isthmus itself. Note from figure 15 that the girdle initially appears at the isthmus roughly adjacent to the region of wall microtubules. Kiermayer (1968b) supports the idea that these microtubules are an important positioning factor in septum formation. Note the vesicles collected about the girdle in figure 14 (fig. 14c and f); these could be taking part in girdle thickening or septum initiation. And microtubules thus associated with vesicles at a time of girdle thickening could be acting as guide elements in girdle deposition and septum initiation much as they are thought to do in secondary wall thickening.

Microtubule bands are characteristic isthmus structures, but the isthmus also lacks the pores found throughout the rest of the secondary wall (fig. 17). Slime contained in these pores coats both the inside and outside of the wall, hence the isthmus may be the only part of the cell at which completely naked secondary wall is exposed to the adjacent cytoplasm. Figure 16 shows the primary wall initial stopping abruptly at the first pore body. Microtubules in this region thin out only gradually and lie over wall which is not accumulating primary wall material. The spatial correspondence between initial

girdle deposition and wall nakedness is, therefore, better than that between deposition and microtubule position. The wall could be offering, at the isthmus, a substratum for wall deposition found nowhere else in the cell. And the argument could be made that microtubules are only one of several contributing factors, that the substratum upon which deposition occurs is also very important, being in this case the limiting factor.

Within the usual limitations of micrograph interpretation, golgi vesicles are generally thought of as a means of packaging and transporting cell wall substances in plants (O'Brien, 1972). It is not surprising then, that in Micrasterias the dictyosome cisternae become very active just as primary cell wall is first being laid down. The product of the dictyosome cisternae, large vesicles with rather fragile-looking membranes, appear very little different from the large vesicles present throughout interphase. These two sorts probably correspond to the single population of L vesicles found by Kiermayer (1970a) in M.denticulata. I have not seen clear evidence of incorporation of large vesicles into primary wall and neither has Kiermayer. He feels that the L vesicles may supply slime to pores in the secondary wall rather than being precursors of primary wall. This conclusion is strengthened by my observation (fig. 10b) that the inner edge of the growing septum, most likely its growing edge, is surrounded by cytoplasm densely packed with dark vesicles but from which large vesicles are excluded. Kies (1970) has shown that the zygote of M. papillifera forms a primary exospore very like the vegetative primary wall in structure. Small, dark vesicles are present in

large number and appear to be associated with the dictyosomes; but no large slime vesicles are present, and the dictyosome cisternae are inactive. Related freshwater algae; Closterium (Pickett-Heaps and Fowke, 1970a), Triploceras (Gerrath, 1968), Spirogyra (Fowke and Pickett-Heaps, 1969) and Oedogonium (Pickett-Heaps and Fowke, 1970b), all have large vesicles in both interphase and division stages similar to those I found in M.rotata. Fowke and Pickett-Heaps (1969) call these "fuzzy" vesicles and suggest that they contain slime which is contributed to the cell cross-wall when it forms. Kiermayer is the only microscopist who has managed to fix these vesicles so as to maintain their membranes. In Closterium and Spirogyra the vesicles show fusion profiles with growing primary wall, and in both cases these can be attributed to fixation artifact. In both Closterium and Spirogyra there is a large population of smaller vesicles associated with wall growth. In Spirogyra this association is particularly clear as it is small vesicles rather than large ones which form the phragmoplast or cell plate.

From the evidence above, one might conclude that large vesicles are not as important in their contribution to primary wall as are other golgi products despite the considerable production of large vesicles occurring at each cell division. But large vesicles are consistently associated with primary wall, particularly at stages of girdle thickening (fig. 14c and f), and so it is difficult to imagine these vesicles having no role at all. Dictyosomes in other plant cells have been shown to produce two sorts of vesicles

simultaneously, both associated with polysaccharide synthesis and transport (Pickett-Heaps, 1968); the tubular system surrounding distyocome cisternae may produce its own product distinct from that of the cisternae (Mollenhauer and Morre, 1966). In M.rotata the role of the several sorts of golgi vesicles in production of primary cell wall has yet to be precisely determined.

In conclusion, electron microscopy reveals some of the cytoplasmic machinery responsible for cell wall synthesis, but provides no facile explanation for the various patterns of wall morphogenesis. As a possible exception to this, during girdle initiation, primary wall deposition correlates spatially with both microtubules and with regions of wall lacking pores. Both may participate in directing wall deposition at this particular stage of development.

Figure 8 Fine-structure of interphase cells
(a) Thick section of an entire cell. The nucleus with its nucleoli is at the center. The very dark areas are chloroplast and contain occasional pyrenoids. Most of the light areas are either vacuole or are packed with large vesicles and contain very little cytoplasm. Stained with toluidine blue.
(b) A pore in the secondary wall having a canal through which pass threads of slime. These exit the canal on both outside and inside, on the inside forming a pore bulb. Stained with uranyl acetate and lead. x 52,200
(c) Part of the cell isthmus. The two semicells come together here, and their secondary walls can be seen to hook into one another (arrow). A row of microtubules are seen in cross-section along the wall; they encircle the isthmus. Stained with uranyl acetate only. x 20,800
(d) Mitochondrion in longitudinal section. Mitochondria are seen as threads in living cells but are most frequently cut across in sections. Stained with uranyl acetate and lead. x 16,000
(e,f) Interphase dictyosomes showing tight stacks of flattened cisternae. Dictyosomes are closely associated with chloroplast and lie within the thin layer of cytoplasm adjacent to the chloroplast. Elements of ER, both cisternae and tubules, are found immediately adjacent to the chloroplast membrane lying between chloroplast and dictyosomes. Vesicles of various sorts including large vesicles and dark vesicles are present throughout the cytoplasm. Smaller vesicles and tubular elements are particularly abundant at the margins of dictyosome cisternae. Stained with uranyl acetate and lead.
(e) x 18,700
(f) x 16,400

Abbreviations: Nucleus (N), mitochondria (M), secondary wall (SW), chloroplast (Ch), large vesicles (LV), dark vesicles (DV), slime threads (Th), pore canal (C), pore bulb (B), microtubules (Mt)

Figure 8

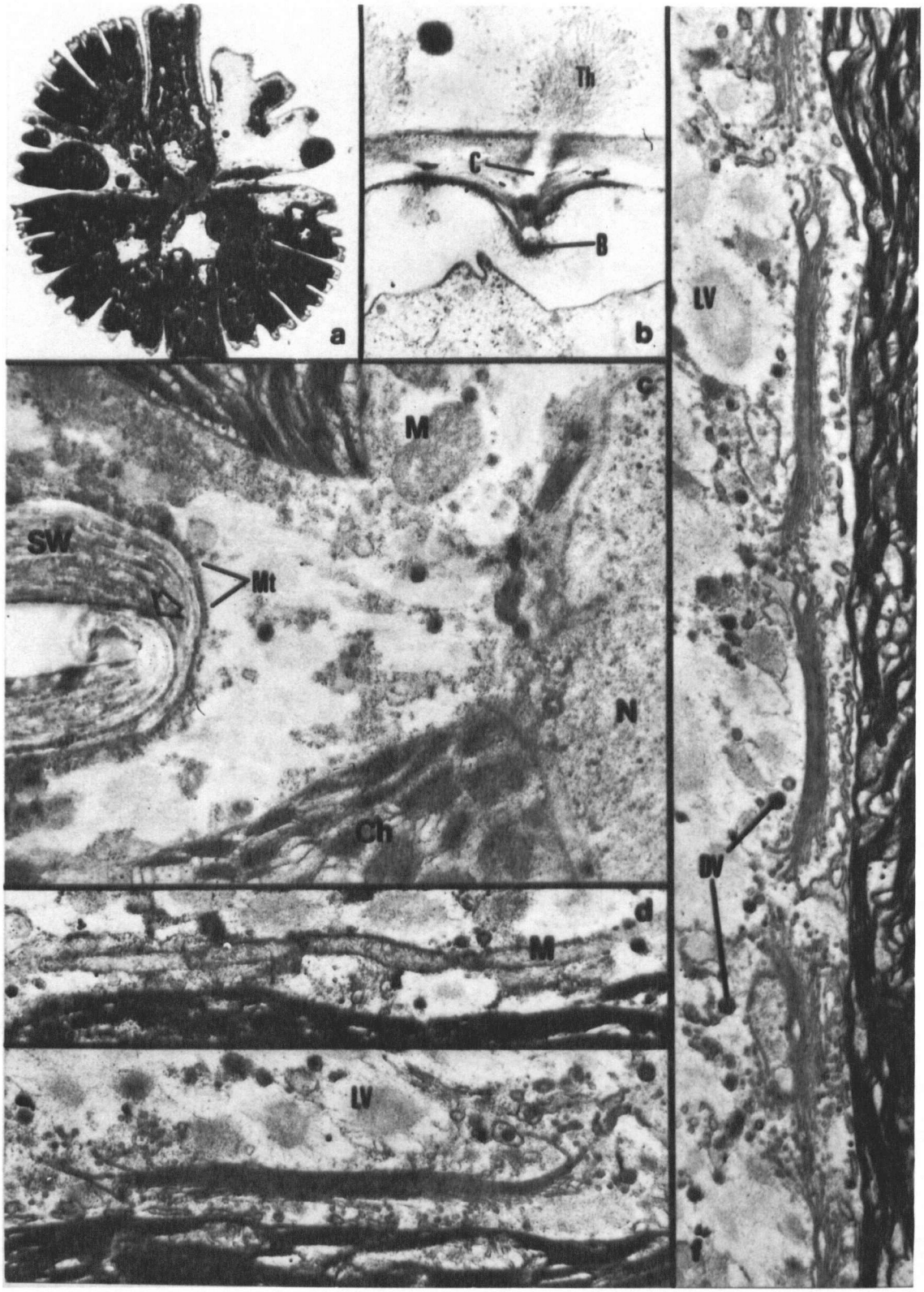


Figure 9 Fine-structure of prophase cells
(a) Thick section of an entire cell very little changed from the interphase condition. The nucleus is perhaps slightly larger and the chloroplasts have withdrawn to some extent from the isthmus area. Stained with toluidine blue.
(b) Cell isthmus showing the semicell walls no longer as tightly hooked as in interphase. A thin girdle of primary wall material lines the inside of the secondary wall. A tuft of similar material is also present between the two semicell walls and is probably primary wall remaining from a previous cell division. Possible wall microtubules are visible. Stained with uranyl acetate only. x 19,300
(c) A later prophase isthmus showing the thickened girdle of primary wall material. Microtubules are not evident. Stained with uranyl acetate and lead. x 26,700
(d) Dictyosomes lying loose in the cytoplasm. The cisternae are active; that is, they are inflated to form large vesicular spaces. Numerous dark vesicles are present (large arrow) and frequently one dictyosome cisterna stains more darkly than the others (small arrow). Stained with uranyl acetate and lead. x 22,000

Abbreviations: Nucleus (N), microtubules (Mt), primary wall (PW), secondary wall (SW)

Figure 9

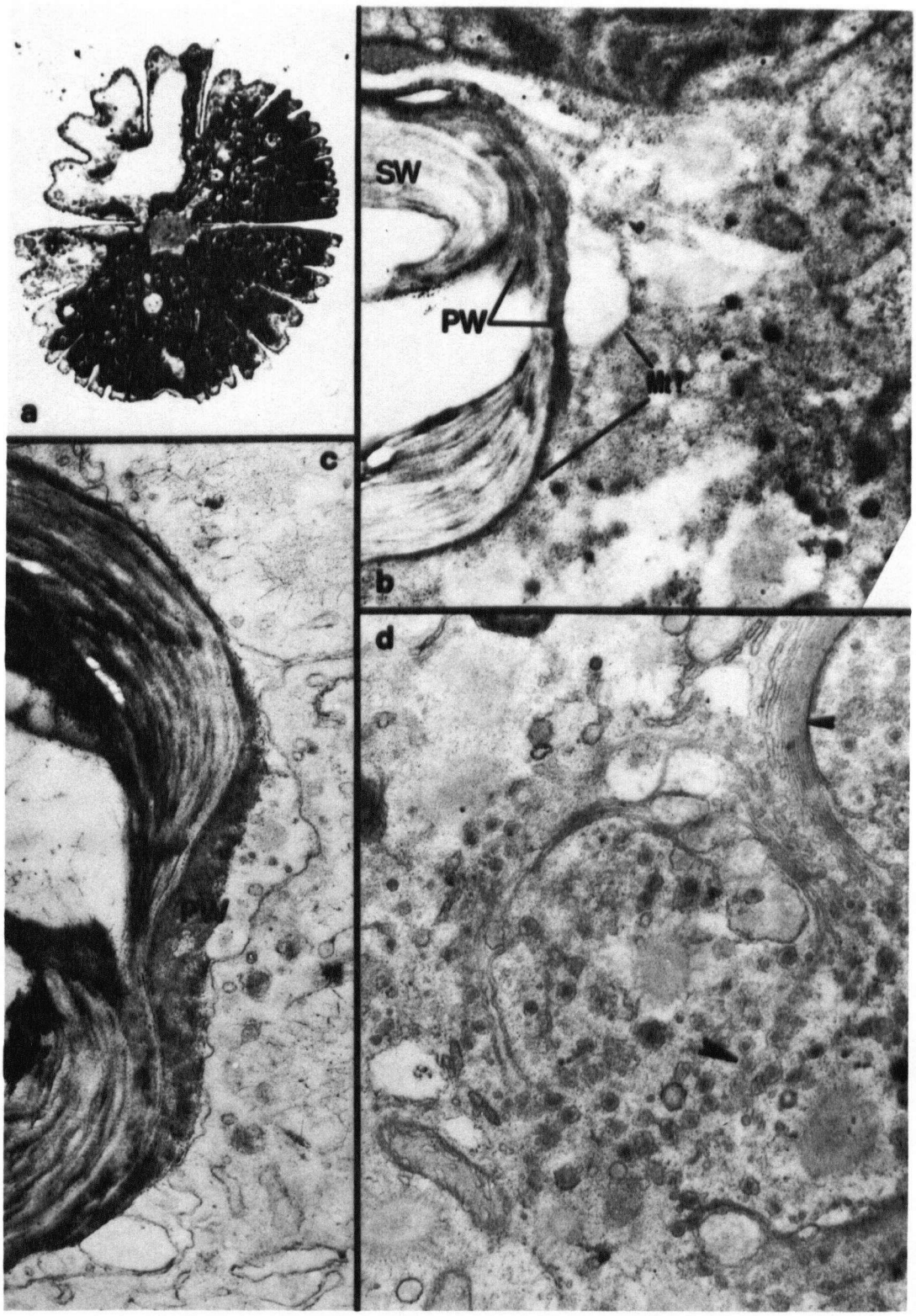


Figure 10 Fine-structure of telophase cells
(a) Thick section of a whole cell. The septum is complete except for a small gap at the center; some bulging of the septum has occurred. Both daughter nuclei are visible. Stained with toluidine blue
(b) Growing primary wall septum with one end anchored to the secondary wall and the other lying free in the cytoplasm (arrow). This leading edge of the septum is surrounded by a region of dense cytoplasm (double arrows) rich in small vesicles and from which large vesicles are excluded. Stained with uranyl acetate and lead. x 19,400
(c) Central region of the isthmus. Note the leading edge of the growing septum (arrow) and the surrounding area of dense cytoplasm. No cell plate structures are seen associated with septum formation and no microtubules are seen in the cytoplasm adjacent to the septum. Stained with uranyl acetate and lead. x 14,200
(d) Telophase nucleus with condensed chromatin and an incompletely reformed nuclear envelope (arrows). Stained with uranyl acetate and lead. x 12,600

Abbreviations: Nucleus (N), large vesicles (LV), dark vesicles (DV), secondary wall (SW), primary wall (PW)

Figure 10

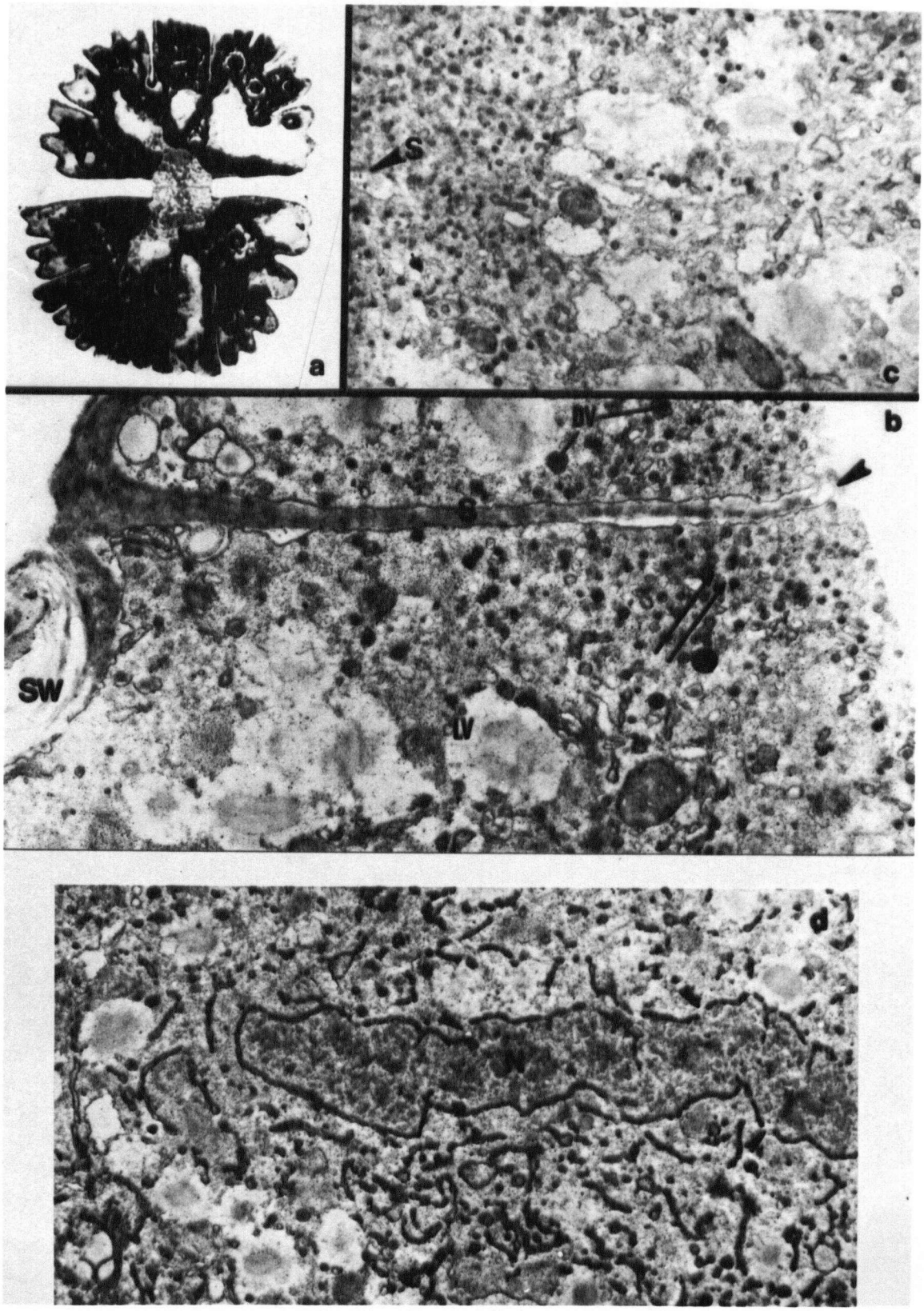


Figure 11 Fine-structure of telophase cells. Sections stained with uranyl acetate and lead.
(a) Endoplasmic reticulum showing the large stacks of cisternae present in dividing cells. These usually appear first in prophase and telophase in association with the chloroplast. x 24,800
(b) Dictyosomes with very active cisternae. x 23,000

Abbreviations: Chloroplast (Ch), starch grains (St)

Figure 11

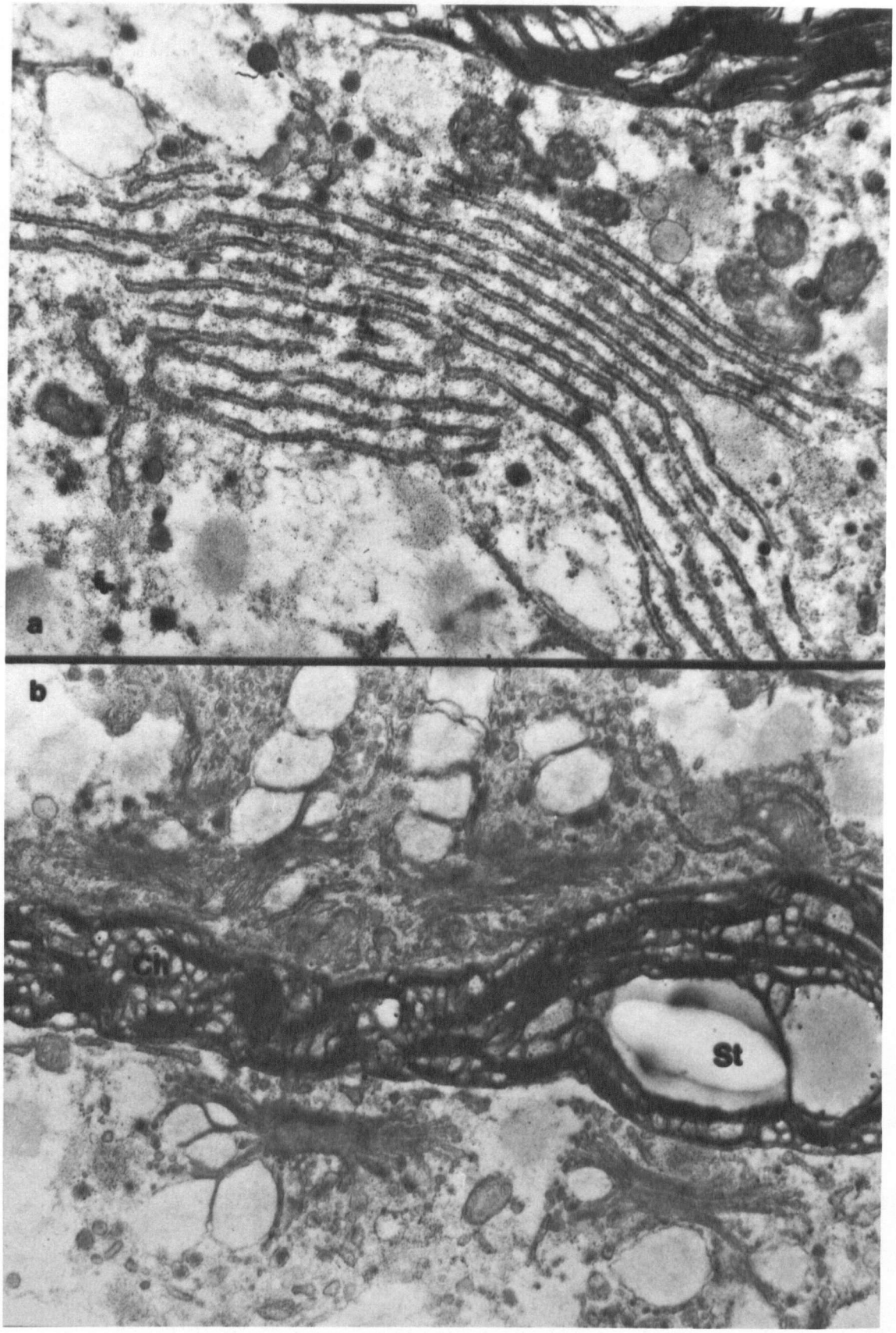


Figure 12 Fine-structure of a developing semicell, stage 7. Sections stained with uranyl acetate and lead. Note the central nucleus and invading chloroplast with surrounding cytoplasm rich in dictyosomes. Dark vesicles and the spaces indicating large vesicles are present throughout the cytoplasm and take up a major portion of the tips of lobes. Growing lobes are also rich in ER and mitochondria. x 3300
Inset: Primary wall and adjacent cytoplasm of a developing semicell. x 92,000

Abbreviations: Nucleus (N), vacude (vac), regions rich in dictyosomes (g), regions rich in ER (er)

Figure 12

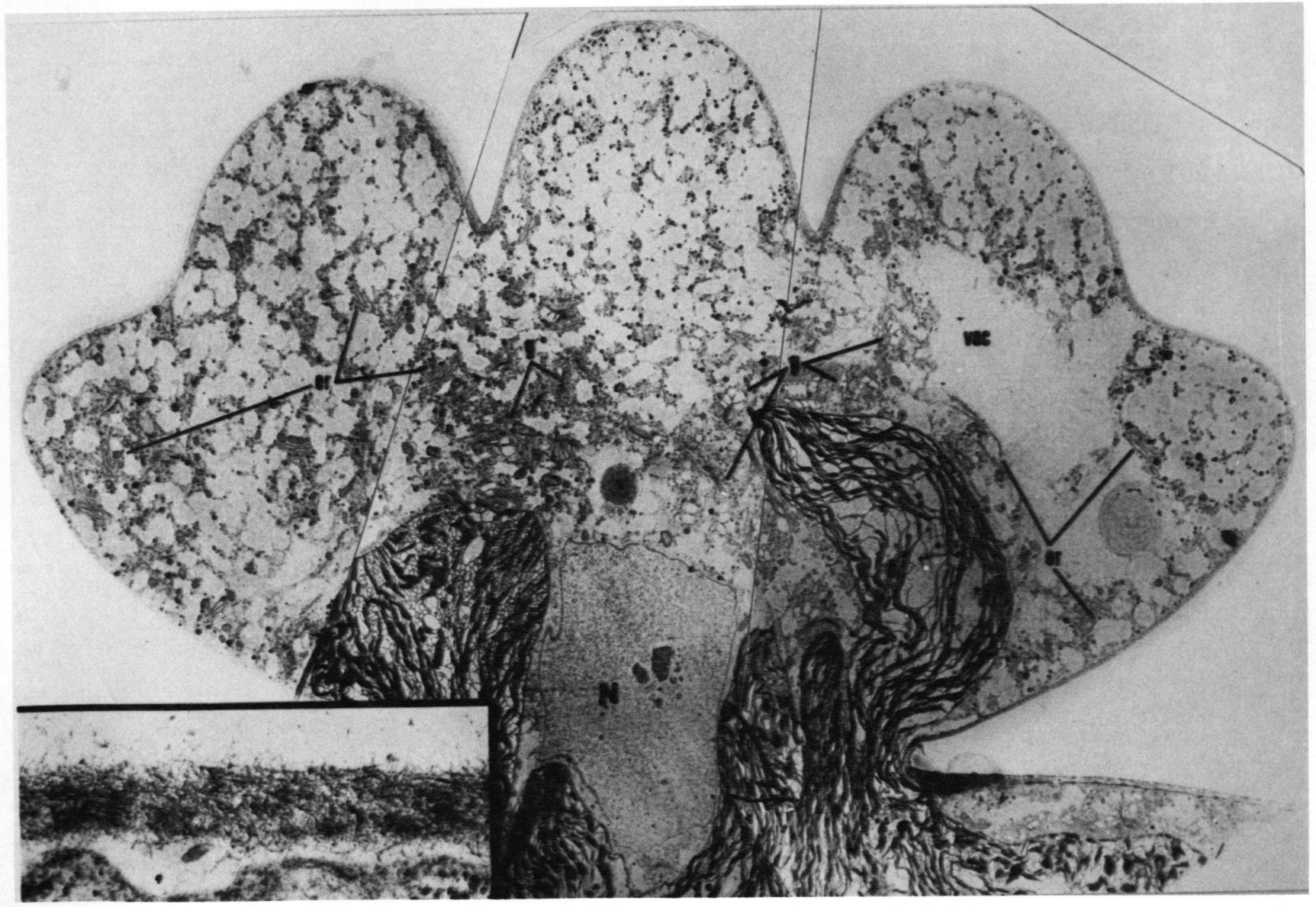


Figure 13 Fine-structure of a developing semicell, stage 7. Sections stained with uranyl acetate and lead.
(a) Tangential section through the nucleus. Nuclear pores are evident; note microtubules flanking the nucleus on either side (arrows). x 18,400
(b) Cytoplasm near the nucleus containing dictyosomes with very active cisternae. Note the abundance of dark vesicles and their association with the outer tubular margin of dictyosome cisternae (double arrows). Occasional microbodies are present. x 18,200
(c) Lamellar structure found in the cytoplasm. Osmophillic patches are not due to stain contamination but are consistent from section to section. x 16,000
(d) Growing tip of a semicell lobe. A lamellar structure (arrow) and vesicle appear between the membrane and cell wall. x 14,000

