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TITLE OF THESIS....Conidial development, mitotic cycle and cleistothecial Development in Powdery Mildews.

UNIVERSITY..........Alberta..........................

DEGREE FOR WHICH THESIS WAS PRESENTED....Ph. D..........

YEAR THIS DEGREE GRANTED..........1971......................

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THE UNIVERSITY OF ALBERTA

CONIDIAL DEVELOPMENT, MITOTIC CYCLE AND CLEISTOTHECIAL DEVELOPMENT IN POWDERY MILDEWS

by

RINA VARMA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

SPRING, 1971

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled 'Conidial Development, Mitotic Cycle and Cleistothecial Development in Powdery Mildews' submitted by Rina Varma in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The conidia of *Erysiphe graminis* DC. develop in chains as the result of the activity of a bulbous generative cell or of a cell produced by it. In such chains of conidia, the oldest conidium is the one farthest from the generative cell. The sequence of nuclear events during mitosis was studied, the interpretation of the sequences being related to the age of each conidium.

Nuclear division in this fungus is different from that found in flowering plants and many other organisms. The principal distinctive division phase in *E. graminis* is that at metaphase the nuclei appear as beaded linear strands which split longitudinally during anaphase.

Cleistothecial development in *Uncinula necator* (Schw.) Burr. and *U. salicis* (DC.) Wint. is characterized by the early appearance of antheridia and ascogonia. No passage of nuclei from antheridia and ascogonia was observed. Asci developed from the cells of the ascogonium at the multicellular stage of the latter but no clue was found as to the origin of the nuclei that pair in the ascus.

The cleistothecial centrum developed through the mediation of periclinal divisions of the antheridial cells.

Hyphal outgrowths from the centrum seemed to serve for anchorage of the cleistothecium rather than as nuclear acceptors as has been suggested.

ACKNOWLEDGEMENTS

The author is deeply indebted to Dr. H. J. Brodie for his guidance and encouragement during the entire course of this investigation. The author also takes this opportunity to thank Dr. L. L. Kennedy and Dr. E. A. Cossins for their interest in the present study. Sincere appreciation is extended to Mrs. Betty Ford for her care and patience in typing this thesis.

Financial support from the University of Alberta and National Research Council (from grant to Dr. H. J. Brodie) are acknowledged.

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INTRODUCTION

This investigation is intended to give information chiefly on two distinct, although related, aspects of the development and morphology of some fungi belonging to the order Erysiphales; the first problem considered concerns nuclear development and the somatic mitotic cycle; the second concerns the manner of development and maturation of the sexual stage, the cleistocarp.

Although interpretation of nuclear behavior is always fraught with pitfalls, the interpretation of serial sections necessary for the study of morphogenesis of cleistothecia is even more so. Every effort has been made to be objective but it is realized that other interpretations are possible for the facts presented herein.

Regarding the sequence of nuclear events, at least some of the Erysiphales present a unique opportunity for objectivity. In *Erysiphe graminis* for example, conidia are formed by the activity of a single basal generative cell. The age sequence in a chain of conidia is therefore acropetal, and the various nuclear conditions observed in a chain of conidia can literally be taken as sequential.

No such aid to objectivity exists when serial sections of young cleistothecia must be used to try to discover the sequence of developmental events. Spatial relations between hyphae tend to be lost, and the continuity of hyphae must be partly conjectural.

For obvious reasons, the number of species examined in the present study is limited. It could be unsafe, therefore, to presume that all members of the Erysiphales will conform to the pattern exhibited by the species studied.

Despite these difficulties, a fairly clear and logical picture seems to have emerged as to the form and behavior of somatic nuclei and as to the progress of cleistothecial development. It is hoped that this contribution may help to throw light on the rather conflicting reports that exist in the literature regarding the cytology and the morphogenesis of an important group of obligate parasitic fungi, the Erysiphales.

It may be best at this point to present a brief account of the Erysiphales in order that the review which follows may be considered against a common background.

The Erysiphales (Class Ascomycetes) comprise about six genera and 60-185 species (Yarwood, 1957). All are obligate parasites and all produce the bulk of their mycelium on the surface of their host platns. The mycelium is course, mostly colourless, strongly septate with perforated septa and the mycelium usually has a single nucleus in each cell. Host epidermal cells are penetrated by haustoria which may be bulbous or digitate (Yarwood, 1957). Under favorable light, nuclei can sometimes be seen in the living condition. Stained interphase nuclei are conspicuous and usually appear to be about half the hyphal diameter, that is the nuclei are approximately 2.5μ in diameter.

Conidia are mostly the shape of an oblate spheroid, uninucleate and papillate. They may be abstricted singly as in *Erysiphe polygoni* or remain in chains as in *Erysiphe graminis* (Brodie, 1942). Conidia of many species have been shown to be capable of germinating at low relative humidity (Brodie, 1942a).

Cleistothecia are usually formed in the late summer and autumn in temperate climates but are rarely formed in the tropics. Fruit bodies are tan to brown or black when mature, and are provided with branched or unbranched appendages. The form of the appendages and the number of asci per cleistothecium are the chief bases for determination of the genera. Ascospores are discharged by rupture of the cleistothecia, mostly in the spring in temperate zones.

REVIEW OF LITERATURE

A. Somatic Nuclear Division

Many excellent reviews concerning fungal cytology are available dating from the 1930's to the present (Cutter, 1951; Martens, 1946; Namboodiri, 1964). However, scant interest in nuclear division and cytology of the somatic hyphae during this period is evident. Of the available reviews, Martens (1946) presented a monumental review of the research concerning life cycles and sexuality throughout the Ascomycetes from as far back as the late 19th century.

Pinto-Lopes (1949) in a comprehensive review of the nuclear structure of fungi points out that, because of the smallness of the nucleus, investigators have used techniques which tended to favor the mere detection of fungal nuclei at the expense of the nuclear detail. Pinto-Lopes (1949) using Feulgen stain methods, found that the structure and composition of the nuclei of fungi apparently is not the same as in higher plants. Three types of fungal nuclei were described: homogeneous, disperse- and thread-nuclei.

Olive (1953), in his review, discussed Wakayama's Karyosome (1931) and stated that "this type of structure is frequently attributed to vegetative nuclei throughout the fungi". Olive (1953) believed the so-called Karyosome to be due to improper fixation and staining. He concluded that Wakayama's (1931) occasional observations of a nuclear reticulum associated with chromosomal formation was evidence

that "a few of the resting nuclei were properly fixed".

Smith (1923), visualized a 'direct division' of the somatic nucleus of Saprolegnia. A nuclear membrane was reported to be present and to remain intact throughout the division. The nucleus became increasingly dumbbell-shaped as the chromoblast (stained part of the nucleus) pulled apart until the membrane constricted in the middle and the daughter nuclei separated. Varitchak (1931) diagrammed a different form of non-mitotic nuclear division in the vegetative nucleus of Ascoidea rubesens showing chromosomes (n-2) attached end-to-end and a centrosome attached to one of the chromosomes constituting a chain of three bodies. Division began at the centrosome and continued along the chain as far as the end chromosomes, which remained attached, so that a linear chain of five bodies was formed. This was acutely angular at first but later extended into a straight line. At this point, the end chromosomes separated and moved apart. The daughter nuclei resumed a spherical interphase shape with the centrosome remaining outside and attached by a thread to the sphere.

Robinow (1956) reviewed non-mitotic nuclear division in various taxonomically "lower" forms of life including fungi. Citing the work of Smith (1923) and Robinow's (1956) own observations, he mentioned that somatic nuclei of fungi divide by "direct division". It was postulated that evolution from unicellular to multicellular forms of life was correlated with a parallel evolution sequence in forms of nuclear division. Three types of division were outlined:

1) mitosis; 2) endomitosis and 3) amitosis. Mitosis was defined as nuclear division associated with well-defined chromosomes aligned on a fibrous spindle which may or may not be anchored in a centriole. The most important criterion, however, was a recognizable resting and dividing state of the chromosomes. Endomitosis was defined as division of the chromosomes, without the aid of a spindle apparatus. This latter type of chromosome separation (as observed in higher plants and animals) is usually not followed by division of the nucleus itself and hence gives rise to varying degrees of polyploidy. Amitosis was defined as division of a nucleus without the emergence of definable chromosomes.

Olive (1953), in his review of fungal cytology, maintained that division in the somatic hyphae of fungi is similar to that of higher plants.

The publication of these concepts of amitotic nuclear division in fungi initiated a controversy among fungal cytologists. Many schools of interpretation have subsequently arisen either rejecting or supporting the concept of 'classical' mitosis in the somatic nuclei of fungi.

Bakerspigel (1959), using HCl-Giemsa stain, described somatic nuclei as being "spherical to oval in shape and are composed of granular chromatin which envelopes unstained areas, the sites of the Feulgen-negative central bodies". In a later publication, Bakerspigel (1962) defined the central body as a **nucleolus.** At division, the chromatin becomes increasingly granular and forms into a "complex of chromosomal filaments in various arrangements".

This complex somehow takes the shape of an "irregular crescent mass" around the central body (nucleolus). The central body stretches into a dumbbell-like body, pushing the ends of the crescent-shaped chromatin apart. When the central body reaches its maximum length the mid region constricts, cutting off the two daughter nuclei. A nuclear membrane was not observed.

A complete review of other fungal species (mainly Phycomycetes) studied by Robinow, Bakerspigel and co-workers appears in a paper by Robinow and Bakerspigel (1965). In most of the fungi investigated, somatic nuclear division was found to be quite similar to the direct division described by Smith (1923) and Bakerspigel (1959). The one exception was found in Basidiobolus ranarum (Robinow, 1965). In this zygomycete, a large barrel-shaped spindle is observed at mitosis and division is considered to be classically mitotic.

Somers et. al. (1960) fixed Neurospora hyphae in acetic acid:alcohol, (1:3) and stained with an HCl-acetic-orcein method. Spindle fibers were not discussed although all the classical mitotic nuclear figures were described, with the exception that, (at telophase) "during the stage of despiralization of the chromosomes the daughter groups assume a much elongated, instead of the regular roundish shape". Also in one-fourth of the observations, an unexplained "tiny, eighth" chromosome was noted. Other non-mitotic nuclear figures were discounted as "disintergrating nuclear material". Nucleoli were seen arising at telophase and, at late telophase, the nucleolus was located centrally with a nuclear

ring surrounding it. Somers et. al. (1960) concluded that their observations differ from those of Bakerspigel (1959) only in interpretation, for "the division figures recorded in Bakerspigel's photographs are similar to those presented in this report".

Ward and Ciurysek (1962) used HCl-Giemsa stain. Although somatic nuclear division was described as mitotic a centriole was seen to be closely associated with the resting nucleus. Non-mitotic nuclear configurations were considered to be degenerating nuclei as noted by Somers et. al. (1960). Although Ward and Ciurysek (1962) did not observe well-defined spindle-fibers, they stated that "many configurations were seen which suggested the presence of a spindle and the separation of the daughter chromosomes to spindle poles".

Weijer and co-workers (1965) have developed a new concept of somatic nuclear division (Karyokinesis) in Neurospora crassa. This work was originally based on the observations of Varitchak (1931) of a linear nucleus and centriole in Ascoidia rubescens. McGinnis (1953, 1956) observed in Puccinia graminis (a basidiomycete) linear nuclear configurations which he called "spiremes".

Three distinct karyokinetic cycles were observed in Neurospora: (1) Juvenile cycle in young undifferentiated hyphae, (2) maturation I, leading to the formation of macro-conidia, (3) maturation II, leading to the formation of micro-conidia.

In the Juvenile cycle, karyokinesis proceeds from a

globular interphase nucleus to which a triangular plate-shaped centriole is attached by a weakly Feulgin-positive thread. The interphase-nucleus gradually becomes ring-shaped, composed of seven particulate chromosome bodies plus the deeply stained centriole. The chromosome bodies appear to be attached to one another by a weakly Feulgen-positive thread. At division the ring breaks, forming a double-stranded beaded nucleus which divides longitudinally.

Strongly Feulgen-positive centrioles were attached to the nuclear ring or to one end of the nuclear filament by a weakly Feulgen-positive thread. A centriole was seen in the *Neurospora* ascus by both Dodge (1927) and Singleton (1953) and the presence of DNA in this body has recently been established (McDonald and Weijer, 1965).

The concept (Dowding and Weijer, 1962) based upon the chromosomes existing in an end-to-end attachment and the longitudinal division of this linear nucleus, has been very unacceptable to many authors, Somers *et*. *al*. (1960); Ward and Ciurysek (1962). These latter authors have described linear nuclei as "migrating nuclei" and other non-mitotic figures as "degenerating", or "autolysing" nuclei without attempting to demonstrate that hyphal nuclei do degenerate at any particular stage. Their own observations of classical mitosis in *Neurospora* have excluded demonstration of a well-defined spindle apparatus, such as was suggested as a criterion for mitosis by Robinow and Bakerspigel (1965).

Wilson *et*. *al*. (1966) described, in *N*. *crassa*, hyphal chromosomes as being "associated linearly into a bar-shaped

metaphase plate". Anaphase occurs when the chromosomes within the metaphase are seen only between "chromatids or groups of chromatids which have already separated". Division occurs within an "enlarged envelope". After division, new membranes are formed around the daughter nuclei and these migrate out of the old membrane, which disintegrates.

In a later publication, however, Aist and Wilson (1968) remark that their earlier interpretation of vegetative nuclear division in fungi was confusing. In this latter publication, observations presented in earlier papers are reinterpreted, although no alternative conclusions are offered. It is suggested that overstaining may explain the "taffy-pull" and "amitotic figures" observed by workers. It was also concluded that, "some of our figures illustrated as telophase may have been caused by fracture of nuclei during fixation and staining". The difficulty encountered in defining the nuclear envelope and thus distinguishing nuclei and chromatin is also discussed. In their work, the existence of a centriole is indicated, together with the occurrence of a spindle-like apparatus situated between daughter nuclei which are enclosed in a common nuclear envelop.

Bianchi and Turian (1967) fixed Neurospora material in acetic acid, chloroform and ethanol (1:3:6), hydrolysed in perchloric acid and stained in buffered Giemsa. In active, rapidly growing parts of the hyphae and in hyphae constricting to form conidia, it was observed that "the

dividing nuclei are often forced out and may display an elongated appearance". However, it was also noted that: "In older hyphae where movement of the cytoplasm is not as rapid, and in large hyphae, the distortion of nucleus is not as pronounced and a more typical mitosis is observed".

Bianchi and Turian (1967), therefore, concluded that for certain developmental stages of Neurospora, a facultative mechanism of longitudinal division of filamentous nuclei as advanced by Dowding (1966) and Weijer et. al. (1965) must therefore be considered.

Namboodiri and Lowry (1967) state that in the somatic nuclei in Neurospora the elongated nucleus is a "distinct nuclear form and not just a stage in division", and that the division of these elongated nuclei and rounded (ring) nuclei are not sequences in the same cycle but "follow independent, though parallel, pathways".

Hall (1963) investigated the cytology of the asexual stage of Monilia fructicola. Although elongated nuclei were noted in the hyphae, Hall described the division of the vegetative nuclei as classically mitotic.

Moor (1964) in an electron microscopic study of Cordyceps militaris proposed a new non-mitotic mode of somatic nuclear division called karyochorisis. The main feature of karyochorisis (or nuclear sundrance) is that, during division, the nuclear membrane remains intact and invaginates at division to separate the daughter nuclei. At first, the inner

membrane invaginates producing two daughter "karyomes". This is followed by invagination of the outer membrane along the same axis, completely separating the karyomes to give rise to daughter nuclei.

Duncan and MacDonald (1965) examined somatic nuclear division in the basidiomycete Marasmius using Feulgen and Aceto-orcein staining methods. The authors maintain that the "individual beads on the strands are not individual chromosomes, as thought by other workers, because they are greater in number than the chromosome complement or even more numerous than twice the complement".

Yomasaki and Niizeki (1965) studied the hyphal nuclear cytology of Piricularia oryzae (Moniliales, Ainsworth and Bisby, 1945). It was found that nuclear division in germinating conidia appeared to be typically mitotic. Yamasaki and Niizeki (1965) stated however, that "detail of the nuclear division in the mycelium is still obscure. It is not considered to be that of a typical mitosis." In the explanation of their photographs (Plate 1-D) it is stated that "chromatin masses connected in irregular rosary-form split longitudinally and separate into two groups".

Knox-Davies (1967) investigated the cytology of the vegetative hyphae of Macrophomina phaseoli with HCl-Giemsa and orcein staining methods. They described the division in nuclei of the vegetative hyphae of this member of the Deuteromycetes as being mitotic. No centrioles or spindles were resolved although all mitotic stages were described.

Hejtmankora-Uhrova and Hejtmanek (1967) and Hejtmanek

et. *al*. (1967) investigated the division of the hyphal nuclei of several *Microsporum* species (*Nannizzia* - perfect stage), a dermatophytic member of the Ascomycetes. They observed the same beaded structure described by other authors, and the centriole is not discussed.

Heale *et*. *al*. (1968) investigated nuclear division in conidia and hyphae of *Verticillium albo-atrum*. Linear nuclear configurations were observed. However, the nucleolus was described as disintergrating before nuclear division and daughter nucleoli arise *de novo* after division of the nucleus. A fiber (demonstrated with Acid Fuchsin) was believed to control the spatial distribution of the chromosomes, but whether or not the chromosomes were attached to this fiber was not determined.

B. Diurnal Periodicity

Diurnal periodicity in spore development and release occurs in many species of Erysiphaceae. Daytime release is of general occurrence (Yarwood, 1957), but night time release has also been reported (Massee, 1905). Hammarlund (1925) did not find diurnal periodicity in *Erysiphe polygoni* DC., but other workers have reported periodicity, with mid-day peaks (Childs, 1940; Yarwood, 1936). In *E*. *cichoracearum* DC., diurnal periodicity has been reported by Childs (1940) the release of conidia taking place from 10 A.M. - 2 P.M., Schnathorst (1959) from noon-4 P.M.; and by Cole (1966) 1-3 P.M. Such a diurnal cycle of development and spore release in *E*. *graminis* DC. has not been clearly demonstrated.

Some workers have failed to find any evidence of a cyclic pattern (Cherwick, 1944; Yarwood, 1936), but others recorded maximum spore release in the afternoon (Hirst, 1953; Last, 1955). Nair et. al. (1965) studied E. graminis in a growth chamber and found no evidence of a pattern of periodic spore release in that species. Pady et. al. (1969) found that in E. polygoni one spore was produced in 24 hours, which was released 2 to 5 hours after the onset of the light period to which the fungus was exposed. Matured spores were produced continuously in chains, through both the dark and light period, but were released only in the light. According to Pady et. al. (1969) this species has periodicity only in spore dispersal. Pady et. al. (1969) however did not observe any diurnal periodicity either in both spore maturation or release.

C. Development of Cleistothecia

Various accounts are available of the development of cleistothecia of the so called 'pyrenomycetes'.

A considerable amount of research has been conducted on the cleistothecia of the Erysiphales. Detailed descriptions concerning heterothallism, sexuality, the function of the ascogonium and antheridium, apogamy, ontogeny of the centrum, and formation of asci are given by various authors. Some references to nuclear division and nuclear passage are also given. Yet the morphogenesis of cleistothecia is as uncertain as ever. From the days of early work to the modern day, investigators have been giving controversial accounts of

cleitothecial development of various members of the Erysiphales.

A few of these investigations will be reviewed.

1. Heterothallism

Homma (1933) was the first to report on sexuality in Sphaerotheca fuliginea (Schlecht). This species was found to be homothallic.

In Erysiphe graminis both homothallism (Cherewick, 1944) and heterothallism (Powers and Mosman, 1956) have been reported. The report that Erysiphe cichoracearum is heterothallic (Yarwood, 1934) rests mainly on field observations as to the position of the cleistothecia in the fungal colonies. They are found only at the intersection of two neighboring colonies. This observation has been supported experimentally by data from single spore culture.

2. Structure and function of sex organs

Homma (1937) described the ascogonia and antheridia as specialized hyphae formed in the center of the fungal mat at right angles to the horizontal body of mycelium. The antheridium is smaller than the ascogonium and each structure contains one nucleus. Both hyphae grow side by side or coil around each other. The antheridial nucleus divides once and one daughter nucleus migrates into the ascogonium through a small pore. The antheridium then withers and disappears. At the time when two layers of the outer cleistothecial wall are developed, the male and female nuclei in the ascogonium conjugate.

de Bary (1887), Bezzsonof (1914), Blackman and

Fraser (1905), Harper (1895, 1896, 1905) all agree with Homma's description.

In a broad range of Ascomycetes, the occurrence of well defined antheridia and ascogonia has been recorded (Baker, 1903; Betts, 1926; Claussen, 1905, 1906, 1907; Dodge, 1920; Elliott, 1925; Gwynne-Vaughan, 1931, 1932, 1934; Harper, 1896, 1900, 1910; Schweizer, 1931; Varitchak, 1933). Antheridia and ascogonia differ in form in different species but are fairly uniform for a given species.

Winge (1911), reported that, in the powdery mildew Sphaerotheca no antheridial nuclei enter the ascogonium. The latter nevertheless develops further and a normal ascocarp forms. Dangeard (1907) took the extreme stand that, while antheridia and ascogonia are of general occurrence in Ascomycetes, it is only in a few of the most primitive that one or more nuclei pass from the antheridium to the ascogonium. In all others, cleistothecial development is apogamous.

