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RECONNAISANCE OF VEGETATIVE NUCLEAR STRUCTURES

AND

THEIR MODE OF DIVISION IN <u>CYATHUS</u> SPECIES

BY

(C)   BRIAN RAYMOND McDONALD

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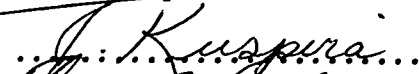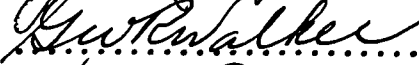DOCTOR OF PHILOSOPHY

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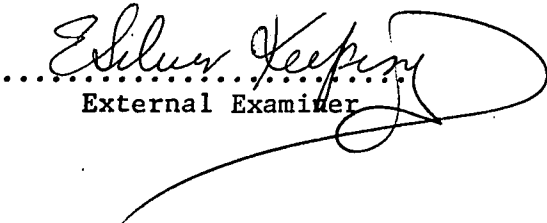
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The undersigned certify that they have read,
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ABSTRACT

Somatic karyokinesis in the homokaryotic and dikaryotic hyphae of Cyathus olla, C. setosus and C. bulleri was investigated using Feulgen, crystal violet and cotton blue lacto-phenol stains. The Feulgen staining technique was tested and found to be DNA specific.

In the homokaryotic hyphae a nucleolus develops within a spherical condensed nucleus consisting of a folded up filament. As the nucleolus increases in size, the nucleus unfolds and can assume a ring, horseshoe or filament configuration. The filament duplicates and, usually when unwound from the nucleolus, divides longitudinally. Occasionally strand separation occurs while the filament is wrapped in the form of a ring around the nucleolus. The daughter nuclei may then be able to undergo further divisions without condensing into a spherical form or they may condense before the next division.

In the dikaryotic hyphae the same nuclear cycle occurs as in the homokaryons except that an extra nuclear condensation to the spherical form can occur in both the clamp nucleus and the tube nucleus. Sometimes the tube nucleus does not condense but remains filamentous and divides as such. Although it is not known for certain that the clamp nucleus must eventually condense, this is suspected because of the limited space available in the clamp. The division of the clamp nucleus and the tube nucleus is not always synchronous or conjugate. Synchronous division of the tube and clamp nuclei is most likely to occur when both nuclei are in the condensed state. The stage of karyo-kinesis of the clamp nucleus is not closely synchronized with the formation of the clamp connection.

A configuration consisting of two spheres connected by a thin strand was occasionally observed. Although reminiscent of the Bakerspigel phenomenon of indirect division, such a configuration may be a persistent telophase in which both daughter nuclei, though still connected, have begun to condense.

A deeply stained granule believed to have a centriole-like function is associated with all the configurations that occur in the nuclear cycle of Cyathus. Sometimes this centriole-like body is connected to the nucleus by a faintly Feulgen positive thread. This body is thought to direct the nuclear elongation process. The centriole-like body may divide prior to or after division of the nuclear filament.

Numerous long smooth filaments of uniform density were observed in living hyphae and are thought to be mitochondria. Such filaments can occur in a Y configuration which may be indicative of longitudinal division or may be merely a branched structure. The filaments were observed to fragment into or be replaced by small spherical bodies.

ACKNOWLEDGEMENTS

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INTRODUCTION

Somatic nuclear division in fungi is a controversial subject.

A variety of descriptions pervades the literature. In many cases,

cytologists, having examined the same organism, reported entirely

different series of nuclear events. Much of the work was based on

observations of Giemsa or hematoxylin stained material and occasionally

Feulgen staining was used, but only as a test to show that the object

stained by some other method was, indeed, the chromatin of the nucleus.

Many investigators found that their Feulgen techniques did not stain

the nuclei intensely enough for observations of all stages of nuclear

division.

The conflicting descriptions in the literature made obvious

the need for a more accurate staining technique. Weijer and Koopmans

(1964) developed a DNA specific Feulgen staining schedule that gave

intensive staining of the chromatin in the Ascomycete Neurospora crassa.

A year later, Weijer et al. (1965) enhanced the staining density by

omitting an acetic alcohol fixation step from the Feulgen schedule.

Using this modified technique, they (loc. cit.) were able to observe

three nuclear cycles in N. crassa. The same technique was used to

observe the nuclear cycles in the Ascomycete Aspergillus nidulans

(Weijer and Weisberg, 1966; Weisberg and Weijer, 1968). The nuclear

cycles in these two Ascomycetes (Neurospora and Aspergillus) were found

to be similar, although not identical.

The question can be raised as to whether such nuclear cycles

are specific to the Ascomycetes or are to be found in other Classes of

the fungi. The present study of several species of the Basidiomycete

Cyathus is an attempt to answer, in part, this question.

LITERATURE REVIEW

Much of the work on Cyathus, a basidiomycete commonly called the bird's nest fungus, has been done by Brodie (1948a, b, 1949, 1950, 1951, 1952, 1956, 1958, 1962a, b, 1967, 1968). Further background information can be found in publications by White (1902), Lloyd (1906), Walker (1920), Martin (1927), Fulton (1950), Lu and Brodie (1962, 1963), Lu (1964) and Alexopoulos (1962).

The literature includes a number of reviews of fungal cytology (Karling, 1937; Guilliermond, 1940; Martens, 1946; Pinto-Lopes, 1949; Cutter, 1951, Olive, 1953, Moreau, 1953; Macdonald, 1956), including reviews on the structure of haploid nuclei and somatic nuclear division (Namboodiri, 1964; Robinow and Bakerspigel, 1965; Moore, 1965; Bracker, 1967; Weisberg, 1968). An outline of the literature in this area would be repetitious and, therefore, this thesis concentrates mainly on publications that deal in depth with somatic nuclear division of the fungi.

Unless otherwise stated, the literature reviewed concerns light microscopy studies.

Some common themes regarding somatic nuclear structure and division exist in literature. One such theme involves a nucleolus which, during interphase, has the chromatin distributed on its surface, either as a sphere, ring, crescent or patch. Nuclear division occurs in either of two ways, depending on whether the nucleolus divides by elongation and constriction or just disintegrates. In either case, a spindle apparatus or metaphase plate is not involved and, except where noted, chromosome-like structures are not formed.

Wager and Peniston (1910) studied a yeast obtained from a brewery. According to them, the resting nucleus consists of a nucleolus with a peripheral layer of chromatin and lies adjacent to a "nuclear" vacuole. Some of the chromatin forms a network around this vacuole. Preceding division, the chromatin network contracts into chromatin granules around the nucleolus. In the process of bud-formation the nucleolus becomes elongated and constricted and divides into two masses, one of which, together with a part of the nuclear vacuole and chromatin granules, passes into the young bud. The granular chromatin mass around the nucleolus is more or less equally divided along with the nucleolus.

In 1923, Smith reported that in a hybrid species, closely resembling Saprolegnia dioica, nuclear division involved nucleolar division. The resting nucleus is spherical or slightly torpedo-like in shape, and consists of a central body surrounded by a layer of nucleohyaloplasm. Granules or mounds of chromatin are present along the inside of the nuclear membrane in the nucleohyaloplasm. The central body is suspended within the nucleus by fine linin threads arising in most cases from the chromatin on the membrane. The shape of the central body is usually spherical or ovoid, but in a few cases it is slightly flattened to a disc-like form.

The division begins with an elongation of the central body. The nuclear membrane, with the attached chromatin granules and linin threads, remains intact throughout the division. A median constriction appears in the central body, and after a short time a similar constriction begins in the membrane. The two portions of the central body, now only connected by a thin strand, separate and round off.

The membrane constricts until the two sides touch and then the two nuclei pull apart.

The chromatin on the membrane does not seem to increase in quantity until the final stages, so that when the nuclear membrane first elongates, these chromatin masses become stretched and, therefore, thinner. It is only after the daughter nuclei start to round off again that the chromatin regains its original density.

Smith (loc. cit.) stated that the central body, which he termed the "chromoblast", first appears to be a nucleolar body, but that it has not a true nucleolar function since it takes an active part in the division.

In 1895, Trow published his investigation of Saprolegnia Thureti and S. mixta. Using haematoxylin staining, he seems to have mistaken the nucleolus for the chromatin. His description can be summarized as follows. In the center of the spherical resting nucleus is a spherical mass which has the appearance of a network. This network never becomes resolved into threads or rods of any kind and cannot consequently correspond to the nucleus of higher plants. This body may be regarded as a chromosome. The chromosome is suspended in the middle of the nucleohyaloplasm by a number of threads.

The nucleus undergoes direct division. The chromosome loses its spherical character, becomes oblong, and soon constricts medially. The two halves move apart. The nucleus as a whole increases in size and alters in shape to accommodate itself to these internal changes. Constriction of the nucleus takes place and the two daughter nuclei separate.

Schmid (1958) examined the nuclei in the hyphae and germinating spores of <u>Geotrichum</u> <u>magnusii</u> and <u>G</u>. <u>candidum</u>. She observed that the nuclei consist of a dense Feulgen-negative nucleolus surrounded by a less dense shell of Feulgen-positive particles. The mass of chromatin and the nucleolus divide at the same time by elongation followed by constriction. Chromosomes are not evident in either resting or dividing nuclei.

Robinow (1957a, b, 1962) reported that in <u>Mucor</u> <u>hiemalis</u>, <u>M</u>. <u>fragilis</u> and <u>Phycomyces</u> <u>blakesleeanus</u> the chromatin shell breaks into two half shells or cups - crescents in optical section - when the nucleolus constricts and divides. He (1957a) proposed that immediately after the division, when the chromatin forms a compact crescent on one side of the nucleolus of the resting daughter nucleus, the chromosomes divide and sister chromatids segregate to opposite sides before the next constriction and division of the nucleus occurs.

A somewhat different behavior of the chromatin was reported by Bakerspigel (1958, 1959b, c, 1960, 1962) in <u>Endogone</u> <u>sphagnophila</u>, <u>Gelasinospora</u> <u>tetrasperma</u>, <u>Neurospora</u> <u>crassa</u>, <u>Saprolegnia</u> <u>parasitica</u>, <u>S</u>. <u>ferax</u> and <u>Achlya</u> <u>racemosa</u>. The chromatin shell condenses into a bar or crescent which constricts at the mid-region and separates into two while the nucleolus, to which the chromatin is still attached, constricts and divides. The elongating nucleolus is thought to aid the separation of the chromatin masses. In <u>N</u>. <u>crassa</u> and <u>G</u>. <u>tetrasperma</u>, the chromatin shell forms a complex of chromosomal filaments which then clump together to form the bar or crescent. A densely stained granule is associated with the chromatin of the interdivisional nucleus and is usually doubled by the time the dividing chromatin is crescent or bar shaped. These

granules separate as the bar of chromatin pulls apart so that each sister nucleus contains one of these granules. The granules are thought to play an important part in the division of the chromatin and, where applicable, in the division of the·elongating nucleolus. .

Turian (1959) on the other hand, preferred to be less definite on the mode of karyokinesis, stating that in <u>Allomyces</u> <u>macrogynus</u>, the chromatin on the surface of the elongating nucleolus separates into two masses.

Turian and Cantino (1960) reported that in <u>Blastocladiella</u> <u>emersonii</u> the shell of chromatin localizes into a beaded ring around the nucleolus. Then the chromatin forms a crescent which later divides, along with the elongated nucleolus, by constriction. They (<u>loc</u>. <u>cit</u>.) hypothesized that the elongating nucleolus provides the mechanical force for separating the two chromatin masses.

Schopfer <u>et</u> <u>al</u>. (1963) noted that in the vegetative cells of <u>Schizosaccharomyces</u> <u>pombe</u> the nucleus consists of a central nucleolus surrounded by a clear halo or nuclear vacuole. At prophase a ring of chromatin granules surrounds the nucleolus. At metaphase these granules or chromosomes are regularly grouped in the clear zone surrounding the nucleolus. These chromosomes, in a minimum number of three at advanced prophase, double to pass on to metaphase in a minimum number of six.

During this division, the nucleolus elongates and divides by a median constriction.

In anaphase or meta-anaphase, two granules, probably centrosomes, are connected to the two separating chromosomal portions.

Telophase is characterized by the elongation of the two chromosomal portions which are still connected by chromatin material (chromosomal bridges). This is indicative of a stretching process. In each of the daughter cells three or four chromosomal granules can be distinguished.

Thyagarajan and Naylor (1962) reported that in the yeast Rhodotorula glutinis the nucleus consists of a central nucleolus surrounded by a shell of chromatin. The nucleus appears to divide by a process of elongation and constriction during which roughly half of the nucleolus, along with the surrounding chromatin, passes into the bud. The authors (loc. cit.) mentioned that in Feulgen stained preparations, the chromatin is in the shape of a ring. No attempt was made to integrate this ring stage with the nuclear sequence.

Yoneda (1961a, b, 1963) noted that mitosis, in a strain of Saccharomyces is different from that in higher organisms. A chromatin patch on one side of a large nucleolus rearranges itself in the form of a ring with a granule in the center. The ring of chromatin, still on the one side of the nucleolus, condenses into a ring of small globular chromosomes. The central granule divides and metaphase, anaphase and telophase occur on the one side of the nucleolus. The chromatin condenses at telophase. Then the nucleolus elongates, constricts and divides with chromatin going to each daughter nucleus.

In the yeast Schizosaccharomyces pombe (Yoneda, 1963) rings and chromosomal bodies are not formed. The chromatin, located on one side of the nucleolus, merely divides into two masses before the nucleolus divides.

In _Saccharomyces_ and _Schizosaccharomyces pombe_, Yoneda (1961a) observed a granule that may be compared to the centriole with regard to its position. However, only one such granule was found in the polyploid strain of _Saccharomyces_. Later, Yoneda (1963) considered the granule to be an internal constituent of the nucleolus.

Slifkin (1967) claims that her observations of nuclear division in _Saprolegnia delica_ are identical to those of _Bakerspigel_ (1960) for _S. parasitica_ and Smith (1923) for a variety of _S. dioica_.

Earlier, some investigators (Beams _et al._, 1940; Sinoto and Yuasa, 1941) mistook the nucleolus to be the complete nucleus in _Saccharomyces cerevisiae_. Beams _et al._ (_loc. cit._) reported observing a dark body against a light background. The dark body underwent amitotic division by elongation and constriction.

Sinoto and Yuasa (_loc. cit._) reported that the nucleus contains a large karyosome (a spherical mass of chromatin in a nucleus, distinct from the nucleolus) around which there is a hyaline zone. They (_loc. cit._) also reported observing small granules around the nucleus. These granules were often separated _en masse_ from the nucleus, giving a dumbbell-shaped appearance which may simulate an amitotic form of behavior of the nucleus.

When the nucleus enters into division, it becomes irregular in shape and entangled chromatin threads appear and become transformed into four rod-shaped chromosomes while a spindle is being formed. Fibers can often be observed between the two poles of the spindle. Each chromosome divides, perhaps longitudinally, and the resulting halves go

to the opposite poles to form two daughter nuclei.

However, in 1958 Yuasa reported that in _Saccharomyces_
cerevisiae there is a nucleolus-like body in the vacuole. The nucleus
is attached to one side of the vacuole and contains centrochromatin.
At prophase the centrochromatin usually changes into two chromatin
bodies, but sometimes it appears as a spireme structure. At metaphase
the nucleus changes into four chromosomes which sometimes conjugate two
by two. The chromosomes appear in a spindle which is situated on the
surface of the vacuole. A centriole was not evident.

In the second type of nuclear division, the nucleolus disin-
tegrates and a new nucleolus is formed in each daughter nucleus.
Pontefract (1956) reported that in several species of _Penicillium_ the
conidial nucleus consists of a small, compact mass of chromatin without
a visible central body. Prior to germination, the nucleus enlarges
into a characteristic vegetative hyphal nucleus in which an outer ring
or crescent of chromatin surrounds but does not touch a large central
body.

In growing hyphae and conidiospores, nuclear division is
preceded by contraction of the normally loosely organized chromatin into
a relatively small dense body. This chromatin then divides by a
simultaneous process of constriction in the middle and elongation of
the distal parts until complete separation is achieved. Arrangements
suggesting mitosis, such as a metaphase plate and spindles, were never
observed. The central body was released into the cytoplasm during
division and left there to disintegrate. The daughter nuclei rapidly
formed new central bodies.

Bakerspigel reported (1957, 1959a, d, 1960b, 1961) that in
Blastomyces dermatitidis, Phyllosticta, Schizophyllum commune,
Scopulariopsis brevicaulis and Ophiostoma fimbriata the chromatin shell
condenses and withdraws from the nucleolus. The chromatin forms a bar
or crescent which constricts at the midregion and divides into two. The
nucleolus, lying free in the cytoplasm, decreases in size and disappears.
As noted for the first type of nuclear division (Bakerspigel, 1958,
1959b, c, 1960a, 1962), deeply stained granules are associated with the
chromatin and its division in B. dermatitidis, Phyllosticta, Schizo-
phyllum commune and Scopulariopsis brevicaulis.

In Scopulariopsis brevicaulis and Ophiostoma fimbriata, the
chromatin shell becomes arranged into tightly bound complexes of
chromosomal filaments which later contract to form the bar or crescent.
In O. fimbriata the chromatin appears as an irregularly-shaped double
bar or strand-like configuration. These strands, which lie almost
parallel to each other, do not slide apart in opposite directions, nor
do they separate to opposite poles, but they divide transversely at
their midregions, usually one before the other, and then their
extremities move apart.

In the basidiomycete Ceratobasidium pratiolum (Pellicularia
pratiocola), Saksena (1961) observed that the nucleolus, cupped within
the concavity of the chromatin crescent, decreases in size and
disappears. The chromatin then elongates to become bar-shaped and
constricts at the midregion. New nucleoli appear in the two attached
portions of the constricted chromatin. The two portions of chromatin
pull apart to form two sister nuclei.

A few nuclear figures suggested that the chromatin becomes concentrated on the periphery of the nucleus in the form of a ring at the beginning of division and divides into two before constriction of the nucleus (Saksena, loc. cit.).

Robinow (1962) reported a somewhat different nuclear occurrence in Allomyces arbuscula. The chromatin shell condenses into filamentous chromosomes arranged in a cup- or crescent-shaped plane at the periphery of the nucleolus. He (loc. cit.) suggested that the chromosomes are paired. The chromosomes sort themselves out into two tight clusters either at each end or on the equator of an elongating nucleolus. The nucleolus becomes vacuolated and disintegrates.

Yoneda (1961a, b, 1963) found that in the yeasts Lipomyces starkezi, Torula utilis and T. ruba chromatin division is carried out on one side of a large nucleolus. In T. rubra the patch of chromatin on the surface of the nucleolus sometimes becomes arranged in a ring with a central granule and behaves like the chromatin ring in a strain of Saccharomyces (Yoneda, loc. cit.). Other times, the chromatin patch in T. rubra will behave like that in L. starkezi and T. utilis and will condense into globular chromosomes without ring formation and then will undergo metaphase and anaphase. The divided chromatin in these three species condenses at telophase and the nucleolus is left behind to disintegrate.

As in the strain of Saccharomyces and Schizosaccharomyces pombe, a granule was also observed in Torula rubra (Yoneda, loc. cit.).

In T. utilis and L. starkezi a slender structure is found that connects the two masses of separating chromatin. Yoneda (1961b) suggested that the chromatin is separated by the elongation of this structure and that the structure is possibly a spindle mechanism.

Duncan and Macdonald (1965) investigated nuclear division in the dikaryotic hyphae of the Basidiomycetes Marasmius androsaceus and M. rotula. The chromatin shell consists of chromatin granules interconnected by a faintly stained Feulgen positive thread. At the beginning of division, the centrally located nucleolus shifts so as to lie attached to the end of the chromatin thread which is condensing. The chromatin thread is double, indicating that replication and separation of the chromatin occurred before the onset of nuclear division. The nucleolus becomes indistinct and disappears while the chromatin mass becomes denser and appears beaded. The two strands of the double stranded chromatin thread separate from each other except at the ends to form a ring. Sometimes the ring is broken, the two strands being connected at only one end. The chromatin thread, whether being an entire ring or having a break within it, becomes twisted into a figure of eight which then doubles over on itself to form a beaded double ring. A break occurs in each ring and the arms extend linearly, giving two beaded rods of chromatin which may appear either crossed upon each other or lying side-by-side. Both rods constrict at their midregions and two chromatin "bridges" are formed as two of the halves of different rods go to one pole and the other two halves go to the opposite pole. Each of the daughter nuclei rounds off and a nucleolus is reorganized in each.