Abbreviations: Nucleus (N), chloroplast (Ch), microbody (Mb), vesicle (V)

Figure 13

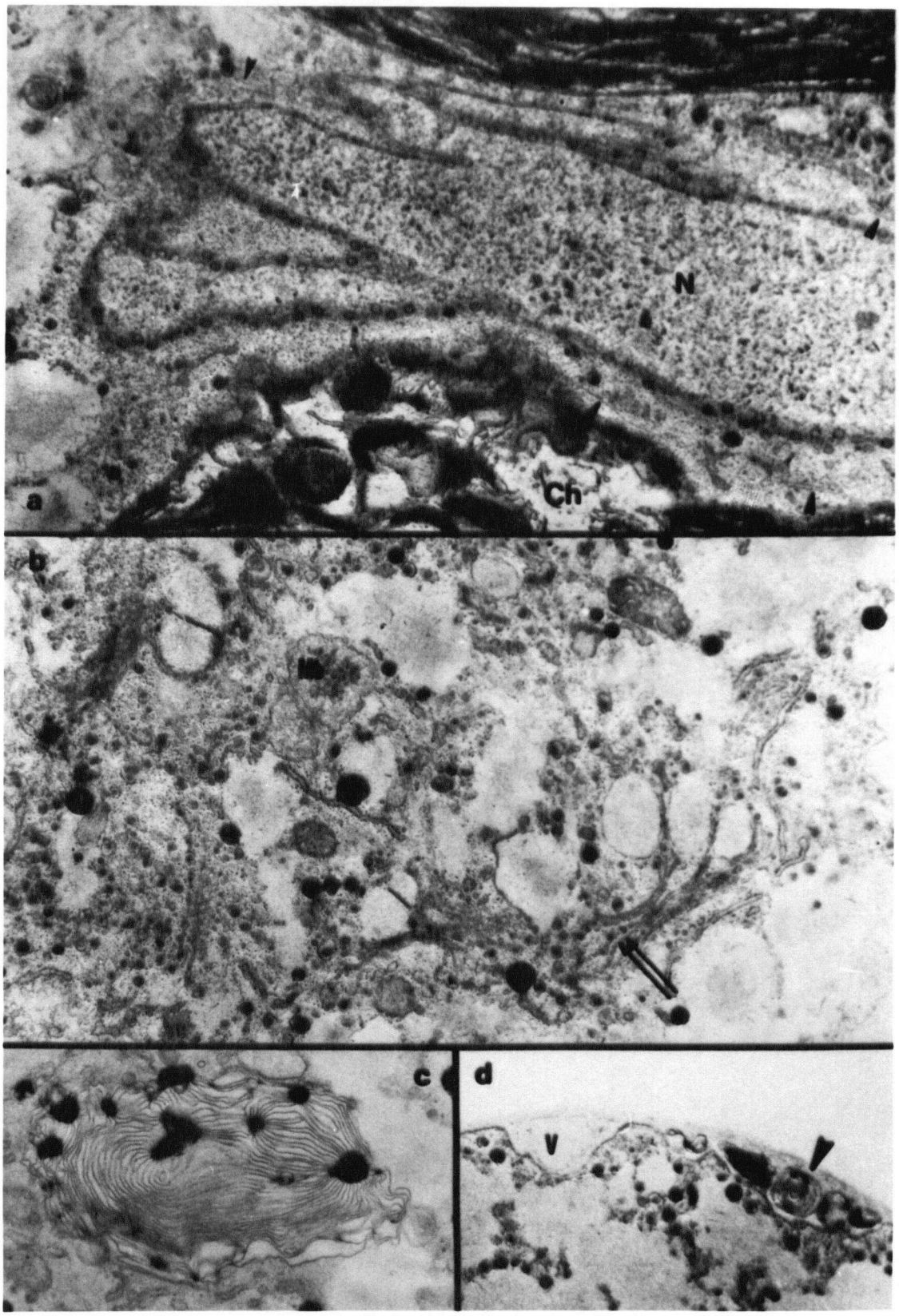


Figure 14 Fine-structure of an early prophase cell
(a) Cell isthmus showing secondary wall and associated vesicles and microtubules. A thin layer of primary-wall-like material lies along the secondary wall (arrow). Stained with uranyl acetate and lead. x 29,000
(b) Cell isthmus similar to that in (a), but stained with uranyl acetate only. x 34,000
(c) Cell isthmus showing the extent of the primary-wall-like material. The layer of material is uniform in thickness and stops abruptly (arrows), in one case (upper arrow) abutting onto the bulb area of a pore structure. Stained with uranyl acetate and lead. x 18,400
(d,e) Dictyosomes from the early prophase cell showing an indication of cisternal activity (arrows). Stained with uranyl acetate and lead.
(d) x 26,000
(e) x 40,800
(f) Tangential section of cell isthmus. Vesicles and microtubules can be seen to encircle the isthmus in a continuous band. Stained with uranyl acetate and lead. x 22,800

Abbreviations: Secondary wall (SW), microtubules (Mt), pore structure (P)

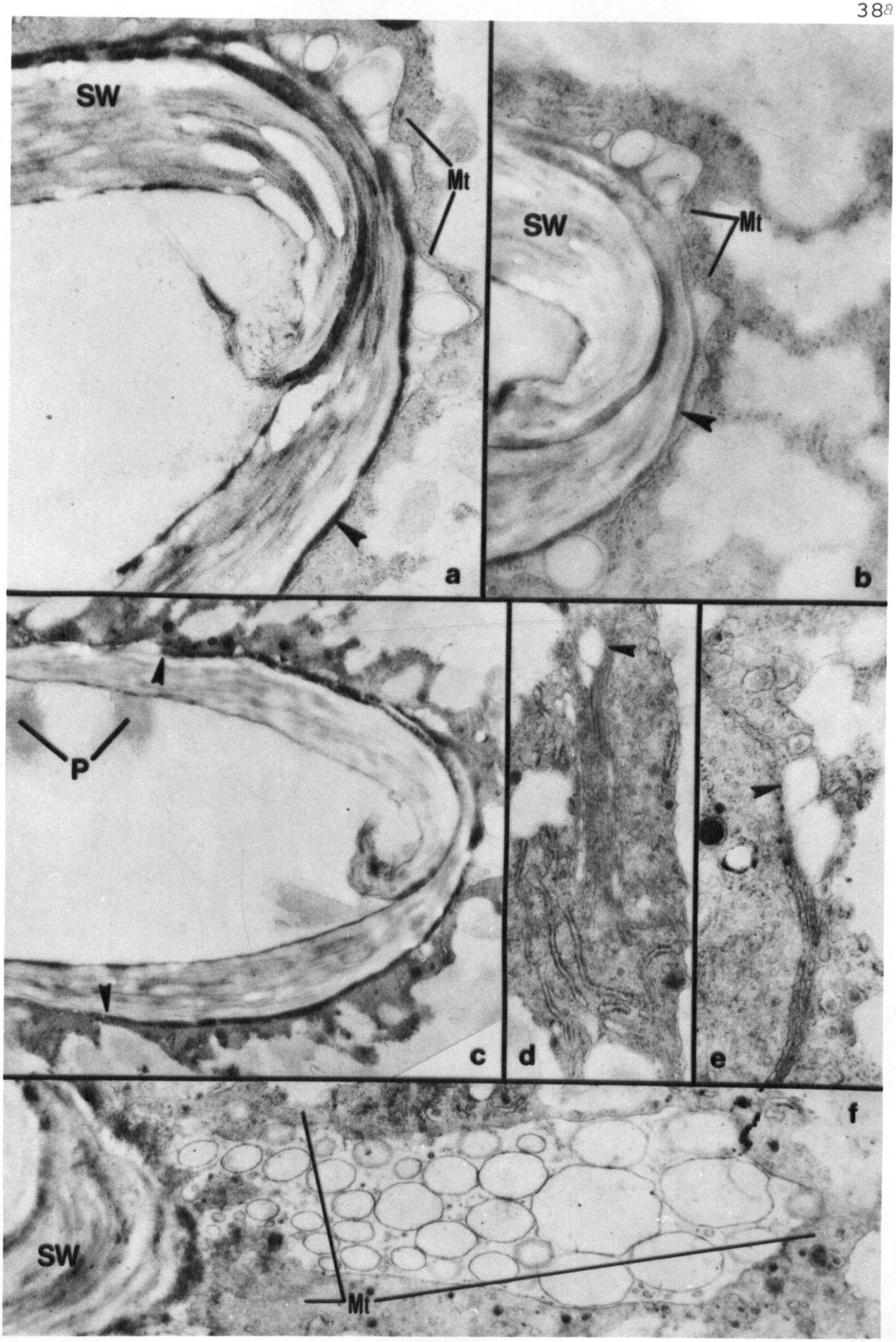


Figure 14

Figure 15 Semidiagrammatic reconstruction of the events of mitosis and cytokinesis compiled from EM information. Sections of the isthmus are shown.
(a) Interphase isthmus with nucleus, nucleoli and chloroplast (hatched) with associated dictyosomes. Mitochondria and large vesicles are shown as is the isthmus band of wall microtubules.
(b) Early prophase isthmus showing the first appearance of primary wall as a thin uniform girdle along the secondary wall. Dictyosomes become active.
(c) Prophase isthmus showing thickening of the primary wall girdle.
(d) Telophase isthmus in which the septum has initiated and grown inward. Its inner leading edge is surrounded by an area of dense cytoplasm. No cell plate or similar structure appears to be present and microtubules are seen in longitudinal array associated with the spindle and not with the isthmus wall.
(e) Late telophase isthmus in which the septum has completed its separation of the two daughter cells.

Abbreviations: Microtubules (Mt), primary wall (Pw)

Figure 15

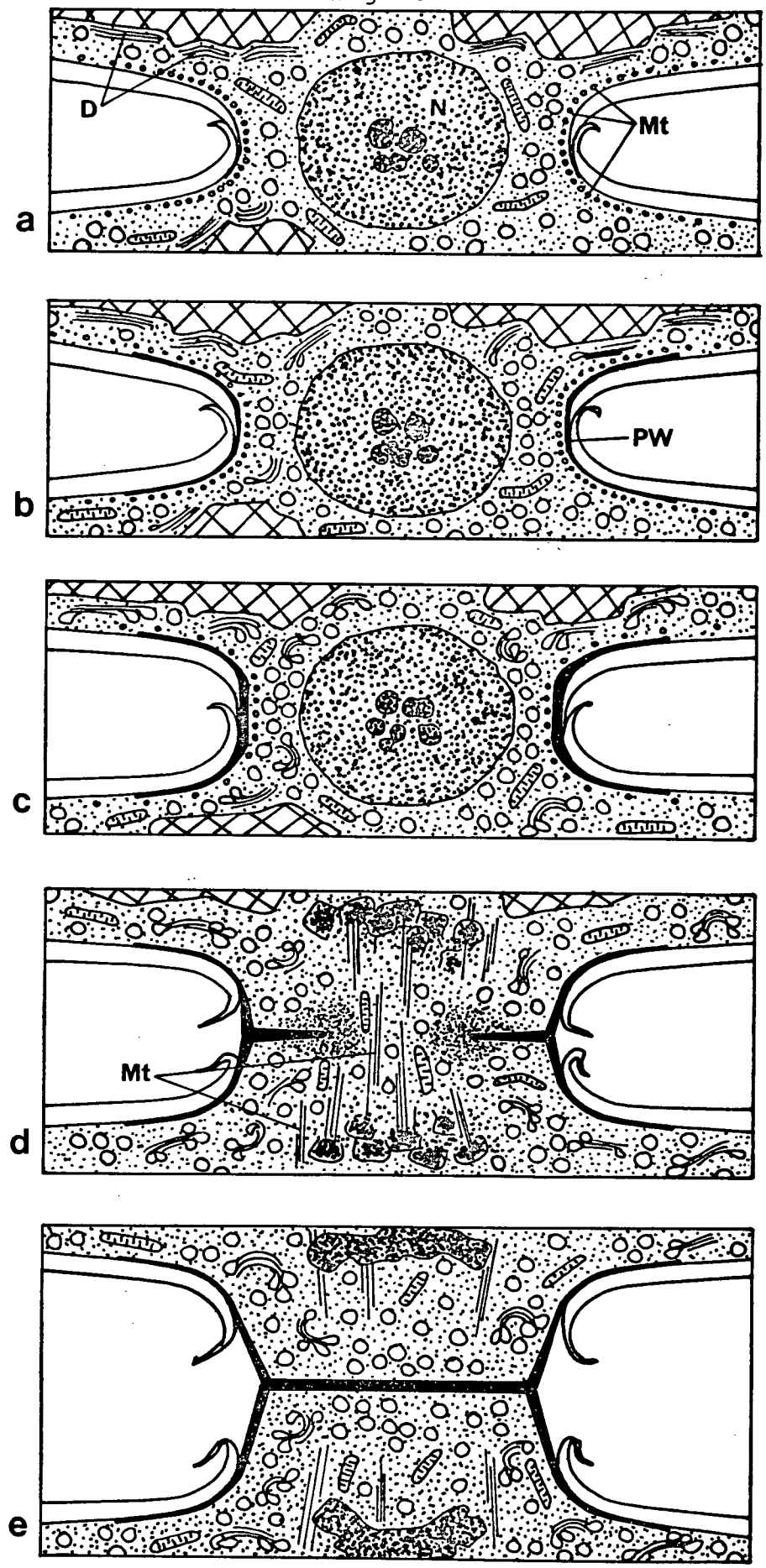
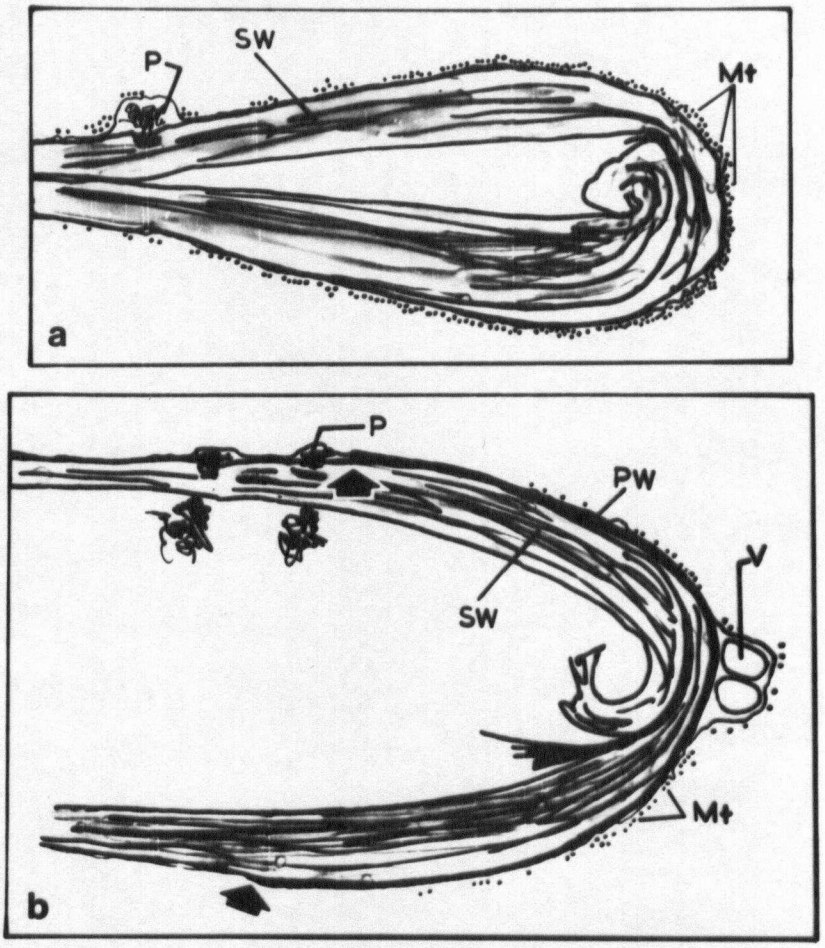


Figure 16 Microtubule positioning compared with the site of primary wall initiation. Diagrams are composites, each traced from several micrographs.
(a) Part of the isthmus of an interphase cell. Note that microtubules thin out gradually and extend into the area of wall containing pores. x 14,900
(b) Part of the isthmus of an early prophase cell. The primary wall girdle stops abruptly (arrows), particularly where pores are present, and does not extend into all areas which in interphase have associated microtubules. x 14,800

Abbreviations: Secondary wall (SW), primary wall (PW), microtubules (Mt), vesicles (V), pore structure (P)

Figure 17 Scanning micrographs of cell isthmuses. Note that there is a sharp boundary (arrows) between those areas of the wall containing pores and the isthmus area which does not. A pore is indicated by each of the small blebs, the blebs probably being tufts of slime threads.
(a) Isthmus of M.rotata showing a particularly sharp boundary. x 750
(b) Isthmus of M.torreyi. x 1,500

Figure 16



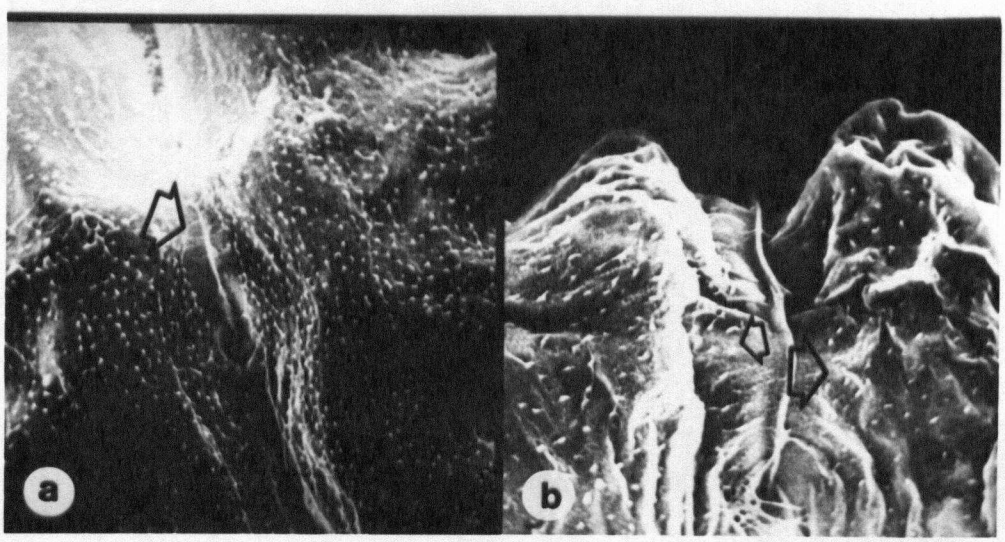


Figure 17

Results and Discussion

II. Observations on Living Material

Figure 18 shows the progress of normal morphogenesis in a cell of M.rotata. A series of stages are shown and numbered 1 to 14 for future reference. The pattern of stages is constant as long as cells are sufficiently healthy to achieve a fully dentated profile. Cells subjected to stress may deviate from this pattern, but usually only very slightly and only if morphogenesis is greatly retarded. The normal pattern I observed is identical to that recorded by Kiermayer (1964), but the time scale is slightly different, probably due to temperature effects. Figure 18 plots development at 20° C with intervals of 20 minutes between stages, the whole morphogenetic process taking about 4 hours. Most of my experiments were done at temperatures close to 20° and so when stages are mentioned, both development time and cell shape are being referred to. Figure 19 compares the rate of development at 30° with that at 20°. Development is more rapid at higher temperatures and morphogenesis is normal at 30° and 35°. At 38° a temperature limit is reached and no development occurs, while at 37° to 37.5° there is growth but in abnormal patterns (fig. 20 and 25a-c). The lobes produced are frequently narrow and unbranched.

M.radiata has a shape somewhat distinct from that of M.rotata, though the two clearly represent variations on a single pattern. M.radiata is smaller with slender lobes and wide notches, whereas M.rotata has rather broad lobes and

very narrow notches. M.torreyi represents an intermediate situation. In healthy, actively dividing cultures, cells of M.torreyi resemble M.rotata and have broad lobes. In aging cultures, cells frequently have narrowed lobes and broad notches and the round profile of the cell is much less obvious (fig. 21). Slight modulations in growth pattern are responsible for the shape differences between Micrasterias species. This becomes obvious if one compares the growth stages of M.radiata (fig. 22) with those of M.rotata (fig. 18).

The culture medium contains about 0.003 M salts. M.rotata cells develop normally over a fairly wide range of osmotic conditions. Development is normal in distilled water and in solutions to which sucrose is added up to a concentration to about 0.06 M. In sucrose concentrations between 0.06 and 0.10 M, development is abnormal; some lobes develop quite normally while others on the same cell arrest at early stages (fig. 23a). At sucrose concentrations between 0.10 and 0.20 M, growth is completely prevented and cell wall thickenings may develop on the inside surface of the wall at lobe tips. Kiermayer and Jarosch (1962) have described this phenomenon and commented on the pattern of these wall thickenings which is similar to that sometimes produced when cells are allowed to develop in solutions of pectin methyl esterase (fig. 25 1). In solutions of 0.2 M sucrose, cells show visible plasmolysis, the protoplast pulling away from the cell wall and the wall itself showing some shrinkage. Cells behave in salt solutions much as they do in sucrose solutions and will develop

normally in deionized water to which NaCl, LiCl or KCl have been added up to concentrations of about 0.05 M. Cells will not develop in deionized water alone.

The culture medium has a pH of 6, but cells will develop normally within a pH range of 4 to 9. In acid solutions (pH 2 - 3) cells invariably rupture within 1/2 hour. Rupture generally occurs at the tip of a lobe, particularly at the polar lobe tip in early stages. If the rupture is small, the cell can be rescued, transferred to culture medium at pH6 and growth resumes. All lobes show normal growth except the lobe suffering rupture which does not grow (fig. 25 d-f). The rupturing was first observed when cells were placed in 0.001 M glucuronic acid, but cells behaved similarly in solutions of galacturonic, citric and acetic acids.

Plasmolysis invariably upsets morphogenesis, but the subsequent patterns of growth are not clearly of any single type. Effects are frequently but not always similar to those of turgor reduction. In a typical experiment, cells were taken from culture medium by steps to 0.2 M sucrose, the process taking several minutes. Cells were left for up to one hour with their protoplasts visibly plasmolysed, then returned to culture medium by steps and allowed to develop. In a sampling of 60 cells, half died without developing further; and of the other half, 10 showed no further growth and 20 produced short lobes of reduced diameter at various points over their surface (fig. 23b).

Various enzymes and chemicals have been tested for their effects on Micrasterias morphogenesis. RNAase, puromycin, mitomycin and other compounds which inhibit transcription and translation have effects similar to those of enucleation (Kallio, 1963). Indoleacetic acid has no specific effects not found with other acids (Kiermayer and Jarosch, 1962). I tried to produce specific pattern effects in M.rotata using enzymes, particularly those enzymes affecting cell wall metabolism. Cellulase has no effect on growing cells even though it will solubilize primary wall preparations. Pectinase has effects similar to those of turgor reduction but less marked, cells seldom develop completely and lobes retain rounded tips (fig. 24a and 25 g, h). Occasionally one lobe may be much less developed than its neighbors. Hemicellulase has more striking effects; many lobes develop fully but specific ones are consistently inhibited (fig. 24b and 25i). The resulting pattern resembles that seen in unhealthy cells of M.torreyi (fig. 21), inhibited lobes are frequently on the upper wing lobe and may face each other across a notch. Treatment with trypsin causes cells to arrest at early stages (fig. 24d), and small refractile areas may appear at the lobe tips. Pectin methyl esterase is particularly likely to cause cells to rupture within several hours; this is not the case with the other enzymes mentioned. In pectin methyl esterase, lobes are rounded much as in pectinase (fig. 24c), but may also become distinctly bulbous or show patterns of cell wall thickening (fig. 25j - m).

I have watched for the appearance of new symmetry types in my cultures. I isolated one triradiate cell from a culture of M.rotata and established a clone. Cells of this clone occasionally reverted to the biradiate type by first forming a semicell which was only partially triradiate (fig. 26b). A partially triradiate semicell might then produce a biradiate daughter semicell on subsequent division, though it could also produce a partial or full triradiates. Cultures of biradiate M.thomasiana produced no abnormal symmetry types, but uniradiate cells reverted routinely to the biradiate form, usually through a partially uniradiate intermediate (fig. 26a). Partial wings may appear at various points on these cells and frequently lie out of the normal plane of symmetry (fig. 26c, d). Cultures of M.torreyi gave rise to uniradiate cells on at least a dozen separate occasions. Clones were established from these cells and some tended to revert more than others. Uniradiate and aradiate cells were found in cultures of the pygmy strain of M.radiata, but only in very old cultures in which cells were small and their shapes much simplified. When placed in fresh medium, these cells reverted to biradiate forms.

I observed live growing cells under the light microscope with particular attention to the actively streaming cytoplasm. I noted nothing that does not already appear in published reports.

In general, the study of cells in culture revealed the natural and experimentally induced modulations of cell shape which the morphogenetic machinery of Micrasterias is capable of

producing. Any good explanation of morphogenesis will have to be able to account for these in addition to dealing with normal morphogenesis.

Figure 18 Morphogenetic stages of M.rotata showing the change in semicell perimeter with time. Fourteen stages are distinguished following one another at 20 minute intervals. Stage 1 is placed just prior to septum completion.

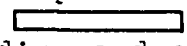
Figure 19 Rate of cell development showing the number of developmental stages that various cells were able to advance in a 2 hour period. Data is shown for development at 20° C () and 30° C () in normal culture medium and at 20° C in medium diluted with a 10x volume of distilled water (). An arrow indicates the developmental rate reported for M.rotata by Kiermayer and Jarosch (1962) for which the the temperature is not reported.