It was believed that the passage of a nucleus (or more than one when antheridia are multinucleate) from the antheridium to the ascogonium was immediately followed by nuclear fusion (Blackman and Fraser, 1905; Claussen, 1905, 1906; Harper, 1895, 1896, 1900, 1905).

The puzzling fact was often discussed, however, that there is also a nuclear fusion in the ascus. The existence of two nuclear fusions in one life cycle demanded an explanation. In 1907, Claussen discovered that in Pyronema confluens Tul. the male nuclei entering the

ascogonium pair with the female nuclei but do not fuse with them, that this pairing is followed by the formation of ascogenous hyphae having paired nuclei and that these give rise to binucleate asci in which finally nuclear fusion takes place.

Although later writers still believed in the existence of nuclear fusions in both ascogonia and the ascus (Gwynne-Vaughan, 1934; Harper, 1910) the majority believed that in the Ascomycetes, as in the Basidiomycetes, the initial cell fusion is not accompanied by nuclear fusion but leads into a more or less prolonged binucleate phase of development with conjugate division of paired nuclei terminated in the ascus or basidium by nuclear fusion.

Ruth Allen (1936) believed that in _Erysiphe_ _polygoni_ any two young branches may twist around each other and initiate cleistothecium formation, although a difference may exist between the two; one may be shorter, thicker and less twisted than the other. Quite often more than one slender hypha twists around one thick short branch.

The cells of the sexual branches fuse and a nucleus from the male cell migrates into the tip cell of the female branch.

In _Phyllactinia_ _corylea_ Lev., as reported by Barbara Colson (1938), definite sexual branches, antheridial and ascogonial, are formed perpendicular to the horizontal mycelium. Every cleistothecium begins with the development of such a pair of sexual branches. The female branch is stouter than the male and more curved.

Each sexual branch soon becomes cut off from its parent hypha by a transverse wall. The ascogonial and the antheridial branch both divide into two, giving rise to an upper ascogonial cell and an antheridium, respectively, and a lower stalk cell for each. The ascogonium is much stouter than the antheridium and has a bluntly rounded tip across which the antheridium lies.

Gordon (1966) reported that 'pseudoantheridia' and 'pseudoascogonia' arise from separate hyphae. Migration of a nucleus from the pseudoantheridium to the pseudoascogonium takes place, followed by nuclear fusion in the pseudoascogonium. The pseudoascogonium then undergoes cell division, resulting in a multicellular structure. No further development of the pseudoascogonium was reported by Gordon.

3. Formation of sheath and centrum

Luttrell (1951) believes that variation in the ontogeny of centrum, along with variation in ascus structure, are the most important criteria for classification of Pyrenomycetes.

Homma (1937) believed that the primary cleistothecial wall cells are formed from the twisted hyphal cells arising from the stalk cell of the ascogonium. The secondary cleistothecial wall cells develop between the ascogonium and the primary cleistothecial wall. The outer two wall layers of the cleistothecium are composed of larger cells whose walls gradually turn brown. The inner two or three layers make up the inner wall whose cells are hyaline and binucleate.

In young cleistothecia, many writers reported that no

well-defined ascogenous hyphae are seen. There is a central "fertile area" composed of multinucleate cells. For example, in Neurospora, Colson (1934) reported that "cross walls are rare and difficult to see". From the central fertile area asci arise. Harper (1895), Winge (1911) and Homma (1934) have illustrated cells with more than one nucleus in vegetative parts of the cleistothecium of different species of Sphaerotheca.

According to Ruth Allen (1936), in Erysiphe polygoni, fertilization is followed by growth in which the septa remain incomplete for a considerable period, so that a large number of the cells of the cleistothecium are in open communication with one another for a time, and many cells in all parts of the cleistothecium contain more than one nucleus. There is no limited set of ascogenous hyphae embedded in sterile tissue; much of the cleistothecium including even part of its peripheral layer, is sporophytic in character.

If the irregular number of nuclei in the cleistothecial maze is the result of the migration of surplus nuclei from the mycelium, then the nuclear content of mycelial cells would be irregular. Allen's actual observations on the number of nuclei per cell in the mycelium are almost invariably one per cell (88 uninucleate; 12 binucleate).

Following the fusion of two initial cells, growth takes place with incomplete closure of the septa and through the mazes thus established, nuclei presumably both (+ and -) become widely distributed, thus diplodizing a large part of both the outer and inner layers of the cleistothecium.

Barbara Colson's (1938) description of the development of the cleistothecium differs markedly from that of Ruth Allen (1936). According to Colson (1938) after the ascogonial branch divides into two cells (ascogonium and stalk cell) the sheath hyphae now begin to grow out from the stalk cell of the ascogonium and sometimes from the parent hyphae also. The male stalk cell sometimes contributes sheath cells. The primary sheath hyphae are developed separately from the ascogonial cell and are never in communication with it. Gradually the sheath hyphae become septate.

While the ascogonium divides into three cells, the basal cell produces hyphae which become the inner layers of the sheath.

The centrum is comprised of ascogenous hyphae. The binucleate middle cell of ascogonium becomes multinucleate; cell walls appear later. Also, cells with a variable number of nuclei are formed. Short branches grow out from the upper side of the multinucleate fertile cell and same of the nuclei pass into the short branches. These branches are young ascogenous hyphae. Ascogenous hyphae increase in length, branch and grow upward.

According to Gordon (1966), pseudoantheridial cells arise centrifugally from the mother cells and form the peripheral cell layers of the developing cleistothecia. The centrum is made up of the above-mentioned pseudoantheridial cells. Luttrell (1951) believes that although the function of sterile tissue of centrum is assumed to be the nourishment of the asci, the most important function of the centrum

is primarily that of a space-making tissue. He concluded that the expansion of the ascocarp and the creation of cleistothecial cavities are brought about, at least in part, by internal centrifugal forces resulting from the growth of these tissues.

4. Formation of asci and ascospores

Homma (1937) believed that the ascogonium, after the conjugation of male and female nuclei, divides into four or five cells, the penultimate of which becomes the ascus.

The nucleus in the penultimate cell divides three times and eight ascospores are cut out by free cell formation. Many other workers have reported essentially the same observations as Homma (1937), namely de Bary (1887), Blackman and Fraser (1905) and Harper (1905).

Ruth Allan (1936) states that in *Erysiphe polygoni* the ascogonium and a few of the cells most closely associated with it usually die when the cleistothecium is seven or eight cells in diameter. After the death of the ascogone and associated cells, some cells (usually multinucleate) of the inner layer of the cleistothecium wall grow centripetaly. Harper (1896) has observed a similar phenomenon in *Erysiphe graminis* (his *E. communis*). These conclusions are the exact opposite to those reached by Gordon (1966) who felt that development of the cleistothecium is centrifugal.

From the lower central area, five or six cells grow up to form the asci. Each ascus contains at first two nuclei which soon fuse.

In *Phyllactinia corylea* (Colson, 1938) the formation of

asci is quite different from that of Erysiphe polygoni.

The nuclei in ascogenous hyphae divide and cell walls are formed, resulting in a row of cells in each branch with a uninucleate tip cell, and several binucleate cells occupying the middle region of the branch. There is also a uninucleate basal cell. Later the topmost of the binucleate cells pushes out to form an ascus. Other binucleate cells may develop also into asci, but all of them do not mature. As the asci increase in size they push aside and absorb the remains of the ascogenous hyphae and the inner layers of the sheath, and the center of the cleistothecium, becomes filled with a group of young asci which, by adjustment in the growth of their bases, then all lie at one level.

Nuclear fusion occurs before the asci are fully grown. Twenty or more asci are formed in Phyllactinia corylea. Following the nuclear fusion, miotic divisions proceed and eight nuclei are formed, but only two are incorporated into two ascospores in each ascus; the rest of the nuclei degenerate. Luttrel (1951) accepted Colson's (1938) views with regard to the development of cleistothecia and the formation of asci.

Gordon (1966) believes that the centrum cells are 'fertilized' by the vegetative nuclei, the latter migrate through the 'receptive' hyphae, which develop from the peripheral cells of the centrum. Asci arise from the cells of the centrum and are more or less isolated by lysis. As the asci grow larger, the contents of lysed cells are reabsorbed by the asci.

Björling (1946) also reported the phenomenon of reabsorption of centrum cells by developing asci.

This review of the literature indicates that two major problems remain unsolved:

(1) What is the correct sequence of nuclear events and nuclear configurations during mitosis in fungi?

(2) What exactly is the role of the antheridium and ascogonium in the development of cleistothecia?

It was in an attempt to answer each of these questions that the present investigation was undertaken.

MATERIALS AND METHODS

A. Materials

A few species of Erysiphaceae were chosen for the present investigation concerning the development of cleistothecia. Plant materials infected with powdery mildew were collected over a period of seven months (May - November) once a week from the Edmonton area and from the Botanical Garden of the University of Alberta, situated near Devon.

The materials were collected from as many different hosts as possible. This was done to allow investigation of any differences in the morphogenesis of cleistothecia growing on different hosts.

The hosts and the pathogens were identified according to Host Index (Seymour, 1929) and the Index of Plant Diseases in the United States, U.S.D.A., 1960, and an Annotated Index of Plant Diseases in Alaska, Canada and Greenland (Conner, 1967).

A list of the materials with their respective hosts is given below:

1. _Uncinula salicis_ (DC. ex Merat) Wint. on _Salix_ spp. on _Populus tremuloides_ Michx and _P. balsamifera_ Muench.
2. _Uncinula necator_ (Schw.) Burr on _Parthenocissus quinquefolia_ (L.) Planch.
3. _Sphaerotheca pannosa_ (Wallr) Lev. var. _rosae_ Wor. on _Rosa nutkana_ Presl.

4. Podosphaera oxyacanthae (DC) de By. on Prunus virginiana L.
5. Microsphaera alni DC ex Wint. on Lonicera involucrata (Richards) Banks ex Spreng and L. canadensis March.
6. Erysiphe cichoracearum DC on Mertensia paniculata (Ait.) G. Don., Mentha arvensis L., Delphinium ajacis L., Asper spp.
7. Erysiphe polygoni DC on Polygonum aviculare L., Polygonum erectum L., Lathyrus spp., Pisum sativum L.
8. Erysiphe graminis DC. f. sp. hordii Em. Marchal., on Hordeum vulgare L..

In their parasitism some of the Erysiphaceae have a wide range of hosts, as shown by such a species as Erysiphe polygoni which Salmon (1900) reports as having been recorded on 352 host species. On the other hand, Podosphaera leucotrica parasitizes only apple, and there are a few more species of powdery mildew, each of which is known to attack only its own special host (Salmon, 1900).

Sometimes, one species of powdery mildew was collected from several hosts to see if there is any variation in the development of cleistothecium due to variation in hosts. Some species were collected from a single host only.

To investigate the conidial stages, where vegetative nuclear divisions were to be studied, Erysiphe graminis was chosen as the most suitable material. The following parasites were the ones chosen for study:

Erysiphe graminis from barley, wheat, quack grass, and Kentucky Blue Grass.

B. Methods of study

1. Collection and fixation.

Fresh materials were collected on bright sunny days once a week. Powdery mildew growing on vigorous leaves was selected. The leaves were cut into a suitable size and fixed in several fixatives, viz:

1. Formalin-Aceto-Alcohol - FAA
2. Formalin-Propiono-Alcohol - FPA
3. Belling's Modified Navashin Fluid - Navashin
4. Acetic acid - Alcohol - AA
5. Lu's Butyl alcohol-Acetic acid-Chromic acid - BAC

The first three fixative fluids were prepared according to Johansen's Plant Microtechnique (1940). AA was prepared by mixing one part glacial Acetic acid and three parts absolute Ethyl alcohol. Lu's BAC fixative is composed of 9 parts of n-Butyl alcohol, six parts of glacial Acetic acid and two parts of 10% aqueous Chromic acid (Lu, 1962).

Materials were fixed either in the field or brought into the laboratory within two hours of collection and fixed. They were placed in a vacuum dessicator and aspirated to free all trapped air and for quick penetration of the fixative. Care was taken not to mutilate the material in any way before putting it into the fixative.

The fixed materials were stored for 24 hours to several days days in the refrigerator and used for embedding or the squash method as required.

For conidial study, material of *Erysiphe graminis*

growing on leaves of barley, wheat, quack grass and Kentucky Blue grass in the greenhouse, was harvested every half hour for 24 continuous hours, extra collections being made every 15 minutes at certain times of the day and night. These were also fixed using the method mentioned above.

Navashin's fluid, although used extensively for smear preparations, was found to be slow in penetrating and did not give clear images of internal cellular structures. Therefore, this fixative was later discarded. It was found that FAA and FPA penetrated the tissues rapidly. FAA and FPA were very satisfactory in showing cell wall structure clearly. FPA was also good for chromatin and cytoplasm fixation. These two fixatives were used extensively to fix materials used for embedding and gave excellent results with stains such as safranin-lightgreen combination and crystalviolet-safranin combination.

Materials fixed in AA have been used for staining with Feulgen. Lu's BAC fixative was used in materials to be stained with Lu's propionocarmine squash technique.

2. Preparation of stained slides.

a. Embedding and rotary microtome methods.

The embedding technique of Johansen (1940) was followed with slight modifications and satisfactory results were obtained. From the fixatives FAA and FPA containing 70% ethanol, the material was carried through the following series. With AA fixative the process began with step 3 (95% TBA).

1.	70% tertiary butyl alcohol solution	Overnight
2.	85% tertiary butyl alcohol solution	2 hours
3.	95% tertiary butyl alcohol solution	2 hours
4.	100% tertiary butyl alcohol solution	2 hours
5.	Absolute tertiary butyl alcohol	2 hours
6.	50/50 TBA/Paraffin oil in oven, 60° C	Overnight
7.	Transfer of the material into a specimen tube 2/3 filled with semi-solid tissue mat (m.p 56.5°C) and 1/3 filled with 50/50 TBA/Paraffin oil in warming oven	Overnight
8.	Pure tissue mat	4 hours
9.	Pure tissue mat	4 hours
10.	Pure tissue mat	4 hours

The hot 'Tissuemat' containing the material was then quickly poured into paper boats (1.5 x 6 cm). The material was then arranged by means of a hot needle and the Tissuemat was allowed to cool until a thin film appeared over the surface of the wax. The boat was then submerged in ice water to solidify the Tissuemat. The embedded material was mounted on wooden blocks and the excess Tissuemat trimmed with a scalpel in preparation for microtoming.

To study the morphogenesis of cleistothecia, the material was sectioned with a rotary microtome (American Optical Co., Model 820). Sections were cut at right angle to the leaf surface at thicknesses of 12, 10, 8 and 5 microns. The sections were affixed to microscope slides with Haupt's adhesive.

Several combinations of stains were used with the sectioned material. Stains investigated were combinations of: 1, crystalviolet safranin; 2, safranin and lightgreen; and 3, Feulgen and fastgreen. Crystalviolet and Feulgen were quite satisfactory for internal cell structures especially for chromosomes and safranin and lightgreen were very useful for cell wall study.

In staining the slides with crystalviolet and safranin, the following schedule was used.

1. Absolute xylene (until Tissuemat disappeared)
2. 50/50 absolute xylene-absolute ethanol, 4-5 minutes
3. Absolute ethanol, 4-5 minutes
4. 95% ethanol, 5 minutes
5. 85% ethanol, 5 minutes
6. 70% ethanol, 5 minutes
7. Stain in safranin dissolved in 70% alcohol, 1 hour
8. 70% ethanol, 5 minutes
9. 80% ethanol, 5 minutes
10. 90% ethanol, 5 minutes
11. 100% ethanol, few seconds
12. 50/50 absolute xylene-absolute ethanol
13. 25/75 absolute xylene-absolute ethanol
14. Stain in crystalviolet dissolved in clove oil, 1.5 minutes
15. Clove oil, a dip
16. Absolute xylene - 3 changes.

On completion of staining, the material was mounted in Canada Balsam.

For staining with safranin and lightgreen, the abovementioned schedule was followed except in step 14, where lightgreen dissolved in clove oil was used.

For Feulgen staining, Johansen's method was used with very little modification. Staining was carried out as follows:

Slides were brought down to distilled water and rinsed in cold 1/N hydrochloric acid. Then the slides were transferred to a Coplin's Jar full of 1/N HCl preheated to 60°C in an oven, and kept in that temperature 5 to 7 minutes. They were then rinsed in cold 1/N HCl, and then in distilled water.

A different method of hydrolysis was also tried and found to give better results. Instead of 1/N HCl heated to 60°C, 5N HCl in room temperature for 30 minutes was used.

Following hydrolysis, slides were transferred to the colorless staining solution and were left in the solution for 1½ hours. They were passed quickly to the first of three closed Coplin jars, each containing the following differentiating solution: 1/N HCl, 5 cc; 10% aqueous potassium metabisulphite, 5 cc; distilled water, 100 cc. The slides were washed for 10 minutes in each of the three jars, then washed in distilled water. After Feulgen stain these slides were counterstained momentarily with 0.05% fastgreen in 95% alcohol. They were then dehydrated in the usual manner and mounted in Euparal.

b. Squash method.

To study the mitotic nuclear division in growing

conidiophores, the squash method was found to be best.

Also some study of the asci and ascospores was made by squash method.

Materials fixed with Lu's BAC fixative were used for staining with Lu's propionocarmine squash technique. For Feulgen staining, materials fixed with AA were chosen.

(i) Lu's staining procedure

Hydrolysis

A very small piece of mycelium with conidiophores carrying conidial chains was lifted from the surface of the host leaf and placed in a watch glass containing a few drops of HCl-alcohol mixture composed of one part of 95% ethyl alcohol and one part of HCl. The watch glass was kept at 60°C for about 10 minutes inside an incubator (oven) leaving further dissection until later.

The mycelium was then picked up and placed on a microscope slide in a drop of fresh HCl-alcohol mixture. The mycelial mat was teased out with a pair of very fine needles.

The HCl-alcohol mixture was then drained off and the material washed with Carnoy's fixative for a minute.

Staining

The staining solution was prepared according to Lu's method (1962). The procedure of staining is as follows:

A few drops of staining solution were added to the material and the slide was heated gently several times over a gas burner. At the same time a very clean needle was used to stir the stain as a source of a trace of iron for mordanting. When the staining solution had become purplish

brown, a coverslip was put on the material and excess stain was blotted off with filter paper. The slide was quickly heated almost to boiling and was then pressed down with several thicknesses of filter paper.

Destaining and permanent mounting

To all corners of the coverslip of the stained and pressed slide, a drop of 45% acetic acid was applied to remove the excess carmine from the preparation and the edges of the coverslip. Finally to one side of the coverslip, a drop of glycerine-acetic acid mixture was applied and was allowed to be drawn under as a mounting medium. All sides were cleaned with a wet tissue paper and were then sealed with fingernail polish.

(ii) Feulgen squash method

The stain was prepared as follows:

1. 1 g basic fuchsin
2. 1.0 g $Na_2S_2O_5$
3. 100 cc 0.5 N HCl
4. Shake the above for 2 hours
5. Add 0.5 g activated carbon
6. Shake for 2 minutes
7. Filter

This solution should be colourless to start with, and it should be used within a few days.

Hydrolysis

Materials that were fixed with acetic alcohol (1 glacial acetic acid:3 absolute alcohol) were put in 5N HCl in room temperature for 30 minutes.

Staining

The materials were then placed in Feulgen stain in a covered petri dish for 1½ hours, and washed with lukewarm tap water for 10 minutes. A small piece of stained material was then placed on a slide and teased out carefully with a pair of fine needles. Then a drop or 2 of absolute alcohol was placed directly on the material to bleach the excess stain and also to dehydrate the material. It was then mounted in Euparal.

3. Photography.

Direct observations were made and photographs taken with a Leitz Phase-Contrast Microscope (No. 595655) oil immersion lens (N.A. 1.40). A green filter was used. Photographs were taken with a Leitz-Wetzlar camera using Kodak Contrast Process Ortho film, developed with Eastman D-11 developer, and printed on Kodabromide paper.

OBSERVATIONS

A. Development of Conidia

The development of mycelial colonies starts as the conidia germinate and send out haustoria which penetrate the epidermal cells of the host (Figure 3). The mycelial network spreads superficially. Typical hyphae are slightly curved and bear short bumps (Figures 6, 9, 13).

The mycelia and the conidia appear white in color as seen by reflected light; however the mycelium and conidia of Erysiphe graminis growing on wheat, barley and quack grass are cream-colored. Mycelium of E. graminis on Blue Grass appears pure white.

Only the conidial stage of E. graminis was found growing on the above-mentioned hosts in the greenhouse; the cleistothecial stage was never observed growing in the greenhouse (Figure 2).

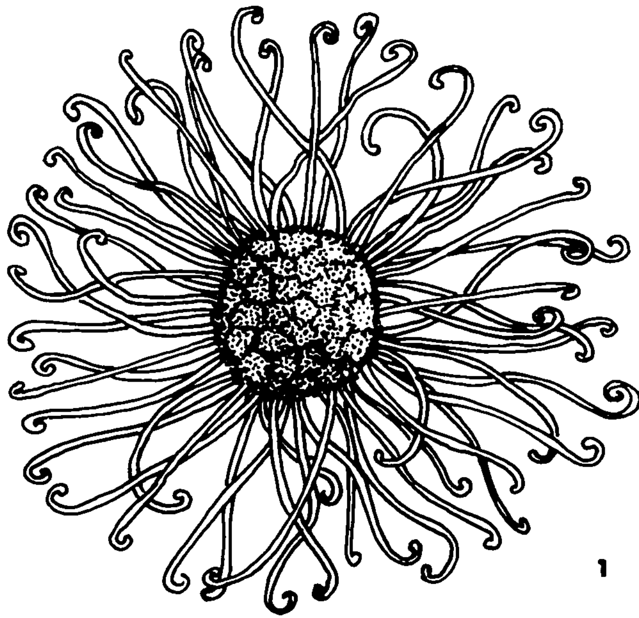
Eventually conidiophores are produced on the mycelia. Small button-like upward projections develop into flask-like 'generative cells' at right angles to the horizontal mycelium. A cell wall is formed between the horizontal mycelium and the upright generative cell. Figure 9 depicts the various stages of conidiophore formation within a young colony.