Nuclear events altogether different from the preceeding
occur in the vegetative nucleus of the yeast <u>Lipomyces</u> <u>lipofer</u> as
reported by Robinow (1961). According to him, a small compact resting
nucleus contains slender chromosomes and a nucleolus. The chromosomes
duplicate in an intact and expanding nucleus. The chromosomes contract
and pair. Robinow (<u>loc</u>. <u>cit</u>.) suggested that these paired chromosomes
are sister chromosomes that never completely lost contact after
duplication. The pairs of chromosomes arrange themselves parallel to
one another and form a stack or palisade. There is no evidence of any
spindle apparatus. The chromosomes come closer together and appear to
become fused into a single solid core. The nucleolus, which previously
had been attached to one or two of the chromosomes, becomes free in the
cytoplasm and gradually decreases in size and disappears. The solid
core of chromatin divides into two drop-shaped solid masses which remain
connected for a while by a thin strand. The dense sister nuclei unfold
into small spheres, each complete with a nucleolus and filamentous
chromosomes.

In the uninucleate, mycelial fungus <u>Basidiobolus</u> <u>ranarum</u>,
Robinow (1963) reported that the nucleus divides by an ordinary form
of mitosis involving a spindle and metaphase plate. The resting nucleus
is composed of a huge vacuolated nucleolus surrounded by a sponge-work
of chromatin. In division, partial dissolution of the nucleolus occurs
and the chromosomes sink into the swollen nucleolus. Some of the
substance of the nucleolus is transformed into a mass of parallel
spindle fibers and the remainder of the substance forms a pair of polar
caps or end-plates over the ends of the spindle cylinder. Nothing that
would suggest the presence of centrioles inside or outside the end-plates

was observed. A disc of closely packed chromosomes forms at the equator of the spindle and eventually forms a ring or pierced disc in the spindle. In some nuclei, the chromosomes remain at the surface of the disintegrating nucleolus, spread out in variously curved flat sheets. The chromosomes gather along one edge of the sheet and finally form the metaphase ring plate. Robinow (loc. cit.) suggested that the difference between these two patterns may be due to different degrees of softening or rate of dissolution of the nucleolus. The chromosomes separate as daughter plates and travel a short distance on the spindle to become submerged in the substance of the end-plates. As the end-plates are pushed farther apart by the growing spindle, they become rounded and the chromosomes inside occupy a smaller area than they did at metaphase. This causes the spindle fibers to diverge and produce two separate conical half spindles. The spindle fibers are gradually dissolved. Reconstruction of the daughter nuclei occurs. The end-plates disintegrate and some of their substance is probably utilized by cell division, thus maintaining the uninucleate condition of the mycelium.

In 1931 Varitchak gave an extensive account of vegetative nuclear division in the ascomycete Ascoidea rubescens. Each resting nucleus has within it an eccentrically located nucleolus. The rest of the nucleus appears homogeneous. The first indication of division is the appearance of a centrosome in the nucleus. The nucleus becomes more chromophilous and the centrosome travels towards the periphery of the nucleus. The nucleus becomes slightly elongated with the centrosome at one pole, the nucleolus at the other and the chromatin mass in the middle. The centrosome increases slightly in size. The nucleus becomes more spindle-shaped at the pole occupied by the centrosome. The

chromatin mass becomes differentiated into two chromosomes which become placed one beside the other. At this stage the centrosome is linked to the chromosomes by a trabecula (small bar, rod or bundle of fibers). The nucleolus still occupies the opposite pole. The centrosome divides into two. Each of these daughter centrosomes is attached by a trabecula to one of the chromosomes, and the nucleus takes on the shape of a sharp angle. The size of this angle increases up to 180° as the centrosomes separate, thus forming a spindle with a centrosome at each pole. Two chromosomes can be distinguished on the spindle and the nucleolus can be seen at the side of the spindle. The two chromosomes line up parallel or perpendicular to the axis of the spindle. Each of the two chromosomes divides into two and four chromosomes and can be seen lined up along the spindle and parallel to the axis of the spindle. The nucleolus disappears in the cytoplasm. The chromosomes travel towards the poles and condense into chromatin masses. The spindle ruptures in the middle and disappears progressively. A centrosome is visible at the side of each rounded daughter nucleus. Then the centrosomes disappear as each of the nuclei returns to the resting stage.

In the developing pycnospores of <u>Macrophomina phaseoli</u>, Knox-Davies (1966) observed that the interphase nucleus consists of thread-like chromatin material enclosing a single large nucleolus. At the beginning of division, the chromatin threads become thicker and chromosomes can be distinguished. The nucleolus completely separates from the chromosomes. Centrioles are usually elongate, sometimes punctiform. During the first division in the spore, the centrioles are large and easily recognized, but during later divisions they are smaller

and difficult to distinguish. The spindle is top-shaped and asters are not present. All that can be distinguished of the nucleolus is a thin crescent closely associated with the spindle fibers. The chromosomes clump and separate into two masses. Trailing ends of chromosomes and lagging whole chromosomes are often observable. A band of interzonal fibers extends between the two chromosome clumps, and the nucleolus is observable in the zone between the two separating masses. The interzonal fibers disappear. The daughter nuclei enlarge and become more rounded. Knox-Davies (loc. cit.) favors Robinow's (1963) suggestion that the nucleolus is in some way involved in spindle formation.

Knox-Davies (1967) later described a similar nuclear division in the vegetative hyphae of M. phaseoli. However, he noticed that at prophase the nucleolus is attached to one of the chromosomes. A spindle is not observable, but centriole-like bodies can occasionally be seen. The nucleolus is discarded and fine strands occur between the separating daughter chromosome groups.

In 1896 Sappin-Trouffy published a lengthy and detailed report on ten genera of rusts and included in his report descriptions of vegetative nuclear divisions. He studied the following: Uromyces erythronii, U. betae, U. striatus, U. rumicis, U. ficariae, U. geranii, Puccinia graminis, P. coronata, P. rubigo-vera, P. borri, P. violae, P. liliacearum, P. menthae, P. fusca, P. boarum, P. caricis, P. polygoni, P. buxi, P. malvacearum, Gymnosporangium sabinae, G. clavariaeforme, G. juniperinum, Triphragmium ulmariae, T. isopyri, Phragmidium rubi, P. subcorticium, Melampsora Helioscopiae, M. farinosa, M. vitellinae, M. tremulae, M. populina, M. betulina, Thecopsora areolata, Cronartium

flaccidum, Endophyllum euphorbiae silvaticae, Coleosporium Senecionis,
C. Sunchi. He noted that the nuclear structures and modes of division
are common to all the species.

The resting nucleus is spherical or elliptic with a small
nucleolus at the center surrounded by a more or less dense hyaloplasm.
The hyaloplasm contains a number of chromatin coils near the periphery
thus leaving a clear zone around the nucleolus. The nucleus is limited
by a thin achromatic membrane. When the nucleus attains a certain
volume, the chromatin coils can be seen to consist of a wound up nuclear
filament within which can be distinguished chromatin granules.

The resting nucleus can take on an irregular shape. It can
elongate in the form of a rod and when it traverses a hyphal constriction,
it can modify its form to that of a pear and then a dumbbell. During
such a passage of the nucleus through a constriction, the nucleolus
generally remains placed at the back end of the nucleus. The swollen
part or parts of the pear- or dumbbell-shaped nucleus are not compact,
but granular, typical of a resting nucleus.

Sappin-Trouffy (loc. cit.) stated that there are two kinds of
division, direct and indirect. Direct division is not as frequent as
indirect division and is observed only in the aged cells of the thallus.
The nucleus elongates and constricts in the middle. The two nuclear
extremities swell and are no longer united except by a fine trabecula
which finally breaks. Occasionally, the new nuclei undergo a further
division before the trabecula has ruptured, resulting in three or four
masses being held together for a while by the constrictions. During
direct division the nucleus does not change in coloration but maintains

the same affinity for stains as it does in the resting state. Sappin-Trouffy (loc. cit.) considers the direct division to be a phenomenon of senility.

At the onset of indirect division the nuclear membrane disappears, the granular chromatin substance becomes condensed or fused into a small mass in the form of a crescent, arc, horseshoe or "S" which is wound around the nucleolus. Subsequently, the chromatin straightens out into a small cord and the nucleolus is abandoned to one side where it eventually disappears. A line of transparent substance longitudinally divides the chromatin cord into two strips or chromosomes which slowly elongate. This stretching or drawing out continues in such a way that each chromosome constricts at its middle and swells at its two extremities. Each chromosome divides into two, the cleavage occurring at the middle of the constriction. The two half chromosomes at each pole unite laterally to form two sister nuclei, each of which forms an arc similar to that formed at the onset of division. The achromatic substance which served as the axis for the longitudinal division elongates, the middle part is destroyed and the sister nuclei slowly move away from each other. Each of the sister nuclei assume the characteristics of a resting nucleus.

There exist , however, nuclei which do not separate longitudinally into two chromosomes but as single masses elongate, constrict in the middle and divide transversely. This type of division is the same as the indirect division except for the fact that the two chromosomes remain united.

Blackman (1904) noted that nuclear division in most of the cells of the Uredineae is of an exceedingly simple type. The nucleolus is extruded from the nucleus and the chromatin condenses into one mass. This mass becomes spread upon a rudimentary spindle and is drawn apart into two masses which form daughter nuclei.

He (loc. cit.) noted the formation of a coiled chromatin spireme thread that breaks up into narrow elongated chromosomes prior to nuclear division in Gymnosporangium clavariaeforme.

In 1953 McGinnis reported the occurrence of a spireme in the rust Basidiomycete Puccinia graminis. At late prophase of the nucleus in germinating basidiospores, the chromosomes form a continuous chain or spireme. Metaphase and anaphase nuclei were very rare and McGinnis (loc. cit.) suggested that these stages of division were of short duration. At metaphase the six chromosomes were associated in pairs. At late metaphase the chromosomes, completely separated with no apparent pairing attractions, split longitudinally. Though not studied in detail, anaphase movement of the chromosomes was thought to be in the conventional manner as observed in higher plants.

Craigie (1959) and Craigie and Green (1962) studied nuclear behavior leading to conjugate association in haploid infections of Puccinia graminis and in P. helianthi, respectively. In both studies it was noted that the nucleus exists in an expanded and unexpanded form. Shortly before an expanded nucleus divides, the seemingly tangled chromosomes collect towards one side of the nucleus and the nucleolus takes up a position at the opposite side. Extrusion of the nucleolus and its disintegration follow , and the nucleus contracts and assumes

the unexpanded form. It then divides into two daughter nuclei which increase in size to become expanded nuclei.

McGinnis (1954, 1956) studied nuclear division in the germinating basidiospores of seven other species of Puccinia: P. xanthii, P. malvacearum, P. asteris, P. coronata calamagrostis, P. sorghi, P. carthami and P. helianthi. With the exception of P. helianthi, all the species studied were very similar in behavior during nuclear division. Fairly frequently, the chromosomes would unite end-to-end to form a complete ring. Such chromosome unions were thought to be similar to those observed in P. graminis (McGinnis, 1953), although in P. graminis the chromosomes failed to complete the ring. Ring configurations were also observed in P. minussensis and Melampsora lini (unpublished data, see McGinnis, 1956). McGinnis (loc. cit.) stated that the ring stage may constitute a normal stage in the nuclear division but he did not attempt to place it in the sequence of events.

Well defined chromosomes were observed at metaphase and anaphase. The metaphase configuration was often in the form of a square.

The first, and occasionally the second, nuclear division in the basidiospores of Puccinia helianthi appeared to proceed as described above in that the chromosomes condensed at metaphase. However, most often in the second division and in all of the subsequent divisions, the chromosomes appeared as stringy masses of unusually large size. Anaphase was never observed in such divisions. Even after division, the chromosomes seemed to retain their individuality, migrating into the germ tube as a bundle of long chromatin strands.

Heim (1952, 1954) studied 32 species of Ascomycetes and 50 species of Basidiomycetes and observed that the nucleus and its division in the Basidiomycetes resembles that in the Ascomycetes. A particulate karyokinetic structure was noticed which constitutes a filament. The number of Feulgen positive chromatin particles was found to be close to the number of chromosomes reported for the numerous species investigated.

Heim's investigations (1952, 1954) confirmed the earlier observations of Savile (1939) who classified the different appearances of nuclei present in the following species of rusts: Uromyces fabae, U. lespedezae-procumbentis, U. hyperici, Puccinia sorghi, P. malvacearum, P. hieracii, Melampsora bigelowii and Tranzschelia fusca. According to his observation, a condensed ("unexpanded") nucleus seems to be prevalent together with a very detailed nuclear structure for which he proposed the term "expanded". The expanded nucleus is similar to the filamentous nucleus as reported by Weijer et al. (1965). Savile (loc. cit.) also reported observing nuclear configurations that were intermediate between the expanded and unexpanded states.

The occurrence of nuclear filaments seems to be widespread among fungi of different genera. Heim (1956) found nuclear filaments in the parasitic fungus Synchytrium endobioticum during meiosis.

Pavgi et al. (1960) studied the nuclear divisions in the basidiospore of P. sorghi at or prior to germination. According to their report, the interphase nucleus increases in size and elongate chromosomes are formed. The nucleolus disappears, the nuclear membrane dissociates and the chromosomes move onto a spindle and become oriented at the equatorial plate. No astral rays or centrioles are discernible at the poles. The chromosomes split longitudinally and the two sets move poleward. The chromosomes remain fairly distinct through late anaphase or early telophase and later aggregate into a dense mass at each pole. The daughter nuclei, after a short interphase, infrequently enter into a second mitosis. In such cases, polar views of the metaphase plates show ring-like configuration. The chromosomes, however, are individually distinct.

DeLamater (1948, 1949b) was of the opinion that the nuclei in the mycelial stage of Blastomyces dermatitidis undergo division in a manner comparable to other organisms. According to him, after telophase the chromatin granules or chromosomes form a dense mass. This separates to become distinct granules which are integral parts of delicate threads in the resting nucleus. The resting nucleus then enlarges and the granules become larger and the linin net connecting them becomes more distinct. Frequently a nucleolus is visible. The chromatin threads condense and aggregate at one side of the nucleus and a granule appears opposite this mass. The granule enlarges and doubles. The two granules separate and migrate to opposite poles. The chromosomes line up between them. The chromosomes double and are drawn to the two poles, forming dense masses.

A similar description was given by the same author (DeLamater, 1949a, b) for the nuclear cycle of the diploid vegetative phase of Saccharomyces cerevisiae. Again, the chromatin threads condense and aggregate at one side of the nucleus and a granule, designated the centriole, appears on the nuclear membrane opposite this condensed chromosomal mass. The centriole divides and each daughter centriole moves to opposite sides of the nucleus, forming a typical spindle. The chromosomes then divide and migrate to the two poles. As this occurs, the chromosomal masses become less distinct and the whole appears to stretch out and pull apart like a piece of taffy.

Mundkur (1954) studied a haploid, diploid, triploid and tetraploid series of Saccharomyces. He reported that the nucleus is an extravacuolar, optically "empty", near-spherical vesicle appressed to the vacuole. In exceptional instances the nucleus occurs within the vacuole. He suggested that the chromatin particles are submicroscopic and in uniform dispersion in the nucleus. Chromosomes were not detected at any period and a nucleolus was not recognizable.

The pattern of nuclear division in vegetative cells is identical in all members of the polyploid series. It involves an elongation of the nucleus into an "hour-glass" form and closure of the medial constriction. The maximum number of Feulgen-positive spheres in a single cell, regardless of its degree of ploidy, is two but their volumes are ploidy-dependent and they are the products of division of a single Feulgen-positive sphere. Therefore, these bodies are daughter nuclei, not chromosomes.

"Centrioles" which stain with hematoxylin and are Feulgen-negative, are sometimes associated with the nucleus and exhibit positions suggestive of an orientating influence during nuclear division. Their role, however, is not consistent and they are not always present.

A spindle or spindle-like organization is not evident.

Lindegren et al. (1955) reported that in a tetraploid strain of Saccharomyces the nuclear apparatus in the dormant yeast cell consists of a hyaloplasmic nuclear vacuole and a solid spindle, both enclosed in the nuclear membrane. The spindle which appears to be adjacent to the nuclear vacuole is actually within, but to one side of the vacuole. The chromosomes lie on the surface of the spindle either in the form of a network of long, slender filaments, or more condensed rods, which often appear to radiate from a central point. The chromosomes, attached to the spindle, extend into the hyaloplasm. These extending chromosomes may shrink back onto the spindle and coagulate to form a crescent closely applied to a small portion of the external surface of the spindle.

A deeply staining spherical granule surrounded by a clear area is associated with the chromosomes on the surface of the spindle. Lindegren et al. (loc. cit.) provisionally identified the granule as a centriole and the surrounding clear area as the centrosome.

Division of the chromatin into two separate masses, each held together by a centriole, occurs on the surface of the spindle. Then a bud is formed by the extrusion of the spindle into the cell wall. This occurs without the transfer of chromatin into the bud. As the bud enlarges, the two chromatin masses form a confluent mass or hollow cone over the surface of the spindle with the chromosomes of the genome of

the mother cell extending into the vacuole of the mother cell. The chromatin in the vacuole remains polarized to the spindle. A process from the mother vacuole into the bud initiates a new bud vacuole. As the bud enlarges and elongation of the spindle occurs, the chromatin appears in the form of two hollow cones connected by their apices conforming to the hour-glass form of the spindle. The two masses separate, the spindle and vacuole pinch off into two and the bud is cut off from the mother cell. The nucleolus divides often at the same time as the chromosomes, and one nucleolus appears in the mother vacuole and another in the bud vacuole.

Widra (1959) investigated nuclear division in Candida albicans, C. tropicalis and C. guilliermondi. Nuclear division was found to be the same in both the yeast-like phase and mold phase of these species. The interphase nucleus consists of semi-lunar condensed chromatin mass polarized to a single deeper staining granule, presumably the central body. During division, the cell (in the yeast-like phase) enlarges and its chromatin material swells up to fill the nuclear vesicle. The granule is sometimes still distinguishable at this time although in most cases it is not evident. Subsequently the chromatin duplicates, and takes a position peripherally against the nuclear membrane of the turgid sphere-like nucleus to form the metaphase "ring" stage. This metaphase ring may open eccentrically to one side or at two points equationally across a diameter as anaphase movement begins. Two chromatin masses separate within the nuclear vesicle. The granule or central body is again visible. The nuclear membrane breaks and the chromatin masses separate completely and each mass reorganizes into an interphase nucleus.

Chromosomes are not evident and a spindle external to the dividing mass is not visible. The dark-staining nucleolus-like central body or granule appears to function as a centriole. Widra (loc. cit.) suggested that in Candida the central body and a spindle apparatus operate within the nuclear membrane since the granule is seen leading the chromatin mass into the daughter cell during anaphase in the yeast-like phase of these species.

Hall (1963) studied the somatic nuclear division in the brown rot fungus Monilinia fructicola. All interphase nuclei, whether in mature conidia, the hyphae or young conidia, have a small nucleolus situated against the nuclear membrane which surrounds the nucleoplasm. During prophase the chromatin becomes densely granular and coalesces, producing fewer large granules. Individual chromosomes could not be resolved throughout the nuclear division. The chromatin material is arranged on a metaphase plate. At anaphase two masses of chromatin separate. Spindles were not mentioned or shown in a photograph in this report, but an internal spindle was portrayed in a drawing.

MacGarvie and Isaac (1966) studied germinating conidia of five species of Verticillium: V. dahliae, V. albo-atrum, V. nigrescens, V. nubilum and V. tricorpus. The small spherical resting nucleus becomes granular and irregular in shape. Five to six discrete spherical or slightly elongated granules become distinguishable. These well separated granules draw close together, usually in two rows of three. It was inferred that the granules split into two groups. After the division was complete, the two nuclei became small, compact and spherical. A spindle was not observed.

Kowalski (1966) claims that two types of nuclear division occur in the vegetative hyphae of Preussia funiculata. Nuclear division in the hyphae in contact with the growing medium resembles mitosis in higher plants. The interphase nucleus is solid and spherical. In prophase the nucleus increases in size and long chromosomes become distinct. The nucleolus is visible at this stage. A typical metaphase, as found in higher plants, is lacking. The chromosomes do not shorten or line up on a medial plane; they simply separate and move apart as in late anaphase. When nuclear division is complete, the nucleus becomes solid and spherical again.

In the aerial hyphae the resting nucleus is narrow and elongated. When the nucleus divides, it elongates slightly and splits in half longitudinally.

Yamasaki and Niizeki (1965) studied the rice blast fungus Piricularia oryzae and concluded that the nuclear divisions in germinating conidia are typically mitotic whereas the divisions in the mycelia are not.

Dowding and Weijer (1960) proposed the following sequence for the vegetative nuclear division in Neurospora crassa. Starting with a coiled filament within a spherical nuclear membrane, the filament becomes free of the membrane and elongates to become a narrow thread which splits longitudinally. The daughter threads separate and each shortens and thickens to become a filament with distinct chromosomes. This filament coils and is again enveloped by a nuclear membrane.