Figure 18

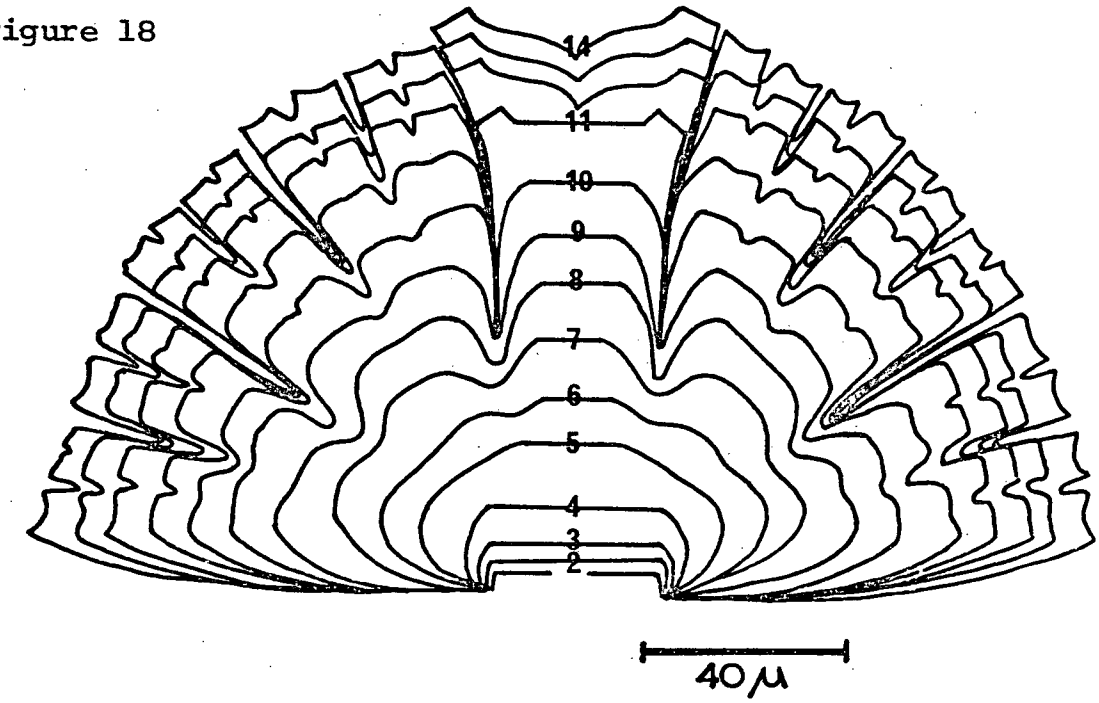


Figure 19

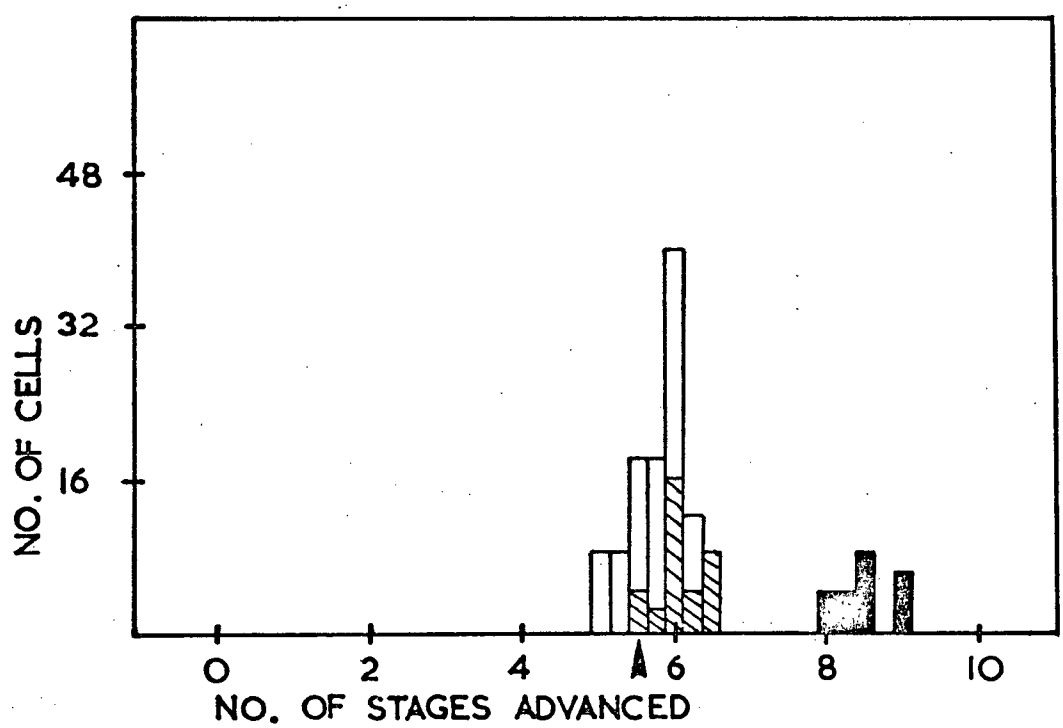


Figure 20 Semicell shape in cells placed at different temperatures at stages 4-5 and allowed to develop. Compare these with the normal semicell (inset).
(a) Development at 35° C
(b) Development at 37° C. Note long, unbranched lobes (arrow).
(c) Development at 37.5° C. Note long, unbranched lobes (arrows).

Figure 20

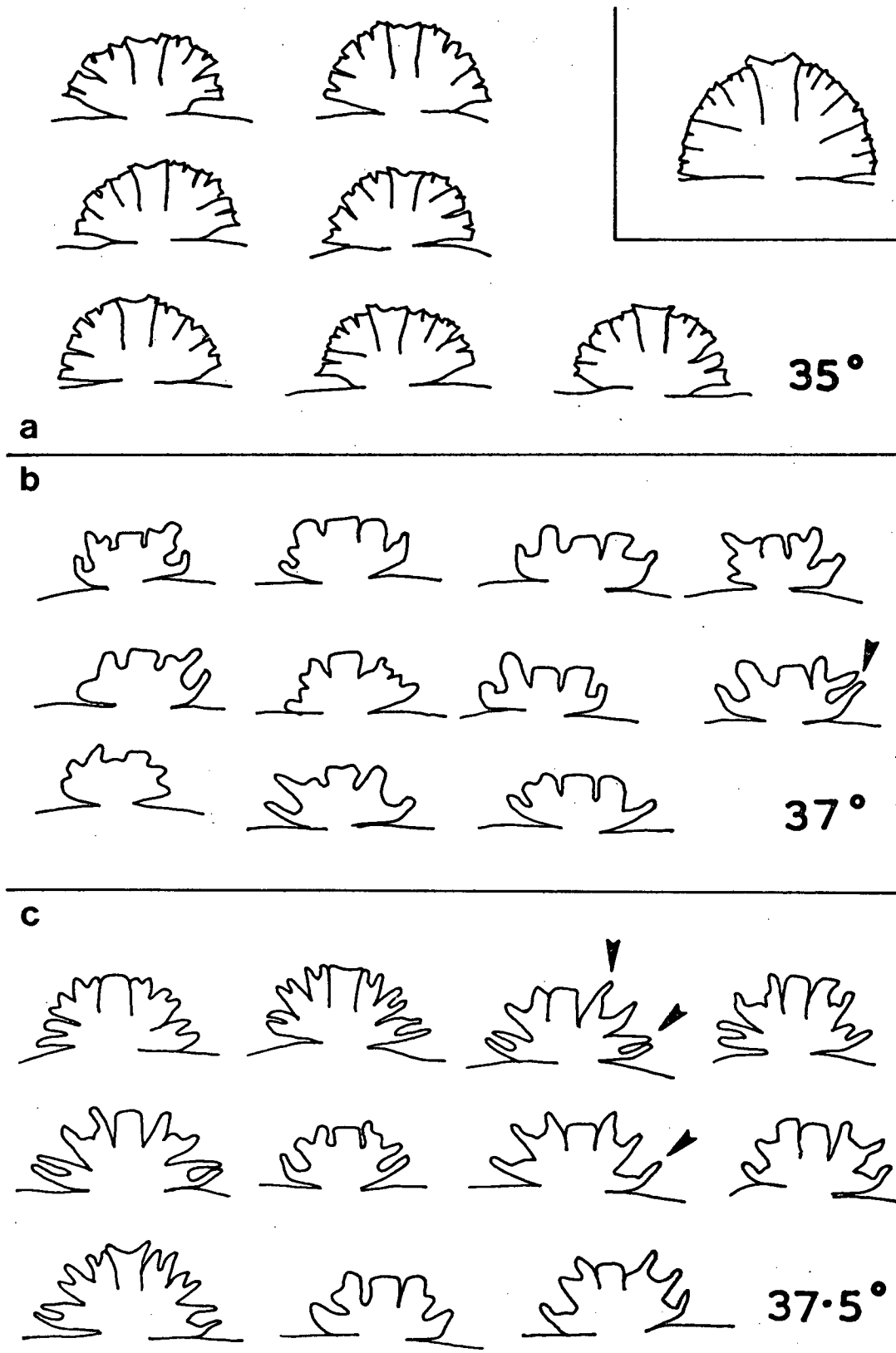
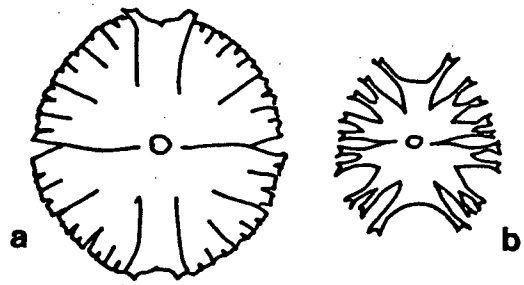


Figure 21 Cell shape in several species of Micrasterias, all to the same scale. One cell each is shown of M. rotata (a) and M. radiata (b). The remaining cells are all examples taken from a single aging culture of M. torreyi and show considerable variation.

Figure 22 Morphogenetic stages of M. radiata shown in three parts for greater clarity. Stages are numbered 1 to 9 and are separated by 20 minute intervals.
(a) Stages 1 - 7. Note that notches remain fixed in position once formed while tips change.
(b) Stages 7 - 9. Note the more complex changes in notch position.
Inset: A comparison of one wing at stage 7 with the same wing at stage 9. Note that the notches change position and that the width of each lobe (arrows) increases with later stages.



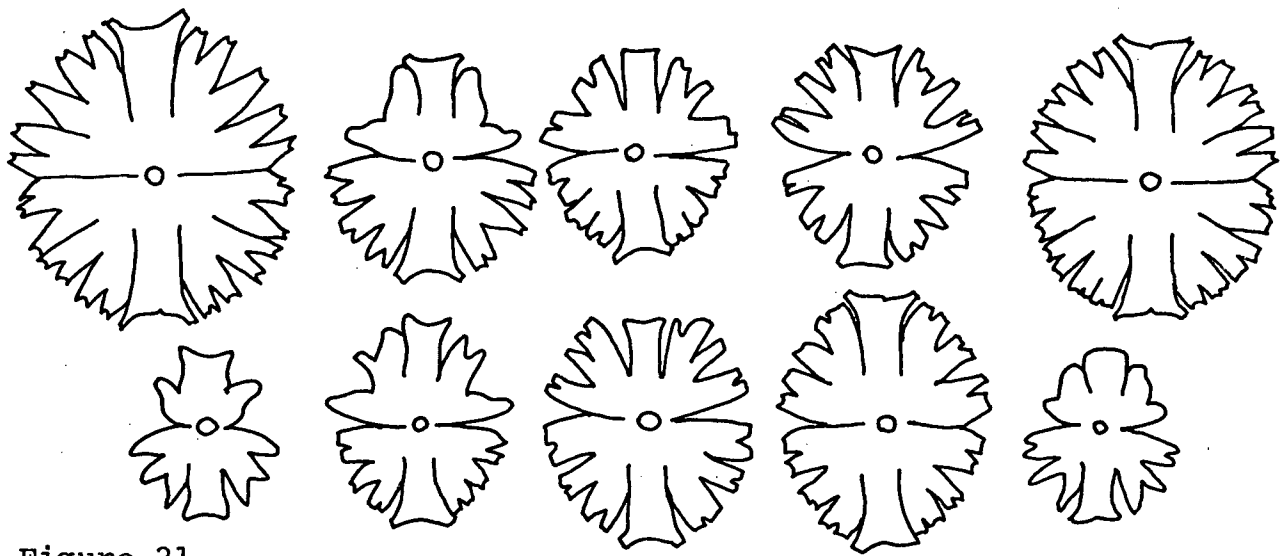
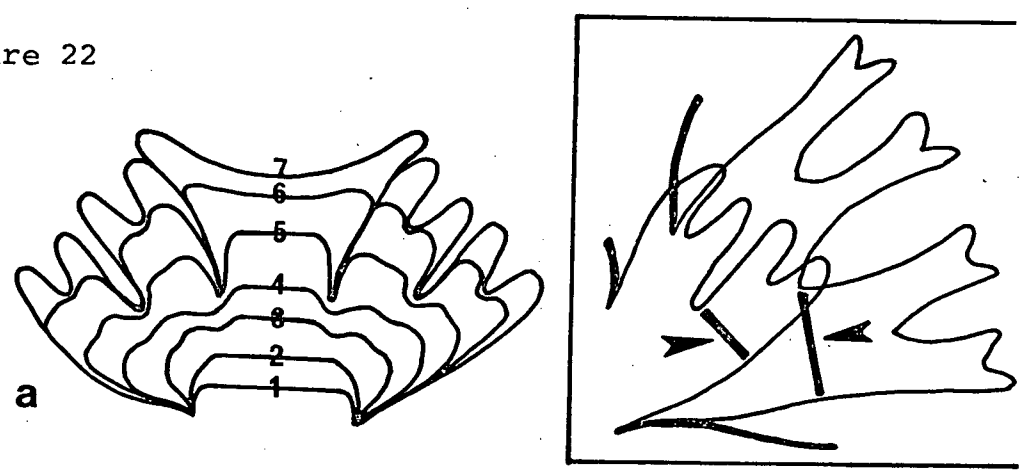


Figure 21

Figure 22



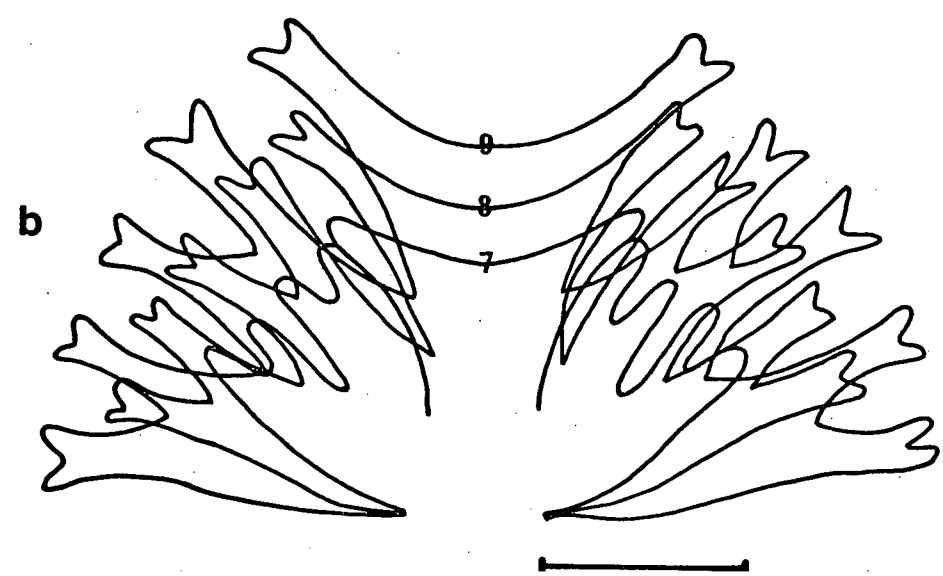


Figure 23 Semicell shape in cells grown from stages 6-7 under different osmotic conditions. Compare these with the normal (inset).
(a) Development in 0.06 to 0.1 M sucrose solutions
(b) Development in culture medium after 40 min. to 1 hr. plasmolysis in 0.2 M sucrose

Figure 23

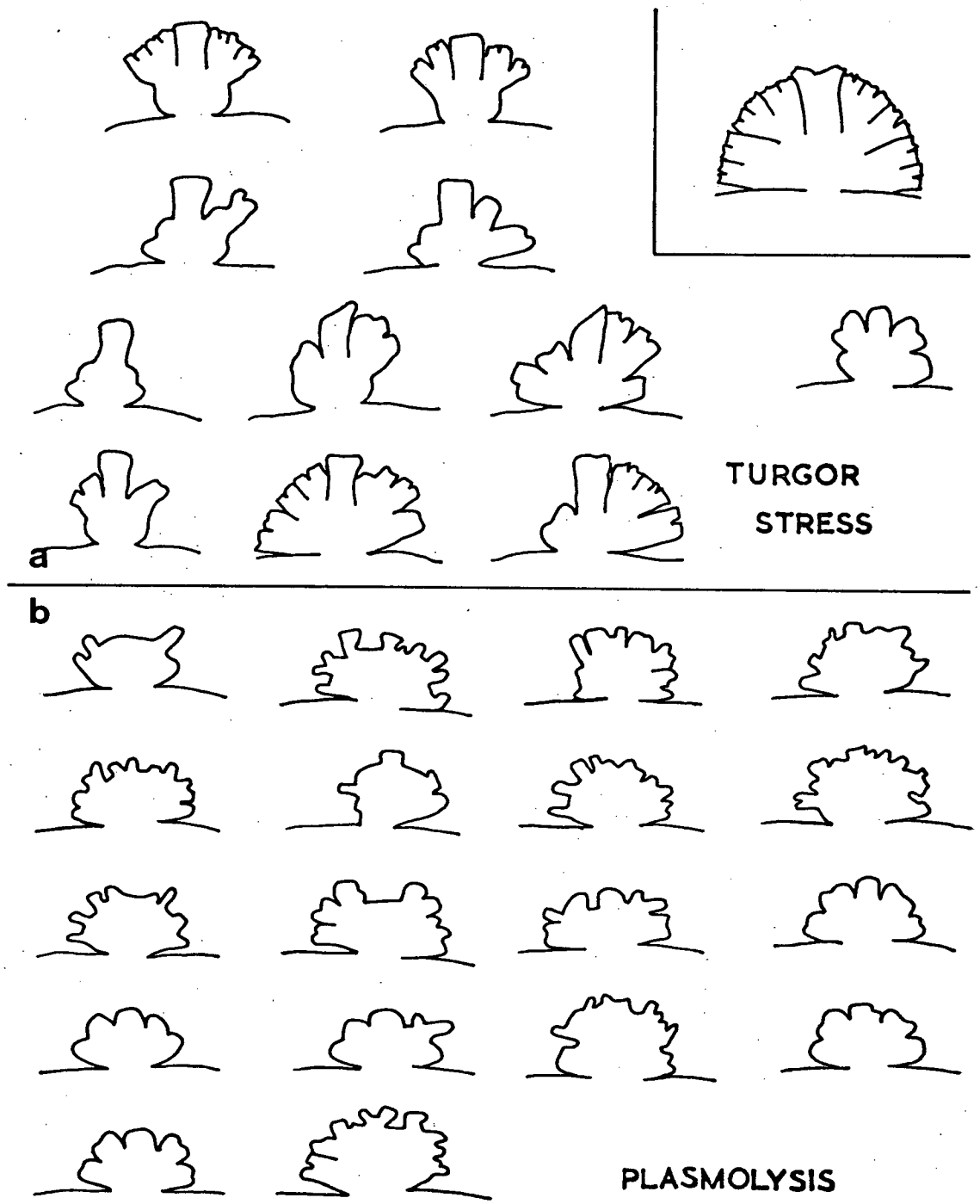


Figure 24 Semicell shape in cells allowed to develop from stages 3-4 in solutions of enzymes. Compare these with the normal (inset).
(a) Development in 0.2 to 2 mg/ml pectinase
(b) Development in 2 to 4 mg/ml hemicellulase. Note characteristic abnormalities of lobes, particularly of upper wing lobes (arrows).
(c) Development in 0.02 to 2 mg/ml pectin esterase. Bulbous lobes are evident in a few cells (arrow).
(d) Development in 0.02 mg/ml trypsin

Figure 24

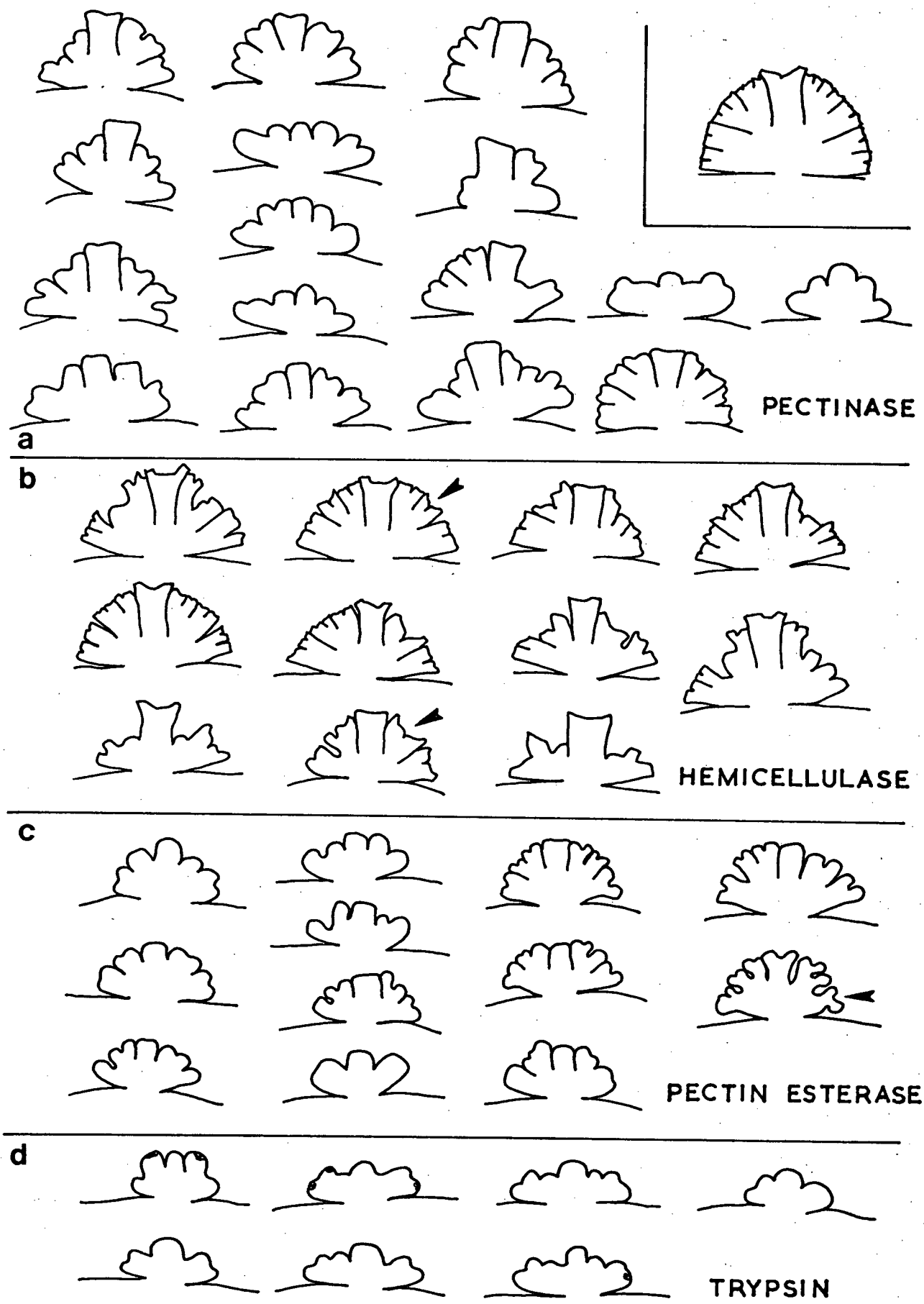


Figure 25 Experimental manipulations of developing cells
(a-c) Cell development at different temperatures. Note long, unbranched lobes (arrows).
(a) Cell placed at 35° C at stage 3, photo taken after 8 hrs.
(b) Cell placed at 37° C at stage 3 for 8 hrs., photo taken after 24 hrs.
(c) Cell placed at 37.5° C at stage 4 for 8 hrs., photo taken after 24 hrs.
(d,f) Cells ruptured in acid solutions (pH 2.5)
(d,f) Cells placed in 0.001 M glucuronic acid for 1/2 hr. and then returned to culture medium. The cells each show rupture at one tip.
(e) Cell from (d) after 24 hr. Ruptured tip has not developed further.
(g-m) Cells allowed to develop in the presence of various enzymes
(g) Cell placed in 1 mg/ml pectinase at stage 5
(h) Cell placed in 0.2 mg/ml pectinase at stage 5
(i) Cell placed in 3 mg/ml hemicellulase at stage 4. Note the characteristic abnormality of the upper wing lobe (arrows)
(j) Cell (M.radiata) placed in 0.2 mg/ml pectin esterase at stage 2
(k) Cell (M.radiata) placed in 1 mg/ml pectin esterase at stage 2
(l) Cell placed in 1 mg/ml pectin esterase at stage 5. The cell is ruptured, but shows a pattern of cell wall thickenings similar to that caused by turgor stress (arrows).
(m) Cell placed in 2 mg/ml pectin esterase at stage 6. This cell also is ruptured, but shows the development of distinctly bulbous lobes (arrows).