Generally all the conidia in a conidiophore are derivatives of the generative cell. The generative cell divides mitotically to give rise to conidia. Before division, the neck of the generative cell elongates to twice its original size (Figures 9, 12, 15).

FIGURE 1. Mature cleistothecium of Uncinula salicis (DC.) Wint. X 140.

FIGURE 1a. Rosette of asci with ascospores of Uncinula salicis. X 478.

FIGURE 2. Conidiophores of Erysiphe graminis DC. with conidia on superficial mycelium which is growing on barley leaf. X 400.



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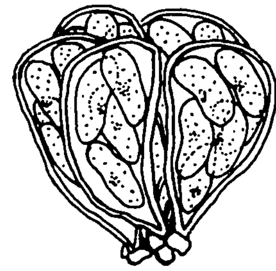

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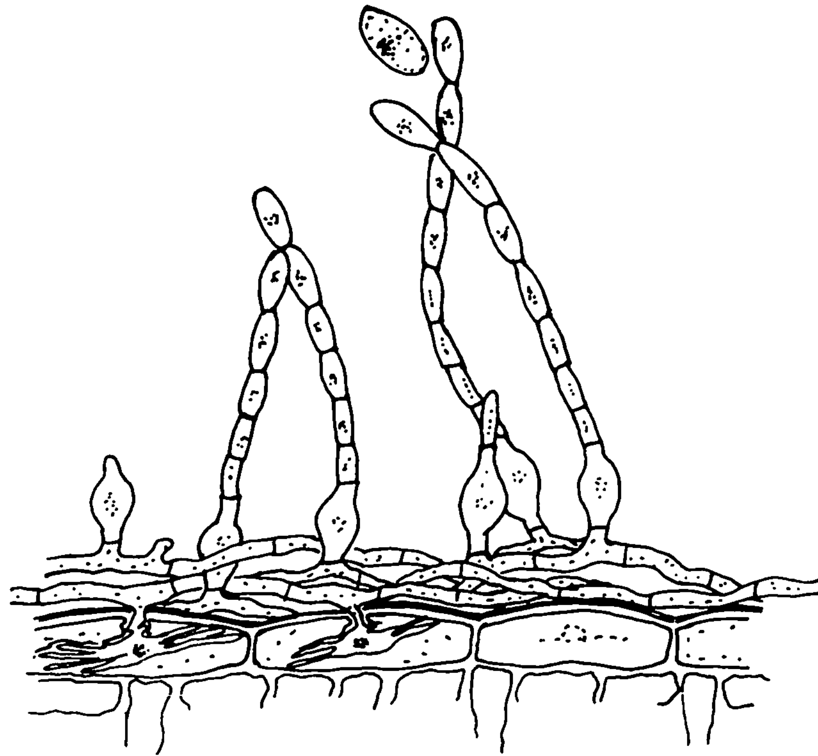

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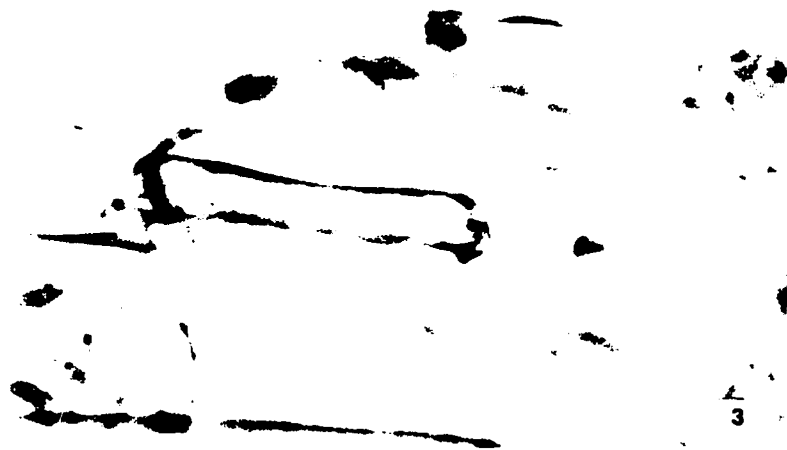
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The undividing nucleus remains mostly in the wide 'bowl' part of the generative cell and is quite condensed and round in shape (Figures 10, 11, 12a, 24, 26). The nucleus moves upwards into the neck prior to division (Figures 12b, 13, 14, 15).

Occasionally, when growth is vigorous, the generative cell has produced a succession of conidial chains, the cell of the conidiophore adjacent to the generative cell divides again to produce conidia; in other words, the latter cell then assumes the role of generative cell, although it does not change into a flask-shaped structure. For simplicity in describing this cell and to differentiate it from the original generative cell it will henceforth be mentioned as the Secondary Generative cell or 'SG' cell (Figures 9, 17, 18, 20).

The original generative cell stops dividing when the 'SG' cell becomes active. As is true for the generative cell, the 'SG' cell also increases to twice its original length prior to nuclear division (Figure 18).

It has never been observed that both the generative cell and the 'SG' cell are active in the production of conidia in a single conidial chain.

Of the various staining methods (as described in Materials and Methods) used to find a suitable staining technique for these organisms, Feulgen stain (Johansen, 1940) was taken as indicating the presence of DNA in the cells. Lu's Propionacarmine method (Lu, 1962) was equally satisfactory in this regard.

Staining the fungi while they were still attached to the host proved unsatisfactory. Therefore, the superficial mycelia were carefully scraped from the host with a scalpel or peeled off with a pair of forceps and then stained.

The somatic nuclear divisions during the formation of conidia among various physiologic forms of *Erysiphe graminis* appear to be similar. Therefore, instead of reproducing photographs of all the forms, the best pictures were used to represent the various stages. For the sake of uniformity, *Erysiphe graminis hordei* on barley was chosen as the material for microphotographs.

Since nothing was known about the exact time of active nuclear division, that is the onset of division and different phases of the process of division, samples were taken frequently and fixed in fixative fluids at different times of day and night. (Refer to Materials and Methods).

B. Description of Mitotic Division

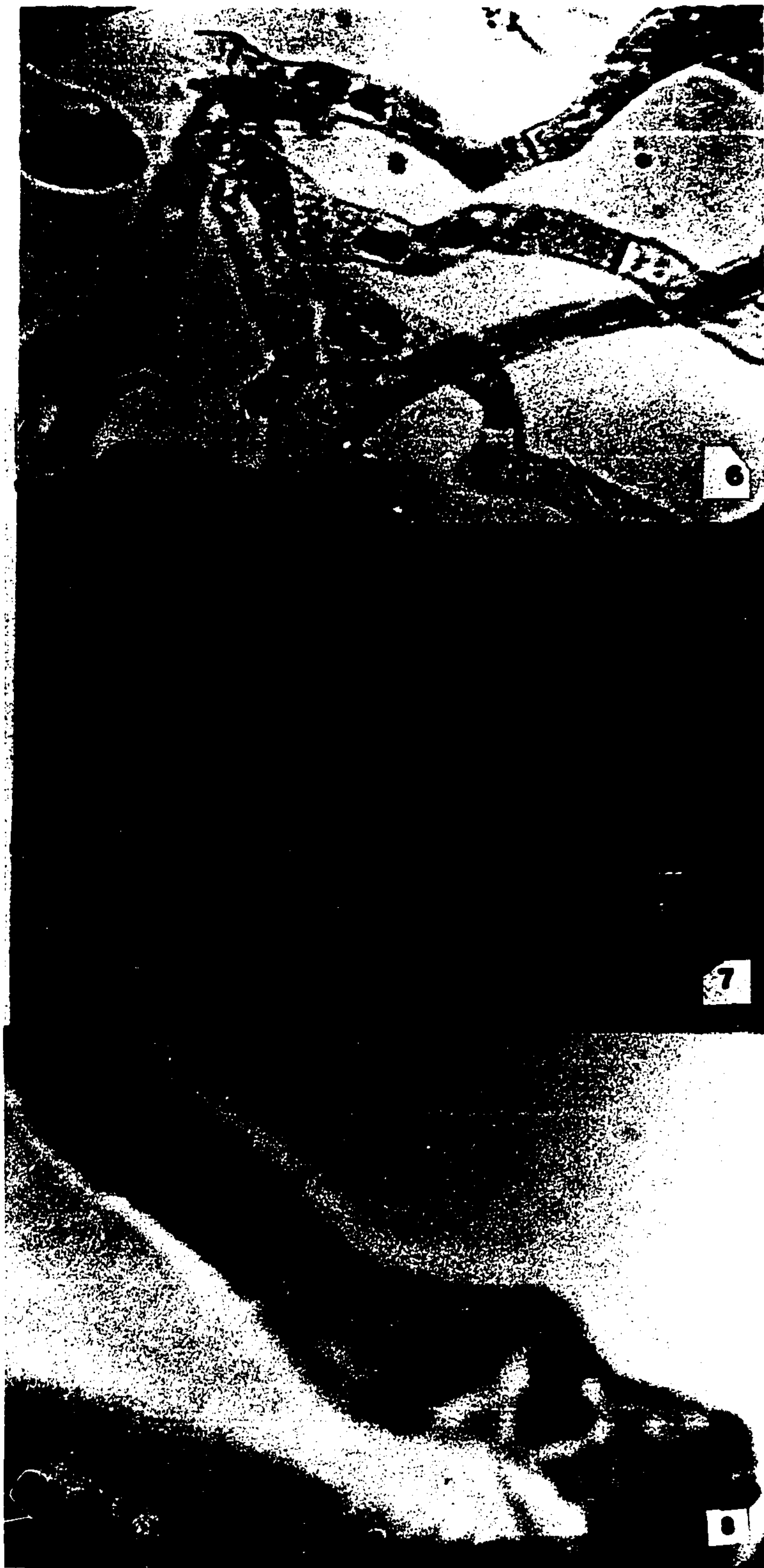
1. Interphase Nuclei

Interphase nuclei, either spherical or conical in shape are frequently observed in the hyphae of all species of powdery mildew studied. Conspicuous deeply stained satellite bodies are often associated with the Interphase nuclei (Figure 25). Such granules are mentioned in the literature as having been observed by previous investigators. Many of these scientists described these organelles as centrioles. In this report such structures are also referred to as 'centrioles'.

FIGURE 6. Several non-dividing nuclei in the vegetative mycelium. X 1400.

FIGURE 7. Double thread configuration of recently divided nucleus in the vegetative hypha. X 2000.

FIGURE 8. Condensing nuclei after the splitting of thread nucleus in the vegetative hypha. X 3000.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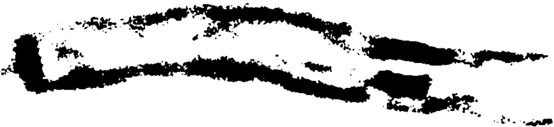

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FIGURE 9. Formation of conidia from conidiophores. Conidia of different stages of maturation are seen here. Different stages of growth of conidiophores with bulbous generative cells are depicted here. X 600.

9

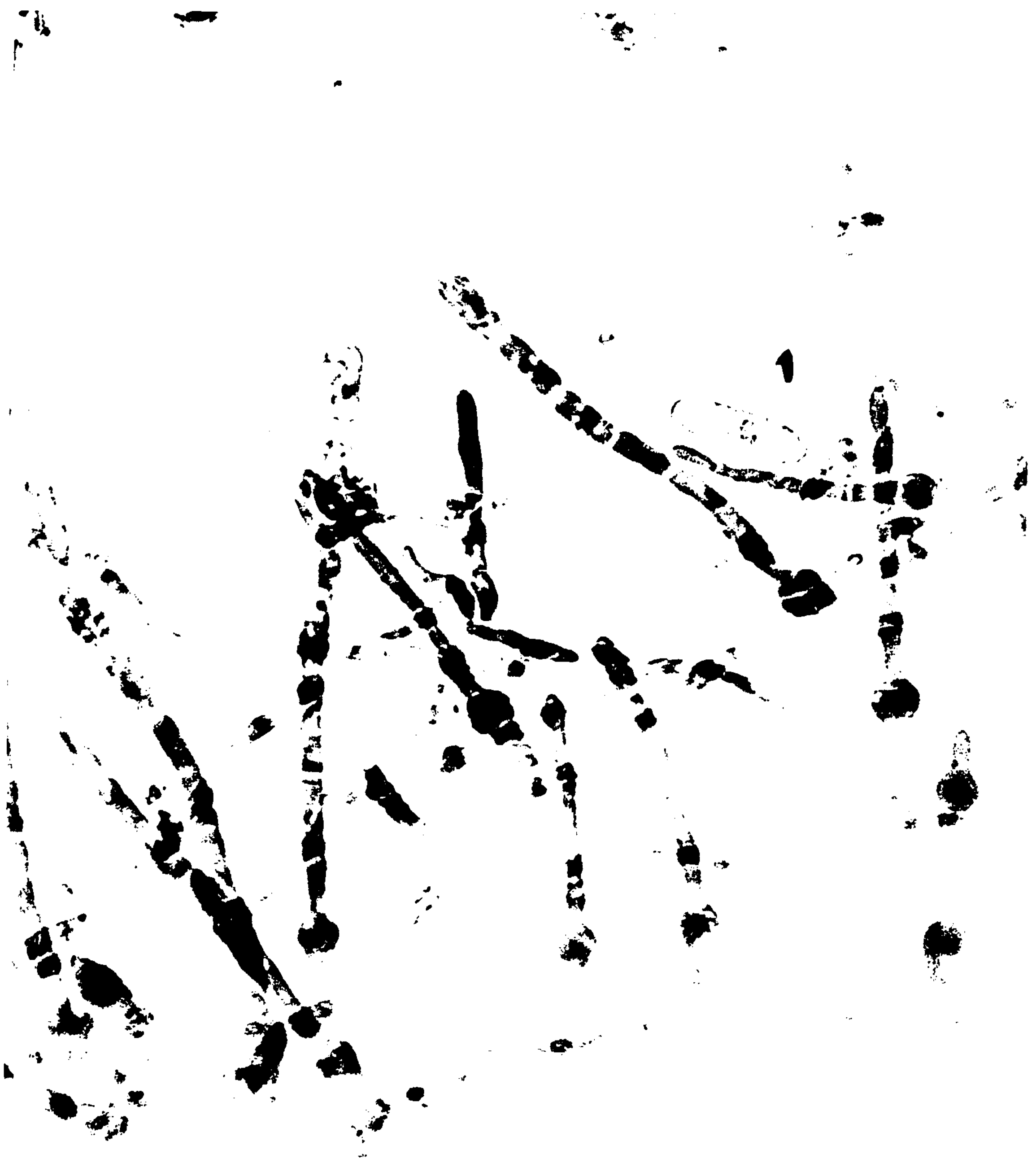
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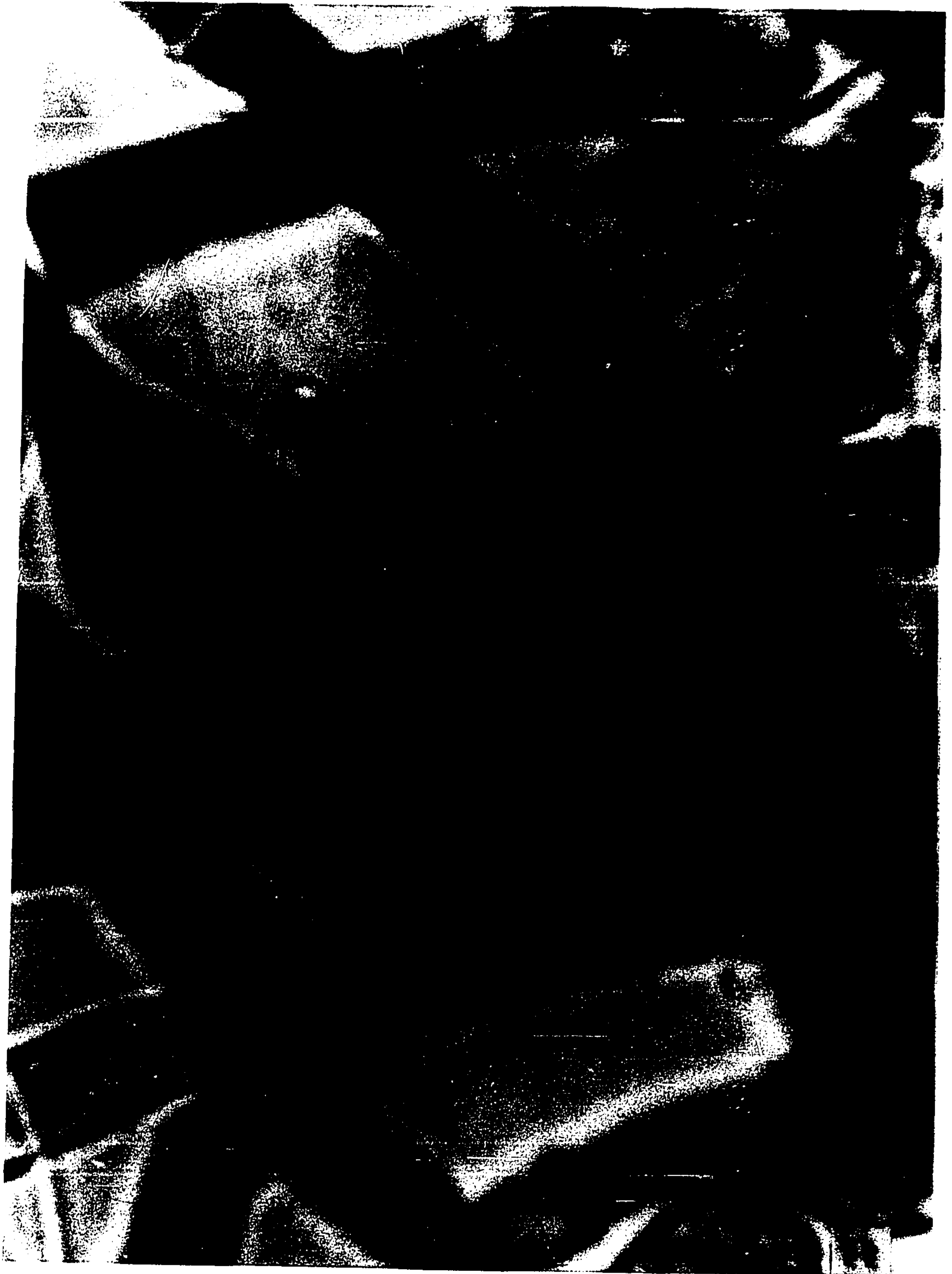
FIGURE 10. A young generative cell. The nucleus is in interphase stage. X 4200.

FIGURE 11. A young generative cell about to give rise to conidial chain. The nucleus is at an early stage of mitotic division. X 3500.

FIGURE 11. A young generative cell about to give rise to conidial chain. The nucleus is at an early stage of mitotic division. X 3500.



Interphase nuclei are abundantly seen located in vegetative hyphae, in the wide 'bowl' of the generative cell (Figures 5, 6, 10). Of these interphase nuclei there appear to be two types, as follows:

(1) Round and relatively condensed nuclei are seen more often in thick-walled hyphae, where conidiophore formation has ceased or has slowed down considerably, typical of aging hyphae. This type of nucleus is also seen in mature conidia. The above-mentioned locations would indicate that these nuclei are in a 'resting'state (Figure 27).

(2) Nuclei of a more conical type (not spherical) are observed mainly in growing hyphae. Many are seen in the very tip of growing hyphae. Some are seen moving toward new hyphal projections, which are budding generative cells (Figures 5, 10). On the other hand, conical nuclei are never seen in conidia.