In 1962, investigating <u>Neurospora</u> <u>crassa</u> and <u>Gelasinospora</u> <u>tetrasperma</u>, Dowding and Weijer elaborated on their earlier (1960) description of nuclear division. They reported that in the rounded resting nucleus, a rather coarse reticulum builds up within itself a long coiled protoplasmic thread and that along the length of this thread small chromatin bodies are deposited from some of the contents of the nucleus. This precipitation of chromatin also occurs on the threads after they have divided, allowing the chromosomes to become recognizable as such. They (<u>loc</u>. <u>cit</u>.) reported the occurrence of a centriole situated outside the nucleus, sometimes appearing as a long thread extending from the nucleus to the cell wall where it terminates as a disc. Sometimes the centriole appears as an angular plate, rod-like when seen edge on.

Dowding (1966) observing <u>Neurospora</u> <u>tetrasperma</u> mycelium, re-affirms the above descriptions (Dowding and Weijer, 1960, 1962) of nuclear division except for the postdivisional phase of which she found the chromosomes to be uncoiled, polarized and apparently unconnected by any filament.

Weijer <u>et</u> <u>al</u>. (1963) examined <u>Neurospora</u> <u>crassa</u> and <u>Gelasinospora</u> <u>tetrasperma</u> and proposed a more elaborate scheme of division consisting of 32 stages grouped under three main phases: the DNA replication phase, the division phase and the chromosome phase.

During the DNA replication phase, the small spherical interphase nucleus transforms into a filament. A medially located nucleolus develops on the filament and the filament eventually wraps around the nucleolus to form a ring. The ring duplicates but does not separate,

giving rise to a double ring which breaks at one point leaving a gap through which an enlarging nucleolus protrudes. At this stage a delta shaped centriole can be seen attached to the nucleus by a thread-like structure. As the nucleolus enlarges, the nuclear material condenses and finally appears as a polar cap on the nucleolus. The centriole and its thread duplicate and remain attached to the nucleus. The nuclear cap separates from the nucleolus and the nucleolus later disintegrates. A second nucleolus develops and enlarges inside the nuclear cap such that the nucleus becomes of variable shape. Eventually, the nucleus becomes a large long structure with the two centrioles attached.

The second major phase, division phase, starts when this long nuclear structure begins to divide longitudinally into two filaments which are held together at one end where they share a common nucleolus which soon divides. The strands separate, each with a nucleolus and with a centriole attached by a thread-like structure. The nucleolus in each daughter nucleus increases in size. The nuclear material flows over the surface of the nucleolus. This structure then transforms into a hyaline sphere within which the chromosomes, nucleolus and centriole can be distinguished. This marks the beginning of the third major phase, the chromosome phase.

The chromosomes, nucleolus and centriole, interconnected by a thin strand, are released from the hyaline sphere. The chromosomes condense and their interconnecting strands shorten, resulting in a nucleus that appears as a small dense spherical body attached to the nucleolus. The spherical nucleus detaches from the nucleolus to become an interphase nucleus.

Weijer et al. (1963, 1965) suggested that the thread interconnecting the chromosomes, nucleolus and centriole is the spindle and that this thread is, therefore, multi-stranded between the centriole and the sixth chromosome and single-stranded between the seventh chromosome and the nucleolus. The centriole is thought to have rotated through 90° in the vegetative nucleus so that it is in line with the equatorial plate and the fibers extending from it are parallel to one another. Such a spindle or interconnecting thread is necessary in the multinucleate coenocytic vegetative phase to maintain the integrity of each nucleus amidst the streaming cytoplasm.

In 1965, Weijer and co-workers advanced an even more elaborate scheme of nuclear division in the hyphae of Neurospora crassa. They reported three distinct karyokinetic cycles: (1) Juvenile cycle of young undifferentiated hyphae (e.g. hyphae of germinating conidia), (2) Maturation cycle I, leading to the formation of macroconidia, and (3) Maturation cycle III, leading to the formation of microconidia.

In the Juvenile cycle, karyokinesis proceeds from a globular interphase nucleus accompanied by a centriole which is attached to the nucleus by a thin thread. The nucleus elongates into a filament consisting of seven chromosomes and one centriole, all interconnected by a thread-like structure.

From this point on, various configurations can occur depending on whether or not the nuclear filament forms a ring around the nucleolus. If a ring is formed, the resulting configurations depend on the time of ring breakage, especially whether this breakage occurs before or after duplication or strand separation. The nuclear filament may only

partially wrap around the nucleolus and a horseshoe configuration results. Even this may not occur, the filament remaining relatively free but still attached to the nucleolus.

The important point Weijer et al. (1965) advanced is that nuclear division occurs by a longitudinal splitting of the filament, whether this filament be in a ring, horseshoe or free configuration. The final result is two daughter nuclear filaments.

In subsequent divisions, these filaments do not return to the globular interphase configuration, but proceed directly into ring or horseshoe configurations, or remain in the free configuration.

This Juvenile cycle has also been observed by D.L. Weijer (1964) in germinating conidia of Neurospora crassa and by Weijer and Weisberg (1966) in germinating conidia of Aspergillus nidulans. D.L. Weijer (1964) did not mention a horseshoe or crescent configuration. According to her, duplication (i.e. strand separation) of the genetic material can occur either during filament phase to give a double filament or during ring phase to give a double ring. If a double filament results, this configuration will eventually close to form a double ring. So in either case, a double ring is formed.

An interesting aspect of the ring configuration has been noted in Neurospora crassa by D.L. Weijer (1964) and Weijer et al. (1965), and in Aspergillus nidulans by Weijer and Weisberg (1966). The centriole is associated with the ring, either being incorporated into the ring or attached to the ring by a thin thread-like structure. During ring phase the centriole divides and the two daughter centrioles move around the nuclear ring so as to occupy polar positions opposite to one

another. According to Weijer et al. (1965) and Weijer and Weisberg (1966), centriole division always precedes ring division, whereas D.L. Weijer (1964) reported that centriole division can occur during either single or double ring phase.

Weijer and Koopmans (1964) concluded that in the Juvenile cycle, DNA synthesis starts during late filament and early ring phase and that since centriole division occurs at late ring phase, the formation of DNA does not depend on the antecedent duplication of the centriole. D.L. Weijer (1964) found that the appearance of the Juvenile filament and ring phases was paralleled by an increase in radiosensitivity. Such an increase would be expected to parallel DNA replication because of the increase in nuclear target size.

Maturation cycle I of Neurospora crassa (Weijer et al., 1965) precedes formation of the macroconidiophore and arises from the late Juvenile nucleus. Again, depending on the various configurations possible, the filament doubles to give either a double ring which will eventually break, or a double horseshoe, or a relatively free double filament. The nucleolus increases in size and the chromatin condenses to appear as a cap on the nucleolus. The nucleolus, which later dis-integrates, separates from the nucleus. A second nucleolus begins to develop inside the nucleus giving rise to a variety of shapes, finally resulting in a configuration in which seven chromosomes, the centriole and the nucleolus form a long continuous filament, all interconnected by a Feulgen-negative strand. The chromosomes increase in size. This filamentous nucleus divides longitudinally, each chromosome dividing individually, not in a sequence. The nucleolus also divides such that each daughter filament possesses one. In separation of the filaments

no spindle or polar movement of the chromosomes was observed. Weijer and co-workers (Weijer et al., 1963, 1965; D.L. Weijer, 1964) thought that separation is guided by cytoplasmic streaming only. The daughter filament condenses into a short string and folds up into a globular interphase nucleus which is incorporated into a macroconidium (Weijer et al., 1965).

Maturation cycle II precedes microconidial formation. Only a few nuclei of Maturation cycle I develop into Maturation cycle II. The link between Maturation cycles I and II is not clear. Maturation cycle II proceeds either from the post-division Needle phase nucleus (daughter filament configuration) of Maturation cycle I or from the condensed pre-division nucleus that has just separated from its nucleolus (Global phase). The latter is thought to be more likely, the nucleus developing a second nucleolus within and then unfolding from around the nucleolus into a thin reticulum which condenses into a single long particulate filament still attached to the nucleolus. The nucleolus decreases in volume and disappears. The filament becomes double and undergoes further condensing. A nucleolus again appears, to which the two daughter filaments are attached. The nucleolus divides, resulting in two separate daughter filaments, each with its own nucleolus. One of these two daughter filaments condenses into a spherical nucleus and is incorporated in a microconidium. The remaining daughter filament prepares for a further division.

Weisberg and Weijer (1968) studied the nuclear events during hyphal differentiation (Maturation cycle) in Aspergillus nidulans. Though the Juvenile cycle was reported to be the same as that in

Neurospora crassa (Weijer and Weisberg, 1966), the Maturation cycles
of these two organisms have been found to differ.

After the Juvenile cycle, when the nutrient medium is
exhausted, the nuclei of Aspergillus nidulans condense into a spherical
shape. Upon further incubation, these spherical nuclei enter the
Maturation cycle by enlarging and developing into long filamentous
nuclei. They appear in progressive stages of polyteny and a lengthening
of interchromosomal connections occurs, such that a nuclear filament
may exceed 100μ in length. The formation of these large polytene nuclei
occurs just prior to the differentiation of the foot cell and of the
conidiophore.

Unlike in Neurospora, in Aspergillus nidulans the Juvenile
and Maturation cycles overlap. At the time of differentiation of the
foot cell, only Juvenile nuclei are observed in its lumen, whereas the
remainder of the hyphal structure may or may not contain Maturation
nuclei. Even later, when the secondary phialides are formed and a
Maturation nucleus is present in the conidiophore, only Juvenile nuclei
are supplied to developing primary phialides.

The long polytene hyphal nucleus divides by independent trans-
verse separation of the chromosomes and centriole. Centriole separation
does not (as in the Juvenile cycle) necessarily preceed chromosome
separation.

These polytene Maturation nuclei are thought to result from
endomitotic replication of haploid juvenile nuclei. In addition to
these nuclei, diploid maturation nuclei occur. These diploid nuclei

are characterized by excessive polyteny and have chromosomes that are

approximately twice the size of the regular maturation cycle chromosomes.

These diploid nuclei are thought to be part of the parasexual cycle as

described by Pontecorvo (1958).

It is not clear whether the polytene maturation nucleus of a

conidiophore is formed from fusion of the remainder of the Juvenile

nuclei after primary phialide formation or from endomitotic replication

of a single Juvenile nucleus.

Weisberg and Weijer (1968) did not investigate in detail the

nuclear division within the phialides and do not rule out the possibility

that such division might be classically mitotic, since the phialide is

a uninucleate cell.

Karyokinesis of the somatic nuclei of the ascomycete <u>Nanizzia</u>

<u>incurvata</u> (St. conid. <u>Microsporum</u> <u>gypseum</u>) was studied by Hejtmánková-

Uhrová and Hejtmánek (1967a, b). They found certain analogies with the

nuclear division in <u>Neurospora</u> <u>crassa</u> as reported by Weijer <u>et</u> <u>al</u>.

(1965). The ascospore contains one spherical resting nucleus.

Germination of the ascospore is preceded by a differentiation of six

to seven Feulgen positive bodies forming a ring. The center of the

ring is thought to be probably filled by a nucleolus. The nucleus, in

this ring phase, passes into the germ tube. The ring nucleus opens to

change into a horseshoe-like structure which in turn straightens out

into a filamentous nucleus containing the six or seven chromosomes in

tandem. These chromosomes are interconnected by a Feulgen negative or

very weakly Feulgen positive thread. The filamentous nuclei divide

longitudinally into two. Separation of the two daughter filaments begins in the center or at the ends and depends on the independent division of the individual chromosomes. The daughter filamentous nuclei can enter directly into a new longitudinal division or pass into a spherical interphase nucleus. No typical spindles or centrioles were visible. It is assumed that the spindle is intranuclear and that the spindle fibers form the linear axis of the filamentous nuclei, interconnecting the chromosomes in tandem and so ensuring the integrity of the haploid nucleus in the multinucleate and streaming cytoplasm.

A similar description of nuclear division was reported by the same authors (Hejtmánek and Hejtmánkova-Uhrová, 1968) in the dermatophyte Tichophyton vanbreuseghemii (St. perf. Arthroderma gertleri).

Heale et al. (1968) described nuclear division in Verticillium albo-atrum. In the conidium a densely staining circular resting nucleus develops a pouch-like opening thus giving rise to a horseshoe configuration. This configuration is probably due to the formation of a nucleolus in the pouch. The nucleus then forms a ring in which the chromosomes are linked by a fine thread. The chromosomes divide while still linked forming a double ring. The centriole divides to produce daughter centrioles. The rings separate and later condense. Spindle fibers are not evident.

In the hypha the resting nucleus is not densely stained as in the conidium but is a reticulate mass. This mass condenses into four chromosomes connected by a fine thread in a complex configuration. The four chromosomes divide while still linked together. The centriole divides and a spindle fiber is formed between the daughter centrioles.

Rapid elongation of the spindle fiber draws out the divided nucleus forming a double filament composed of two parallel rows, each of four chromosomes connected by a fine thread. The spindle fiber lies between the two filaments. Breakage occurs probably across the middle (transversely) of the parallel strands, now composed of condensing chromatin. The daughter nuclei undergo further condensation as polar movement is brought to completion by the spindle fiber. Nucleoli arise de novo. Ring stages are not evident in the hypha.

According to Ferreira and Phaff (1959), in a newly discovered species of Schwanniomyces, the first stage of division consists of the formation of a beaded ring-like structure. Presumably during this stage the chromatin material duplicates itself. Often the beaded bodies in the ring seem to occur in pairs. The ring breaks and the resulting strand may assume a number of characteristic shapes. The strand shortens and two terminal bodies, which are to become two daughter nuclei, approach each other very closely. The strand breaks and the two daughter nuclei separate.

McLarty (1941) studied Olpidiopsis Achlyae and reported that the spherical resting nucleus contains a centrally placed nucleolus. The appearance of delicate chromatin strands radiating out from the nucleolus indicates the beginning of prophase. In later stages, the chromatin is evident as globular or rod-like masses. McLarty (loc. cit.) associated the formation of these masses or chromosomes with the early disappearance of the nucleolus and suggested that the nucleolus in this species functions as a storehouse of chromatin.

Concomitant with the formation of the chromosomes is the
production and division of a centrosome-like body and the formation of
a spindle. The chromosomes become arranged in a ring about the margin
of the equator of the spindle. Astral rays are not evident. The ring,
most often viewed from its side, appears as a dark line of crowded
chromosomes. This dark line becomes two parallel lines. The spindle
elongates, forcing the two masses of chromatin farther apart. The
stages of interphase reorganization of the daughter nuclei were not
followed.

Olive (1952) reported that in the hyphae of the basidiomycete
Itersonilia perplexans the resting nucleus consists of a nucleolus
surrounded by a nuclear vacuole. In division, a small spindle appears
beside the large nucleolus in the nuclear vacuole. Chromosomes, which
cannot be distinguished individually, appear as a band of dense material
across the equator of the spindle. A centrosome appears to be at each
spindle pole. The band of chromatin separates into two equal parts
which move towards opposite poles of the spindle. The nucleolus
decreases rapidly in size and disappears. The two chromatin masses
round up.

Laane (1967) studied somatic nuclear division in Penicillium
expansum. He observed that the dividing nuclei were composed of five
chromosomes or four chromosomes and one centriole interconnected by a
Feulgen positive material and that the nucleus was wound, in the shape
of a V, around a disk-like nucleolus. Subsequently the five bodies
arranged themselves in a linear fashion to form a filament. The fila-
ment splits longitudinally into two daughter filaments which separate.
Separation may start at one end or it may start at the middle giving

rise temporarily to a ring structure. No spindle apparatus was detected and it is thought that separation is aided by cytoplasmic streaming. Each daughter filament folds up in a zig-zag fashion, giving rise to an interphase nucleus.

Kwon-Chung (1969), studying Coccidioides immitis, found a V-shaped configuration similar to that found by Laane (1967). Kwon-Chung (loc. cit.) also observed two distinct nuclear cycles, one occurring in actively growing young hyphae and the other in old thin hyphae or during arthrospore formation.

In the first cycle, the resting nucleus is a round dense chromatin mass. The nucleus proceeds to a ring stage in which three chromatin bodies are apparent. The ring is opened, forming a V-shape. The V-shape proceeds to form a rod-like configuration which extends to become a filament with three beads. The filament divides longitudinally to produce two daughter nuclei. The separated daughter nuclei reorganize into round, dense resting nuclei.

In the old part of the colony, some thinner hyphae showed a simpler cycle of nuclear division. The round resting nucleus becomes a short rod which in turn divides transversely at the middle, producing two spherical daughter nuclei. Spindles or metaphase plates were not observed in either cycle.

Namboodiri and Lowry (1967), using electron as well as light microscopy, investigated vegetative nuclear division in the "clock" mutant and wild type strains of Neurospora crassa. They concluded that division can occur in two forms. The rounded resting nucleus can

either become elongated and divide or it can form a ring and divide. In the first case, the pre-division elongated nuclei were observed, through electron microscopy, to have a knob-like terminal portion that was correlated to a deeply stained knob in light microscopy observations. This knob was noted to undergo displacement from the elongated nucleus, but still remain attached by a weakly stained thread. Ultra thin sections showed that the nuclear envelope is still intact at this stage but disintegrates later on.

In the early stages of division, the nucleus coils. Then despiralization occurs and the nucleus appears visibly duplicated into two strands. Usually the separation of the strands begins from the end opposite the one with the darkly stained knob or from the middle. Sometimes the daughter strands are wrapped around each other and have to rotate at their ends to separate.

A variable number of bead-like bodies appear on the daughter strands, and two or more such beads may merge to form chromosomes.

In some cases, before duplication is completed, a terminal or intermediate chromosome is displaced from the beaded chain, but is still attached by an interchromosomal strand. It is suggested that this displaced chromosome acts as a focal point for the other chromosomes to follow and thus may act as a mitotic center.

Displaced chromosomes were not detected in all separating chromatin strands, especially in cases where the separating strands have "interzonal" connections between them.

During the terminal phase of division, the chromosomes become less distinct. Electron micrographs show that the nuclear envelope is reconstituted at this stage. Each of the elongated daughter nuclei undergo slight linear condensation. From this stage, the elongated nucleus may pass into the next division cycle without reverting to the rounded form, it may transform into forms of varying degrees of annularity (e.g. crescent, semi-ring and ring-shaped nuclei), or it may undergo linear condensation to form a rounded resting nucleus.

In the second mode of division, the rounded resting nucleus can assume a ring-like form. The nucleus will duplicate to form a double ring, sometimes connected together by "interzonal" connections. If the rings are interlocked, separation is effected only when at least one of the rings breaks. Distinct chromosomes are apparent only after separation of the daughter rings. In any case, the rings eventually break, chromosomes become indistinct, and the rounded resting nucleus is again formed.

Robinow and Caten (1969), using light and electron microscopy, investigated mitosis in the hyphae of haploid and diploid _Aspergillus nidulans_. Nuclear division occurs within an intact nuclear envelope and commences with the condensation of a chromatin network into distinct chromosomes. The chromosomes become arranged in a double bar which is resolved into daughter nuclei by a transverse separation. Lying within the chromatin mass and elongating with the separation of the daughter complements is an acidophilic fiber which develops from a granule present in the resting nucleus. This fiber is composed of a bundle of fibrils and is believed to be the equivalent of a mitotic spindle.

Aist and Wilson (1965, 1966, 1967a, b, 1968), Wilson et al.
(1966) and Brushaber et al. (1967) described an asexual nuclear division
common to Ceratocystis fagacearum, C. ulmi, C. virescens, C. ambrosia
C. coerulescens, Fusarium oxysporum, Neurospora crassa, Alternaria
cucumerina, Colletotrichum gossypii, Fomes annosus, Lenzites saepiaria,
Rhizoctonia solani, Sclerotium rolfsii, Thielaviopsis basicola and
Verticillium albo-atrum. According to these authors, the nucleolus is
usually on the periphery of the chromatin in the interphase nucleus.
In division, the chromosomes contract and thicken, and assume a linear
end-to-end arrangement, forming a bar or filament of chromatin that lies
against the hyphal wall. Prior to division this bar of chromatin becomes
double stranded. The chromatids begin to separate and a spindle develops
between the separating chromatids and pushes them apart. This anaphase
movement is unilateral and not synchronized. In other words, the
chromatids of one strand are pushed asynchronously away from the
chromatids of the other strand such that the migrating chromatids will
eventually form a second chromatin bar against the hyphal wall opposite
the stationary chromatin bar. The spindle disappears and the daughter
nuclei migrate out of the old enlarged nuclear envelope one at a time.
The old nuclear envelope disappears. The nucleolus may fade from view
during division, or it may remain visible until division is complete
and then disappear. New nucleoli form in the maturing daughter nuclei.

Brushaber et al. (1967) commented on the high frequency of
occurrence of double stranded nuclei with paired chromatin and concluded
that all of these could not be metaphase plates. They (loc. cit.) also
mentioned that a centriole was not positively identified although
bodies that might have been centrioles were frequently observed.