Figure 25

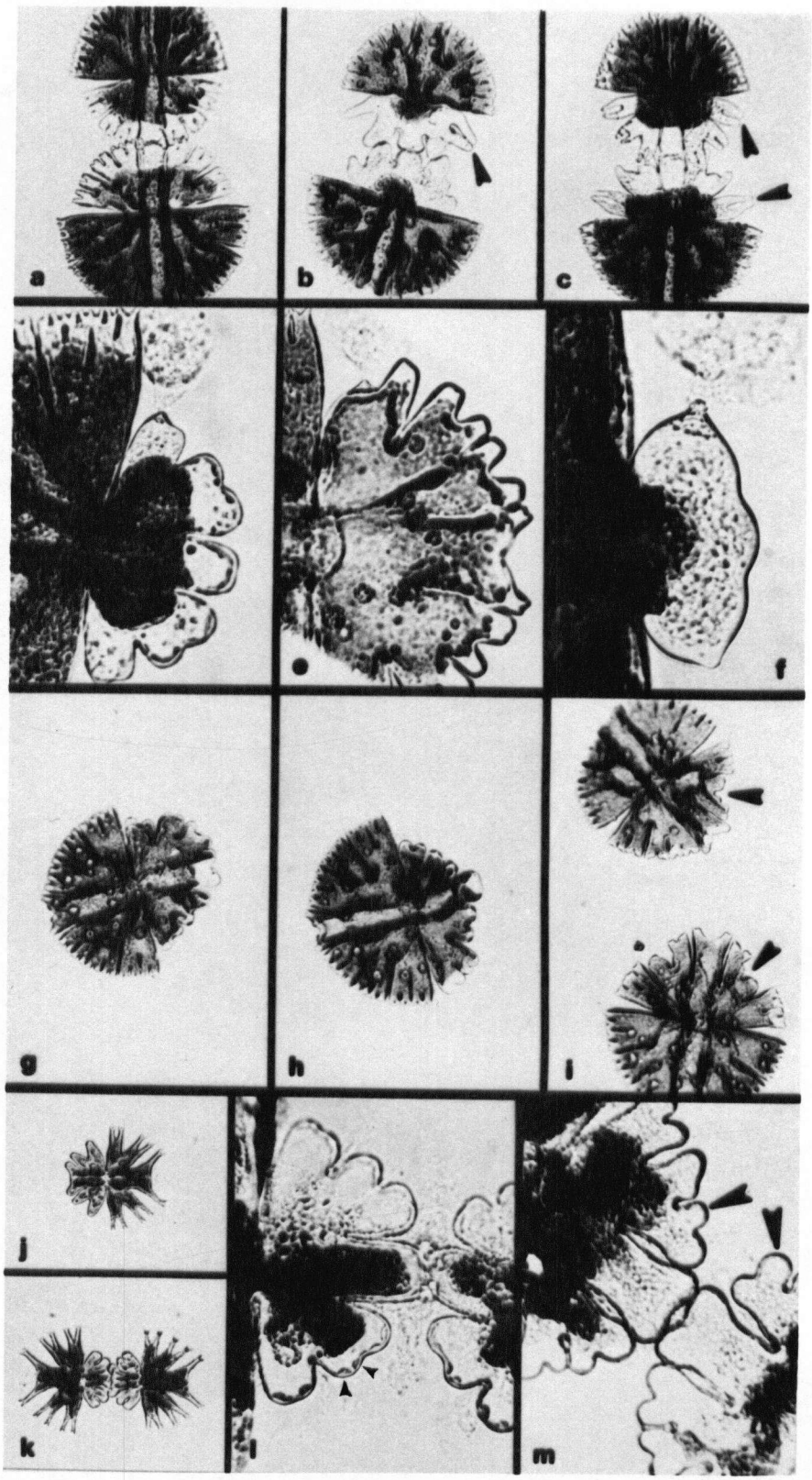
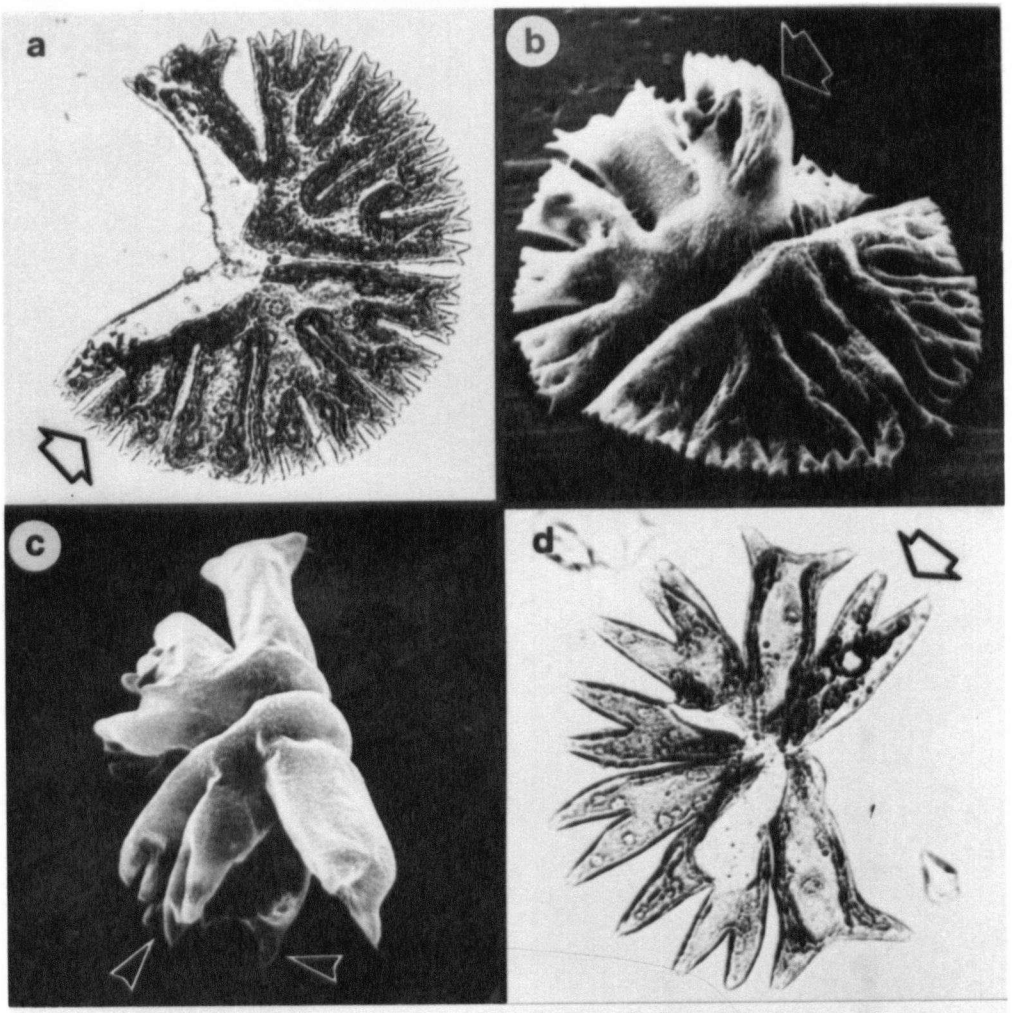


Figure 26 Scanning and light micrographs of intermediate facies in three species. In each case an arrow indicates the partial wing.
(a) Uniradiate M.thomasiana with a partial wing
(b) Biradiate M.rotata with a partial wing. The cell has probably reverted from a full triradiate in the following way: The partial semicell arose from division of a triradiate cell and at a later division, this semicell produced a biradiate semicell.
(c) Uniradiate M.torreyi showing a lateral duplication of one wing. In some cells two such wings may take up positions completely perpendicular to one another.
(d) Uniradiate M.torreyi with a partial wing

Figure 27 Scanning micrographs of double cells found in old cultures
(a) A double cell of M.rotata
(b) A triple cell of which two isthmuses are shown (arrows).

Figure 26



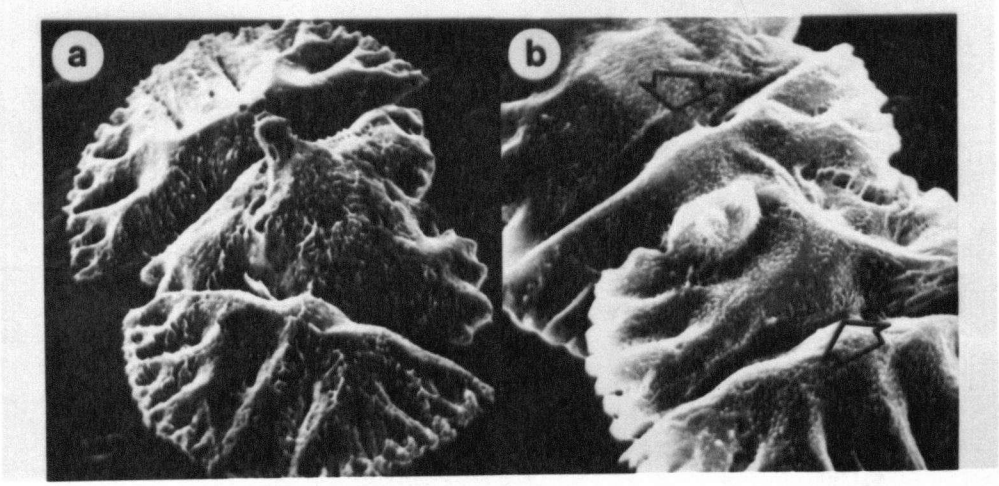


Figure 27

Results and Discussion

III. Primary Cell Wall Composition

Cell wall ghosts from M.rotata were mounted on slides, extracted in various ways and stained for polysaccharide (PAS), acid fractions (alcian blue) and slime (crystal violet). Parallel extractions were done on radiolabeled walls and autoradiograms made. Table IV presents a summary of the results of these experiments.

The primary wall shows three distinct fractions. There is a "loosely bound" component which is removed by all extractions with alkali and which stains with crystal violet. The staining is similar to that of pores in the secondary wall suggesting that this component is a slime, either similar to or identical with the pore slime, though the stain is relatively non-specific. The second component stains with alcian blue at pH 2.5 and is not removed by alkalis. There is no staining at pH 0.5, indicating that this acidic fraction is rich in uronic and not sulfonic acids. The acid groups can be methylated with loss of staining and the staining returned by saponification. Hot acid alcohol and chromic acid will extract the alcian blue positive fraction leaving behind visible walls which stain with PAS, suggesting that a third and more stable component exists.

Mix (1961) found that chromic acid removed all matrix substances from the primary wall of a closely related desmid leaving a mat of randomly oriented microfibrils. Desmids are known to possess microfibrils which are cellulose-like but contain several sugars in addition to glucose

Table IV

Histochemical Analysis of Primary Cell Walls*

Treatment	Results				
	Alcian blue	Crystal violet	PAS	Glucose Cl-^{3}H	Methionine methyl-^{3}H
Control	+	+	+	+	+
Mild alkali extraction	+	-	+	+	-
Alkali extraction	+	-	+	+	-
Strong alkali extraction	+	-	+	+	-
Chromic acid	-	-	+	+	-
Strong methylation	-	-	+	+	-
Strong methylation plus saponification	-	-	+	+	-
Mild methylation	-	±	+	+	+
Mild methylation plus saponification	+	±	+	+	-
Pectin esterase					-
Pectin esterase Control					±
Trypsin					+
Pronase					+
Cellulase	-	-	-	-	-
Cellulase control	+	+	+	+	+
Pectinase	-	-	-	-	+
Pectinase control	+	+	+	+	+
Hemicellulase	+	+	+	+	+
Hemicellulase control	+	+	+	+	+

*Staining or autoradiographic labeling is either present (+) absent (-) or reduced (±) following the various treatments.

(Klein and Cronquist, 1967). The third wall component is therefore most likely a network of cellulose-like microfibrils which may be oriented randomly or aligned in some fashion. The alcian blue and crystal violet positive fractions represent two matrix substances. The alcian blue fraction could be called hemicellulose-like because it is alkali stable. It is probably the structurally more important of the two.

Radiolabeled, externally-supplied glucose is clearly incorporated into the microfibrillar fraction and does not contribute greatly to the matrix, as considerable radioactivity remains after chromic acid treatment. Methyl-labeled methionine activity is removed by alkali, by pectin methyl esterase and to some extent by boiled esterase. The label is not removed by trypsin or pronase. This indicates, but does not prove, that label is present in esterified methyl groups bound probably to the uronic acid rather than microfibrillar fraction, and that it is not present in loosely bound protein as methionine.

The synthetic activities associated with incorporation of glucose and methionine probably occur at the cell wall and not in cytoplasmic structures such as Golgi. Golgi vesicles probably transport acid polysaccharides but are not thought to be involved in cellulose synthesis or transport except in very special cases (Brown et al., 1970). Cellulose synthesis probably occurs right at the cell surface (Roelofsen, 1965; Northcote and Pickett-Heaps, 1966), and particles perhaps responsible for this synthesis have been found on the plasma membrane (Robertson and Preston, 1972). The glucose label is contained in a microfibrillar component

which is probably synthesized in this fashion also. Methylation of polysaccharides is thought to parallel methylation of other biological macromolecules. Rather than building from methylated precursors, the assembled macromolecule is methylated as a final step (Lamport, 1970).

Label appears in the cell wall within about 10 minutes. This is not so rapid that it could not have come through the golgi (Northcote and Pickett-Heaps, 1966), but the timing is similar for glucose and methionine and there are independent reasons in both cases for supposing that incorporation occurs at the wall itself. I therefore conclude that none of the label arrives via the golgi. Golgi contributions to the wall, probably a matrix precursor contained in small dark vesicles and a certain amount of slime contained in large vesicles,are probably not labeled in these experiments. This is consistent with their presence in separate cytoplasmic compartments which turn over very slowly or into which label does not penetrate (Oaks and Bidwell, 1970).

In conclusion, primary wall comprises three distinct fractions; a slime matrix substance, an acid matrix substance and microfibrils. The microfibril fraction incorporates label from tritiated glucose; one of the fractions incorporates label from methyl-labeled methionine, probably as methyl esters. Both incorporations are rapid and probably are not associated directly with golgi transport.

Results and Discussion

IV. Laser Experiments

Some means of damaging selected parts of the Micrasterias cell was required so as to determine their contribution to morphogenesis of the whole. A laser microbeam was chosen as it can deliver large amounts of energy to a very small area. I mounted dividing cells as described and subjected them to laser irradiation. The clear cytoplasm was found to be quite insensitive to laser radiation, though cells responded to lasings of their chloroplast by turning brown and dying. I then tested a number of blue and green dyes to see if they would be taken up by cells without killing and whether they would promote the absorption of laser energy. Of about 20 dyes tested, 5 were clearly taken up by the cells (malachite green, alcian blue, janus green, nile blue and methylene blue) and of these 5, alcian blue promoted the most specific and interesting sorts of laser damage.

Routinely, cells were colored in a 0.001% solution of alcian blue and lased with a 2 to 5 μ spot for no more than 5 seconds. At the concentrations used, alcian blue was not toxic and did not affect the pH of the culture medium. Cells developed normally if left in a 0.001% solution of alcian blue and could survive there for several days. At 0.010%, cells did begin to show contracted chloroplasts and other signs of toxicity.

Lasing at the point between two developing semicells prevented formation of a polar lobe while the wings developed normally (fig. 28a, b). Lasing this same point at an earlier stage produced a similar loss, but occasionally caused two polar

lobes to develop rather than one (fig. 28c, d). As for wing development, the effects were quite striking when the tips of lobes were lased (fig. 30), but less so when other parts of the cell wall were lased. A lobe could be damaged so as to only retard its growth (by lasing a notch as in figure 31 for example) and the lobe would continue to grow and branch. These simple experiments demonstrated clearly that lobes can grow and develop independently of one another and suggested that laser damage of this sort is quite localized. The experiments also demonstrated that certain points on the cell wall play a far more crucial role in lobe growth than others. The participation of these points is required in some way for morphogenesis.

In a series of laser experiments involving about 300 cells, I mapped out the position on the cell wall of these sensitive points or "singularities" as I shall call them. The word "singularity" I have borrowed from Tokunaga and Stern (1965). They use the term in a broad sense to indicate only that something interesting occurs at one point of an otherwise undistinguished surface and not elsewhere (Stern, 1972). I will use the word in a similar fashion so as to indicate position while implying nothing about structure.

Fate-mapping experiments are summarized in figure 32. Lasings in which the lased lobe failed to develop are separated from those in which it recovered sufficiently to produce a normal pattern of dentation. In each lasing, one daughter was the experimental cell, and the other served as a control. All cells were kept and followed through one further division, and the most malformed cells were followed through several. In no

case were laser-induced shape abnormalities inherited. Cells lacking both polar lobe and one wing produced normal biradiate daughter semicells at each division.

An idealized plot can be constructed showing the position of singularities on the cell wall at different developmental stages. Such a plot, with the points connected by a line, is shown in figure 33. Singularities are generally located at lobe tips, but some lobes may show two in association with branch formation. The implication, of course, is that each singularity is responsible for the growth of a lobe; and when singularities duplicate, lobes become branched. I shall present further evidence before arguing this point.

Having surveyed stages 3 to 8 in this fashion, I was particularly interested in tracing the position of laser singularities in very early stages. I wanted to find their points of origin; presumably three existed, one each associated with the polar lobe and two wings. In fact, early stages recover far more readily from laser irradiation, lasing may slow development of stage 1 to 3 cells but in all cases a normal semicell is produced. Irreparable damage may occur if much larger areas of cell wall are irradiated at these early stages or more concentrated solutions of alcian blue used to color the cells. I chose to use a 0.005% dye solution and retain the 2 to 5 μ beam diameter for studies of stages 1 to 3. Cells were again lased for 5 seconds or slightly longer and frequently showed retarded development before recovering. Some of the cells developed a mark on the cell wall corresponding to the point of lasing. In cells which recovered completely, this

mark served to delimit the area from which lobes could have arisen. Of 65 cells lased at stages 1 to 3, about 25 show sufficient marking that I could determine the point of origin of lobes relative to the lasing. Two of the more clear-cut examples are shown in figures 34 and 35.

The following important conclusion emerged from this work; at an early stage the side lobe can arise from any of several different places, its origin is not tied to a single point. Note in figure 36 that stage 3 cells are well-behaved. Lasing small spots within an area, one can pick out singularities for upper and lower wing lobes or hit between the two. Lasing the large area including these prevents formation of one whole wing (as in fig. 29) though frequently the unlased wing appears to compensate for the missing wing opposite by hypertrophy. At stage 1 and 2 laser experiments become ambiguous (fig. 37). Lasing at A (fig. 37b) leaves a mark, and the developing lobes must be assumed to have arisen from B (as in fig. 35). Lasing at B (fig. 37c) leaves a mark and suggests that lobes arose at A or beyond (as in fig. 34). Lasing at A and B both (fig. 37d) leaves a mark and again a normal lobe which must have arisen from an area separated from B by at least the distance across A. Lasing at A and B together may also cause the lobes to grow out oblique to their normal plane, implying an origin lateral to the lasing rather than above or below it. So a rather large area of the stage 2 cell is competent to produce a normal wing, certainly the competent area is larger than the area of a singularity as defined by lasing later stages. The morphogenetic machinery of M.rotata, whatever its

material basis, is therefore able to regulate to a considerable extent at early stages, but loses this ability as development proceeds.

A note is required here concerning the mechanism of laser effects. Power output of my helium-neon laser is on the order of 10^{8} times less than that of a pulsed ruby laser. This output is still sufficient to produce cell damage if the energy can be absorbed. Absorption is of a normal photochemical sort so that blue, green or black substances are required if the laser produces red light. Energy absorbed by the dye may then be transferred to associated biological molecules, and proteins may be good candidates for this. A number of dyes bind loosely to proteins in solution, and model studies show thermochemical and free radical effects when solutions of lipase and methylene blue are lased (Saks, 1971).

In cells then, one would expect very rapid local heating and either protein denaturation or the formation of free radicals and molecular cross-links. In M.rotata, alcian blue probably serves to color the cell wall. The wall appears slightly tinted, and alcian blue is known to bind very tightly to cell walls (Benes, 1968) and is used as a histochemical stain for acid polysaccharides (Stadelmann and Kinzel, 1972). If a lased cell is immediately plasmolysed, the cell membrane and wall stick together in the area of lasing. When the protoplast breaks away, a small bit of membrane is left behind fused to the cell wall (fig. 38). Lased portions of cell wall do not participate fully in cell morphogenesis. Note in figure 30c that the

lased portion of wall retains its position in rigid indifference to the changes going on about it. The wall is not marked immediately by lasing. Marks appear over a 10 to 15 minute period as surrounding wall continues to develop. Marks appear to be due to optical refraction, the rigid lased area having different refractile properties from the surrounding, more plastic wall.

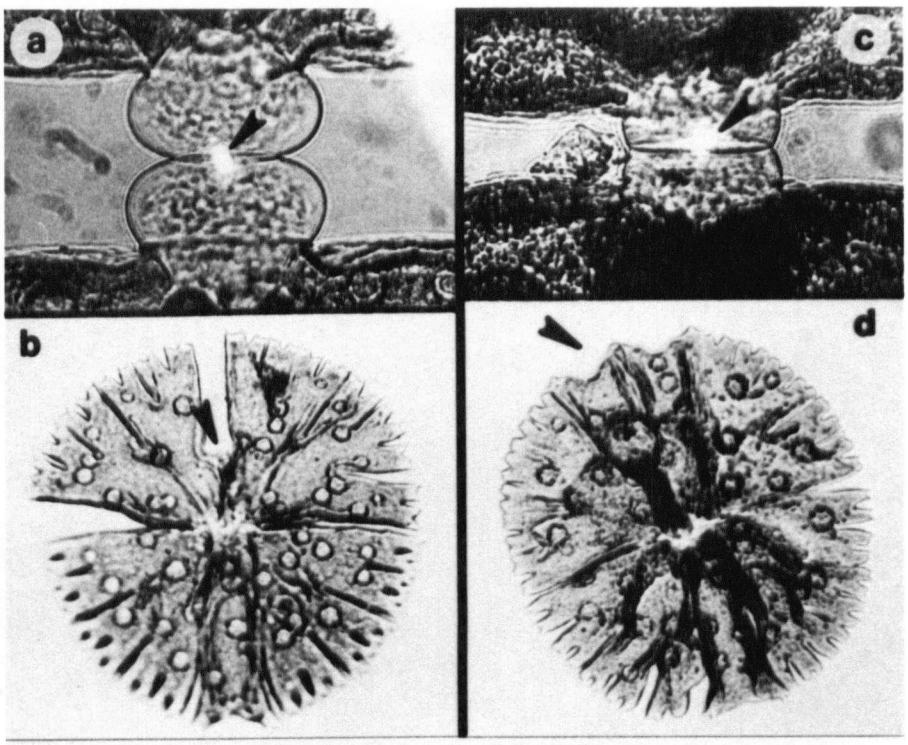
Laser experiments do not distinguish the morphogenetic role of cell wall from that of cell membrane as both are affected by lasing. The laser may act solely on wall, making parts of the wall physically too rigid to participate in morphogenesis for example. It may act on membrane alone or perhaps interfere with the association between wall and membrane. It is appropriate then to say that the laser has its effect on the cell cortex; meaning by cortex the cell wall, the membrane and the first micron or so of cytoplasm with whatever structures this may contain.

In conclusion, laser experiments demonstrate particular regions of the cell cortex (singularities) which are especially important for maintaining morphogenesis. These regions first become fixed and well-defined at about stage 2 and subsequently are associated with the tips of developing lobes.

Figure 28 Laser damage to polar lobes
(a,b) Cell 498. An 8 sec. lasing of the region shown results in the absence of the polar lobe in both daughter cells.
(a) lasing at stage 4
(b) One daughter on the following day. Both daughter cells were similar.
(c,d) Cell 387. An 8 sec. lasing of the region shown results in a duplication of the polar lobe in both daughter cells.
(c) Lasing at stage 3
(d) One daughter on the following day. Both daughter cells were similar.

Figure 29 Laser damage to wings, cell 506. A 10 sec. lasing of the region shown with a somewhat larger spot results in the absence of one wing in each daughter. At an intermediate stage of development (b), a refractile area can be seen indicating the region of laser damage (arrow).
(a) Lasing at stage 3
(b) Development 2 hrs. after lasing
(c) and (d) Daughter cells on the following day

Figure 28



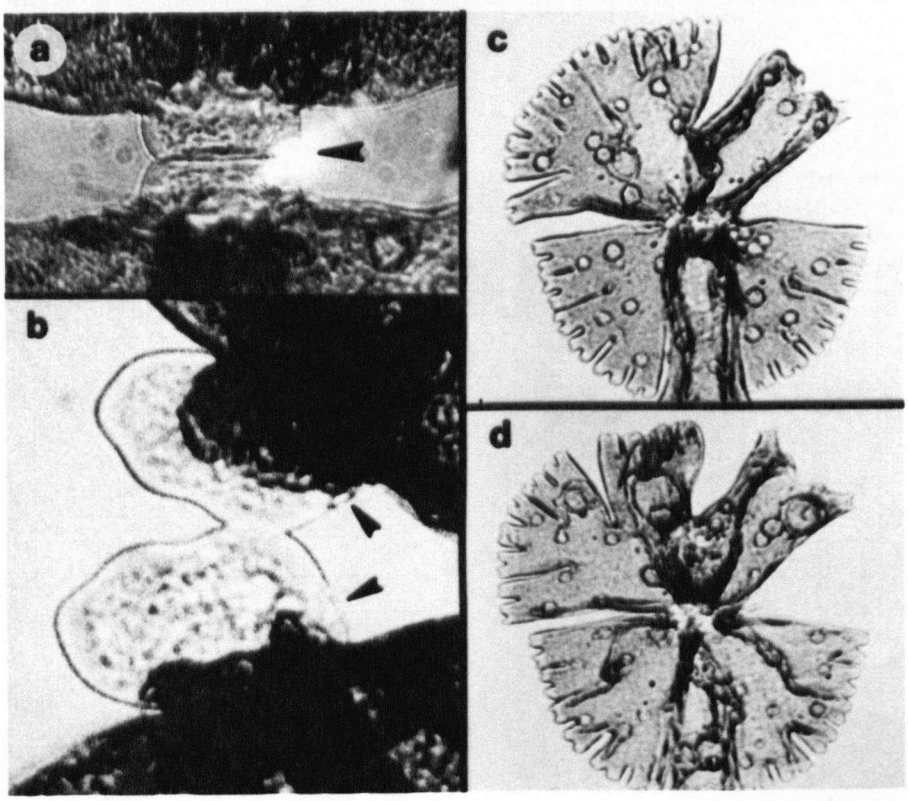


Figure 29

Figure 30 Laser damage to lobe tips, cell 590. A 2 sec. lasing of the lobe tip results in complete arrest of that lobe's subsequent development while other lobes remain unaffected. Compare the control cell (d) with the lased cell (c)
(a) Lasing at stage 7
(b) Develpment 1 hr. 30 min. after lasing
(c) and (d) Daughter cells on the following day

Figure 31 Laser damage to lobe notches, cell 595. A 2 sec. lasing of the side of the lobe does not prevent formation of a complete pattern of lobe dentation, but does leave a slight abnormality (arrow in c).
(a) Lasing at stage 7
(b) Development 1 hr. 40 min. after lasing
(c) and (d) Daughter cells on the following day

Figure 30

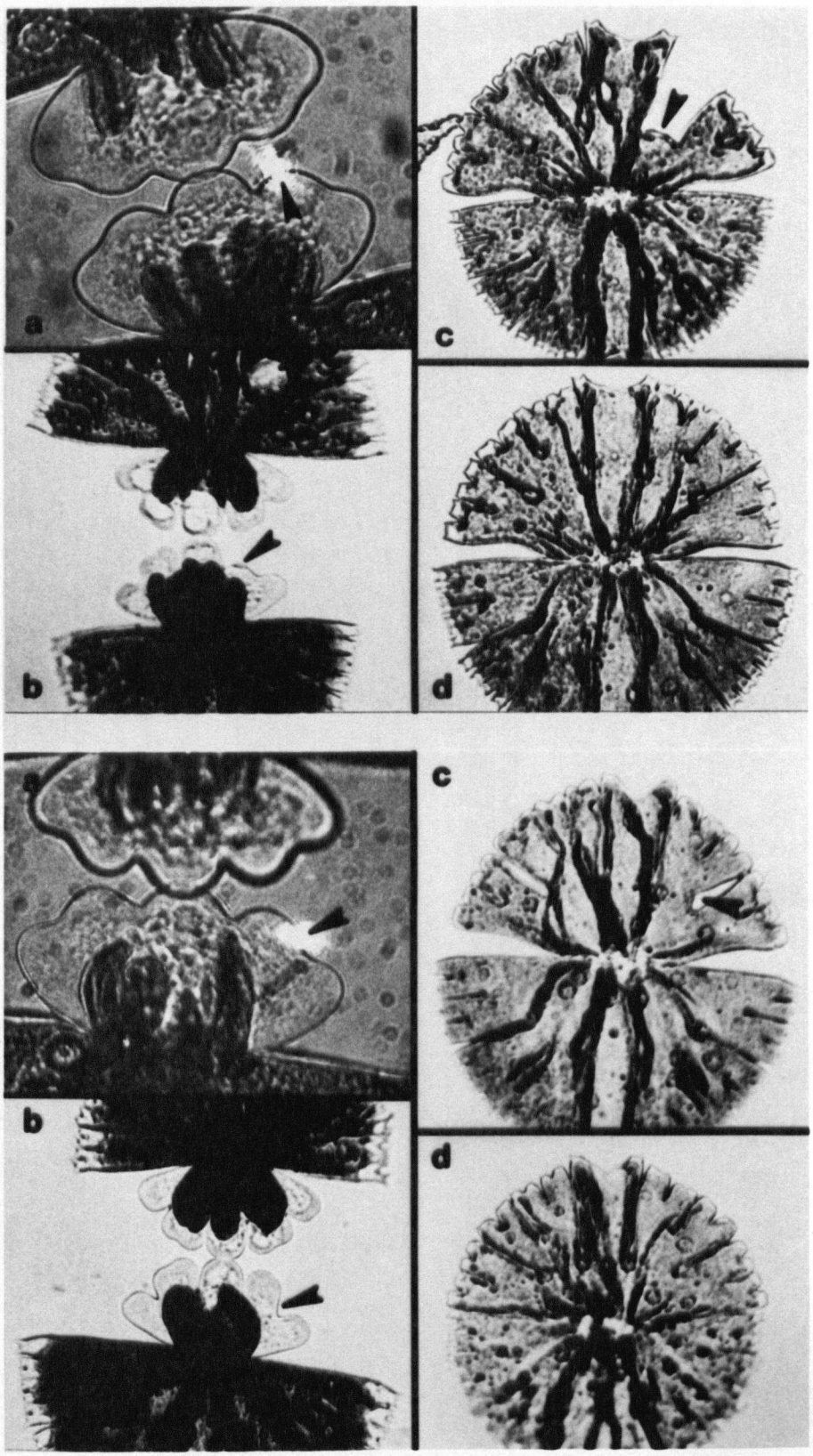


Figure 31

Figure 32 Composite of laser experiments in which regions of developing cell wall were irradiated. Representative growth stages from 2 to 6 are shown. Each circle represents the position of a lasing of the cell wall in one experiment; lasings are for 5 sec. with a beam diameter of 2-5 μ.
(a) Positions of lasings which in each case failed to produce shape abnormalities. All lobes were present in the fully developed semicell.
(b) Positions of lasings which in each case resulted in lost morphogenetic capacity made evident by the absence of the associated lobe or lobes.