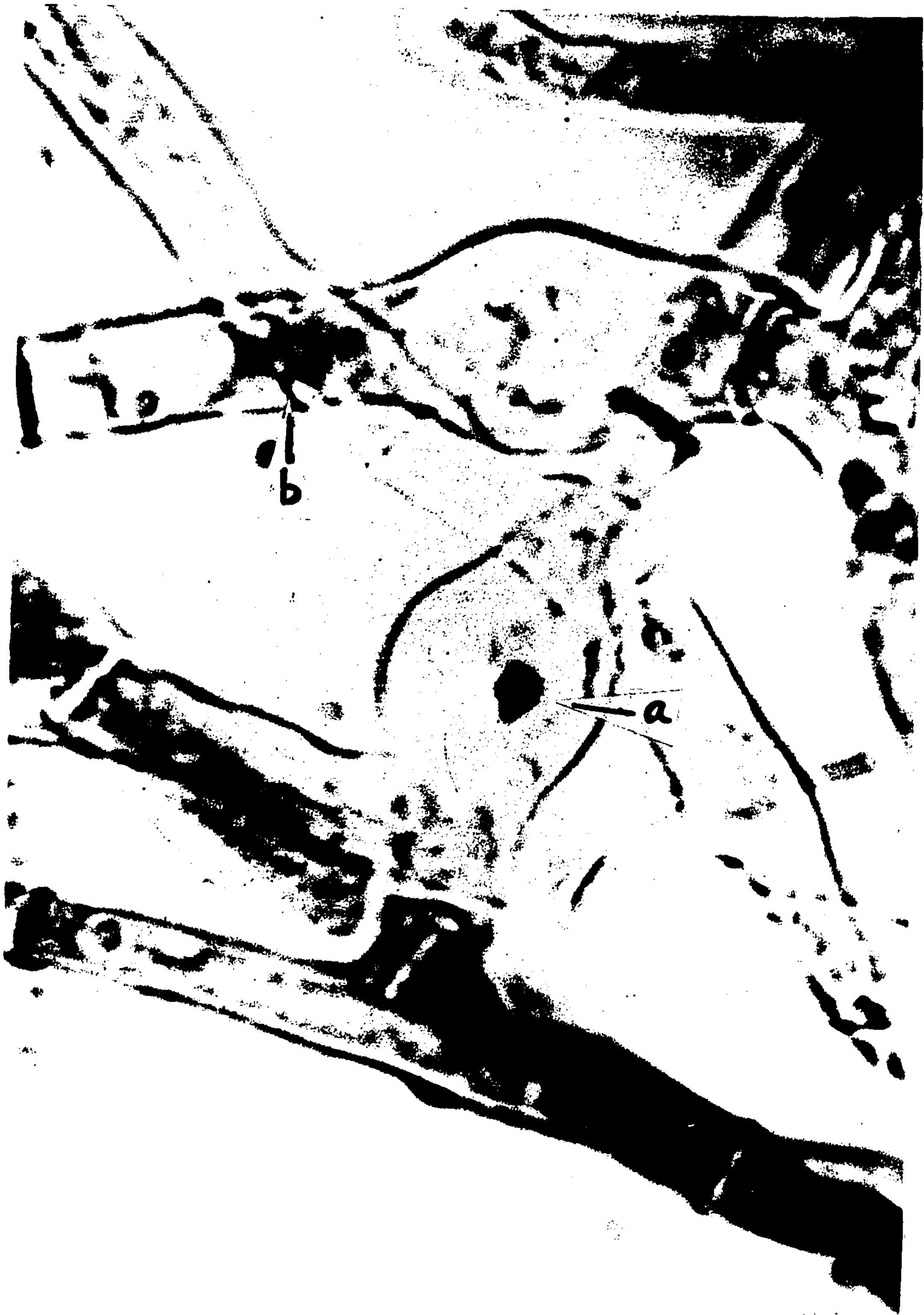
The interphase nucleus has a large nucleolus with a large amount of chromatin network which makes up most of the cone within a nuclear membrane. Often there is an unstainable 'halo' between nucleolus and chromatin materials (Figures 4, 5, 6, 10). Chromatin material in interphase is finer than that in the dividing stage. The chromatic materials are deeply stained and Feulgen-positive. The centriole is situated at the end opposite to the nucleolus (Figure 25). Whether the centriole has a role in the movement of the nucleus is not clear.

There is always one nucleus present in each mycelial cell except where, obviously, the nuclei are the result of

FIGURE 12.

a. Early stage of nuclear division in the generative cell. The nuclear membrane is disappearing and the shape of the nucleus is becoming irregular. Nucleolus is disintegrating. X 2900.

b. Nucleus has moved in the neck region of the generative cell. Nucleus is in a much more advanced stage of division. A distinct ring of beaded chromatids is formed. X 2900.



12

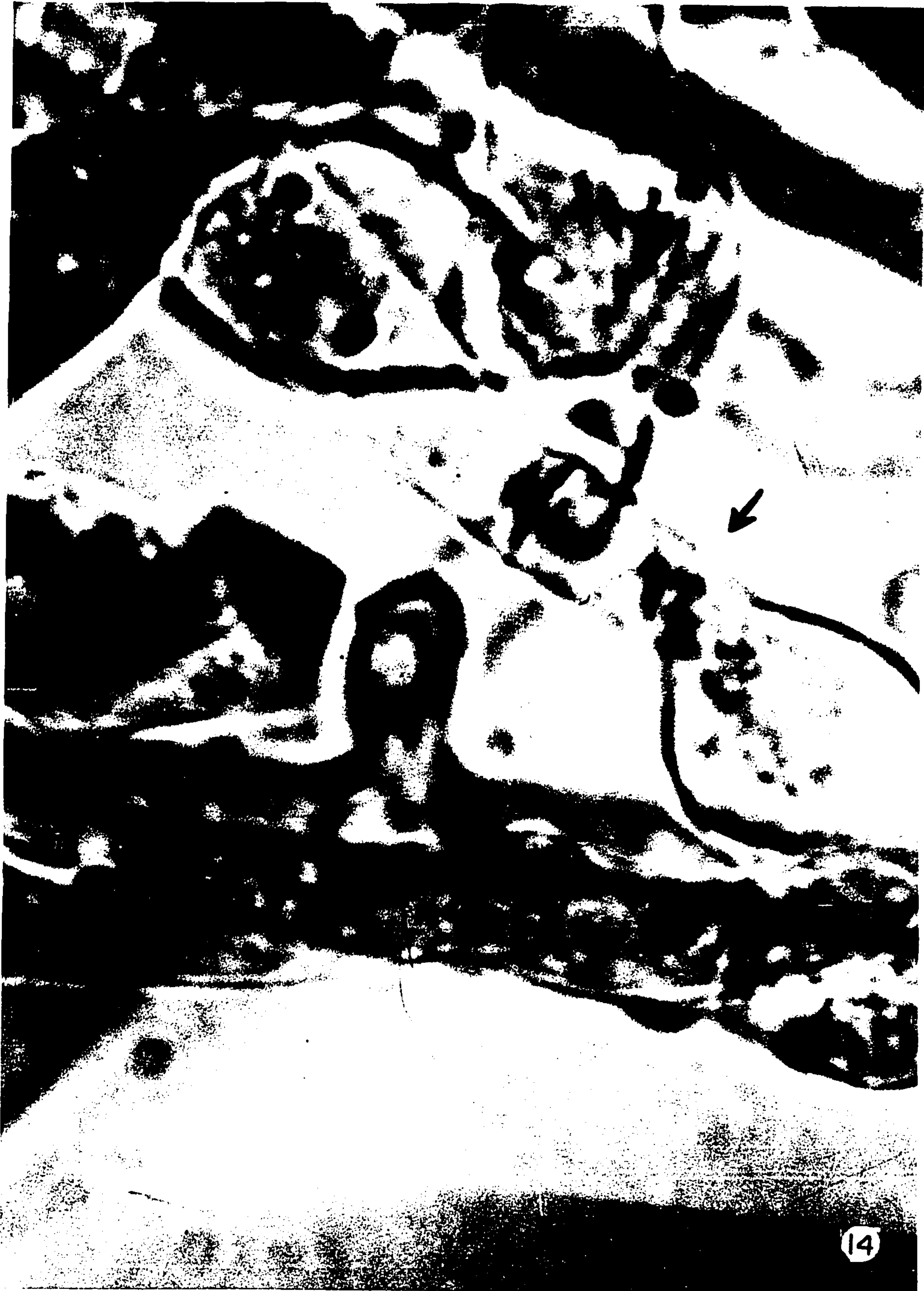
b
a
12

FIGURE 13. The nucleus is much diffused. The nuclear membrane is dissolving. This is a stage in mitotic division slightly advanced from the stage of the nucleus in Figure 12a. X 3500.

13

13

FIGURE 14. The nuclear membrane and nucleolus have disappeared. The nucleus is quite irregular in shape. This stage is comparable to 'late prophase' of 'standard mitosis' of higher organisms. X 3500.


14

nuclear division.

Figure 6 shows vegetative interphase nuclei with their centrioles at opposite end of nucleolus. Figure 10 shows a budding generative cell with a typical Interphase nucleus.

2. Division phases

The onset of karyokinesis begins when the round somewhat condensed nucleus begins to expand and increase in volume. The fine granules of the chromatin body begin to change into a coarse reticulum. The nucleolus begins to diffuse and dissolve and takes very little stain. At this point, the nuclear membrane has not yet disappeared. In the generative cell, at this dilated stage, the nucleus begins to move towards the neck which has already been elongated. Although the shape of the nucleus is round it does not resemble the Interphase or condensed nucleus of the resting stage (Figure 11, 12a).

As the nucleus continues to move towards the neck, it becomes more expanded and the nucleolus eventually disappears. The chromatin material takes a deeper stain. The nuclear membrane has not completely disintegrated. The whole mass of nuclear material still retains the rounded shape. Chromatin material is visible, mainly in two Feulgen-positive masses (Figure 13).

In Figure 14, the nuclear membrane is seen to have been lost; the nuclear materials are more diffused or scattered than in the previously mentioned Figures 12a, 13. Thus the round shape of the nucleus is replaced by irregularly-shaped

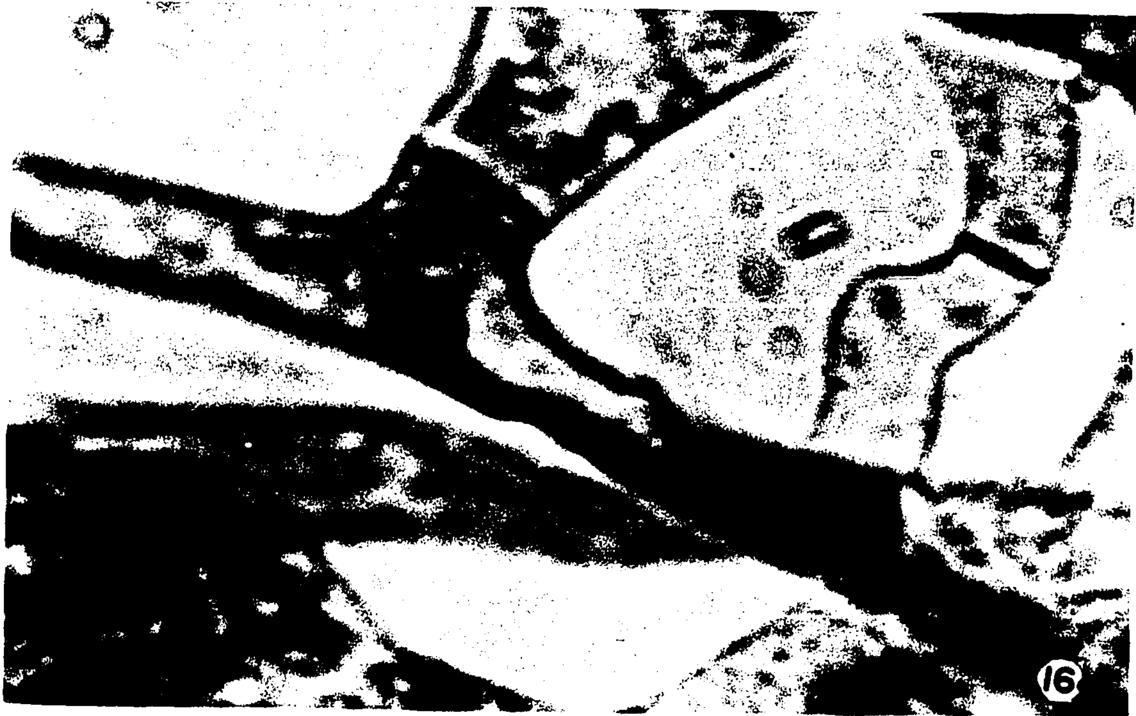
FIGURE 15. The splitting of the ring of beaded chromatid material is almost complete. Each bead is considered to be a chromosome. Beads are connected longitudinally by fine fibrils (b) Cross-fibrils (a) connecting the homologous beads are distinctly visible. Number of chromosomes are observed to be seven (n=7) with a Feulgen-positive centriole. X 4000.

15

15

FIGURE 16. Elongated thread nucleus appeared to be splitting longitudinally from one end. X 2800.

a. Nuclear threads are drawn in to clarify their position. X 2800.


16

16

16a

nuclear materials (Figure 14). This stage is more or less equivalent to the prophase stage of 'standard' karyokinesis.

As division proceeds, the nuclear material becomes more diffused and occupies a larger volume in the cytoplasm.

After uncoiling from the reticulate stage, the nuclear material forms a beaded, oval ring. Each bead is here interpreted as a chromatid. At this beaded-ring stage, more chromatin material has accumulated on the chromosomes (Figure 12b).

A ring is formed as a result of separation of homologous beads from each other. Observations indicate that homologous beads separate at the center first and progressively from the center to the ends, resulting in a ring-configuration.

Instead of separation of homologous beads at the center, which results in ring formation, the separation occasionally begins at one end which results in a split-end elongated thread-like appearance (Figure 16).

Although this stage has many characteristics of 'standard' metaphase (*e.g.* of higher plants), it cannot be considered a true metaphase. There is no stage that structurally resembles standard metaphase.

Figure 15 represents the stage in which the ring has changed into a long double thread. The separation of beaded chromosomes is complete except at one end where the pair of homologous beads did not separate. The beads in a string are variable in size, some being small and some large. This kind of configuration is seen in the elongated neck region of the generative cell. 'SG' cells when actively dividing

FIGURE 17. Thread chromatids are almost separated except at one point where one pair of homologous beads are not separated as yet. X 5000.

17

FIGURE 18. Two threads are completely separated. The cross-fibril is connecting the parallel threads at one point only. X 4400.

18

18

also have the same kind of configuration.

At this particular stage the nuclear material is spread out clearly. The individual beaded chromosomes are distinctly separated from each other. The homology of the beaded strings is obvious. The fibrils that connect the beads which results in the beaded string appearance of nucleus are clearly visible here. These fibrils are faintly Feulgen-positive.

In addition to the latter fibrils, another kind of Feulgen-positive fibril is seen connecting the homologous beads. The structure of these cross-fibrils will be discussed later.

This stage (Figure 15) is believed to be comparable to late metophase or early anaphase stage of standard mitosis. Standard metaphase configurations are never observed in this investigation. What appeared to resemble metaphase is in fact two threads of nuclear material laying side by side which is comparable to early anaphase. At this stage, chromosome counting is relatively easier (n=7 - result of investigation). Chromosome counting from other stages is extremely difficult. During prophase-like stages chromatin material is extremely diffused and duplication of chromatin material is in progress. Therefore, accurate counting of chromosomes is impossible.

Telophase is a stage where various degrees of condensation of nucleus is going on, and is, therefore, not suitable for chromosome counting.

Considering the facts that the beaded chromatids are

distinctly separated from each other and the homology of the two sets of beads is clearly visible, this is the most suitable stage for the counting of chromosomes with certainty (Figure 15).

The same sequences of nuclear division may be readily observed in the vegetative mycelium (Figures 6, 7, 8). As separation proceeds, the threads can be bent or twisted, resulting in several different conformations which are shown in Figures 16, 17, 18.

In Figure 17, the arrow shows that, in a 'SG' cell, the nuclear threads are separated except at one point where the homologous beads are not yet separated, resulting in a tuning-fork like configuration.

In Figure 18, the arrow points out much the same stage except that separation of the nuclear threads is more advanced in that the homologous beads are completely separated, except at one end where the cross-fibril is still attached to one pair of homologous beads. This is in a 'SG' cell, which is about to divide into two and hence produce a conidium.

After the splitting is complete, the two newly-formed thread nuclei lie side by side for a time and then move toward the two ends of the cell and a cell wall is formed between them.

3. Condensation phase

After separation, the thread nuclei begin to condense and eventually assume a conventional round form. This change in nuclear shape is gradual and will now be examined in somewhat more detail.

FIGURE 19. A septum is formed between the two threads after they are separated. X 3000.

19

'19'

FIGURE 20. Elongated thread nuclei after nuclear division - in SG cell. A partition wall is formed to produce a conidium. X 4000.

20

FIGURE 21. Three conidia are shown in a chain. The thread nuclei are in the process of condensation. The oldest of the three conidia is seen with the shortest nucleus and the youngest conidium with the longest nucleus. X 3000.

21

FIGURE 22. Condensing nuclei in maturing conidia.

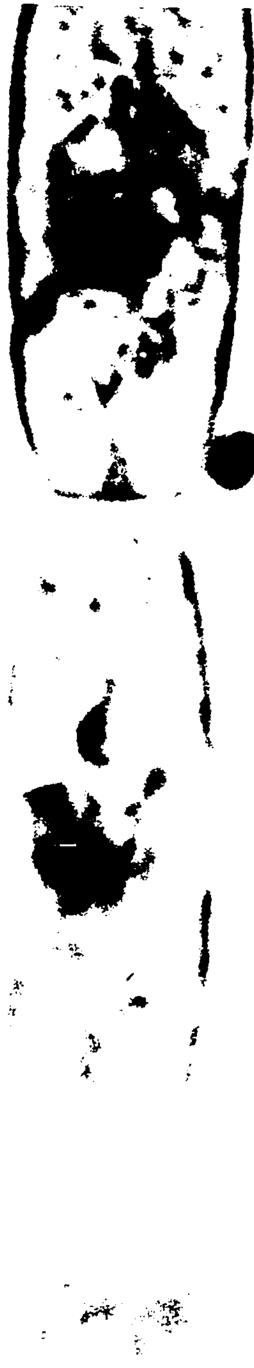
(a) Condensing thread nucleus with chromosome beads.

(b) The conidium is more mature than 'a' and the nucleus is more condensed than the nucleus in 'a'.

(c) The conidium is quite mature being at the tip of the conidiophore. The nucleus has condensed into a round form and the nucleolus has re-appeared. X 3000.

a
b
c
22

a


b

c
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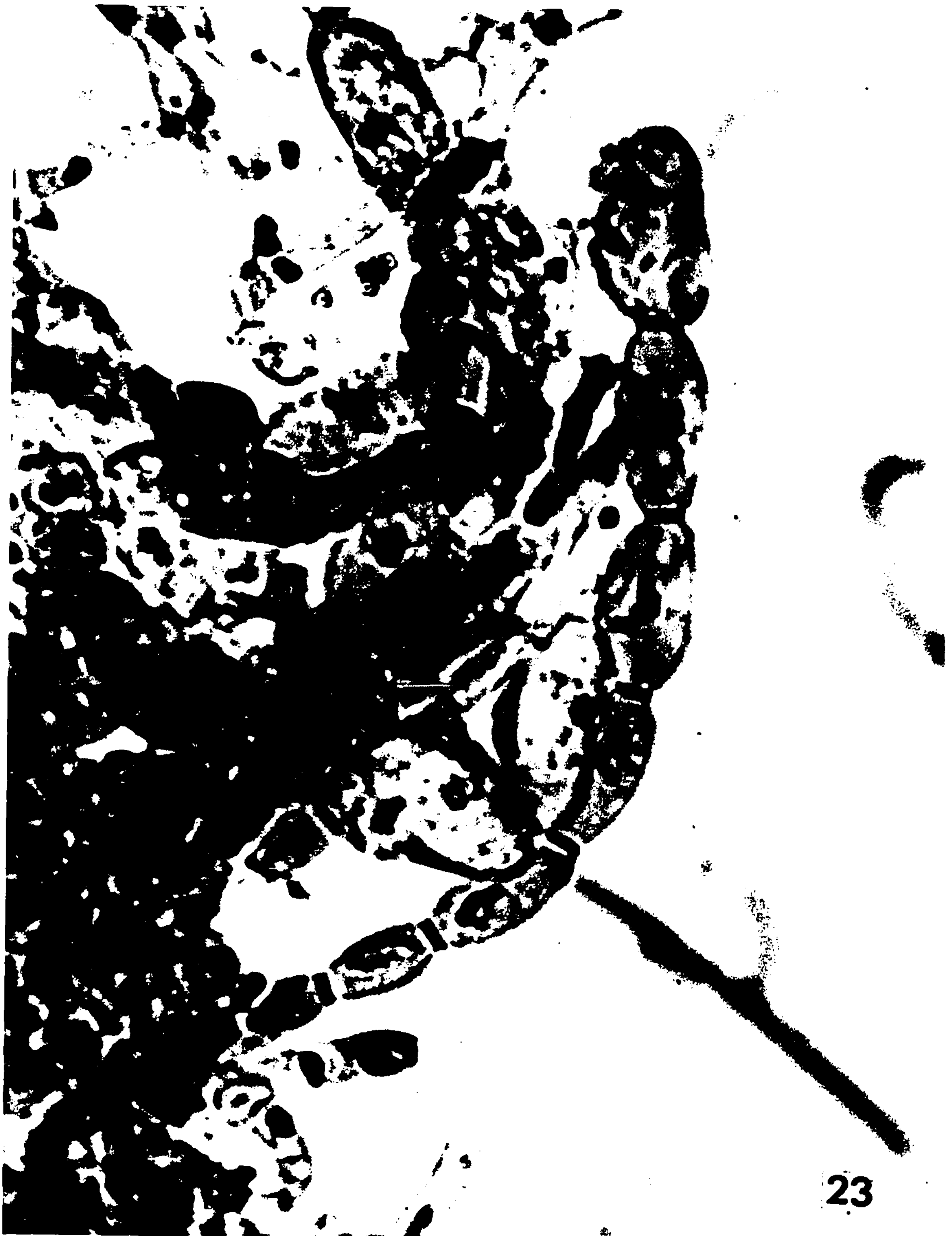
FIGURE 23. Progressive stages of nuclear condensation from long beaded thread in the youngest conidium at the bottom of the chain, to round condensed nucleus in the oldest conidium at the tip of the chain. X 1130.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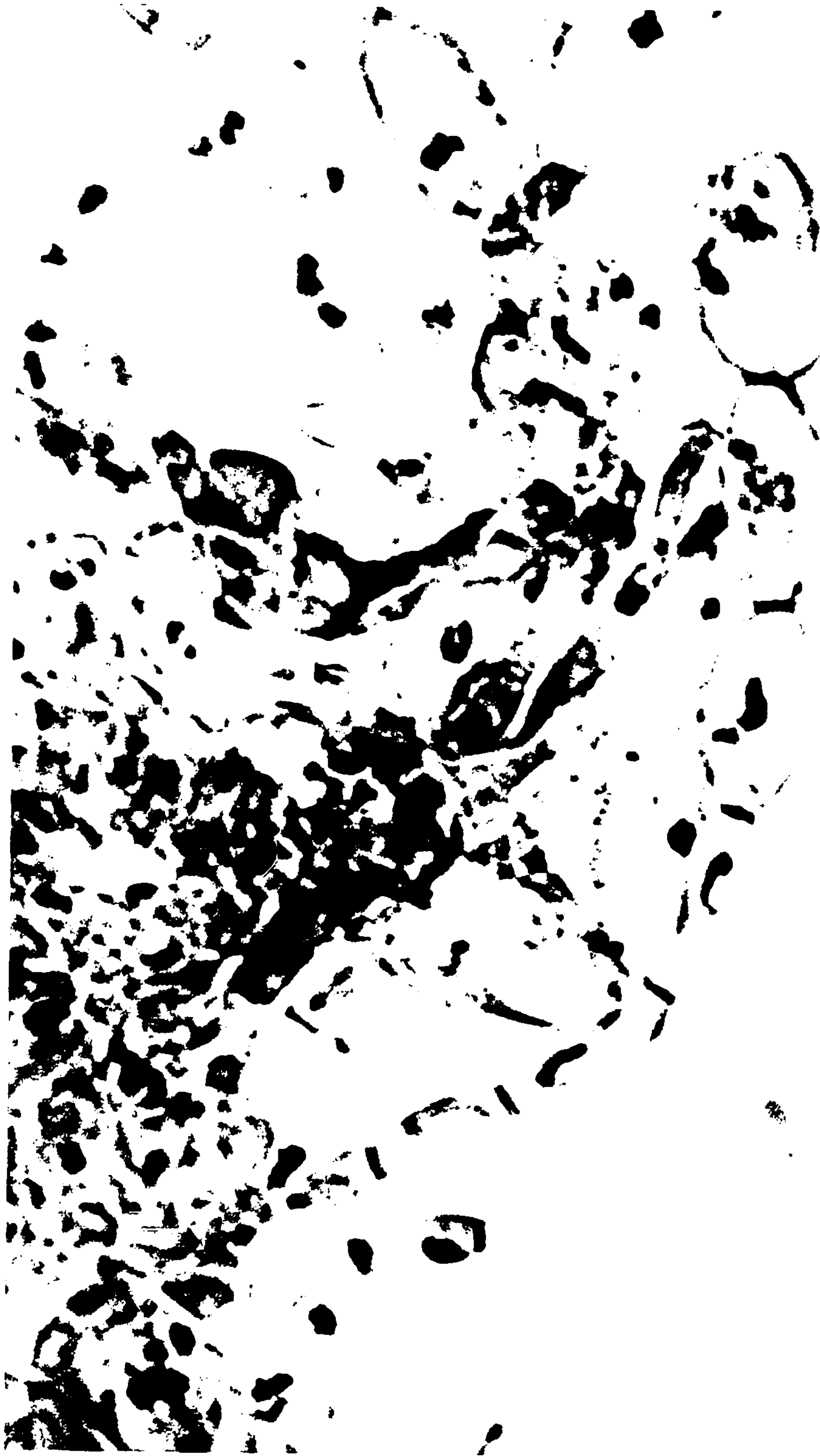