Later, Aist (1969) published a completely different description
of nuclear division in Ceratocystis fagacearum and Fasarium oxysporum.
He noted the occurrence of a centriole in the interphase nucleus. In
division, the centriole divides and a spindle is formed between the
separating daughter centrioles. Chromosomes become visible and attach
to the spindle at different points. The chromosomes split longitudinally
and the sister chromatids move along the spindle to opposite poles.
This anaphase movement is asynchronous. The chromosomes fuse into a
homogeneous mass at each end of the still-existing spindle. Continued
elongation of the spindle further separates the daughter nuclei. The
spindle then breaks and disappears. The behavior of the nucleolus is
quite variable, but eventually it is lost in the cytoplasm and disintegrates.
The daughter nuclei produce new nucleoli.

This description given by Aist (1969) closely fits the descrip-
tions given earlier by Motta (1967), Girbardt (1968) and Ichida and
Fuller (1968), and in the same year by Motta (1969) which were based on
electron as well as light microscopy.

Girbardt (1960, 1961a, b, 1962, 1968) studied the ultrastructure
of the nuclear division occurring in the basidiomycete Polystictus
versicolor (Trametes versicolor). He noted that nuclear behavior in
monokaryotic and dikaryotic hyphae is the same. At the first stage of
division, the nucleus increases in size. Then the chromatin contracts
and the nucleolus dissolves in the cytoplasm, resulting in a very small
nucleus. Connected to the outside of the external membrane of the
nuclear envelope is a bipolar activity center of the nucleus called the
KCE (kinetochore equivalent) which lacks the typical centriolar

ultrastructure. At this early stage of division, microtubules are in a scattered or unspecific arrangement in the cytoplasm throughout the cell and rarely do they connect with the bipolar KCE. Later, many microtubules appear, radiating from the globular parts of the bipolar KCE into the cytoplasm. Their appearance is correlated with nuclear movement in the cell. The nuclear envelope breaks down, connection with the KCE is lost and the bipolar KCE enters the condensing chromatin inside the nucleus. While the KCE is inside the nucleus, microtubules are not found in the cytoplasm. A bundle of growing microtubules push apart the globular poles of the KCE, thus forming a central cord (Zentralstrang).

All of the condensing chromatin collects around the central cord. A new envelope is formed and microtubules are again extending from the KCE into the cytoplasm. Both globular ends of the KCE, now lying at opposite poles of the dividing nucleus, are enclosed by a perforated cap of an endoplasmic reticulum cisterna which is continuous with the nuclear envelope. The two poles of the KCE move in opposite directions and the central cord is elongated. The microtubules of the central cord are torn centrally and the nuclear envelope also tears in this region and immediately closes, surrounding the chromatin of the two daughter nuclei.

The young daughter nucleus has no intranuclear microtubules, but microtubules may extend from the unipolar KCE through the endoplasmic reticulum cap into the cytoplasm. The unipolar KCE remains connected to the nuclear envelope. A new bipolar KCE is formed, developing from a small double granule at the base of the old unipolar KCE which then disappears.

Robinow (Robinow and Bakerspigel, 1965), using light microscopy, claimed to have discovered in Schizosaccharomyces versatilis the same kind of "Zentralstrang" as Girbardt. During division, a straight fiber is seen in the nucleus to one side of the nucleolus. The fiber lengthens and pushes the poles of the nucleus apart. As the nucleus expands, it assumes a spindle shape due to the slow moving disintegrating nucleolus at the center. The inertia of the nucleolar material is soon overcome and the nucleus is pulled out into a long narrow shape with two masses of nucleolar material at each end. With elongation continuing, the nucleus snaps in two.

Motta (1967, 1969) examined the ultrastructure of the dividing nucleus in the rhizomorph meristem of the basidiomycete Armillaria mellea and found that a spindle apparatus, similar to that reported by Girbardt (1968), was involved. A large nucleolus was found in most of the interphase nuclei. Prior to division, chromatin aggregates at the periphery of the nucleus and two spherical uniform dense centrosomes appear at the poles in the cytoplasm adjacent to the nuclear membrane but not in contact with it. These polar bodies or centrosomes do not show the regular centriolar ultrastructure. There is some evidence that the centrosomes arise singly and duplicate during the early stages of division. A centrally located, cylinder-like bundle of microtubules is formed, extending through the nucleus to the centrosomes in the cytoplasm. The nuclear membrane is intact except for discontinuities which occur at the points of penetration of the spindle. The chromatin material becomes distributed about the periphery of the spindle. The chromatin condenses and aggregates into chromosomes which can be seen to attach to the spindle, though kinetochores were not observed. The nucleolus

and nuclear membrane begin to disappear. The spindle becomes fusiform in shape and asynchronous separation of the chromatids and polar migration of the chromosomes takes place. Remnants of the spindle apparatus persist between the chromatin aggregations at the poles. Resynthesis of the nuclear membrane, in close association with the centrosomes, begins at the poles and eventually delimits the sister nuclei. The sister nuclei, lying close together with parallel, adjacent membranes in pore-to-pore association, separate.

Ichida and Fuller (1968) studied the ultrastructure of nuclear division in the zoosporangia of the aquatic fungus Catenaria anguillulae. The interphase nucleus has a nucleolus, and a single pair of centrioles is found exterior to the nuclear envelope and is accompanied by microtubules which radiate from the region of the centrioles. An ultra thin section of the centriole shows nine triplets of microtubules arranged in a circle. One of the centrioles migrates to the opposite side of the nucleus. The two centrioles will become double again at some point between their polarization and metaphase. After the centrioles become polarized, the nucleolus disappears, an intranuclear spindle is formed and chromosomes become clearly evident. During metaphase and anaphase, continuous spindle fibers traverse the length of the nucleus. Chromosomal fibers extend from the chromosomes to the polar area of the nucleus. However, there are no distinguishable kinetochores where the fibers connect with the chromosomes. Interzonal connections or fibers which extend between chromosomes moving in the opposite direction are not evident. The spindle elements do not pene-trate the nuclear membrane but terminate in a band of dense material which is opposite each of the centrioles and inside the nuclear membrane.

The nucleus elongates as the continuous spindle fibers elongate and the poles become more distant from each other. However, the length of the chromosomal fibers varies little and the two sets of chromosomes are pulled apart as the poles move apart. After the chromosomes have separated, the two daughter nuclei round up, leaving a large spindle-shaped structure between them. The two daughter nuclei pinch off and separate from this spindle-shaped structure and the membrane around the structure breaks down and the structure becomes part of the cytoplasm.

Berlin and Bowen (1965) observed that in nuclear division in vegetative hyphae and sporangiophores of Albugo candida and A. bliti, centrioles apparently organized an internal spindle through an intact nuclear envelope. This report is in the form of an abstract and whether or not it is based on ultrastructure studies is not known.

From the ultrastructure study of Saccharomyces cerevisiae, Hashimoto et al. (1958, 1959) concluded that the nuclear membrane did not disappear during nuclear division. A sharp wedge-like constriction of the nucleus is formed by the nuclear membrane. The constriction deepens, resulting in complete separation of the nucleus into two. A structure corresponding to a centriole was not observed; however, this does not negate the presence of such a body. Structural units, comparable to the chromosomes found in higher organisms, are lacking. The authors (1959) suggested that submicroscopic units of chromatin function as chromosomes. They (1958) also suggested that there is some mechanism which regulates the constriction of the nucleus other than the simple pressure of the constricting cell wall. The presence of an internal or external spindle was not reported, nor is such a

structure evident in the electron micrographs that they (1958, 1959) published.

Moore (1963, 1964, 1965), observing the ultrastructure of Cordyceps militaris, described what he has termed "karyochorisis" or nuclear sundrance. Karyochorisis is a type of somatic nuclear division that appears to be similar to cell division. Karyochorisis in Cordyceps appears to be initiated by an invagination of the inner nuclear membrane that upon completion divides the nucleoplasm into discrete subunits to which Moore (1964) has applied the new term "karyome". The invagination of the outer nuclear envelope commences after the completion of karyome formation and separates them into individual daughter nuclei.

During karyochorisis more than two karyomes may be produced. Karyomes are indiscernible in the light microscope and such nuclei would appear to be in interkinesis. Distinct chromosome phases are not recognizable in karychorisis.

In 1967 Moore suggested that the karyochoritically dividing nucleus may require rigidizing or girdering "microcables" to assure that the increase in nuclear envelope material will lead to consecutive infoldings that effect karyome formation and separation.

Rogers (1965) noted that in Coniochaeta ligniara the round interphase nucleus in an actively growing hypha displays a prominent nucleolus and thread-like chromatin material. Prophase nuclei take a variety of shapes and the prominent nucleolus is most frequently located eccentrically. The chromosomes are thread-like and as they become

thicker, one of them can sometimes be seen in close association with the
nucleolus. Other times, no connection between the nucleolus and
chromosomal mass is seen. The chromosomes become very short and the
nucleolus is either not visible or very much diminished in size. At
metaphase the chromosomes appear as short rods or as dots arranged in a
compact group. A spindle and what are probably centrioles can be seen.
The chromosomes, not individually distinguishable, asynchronously
migrate to the poles. Occasionally lagging chromosomes can be seen.
In several instances Rogers (loc. cit.) has seen material lying between
the two sister nuclei and attached to both. He (loc. cit.) suggested
that perhaps this was chromosomal material that was not incorporated into
either nucleus. Rogers (loc. cit.) maintained that the familiar form
of mitosis is operative in the hyphae of this species.

Callen (1940) reported that in Rhizopus sexualis the mitotic
divisions are intranuclear and that a clear non-staining area surrounds
the dividing chromatin mass. Prophase is indicated by an increase in
size of the chromatin mass, which assumes an irregular oval shape. At
metaphase, a spindle assumes a more or less central position over the
mass of chromatin. Centrosomes appear to be absent, though a small body
can sometimes be observed at one of the apices of the spindle. The
spindle itself is pointed at both ends and possesses a uniform structure
in which no trace of fibrils can be discerned. The chromatin divides
into two oval masses, each of which can sometimes be seen to be divided
into two portions. With the beginning of anaphase the two masses move
to the poles, and in telophase they are still seen to be connected by the
now thread-like spindle. The nuclear membrane elongates as they move
apart and begins to constrict in the midline, and ultimately the two
daughter nuclei separate completely.

A curious structure may often be seen in what would be late anaphase. There are two chromatin masses joined together by the spindle, the whole being crescent-shaped with the chromatin masses at the apices of the horns. In several cases the whole chromatin mass appeared to have moved to one pole, the meaning of which is not clear.

In 1908, E.W. Olive described vegetative nuclear division in the rust Triphragmium ulmariae. The nucleus consists of a small nucleole (nucleolus), a chromatin network and two centrosomes lying close together on the outer surface of the nuclear membrane. Each centrosome has attached to it a well-defined chromatin filament. The two centers move apart on the nuclear membrane and between these diverging bodies a filamentous structure or spindle appears. The nuclear membrane breaks down, the nucleolus is cast out into the cytoplasm where it disintegrates, and the spindle comes to lie at one side of the chromatin mass. Either, mantle fibers extend out from the centrosomes to the chromatin, or the chromatin is drawn into the central spindle. The chromatin separates into two and moves to opposite poles. During the later stages of division a few asters appear at the poles.

According to Schurhoff (1907), the nuclear division in the hyphae of Penicillium crustaceum takes place in the following manner. The nucleus increases in volume and two comma-shaped chromosomes become evident. The nucleolus gradually loses its ability to be stained with hematoxylin which may indicate a reduction in its contents. Soon the nucleolus disappears completely. The two chromosomes split longitudinally into four rather plump rod-like daughter chromosomes. Occasionally two connecting spindle bundles can be observed between the chromosomes.

Sometimes only one bridge can be observed due to the fact that the interconnecting spindle bundles are overlapping or situated adjacent to one another. In addition, an achromatic spindle configuration resembling that in classical mitosis can sometimes be seen, especially in the plasm-rich sterigmata. The daugher nuclei soon take on the appearance of resting nuclei and a nuclear indentation is evident. The nucleolus reappears.

According to Fuller (1962), mitosis in Rhizidiomyces apophysatus occurs in a normal manner except for the persistence of the nuclear membrane. During prophase the nucleolus gradually disappears. At metaphase four distinct chromosomes align on a metaphase plate and are attached to a spindle. At telophase, when the daughter chromosomes have separated to opposite poles, the persisting nuclear membrane constricts between the two poles and surrounds the daughter nuclei.

Cole and Couch (1959) observed mitotic nuclear divisions in conidial and mycelial growth forms of Kabatiella caulivora. They saw equatorial plates, spindle fibers and distinct chromosomes during division.

Evans (1959) investigated nuclear behavior in a large white variety of cultivated mushroom which is grown on a commercial scale in Britain. The resting nuclei are of two sizes. The large nuclei are granular in appearance and the smaller nuclei appear as dense bodies. When mitosis commences, the resting nuclei increase in volume. The chromosomes first appear at prophase as long threads, some of which have swollen heterochromatic regions which stain intensely. These nuclei are haploid and contain a single nucleolus which is especially prominent

a spindle. However, these configurations become distorted due to the movement of the cytoplasm during growth, especially in germ tubes and thin actively growing hyphae where the dividing nucleus may display an elongated appearance. The same can be said of hyphae constricting to form conidia.

In older hyphae where movement of the cytoplasm is not as rapid and in large hyphae, the distortion of the nucleus is not as pronounced and a more typical mitosis is observed.

Bianchi and Turian (1967), however, mention that for certain developmental stages of Neurospora, a facultative mechanism of longitudinal division of filamentous nuclei as advanced by Keeping in the Neurospora Newsletter (No. 8, p. 27, 1965) and by Weijer et al. (1965) must be considered.

Somers et al. (1960), Ward and Ciurysek (1962) and Lu (1962) have reported classical mitosis in the vegetative hyphae of Neurospora crassa, however, they were not able to detect any spindles. Somers et al. (1960) and Ward and Ciurysek (1962) claimed that the presence of spindles is suggested by late anaphasic and early telophasic configurations.

Ward and Ciurysek (1961) reported having observed typical stages of mitosis, with the exception of early prophase, in the dikaryon hyphae of an unidentified basidiomycete. The resting nuclei are of variable shape. At the onset of mitosis, four chromosomes can be distinguished in the nucleus. The chromosomes line up on a metaphase plate. A spindle is present. The chromatids separate towards the

spindle poles. At telophase the chromosomes can no longer be resolved.

Hartman (1964) reported that in <u>Alternaria</u> <u>tenuis</u> the hyphal and conidial nuclei undergo typical mitosis. In the early stages of division the chromatin network becomes denser and discrete strands become visible. By late prophase the dense nucleus assumes a particulate appearance. A metaphase plate with a spindle, but no visible centrioles, is formed. The chromosomes separate and move towards the spindle poles. Individual chromosomes cannot be distinguished. At telophase each chromosomal cluster becomes tightly compacted and loses its particulate nature. A rather faint hint of a spindle apparatus can sometimes be seen in the telophase nucleus.

Shatla and Sinclair (1964) reported that nuclei in the mycelium of <u>Rhizoctonia</u> <u>solani</u> divide by ordinary mitosis. Prophase, metaphase and anaphase, as well as rod-shaped chromosomes, were observed.

Lu (1964) concluded that the vegetative nuclei of <u>Cyathus</u> <u>stercoreus</u> divide by mitosis essentially similar to that of higher organisms and that this mitosis is essentially the same in the homo- karyotic and dikaryotic mycelium. He found that at the onset of nuclear division, the nucleus expands somewhat and that long, thread- like chromosomes appear (early prophase). The chromosomes coil and become contracted and visibly distinct. A centriole is present. At metaphase the chromosomes align themselves on the equatorial plate. The individual chromosomes cannot be distinguished at metaphase. The nucleolus is freed into the cytoplasm where it disintegrates. At anaphase the chromatids split apart, by-pass their sister chromatids

and move asynchronously to opposite poles. In each daugher nucleus,
the chromosomes uncoil and a new nucleolus is formed. Lu (<u>loc</u>. <u>cit</u>.)
claimed that the by-passing of the sister chromatids and the
asynchronous chromosome movement causes the anaphase configuration to
often appear like a chromatin cord.

MATERIAL AND METHODS

Preparation of Specimen

Three species of the Basidiomycete _Cyathus_, commonly called

the bird's nest fungus, were used throughout this study. The species

used were _C. olla_, _C. bulleri_ and _C. setosus_ and were obtained from Dr.

H.J. Brodie of the Department of Botany, the University of Alberta.

These species, which have a tetrapolar mating-type pattern, were studied

in their homokaryotic and dikaryotic forms. The homokaryons of each

species were crossed in all directions to make sure that all four of

the homokaryons of each species had, indeed, been obtained and that

none were duplicates of the others.

"Brodie medium" (Brodie, 1949) was employed for maintenance

of stock cultures.

In preparation for staining, cultures were grown on 75 x 25

mm glass microscope slides which, prior to inoculation, had been auto-

claved and, while still hot, quickly dipped once into a hot solution

of Brodie medium at one-third strength. Each inoculated slide was

kept in a Petri dish (20 mm in depth and 100 mm in diameter). Each

Petri dish had a No. 1 Whatman filter paper circle (90 mm in diameter)

placed in it. Approximately 5 - 10 ml of distilled water was added

to each dish to moisten the filter paper. Then two glass rods were

placed in the dish. The Petri dish and contents were autoclaved. The

dipped microslide was placed in the Petri dish on the two glass rods

so that it did not make contact with the moist filter paper. After

cooling the slide was inoculated.

Synchrony of nuclear divisions was attempted by keeping some 15 day old cultures at 4°C for three months.

In preparation for live phase contrast observation, the well-slide technique of Weijer et al. (1963) was used. Thin 75 x 25 mm depression slides with a well 18 mm in diameter were used. These slides were cleaned in absolute alcohol, wiped with lens paper and allowed to dry. Each slide was placed in a Petri dish (20 mm in depth and 100 mm in diameter) which contained a No. 1 Whatman filter paper circle (90 mm in diameter) at the bottom of the dish. The Petri dishes and contents were sterilized. Corning 24 x 50 mm No. 1 1/2 coverslips were cleaned in absolute alcohol, wiped with lens paper, dipped in clean absolute alcohol and flamed to remove any wax. As with the depression slides, each coverslip was placed in a Petri dish that contained a filter paper circle. The Petri dishes and contents were sterilized. Disposable Pasteur pipettes, used to dispense the culture solution, were plugged with cotton at the wide end, placed in test tubes which were capped and were sterilized. The inoculation was carried out in a room that had been exposed to ultra violet light for several hours beforehand.

A drop of liquid paraffin was placed in the well to almost fill the depression. A small drop of 2% (w/v) malt extract solution was placed on the Corning 24 x 50 mm No. 1 1/2 coverslip. To this droplet was added an inoculum of mycelial growth from a Cyathus stock culture. The inoculum was then spread out by careful teasing with two needle probes. The coverslip was inverted and placed over the paraffin on the depression slide. Air bubbles were avoided as much as possible by adjustment of the amount of paraffin in the well. The slides were placed in Petri dishes and incubated at 25°C in a Millipore incubator.

Staining and Mounting of Preparations

Feulgen

The Feulgen stain was prepared as follows (Jensen, 1962, p. 199):

Basic fuchsin	4 g
$Na_2S_2O_5$	7.6 g
0.15 N HCl	400 ml (room temperature)

The resulting solution was shaken for two hours. Two grams of activated Norit A powder charcoal was added and after two minutes of shaking the suspension was filtered through a Whatman cellulose extraction thimble to remove the carbon. The filtrate was stored at 4°C in an air tight amber bottle.

The medium-dipped microslide with a growing mycelium was dipped into liquid nitrogen and then placed in a vacuum for one hour. A Virtis freeze drier was used for this purpose. The microslide was then placed in 5 N HCl at room temperature (Decosse and Aiello, 1966) for 75 minutes. During this hydrolysis the mycelium was carefully removed from the microslide by a scraping action with the glass edge of the small end of a glass capillary Pasteur pipette. Then the mycelium, without the microslide, was transferred serially to 2 1/2 N HCl (1 minute), 1 N HCl (1 minute), distilled water (15 minutes - 4 changes) and Feulgen solution (75 minutes). The preparation was then washed in several changes of tap water until the water remained clear and not pink (5 - 10 minutes). The stained mycelium was floated onto a clean microslide and mounted directly in Gurr's water mounting medium. Corning No. 0, 22 x 22 mm coverslips were used.

Crystal Violet

The medium-dipped microslide with a growing mycelium was fixed in Navashin's fluid (Conn et al., 1965, p. 9) for 24 to 36 hours and then washed in tap water for 6 to 12 hours.

Staining was as follows (Weisberg and Weijer, 1968):

1% Aqueous crystal violet	10 min.
Lugol's solution (1% KI and 1% I in 80% ethanol)	4 min.
95% Ethanol (2 changes)	2 min.
98% Ethanol (2 changes)	2 min.

The stained microslides were then cleared in three changes of xylol and mounted in Canada Balsam.