Figure 33 Idealized fate map of the morphogenetically important regions of cell wall (singularities) as shown by laser experiments. Stages 4 to 8 are represented.

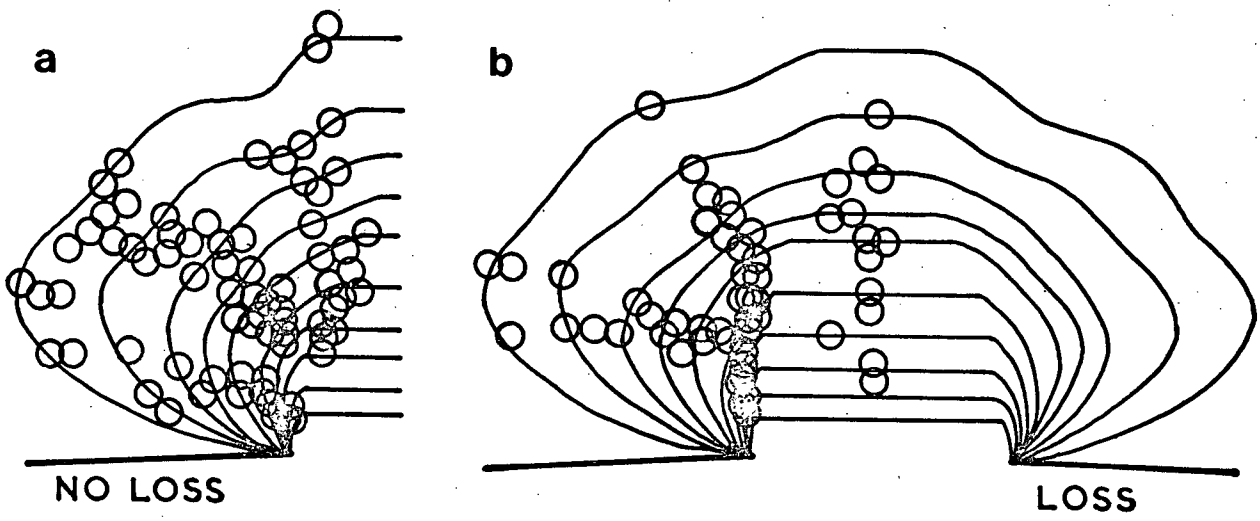


Figure 32

Figure 33

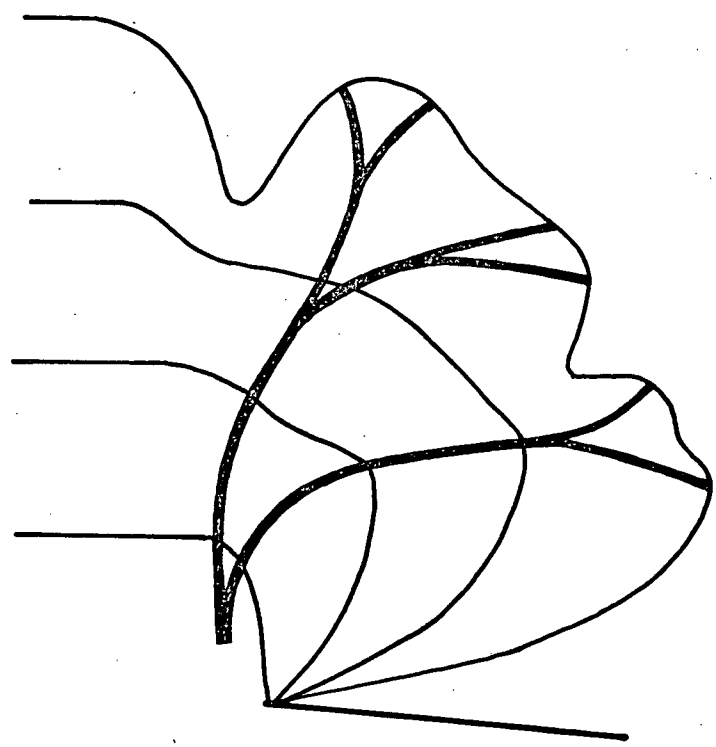
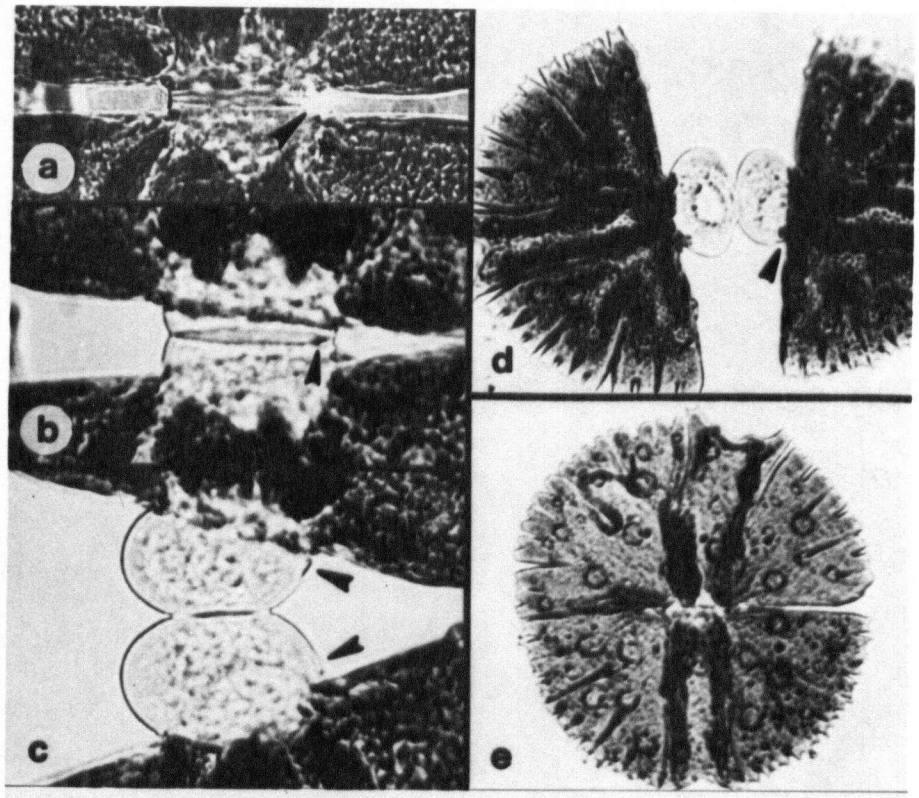


Figure 34 Laser damage to young semicells, Cell 556. An 8 sec. lasing leaves its mark on the semicells (arrows); but does not prevent a complete complement of lobes and notches from being produced, though the "recovered" wing is abnormally small and the opposite wing somewhat enlarged. Note that the recovered wing must have originated from the cell wall at a point above the lasing.
(a) Lasing at stage 1
(b) Development 45 min. after lasing
(c) Development 1 hr. 15 min. after lasing
(d) Development 1 hr. 45 min. after lasing
(e) One daughter on the following day. Both daughter cells were similar.

Figure 35 Laser damage to young semicells, Cell 554. An 8 sec. lasing leaves its mark, but a partial lobe is able to develop from below the point of lasing. The lobe is small but shows branching and dentation of a fairly normal sort.
(a) Lasing at stage 2
(b) Development 20 min. after lasing
(c) Development 1 hr. after lasing
(d) and (e) Daughter cells on the following day

Figure 34



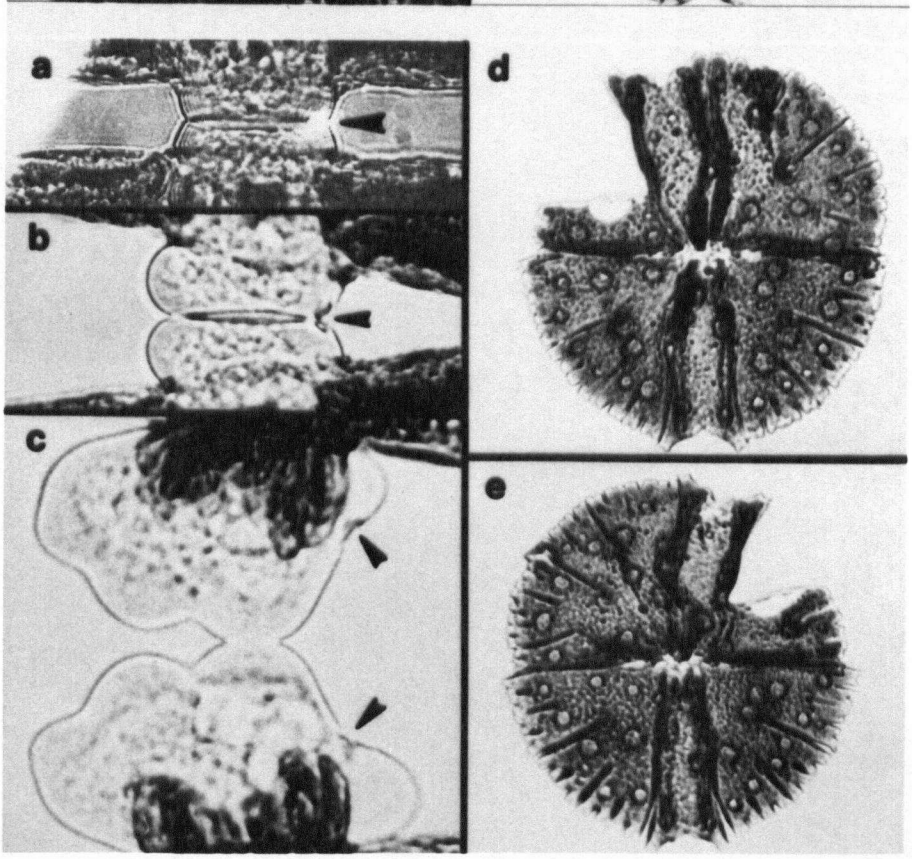


Figure 35

Figure 36 Diagrammatic summary of laser experiments on young semicells. The semicell represented is stage 2 or 3. Lasing with a large spot can prevent one entire wing from forming (as in fig. 29). Smaller lasings within this large area can selectively cause the absence of the upper or lower wing lobe or can hit between the two.

Figure 37 Diagrammatic summary of laser experiments on very young semicells
(a) Diagram of a stage 1-2 isthmus distinguishing two general regions, A and B.
(b) Results of lasing at A. A lobe develops below the lased spot (as in fig. 35) and hence must have developed from region B.
(c) Result of lasing at B. A lobe develops above the lased spot (as in fig. 34) and hence must have developed from region A.
(d) Results of lasing a large area including both A and B. A lobe will still develop in many cases from an area which is neither B nor A. A lobe may also develop not above or below the lased spot but lateral to it on either side, producing a wing projecting somewhat out of the normal plane of symmetry. These experiments roughly define the area from which lobes can be induced to form (shown in fig. 47).

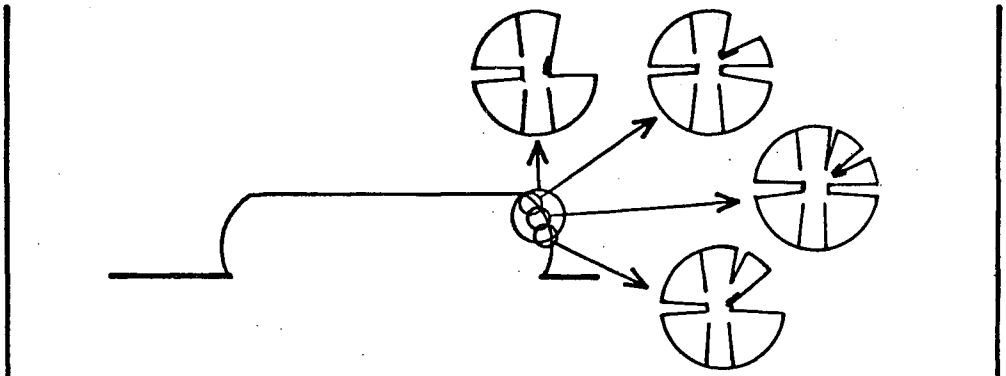


Figure 37

Figure 36

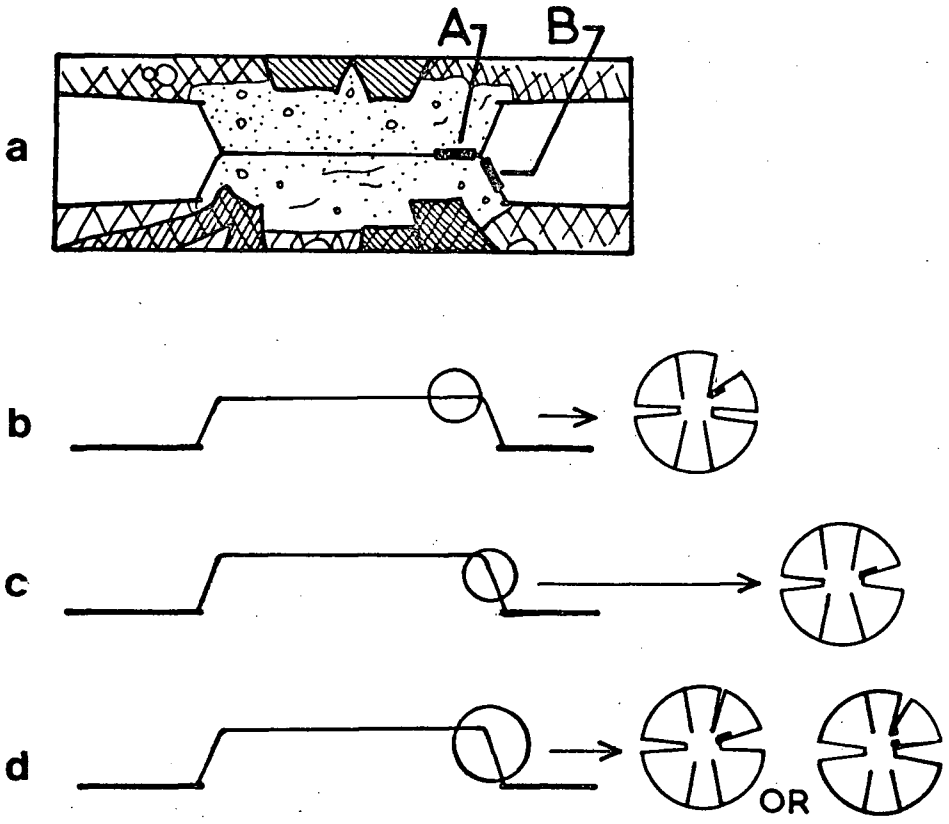
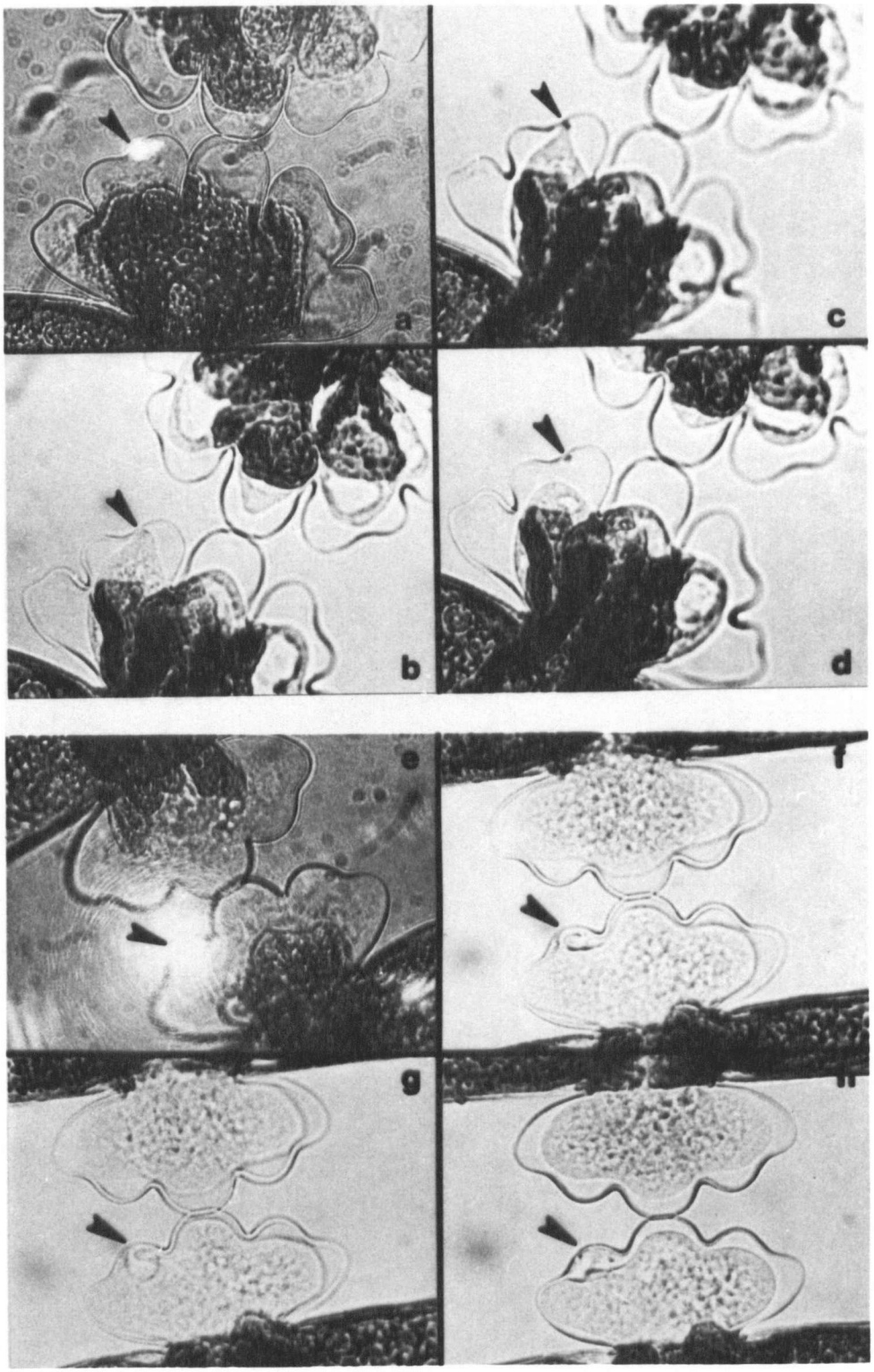


Figure 38 Laser fusion of cell membrane to cell wall. Fusion is shown by plasmolysing cells in 0.2 M sucrose immediately after lasing of the wall. Protoplast sticks to the wall at the point of lasing and eventually is torn away leaving a remnant of membrane behind.
(a-d) Small laser spot, 3 sec. lasing followed by plasmolysis
(e-h) Large laser spot, 3 sec. lasing followed by plasmolysis

Figure 38



Results and Discussion

V. Autoradiography

Autoradiography was used in order to reveal patterns of cell wall synthesis in M.rotata. Cells at different developmental stages were incubated for fixed times with radioactive compounds and preparations made from the primary walls. In all cases, autoradiograms are of cell wall ghosts only. The protoplast has been removed by rupturing the cells in distilled water and the preparations washed to remove small molecules (see Methods). Tritiated water, proline, glucose and methyl-labeled methionine were tested as possible wall precursors. The former two did not show any incorporation into walls whereas methionine and glucose produced heavily labeled preparations and revealed a distinct and consistent pattern of labeling. With both methionine and glucose, incorporated label was extractable under conditions consistent with its presence in cell wall polysaccharides and not in cytoplasmic or membrane residue or as small molecules (see Table IV and associated discussion); glucose incorporated into microfibrils, and methionine probably incorporated into matrix substances in the form of methyl esters.

Glucose showed very little incorporation into cells stage 1 to 6, but did label the tips of lobes in later stages. In stages 11 to 14, tips were heavily labeled; but in addition, label appeared in a pattern over the cell wall resembling somewhat the veining in leaves (fig. 39a-i). In very late stages, labeling was frequently uniform over the whole semicell and considerably denser (fig. 39j-l). This I concluded to

represent incorporation into secondary wall laid down by apposition in cells which had completed morphogenesis. Most of the completely dentated primary wall ghosts labeled in this fashion, and frequently labeled portions appear to have been torn away from the primary wall. All three patterns of label were seen in cells treated for as little as 10 minutes (including the 5 minute washing procedure). Label was denser and more extensive in cells exposed for longer times, but the patterns were the same.

Methionine-treated cells showed similar tip and vein-like label patterns. The tip pattern was noticeable in stages as early as 4, and in general all labeling was much denser and the pattern more distinct (fig. 40a-k). Of 30 fully developed primary walls in this experiment, none showed the uniform pattern characteristic of glucose labeling (fig.40 1). Cells treated 10 minutes with methionine showed a pattern of label somewhat different from that seen in cells treated with glucose for a similar length of time (fig. 41a,b). It is clear that the methionine label is more localized, and in particular, localized to the very tip area of lobes. When glucose label first appears, it occupies a much larger area around and including the tips of lobes. It is characteristic of these experiments that density of label may vary greatly from wall to wall, even though the autoradiographic technique was standardized. For a given length of exposure to label, however, the area of labeling for any given stage is always very constant (fig. 41c, d). Hence it was easy to construct the diagrams in figure 43. For these I drew the upper side lobes of about 30 stage 10

cells, using a camera lucida, and outlined the area of radiolabeling. As I selected cells labeled for longer lengths of time, the area showing incorporation increased progressively in size as shown. For glucose and methionine the patterns were different.

Note now figure 42, representing a model of cell wall fate constructed on the assumption that all increase in wall perimeter from stage to stage originates as new wall formed at the tips. The lines mark off portions of the wall which, by the assumption, must be less than 10 minutes old, less than 20 minutes old and so on. The time scale here is appropriate to development at 20° C as the model is constructed from measurements done on figure 18.

Compare figure 42 with figure 33 showing the fate of cell wall singularities as demonstrated in laser experiments. If growth originates at the tips of lobes only, these alternative forms of fate mapping become complementary. Figure 33 is derived from experimental work and figure 42 shows patterns identical to those observed for methionine incorporation into cell walls (fig. 43b). I take this as good evidence that lobes in M.rotata increase their size by growth originating at the tips of lobes (tip growth), and that methionine label acts as a good indicator of the areas into which new wall material is contributed.

In conclusion, autoradiograms reveal specific tip and vein-like patterns of incorporation into primary wall indicating localized synthesis of microfibrils (in the case of glucose label) and probably of matrix (in the case of methionine).

Methionine incorporation is particularly localized and follows the pattern predicted for tip growth.

Figure 39 Glucose autoradiography of cell walls. Times listed include the time cells were left in label plus 5 min. for the standard washing procedure.
(a-c, j) Walls of cells labeled for 10 minutes
(d-f, k) Walls of cells labeled for 30 minutes
(g-i, l) Walls of cells labeled for 1 hour
Note the tip label pattern in a-i and the veined pattern in f, i and h. Walls j-l show label patterns probably associated with secondary wall synthesis.

Figure 39

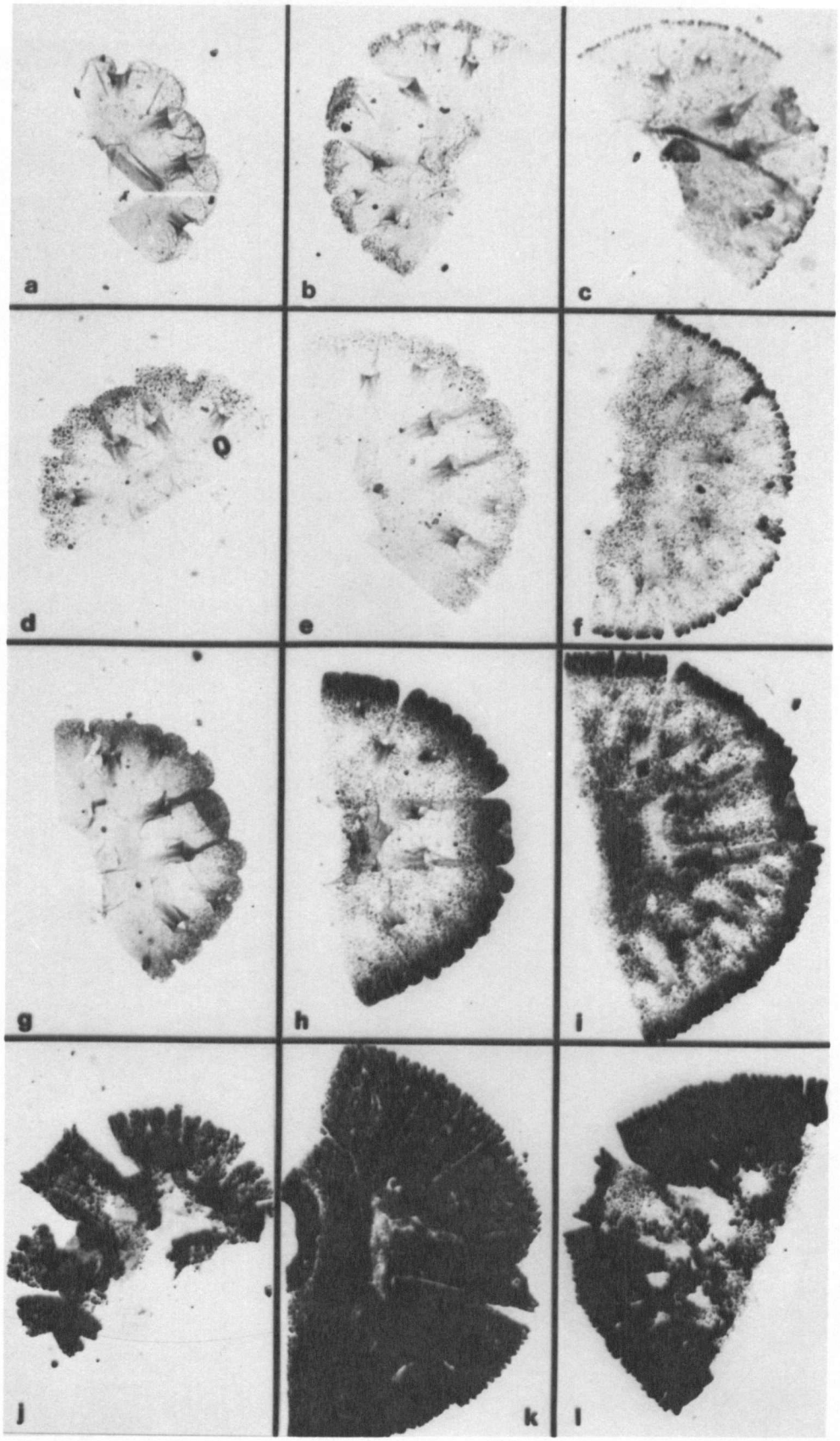


Figure 40 Methionine autoradiography of cell walls. Times listed include the time cells were left in label plus 5 min. for the standard washing procedure.
(a) Wall of a cell labeled for 10 minutes
(b,c) Walls of cells labeled for 15 minutes
(d,f) Walls of cells labeled for 20 minutes
(g-l) Walls of cells labeled for 35 minutes
Note the tip label pattern in a-k and the veining in j and k. Cell wall l is typical of a number of walls probably in stages of secondary wall synthesis.