23

FIGURE 24. A nucleus is seen in the wide bowl of the generative cell in the process of condensation following division. X 3920.

24

FIGURE 25. An interphase nucleus with a conspicuous nucleolus is seen. A Feulgen-negative 'halo' is present between the nucleolus and the chromatin material. A pair of centrioles is protruding from the nuclear material. X 6000.

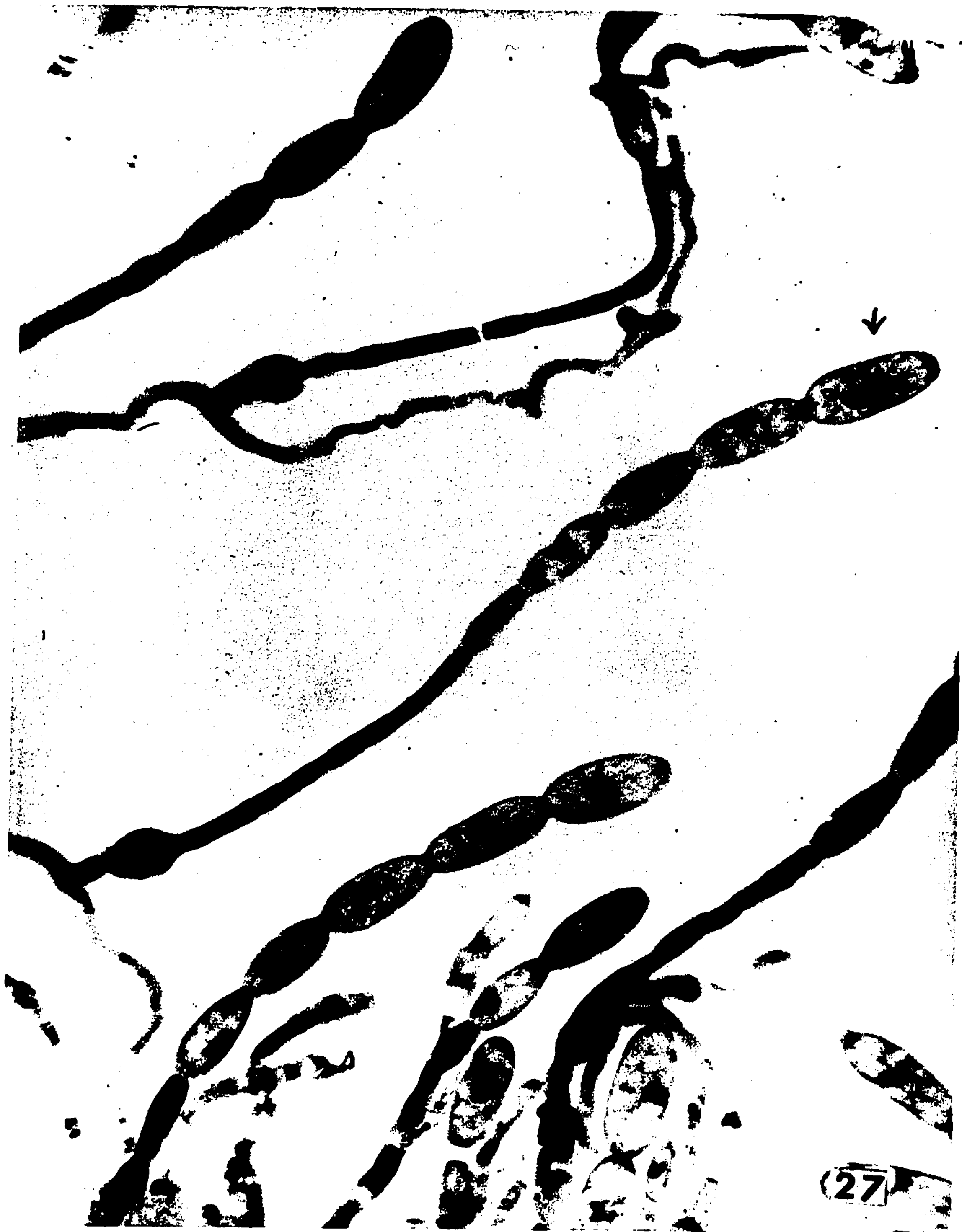
25

FIGURE 26. Condensed thread nuclei in the conidia and a round condensed nucleus in the generative cell are shown here. X 5000.

26

26

FIGURE 27. Conidial chains. Each chain containing 5 to 6 nearly mature conidia is shown. A round, not filamentous nucleus can be seen in all of the conidia (in various stages of development) as opposed to Figure 23. X 740.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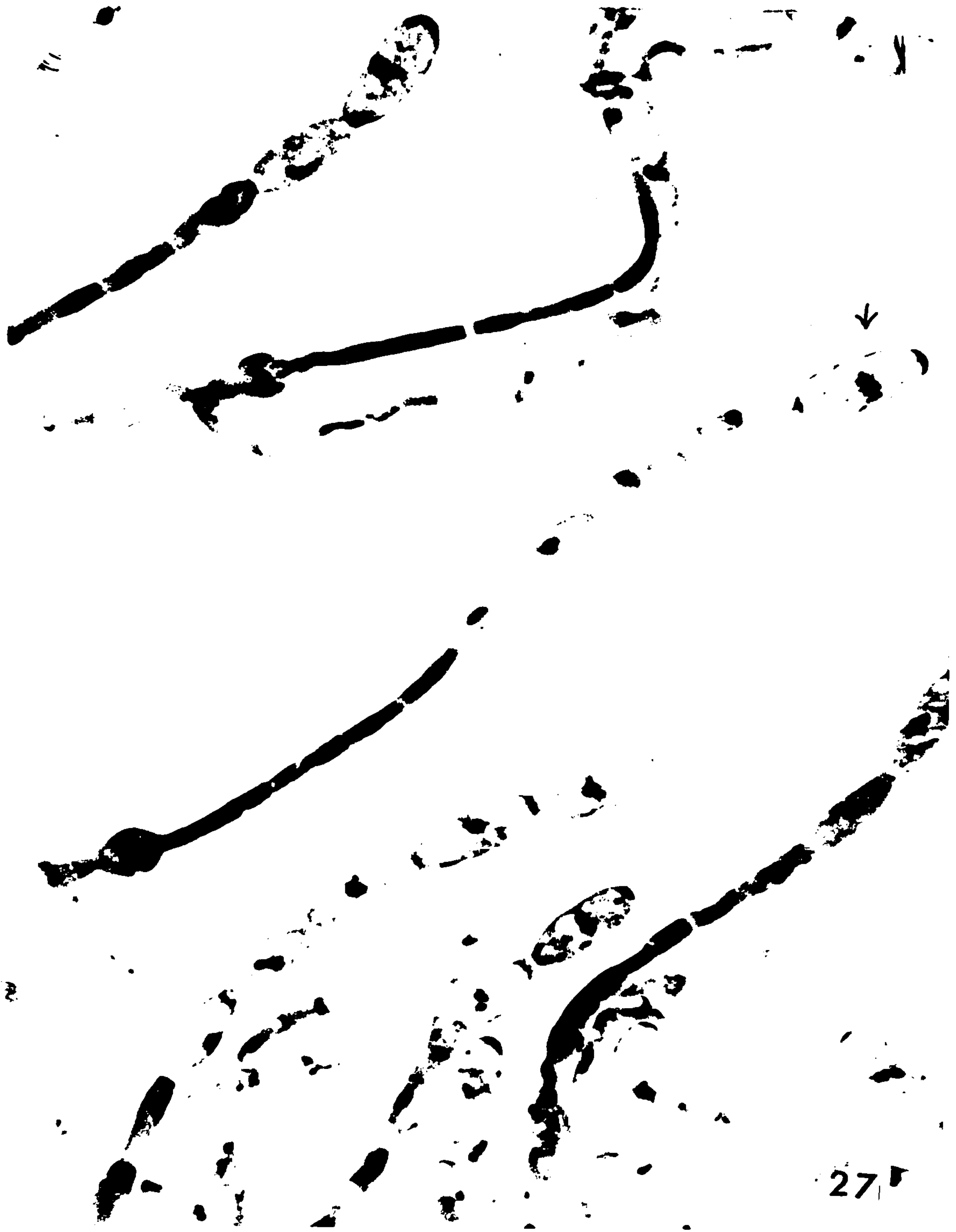

27

A cell wall is formed between the two threads after they separate and move toward the opposite ends of the cell. Elongated thread nuclei are seen after the cell walls are formed. Different stages of condensation of thread nucleus to a round resting nucleus are depicted in Figures 19, 20, 21, 22, 23, 24, 26, and 27.

Figure 20 shows the conidial wall being formed while the nucleus is still elongate. The width of the conidium itself is not larger than that of the neck of the generative cell, indicating a recently formed conidium. As the conidium starts maturing, the nuclear thread shortens.

The gradual condensation of the thread nuclei can be seen in Figures 19 and 20. The nuclei, following cell wall formation, are very long and thin in Figure 19. Some condensation occurs resulting in a somewhat short and a slightly thicker nuclear thread, exemplified in Figure 20.

Further evidence for the gradual condensation of thread nuclei is seen in Figure 21, which shows a chain of 3 conidia in progressive stages of maturation, the bottom one being the youngest. The correlation of the condensation of the thread nuclei with age of the conidium is evident in that the youngest conidium has the longest thread and the relatively mature conidium contains a much shorter and thicker nuclear thread (Figure 21).

In Figure 22, three separate conidia at different stages of development have different degrees of nuclear condensation. Conidium 'a', is the youngest as is shown by its position in the chain. This young conidium has a long

beaded thread. The nuclear thread is composed of seven chromosomes and a satellite. In conidium 'b' which is more mature than conidium 'a' the beaded nucleus is more condensed than it is in 'a'. At this stage the nucleolus begins to appear. Conidium 'c' is the most mature of the three as is shown by its terminal position. The nucleus of the latter conidium is not only more condensed but also has a spherical shape. The nucleolus is very distinct and a nuclear membrane is forming.

The re-appearance of the nucleolus and the formation of the nuclear membrane is illustrated in Figure 24. Although the nucleus has not taken a fully spherical shape it has begun to assume a somewhat rounded form; the nuclear membrane is developing and the nucleolus has re-formed.

Figure 23 depicts all the stages of condensation of the divided nucleus from the thread stage to the condensed round nucleus enclosed in a nuclear membrane and containing a nucleolus. This sequence starts in a newly-formed conidium and progresses to the mature one.

Figure 27 graphically illustrates the final stage of nuclear condensation. Here two conidiophores have 5 mature conidia in each, each conidium with a condensed 'resting' nucleus. The cytoplasm of the conidia contains vacuoles and what may be oil, indicating the maturity of the cell. Figure 28 diagrams the events of mitotic cycle.

FIGURE 28. Diagrammatic representation of the cycle of mitotic division showing all the different stages.

a. Interphase nucleus.

b. Dilation of nucleus.

c. Degeneration of nuclear membrane.

d. Disappearance of nucleolus; chromatin materials in large masses; increased volume of nuclear material.

e. Chromatin materials in a beaded ring, separation of homologous beads at the center of the ring.

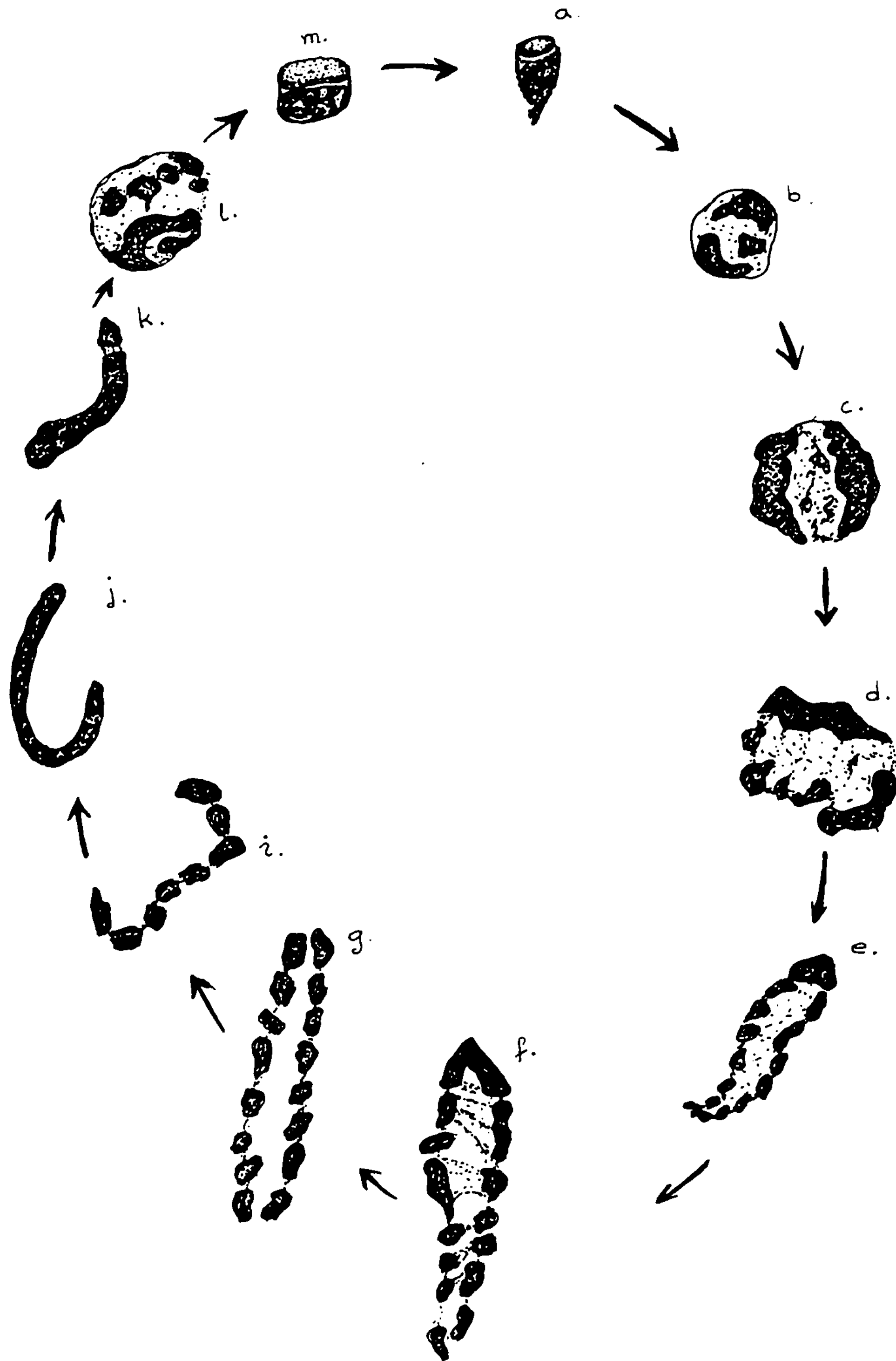
f. Elongation and splitting of the ring.

g. Separated threads.

i-k. Condensation of thread nucleus.

l. Nucleus assumes a spherical shape.

m. Reappearance of nuclear membrane and nucleolus.


a.
b.
c.
d.
e.
f.
g.
i.
j.
k.
l.
m.

C. Time Sequence of Cell Division

It appeared that the full cycle of division is repeated several times within a twenty-four hour period. It was found that at 3 A.M. the nucleus in the generative cell is condensed which shows that no active cell division is in progress (Figure 27). At 4 A.M. the nucleus in the generative cell appears somewhat dilated. Long thread-like nuclear material is often seen in many of the generative cells and 'SG' cells (Figures 19 and 20). In places, the nuclear material is seen to be divided into two linear masses either in the generative and 'SG' cells, depending upon which cell is undergoing cell division.

Division of nuclei in the generative cells is still going on at 5 A.M. In some cases, telophase-like configurations are observed in 'SG' cells. The nuclei of newly-formed conidial cells have a more or less elongated thread-like structure (Figure 17).

At 6:30 A.M. the nuclei in the generative cells are still dividing. In some cases the nuclei have divided and a new cell wall has formed - separating the 'SG' cell from the generative cell. The nucleus in the generative cell is located in the neck region rather than in the wide 'bowl' (Figures 12, 13, 14, 15).

At 7 A.M. the nucleus in the 'SG' cell is in an advanced post-division stage. The nucleus in the generative cell is spherical and condensed and is located in the wide

bowl of the cell rather than in the neck. Also the conidial cells (including 'SG' cells) do not have thread-like nuclei (Figure 24).

Further study reveals that the cycle of nuclear division is repeated several times within 24 hours. Observation shows that at 7 A.M. the nuclei of generative cells and SG cells are condensed and more or less rounded. Materials fixed at 8 A.M. show that the nuclei in the generative cells and SG cells are enlarged and much diffused; a very few of such nuclei are seen to be dividing (Figure 12).

At this point it should be mentioned that a preparation made from material fixed at a specific time, when observed under the microscope, does not reveal a single stage of cell division exclusively, but a mixture of various stages at the same time is seen. However, there was, in general, an abundance of a particular stage of nuclear division at any definite period of development.

At 9:10 A.M. the number of dividing nuclei increases in the generative cells, and elongated threads are seen in SG cells (Figure 18). Long thread-like nuclei are abundant even in the immature conidia, indicating their recent formation (Figure 21). At 10:30 A.M. SG cells are more active and generative cells are dividing at a slower rate although the nuclei in the generative cells are larger (apparently indicating that these nuclei are still in a dividing stage) (Figure 26).

The number of spherical nuclei in the generative cell at 11:15 A.M. is greater than the number of elongated nuclei. The SG cells are no longer dividing but a few generative cells are still dividing (Figure 24).

At 12 A.M. very few nuclei in the generative cells are short and round; most of the nuclei are dividing. A few that are not dividing are very much enlarged; these are at a stage just prior to division. A prophase-like stage is very frequent (Figure 12).