Cotton Blue   Lacto-phenol

The cotton blue   lacto phenol stain was prepared as follows (Darlington and LaCour, 1962, p. 146):

	Lactic acid
Equal	phenol
parts	glycerine
	water

0.08% to 1% cotton blue is dissolved in the above.

A drop of cotton blue · lacto-phenol stain was placed on a microslide. A small amount of mycelium was transferred with an inoculating needle from a stock culture to the drop of stain. The mycelium was teased apart and spread out in the drop with the aid of two inoculating needles. A coverslip was placed over the preparation and a gentle pressure was applied to the coverslip.

Giemsa

The Giemsa stain was prepared as follows (Conn et al., 1960; A.H. Grynchenko, personal communication):

0.6g of Giemsa powder was dissolved in 50 ml glycerol by allowing the mixture to stand from one and one half to two hours at a temperature of 55 to 60°C. Then 50 ml of methanol was added and the preparation was allowed to stand at least one day before use.

Singleton's fixative was prepared by mixing six parts absolute alcohol, one part glacial acetic acid and one part lactic acid (Singleton, 1953).

The buffer solution was prepared by mixing a 0.5% (w/v) KH_2PO_4 solution and a 1% (w/v) Na_2HPO_4 solution so as to obtain a pH of 6.8.

A medium-dipped microslide with a growing mycelium was fixed for 10 minutes, after which it was quickly dipped in 95% ethanol (5 seconds), and subsequently in 75% ethanol (10 seconds) and distilled water (10 to 15 seconds, or until the water adheres to the slide). The slide was then transferred to 1N HCl at room temperature for five minutes and then to 1N HCl at 60°C for six minutes. The material was washed in five changes of distilled water, one minute in each change, and then placed in the buffer solution for five minutes. The slide with the mycelial growth was placed in a Giemsa stain-buffer mixture (10cc of buffer solution and 20 drops of Giemsa stain) from 30 minutes to 12 hours. The preparation was washed by dipping it into distilled water. Then it was dipped in buffer solution and mounted in a drop of Abopon (Valnor Corp., Brooklyn).

DNA Specificity Test for Feulgen Staining

An enzyme experiment with deoxyribonuclease (DNase) was run to eliminate any doubt that Feulgen positive material in a stained preparation was DNA. The technique followed is that outlined by Leuchtenberger (1958) and used by Namboodiri (1964) on Neurospora. Twenty mg rather than the suggested 8-10 mg of DNase was used per 50 ml buffer solution.

The DNase reagent and buffer were prepared as follows (Leuchtenberger 1958, p. 273):

1. The buffer was made from solutions of NaOH (0.4 g in 150 ml of distilled water) and KH_2PO_4 (1.3 g in 150 ml of distilled water). The solutions were mixed in the ratio of 1 (NaOH): 2 (KH_2PO_4) and diluted with an equal quantity of distilled water. The pH of the buffer was checked with a pH meter and was found to be 6.9 (a pH of 7 was desired).

2. To 100 ml of this buffer, 100 mg of gelatin (bacteriological grade) was added and the mixture was heated very slightly with constant shaking until the gelatin was in solution.

3. Thirty-six mg anhydrous $MgSO_4$ was added and allowed to dissolve.

4. The buffer was allowed to cool to room temperature and was poured into two 50 ml lots. To one of these 50 ml lots, 20 mg of DNase II (bovine spleen, Calbiochem Corp.) was added.

Three medium-dipped microslides with fungal growth were used. Two of the microslides were incubated at 37°C for four hours - one slide in the buffer solution and the other in the buffer solution with the DNase added. The third slide was left at room temperature for the four hours.

All three slides were then dipped in liquid nitrogen, dried in a vacuum, hydrolyzed, Feulgen stained and mounted according to the preceeding schedule.

Photomicrography

Observations and photomicrographs were made with a Leitz Orthoplan microscope (tube factor 1.25) fitted with 10X periplan hi-point compensating oculars. A Leitz xenon lamp was used as a light source for both brightfield and phase contrast micrography.

A Leitz aplanatic condenser fitted with oil cap (N.A. 1.40) and Leitz oil immersion plano objective (100X, N.A. 1.32) were used in brightfield micrography. A Leitz phase apochromatic oil immersion objective (90X, N.A. 1.32) and Leitz variable phase (Heine) condenser were used in phase contrast micrography.

Photomicrographs were taken with a Leitz Orthomat 35 mm automatic camera. Photomicrographs of Feulgen stained preparations were taken with a 540 (green) interference filter. A 576 (yellow) interference filter was used for crystal violet stained preparations. Phase contrast photomicrographs of living preparations were taken with a Leitz Mikroblitz 300 flash lamp set at 300 watt-seconds and no interference filters were used.

Kodak High Contrast Copy extreme resolution panochromatic 35 mm film was used for photomicrographs of the Feulgen and crystal violet stained preparations. The film was developed with Kodak D 19 developer for 6 minutes at 20°C. Adox KB14 fine grain 35 mm film was used for phase contrast photomicrographs of living preparations. The

film was developed with Kodak D 19 developer for 6 minutes at 20°C.
Adox KB14 fine grain 35 mm film was used for phase contrast photo-
micrographs of living preparations. The film was developed with
Neofin-Blau (Tetenal-Photowerk, Hamburg-Berlin) for 12 minutes at 20°C.
Enlargements were printed on Gevagam variable contrast paper and
developed in a suitable developer.

For publication these prints were mounted on cardboard and
photographed on Kodak Pan X 35 mm film. Enlargements were then made
on 8 1/2" x 11" Gevagam variable contrast paper and bound directly into
the thesis.

OBSERVATIONS

General

Mycelial growth on medium coated microslides was found to be too dense for cytological examination when full Brodie medium was used. Brodie medium at one-third strength was found to be satisfactory.

The mycelia of the three species studied in the above manner appeared white in color, however, some mycelia of the stock cultures of Cyathus olla and C. setosus homokaryons and dikaryons darkened to deep brown colour after four to six months of cultivation. Cultures developing a brown colour were not employed in cytological studies. The C. bulleri homokaryon and dikaryon stock cultures maintained an almost white mycelial color, except for one culture which turned a cream color.

The homokaryotic mycelia of these species were observed to be very 'matty' with the hyphae intertwined, whereas the dikaryotic hyphae were less intertwined and grew out or radiated from the point of inoculation.

An attempt to synchronize nuclear divisions by placing 15 day old cultures at 4°C for three months, failed. Examination of Feulgen stained material revealed the presence of spherical, filamentous and intermediate nuclei. However, the spherical nuclei were more frequent than in the non-cooled mycelia.

The intensity of the Feulgen staining was increased by removing the mycelium from the agar medium coated microslide and

placing the isolated mycelium into the Feulgen reagent, rather than placing the mycelium, while still attached to the microslide, into the reagent.

Cultures were grown on cellulose circles covering Brodie medium in Petri dishes. The cellulose circle with the growing mycelium was removed and run through the Feulgen staining technique. Since the staining was not intense enough for cytological examination, this method was discarded.

Unless otherwise stated, the photographs are of Feulgen stained preparations.

Similar nuclear configurations have been observed in the homokaryotic and dikaryotic forms of all the three species of Cyathus investigated. This finding suggests that these species (either in the homokaryotic or dikaryotic state) have comparative nuclear cycles.

The nuclei were observed to exist in a condensed state, a filamentous state, and a variety of forms which seemed to be intermediates of the first two states.

Condensed Nuclei of the Homokaryon

Condensed spherical nuclei are frequently encountered in the hyphae of all the three Cyathus species studied. Conspicuous deeply stained satellite bodies are often associated with the condensed nucleus (Fig. 88, C. bulleri). Similar configurations are evident in C. setosus (Figs. 40, 48 and 60) and C. olla (Fig. 3). Such granules are noted in the literature review of this thesis as having been observed by many investigators, some of whom attribute a centriole-like function

to these organelles. Hence, in this report such structures are referred to as "centriole-like bodies".

Since condensed nuclei seem to be located in very wide hyphae (Fig. 96), the question can be asked as to whether or not condensed nuclear configurations are a sign of a gerontic condition of the hyphal structure, therefore constituting a nucleus in a resting stage. Although hyphae with large diameters are laid down by the fungus, it is common to find condensed nuclei occupying extremely thick-walled hyphae with a brownish discoloration typical for aging hyphal structures.

Intermediate Nuclei of the Homokaryon

Early karyokinetic stages such as ring and horseshoe nuclei are evident in all three Cyathus species. Figs. 94 and 96 depict these structures in C. bulleri. Simultaneous occurrence of condensed spherical and ring nuclei, indicating non-synchrony of karyokinetic development, can be observed in Figs. 89, 90, 95 and 97. Similarly, late stages of karyokinesis, such as filament stage and separation stage, can occur simultaneously (Fig. 60, C. setosus). It is at this particular point that the Ascomycete Neurospora differs from the Basidiomycete Cyathus. Whereas in Neurospora nuclear synchrony is maintained over a long distance of hyphal structure (Weijer et. al., 1965), compartmentization of the Cyathus hyphae seems to allow for a different timing of karyokinesis of nuclei in adjacent nuclear compartments. Observations on C. setosus indicate that filamentous nuclei may occur adjacent to condensed spherical nuclei located in different hyphal compartments (Fig. 38). Rarely does one see within the same hyphal

segment of the homokaryon different karyokinetic stages. In comparing

the homokaryon with the dikaryon (see p.77), it can be noted that in

the dikaryon the simultaneous occurrence of filamentous and condensed

spherical nuclei within the same hyphal compartment is extremely

common especially during the division of the clamp nucleus.

In C. setosus identical ring and horseshoe-like nuclear

structures are present. Fig. 47 indicates that ring configurations do

occur simultaneously with short filaments which are still of a rather

condensed but yet particulate nature. A horseshoe configuration with

a conspicuous triangular-shaped centriole-like body can be observed

in Fig. 43 (C. setosus). Fig. 45 (C. setosus) closely resembles a

nuclear configuration observed in N. crassa (Weijer et al., 1965)

in which the centriole-like body is located at quite some distance

from a ring-shaped nucleus. A very early phase of nucleolar development

is depicted in Fig. 44. The developing nucleolus appears as a Feulgen

negative "central body" within the condensed spherical nucleus. Con-

densed spherical nuclei often occur simultaneously with early developing

ring and horseshoe nuclei. In Fig. 39 a horseshoe or an incomplete ring

nucleus is shown adjacent to a condensed spherical nucleus. The

significance of this information is that it may constitute the develop-

mental relationship: spherical nucleus - horseshoe nucleus - ring

nucleus, as is the case in N. crassa. Such a sequence of events would

facilitate the explanation of the progressive development of the

nucleolus during these stages, as is evident in Figs. 46, 48, 49 and 50

for ring nuclei and in Figs. 41 and 42 for somewhat later stages (early

filament stage). In C. olla early nucleolar development is evident in

Figs. 3 and 4.

C. olla homokaryons do not differ from those of C. setosus and C. bulleri. Condensed spherical nuclei as well as ring nuclei are encountered in the same vegetative hyphae (Fig. 5).

Filamentous Nuclei of the Homokaryon

The filamentous nucleus is characteristically long and particulate. Figures 89, 97, 99, 100, 101, 102, 103, 104, 105, 106 and 108 depict such filaments in the Cyathus bulleri homokaryon. Fig. 103 reveals seven Feulgen positive (chromatin) bodies, though when longer filaments are encountered the chromatin bodies seem to be more numerous. For example, the structure in Fig. 99 has 12 chromatin bodies, but whether the structure represents a single or a double nucleus is not clear. Neither is it clear, at this time, whether these chromatin bodies constitute entire chromosomes or chromosomal structures in terms of satellites and sub-terminal appendices. Fig. 104 would indicate that there are at least seven main chromatin bodies in C. bulleri. The number of chromatin bodies exceeds seven in Figs. 105 and 106. Approximately 15 bodies can be counted in Fig. 108, however, the structure may consist of two filaments in tandem. In live phase contrast observations (as depicted in Figs. 125 and 126) a good concordance with the Feulgen stained configurations is evident.

Figs. 47, 51, 52 and 53 show filamentous nuclei in a C. setosus homokaryon. In some instances (Figs. 51 and 52) nuclear bundling due to septal obstruction was noted. These configurations may confuse the observation of the progression of divisionary stages since, in this manner, structures may originate which closely resemble established karyokinetic structures such as duplication and separation stages.

Filamentous nuclei of C. olla are depicted in Figs. 3, 4, 5 and 6.
Phase microscopy of freeze-dried but unstained material reveals
within the same hyphal structure (Fig. 8) the presence of a long
nuclear filament and a partially condensed nucleus.

Figs. 98, 99, 100, 101 and 102 depict the centriole-like
body at one of the ends of the filamentous nuclei in a C. bulleri
homokaryon. From a comparison of Figs. 99 and 100, one would deduce
that the structure is plate-like, that is to say, thinner on edge
than when seen face on. If, as suggested earlier, the structure in
Fig. 99 is actually composed of two filamentous nuclei, then the center
darkly stained body possibly constitutes the second centriole-like
body. The connections between the nuclei and their centriole-like
bodies are faintly Feulgen positive, indicating that some chromatin
is present.

As in C. bulleri, these centriole-like bodies are seen in C.
setosus (Fig. 53). In the C. olla homokaryon, the development of a
centriole-like body is depicted in Fig. 6 in which the filamentous nucleus
is in the process of unfolding.

Filamentous nuclei have been observed in a state of replication
and longitudinal separation in the homokaryotic forms of Cyathus. Fig.
119 pictures an early duplication configuration of the nucleus of C.
bulleri, whereas nuclear structures in Figs. 111, 114 and 120 (C. bulleri)
may be indicative of longitudinal division, as is the case in Fig. 115
in which two nuclear strands with a reasonable amount of chromatin body
homology are present. A nuclear double structure with perfect homology
is evident in Fig. 116.

The onset of the actual separation of daughter nuclear filaments is pictured in Fig. 109, showing the separation of nuclear strands at the terminal point of the nuclear double structure. Fig. 112 can be interpreted as an end-state of division in which the two daughter strands hold together at a terminal point and twist around each other by the free ends. A similar situation is evident from Fig. 113, but in this latter case the free ends of the daughter filaments are clear of each other. The particulate nature of the divisionary structure can be observed in Fig. 118.

Early division of the centriole-like body, prior to nuclear division is depicted in Fig. 107, whereas Fig. 121 represents an early division in which nuclear strand separation seems to precede the division of the centriole-like body. Simultaneous division of both the centriole-like body and the nucleus is indicated in Fig. 117.

Filamentous divisionary configurations are also evident in C. setosus and C. olla homokaryons. For instance, karyokinetically early "Y" shaped configurations are present in Figs. 8, 63 and 110. Characteristic chromatin body homology, expected to occur late in karyokinesis, is evident in Fig. 54.

From observations it is evident that late stages of karyokinesis are less stainable than early stages. Figs. 56 and 58 picture a late stage of somatic karyokinesis with individual chromatin bodies faintly visible. Similar observations can be made from Fig. 55. In comparing Fig. 56 (late stage) with Fig. 57 (early stage of karyokinesis) the difference in stainability becomes obvious. Fig. 61 depicts a stage in which division of the centriole-like body does not precede the division

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of the nucleus, a phenomenon reported for other fungi (N. crassa, Weijer et al., 1965).

The formation of the nucleolus is sometimes evident in Feulgen stained preparations of filamentous nuclei. In Figs. 92 and 93 a subterminal nucleolus can be seen. By comparing these structures with those present in literature for N. crassa, one can see that these pictures resemble the Needle phase as they were observed in living material of Neurospora (Weijer et al., loc. cit.).

Fig. 122 (C. bulleri) is of special interest. Two spherical nuclei with a slightly Feulgen positive connecting strand can be observed. Such a configuration may represent the Bakerspigel phenomenon of indirect division, although its frequency is extremely rare. Its origin, on the other hand, may be related to that of a persistent telophase as is sometimes found in higher organisms. Similarly, in C. setosus the significance of the double spherical nuclear structure, as depicted in Fig. 38, is not clear. This structure may be indicative of nuclear division according to the Bakerspigel theory or, may be caused by overlapping of two adjacent nuclei. The criterion for distinguishing between the two alternatives would be the existence of an internal spindle, which unfortunately is not visible by the light microscopy methods employed.

Nuclear Configurations of the Dikaryon

In the Cyathus bulleri dikaryon the condensed spherical nucleus elongates (Figs. 134, 135 and 136) thereby yielding a condensed nuclear filament. This structure may become elaborate, giving rise to a long single stranded filament (Figs. 139, 140 and 141) Subsequently,

duplication of the nuclear filament occurs (Fig. 142) after which the
double filament separates longitudinally (Figs. 143, 144, 145, 146 and
147). Filamentous daughter nuclei may coil up yielding ring-like
structures as can be seen in Fig. 198 (phase contrast microscopy,
Feulgen stained).

Condensed and intermediate nuclear configurations were observed
in the dikaryotic stages of C. olla, C. setosus and C. bulleri. Fig. 11
shows a centriole-like body to be some distance from a globular nucleus
in C. olla. These connections between nuclei and centriole-like bodies
are densely stained in the crystal violet staining procedure (Figs. 25
and 26). In the C. olla dikaryon ring configurations are frequent (Figs.
181 and 182). Often a dark stained centriole-like body can be noticed
associated with the ring, either attached to the ring (Fig. 182) or at
some distance from it (Figs. 183 and 184). Although ring divisions are
frequent in other fungi (N. crassa, A. nidulans, Weijer et al., 1965;
Weijer and Weisberg, 1966), ring divisions occur infrequently in Cyathus
(fig. 185).

During karyokinesis the ring enlarges considerably as is
evident in Figs. 186 and 197, the latter being of a Giemsa stained
preparation. Figs. 34 and 35 (the latter is the same as the first but
photographed using phase contrast microscopy) indicate that, most likely,
the spherical nuclear stage consists of a rolled up filament. In Figs.
194 and 195 ring nuclei can be observed, although the density of the
crystal violet staining obscures the fine detail. Division of centriole-
like bodies is evident in Fig. 195, which shows a ring with two centriole-
like bodies which have divided in frontal view. In Figs. 32 and 36
the centriole-like bodies are at some distance from the nucleus and

some nucleolar development is occurring.

In the C. setosus dikaryon a conspicuous dark stained centriole-like body is present, either attached to or at some distance from the nucleus (Figs. 70, 71 and 72) In a young mycelium,ring structures and horseshoe-like structures are common (Figs. 80 and 81). Condensed nuclei are apparent in Figs. 74 and 79.

Often nucleolar development can be observed in the early structures in C. setosus. For instance, in Figs. 71 and 72 the nucleolus can be seen as an area with decreased density within the nucleus. This nucleolar development becomes more pronounced at a later stage when the spherical nucleus unfolds (Figs. 72, 73, 75 and 76).

In the C. bulleri dikaryon spherical nuclei are slightly larger than in the other two species (Fig. 130). Sometimes a dark stained centriole-like body is attached at some distance to the nucleus (Figs. 131, 132 and 133). As was mentioned for the C. bulleri homokaryon (Fig. 122), rare examples of indirect division in accordance with Bakerspigel's theory can sometimes be seen in the C. bulleri dikaryon (Figs. 148 and 149). However, one has to remember that these configurations may originate at post-division, similar to what is known in higher plants as a persistent telophase.

Ring structures are somewhat less frequent in the dikaryon than in the homokaryon of C. bulleri. Fig. 138 depicts a ring configuration with a conspicuous nucleolus, whereas in Fig. 137 a nucleolus is indicated by a decrease in the density of the Feulgen stain.

As in the homokaryons, the dikaryotic form of Cyathus has filamentous, condensed and intermediate nuclear configurations. However, clamp connection formation in the dikaryon can be used as a developmental "marker" in studying nuclear division, an advantage that is not available in the observation of homokaryons.

In the C. olla dikaryon there is an abundance of filamentous structures (Fig. 10). A conspicuous dark stained centriole-like body can be distinguished at the end of the nuclear filament (Figs. 10, 12 and 13). In Fig. 14 a filamentous nucleus in a folding position is recorded. A single strand at approximately a length of 6μ is depicted in Fig. 15.

The filament divides longitudinally without a site preference for strand separation (Figs. 16, 17, 18, 19, 20 and 22). Duplication of chromatin material is pictured in Figs. 21 and 24. Chromosome homology of separated strands in a clustered configuration are pictured in Fig. 23.

Giemsa stained preparations show approximately the same configurations, although with Giemsa much of the detail is lost and the particulate nature of the filament is often obscured (Figs. 28 and 29). Figs. 30 and 31 depict strand separation in a Giemsa stained preparation.

Crystal violet staining confirms observations made on Feulgen stained material. Post division filaments arise with a characteristic centriole-like body at the terminal position (Fig. 27). Elongated nuclei can be seen in Figs. 187, 188, 189 and 196. Occasionally the crystal violet staining will bring out the Woronin bodies (Figs. 188, 190 and 191). Figs. 192 and 193 show filaments dividing. In Fig. 192 the centriole-like bodies have divided as well.