Figure 40

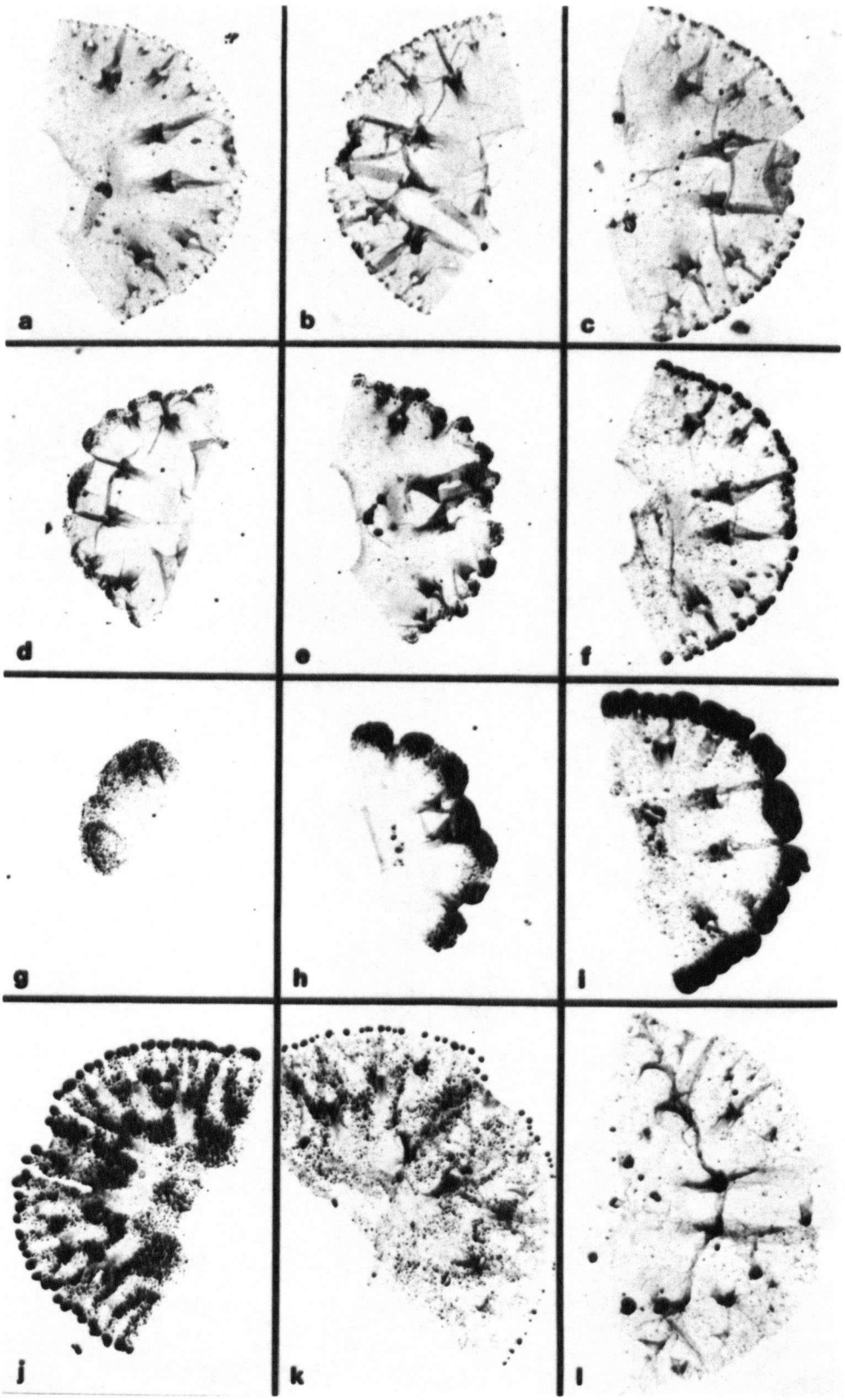


Figure 41 Tip labeling as shown by autoradiography
(a,b) Two stage 11 cell walls both labeled for 10 minutes and showing patterns of tip labeling. The area of uniform tip labeling with glucose (a) is much larger than that with methionine (b).
(c,d) Two stage 9 cell walls labeled for 10 minutes with glucose and showing tip labeling. The amount of labeling is somewhat different, but the area labeled is roughly the same.

Figure 41

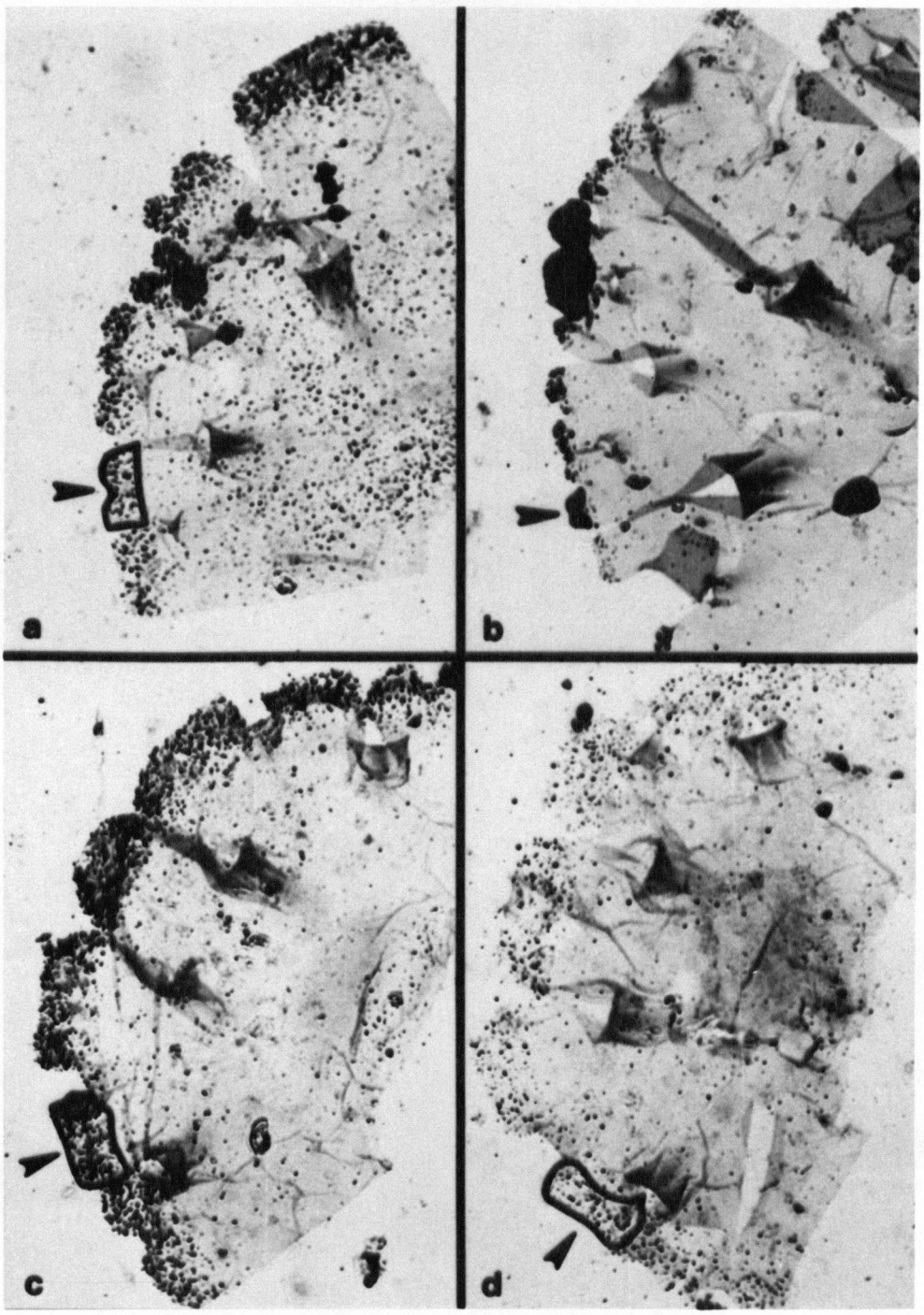


Figure 42 Idealized map of cell wall fate for the upper wing lobe (arrow) of a stage 10 semicell. The model assumes that all new wall starts at the tips of lobes and is pushed back by subsequent tip addition of wall as rapidly as wall perimeter is added to (this rate measured from fig. 18). Contours divide the wall into age categories; all wall within the first contour is less than 10 minutes old, all within the second contour is less than 20 minutes old and so on.

Figure 43 A composite diagram of labeling experiments, the tracings made from the upper wing lobes of about 30 stage 10 semicells using a camera lucida. The area of uniform and dense tip label is shown for cells labeled for 10 minutes, 20 minutes and so on.
(a) Patterns of tip labeling with glucose
(b) Patterns of tip labeling with methionine. Note the close correspondence between this methionine pattern and the model pattern (fig. 42).

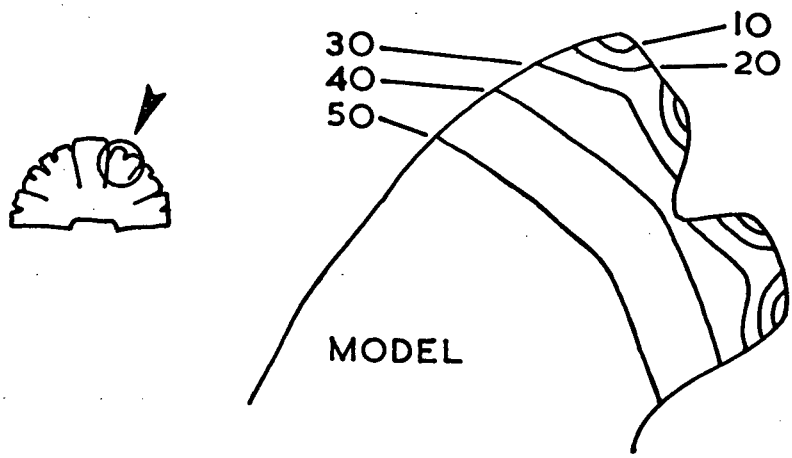
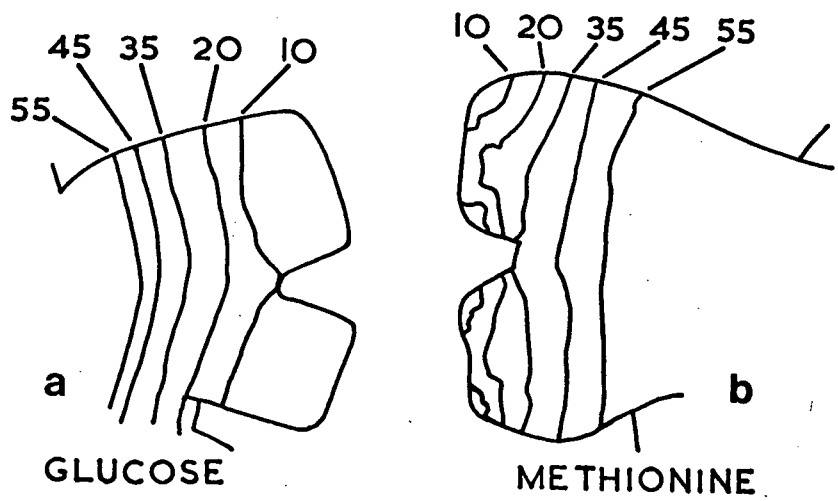


Figure 42

Figure 43



Results and Discussion

VI. Sections through the Isthmus

I have commented already on the occurrence of different symmetry types in Micrasterias. I have also observed that some species have a marked tendency to produce cells of abnormal symmetry types whereas others do not. Kallio has noted differences in both frequencies of spontaneous symmetry mutation and the ease with which these changes can be induced. Table V summarizes my observations and the information available from the literature. It is clear that some species and clones are notoriously mutable (M.torreyi and uniradiate M.thomasiana) and others extremely stable (M.rotata and M. denticulata). I reasoned as follows: If there is a structure in the parent semicell responsible for continuity of symmetry in daughter semicells, in mutable species or clones the structure must be either more labile or its disposition is in some way radially more uniform than in very stable species.

I began looking at cross-sections through the cell isthmus hoping that in mutable species or particularly mutable clones the cross-section would be more nearly round than in stable species. In a preliminary study of 3 species (including 6 symmetry types) this proved to be the case. About 70 cells were sectioned, and for 4 to 5 cells in each of the six groups I got appropriate sections in good condition (fig. 44). My prediction was borne out as follows: Biradiate M.torreyi, uniradiate M.thomasiana, partially

Table V

Stability of Different Symmetries (facies) in Haploid Micrasterias

Species and Facies		Personal Observations	Published Reports	
			Comments	Reference
M. rotata	biradiate	Stable	Stable	Kallio (1972)
	uniradiate		Single occurrence in pygmy clone, very unstable	Kallio (1954)
	triradiate	Single occurrence, unstable, perhaps diploid		
M. denticulata	biradiate	Stable	Very stable	Kallio (1972)
M. radiata	biradiate	Stable	Very stable	Kallio (1972)
	uniradiate	Occurs only in unhealthy pygmy strain		
M. sol	biradiate	Stable	Stable	Kallio (1968)
	uniradiate		Easily induced by UV	
	aradiate		Easily induced, somewhat non-viable	
M. thomasiana	biradiate	Stable	Stable	Kallio (1951)
	uniradiate	Unstable	Occurs only rarely, is unstable	Kallio (1960) Waris (1950b)
M. americana	biradiate		Stable	Kallio (1960)
	uniradiate		Occurs only rarely, is unstable	Kallio and Heikkila (1969)
	aradiate		Stable	
M. fimbriata	biradiate			Kallio (1972)
	uniradiate			
M. torreyi	biradiate	Unstable	Unstable, easily induced	Kallio (1957)
	uniradiate	Occurs frequently, is unstable	Easily induced by UV, is unstable	Kallio and Heikkila (1969)
	aradiate		Induced by UV, is unstable	

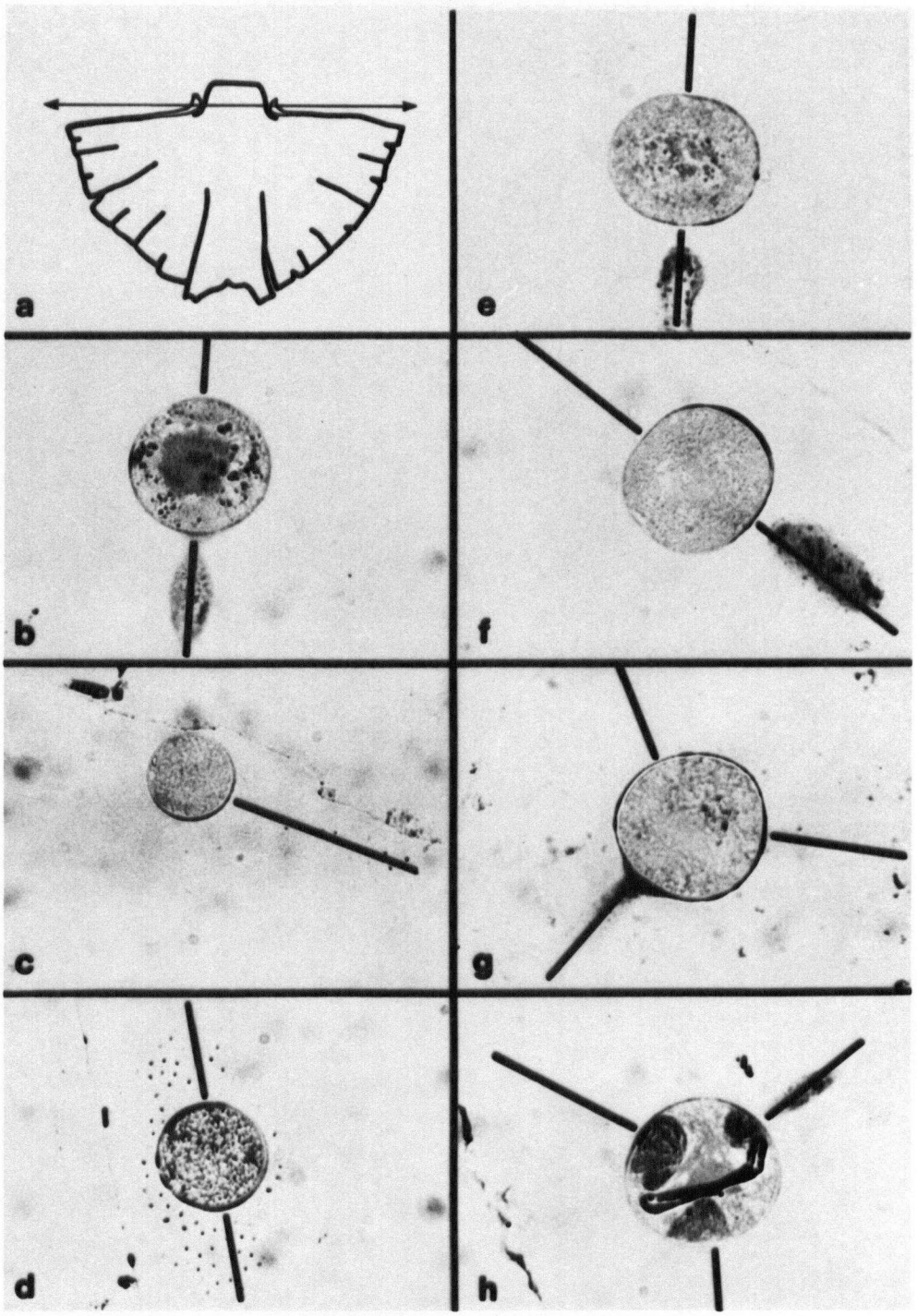
uniradiate and revertant biradiate M.thomasiana all have isthmus cross-sections which appeared to be round. The cross-sections for biradiate and triradiate M.rotata were clearly not round. As a rule, in M.rotata the isthmus is flattened slightly at points from which wings arise. In biradiate cells this gives the isthmus the cross-sectional shape of a slightly squashed circle, a shape asymmetry which could be related to preformed template required by theory to impose or transmit symmetry information to daughter semicells.

Other structural components of the parent semicell do not show appropriate asymmetries, do not maintain a fixed asymmetric position or have an asymmetry which has proven to be irrelevant. In the latter category are included semicells lacking one or more wings or the chloroplast contained therein. Wings are not necessary for normal morphogenesis (see fig. 29), and cells also develop normally though lacking large pieces of chloroplast, all the chloroplast of one wing being absent for example.

In conclusion, parent and daughter semicells in M.rotata share a biradial symmetry with the isthmus cross-section. M.torreyi and M.thomasiana are more mutable and hence may have a less determinate template. These species lack the isthmus asymmetry thus suggesting a possible link between isthmus cross-section and the morphogenetic template.

Figure 44 Isthmus cross-sections. Sample sections are taken from Epon-embedded cells sectioned as shown in (a) so that secondary and primary wall are both included in the section. Lines are drawn in to show the orientation in which lateral wings would lie if deeper sections were taken. All photos are X650.
(b) M. torreyi biradiate
(c) M. thomasiana uniradiate
(d) M. thomasiana biradiate reverted from a uniradiate culture
(e,f) M. rotata biradiate, two different cells
(g,h) M. rotata triradiate, two different cells

Figure 44



Discussion

I. Tip Growth in Micrasterias

Area growth in cell walls generally occurs in walls of a particular sort; they are thin and constructed of loosely packed microfibrils with matrix filling the spaces. Such walls are called primary walls and grow in substance by the intussusception of material into existing wall.

Various physical, chemical and biochemical processes contribute to primary wall growth. Wall stretching, wall loosening and wall deposition are all important aspects of growth, and one would like to know if any one of these is more important than the others. For example, growth might occur because the fabric of the wall is loosened, for this would allow the wall to be stretched by turgor and new material would fill up the spaces. This is a difficult hypothesis to prove (Cleland, 1971) for the processes contributing to wall growth are as difficult to separate from one another as are Kiermayer's five causal aspects of morphogenesis in Micrasterias. In general it does appear that turgor stretching alone cannot drive the growth process (Roelofsen, 1965), but may act to align growth if the wall is not isotropic. I tend to accept the argument that cell wall deposition is rather important, particularly the deposition of matrix substances (Setterfield and Bayley, 1961; Frey-Wyssling, in Preston, 1964). Setterfield and Bayley (1961, pg. 56) view growth as follows:

> "Dependence of wall deposition on extension might be visualized as some process by which stretching of the wall frees active surface areas required for addition of new materials ... The alternative relation, dependence of wall elongation on deposition, might be explained on the basis that a given amount of wall material is only capable of a certain amount of stretching by turgor, and for continued expansion to occur, new material capable of being stretched must be added."

Obviously we are faced with multiple causation (Castle, 1953) and want to discover how directional information is contributed.

Two categories of primary wall growth may be contrasted; extension and tip growth. Extension growth is the means by which cylindrical cells elongate uniformly along their length. The multi-net hypothesis (Roelofsen and Houwink, 1953) explains changes observed in microfibril orientation during extension growth, but does not explain the mechanism of growth itself. Setterfield and Bayley, as quoted above, come closer to that.

Tip growth is demonstrated when marking experiments or radiolabeling show that wall extension and deposition are localized at a small area of the wall. This area may be hemispherical and act so as to produce a cylinder of non-growing wall behind as in the extensively studied case of hyphal growth in fungi (Robertson, 1959, 1968; Grove et al., 1970). Tip growth also occurs in algal rhizoids and moss protonema (Sievers, 1967) and in higher plants; in root hairs (Sievers, 1963; Bonnett and Newcomb, 1966), pollen tubes

(Rosen et al., 1964; Franke et al., 1972) and fibers and sclerids (Esau, 1967). It is to be expected that in some cases tip and extension growth could both occur as the mechanisms of each may be similar. Mix (1961) looked at microfibril orientation in the primary walls of three desmid species. Microfibrils were unoriented in walls of Cosmarium but longitudinally oriented in parts of the elongated wall of Pleurotaenium. Longitudinal microfibril orientation is usually associated with extension growth while in all cases so far studied, growing tips show randomly oriented microfibrils (Houwink and Roelofsen, 1954; Sassen, 1964; Green and King, 1966; Bartnicki-Garcia, 1972). The apical cell in Nitella has randomly oriented microfibrils at its tip, but these become transversely rather than longitudinally aligned at the base of the growth hemisphere. Microtubules are implicated in primary wall extension growth but have not yet been found in the cytoplasm of growing tips (Newcomb, 1969).

Cytoplasmic polarity is a striking ultrastructural feature of cells showing tip growth. Tip cytoplasm is usually packed with vesicles to the exclusion of all other organelles. Golgi and ER lie at some distance from the tip and larger structures, such as nuclei, are even more distant. With phase microscopy, vesicles can be seen to wander from the golgi region to the vesicle-filled tip and to remain there. As the cell wall is differentiated into only two areas, a rapidly growing one (the tip) and a non-growing one, it is frequently argued that the observed cytoplasmic polarity is sufficient to explain tip growth of the wall.

In the various mechanisms proposed for tip growth, cytoplasm is responsible directly for supporting areas of rapid cell wall synthesis or indirectly through cytoplasmic polarity, allowing a spatial differentiation of cell wall functional activity to exist. Mechanisms appropriate to the former argument include;

(1) directional transport of vesicles to certain regions of the cell wall by microtubules or oriented cytoplasmic streaming,

(2) regional specialization of the membrane to attract vesicles, or

(3) the elaboration of a special organelle responsible for tip growth. Some of these organelles can actually be seen, such as the spitzenkörper found in some hyphae (McClure et al., 1968; Grove and Bracker, 1970); others are purely hypothetical (Bartnicki-Garcia and Lippman, 1969).

The growth of lobes in M.rotata shows many parallels with tip growth in these other plant and fungal systems. Lobe cytoplasm shows a similar polarity in distribution of organelles with vesicles only at the tip, then ER and golgi. If growth is arrested, lobes deposit a thick layer of cell wall at their tips as is characteristic of root hairs (Schröter and Sievers, 1971). The laser serves to mark M.rotata wall in the same way that dyes and various particles have been used to mark other cells for wall fate studies (Rosen et al., 1964; Green and King, 1966), and autoradiographic studies similar to mine are common in the literature

on tip growth (Bartnicki-Garcia and Lippman, 1969; Galun, 1972). Hyphae will rupture at their tips much as M.rotata lobes do in acid solutions. Hyphal tips will begin to harden over if growth is arrested, for if growth is then allowed to resume, tips produce hyphae of a much reduced diameter (Robertson, 1959). M.rotata shows similar narrowing of tips after plasmolysis (fig. 23b), but the time course of tip hardening could not be determined as it has been in hyphae. Pollen tube growth is enhanced by the presence of pectinase and cellulase (Roggen and Stanley, 1969); M.rotata lobes do not show this effect.

In short, I have fairly good evidence that Micrasterias lobes offer an example of tip growth comparable with the growth of fungal hyphae, root hairs and perhaps pollen tubes. And as well as being just another example, Micrasterias offers potentially interesting information not available for other tip growth systems. In particular, M.rotata cell wall preparations are very thin and autoradiograph can be done close to the theoretical limit of resolution (Schultze, 1969).