During the day the cycle is repeated slightly faster than it is during the night. The activity of the generative cell is slightly slower at night; however, growth of vegetative hyphae is quite vigorous during the night. The spreading of the mycelial colony during the night is observed to be quite fast. Growing on a vigorous host, colonies no bigger than pin heads (.5 mm) at 9 P.M. increased in diameter 3 to 5 times (2.5 mm) by 3 A.M. in the morning.

At 12:50 P.M. nuclei are condensed but not rounded. These nuclei have a beaded appearance and are not homogeneous. This is seen in SG cells, generative cells and in maturing conidia, indicating that the nuclear division is recently over and the process of condensation is to be followed (Figures 19, 22). At 2 P.M., nuclei in the generative cells and SG cells are not dividing. Some nuclei become rounded whereas others have not

and are still elongate (Figure 21).

Observable active divisions of the nuclei in the generative cells are considerably reduced by 3:00 P.M. At this time, the nuclear configuration is round and compact. The nucleus is present in the 'bowl' of the generative cell (Figure 24).

The cycle begins to repeat at 4 P.M. Great numbers of enlarged nuclei are seen in generative cells and SG cells (Figure 13).

By 5 P.M. the cycle has almost been completed. Hence, in the generative cells few nuclei are dividing but some are enlarged. In many cases, post-divisional stages are observed. For example, double-thread nuclei, condensing nuclei and condensed nuclei are all present (Figure 17).

At 6 P.M. very few nuclei in the generative cells are dividing but most of them are enlarged, which indicates the beginning of another cycle of division. At 7 P.M. nuclei in the generative cells are in 'prophase' stage. SG cells are also dividing.

The cycle of division which began at 6 P.M. is completed by 8 P.M. At 9 P.M. the cycle begins to be repeated but the numbers of dividing generative cells and SG cells observed are fewer. Still another cycle of division was observed to start at 11:45 P.M. but this proceeded at a much slower pace.

FIGURE 29. L.S. of a mature cleistothecium showing asci with ascospores. (*U. necator*) X 1100.

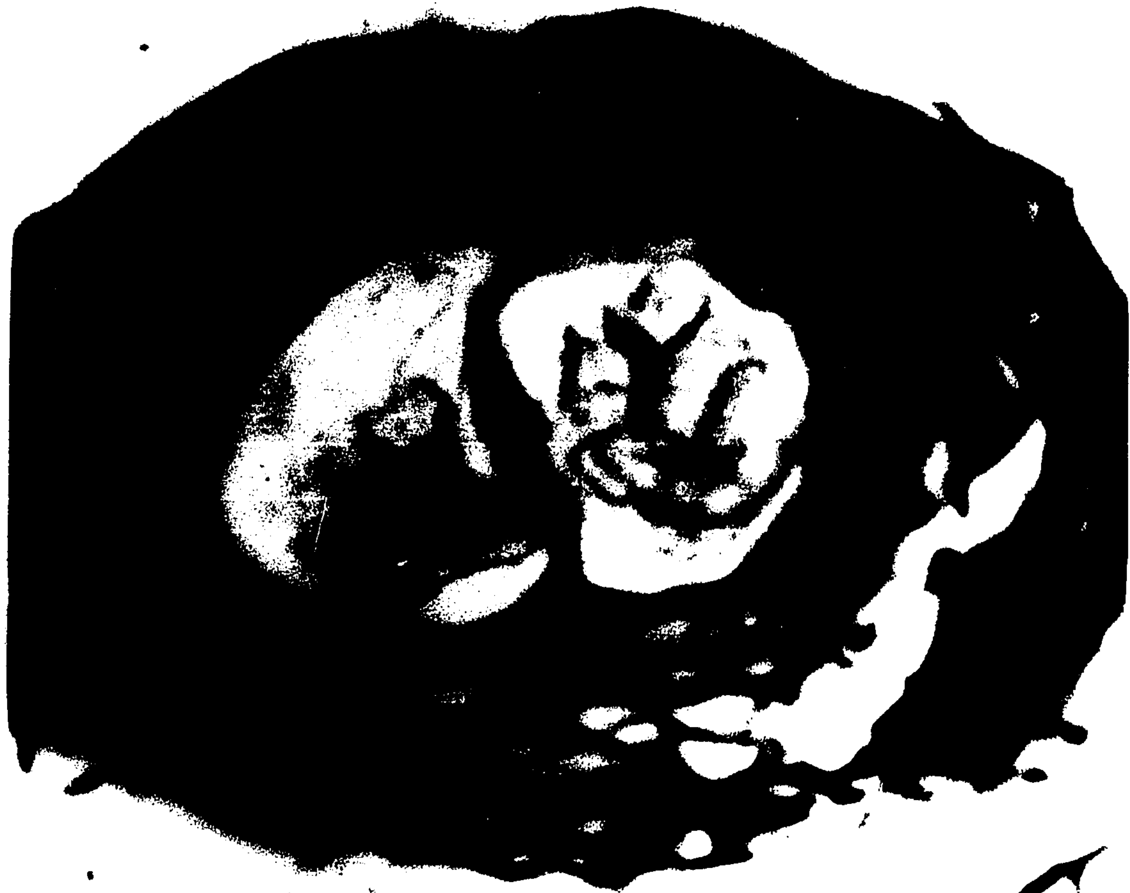


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FIGURE 30. Initiation of cleistothecium formation;

a. ascogonium.

b. antheridium. (_U_. _necator_) X 1200.

FIGURE 31. a. ascogonium.

b. antheridium coiled around the ascogonium. X 1200.

FIGURE 32. a) ascogonium encircled by the b) antheridium one and a half times. (_U_. _necator_) X 1000.

FIGURE 33. a) young ascogonium.

b) antheridium elongated and coiled around the ascogonium; cross walls are visible in the antheridium. X 1200.

FIGURE 34. (a) ascogonium and antheridium (b) are in similar developmental stage as is shown in Figure 33. X 1100.

FIGURE 35. Ascogonium (a) circled by more than one antheridial branch - (arrow). (b) Antheridial branch with binucleate cells. X 1000.

FIGURE 36. Ascogonium (a) surrounded by pseudoparenchymatous cells (b) originated from antheridium. Ascogonium is binucleate. X 800.

FIGURE 37. Binucleate ascogonium (a) encircled by antheridial cells (b). X 800.

b
a
30
a
b
31
a
b
32
b
a
33
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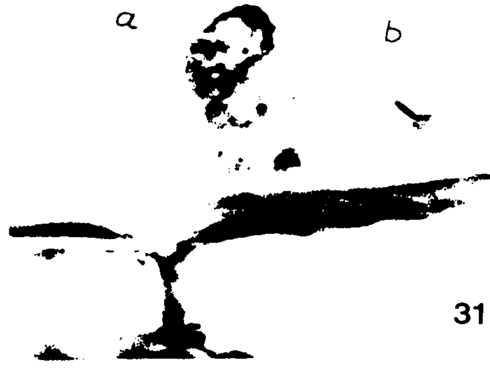
b
34
b
35
a
b
36
b
a
37

30



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D. Development of Cleistothecium

a. Initiation of cleistothecium.

Although the other species of the Erysiphaceae listed in Materials and Methods were also studied, observations merely confirmed the observations recorded herein for _Uncinula_ _salicis_ and _U_. _necator_. Details regarding species other than the latter two are not recorded in this thesis.

The initiation of the fruiting body begins when the antheridium and ascogonium are formed from the superficial vegetative mycleium. Development of the ascogonium begins when the tip of a hypha increases in diameter and curves upward. The cytoplasm appears denser than in the surrounding hyphae probably due to food materials. A uninucleate gametangium is formed when a septum develops thus separating the gametangium from the vegetative hyphae. The female gametangium, which is the ascogonium in this case, is slightly curved and fatter than the male gametangium which is the antheridium (Figure 30).

The antheridial branch arises from a hypha close to the ascogonium and grows upwards. It is relatively slender compared to the ascogonium. The two gametangia are always formed from nearby hyphae and press closely together, but are never seen arising from the same hyphal branch. The antheridium curves around the ascogonium (Figure 31). This is the action most comonly observed. Sometimes, however, more than one antheridium will encircle the ascogonium (Figure 35). Figure 35 also shows one nucleus in the ascogonium. As the uninucleate antheridium surrounds the

FIGURE 38. (a) Ascogonium with two nuclei, Pseudo-parenchymatous cells developed from antheridial hyphae. (*U. necator*) X 1000.

FIGURE 39. The closeness of the two nuclei in the ascogonium (a) indicating imminent fusion in the future. Binucleate pseudoparenchymatous cells are visible (arrow). X 1200.

FIGURE 40. (a) Large ascogonium with two nuclei.
Centrum cells produced by periclinal division of antheridial cells. Arrows indicate binucleate centrum cells. X 800.

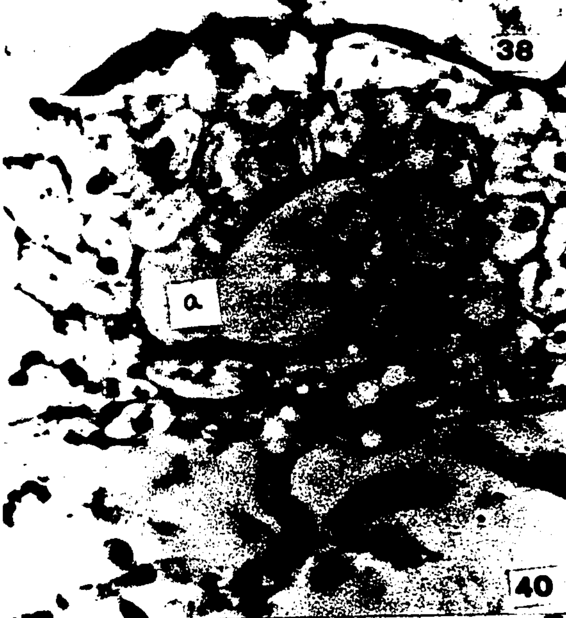
FIGURE 41. (a) Ascogonium
Centrum cells. Arrows showing binucleate cells. X 800.

FIGURE 42. (a) Ascogonium with four nuclei.
Mixture of binucleate and uninucleate centrum cells. Arrow indicates binucleate cell. X 800.

FIGURE 43. (a) Multinucleate ascogonium preceeding cytokinesis.
centrum cells. Arrow indicates binucleate cell. X 1000.

a
38

a
39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a
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a
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a
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a
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a
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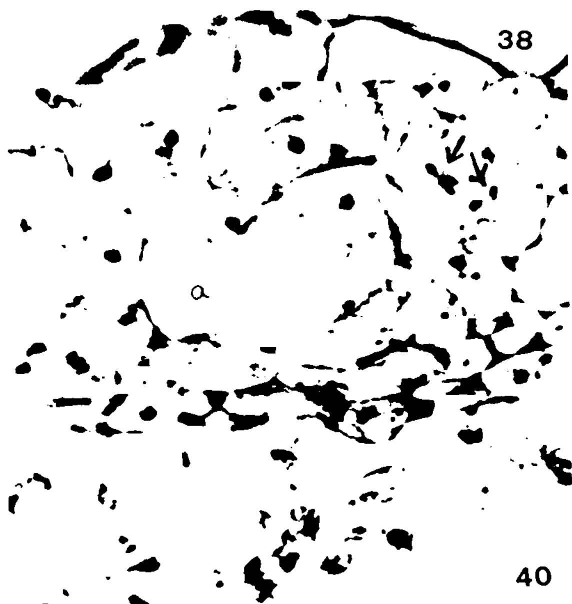
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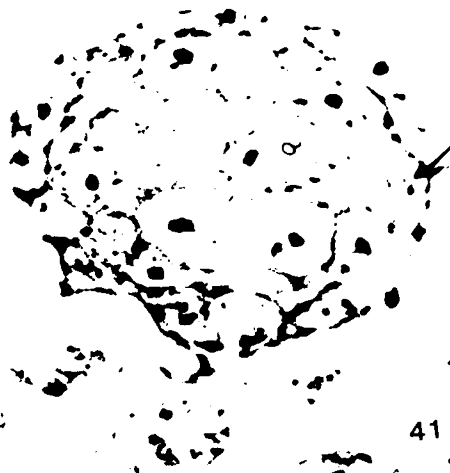
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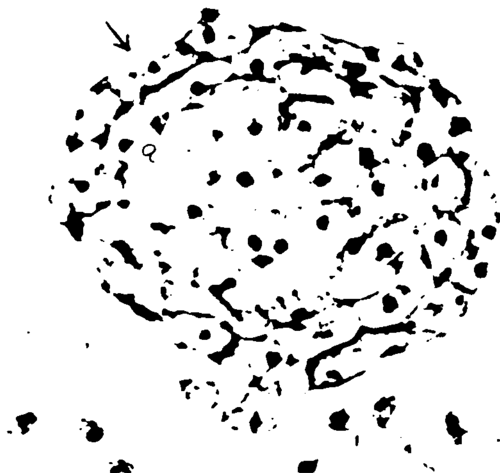
a
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a
a
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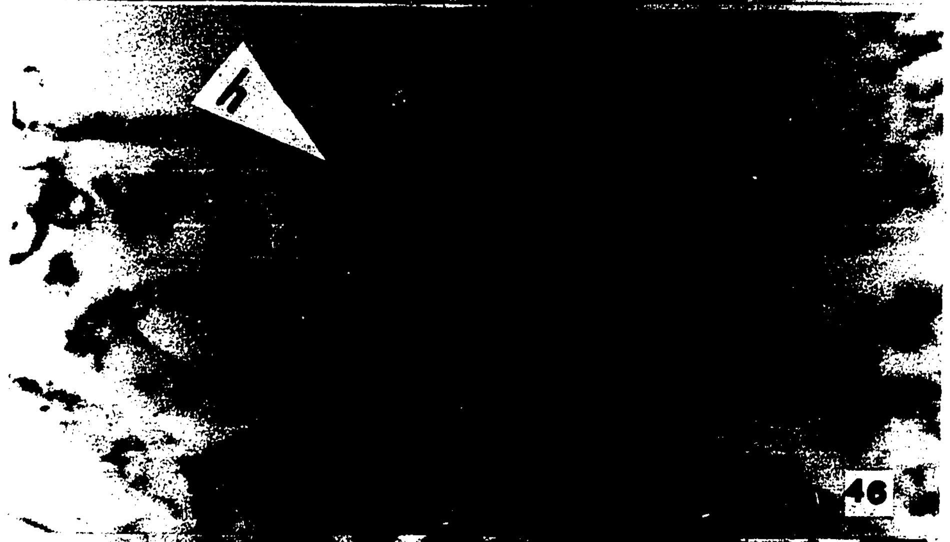
FIGURE 45. Hyphal outgrowth from the peripheral centrum cell are initiated when the cleistothecium is at 1/3 of its mature size. 'h' indicates hyphal growth. X 1600.

FIGURE 46. Further growth of anchoring hypha from the outer centrum cells. 'h' indicates hyphal growth. X 1200.

FIGURE 47. After reaching the surrounding vegetative mycelium the anchoring hyphae develop foot-like structures which encircle the vegetative mycelium (Arrow). 'h' indicates hyphal growth. X 1500.

h
45


h
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h
47

h
h
45
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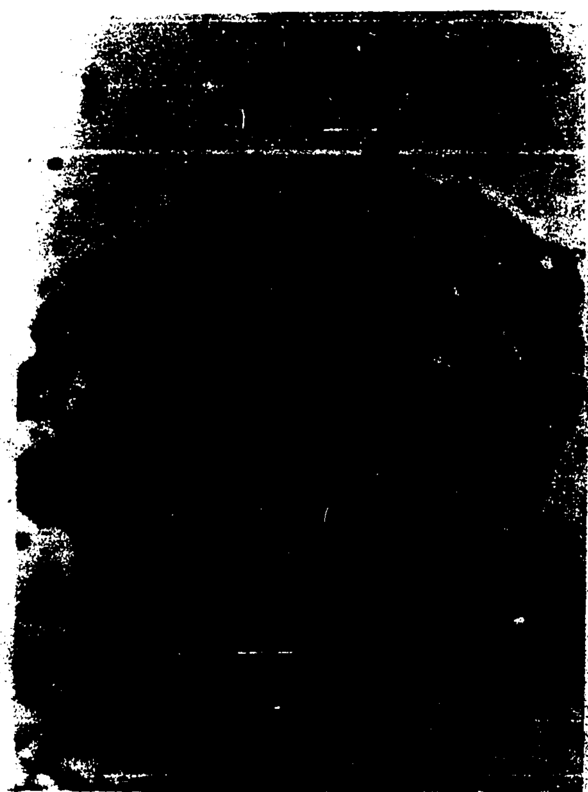
h
47

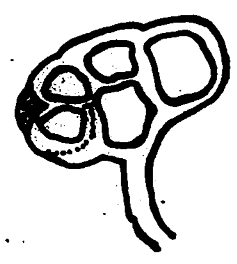
FIGURE 44. Arrow indicating multicellular ascogonium. This stage is found after ascogonium becomes multinucleate (Figure 43). Ascogonium still retaining its original shape. X 1200.

FIGURE 44a. Line drawing showing outline of the multicellular ascogonium in Figure 44. X 1200.

FIGURE 49. L.S. of cleistothecium showing middle region of centrum with dense cytoplasm. X 800.

FIGURE 51. Lysis of the centrum cells is evident. Rows of centrum cells are isolated due to lysis of the surrounding cells. X 600.





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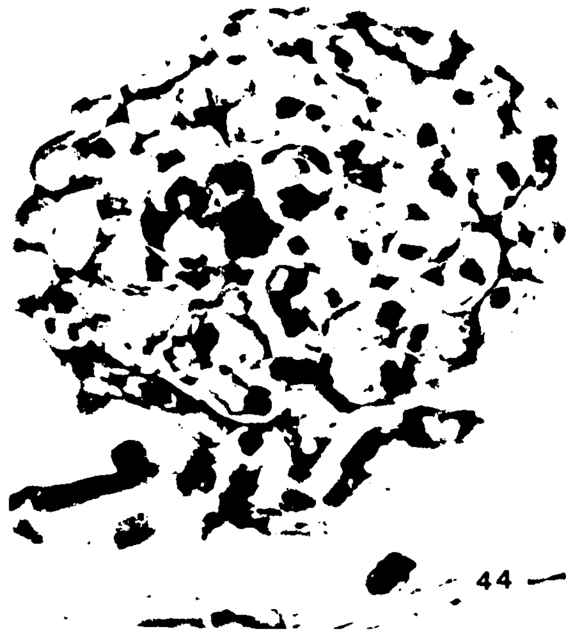

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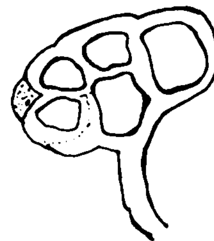

44a

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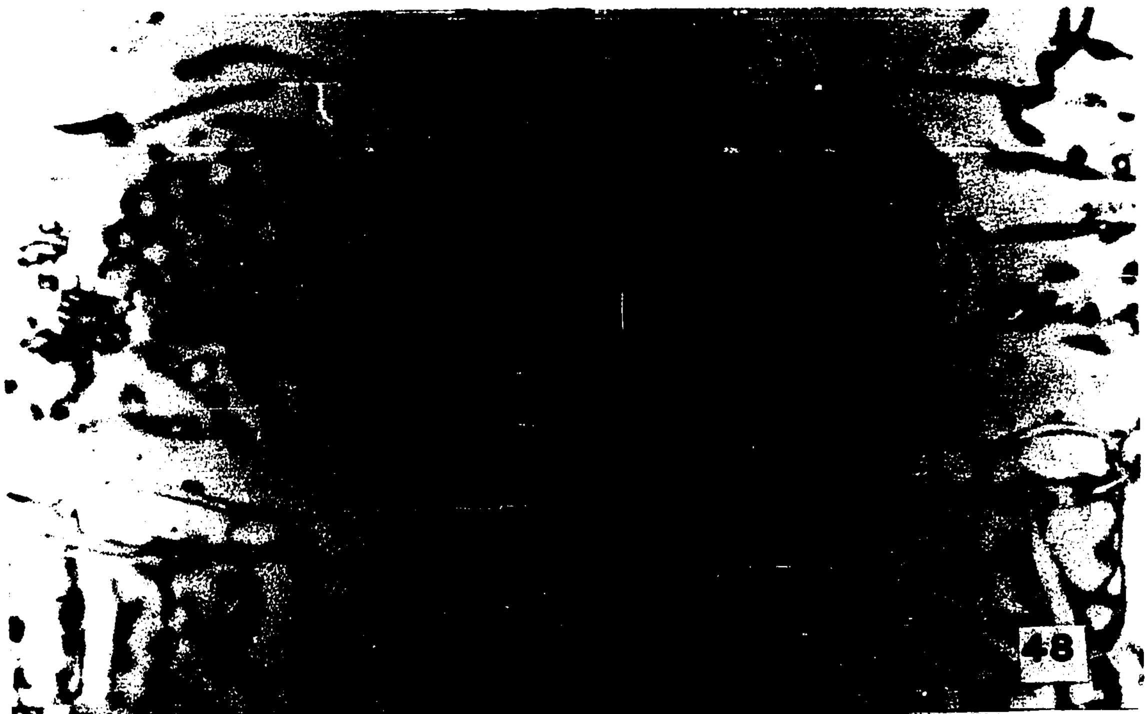
FIGURE 48. L.S. of a group of cleistothecia growing on leaf surface. X 360.