At a somewhat later stage the chromatin bodies become very conspicuous as is shown in Figs. 172, 173 and 174. The average chromatin body count amounts to approximately seven.

Fig. 175 is of interest since it depicts late stages of karyokinesis in which the individual chromatin bodies are visible in great detail. At least 10 chromatin bodies and a centriole-like body are present in these complements. It should be pointed out, however, that the overall chromatin mass greatly exceeds the amount present in the homokaryotic phase of C. olla. A similar situation can be seen in Fig. 176 and, therefore, these configurations could be interpreted as polyneme chromosome stages of C. olla. Moreover, these stages are correlated with a considerable diameter of the hyphal structure, probably indicating a gerontic condition of the structure. By comparing Figs. 177 and 180 with Figs. 175, 176, 178 and 179, the difference is the amount of Feulgen positive material per haploid nucleus becomes evident.

In the C. setosus dikaryon (Fig. 78), the division of the centriole-like body can be observed in the filament stage and long filaments have been observed in Figs. 77, 82 and 83. A more advanced stage of the replication of chromatin material is shown in Figs. 84 and 85. Actual karyokinesis, i.e., longitudinal separation of the nuclear strands, can be observed in Fig. 86. The number of chromatin bodies per filament amounts to approximately 10 (Fig. 87).

Lu and Brodie (1962) reported that the number of chromosomes in Cyathus stercoreus is n=12 and that chromosome numbers of other species of Cyathus are also of the same order and clearly not less than n=8.

Namboodiri and Lowry (1967) observed that in <u>Neurospora</u> <u>crassa</u> a variable number of bead-like bodies appear on nuclear filaments and that two or more such beads may merge to form chromosomes.

Clamp formation in <u>C</u>. <u>bulleri</u> (Feulgen staining) revealed that the nucleus in the clamp takes on a globular configuration whereas the tube nucleus may be filamentous. In Fig. 150 one nucleus has moved into the clamp whereas the filamentous tube nucleus has replicated and is in the stage of division. This is in contrast to information in literature which indicates that synchronized division of the clamp nucleus and the tube nucleus is characteristic for the dikaryon. In <u>C</u>. <u>bulleri</u>, a great variation in the time of nuclear division of the two nuclei was noted. Although these nuclei necessarily divide, there is no evidence for conjugate division in which both nuclei divide in a synchronous manner (Fig. 152). In Fig. 153(a) the tube nucleus is in the process of replication. In Fig. 153(b) a daughter nucleus of the clamp nucleus is pictured, probably in the process of duplication. In the clamp (Fig. 153(c)) the other daughter nucleus is unfolding into a filament, destined to migrate into the penultimate hyphal segment.

Fig. 155 indicates that all four post-division nuclei can be in the condensed spherical phase. Hence, the tube nuclei are not always in a filamentous state.

Conjugate division is depicted in Fig. 157. The tube nucleus (not in a filamentous stage) has divided whereas the clamp nucleus is in the process of dividing. Observations of dikaryons seem to indicate that conjugate division of tube and clamp nuclei occur only when both nuclei are in the condensed state. However, when one nucleus is

filamentous and the other is not, a difference in the timing of the two divisions is likely to occur.

Although, in most cases, the nucleus in the clamp is of a spherical nature, nuclear filaments in clamps have been observed (Fig. 158). Depending on the completion of the clamp, the nucleus may divide in a spherical state or in a elongated state. The nuclear state probably depends on the clamp space available. For example, in Fig. 151 the clamp nucleus seems to be accommodating itself to the space available, i.e., to the curvature of the clamp tip, and does not seem to be a solid sphere.

In Fig. 159 the tube nucleus has replicated but not separated, whereas the clamp nucleus is at a different stage of karyokinesis. The clamp nucleus has not replicated as its chromatin mass is not equal to that of the tube nucleus. Instead, the clamp nucleus, still in a filamentous condition but showing signs of condensation at the terminals, seems to be migrating into the clamp.

Although filamentous tube nuclei are prevalent, spherical nuclei are also encountered. Fig. 161 indicates that the tube nucleus has replicated in a spherical state. This is also evident in Fig. 160 which is the same picture as Fig. 161 except that the Feulgen stained preparation was photographed through phase contrast optics. In Figs. 160 and 161 the clamp nucleus is moving towards the center of the clamp. Fig. 160 indicates that the clamp nucleus is attached by a thin Feulgen-negative connection to a large globular Feulgen-negative body (nucleolus).

In Fig. 156 the tube nucleus has replicated and is separating, whereas the other nucleus, which will probably function as a clamp nucleus, is at an early stage of karyokinesis and has a pronounced centriole-like body.

Fig. 154 shows one of the nuclei to be in a replicated condition while the other nucleus of this dikaryon terminal hyphal compartment is in the process of condensation, probably prior to clamp formation.

Phase contrast microscopy of C. bulleri dikaryotic living material has revealed the presence of two large spherical bodies in most of the cells not apparently undergoing new clamp formation (Figs. 162 and 164). These bodies are not visible in Feulgen stained preparations. During the process of clamp formation these two bodies draw very close together but do not touch (Figs. 166 and 167) and then draw apart again (Fig. 168). At this point these two bodies disappear from view and then a septum is formed across the clamp (Fig. 169). Approximately five minutes later a septum is completely formed across the hyphal tube and a small spherical body becomes visible in the clamp (Figs. 170 and 171).

Numerous long smooth filaments of uniform density have been observed in living material (Figs. 163 and 165). These numerous filamentous structures are not visible in Feulgen stained preparations and are not to be confused with the Feulgen-positive filamentous nuclei which are often beaded and of uneven density and which were never observed to occur in a number greater than two per dikaryotic hyphal segment. These Feulgen-negative filaments, thought to be mitochondria

(Kozar and McDonald, 1970), have been observed in a Y configuration (Fig. 163) which may be indicative of longitudinal division or merely a branching phenomenon. In some cases these filaments were observed to fragment into or be replaced by small spherical bodies of approximately the same diameter as the thickness of the filaments (compare Figs. 162 and 163). Both filaments and spheres are present in Fig. 165. These structures are not visible during clamp connection formation (Figs. 166, 167, 168, 169, 170 and 171).

Although the existence of vegetative spores in C. bulleri, C. setosus and C. olla has not as yet been reported in the literature, such bodies have been observed. Since the critical proof of origin of these structures is lacking at this time, the author wishes to refrain from comment and merely record the observations. Spherical spore-like structures have been encountered in the C. olla dikaryon (Fig. 33). In C. bulleri homokaryotic preparations, cellular spore-like configurations are evident in Figs. 123 and 124. Often the nuclei in these structures appear to be double. These spore-like structures are joined by thick septa which appear very prominently in cotton blue lacto-phenol stained preparations (Figs. 127, 128 and 129).

Cotton blue lacto-phenol staining of homokaryotic C. setosus preparations reveals cellular spore-like structures similar to those observed in C. bulleri (Figs. 68 and 69). However, in addition to these structures, a second type of spore-like structure has been observed in the C. setosus homokaryon. These structures appear to be conidia-like. In Figs. 64, 66 and 67 there is evidence that these structures originate in an intrahyphal manner somewhat reminiscent of the microconidia of Neurospora crassa. Fig. 65 illustrates the formation of this spore-like

structure by constriction. A similar structure was observed in a

Feulgen stained preparation (Fig. 62).

DISCUSSION

Comparison of Nuclear Configurations

The nuclear configurations observed in Cyathus olla, C. setosus and C. bulleri are similar to those observed in Neurospora crassa and Aspergillus nidulans by Weijer and co-workers (Weijer et al., 1963, 1965; Weijer and Weisberg, 1966; Weisberg and Weijer, 1968).

In the Juvenile cycles of N. crassa (Weijer et al., 1965) and A. nidulans (Weijer and Weisberg, 1966), a spherical interphase nucleus elongates into a filament on which develops a medially located nucleolus. Horseshoe and ring configurations may occur depending on whether or not, and to what degree, the filament wraps around the nucleolus. Nuclear division occurs by a longitudinal splitting of the filament, whether this filament be in a ring, horseshoe or free configuration. The final result is two daughter filaments.

In Maturation cycle I of N. crassa (Weijer, et al., 1965) filament, horseshoe and ring configurations in the single and duplicated state are also evident, and nuclear division occurs by a longitudinal splitting of the filament.

Maturation cycle II of N. crassa (Weijer et al., 1965) does not seem to involve any wrapping of the filament around the nucleolus, but consists simply of the filament duplicating and separating longitudinally. The same is true of the Maturation cycle in A. nidulans (Weisberg and Weijer, 1968).

In these two organisms (Neurospora and Aspergillus) the filamentous daughter nuclei eventually return to the spherical

interphase state (Weijer et al., 1965; Weijer and Weisberg, 1966; Weisberg and Weijer, 1968).

In Cyathus olla, C. setosus and C. bulleri spherical nuclei, filaments, horseshoes and rings are evident. The filamentous nuclei have been observed in states of replication and longitudinal separation. Ring division has been observed infrequently (Fig. 185). Therefore, it would seem that a nuclear cycle similar to the Juvenile cycles of Neurospora crassa and Aspergillus nidulans and Maturation cycle I of N. crassa operates within these three species of Cyathus.

In Cyathus, spherical nuclei have been observed in the process of elongation, or alteratively elongated nuclei in the process of condensation: both processes probably occur. Figs. 34 and 35 indicate that the spherical nucleus consists of a rolled up or folded up filament. Such a phenomenon is also reported by Laane (1967) in Penicillium expansum in which each daughter filament folds up in a zig-zag fashion, giving rise to an interphase nucleus. Such a scheme would allow the filament to retain its integrity while in the condensed state. It is less likely that the filament would fragment while condensing into the spherical state and then re-unite while elongating into the filamentous state.

It is interesting to note that Wagenaar (1969) reported a similar folding phenomenon during mitosis in several different genera of higher plants. According to him, chromosomes become attached end-to-end to form a chain which folds up into a small package during late telophase and unfolds during early prophase.

Though the nucleolus is not visible in the Feulgen stained
preparations of Cyathus in which horseshoe and ring configurations were
observed, it is conceivable that the nucleolus occupies the Feulgen
negative space inside the horseshoes and rings. Such an assumption was
also made by Thyagarajan and Naylor (1962) for Feulgen stained
preparations of Rhodotorula glutinis in which the chromatin was observed
to be in the shape of a ring. Hejtmánková-Uhrová and Hejtmánek (1967a,
b) and Hejtmánek and Hejtmánková-Uhrová (1968) thought the center of a ring
of six to seven Feulgen positive bodies to be filled probably by a
nucleolus in Nannizzia incurvata and Tichophyton vanbreuseghemii. They
also noted that the ring opens to change into a horseshoe configuration
which in turn straightens out into a filamentous nucleus. Heale et al.
(1968) observed that in the conidium of Verticillium albo-atrum a circular
resting nucleus develops a pouch-like opening thus giving rise to a
horseshoe and then a ring configuration in which chromosomes are linked
by a fine thread. They (loc. cit.) thought that this development is
due probably to the formation of a nucleolus in the pouch.

Chromatin rings associated with nucleoli have been mentioned
by many investigators. Turian and Cantino (1960) mention that in
Blastocladiella emersonii a shell of chromatin localizes into a beaded
ring around a nucleolus. Schopfer et al. (1963) noted that in
Schizosaccharomyces pombe a ring of chromatin granules surrounds the
nucleolus at prophase. Pontefract (1956) reported the occurrence of
an outer ring or crescent of chromatin surrounding, but not touching,
a large central body in several species of Penicillium. Laane (1967)
observed the nucleus to be wound, in the shape of a V, around a disc-
like nucleolus. Sappin-Trouffy (1896) noted that in ten genera of rusts,

at the onset of indirect division, the chromatin substance becomes fused

into a small mass in the form of a crescent, arc, horseshoe or S which

is wound around the nucleolus. Yoneda (1961a, b, 1963) observed a

somewhat different association in which a chromatin ring with a granule

in the center is located on one side of the nucleolus in a strain of

Saccharomyces and sometimes, but not always, Torula rubra.

Cyathus differs from Neurospora and Aspergillus (as described

by Weijer and co-workers) in that ring division has been observed only

occasionally. A dividing horseshoe configuration has not yet been seen.

It would seem that strand separation does not usually occur until the

filament has unwound itself from the nucleolus. Perhaps the horseshoe

and ring stages in Cyathus are of shorter duration than in Neurospora

and Aspergillus and, consequently, the nucleus is usually in the

filamentous state by the time strand separation is ready to occur.

Ring division is by no means peculiar to Neurospora, Aspergillus

and occasionally Cyathus. Heale et al. (1968) reported that in the

conidium of Verticillium albo-atrum chromosomes, while still linked in a

ring by a fine thread divide to form a double ring. The rings separate

and later condense.

Ferreira and Phaff (1959) have observed that in a newly dis-

covered species of Schwanniomyces the first stage of division consists of

the formation of a beaded ring. Presumably, during this stage duplication

occurs as the beaded bodies in the ring have been noted often to occur

in pairs. The duplicated ring does not separate as such, but instead

breaks and follows another course of action which shall be discussed

later.

McLarty (1941) claimed that in <u>Olpidiopsis</u> <u>achlyae</u> the chromosomes become arranged in a ring about the margin of the equator of a spindle. The ring divides to form a double ring and each daughter ring goes to a pole.

Namboodiri and Lowry (1967), investigators of the "clock" mutant and wild type <u>Neurospora</u> <u>crassa</u>, reported that in one of the two modes of division they observed, the rounded resting nucleus assumes a ring-like form and duplicates to constitute a double ring. The rings can separate as such but will eventually break. They (<u>loc</u>. <u>cit</u>.) even discussed the delay resulting from interlocked rings. Presumably, such an interlocking condition must have required a type of crossing-over to occur between the two daughter rings, as is thought to occur in the catenated circles of mitochondrial DNA (Hudson and Vinograd, 1967; Clayton and Vinograd, 1967; Hudson <u>et</u> <u>al</u>., 1968).

A distinction must be made between ring structures that occur as a result of a filament wrapping itself around a nucleolus and larger ring structures that occur due to medial separation of two daughter filaments (compare Figs. 44 and 182 with 59). Duncan and Macdonald (1965) referred to the latter process in their investigation of the dikaryotic hyphae of the Basidiomycetes <u>Marasmius</u> <u>androsaceus</u> and <u>M</u>. <u>rotula</u>. A chromatin thread condenses and becomes double. The two strands separate, except at the ends, to form a ring which becomes twisted into a figure eight and then doubles over on itself to form a double ring.

Such a double ring is completely different from those previously
described in that it is not a homologous double ring and does not
separate as such. In M. androsaceus and M. rotula a break occurs in
each ring and a completely different course of action occurs.

Widra (1959) noted that in several species of Candida the
chromatin duplicates and takes a position peripherally against the
nuclear membrane of a turgid sphere-like nucleus to form a metaphase
ring stage. In this case, the duplicated chromatin material has formed
a single ring which opens at one side or at two points equationally
across a diameter as anaphase movement begins.

In Cyathus olla, C. setosus and C. bulleri the configuration
most frequently encountered was that of the filament. This is to be
expected as the cultures examined were between fifteen and twenty days
old. Cultures older than this were too dense for satisfactory examination
and even the fifteen to twenty day old cultures could not be examined
at the center or point of inoculation. Therefore, the hyphae examined were,
for the most part, actively growing. This may explain why the filamentous
nuclei outnumbered the condensed, spherical nuclei.

Many investigators refer to condensed spherical nuclei as
resting, interphase or interdivisional nuclei. In this study of Cyathus,
the author wishes to refrain from such labelling. In the Juvenile cycles
of Neurospora and Aspergillus (as described by Weijer and co-workers),
after the first nuclear division in a germinating conidium, which pro-
ceeded from a spherical nucleus, the nuclear filaments do not return
to the globular configuration, but proceed directly to the next division.
In Maturation cycle II of Neurospora (Weijer et al., 1965) one of the
daughter nuclei condenses into a spherical nucleus which is incorporated

into a microconidium while the other daughter filament, without condensing, proceeds to replicate and divide.

Therefore, the term resting, interdivisional or interphase nucleus is ambiguous. In the case of Neurospora the filamentous nucleus, as well as the spherical nucleus, could be referred to as interdivisional.

However, it is interesting to note that in the 15 day old Cyathus culture which was kept at 4°C for three months, there was a considerable increase in the occurrence of globular condensed nuclei. Perhaps if an old culture could be satisfactorily examined, one would find all or most of the nuclei to be in a globular or "resting" state. However, these "resting" nuclei could also be "dying", indicating a gerontic state.

Another explanation for the high frequency of filamentous nuclei compared to the more condensed states is that a nuclear cycle, similar to that in Neurospora (Weijer et al., 1965) and Aspergillus (Weijer and Weisberg, 1966), may exist in Cyathus in which the filamentous daughter nucleus does not have to condense, or even assume ring or horseshoe configurations, before going on to the next division.

In the three Cyathus species studied, filamentous nuclei have been observed in the state of replication and longitudinal separation. Kowalski (1966) noted that in aerial hyphae of Preussia funiculata the narrow and elongated resting nucleus lengthens slightly and splits in half longitudinally. Duncan and Macdonald (1965) reported that in Marasmius androsaceus and M. rotula a chromatin strand, composed of granules interconnected by a faintly stained Feulgen positive thread,

becomes double, but then, as mentioned previously, separates medially
to form a ring. Hejtmánkova-Uhrová and Hejtmánek (1967a, b) and
Hejtmánek and Hejtmánková-Uhrová (1968) observed that in Nannizzia
.incurvata and Tichophyton vanbreuseghemii filamentous nuclei, consisting
of six or seven chromosomes interconnected by a Feulgen negative or
weakly Feulgen positive thread, divide longitudinally into two. The
daughter filamentous nuclei can enter directly into a new longitudinal
division or pass into a spherical interphase state. Laane (1967)
reported that in Penicillium expansum five chromatin bodies interconnected
by a Feulgen positive material arrange themselves in a linear fashion
to form a filament which splits longitudinally into two daughter
filaments. Kwong-Chung (1969) found that in the actively growing young
hyphae of Coccidioides immitis, a filament with three beads divides
longitudinally to produce two daughter nuclei which condense to a round
resting state. Namboodiri and Lowry (1967) concluded that in one of the
two modes of division observed in Neurospora crassa, an elongated
nucleus coils. Subsequently despiralization occurs and the nucleus
appears visibly duplicated into two strands. Each of the daughter
strands undergoes slight linear condensation and may pass into the next
division cycle without reverting to the rounded form.

Aist and Wilson (1965, 1966, 1967a, b, 1968), Wilson et al.
(1966) and Brushaber et al. (1967) described an asexual nuclear division.
common to many species of eleven various genera. In division the
chromosomes assume a linear end-to-end arrangement, forming a bar or
filament which then becomes double stranded. However, the longitudinal
separation movement is unilateral and asynchronous.

Sappin-Trouffy (1896) reported that in the indirect division
which occurs in ten genera of rusts, a line of transparent substance
longitudinally divides a chromatin cord into two strips. However, each
of these strips or chromosomes subsequently proceeds to divide trans-
versely. Also, not all of the nuclei separate longitudinally prior
to transverse division.

A similar sequence of events was suggested to occur in the
hyphae of Verticillium albo-atrum by Heale et al. (1968). A reticulate
resting nucleus condenses into four chromosomes connected by a fine
thread. The four chromosomes divide while still linked together. A
double filament is formed and the two strands are separated by a spindle
fiber which is formed between two separating daughter centrioles. They
(loc. cit.) stated that breakage occurs probably across the middle of
the parallel strands.

Of interest is the work of Wagenaar (1968, 1969) and Wagenaar
and Sadasivaiah (1969) in which end-to-end chromosome attachments
were reported to occur in higher plants. Wagenaar (1968, 1969) studied
mitosis in the root tip cells of Allium cepa, Crepis capillaris, C.
neglecta, C. sibirica, Callitriche hermaphroditica, Nigella arvensis
and Secale cereale. He observed that in late telophase and early pro-
phase, the chromosomes are attached end-to-end to form a chain, or in
the case of Crepis capillaris, a ring of four chromosomes with two
nucleolar chromosomes attached. Wagenaar (1969) assumed that the
chromosome ends remain attached during interphase. He observed that
at late telophase and early prophase the chain of chromosomes is folded
into a small package in the nucleus with the kinetochores all located
at one end of the nucleus and the attached chromosome ends at the

opposite end. He suggested that the kinetochores and chromosome ends
are fixed at certain points on the nuclear membrane, facilitating
chromosome uncoiling and lengthening in any direction. The unwinding
is necessary to enable DNA synthesis to take place during the S period
of interphase.