Figure 45 represents the model for tip growth developed by Robertson (1959) to explain his observations on hyphae. In general, tip growth is a steady state situation. The hemispherical growth zone must maintain itself; new wall material must be supplied to the growth hemisphere as rapidly as completed stable wall passes to the cylinder behind. Wall in the growth zone is therefore plastic as it is both

gaining new substance and enlarging in area. Wall passing from the growth zone cannot be plastic and isotropic both while still contributing to a cylinder of fixed radius. Robertson proposes two fundamental parts to tip growth. Wall area must be increased and new wall incorporated, and these will occur at a maximum rate at the very tip (A in fig. 45). Over the rest of the growth zone wall area may increase, but hardening of plastic wall must also occur (B in fig. 45). The two processes, incorporation and hardening, could occur by any of a number of mechanisms. Plastic wall may be added at A, expand and become strain hardened at B. Plastic wall may be biochemically altered at B, enzymes added at A may be degraded, or chemical bonds altered to affect wall rigidity. Or a second wall component may be added at B strengthening and hardening the plastic component. This latter possibility was envisaged by Robertson for hyphae and appears to be the case in M.rotata, as two distinct patterns of wall incorporation are revealed by autoradiography. The pattern of methionine incorporation fits well with its being associated with synthesis of plastic wall at A. The hardening process at B is then associated with the incorporation of glucose and hence with synthesis of microfibrils. This correlation of two distinct synthetic activities with Robertson's two model processes is an important result.

Note that the two processes must be exactly balanced for tip growth to produce a cylinder uniform radius. If the hardening reaction begins to overtake incorporation

of plastic wall, the cylinder produced will begin to narrow. This appears to be the case in normal lobe development in M.rotata for lobes do become progressively narrower and eventually come to a point. Narrowing is not so marked in cells grown at 37° C and can be completely reversed, as in the presence of pectin methyl esterase. One would expect that chemicals altering methionine metabolism would be likely to affect narrowing or broadening of tips.

I have so far discussed lobe growth in M.rotata as a tip growth system. I wish now to propose that Micrasterias wings are produced by a combination of processes including tip growth and branching. It may strike the reader that M.rotata wings do not much resemble a branched hypha. The pattern is far more obvious in M.radiata, in the cell's shape and its developmental stages. Note figure 22, stages 4 to 7. Here the four wing lobes lengthen while the notches remain fixed; clearly a case of pure tip growth. Note stages 7 to 9 of the same figure; here both tips and notches change in relative position and tips branch. In M.radiata tip growth is only part of normal morphogenesis; a second sort of growth which acts to broaden lobes also contributes (fig. 22 inset). In M.rotata, tip growth and broadening growth are less separable. Throughout development both lobe tips and notches change relative position, though in late developmental stages (stages 11 to 14 in fig. 18) cell size continues to increase after lobe dentation is complete. It is during these stages that the

vein-like pattern of cell wall labeling appears in autoradiograms. The conclusion is inescapable that broadening growth is associated with a synthetic process similar to tip growth, but synthesis occurs in various patterns over the surface of the cell wall rather than exclusively at lobe tips (fig. 46). Note that branching and lobe broadening can be separated experimentally from pure tip growth. Growth of M.rotata lobes at 37° is an example where, as in normal development of M.radiata, branching and lobe broadening are both absent. To this extent branching and broadening appear to be associated with one another.

This discussion has neglected almost entirely the morphogenesis of the polar lobe. Polar lobe growth does not fit nicely into a model depending on localized synthesis. The lobe does not appear to grow at its tip but rather by some more diffuse mechanism, perhaps by extension growth. An EM study of microfibril orientation might clarify this problem.

In conclusion then, the growth patterns observed in Micrasterias wings are associated with characteristic patterns of wall synthesis. This implies that Micrasterias shape should be explained in terms of the patterns of cell wall synthesis and the ways that these arise and are maintained.

Figure 45 A model for hyphal tip growth according to Robertson (1959). New wall is initiated at the tip and grows in area to pass eventually into the cylinder behind. Two processes are associated with this:
(1) Incorporation of large amounts of plastic wall substance in region A and
(2) Hardening of this wall over the surface of the hemisphere (region B).

Figure 46 Morphogenetic variations on tip growth
(a) Tip growth showing some degree of narrowing
(b) Tip growth with branching
(c) Tip growth with branching and broadening (arrows) as occurs in stages 7 to 9 of M.radiata development (fig.22)
Inset: The patterns of label which would be associated with the three variations on tip growth if wall synthesis and localized wall expansion always occurred together. Note that (c) resembles the veined pattern seen in late stages of M.rotata growth.

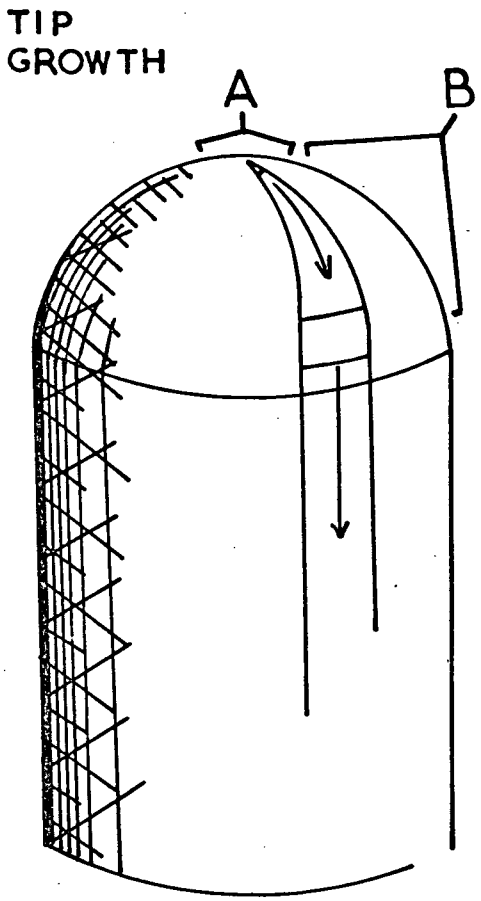
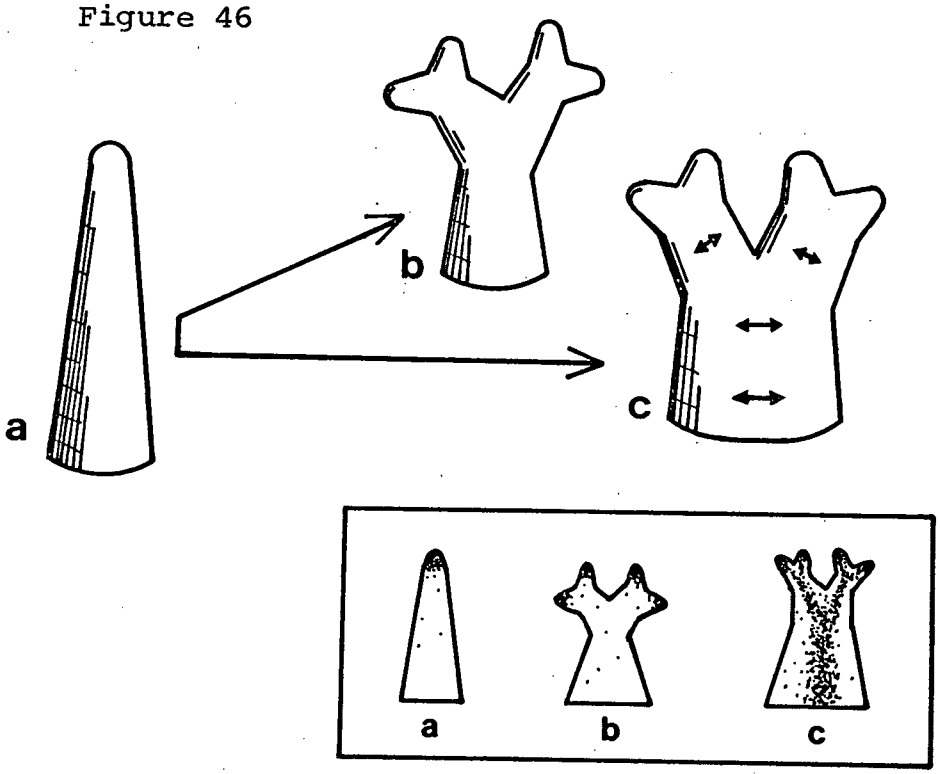


Figure 45

Figure 46



Discussion

II. The Template in Micrasterias

There is some agreement that at least part of Micrasterias morphogenesis depends on the actions of a template (Kallio, 1972b). The template may embody specific information concerning the ultimate position of each semicell lobe and notch (Kiermayer, 1970b) or it may provide only general instructions concerning symmetry. The latter situation is more likely if, as I will argue in part III, the growth of each lobe is independent and self-organizing. The template then need be responsible only for initiation of the wings and polar lobe, presumably by establishing the initial growth singularity for each. As a fairly large area of the stage 2 septum is competent to give rise to wing singularities (fig. 47), any template associated with the septum at this stage probably contains only general information sufficient to initiate singularities in roughly the appropriate symmetry relationship.

An understanding of septum structure and growth may be crucial to any explanation of the Micrasterias template. The mechanism of wall growth in the septum is probably similar to or identical with that occurring at later stages of morphogenesis. Certainly the wall appears to have a similar structure throughout morphogenesis.

If we consider the mechanics of septum growth, we must allow that a number of processes are involved. The septum wall must grow in substance through addition of matrix and the lengthening of existing microfibrils, probably also by the

addition of new microfibrils. The cell membrane must also grow and may do so by the fusion of vesicles or intussusception of membrane subunits. Any of these processes might be disposed in a particular pattern so as to contribute a structural or functional asymmetry to the septum thereby causing it to act as a template.

As we have seen (fig. 15), the septum initiates at the isthmus girdle and grows inward. I presume that synthetic activity is concentrated around the inner edge of the advancing septum and that this edge, with its associated membrane, forms the substratum upon which new wall and membrane must be deposited. If the inner septum edge is differentiated into different regions about its perimeter, synthetic activities could be correspondingly spatially differentiated. If the isthmus cross-section is perfectly circular, this differentiation cannot occur as all parts of the resulting septum edge are identical (fig. 48a). Cells with an asymmetric isthmus cross-section, however, have the opportunity to produce an asymmetric septum. If template formation were related to cross-sectional asymmetry, we would expect cells with the most asymmetric cross-sections to have the least mutable templates, and this appears to be the case.

At an isthmus similar in cross-section to that found in M.rotata (fig. 48b), certain regions of the septum edge (A in fig. 48b) will always have a greater curvature than others. If the efficiency or orientation of membrane addition or wall synthesis depended upon curvature of the substratum, then the septum formed at such an isthmus would have to be

biradially symmetric in structure. This deviation from radial symmetry offers itself as a relatively plausible candidate for a template.

Consider a hypothetical case in which the synthesis of wall microfibrils is proportional to the density of microfibrils in the existing inner septum edge. The more radially disposed microfibrils would converge and become relatively dense at points of greatest curvature (A in fig. 48c). The resulting septum would have a biradially symmetric gradient of microfibril density with points of least density falling roughly where, in M.rotata, the initiation of singularities may be demonstrated. This example is presented to demonstrate that wall structure has, itself, considerable potential as a bearer of morphogenetic information. A case could similarly be made for specific structural organization in the cell membrane or associated cytoplasm.

In conclusion, the septum produced in M.rotata could contain information concerning the symmetry of the parent semicell, possibly as asymmetries built into the structure of the septum during its formation. The septum could fulfill the minimum required of a template for Micrasterias morphogenesis if this information were translatable into instructions for the initiation of three singularities and could thus represent the template sought by other authors.

Figure 47 One daughter of a dividing pair at stage 2 of cell development. Regions of the new semicell competent to produce wing lobes are cross-hatched and labeled (R).

Figure 48 Three models of septum growth. In each case the cross-sectional isthmus space is to be filled in by uniform inward growth of cell wall material.
(a) A perfectly round cross-section such as that found in M.torreyi and M.thomasiana. Note that all points of the inner septum edge (arrows) will be identical regardless of the mechanism by which the septum is constructed.
(b) A cross-section similar to that found in M.rotata showing the regions of greatest (A) and least (B) curvature at the inner septum edge.
(c) A cross-section identical to that in (b) demonstrating that points spaced uniformly at septum initiation will converge non-uniformly as the septum grows inward. If the points represented some real structure, the resulting septum would demonstrate a biradially symmetric gradient in the density of the structure.

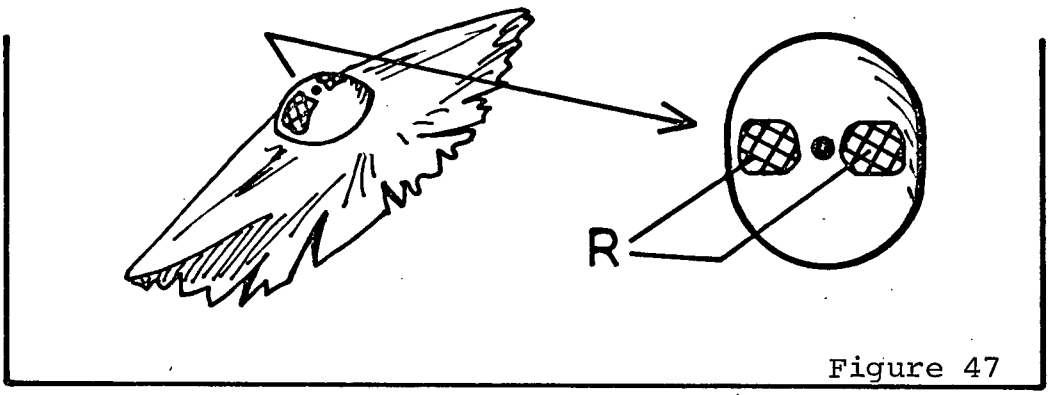
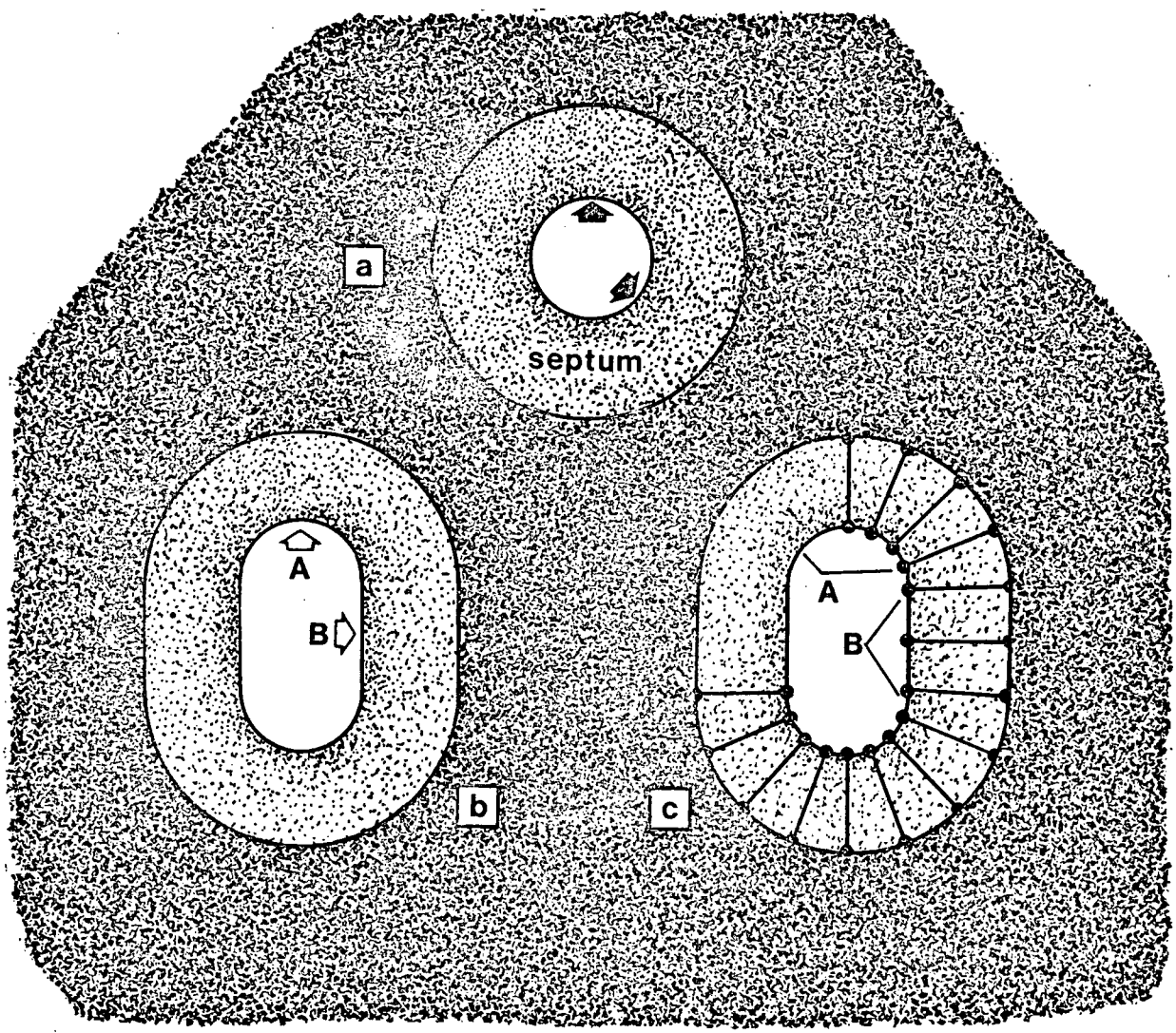


Figure 47

Figure 48



Discussion

III. Morphogenesis and Organization

It is natural to hope for some unity underlying the different morphogenetic contributions to final cell wall shape. For one, the raw material of morphogenesis, the primary cell wall, appears to have the same physical structure throughout cell development, hence the physical and biochemical mechanisms of growth may be the same. Perhaps also the organization responsible for morphogenesis is in some way the same regardless of the pattern of growth, even in two such distinct growth processes as template formation and tip growth.

As mentioned, wall growth is a coordinated activity demanding a number of preconditions, and most of the contributing processes do not also contribute directional information. With tip growth, though it also is a complex activity, we expect to be able to point to some part responsible for directional information; to find, somewhere, organization directly relevant to morphogenesis rather than just to growth.

It is usual to look either to the cytoplasm or to the cell wall itself for hints concerning this organization.

> "Theories of the mechanism of spiral growth (in Phycomyces sporangiophores) fall into two categories: (1) those that attribute the origin primarily to protoplasmic activity of some sort, not always well defined; and (2) those that look to existing oriented wall structure, on the whole or in part, for its immediate source." (Castle, 1953; pg. 365)

Castle's comments are as appropriate to tip growth as to spiral growth; it is fashions in research which dictate where one is most likely to look for organization.

With tip growth in Micrasterias, the choice of cell cortex (cell wall plus plasma membrane) as the seat of organization is fairly easily made. We can first eliminate the nucleus as a director of wall growth. Certainly the nucleus provides something to the growing cell, for the enucleate cell runs down after several hours. But a certain amount of morphogenesis does take place in the absence of a nucleus (Selman, 1966), and the various interesting effects of nuclear ploidy on growth patterns can all be interpreted as due to the increased turgor available to polyploid cells (Green, 1969). The general cytoplasm is also a poor candidate for organization. Oriented structures such as microtubules are absent from areas of active cell wall growth (Kiermayer, 1968b), and the cytoplasm shows very active streaming (Kiermayer, 1964). It is difficult to imagine any cytoplasmic organization or structure maintaining itself in such a situation though Waddington (1962, 1966) has tried to get around the problem of cytoplasmic activity by proposing various dynamic structures, all of which remain hypothetical.

The cell cortex is a much better candidate for the embodiment of organization. First, the cortex (the wall portion, at least) is a fixed structure, and it is easiest to imagine organization in something relatively fixed. Second, morphogenesis is altered in very specific ways when the cell cortex is damaged. Neither of these arguments is entirely compelling, but the third is more so; the cell cortex exhibits a spatial differentiation of activities at a far finer scale

than any seen in the cytoplasm. That is, the cell cortex shows a singularity with particular growth and synthetic capabilities which is of the order of microns in size whereas cytoplasmic polarity is expressed over tens of microns. Hence the organization most relevant to tip growth must reside in the cell cortex, and the seat of organization is roughly identical with the singularity as described from laser experiments.

Note that Robertson's model for tip growth has similar implications. His region of incorporation (A in fig. 45) is self-perpetuating as long as the hardening process (B in fig. 45) does not catch up; the tip growth zone tends to maintain itself once established. Hence in hyphae or in Micrasterias lobes, tip growth is a self-organizing behavior of the cortex.

Tip growth is an organized system, the organization associated with singularities. By using this vague word "singularity," I stress that we know nothing of the structure of these specific sites or even whether they should be thought of in structural terms at all. We can ask in what part of the cell cortex the singularity resides, whether in membrane, in cell wall or whether in both. I have no experimental evidence to support a particular alternative, but I favor cell wall as the most likely place for reasons analogous to those given by Waddington for rejecting cytoplasmic structure as a possible template. That is, the wall is probably the most fixed and stable component of the cell cortex whereas, if animal cells are any indication, cell membrane is probably much more labile (Chapman-Andresen, 1972). In any case, I feel it a worthwhile

exercise to show that cell wall could in theory have structure and organization sufficient to explain morphogenesis, without the imposition of specific structural order from cytoplasm or membrane.

A singularity in Micrasterias could be thought of as a point of instability on the cell wall. If stable wall comprised a feedback-controlled balance of microfibrils and matrix, then the singularity would be a point at which the balance was upset, the initial upset occurring when turgor stretched the wall enough to allow a bit of extra matrix to be incorporated. Fibrils would begin to be synthesized in the area of new matrix incorporation to return the matrix-to-microfibril balance, but would always be just a few steps behind. Matrix and microfibril synthesis would race one another; matrix synthesis could not get too far ahead as a wall of matrix alone becomes unstable. So we have a singularity established, one which perpetuates itself. Figure 49 shows by graphical example the sorts of interactions one would expect in such a system. The singularity is an instability in the sense that wall precursors flow through it on their way to becoming mature wall (Prigogine and Nicolis, 1971). The singularity remains, not as a real structure but as a dynamic one.

One can now imagine how singularities might arise in the Micrasterias septum. When the septum is being formed, there is equal turgor on both sides and no net stress, so areas of different microfibril density can be formed. Only when the septum bulges out and is subjected to internal turgor do these

areas expose themselves as weak points into which extra bits of matrix may be able to sneak. Badly coordinated osmotic events could account for the production of double cells (fig. 27), for regardless of whether the septum were complete when septum bulging occurred, the template would be expressed.

The model presented in figure 49 has a number of biases. It focusses on cell wall as a site of organization ignoring other possibilities. It exploits dynamics at the expense of real microstructure for no specific structural order is required; there are no ordered fibrillar networks or pavements of matrix to which subunits must be added in specific configuration. Events are ordered rather than structures.

The model relies heavily on physical rather than biochemical principles. It assumes that synthetic enzymes and precursor substances are available in abundance as part of the preconditions for growth, and so biochemical regulation does not enter into the arguments. Explanations in terms of instabilities and equilibria are perhaps too simple to endure, but they are appropriate to the limited information available for Micrasterias.

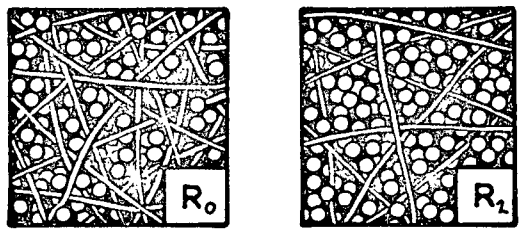
In conclusion, the organization relevant to morphogenesis resides in the cell cortex, perhaps in the cell wall. Organization may be thought of in strictly structural terms, as embodied in a specifically ordered microstructure for example; but plausible alternatives to this view can be offered.

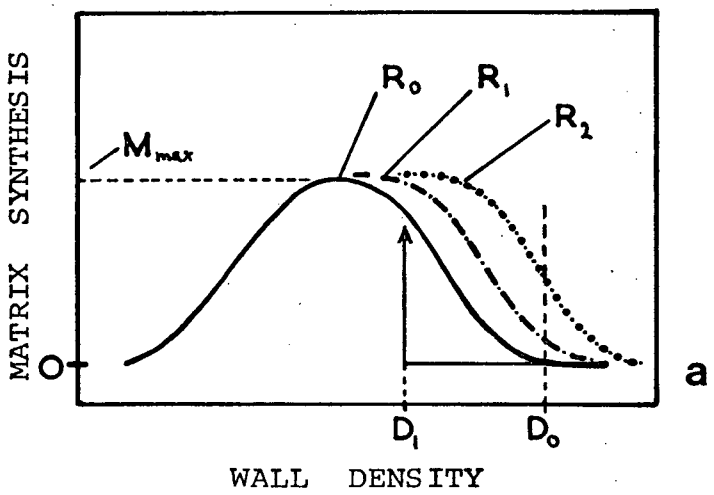
Figure 49 A physical model of tip growth dynamics. The wall is considered to have two major components as shown in the two upper insets; a fibrillar network and a globular matrix. Appropriate physical variables include the following:
(1) R, the ratio of matrix to fibrils (a ratio of mass per unit area of wall for each);
(2) M and F, the synthesis (incorporation)rates for matrix and fibrils respectively (measured as mass per unit time added to a unit area of wall) and
(3) D, total wall density (the sum of mass per unit area of wall of matrix and of fibrils).

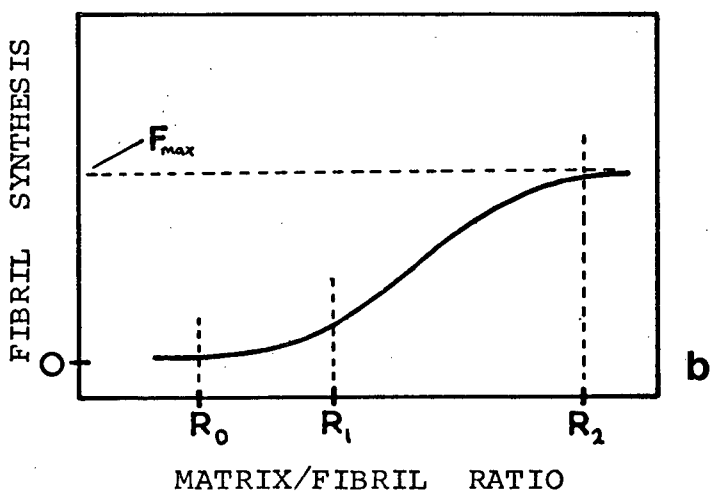
(a) The graph shows that M responds to changes in both R and D. For a given R there is a D at which the wall is stable and no matrix is being added to the wall. If D is lowered, matrix begins being added so as to re-establish a stable D. The response curve shifts as R changes.
(b) The graph shows that F responds to changes in R but not to changes in D. If matrix is added to the wall, thereby increasing R, F will act to re-establish the original R by incorporation of fibrils.
(c) The graph shows that matrix added to walls with different R values contributes differently to increase in wall area. In walls of low matrix content, additional matrix first fills up existing spaces between the fibrils. In walls rich in matrix, additional matrix gets in only by pushing aside existing wall to create space.