FIGURE 50. A cleistothecium at half of its mature size. At this stage, the ascogonial cells are indistinguishable from the surrounding centrum cells. X 600.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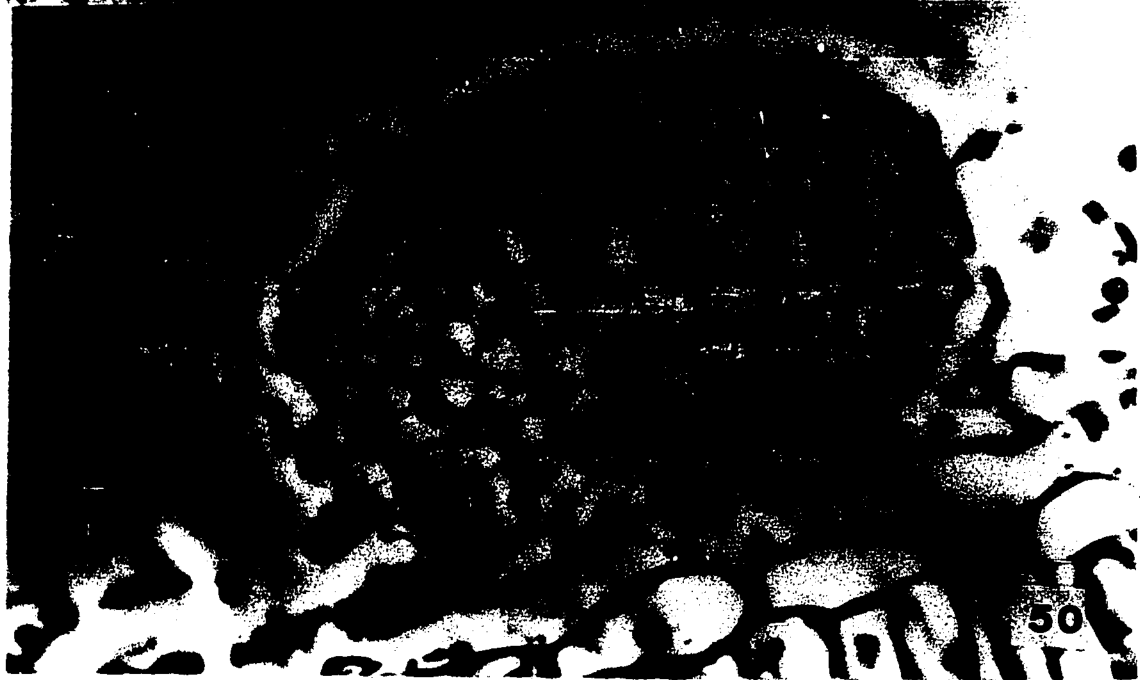
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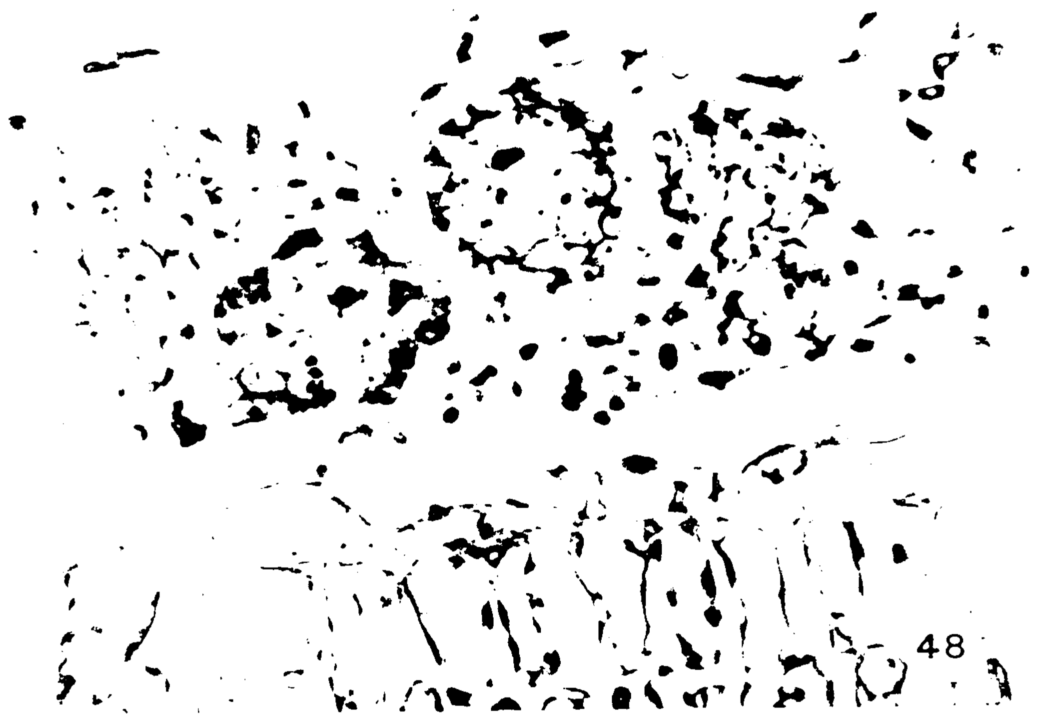
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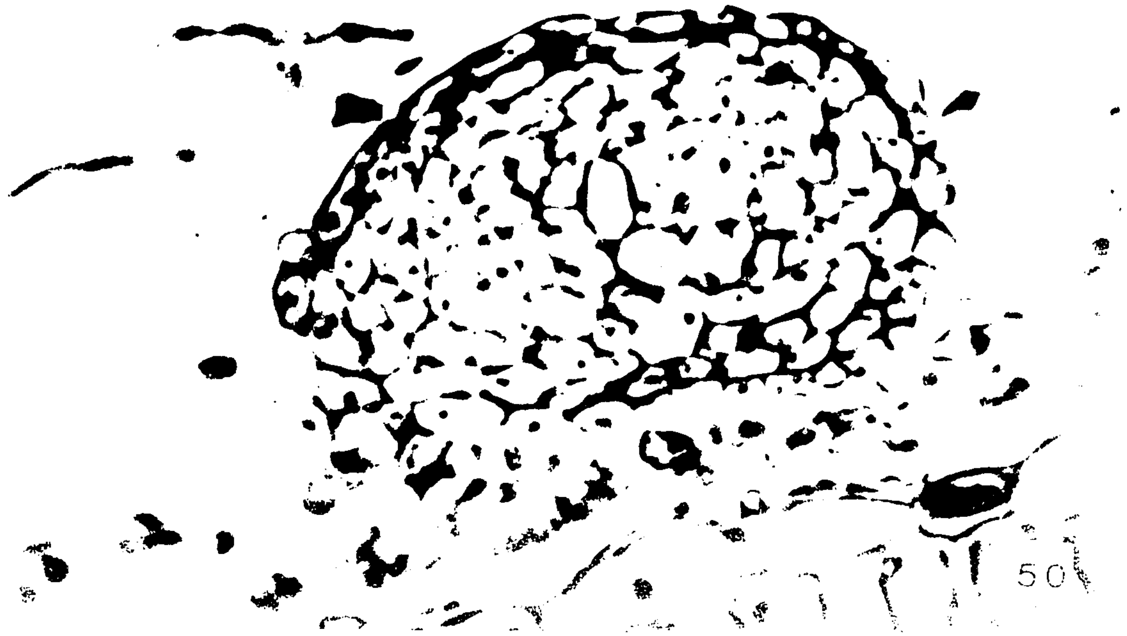
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uninucleate ascogonium, nuclear division and cytokinesis occur in the antheridium (Figure 32). No passage of an antheridial nucleus to the ascogonial cell was observed. Meanwhile, the ascogonium curves and elongates to approximately five times the size of any other cell of the early centrum.

b. Morphogenesis of the ascogonium.

Quite early in the development, although no nuclear passage is seen from the antheridium, the ascogonium is seen to contain two nuclei (Figures 36, 37, 38, 39). Figure 37 is the serial section adjacent to that shown in Figure 36 showing 2 nuclei in the ascogonium. Although no nuclear fusion is observed other cytological changes do occur. Figure 39 shows proximation of two nuclei but actual fusion was never seen. The ascogonium increases in size and nuclear division continues until the ascogonium becomes multinucleate (Figures 40, 41, 42, 43). After the nuclear division the ascogonium undergoes cytokinesis. Cytokinesis of the ascogonium usually occurs in the cleistothecium which is no further developed than those in Figure 43.

Eventually cell walls are formed following cytokinesis resulting in 3 to 5 cells. As a consequence, the curved ascogonium becomes a series of uninucleate cells. Figure 44 shows such a multicellular ascogonium. The individual cells are no larger than the surrounding cells but have denser cytoplasm than other cells. However, the shape of the multicellular ascogonium is still clearly visible (Figure 44). By the time the cleistothecium has developed to over 12

cells in diameter, the density of cytoplasm of these cells decreases and the shape of the ascogonium is no longer recognizable (Figures 48, 49, 50). The largest cleistothecium in which the ascogonium was observed prior to cytokinesis is shown in Figure 40. Lysis of the ascogonium was never evident. The ascogonium apparently does not divide in two planes, nor does it ever give rise to any hyphal structure that penetrates the antheridial cells.

c. Formation of sheath and centrum.

While the ascogonium is undergoing the changes described above, the antheridium is growing in length while encircling the ascogonium (Figure 32). Meanwhile antheridial nuclear division occurs several times, followed by septation of the antheridium. This stage is clearly seen in Figures 35, 36, 37, 38, 39. Passage of an antheridial nucleus into an ascogonium was not observed at any time. In spite of this, at the stage when the antheridium has encircled the ascogonium one and one-half times, the ascogonium already is binucleate (Figure 36). Later the pseudoparenchymatous cells produced by the antheridium divide periclinally and they develop into the centrum. Quite early in the centrum development, bi-nucleate cells are observed (Figure 42). Periclinal division continues, resulting in centrifugal growth of the centrum.

By the time the cleistothecium has grown to 1/3 its mature size, hyphal outgrowths from the peripheral cells of the centrum occur (Figure 45). These hyphae grow downward till they reach the vegetative mycelium on the surface of

FIGURE 52. Fusion nucleus is seen inside each young ascus. The base of the appendage is shown by the arrow. X 367.

FIGURE 53. Three developing asci each containing a meiotic prophase nucleus. X 367.

FIGURE 54. Asci with nuclei in various stages of division. X 367.

FIGURE 55. Ascospores within mature asci. X 500.

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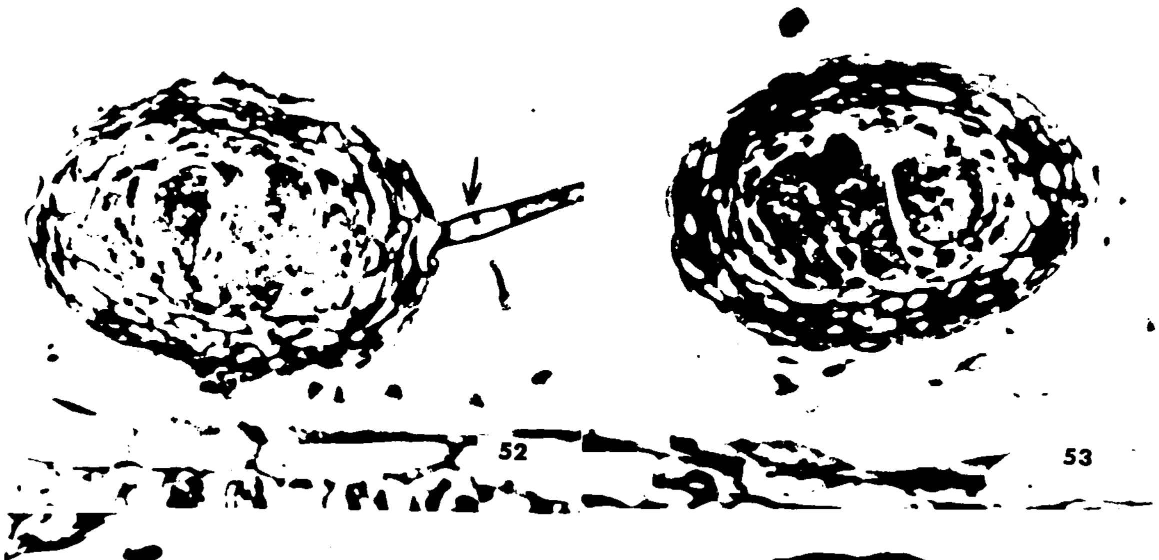
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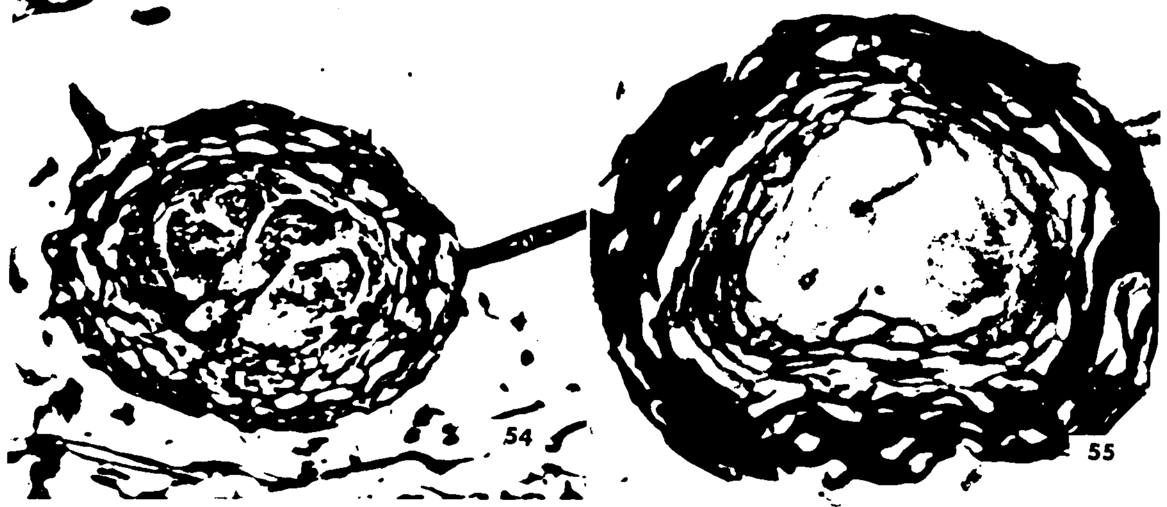
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the leaf (Figure 46). As this happens, nuclear divisions occur and crosswalls form within the hyphae. Each hypha is now 2 to 5 celled with a nucleus in each cell. Having grown downward to the vegetative mycelium, the hypha grows around it to produce a foot-shaped cell that partially encircles the vegetative hypha, thus securely anchoring the cleistothecium (Figure 47). The immature cleistothecium, after reaching its full size, consists of a pseudoparenchymatous centrum, composed primarily of bi-nucleate cells mixed with some uninucleate cells and surrounded by a peridium four to six cells thick. The peridial cells are rectangular and become darkly pigmented; the centrum cells are roughly cuboid.

The next developmental phase occurs when lysis of the centrum begins. This process is initiated in the middle of the centrum and proceeds towards the periphery. Rows of cells lyse from the centre radially towards the periphery (Figure 51). At this time the hyphal outgrowths begin to wither (Figures 50, 51). Maturation of the cleistothecium results in pigmentation and thickening of the cell wall of the peripheral cells (Figure 52).

d. Formation of asci and ascospores.

Into the area resulting from lysis, 3 to 5 cells increases in size eventually developing into asci. These cells are more or less isolated by lysis. Apparently karyogamy occurs within these bi-nucleate cells although actual karyogamy was not observed. Ascus cells enlarge immediately after karyogamy. These cells have the same

FIGURE 56. Asci with fusion nuclei (f). X 800.

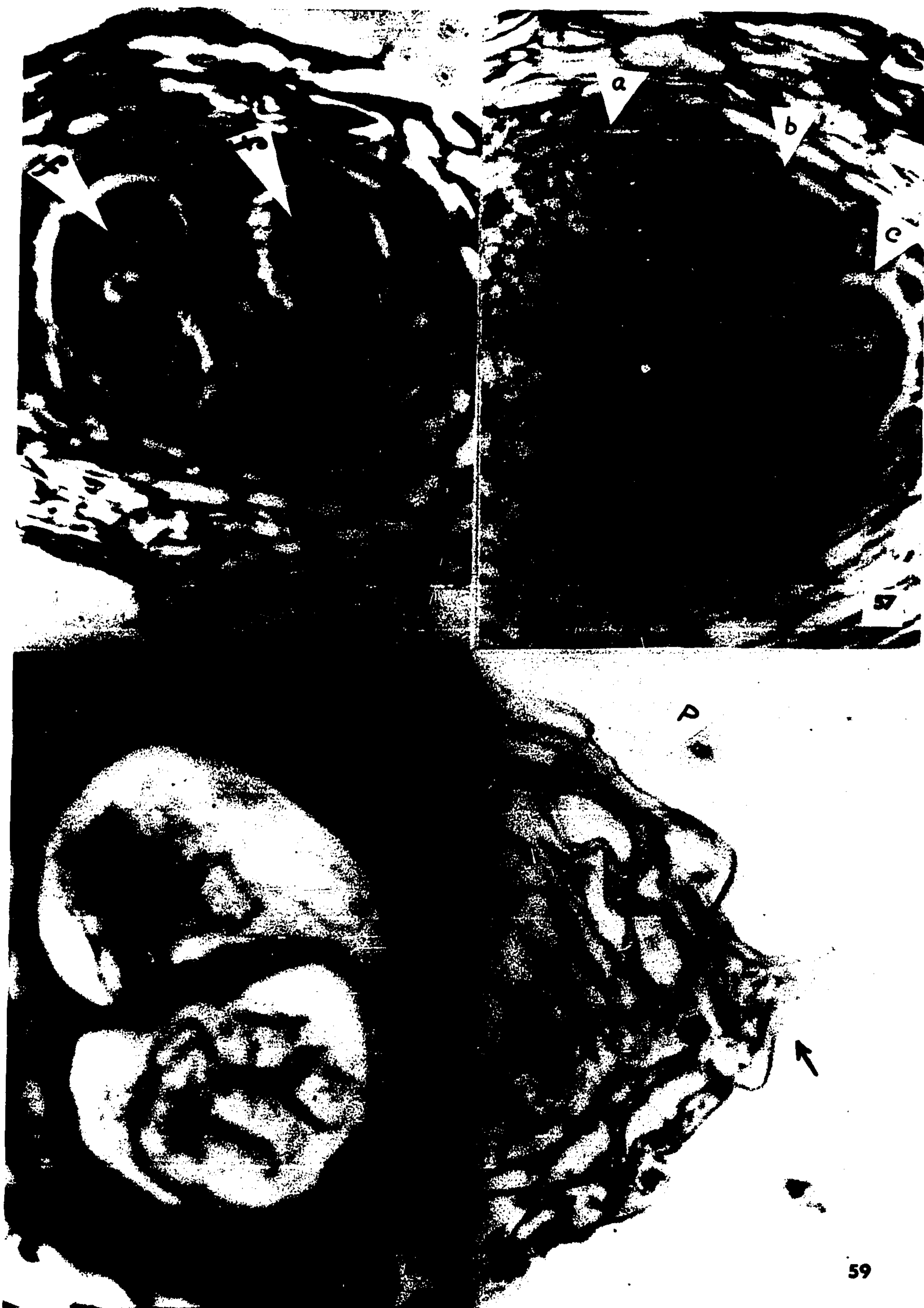
FIGURE 57. (a) Non-dividing fusion nucleus

(b) Metaphase stage of meiotic division in the ascus

(c) Nucleus showing early anaphase of meiotic division. X 953.

FIGURE 58. Mature asci with ascospores. Each ascus contains five to eight ascospores. Three to five asci are generally found in each cleistothecium. (U. necator) X 1100.

FIGURE 59. Arrow showing development of appendages from peridial cells of the cleistothecium. X 1100.


a
b
c
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59

56

a
57

58

cytoplasmic consistency as the surrounding loose pseudoparenchymatous cells. Dissolution of cell walls by contact of the ascus wall and a centrum cell wall was not observed. The enlargement of the asci continues until the asci have filled the central region of the centrum. Mature asci are attached basally to the centrum cells (Figures 52, 53, 54, 55). When the ovate ascus attains its full size, the nucleus divides meiotically to produce a multinucleate stage (Figure 53 shows dilated large nucleus prior to meiosis). Figure 54 shows several stages of the division of the nuclei within the asci. In Figure 57 early prophase (a), metaphase (b) and anaphase (c) of the nuclei within the asci are clearly visible. This is followed by rounding of cytoplasm and by spore wall formation, resulting in ascospores.

At maturation, the cleistothecium contains 3 to 5 asci with ascospores (Figure 58). The remaining few layers of the pseudoparenchymatous centrum are still visible. The outer peridial layer is made up of four to five layers of flattened thick-walled pigmented peridial cells (Figures 55, 58, 59). From these peridial cells, the cleistothecial appendages develop (Figure 59). Appendages were never apparent prior to karogamy and meiosis in the ascus.

DISCUSSION

A. Mitotic Division.

The structure of the vegetative nucleus and the mechanism of nuclear division in fungi still are matters of controversy. As pointed out by Pinto-Lopes (1949) in his review of the nuclear structure of fungi, because of the smallness of the nucleus, investigators have used techniques which are suitable for the mere detection of fungal nuclei at the expense of nuclear details. It is clear that this difficulty in differentiation in nuclear detail has contributed much to the confusion which is so evident in the literature.