Apparently, in prophase the end-to-end attachments usually
break down at the same time as the nuclear membrane. However, during
the subsequent mitosis the chromosomes stay, although loose, in the
same order or relative position that they had in the chain. During
anaphase the daughter chromosomes move toward the poles while maintaining
their relative positions. At telophase the chromosome ends, with their
specific affinities, find each other and become attached.

Wagenaar (1968, 1969) and Wagenaar and Sadasivaiah (1969)
suggested that homologous chromosomes are adjacent to each other in
the chain and that such an arrangement would facilitate synapsis in
meiosis. Wagenaar and Sadasivaiah (loc. cit.) searched the pollen mother
cells at pachytene of young anthers of Crepis capillaris and found
end-to-end attachments of paired chromosomes which constitutes a double
chain. The two investigators (loc. cit.) suggested that end-to-end
chromosome attachments are present in all somatic and meiotic interphase
nuclei.

It is important to note that according to Wagenaar's scheme,
DNA replication occurs while the chromosomes are attached end-to-end.
This proposal is similar to that put forward for Neurospora crassa by
Weijer et al. (1965). If, as Wagenaar (1969) has stated, the mitotic
chromosomes, though unattached, retain the relative position they had
in the chain during division, then such a phenomenon can be considered

an evolved form of longitudinal division as described in N̲. crassa by
Weijer e̲t a̲l̲. (1965).

In conclusion, a nuclear cycle, similar to that in Neurospora
and Aspergillus (as described by Weijer and co-workers), can be
summarized as follows.

A spherical, dense nucleus consisting of a folded or rolled up
filament has a developing nucleolus within its center. (Perhaps the
nucleolus, increasing in size, is responsible, in part, for the unfolding
of the filament.) The filament begins to unfold or unroll in such a way
that a tail protrudes from the main spherical mass of the nucleus. The
nucleolus increases in size and a ring configuration with a tail is
sometimes visible. As the filament unravels it can be of varying length:
very long with many small particles or short with fewer but larger parti-
cles or beads. As the nucleolus increases in size (and depending perhaps
on cytoplasmic streaming) the filamentous nucleus can take on a horseshoe
or ring configuration by wrapping itself around the nucleolus. Perhaps
the nucleus can change directly from a spherical shape to a ring or
horseshoe by enlargement of the nucleolus and localization of the
chromatin as a strand around the nucleolus. In either case, the filament
eventually duplicates, and in its free state, divides. Occasionally
strand separation occurs while the filament is still wrapped around the
nucleolus. But usually this does not occur because by the time strand
separation is to occur the nucleolus has been discarded by or detached
from the nucleus. The daughter nuclei may then be able to undergo
further divisions without folding up into a condensed spherical nucleus
or they may condense before the next division.

The above summary applies to the homokaryons, but only in part to the dikaryons of Cyathus olla, C. setosus and C. bulleri. As in the homokaryons, the dikaryotic form of Cyathus has similar spherical, horseshoe, ring and filamentous nuclear configurations. Nuclei in the process of longitudinal division, and one dividing in the ring stage (Fig. 185), have been observed. However, observations of C. bulleri indicate that the dikaryons are more complex than the homokaryons in that a nuclear condensation to the spherical form seems to occur and is thought to be an adaptation of the nucleus to the space available in the clamp connection (Fig. 151) and is not to be confused with the spherical nuclei in non-dividing cells.

What further complicates matters is that not only does the clamp nucleus become spherical but sometimes the tube nucleus, which has no apparent space limitations, becomes spherically condensed as well. Moreover, it is evident that the division of the clamp nucleus and the tube nucleus is not always synchronous or conjugate (Figs. 150, 159 and 161).

The tube and clamp nuclei can both be in the filamentous state and in the process of duplication before a new clamp connection begins to form (Fig 153 (a) and (b)). After clamp connection formation has started, the clamp nucleus can remain in the filamentous duplicated state (Fig. 158). Although it is not known for certain that the clamp nucleus must eventually condense, this is suspected because of the limited space in the clamp. The clamp nucleus does not need to undergo duplication by the time clamp connection formation occurs. Fig. 159 shows a non-replicated filamentous nucleus migrating into the clamp.

Therefore, it seems that the stage of karyokinesis of the clamp nucleus is not closely synchronized with the formation of the clamp connection. The clamp nucleus in Fig. 159, though still filamentous, shows signs of condensation at the ends. A similar nuclear phenomenon of terminal condensation was reported by Ferreira and Phaff (1959) in a species of Schwanniomyces, except that the two terminal condensations approach each other until they are connected only by a short strand. Then the strand breaks and each of the condensations becomes a spherical daughter nucleus. Although it is tempting to apply the same reasoning to the Cyathus nucleus in Fig. 159, it is, however, though to be single and not duplicated because its chromatin mass is not equal to that of the duplicated tube nucleus. In Fig. 157 the clamp nucleus is in the process of division. Two connected spheres are apparent. The lower sphere, which presumably will migrate back into the tube, is already in the process of unfolding. The top sphere will probably maintain its spherical shape until it has been delivered through the clamp connection to the next cell (Fig. 151). Then it is expected to unfold into a filament, as is evident in Fig. 153 (c).

The tube nucleus can duplicate (Fig. 153 (a)) and divide (Fig. 150) while in the filamentous state or spherical state (Fig. 161) or in states of elongation that are intermediate between the filamentous and spherical states (Figs. 157 and 159). Therefore, as in the case of the clamp nucleus, it is thought that there is a certain degree of independence or asynchrony between the duplication and separation process and the condensing or unfolding process of the tube nucleus. It is not known at this time that the tube nucleus must necessarily condense at some point in time. It could very well be that in some cases the

filamentous tube nucleus and its daughter filaments do not condense.

This nuclear condensation that occurs in the dikaryon can be looked upon as a process that is superimposed upon the process that occurs in the homokaryon. It is felt that, basically, the nuclear cycles in the homokaryon and dikaryon are the same except that in the dikaryon an extra folding occurs to facilitate passage of the clamp nucleus through the clamp connection. The fact that the tube nuclei have been found to condense would lead one to believe that there is no particular "clamp" nucleus and "tube" nucleus, but that both nuclei can play either role.

That there might be a common triggering device for nuclear folding, i.e., the clamp formation itself, is not supported by Fig. 150 in which the tube nucleus is filamentous and duplicated, while the clamp nucleus is spherical and apparently single. The reverse situation is depicted in Fig. 158 in which the nucleus migrating into the clamp is filamentous and duplicated, whereas the tube nucleus is somewhat condensed, but may or may not be duplicated.

A very hypothetical (and unlikely suggestion) regarding tube nuclear condensation may be that formation of the tube septum may require the tube nucleus to be spherical for quick passage into the adjacent cell.

The most likely reason for tube nuclear condensation, as mentioned before, is that the determination of which nucleus shall be the clamp nucleus and which shall be the tube nucleus occurs, at least in some cases, late in the process of karyokinesis.

Overall observations on dikaryons seem to indicate that synchronous (conjugate) division of tube and clamp nuclei most likely occurs when both nuclei are in the condensed state (Figs. 155 and 157). However, when one nucleus is filamentous and the other is not, a difference in the timing of the two divisions is likely to occur.

Fig. 122 of a homokaryon and Figs. 148 and 149 of a dikaryon of C. bulleri are of interest in that in each picture two spherical bodies are connected by a slightly Feulgen positive strand. Such configurations bring to mind the Bakerspigel phenomenon of indirect division (Bakerspigel, 1957, 1958, 1959a, b, c, d, 1960a, b, 1961, 1962). However, if these pictures represent indirect division as described by Bakerspigel, one would not expect to see a configuration with a connecting strand of uniform thickness as is shown in the pictures, but a configuration that looks like two spheres of taffy pulling apart with a connecting strand that is thinnest at the middle and progressively thicker towards the ends.

This description of a strand of uniform thickness connecting two spheres is also given in the work of Ferreira and Phaff (1959) on a species of Schwanniomyces in which a strand shortens and two terminal bodies (which are to become two daughter nuclei) approach each other very closely. Subsequently the strand breaks and the two daughter nuclei separate. Fig. 38 of a double spherical nuclear structure in a C. setosus homokaryon would fit Ferreira and Phaff's description (loc. cit.) of two daughter nuclei approaching each other. Alternatively, Fig. 38 could depict the overlapping of two adjacent nuclei. One has to bear in mind however that such configurations are rarely observed in

Cyathus, though one could argue that the configuration is a regular
but very short lasting occurrence. On the other hand, it is not impossible
that these configurations concern anomalies and have limited cytological
significance. Sappin-Trouffy (1896), in his study of rusts, described
an infrequent "direct division" that was observed only in the aged cells
of the thallus. The nucleus elongates and constricts in the middle.
The two nuclear extremities swell and are no longer united except by a
fine trabecula which finally breaks. Sappin-Trouffy (loc. cit.)
considered this kind of division to be a phenomenon of senility.

Another interpretation of the configuration in Figs. 122, 148
and 149 could be that it constitutes a persistent telophase, as is some-
times found in higher organisms, and therefore does not constitute a
second kind of division. In such a case, the duplicated nuclear filament
could have separated except at or near one end. Nuclear folding of
both daughter filaments would result in two spherical bodies connected
by a uniform strand. The uniform strand could be part of one of the
daughter filaments with the unseparated portion of the duplicated
filament being folded up in one of the spheres. This would cause one
of the spheres to be slightly larger than the other, as can be observed
in Fig. 148.

The Centriole-Like Body

Many fungal investigators have observed a densely stained
granule to be associated with the nucleus. Some observers have attributed
a centriole-like function to this granule. Such a structure has been
observed in the nuclear cycle of Cyathus olla, C. setosus and C. bulleri,
both in their homokaryotic and dikaryotic states. Since it is believed

that this body has a centriole-like function it is referred to as a centriole-like body.

This centriole-like body is associated with all the configurations that occur in the nuclear cycle of Cyathus. When the nucleus is in the condensed spherical state, the centriole-like body can be either attached to (Figs. 26, 70 and 88) or at some distance from (Figs. 11, 71, 72 and 132) the nucleus. Weijer and Weisberg (1966) reported the occurrence of a centriole attached to a resting nucleus in the conidium of Aspergillus nidulans.

The connection between the nucleus and the centriole-like body in Cyathus is usually faintly Feulgen positive, indicating that some chromatin is present. This connection is densely stained in crystal violet stained preparations (Fig. 25).

Varitchak (1931) observed that at a certain stage in the vegetative nuclear cycle of Ascoidea rubescens, a centrosome is attached to the nucleus by a connection which he called a trabecula.

A similar connection has been described by Weijer et al. (1965) in Neurospora crassa. A thin but only slightly Feulgen positive thread connects a highly stainable body (centriole) to the globular interphase nucleus. This thread was also seen in other stages of the nuclear cycle.

Figs. 32 and 36 may indicate that the centriole-like body in Cyathus directs the nuclear elongation process. Such a process was reported to occur in Ascoidea rubescens (Varitchak, 1931) in which the nucleus becomes slightly elongated with the centrosome at one pole.

Perhaps the centriole-like body in Cyathus is terminally located on the nuclear filament and leads in the unfolding process. Figs. 131 and 133 show the centriole-like body to be on the axis of elongation of the nucleus. As with spherical nuclei, the centriole-like body can be attached to (Fig. 131) or at some distance from (Fig. 133) the elongating nucleus.

Namboodiri and Lowry (1967), investigating Neurospora crassa, observed through the electron microscope that pre-division elongated nuclei have a knob-like terminal portion which correlates with a deeply stained knob in their light microscopy observations. This knob, as seen through the light microscope, was noted to undergo displacement from the elongated nucleus, but still remain attached by a weakly stained thread.

Fig. 43 of Cyathus depicts a horseshoe configuration with a conspicuous triangular-shaped centriole-like body attached. Weijer and co-workers (Weijer et al., 1963 and 1965; Weijer and Weisberg, 1966) also reported the occurrence of a horseshoe configuration with an attached centriole in Neurospora crassa and Aspergillus nidulans.

The centriole-like body in Cyathus can be attached to (Fig. 182) or at some distance from (Figs. 45, 183 and 184) the nucleus when it is in the ring configuration. The same phenomenon of a centriole being incorporated into or being at some distance from a ring was reported to occur in N. crassa (D.L. Weijer, 1964; Weijer et al., 1965) and A. nidulans (Weijer and Weisberg, 1966).

Yoneda (1961a, b, 1963) noted the occurrence of a granule in the center of a ring of chromatin located on one side of a large

nucleolus in a strain of Saccharomyces and sometimes, but not always, in Torula rubra.

Fig. 195 is of interest because, even though the density of the crystal violet staining of this Cyathus preparation obscures the fine detail, two centriole-like bodies, which presumably have just divided, can be distinguished side by side in the ring. Division of this body in the ring is not surprising since, as mentioned before, strand separation occasionally occurs while the nucleus is in a ring configuration.

In Neurospora crassa and Aspergillus nidulans, in which ring division is more frequent than in Cyathus, Weijer et al. (1965) and Weijer and Weisberg (1966) have observed centriole division to always precede ring division. However, D.L. Weijer (1964) reported that in N. crassa centriole division can occur during either the single or double ring phase. In both organisms (N. crassa and A. nidulans) during ring phase, the centriole divides and the two daughter centrioles move around the nuclear ring so as to occupy polar positions opposite one another (D.L. Weijer, 1964; Weijer et al., 1965; Weijer and Weisberg, 1966).

Heale et al. (1968) reported that in the conidium of Verticillium albo-atrum a ring nucleus divides to form a double ring and a centriole divides to produce daughter centrioles.

Fig. 6 of Cyathus depicts a filamentous nucleus in the process of unfolding. A centriole-like body is attached to the nucleus by a thin strand and seems to be orienting the axis of the unfolding nucleus. Such an orientation function of a centriole was reported by Heale et al. (1968) in Verticillium albo-atrum. However, in this case the centriole divides and a spindle fiber is formed between the daughter centrioles.

Rapid elongation of the spindle fiber draws out an already divided filamentous nucleus such that the two nuclear filaments lie parallel to each other with the spindle in between.

If the centriole-like body in Cyathus does have an orientation function, one would expect it to be terminally located on filamentous nuclei, as is the case in Figs. 12, 13, 53, 98, 99, 100, 101 and 102. This observation is confirmed in cyrstal violet stained preparations (Fig. 27).

A terminally located centriole on a nuclear filament has been reported in Neurospora crassa by D.L. Weijer (1964) and Weijer et al. (1963, 1965). Weijer et al. (1965) recorded seven Feulgen positive bodies and one deeply stained body which precedes the former seven. All eight bodies have been observed to be interconnected by a very weakly Feulgen positive thread.

An exception to the terminal position of the centriole-like body in Cyathus is depicted in Fig. 156 in which the second nucleus from the hyphal tip has a deeply stained granule located to one side but near the end of a filamentous nucleus.

Namboodiri and Lowry (1967) have reported a similar occurrence in N. crassa. In some cases, before duplication of a filamentous nucleus is complete, a chromosome may be displaced from the beaded chain, but is still attached by an interchromosomal strand. This displaced body is not always a terminal chromosome, but may, at times, be an intermediate chromosome. They (loc. cit.) suggested that this displaced chromosome acts as a focal point for the other chromosomes and thus may act as a mitotic center.

Weisberg and Weijer (1968) noted that in the Maturation cycle of _Aspergillus nidulans_ the centrioles did not always appear to be terminal in linear groupings of chromatin bodies.

A comparison of Figs. 99 and 100 indicates that the centriole-like body in _Cyathus_ is plate-like, thinner on edge than when seen face on. A similar shaped centriole has been observed in _Neurospora crassa_ and _Gelasinospora tetrasperma_ by Dowding and Weijer (1962). They described a centriole that sometimes appears as a long thread extending from the nucleus to the cell wall where it terminates as a disc. Sometimes the centriole appears as an angular plate, rod-like when seen edge on. Weijer _et al._ (1963) reported that in the pre-division phase of _N. crassa_ two protruding filaments, each carrying a small dense delta shaped body (centriole), were attached to a long nuclear structure.

In _Cyathus_ the division of the filamentous nucleus and centriole-like body is not closely synchronized. The centriole-like body can divide prior to the filament (Figs. 78 and 107), at the same time as the filament (Figs. 117 and 192), or after the filament has divided (Figs. 61 and 121).

In the case of _N. crassa_, Weijer _et al._ (1963) observed a long undivided nuclear structure with two centrioles attached. Later, they (1965) observed two strands of seven chromosomes connected to a deeply stained eighth body, and Y configurations (longitudinal strand separation) with a centriole at each of the free ends of the separating strands.

In <u>Aspergillus</u> <u>nidulans</u> Weisberg and Weijer (1968) observed that in the asynchronous separation of linearly grouped chromosomes, the division of the centriole could precede or follow chromosomal separation.

Thus it appears that in <u>Neurospora</u> and <u>Aspergillus</u>, as in <u>Cyathus</u>, the division of the filamentous nucleus and centriole or centriole-like body is not closely synchronized. Asynchronous division of a centriole and nucleus does not necessarily indicate that the replication of these structures is asynchronous.

Ultrastructure studies of nuclear division in fungi has revealed that a body which divides, and whose division products assume polar positions, may or may not have the typical centriolar structure, as described by Brown and Bertke (1969). According to these two authors (<u>loc</u>. <u>cit</u>.), a typical centriole contains nine triplets of microtubules arranged in a circle. A third of the centriole appears to have electron-dense material (cartwheel), whereas the remainder appears "hollow".

Such a typical ultrastructure has been discovered in <u>Albugo</u> <u>candida</u> by Berlin and Bowen (1964) who reported that in the hyphae of this fungus the centriole consists of nine triplets connected to a central tubule by radial elements in a cartwheel arrangement. The same arrangement has been observed in <u>Achlya</u> by F.R. Turner (Brown and Bertke, 1969, p. 395).

Ichida and Fuller (1968) reported that in zoosporangia of <u>Catenaria</u> <u>anguillulae</u>, during interphase a pair of centrioles can be found exterior to the nuclear envelope and accompanied by microtubules

which radiate from the region of the centrioles. An ultra thin cross section of the centriole reveals nine sets of triplets arranged in a cylindrical pattern. It should be noted that during nuclear division an intranuclear spindle is formed which terminates at each end in a band of dense material opposite the centrioles and inside the nuclear membrane. The spindle does not connect to the centrioles which are outside the nuclear envelope. This description is of interest because it brings into question the function of the typical centriole.

According to Brown and Bertke (1969) each centrosome (which consists of a centriole surrounded by a distinctive region of proto-plasm called the centrosphere) causes the formation of microtubules which radiate out and constitute the aster. They (loc. cit.) referred to the work of Dietz (1966) in which experimental displacement of centrosomes and production of secondary spermatocytes without centrioles indicates that centrioles are not necessary for spindle formation or anaphase movement of chromosomes. Brown and Bertke (loc. cit.) suggested that the divisions and movements of the centrosomes are merely for their own perpetuation and distribution to daughter cells so that in certain cells they will be available to form cilia or flagella.

However, Brown and Bertke (loc. cit.) noted that the centrosomes present in fungi may be, in some cases, predecessors of true centrioles and more like nuclear envelope thickenings associated with spindle fiber-like microtubules. The KCE (kinetochore equivalent), as revealed in the ultrastructure study of Polystictus versicolor by Girbardt (1968), is connected to the outside of the external membrane of the nuclear envelope. During division the envelope breaks down. The KCE, which lacks the

typical centriolar ultrastructure, is bipolar and a bundle of micro-
tubules (the Zentralstrang) forms between and pushes apart the
globular poles of the KCE. This bundle appears to be directly connected
to the KCE poles. Condensing chromatin collects around this bundle of
microtubules.

In the case of <u>Armillaria</u> <u>mellea</u> (Motta, 1967, 1969), two
spherical uniform dense bodies that appear at the poles prior to
division are not in contact with the nuclear membrane. During division
a cylinder-like bundle of microtubules extends through the nucleus to
opposite poles where they appear to originate from the two dense bodies
in the cytoplasm. Motta (1967) noted that the position of these bodies
relative to the nucleus and their association with the microtubules of
the spindle apparatus suggests that they may represent centrioles.
However, these bodies do not have the typical centriolar ultrastructure.

The point is, that centrioles or centriole-like bodies vary
not only in ultrastructure, but in function as well. Therefore, the
centriole-like body in <u>Cyathus</u> could very well be called a centriole.
The question is whether the definition of the word centriole is to
depend on the function of a body, or its structure. At present the
literature reveals no decisive answer to this question.

Filamentous Mitochondria

The numerous long smooth filaments of uniform density observed
in living hyphae of <u>Cyathus</u> <u>bulleri</u> are thought to be mitochondria.
Elongated mitochondria were observed by Kozar and McDonald (1970) in an
ultrastructure study of the same organism.