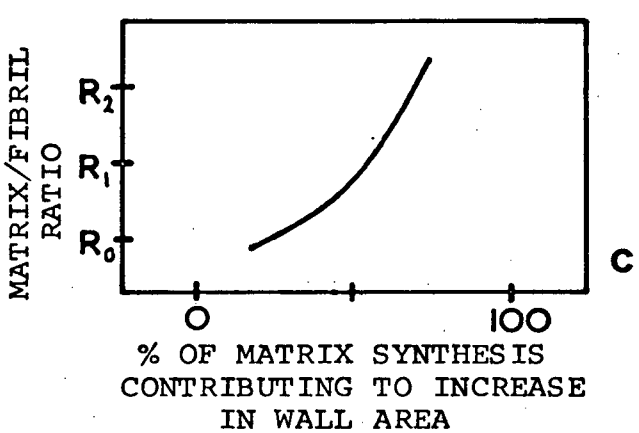
Note now the interesting behaviors of the system defined by these graphs. Take a stable wall of density D_o and ratio R_o and subject it to increased turgor. If parts of the wall can be stretched sufficiently to cause local shifts in wall density - a shift from D_o to D_1 (graph a) - then matrix will be added at these points to fill up spaces. This has the effect of increasing both D and R together. As R increases (to R_1 or R_2) fibril synthesis is brought into play (graph b) and acts to increase D while decreasing R. Thus R can be balanced by conflicting activities while D steadily returns to its initial value. We are returned to R_o and D_o but an increase in wall area has occurred. We could imagine a case, however, in which fibril synthesis was unable to dominate matrix synthesis in this way (a low F_{max}). Local areas could exist at which matrix addition outpaced the effect of F to lower D (and hence to lower M), but would be surrounded by areas in which fibril synthesis was catching up by this same mechanism. The regions of maximum matrix incorporation would have high R values and so substantial local increases in wall area would accompany matrix addition there (graph c). In effect we would have at those regions, all the characteristics required of a tip growth singularity.

Figure 49


R0
R2


MATRIX SYNTHESIS
R0
R1
R2
Mmax
O
D1
D0
a
WALL DENSITY


FIBRIL SYNTHESIS
Fmax
O
R0
R1
R2
b
MATRIX/FIBRIL RATIO


MATRIX/FIBRIL RATIO
R2
R1
R0
O
100
c
% OF MATRIX SYNTHESIS CONTRIBUTING TO INCREASE IN WALL AREA

Discussion

IV. Morphogenesis and Explanation

I have devoted considerable space to a discussion of morphogenesis in Micrasterias and wish now to examine whether our understanding of the process has been thereby improved. On the one hand, I have tried to undermine certain of the published arguments concerning morphogenesis. In particular, I have shown the cell cortex to be far more active in organizing its own morphogenesis than Kallio or Kiermayer appear willing to admit. Waddington is closer to my position in his recognition of the importance of regional specialization of cell surface, but he is at a loss as to the mechanism responsible. I will admit that I have not come to grips with the exact mechanisms, but I have suggested the form that an explanation might take, using by example a relatively plausible argument stated in terms of cell wall microstructure and synthetic dynamics. I have broken morphgenesis of the whole cell into manageable parts, analysed the parts and shown that they can be reassembled. And again, I have tried to state my arguments as I feel an explanation of morphogenesis must be stated. I have avoided discussing "causes" of morphogenesis, for example; I have not asked whether turgor causes morphogenesis or organization causes tip growth. I now want to examine whether my arguments are really appropriate and especially whether we should expect that better explanations exist.

In preparing this section I have consulted a number of works concerned with scientific explanation particularly

as regards developmental biology (Woodger, 1929; Waddington, 1934, 1962; Needham, 1934; Hempel and Oppenheim, 1948; Bonner, 1960; Picken, 1960; Blandino, 1971; Pattee, 1971; Steward, 1971; Rosen, 1970, 1972). Each author deals with the problem in a different way, the most useful I found to be the machine analogy used by Needham (1934). If appropriately developed, this one analogy can embrace all the other arguments I collected.

Therefore let us take a machine, a Swiss watch for example, and try to explain its functioning. If live and mechanical systems have the similarities suggested by Descartes, or more recently by Loeb (Blandino, 1971), then a satisfactory explanation of the watch will suggest how we must best explain biological systems. We would probably want to first take the watch apart and examine its parts, perhaps listing a certain number; springs, gears, shafts and so on, and diagramming the way they fit together. Knowing the important parts and the manner of fitting together, we could describe all the mechanical actions which take place when the watch functions. This gives us an analytic explanation, but the watch is not yet explained as well as it could if we <u>also</u> knew something of the principles of mechanical motion. Knowing about momentum, moments of inertia or simple harmonic motion adds a dimension to the explanation which does not exist in a simple description of the parts and the structural relations between them. What we add to the analytic description is a group of constraints (Pattee, 1971). In naming constraints, inertia and harmonic motion are examples, we are building a nonanalytic

language in which names refer to concepts, though each concept may be associated with its own constellation of specific physical experiments. Without constraint language, a decent explanation of almost anything would become impossibly cumbersome and generalization would be very difficult.

Now an enzyme is a chemical machine of sorts, and enzyme chemists are close to a complete analytic description of their machine. Yet they are not satisfied that enzyme action has been explained. A gap exists; concepts such as active site, microscopic reversibility, entropy, even organization, cannot yet be replaced by analytic description. Some concepts may be discarded or modified when a complete analytic description emerges, because they will then be unnecessary or misleading. But some of the concepts will remain in their abstract form. To deny this is to suggest that, knowing how the Swiss watch works, one need no longer bother about inertia or harmonic motion.

The need for constraint language does not reflect the reality that we wish to explain so much as it does our own intellectual demands. Man cannot systematize nor use his knowledge and cannot learn without the use of abstractions, and abstractions are found only in constraint language. Physicists and chemists cannot get along without such a language and we cannot <u>a priori</u> expect biologists to be any different. A science has the job, not only of analysing its particular machines into their parts, but of sorting through and refining the collection of words that make up its own language, particularly as regards the more abstract parts of that language. To

have the Swiss watch explained only in terms of constraints such as inertia and harmonic motion may be unsatisfying, but this does not mean that the terms themselves are useless or old-fashioned.

> "Things are what they are; and it is useless to disguise the fact that 'what things are' is often very difficult for our intellects to follow." (Whitehead, 1920; pg. 119)

With Micrasterias, all that I observed concerning morphogenesis could have been described using terms borrowed from embryology, all of them essentially constraint terms. Thus the septum has its morphogenetic potential progressively delimited as regions within the semicell field are determined as presumptive polar or wing lobe. The original field is self-organizing and can regulate as one would expect. Subsidiary fields are also self-organizing and may retain the character of the original field (as in the polar lobe) or may not (as in the wings). This description is quite appropriate, but for the purposes of this thesis I agree with Waddington (1962):

> "As all operationally defined terms, they (embryological terms) are useful for describing the results of experiments, but are feeble guides, or perhaps even deceptive ones, to the nature of the underlying elements whose properties bring about the processes which the experiments discovered."

I have found it far more useful to introduce terms such as wall organization and singularity, and to add as a corollary that they show field-like behavior. In a similar fashion I have tried to avoid the term cortical information, though clearly the Micrasterias cell wall and plasma membrane make up a cell

cortex which I have shown to contain information. A biochemist, now, might object to singularity and wall organization as abstractions so fuzzy as to be meaningless, and claim that a biochemical explanation is to be preferred. But biochemistry, even in its study of relations between molecular structures rather than just structures themselves is a discipline heavily committed to analysis and analytic explanations. Analysis in the absence of a carefully framed constraint language will not by itself give the best explanation of Micrasterias morphogenesis, just as it does not do so for enzyme activity or Swiss watches.

The problem of explanation is more precisely stated in the language of systems theory. If we want to work out the dynamics of systems behavior, particular attention must be paid to finding the state variables appropriate to interactions within the system (Rosen, 1970, 1972). Systems theory is especially forceful on this point; the state variables which present themselves to us as most obvious and measurable may not be the ones necessary for describing system interactions. Therefore, there are theoretical reasons for doubting that analytically measureable variables are important in themselves. Rosen (1972) comments that the best state variables are frequently constructed from combinations of the observable variables. These combinations may be rather abstract in appearance and resemble closely the constraints of Pattee's argument.

It would be absurd to suggest that analytic technique will not eventually provide a full description of tip growth

and cell morphogenesis. However, there are good theoretical reasons for doubting that this full analytic description will be chosen as the preferred explanation.

Literature Cited

Bartnicki-Garcia,S. 1972. personal communication.

Bartnicki-Garcia,S. and E.Lippman 1969. Fungal morphogenesis: Cell wall construction Mucor rouxii. Science,165:302-303.

Benes,K. 1968. On the stainability of plant cell walls with alcian blue. Biol.Plant.,10:334-346.

Blandino,G. 1969. "Theories on the Nature of Life," New York, Philosophical Library.

Bloom,A.L. 1968. "Gas Lasers," New York, John Wiley & Sons.

Bonner,J.T. 1960. The unsolved problem of development: An appraisal of where we stand. Am.Scientist,48:515-527.

Bonnett,H.T.,Jr. and E.H.Newcomb 1966. Coated vesicles and other cytoplasmic components of growing root hairs of radish. Protoplasma,62:59-75.

Brown,R.J.,Jr.,W.W.Franke,H.Kleinig,H.Falk and P.Sitte 1970. Scale formation in chrysophycean algae I. Cellulosic and noncellulosic wall components made by the golgi apparatus. J.Cell Biol.,45:246-271.

Castle,E.S. 1953. Problems of oriented growth and structure in Phycomyces. Quart.Rev. Biol.,28:364-372.

Chapman-Andresen,C. 1972. Membrane activity in freshwater amebae. J.Protozool.,19:225-231.

Cleland,R. 1971. Cell wall extension. Ann.Rev.Plant Physiol., 22:197-222.

Crick,F.H.C. and A.F.W.Hughes 1950. The physical properties of cytoplasm. A study by means of the magnetic particle method II. Theoretical. Ex.Cell Res.,1:505-533.

Drawert,H. and I.Metzner-Küster 1961. Licht- und elektronenmikroskopische Untersuchungen an Desmidisceen I. Zellwand- und Gallertstrukturen bei einigen Arten. Planta,56:213-228.

Drawert,H. and M.Mix 1961a. Licht- und elektronenmikroskopische Untersuchungen an Desmidisceen II. Hüllgallerte und Schleimbildung bei Micrasterias,Pleurotaenium und Hyalotheca. Planta,56:237-261.

_____ 1961b. III. Der Nucleolus im Interphasenkern von Micrasterias rotata. Flora,150:185-195.

_____ 1961c. IV. Beiträge sur elektronenmikroskopischen Struktur des Interphasenkernes von Micrasterias rotata. Z.Naturforsch.,166:546-551.

_____ 1961d. V. Uber die Variabilitat der Chloroplastenstruktur bei Micrasterias rotata. Planta,56:648-665.

_____ 1961e. VII. Der Golgi-Apparat von Micrasterias rotata nach Fixierung mit Kalimpermanganat und Osmiumtetroxyd. Mikroskopie,16:207-212.

_____ 1961f. VIII. Die Chondriosomen von Micrasterias rotata. Flora,151:487-508.

_____ 1962a. Die Struktur der Pyrenoide von Micrasterias rotata. Planta,58:50-74.

_____ 1962b. X. Bieträge zur Kenntnis der "Häutung" von Desmidiaceen. Arch.Mikrobiol.,42:96-109.

Esau,K. 1967. "Plant Anatomy," New York, John Wiley & Sons.

Fowke,L.C. and J.D.Pickett-Heaps 1969. Cell division in Spirogyra II. Cytokinesis. J.Phycol.,5:273-281.

Franke,W.W.,W.Herth,W.J.Van Der Woude and D.J.Morre 1972. Tubular and filamentous structures in pollen tubes: Possible involvement as guide elements in protoplasmic streaming and vectorial migration of secretory vesicles. Planta,105:317-334.

Fritsch,F.E. 1935. "The Structure and Reproduction of the Algae," London, Cambridge Univ.Press.

Galun,E. 1972. Morphogenesis of Trichoderma: Autoradiography of intact colonies labeled by (^{3}H)N-acetyglucosamine as a marker of new cell wall biosynthesis. Arch.Mikrobiol., 86:305-314.

Gerrath,J.F. 1968. Studies on the ultrastructure of desmids and its relation to their taxonomy. PhD thesis,Univ. of British Columbia.

Green,P.B. 1962. Mechanism for plant cell morphogenesis. Science,138:1404-1405.

_____ 1969. Cell morphogenesis. Ann.Rev.Plant Physiol., 20:365-394.

Green,P.B. and A.King 1966. A mechanism for the origin of specifically oriented textures in development with special reference to Nitella wall texture. Aust.J.Biol.Sci., 19:421-437.

Grove,S.N. and C.E.Bracker 1970. Protoplasmic organization of hyphal tips among fungi: Vesicles and Spitzenkorper. J.Bact.,104:989-1009.

Grove,S.N.,C.E.Bracker and D.J.Morre 1970. An ultrastructural basis for hyphal tip growth in Phthium ultimum. Am.J.Bot., 57:245-266.

Heimans,J. 1942. Triquetrous forms in the genus Micrasterias Blumea,suppl. 2:52-63.

Hempel,C.G. and P.Oppenheim 1948. Studies in the logic of explanation. Phil.Science,15:135-175.

Houwink,A.L. and P.A.Roelofsen 1954. Fibrillar architecture of growing plant cell walls. Acta Bot.Neerl.,3:385-395.

Jarosch,R. 1962. Die Golgiapparate lebender Pflanzenzellen in Lichtmikroskop. Protoplasma,55:406-410.

Jensen,W.A. 1962. "Botanical Histochemistry," San Francisco, W.H.Freeman.

Johnson,U.G. and K.R.Porter 1968. Fine structure of cell division in Chlamydomonas reinhardi. J.Cell Biol.,38:403-425.

Kallio,P. 1951. The significance of nuclear quantity in the genus Micrasterias. Ann.Bot.Soc.Zool.-bot.fenn.Vanamo, 24(2):1-122.

_____ 1954. Morphogenetic studies in Micrasterias rotata (Grev) Ralfs var. evoluta Turner (Desmidiaceae). Arch. Soc.Zool.-bot.fenn.Vanamo,8:118-122.

_____ 1957. Studies on artificially produced diploid forms of some Micrasterias species (Desmidiaceae). Arch.Soc. Zool.-bot.fenn.Vanamo,11:193-203.

_____ 1960. Morphogenetics of Micrasterias americana in clone culture. Nature,187:164-166.

_____ 1963. The effects of ultraviolet radiation and some chemicals on morphogenesis in Micrasterias. Ann.Acad.Sci. fenn.Ser.A IV,70:1-39.

_____ 1968. On the morphogenetic system of Micrasterias sol. Ann.Acad.Sci.fenn.Ser.A IV,124:1-23.

_____ 1972a. personal communication.

_____ 1972b.article in preparation.

Kallio,P. and H.Heikkilä 1969. UV-unduced facies change in Micrasterias torreyi. Osterr.Bot.Z.,116:226-243.

Kertesz,Z.I. 1951. Pectic enzymes. In "The Enzymes," J.B. Sumner and K.Myrback,eds. New York,Academic Press. 1:745-768.

Kiermayer,O. 1964. Untersuchungen über die Morphogenese und Zellwandbildung bie Micrasterias denticulata Bréb. Protoplasma,59:76-132.

_____ 1967. Das Septum-Initialmuster von Micrasterias denticulata und seine Bildung. Protoplasma,64:481-484.

_____ 1968a. Hmmung der Kern- und Chloroplastenmigration von Micrasterias durch Colchizin. Naturwissenschaften, 55:299-300.

_____ 1968b. The distribution of microtubules in differentiating cells of Micrasterias denticulata Bréb. Planta,83:223-236.

_____ 1970a. Elektronenmikroskopische Untersuchungen zum Problem der Cytomorphogenese von Micrasterias denticulata Bréb. I.Allgemeiner überblick. Protoplasma, 69:97-132.

_____ 1970b. Causal aspects of cytomorphogenesis in Micrasterias. Ann.N.Y.Acad.Sci.,175:686-701.

Kiermayer,O. and R.Jarosch 1962. Die Formbildung von Micrasterias rotata Ralfs und ihre experimentelle Beeinflussung. Protoplasma,54:382-420.

Kiermayer,O. and L.A.Staehelin 1972. Feinstruktur von Zellwand und Plasmamembran bei Micrasterias denticulata Bréb. nach Gefrieratzung. Protoplasma,74:227-237.

Kies,L. 1970. Elektronenmikroskopische Untersuchungen über Bildung und Struktur der Zygotenwand bei Micrasterias papillifera (Desmidiaceae) I.Das Exospor. Protoplasma, 70:21-47.

Klein,R.M. and A.Cronquist 1967. A consideration of the evolutionary and taxanomic significance of some biological, micromorphological, and physiological characters in the Thallophytes. Quart.Rev.Biol.,42:102-296.

Lacalli,T.C. and A.B.Acton 1972. An inexpensive laser microbeam. Trans.Amer.Mic.Soc.,91:236-238.

Lamport,D.T.A. 1970. Cell wall metabolism. Ann.Rev.Plant Physiol.,21:235-270.

Lenzenweger,R. 1966. Zellteilung bei der Zieralge Micrasterias. Mikrokosmos,55:289-293.

McClure,W.K.,D.Park and P.M.Robinson 1968. Apical organization in the somatic hyphae of fungi. J.gen.Microbiol.,50:117-182.

Mix,M. 1966. Licht- und elektronenmikroskopische Untersuchungen an Desmidiaceen XII. Zur Feinstruktur der Zellwände und Mikrofibrillen einiger Desmidiaceen vom Cosmarium-Typ. Arch.Mikrobiol.,55:116-133.

Mollenhauer,H.H. and D.J.Morre 1966a. Tubular connections between dictyosomes and forming secretory vesicles in plant golgi apparatus. J.CellBiol.,29:373-376.

_____ 1966b. Golgi apparatus and plant secretion. Ann.Rev.Plant Physiol.,17:27-46.

Moore,A.R. 1935. On the significance of cytoplasmic structure in Plasmodium. J.cell.comp.Physiol.,7:113-129.

Narahashi,Y. 1970. Pronase. In "Methods in Enzymol.," G.E. Perlmann and L.Lorand, eds. New York,Academic Press. 19:651-664.

Needham,J. 1933. On the dissociability of fundamental processes in ontogenesis. Biol.Rev.,8:180-223.

Newcomb,E.H. 1969. Plant microtubules. Ann.Rev.Plant Physiol., 20:253-288.

Northcote,D.H. and J.D.Pickett-Heaps 1966. A function of the golgi apparatus in polysaccharide synthesis and transport in the root-cap cells of wheat. Biochem.J.,98:159-167.

Oaks,A. and R.G.S.Bidwell 1970. Compartmentalization of intermediary metabolites. Ann.Rev.Plant Physiol.,21:43-66.

O'Brien,T.P. 1972. The cytology of cell-wall formation in some eukaryotic cells. Bot.Rev.,38:87-118.

Parker,B.C. and A.B.Diboll 1966. Alcian stains for histochemical localization of acid and sulfated polysaccharides in algae. Phycologia,6:37-49.

Pattee,H.H. 1970. Physical theories of biological co-ordination. Quart.Rev.Biophys.,4:225-276.

Pearse,A.G.E. 1968. "Histochemistry,Theoretical and Applied," London,J.A. Churchill.

Picken,L. 1960. "The Organization of Cells and Other Organisms," Oxford, Clarendon Press.

Pickett-Heaps,J.D. 1967. The effects of colchicine on the ultrastructure of dividing plant cells, xylem wall differentiation and distribution of cytoplasmic microtubules. Develop.Biol.,15:206-236.

_____ 1968. Further ultrastructural observations on polysaccharide localization in plant cells. J.Cell Science,3:55-64.

_____ 1969. Preprophase microtubules and stomatal differentiation; some effects of centrifugation on symmetrical and asymmetrical cell division. J.Ultra.Res., 27:24-44.

_____ 1972. Variation in mitosis and cytokinesis in plant cells and its significance in the phylogeny and evolution of ultrastructural systems. Cytobios,5:59-77.

Pickett-Heaps,J.D. and L.C.Fowke 1970a. Mitosis, cytokinesis and cell elongation in the desmid, Closterium littorale. J.Phycol.,6:189-215.

_____ 1970b. Cell division in Oedogonium III. Golgi bodies, wall structure, and wall formation in O. cardiacum. Aust.J.Biol.Sci.,23:93-113.

Pickett-Heaps,J.D. and D.H.Northcote 1966. Organization of microtubules and endoplasmic reticulum during mitosis and cytokinesis in wheat meristems. J.Cell Science,1:109-120.

Pigman,W. 1951. Cellulases, hemicellulases, and related enzymes. In "The Enzymes," J.B.Sumner and K.Myrback, eds. New York, Academic Press. 1:725-744.

Porter,K.R. 1966. Cytoplasmic microtubules and their function. In "Principles of Biomolecular Organization, Ciba Found. Symp.," G.E.W.Wolstenholme and M.O'Conner, eds. London, Churchill. pp 308-345.

Preston,R.D. 1964. Structural and mechanical aspects of plant cell walls with particular reference to synthesis and growth. In "The Formation of Wood in Forest Trees, Second Symp. Maria Moors Cabot Found.for Bot.Res.," M.H. Zimmermann, ed. New York, Academic Press. pp.169-188.

Prigogine,I. and G.Nicolis 1971. Biological order, structure and instabilities. Quart.Rev.Biophys.,4:107-148.

Reynolds,E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J.Cell Biol.,17:208-212.

Robertson,N.F. 1959. Experimental control of hyphal branching and branch form in hypomycetous fungi. J.Linn.Soc.Lond. Bot.,56:207-211.

_____ 1968. The growth process in fungi. Ann.Rev. Phytopathol., 6:15-136.

Robinson,D.G. and R.D.Preston 1972. Plasmalemma structure in relation to microfibril biosynthesis in Oocystis. Planta, 104:234-236.

Roelofsen,P.A. 1965. Ultrastructure of the wall in growing cells and its relation to the direction of growth. Adv. Bot.Res.,2:69-149.

Roelofsen,P.A. and A.L.Houwink 1953. Architecture and growth of the primary wall in some plant hairs and in the Phycomyces sporangiphore. Acta Bot.Neerl.,2:218-225.

Roggen,H.P.J.R. and R.G.Stanley 1969. Cell-wall hydrolysing enzymes in wall formation as measured by pollen-tube extension. Planta,84:295-303.

Rosen,W.G.,S.R.Gawlik,W.V.Dashek and K.A.Siegesmund 1964. Fine structure and cytochemistry of Lilium pollen tubes. Am.J.Bot.,51:61-71.

Rosen,R. 1970. "Dynamical System Theory in Biology," New York, Wiley-Interscience.

_____ 1972. On the decomposition of a dynamical system into noninteracting subsystems. Bull.Math.Biophys., 34:337-341.

Saks,N.M. 1971. Cell biology by laser light. In "Laser Applications in Medicine and Biology," M.L.Wolbarsht, ed. New York, Plenum Press.1:67-90.

Sassen,M.M.A. 1964. Fine structure of petunia pollen grain and pollen tube. Acta Bot.Neerl.,13:175-181.

Schröter,K. and A.Sievers 1971. Wirkung der Turgorreduktion auf den Golgi-Apparat und die Bildung cler Zellwand bei Wurzelhaaren. Protoplasma,72:203-211.

Schultze,B. 1969. Autoradiography at the cellular level. In "Physical Techniques in Biol.Res.," A.W.Pollister, ed. New York, Academic Press. vol. III B.

Selman,G.G. 1966. Experimental evidence for the nuclear control of differentiation in Micrasterias. J.Embryol.exp.Morph., 16:469-485.

Setterfield,G. and S.T.Bayley 1961. Structure and physiology of cell walls. Ann.Rev.Plant Physiol.,12:35-62.

Sievers,A. 1963. Beteiligung des Golgi-Apparates bei der Bildung der Zellwand von Wurzelhaaren. Protoplasma, 56:188-192.

_____ 1964. Zur Feinstrukturanalyse of pflanzlicher Zellen mit Spitzenwachstum. Ber.Dent.Bot.Ges.,77:388-390.

_____ 1967. Elektronenmikroskopische Untersuchungen Zur geotropischen Reaktion II. Die polare Organisation des plasma,64:225-253.

Spurr,A.R. 1969. A low-viscosity epoxy resin embedding medium for electron Microscopy. J.Ultra.Res.,26:31-43.

Stadelmann,E.J. and H.Kinzel 1972. Vital staining of plant cells. In "Methods in Cell Physiology," D.M.Prescott, ed. New York, Academic Press. V:325-372.

Staehelin,L.A. and O.Kiermayer 1970. Membrane differentiation in the golgi complex of Micrasterias denticulata Bréb. visualized by freeze-etching. J.Cell Science,7:787-792.

Starr,R.C. 1958. The production and inheritance of the triradiate form in Cosmarium turpinii. Am.J.Bot.,45:243-248.

_____ 1964. The culture collection of algae at Indiana University. Am.J.Bot.,51:1013-1044.

Stern,C. 1972. personal communication.

Steward,F.C. 1971. Plant physiology: The changing problems, the continuing quest. Ann.Rev.Plant Physiol.,22:1-22.

Tartar,V. 1960. Reconstitution of minced Stentor coeruleus. J.exptl.Zool.,144:187-207.

Teiling,E. 1950. Radiation of desmids, its origin and its consequences as regards taxonomy and nomenclature. Bot. Not.,299-327.

Tews,L.L. 1969. Dimorphism in Cosmarium botrytis var. depressum. J.Phycol.,5:270-271.

Tokunaga,J. and S.Bartnicki-Garcia 1971. Structure and differentiation of the cell wall of Phytophthora palmivora: Cysts, hyphae and sporangia. Arch.Mikrobiol.,79:293-310.

Tokunaga,C. and C.Stern 1965. The developmental autonomy of extra sex combs in Drosophila melanogaster. Devel.Biol., 11:50-81.

Waddington,C.H. 1934. Morphogenetic fields. Sci.Prog., 29:336-346.

_____ 1962. "New Patterns in Genetics and Development," New York, Columbia Univ. Press.

_____ 1966. Fields and gradients. In "Major Problems in Devel.Biol.,25th Symp.Soc.Devel.Biol.," M.Locke, ed. New York, Academic Press. pp.105-124.

Walsh,K.A. 1970. Trypsinogens and trypsins of various species. In "Methods in Enzymol.," G.E.Perlmann and L. Lorand, eds. New York, Academic Press,19:41-63.

Waris,H. 1950a. Cytophysiological Studies on Micrasterias I. Nuclear and cell division. Physiologia Plant.,3:1-16.

_____ 1950b. II. The cytoplasmic framework and its mutation. Physiologia Plant.,3:236-246.

_____ 1953. The significance for algae of chelating substances in the nutrient solutions. Physiologia Plant., 6:538-543.

Waris,H. and P.Kallio 1964. Morphogenesis in Micrasterias. Adv.Morphogenesis,4:45-80.

West,W. and G.S.West 1905. "British Desmidiaceae," London, Adlard & Sons for the Ray Soc., vol. II.

Whaley,W.G.,M.Dauwalder and J.E.Kephart 1966. The golgi apparatus and an early stage in cell plate formation. J.Ultra.Res.,15:169-180.

Whitehead,A.N. 1920. "The Concept of Nature," London, Cambridge Univ.Press.

Woodger,J.H. 1929. "Biological Principles," London,Kegan Paul,Trench,Trubner & Co.