Olive (1953), in his review, doubted the nature of Wakayama's (1931) karyosome and attributed it to improper fixation and staining. Olive mentioned that "few of the resting nuclei were properly fixed", (these were classic resting nuclei) and regarded other nuclear configurations as artifacts, caused by the collapse of chromatin around the nucleolus, since the latter nuclei did not resemble conventional dividing nuclei.

However, the results of investigations by various authors on different fungi indicate that, at least in some, the vegetative nuclear division does not follow the pattern of commonly observed mitotic division, for example, Smith (1923), Varitchak (1931). Hall (in _Monilinia_, 1963), Yamasaki and Niizeki (in _Piricularia_, 1965) and Knox-Davies (in _Macrophomina_, 1967) describe somatic nuclear division as 'mitotic', linear nuclear configurations were observed

by all these authors and interpreted as migrating nuclei. Moore's (1964) description of 'Karyochorisis' in Cordyceps is an electron micrographic confirmation of 'direct division' in this species, with particular emphasis on a description of the mechanics of the nuclear membrane during nuclear division. Duncan and MacDonald (1965) in Marasmius and Hejtmanek and co-workers in Microsporum observed with some modification that somatic nuclear division was longitudinal.

Weijer et. al. (1965) described a method of division (Karyokinesis) in Neurospora which is based upon the longitudinal division of a nucleus consisting of a Feulgen-positive triangular plate centriole and seven chromosomes, attached end-to-end in a linear order by a weakly Feulgen-positive thread. Different nuclear cycles have been described for different stages (hyphae, macroconidia and microconidia) in the life cycle. These nuclear cycles are basically similar in their mode of division except that the chromosomes and the centrioles become much larger (polytene) in the developmental stages which lead to the formation of macro- and microconidia.

Other cytologists such as Namboodiri and Lowry (1967), Bianchi and Turian (1967) and Wilson et. al. (1966) have contributed information with regard to the somatic nuclear division of N. crassa. While not agreeing in toto with observations of Weijer and co-workers, their conclusions have favored longitudinal division of a linear and filamentous nucleus.

The present investigation was conducted keeping in mind

that vegetative nuclear division in the Erysiphales may resemble mitosis of the vascular plants, etc., or that it may differ from classical mitosis.

The undividing vegetative nuclei of Erysiphe graminis have the classic configuration of fungal nuclei. The nuclei are somewhat conical or spherical in shape, they have a conspicuous nucleolus attached to a considerable amount of nuclear material. A pair of satellites is also seen attached to the chromatin material (Figures 25, 5). This kind of nuclear structure is described by many authors, e.g. Olive (1953), Varitchak (1931), Bakerspigel (1956), Somer's et.al. (1960) and Lu (1962, 1964).

What puzzled many investigators is that many of the fungi have nuclei that are mostly elongated thread-like or rod-like or consist of masses of chromatin materials in beaded form, the beads attached to one another in a string.

Various authors described these nuclei and explained them as artifacts or as migrating nuclei or as degenerating nuclei.

Many discrepancies in techniques and observations are evident among the studies of fungus nuclei:

1. Somers et. al. noted that although Bakerspigel used the same techniques as they used and obtained the same nuclear configurations, their observations and conclusions were very different;

2. Ward and Ciurysek explained that although Bakerspigel had used the same staining technique (Giemsa) as they had, his observations were the result of extensive overstaining

of the preparations (12 hours) as opposed to their own which were stained for 15 minutes;

3. Bakerspigel, who also used Feulgen technique, obtained results and conclusions which are very different from those reported by Weijer _et_. _al_. (1965), using the same technique.

To understand how these discrepancies might occur, one must appreciate the tremendous difficulties associated with the study of a nucleus as small as the one usually present in the hyphae of fungi. Not only may a particular stain block out the fine details of the nucleus (as suggested by Pinto-Lopes (1949), but it may also vary greatly in density of stain. The variance can be observed within a particular slide preparation as well as between different slides. Moreover, a particular stain may be suitable for staining nuclei in certain structures but unsuitable in others. Cytologists are generally wary of these difficulties and compensate for them, by looking for a 'good nucleus' or a 'good slide'. All other nuclei or slides are rejected as 'poorly stained'. When many observations are made of a particular 'poorly stained nucleus', the cytologists modify either their techniques or their conclusions. It is around these various interpretations of nuclear configurations that the controversy exists.

The reason that there exist so many different views with regard to somatic karyokinesis in the fungi is mainly due to the difficulties encountered in studying chromosomes which are close to the extreme limit of resolution of the light microscope.

With the improvement of staining techniques and with better microscopic facilities, many authors believe beyond doubt that linear nuclei are not artifacts or degenerating nuclei.

Erysiphe graminis DC. was chosen for the present investigation because the generative cell is relatively large with a large nucleus in it. Because of the large size of the nucleus it is not necessary to overstain and thereby obscure nuclear details.

Feulgen DNA reaction has been accepted as standard conclusive evidence of nuclear chromatin.

Crystalviolet stained chromatin, as did Feulgen, but in addition crystalviolet always stained other micro-structures in the cell. Lu's (1962) success in staining fungal nuclei with propionocarmine stain encouraged application of this stain in the present investigation and it was found very satisfactory.

Hydrolysis with 5N HCl at room temperature gave rise to consistently good results with the Feulgen reaction as compared to the inconsistent results experiences with 1N HCl at 60°C (Decosse and Aiello, 1966).

Weijer, et. al. (1965) in describing the cycle of mitotic division in Neurospora, attempted to establish a definite pattern. He described that the condensed round nuclei becomes a double ring which divides into two single-ring nuclei. Next two sequences of events are possible:

(1) A filament phase develops directly into a Ring phase, after which breakage of the single ring takes

place thereby yielding a filament which divides longitudinally, or

(2) A Filament phase resolves into a double-Filament phase and subsequently into a double-ring phase either by (a) separation of individual daughter rings (which after breakage yield two single filaments) or, alternatively (b) the Double ring phase breaks and gives rise to a filament which divides longitudinally.

"The authors are aware of the inelegance of these alternate karyokinetic pathways but the evidence is not sufficient to propose the sequence "Single Filament-Double filament-Double ring-Ring breakage (Horsehose phase-single filaments (separation)." (Weijer, 1965).

Since conidia of *Erysiphe graminis* are formed progressively in chains, the sequence of vegetative nuclear division in the conidia can be easily established. Also the various stages of nuclear division in the generative cells are consistently correlated with the position of the nucleus within the generative cell. The stages of condensation cannot be artifacts because, within a conidiophore, the progressive stages are visible from the youngest conidium with the least condensed thread-nucleus to the most mature conidium with a condensed round nucleus.

When the nucleus in the generative cell is not dividing, it is typically spherical in shape and stays inside the bowl (Figures 10, 12a). This kind of nucleus has been seen by Allen (1936) in vegetative mycelia of *Erysiphe*.

During division, the neck of the generative cell elongates to twice its original size and the nucleus moves upwards (Figures 9, 12b, 15), while the nucleus itself is going through a series of changes.

The SG cell, when dividing, also undergoes changes of size, and the nucleus also undergoes changes (Figures 17, 18). In Erysiphe the nuclear changes prior to the splitting of chromatin materials and condensation of the nucleus that follows are synchronized with the structure of the generative cell, the 'SG' cell and the conidia.

The interphase nucleus is always situated inside the wide bowl of the generative cell. The first indication that the cell is about to divide is the elongation of the neck of the generative cell. As the neck elongates, the nucleus moves upwards. How far the nucleus has moved up into the neck of the cell is correlated with how far the division has advanced. Advanced stages of division (for example, beaded-ring or elongated beaded double threads) are never seen in the bowl or in the basal portion of the neck. They are always situated at the middle region in the neck. The dividing cell wall is formed when the double threads separate and one thread moves upward and the other moves downward.

After the cell wall is formed, producing a conidium, the thread nucleus begins to condense. This process of condensation of the nucleus is also synchronized with the process of maturation of the conidium. Nuclear condensation within a conidiophore is correlated with all stages of conidial maturation, viz., the position in the conidiophore,

the increase in width, the thickening of the cell wall and vacuolation. Hence the sequence of stages of condensation in the nucleus can be determined by the position of the conidia in the conidiophore.

That the SG cell divides into two is also evident from the size of the SG cell; it may elongate to twice its original length prior to division (Figure 18).

As the journey of the nucleus continues toward the neck, the nucleus becomes more expanded and the nucleolus eventually disappears (Figure 13).

Lu (1962) and Weijer (1965), reported that the nuclear membrane disappears during division, although Olive (1953) mentions that fungal nuclear division occurs within a nuclear membrane. Although a nuclear membrane has been demonstrated around hyphal nuclei of *Aspergillus niger* (Tanaka and Yanagita, 1963a, 1963b), its integrity throughout the entire division cycle has not been proven as yet. Moore (1964), in an electron microscope study of *Cordyceps militaris*, was able to demonstrate that the nuclear membrane of this Ascomycete remained intact throughout the division. In *Erysiphe graminis* DC, the nuclear membrane completely disappears before the chromatin material takes on the beaded ring configuration (Figures 14, 12b, 15).

After the disintegration of the nuclear membrane, the nucleus still retains its round shape (Figure 13). Gradually the round shape of the nuclear material gives way to an irregular shape (Figure 14). This stage is interpreted as more or less equivalent to the prophase stage of standard

karyokinesis.

The nuclear material then becomes more diffused and occupies a larger volume in the cytoplasm, and forms a large beaded oval ring. At this beaded oval stage, more chromatin material accumulates on the chromatids (Figure 12b). The ring is formed after the chromatin materials are duplicated and homologous beads are slightly separated from each other in the center of the ring, though separation is not complete. This beaded ring structure was also noted by Weijer et. al. (1965), in Neurospora. However, McDonald (1970), stated that ring configuration of a condensed nucleus is different from the ring which is formed due to median separation of daughter nuclei. Bakerspigel (1962) reported that a ring is formed due to the fact that the chromatids envelop a Feulgen-negative spherical nucleolus at the center, resulting in a ring.

In the present observation, it is shown clearly that the nucleolus disappears at the onset of division (Figures 13, 12b).

Instead of the separation of homologous beads at the center, resulting in ring formation, the separation occasionally begins at one end resulting in a split-end elongated thread-like appearance (Figure 16). Therefore, ring and split-end thread are not two different phases but two different configurations of the same phase of nuclear division. This stage in which chromosomes are duplicated but not completely separated is considered to be comparable to metaphase, although no classic spindle is seen (Figure

12b).

Somer's _et_. _al_. (1960) and Ward and Ciurysek (1962) believed that _Neurospora_ has a classic mitosis and both described anaphase and metaphase figures; however, the criteria as set down by Robinow (1956) of a recognizable spindle apparatus together with a resting and dividing stage of the nucleus, were not met. Bakerspigel (1959) and Robinow and Bakerspigel (1965) described division as being a direct division of the nucleus as depicted by Smith (1923) in _Saprolegnia_; and Smith named this type of mitosis "direct division". The nuclear configurations which he termed "direct division" are similar to the "middle stage" nuclear configuration in _E_. _graminis_. However, in _E_. _graminis_, at the "middle stage", when homologous beads separate from each other there are visible cross fibrils connecting these beads. It is possible that these may act as the spindle fibres. Electron microscopic study could possibly reveal the nature of these fibrils (Figure 15a). A weakly Feulgen-positive thread connecting the chromosomes which gives the appearance of a beaded thread, always appeared to pass through, and not around the chromatin bodies. Since it is connected with the chromosomes, we judge it to have intranuclear continuity and hence may refer to it as a nuclear structure. The fiber was often observed to be double-stranded, with separation between the individual strands, especially where it appeared next to separating daughter chromosomes (Figure 15b).

For counting chromosomes, the most suitable stage is that in which the nuclear beads are clearly separated from

each other in the nuclear thread and the homologous pairs are quite distinct. At this stage, chromosomes can be counted without ambiguity (Figure 15).

After the splitting of threads is complete, the two newly-formed thread nuclei sometimes lie side by side for a time, move toward the two ends of the cell, and a partition is formed between them (Figures 19, 20). Somers *et. al.* (1960) also observed long threadlike nuclei in 'telophase' of *Neurospora*.

As separation proceeds, the threads may become bent or twisted, resulting in several different conformations which are shown in Figures 17, 18. Weijer and others (*loc. cit.*) have also seen this kind of configurations.

After separation, the thread nuclei begin to condense and eventually assume a conventional round form (Figures 22c, 24). Varitchak (1931) observed linear end-to-end nuclear arrangement during division and reported that, after the chromosomes are separated and move apart, the linear daughter nuclei resume a spherical shape. Weijer's (1965) observations are also fundamentally the same as Varitchak's although his description of intermediate stages is different.

Different stages of condensation of the thread nucleus into a round resting nucleus are depicted in Figures 20, 21, 22, 23, 24, 26, 27. Various authors have observed similar stages of condensation (Weijer, 1965) etc., but there is much variation in the published interpretations of such stages. In *E. graminis*, since the chains of conidia grow acropetally, the sequences of nuclear condènsation can be

determined with certainty.

Figure 20 shows the conidial wall being formed while the nuclei are still elongated. As the conidium starts maturing, the nuclear thread shortens progressively. The thread nuclei can be seen in Figures 21 and 22. The evidence for the gradual condensation of the thread-nuclei is clear in Figures 21 and 22. Figure 21 shows where there is a chain of three conidia in progressive stages of maturation, the bottom one being the youngest. The correlation of the condensation of thread nuclei is clearly demonstrated in Figure 21. Figure 23 depicts all the stages of condensation of the divided nucleus from the thread stage to the condensed round nucleus enclosed in a nuclear membrane and containing a nucleolus. This sequence starts in a newly-formed conidium and progresses through to the mature one. Figure 27 shows a conidiophore with about six mature conidia all with round condensed nuclei in them.

Diurnal Cycle of Mitotic Nuclear Division

During the investigation of the structure and mechanism of mitotic division, it was also concluded that the full cycle of division is repeated several times within a twenty-four hour period. From the method of collection of material at frequent intervals during the day and night and from extensive observations, periodicity of cell division, can be concluded beyond doubt (Refer to Materials and Methods and Observations on conidia). In *E. graminis* the conidia develop in chains, several mature conidia remaining attached to the

conidiophore (Brodie, 1945). If several conidia are to develop in a day, the division cycle has to repeat several times to produce that many conidia.

It is observed that the onset of cell division occurs at slightly before 4 A.M., 8 A.M., 12 A.M., 4 P.M., 6 P.M., 9 P.M. and 1:45 P.M. Most cell division occurs between the hours of 4 A.M. and 6:30 A.M. During this interval all stages of division are seen in great abundance. Since nuclear divisions follow in rapid succession, the time required for each cycle must be short. Hence the 2½ hour period (4 A.M. to 6:30 A.M.) is that in which maximum cell division occurs in generative cells and SG cells. This period of rapid cell division begins to end around 7 A.M. at which time the number of dividing nuclear configurations become fewer and condensed nuclei are seen more frequently.

Other periods do not have this rapid and frequent cell division. For example, although an onset of the nuclear division cycle is observed at 11:45 P.M., the number of nuclei seen in dividing stages is considerably less. Therefore, it was concluded that this period of nuclear division has the fewest number of cell divisions. It should be noted that, although the division of generative cells and SG cells slows down, resulting in comparatively fewer conidia, the vegetative nuclear division of hypha increases markedly. It is during this time that the greatest extension of vegetative growth occurs. Since maximum vegetative growth occurs during the period of minimum conidial formation one may postulate that vegetative growth and conidia formation occur in a

rhythm.

During the 14 hours between 8 A.M. and 10 P.M. the cycle of division repeats at least five times but dividing cells are moderate in number - neither few nor many. During this time period, dissemination of conidia is at its greatest, as has been reported by Yarwood (1936) in *Erysiphe polygoni* and by Childs (1940) in powdery mildews including several species which produce their spores in chains. Therefore, it is evident that, during the day time, spore discharge is more prominent whereas spore formation is not.

In summary, the vegetive growth is at its maximum during late night and early morning. Following this, maximum conidial formation occurs in the morning. Later in the day spore discharge is at its maximum. Yarwood (1936) Pady *et. al.* (1969) stated that no detectable diurnal periodicity is found in *E. graminis*. Since conidiophores have a life span of 6-8 days, it is evident that new conidiophores as well as new conidia are constantly developing. Therefore, it is not possible to detect the diurnal cycle of individual conidiophores. Gregory *et. al.* (1952) reported that *E. graminis* continued to display diurnal periodicity in darkness which suggests an endogenous rhythm in spore release. This endogenous rhythm may very well be the reflection of the rhythm of nuclear division. Structurally there is a relationship between nuclear condensation and the maturation of the conidia (Figure 23). Therefore, when several fully condensed nuclei are seen in a conidial chain (Figure 27), those conidia containing

condensed nuclei must be mature. Although the evidence presented herein establishes a diurnal regularity in nuclear division, the relationship between this and the time of dispersal of conidia is a complex one because of the possible adherence of conidia in chains. Any direct relationship between these stages of the life cycle could only be detected by further study.

Development of Cleistothecia

The few species that have been investigated here cannot be considered as representative of the entire order Erysiphales. However, uniformity of the morphogenesis of the cleistothecium is clearly evident among the species studied. This evidence suggests that the typical morphogenesis of the cleistothecium of the Erysiphales may be represented by this investigation.

a. Gametangium

In the present investigation, structurally differentiated antheridia and ascogonia were observed. The ascogonium is identified as such on the basis that it is from this cell that the asci eventually arise; the antheridium is identified as such on the basis that it is the encircling cell. This is in agreement with the observations of many other investigators, namely Colson (1938), Gordon (1966), whose opinions as to the function of antheridium and ascogonium varies greatly. On the other hand, Allen (1936) believes that any two hyphae may function as gametangia. The present study does not confirm Allen's (1936) contention of structural

degeneration of sex organs. A morphologically distinct antheridium and ascogonium are depicted in Figure 30..

Generally the function of the ascogonium is considered to entail fertilization by the antheridium and production of asci. In this study, fusion of the ascogonial and antheridial nuclei was not seen, although a binucleate stage in the ascogonium was seen at a very early stage of development. It may be that the presence of an antheridium stimulates the ascogonium to become active, resulting in the division of the single nucleus within the ascogonium. When the latter becomes a multinucleate cell walls are formed to produce several cells. Dangeard (1907), Moreau and Moreau (1930, Eftimu and Kharbush (1928), and Bezssonov (1914), also believe in the apogamous development of the cleistothecium. Colson (1938) did not observe any sexual fusion of nuclei within the ascogonium. de Bary (1887), Harper (1905), Blackman and Fraser (1905) believed that a fusion of sexual nuclei occurs. Allen (1936), reported fusion of male and female nuclei within the ascogonium; she also reported that the fertilized ascogonium degenerates. Gordon (1966), however, postulates that fusion of nuclei occurs in the ascogonium and that, although the diploid ascogonium does not degenerate, it does not take part in the production of asci. For the latter reason he named the structure a 'pseudoascogonium'.

Since Figures 35, 36, 37, 38, 39, show that the antheridial branch divides periclinally to produce the pseudoparenchymatous cells of the centrum, it is evident that the function of the antheridium is modified. It no longer

supplies a nucleus to fertilize the ascogonium but it does contribute to ascogonium development.

b. Centrum

As has been previously mentioned, the pseudoparenchymatous cells produced by peridinal division of antheridial cells make up the centrum. Gordon (1966), found similar development of the centrum from the antheridium by periclinal division. Therefore, the present investigation is in complete agreement with Gordon, regarding centrifugal development of the centrum. This study further agrees with Gordon that the centrum cells are binucleate.

Gordon postulated a mechanism by which this binucleate state is achieved. He felt that receptive hyphae grow out from the peripheral cells of the centrum and contact the vegetative mycelium. Nuclei from the vegetative mycelium migrate through the receptive hyphae to make centrum cells binucleate. The lack of trinucleate cells in the receptive hypha, as observed in the present investigation, tends to contradict Gordon's theory.

The method of attachment of the receptive hyphae leads one to believe that the function of these hyphae is anchorage, not that of acting as nuclear receptors (Figure 47). Further evidence from this study that these anchorage hyphae do not function to transmit nuclei from vegetative hyphae to the centrum is provided by the observation that the centrum cells are binucleate, long before these anchoring hyphae are produced (Figures 39, 40).

Lysis of the pseudoparenchymatous cells begins in the

middle of centrum when it reaches about half its mature size (Figure 51). Because lysis occurs from the center radially outwards, it gives the impression that cells are growing from the periphery inwards. This would explain the conclusion reached by many workers that growth of the cleistothecium is centripetal (Hein, 1927; Eftimiu and Kharbush, 1928; Homma, 1934).

c. Asci

Figures 52, 53, 54, 55 show ascus development. Asci develop into the region where lysis has occurred. Gordon (1966) believes that asci develop in the upper region of the cleistothecium and grow downward. This downward growth is accompanied by successive lysis of cells adjacent to the basal portion of the asci. Observations of sections and of squash preparations of cleistothecia refute this theory. The asci are stalked and attached basally to the cleistothecium. Squashed preparations show that the asci come out of the cleistothecium in a rosette attached at the base. If Gordon's theory were correct, the asci would be floating without any basal attachment. It seems more logical to suppose that the asci originate from a common region which is the ascogonium.

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