Such structures are by no means unique to Cyathus. Filamentous mitochondria have been observed in living material of Blastomyces dermititidis, Neurospora crassa, Gelasinospora tetrasperma, Schizophyllum commune (Bakerspigel, 1957, 1959b, c, d), Mucor fragilis, Lipomyces lipofer (Robinow, 1957a, 1961), Fomes annosus, Fusarium oxysporum (Armentrout et al., 1968). Filamentous mitochondria were observed in an iron alum hematoxylin stained preparation of Saprolegnia ferax (Bakerspigel, 1960a). Vendrely (1950) traced the affinity of mitochondria for hematoxylin to their contents of lipids and ribonucleic acid.

Ultrastructure studies have revealed the presence of elongated mitochondria in Nadsonia fulvescens (Kawakami, 1961), Polystictus versicolor (Girbardt, 1961c), Sporobolomyces roseus (Prusso and Wells, 1967), Peziza ostracoderma (Hughes and Bisalputra, 1970) and Ustilago hordei (Kozar and Weijer, 1970). Kozar and Weijer (1970) often found the length of elongated mitochondria in diploid sporidia of U. hordei to be in excess of 10μ.

In Cyathus bulleri filamentous mitochondria have been observed in a Y configuration (Fig. 180) which may be indicative of longitudinal division. However, a similar configuration was not observed by Kozar and McDonald (1970) in their study of the ultrastructure of the same organism. Kozar and Weijer (1970) reported that the mitochondria in U. hordei appear to duplicate by binary fission. Also these authors (loc. cit.) have observed multiple branched as well as elongated mitochondria. Therefore, a Y configuration may not, in the case of mitochondria, be indicative of longitudinal division but may be merely a branched structure.

In C. bulleri the filaments were, in some cases, observed to fragment into or be replaced by small spherical bodies of approximately the same diameter as the thickness of the filaments (compare Figs. 162 and 163). Bakerspigel (1959d) reported that filamentous nuclei break up into a number of separate granules in the germinating basidiospores of Schizophyllum commune.

Armentrout et al. (1968) reported that in Fomes annosus and Fusarium oxysporum the mitochondria are pleomorphic, changing in size and shape. The transformations involve fragmentation of filamentous forms into short rods or granules, lengthening of short rods or granules into filaments, and the interaction of these forms with each other. This interaction involves end-to-end fusion of filaments with short rods or granules, fusion of short rods, or fusion of granular forms.

Further study is required to definitely establish the mode or modes of replication of mitochondria in Cyathus. One would expect it to be the same as that in many other fungi.

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Fig. 1   Stage micrometer. Phase contrast microscopy. The distance
         from one line to the next is 0.01mm or 10μ. A measurement
         across five of these distances (0.05mm or 50μ) was 212.5mm.
         Therefore the enlargement is 212.5/0.05 = 4250. All phase
         contrast pictures are of this enlargement.

Fig. 2   Stage micrometer. Bright field microscopy. The distance
         from one line to the next is 0.01mm or 10μ. A measurement
         across four of these distances (0.04mm or 40μ) was 178mm.
         Therefore the enlargement is 178/0.04 = 4450. All bright
         field pictures are of this enlargement. Unless otherwise
         stated, all the pictures are by bright field microscopy.
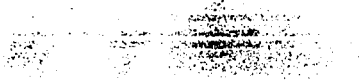
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Fig. 3   Cyathus olla homokaryon. Condensed and filamentous nuclei.
          The Feulgen negative or less dense area in one of the
          condensed nuclei (arrow) is thought to be occupied by a
          nucleolus. Feulgen staining.

Fig. 4   C. olla homokaryon. Condensed and filamentous nuclei. One
          of the condensed nuclei (arrow),which is in the process of
          unfolding or elongating, has a Feulgen negative or less
          dense area that is thought to be occupied by a nucleolus.
          Feulgen staining.

Fig. 5   C. olla homokaryon. Two ring nuclei. The Feulgen negative
          or less dense area in the center of the rings is thought to
          be occupied by a nucleolus. Feulgen staining.

Fig. 6   C. olla homokaryon. A filamentous nucleus in the process of
          of unfolding. A centriole-like body is connected to the
          nucleus by a thin thread. Feulgen staining.

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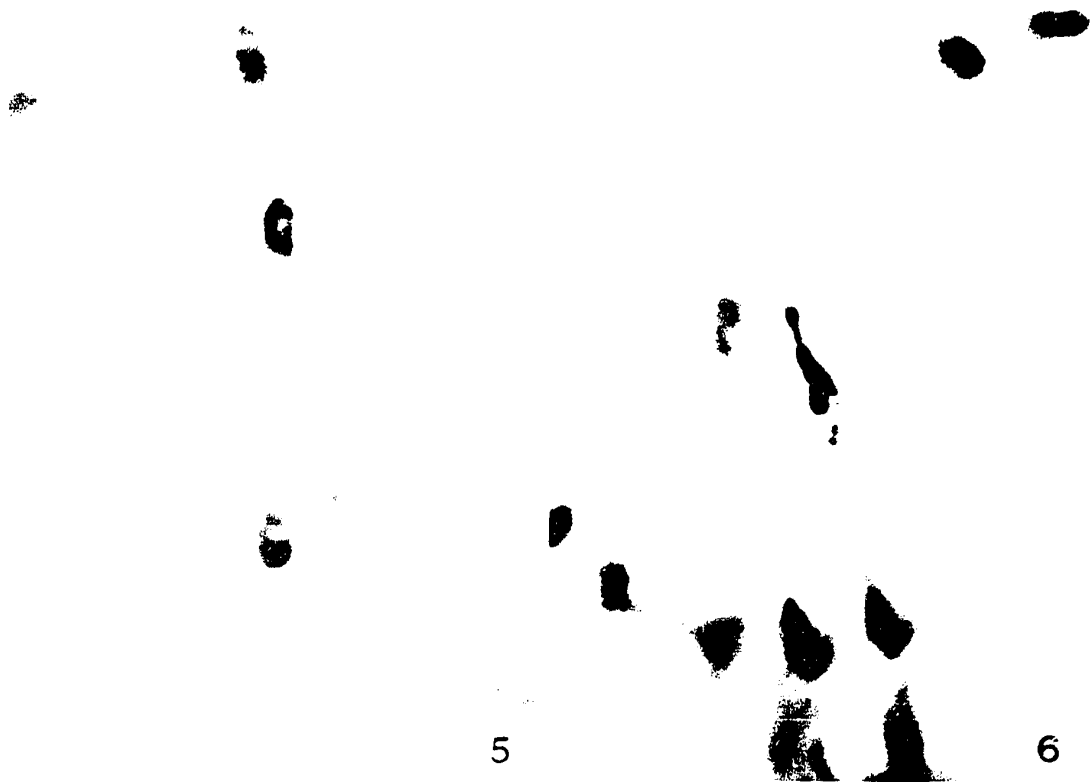
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Fig. 7   C. olla homokaryon.   A nuclear filament which has duplicated.
          Feulgen staining.

Fig. 8   C. olla homokaryon.   A partially condensed nucleus probably
          in the process of unfolding and a long nuclear filament
          which is dividing longitudinally as indicated by the strand
          separation at one end.   Freeze dried and mounted in water
          mounting medium.   No staining.   Phase contrast microscopy.

Fig. 9   C. olla homokaryon.   Nuclear filaments which have duplicated
          (arrows).   Feulgen staining.
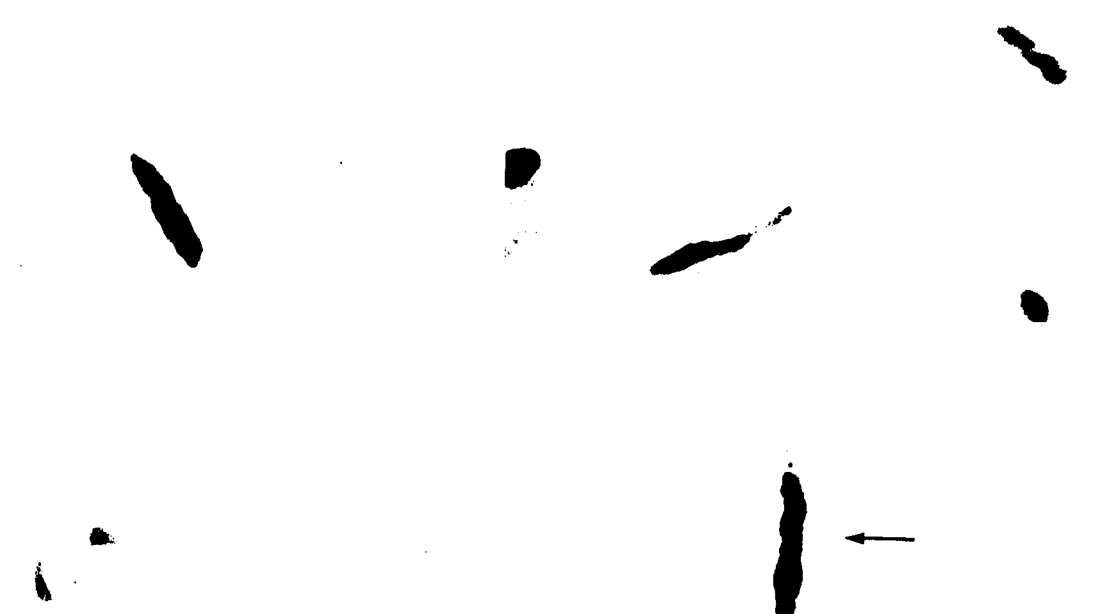
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Fig. 10   C. olla dikaryon. Filamentous nuclei. A centriole-like body
           (arrows) can be distinguished at the end of two of the nuclear
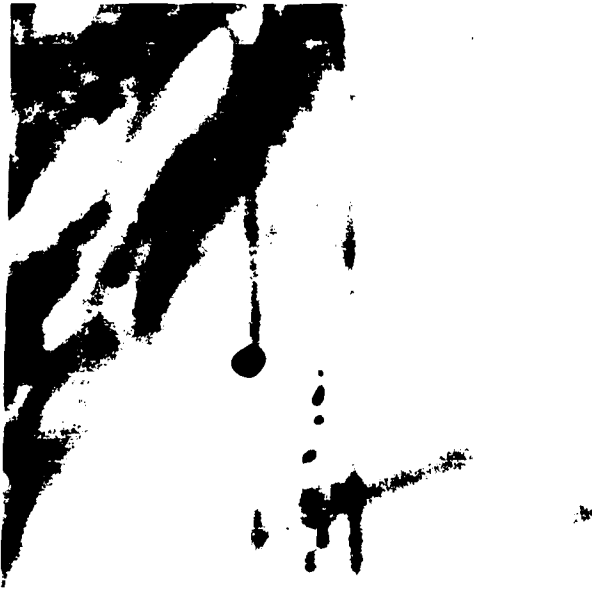   filaments. Feulgen staining.

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Fig. 11   <u>C</u>. <u>olla</u> dikaryon. A condensed nucleus with a centriole-like body (arrow). Feulgen staining.

Fig. 12   <u>C</u>. <u>olla</u> dikaryon. A filamentous nucleus with a terminally located centriole-like body (arrow). Feulgen staining.

Fig. 13   <u>C</u>. <u>olla</u> dikaryon. A filamentous nucleus with a terminally located centriole-like body (arrow). Feulgen staining.

Fig. 14   <u>C</u>. <u>olla</u> dikaryon. A filamentous nucleus in a folding position. Feulgen staining.

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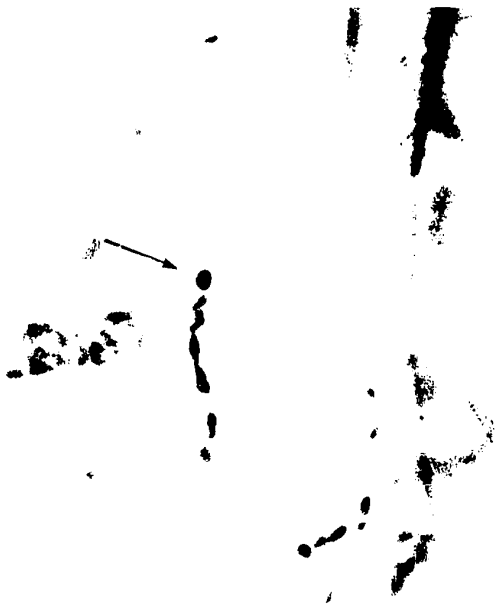
Fig. 15   <u>C</u>. <u>olla</u> dikaryon. A filamentous nucleus. Eight chromatin
           bodies are visible and are thought to be interconnected to
           constitute a nuclear filament. The connections between the
           terminal and subterminal bodies at either end are apparent.
           The large black sphere is an artifact. Feulgen staining.

Fig. 16   <u>C</u>. <u>olla</u> dikaryon. A nuclear filament in the process of
           longitudinal division or strand separation. Feulgen
           staining.

Fig. 17   <u>C</u>. <u>olla</u> dikaryon. A filamentous nucleus in the process of
           longitudinal division or strand separation. Feulgen
           staining.

Fig. 18   <u>C</u>. <u>olla</u> dikaryon. A nuclear filament in the process of
           longitudinal division or strand separation. Feulgen
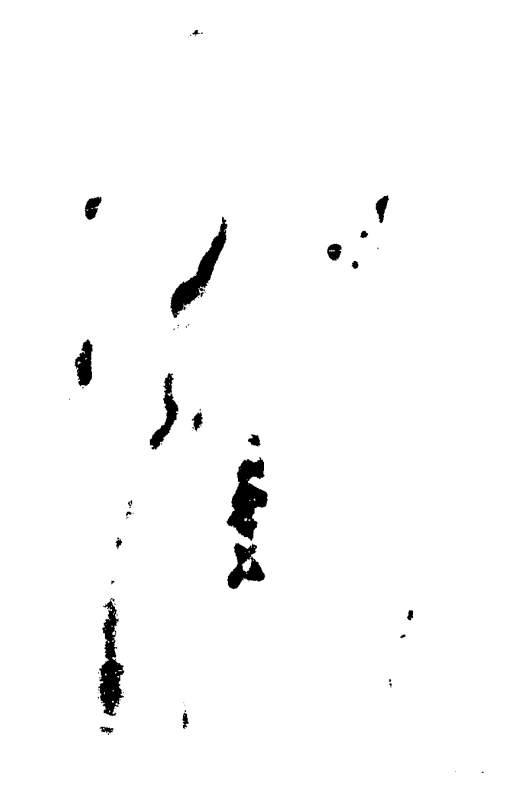   staining.

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Fig. 19 <u>C</u>. <u>olla</u> dikaryon. A nuclear filament in the process of longitudinal division or strand separation. Feulgen staining.

Fig. 20 <u>C</u>. <u>olla</u> dikaryon. A nuclear filament in the process of longitudinal division or strand separation. Feulgen staining.

Fig. 21 <u>C</u>. <u>olla</u> dikaryon. A nuclear filament which has duplicated and is beginning to undergo strand separation (longitudinal division). Feulgen staining.

Fig. 22 <u>C</u>. <u>olla</u> dikaryon. A nuclear filament in the process of longitudinal division or strand separation. Feulgen staining.

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Fig. 23   C. olla dikaryon. Chromatin bodies of separated strands in
           a clustered configuration. The interconnections are visible
           between some of the chromatin bodies. Feulgen staining.

Fig. 24   C. olla dikaryon. Two nuclear filaments (center hypha) which
           have duplicated. The lower nucleus is undergoing strand
           separation (longitudinal division). Feulgen staining.

Fig. 25   C. olla dikaryon. A condensed nucleus (center) with a
           centriole-like body attached by a thin thread. Crystal
           violet staining.

Fig. 26   C. olla dikaryon. Condensed nuclei. A centriole-like body
           (arrow) is connected to the uppermost nucleus by a thin thread.
           A centriole-like body (arrow) is attached directly to the
           middle nucleus. The lower nucleus presents a perplexing
           configuration. Crystal violet staining.

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Fig. 27   C. olla dikaryon.   Two nuclear filaments each with a centriole-
like body at a terminal position.   Crystal violet staining.

Fig. 28   C. olla dikaryon.   A filamentous nucleus.   Giemsa staining.

Fig. 29   C. olla dikaryon.   A filamentous nucleus.   Giemsa staining.

Fig. 30   C. olla dikaryon.   A nuclear filament in the process of
longitudinal division or strand separation.   Giemsa staining.

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Fig. 31   C. olla dikaryon. A nuclear filament undergoing longitudinal
           division or strand separation. Giemsa staining.

Fig. 32   C. olla dikaryon. A condensed nucleus with a centriole-like
           body. A thin thread extends from the nucleus towards the
           centriole-like body and a thread extends from the centriole-
           like body to the nucleus. Although the two threads are not
           visibly connected, their orientation towards one another
           indicates that they are one thread. The thread is probably
           out of focus at the point of separation. The nucleus has
           an area of decreased density that is thought to be occupied
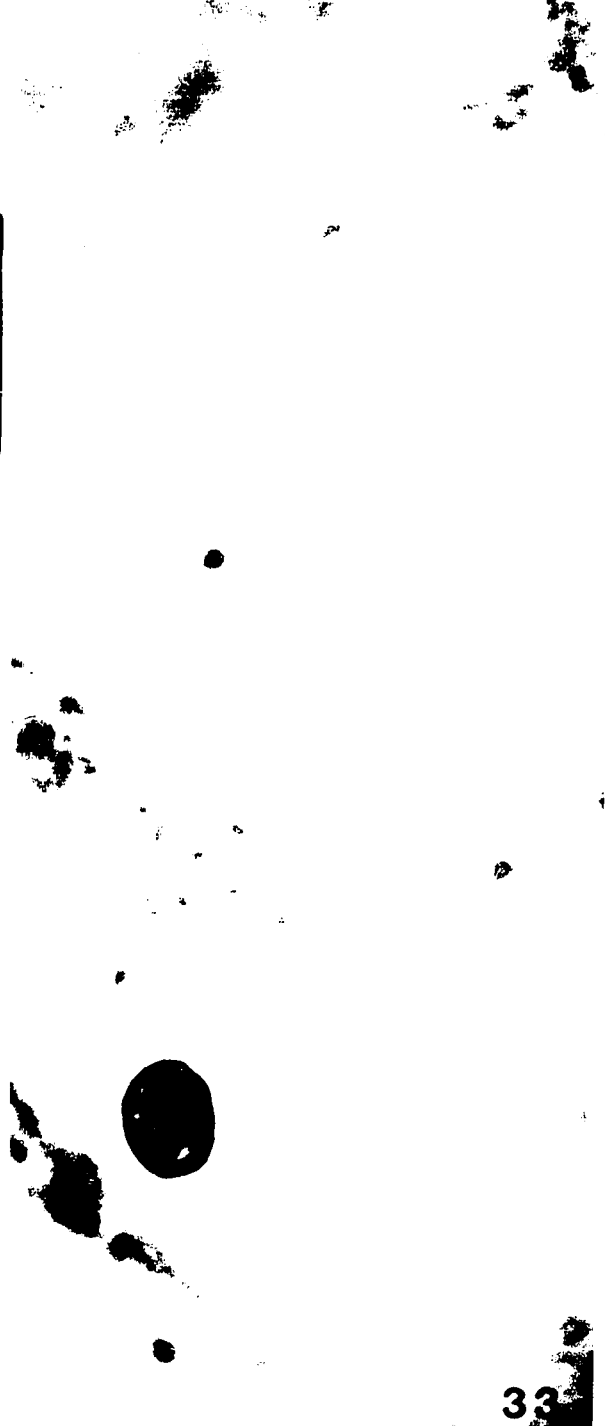   by a nucleolus. Feulgen staining.

Fig. 33   C. olla dikaryon. Two spherical spore-like structures.
   Feulgen staining.

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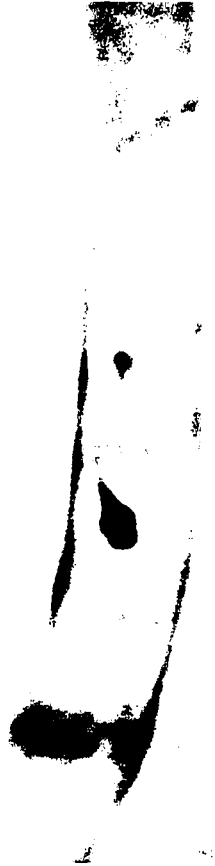
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Fig. 34   C. olla dikaryon. A condensed nucleus which appears to
            consist of a folded up filament (arrow). Feulgen staining.

Fig. 35   C. olla dikaryon. The same nuclear structure as depicted in
            Fig. 34. Feulgen staining. Phase contrast microscopy.

Fig. 36   C. olla dikaryon. A partially condensed nucleus (right) with
            a centriole-like body connected by a thin thread. The nucleus
            has an area of decreased density that is thought to be occupied
            by a nucleolus. Feulgen staining.

Fig. 37   C. setosus homokaryon. Two condensed nuclei. A centriole-
            like body (arrow) is located at a distance from one of the
            condensed nuclei. Feulgen staining.
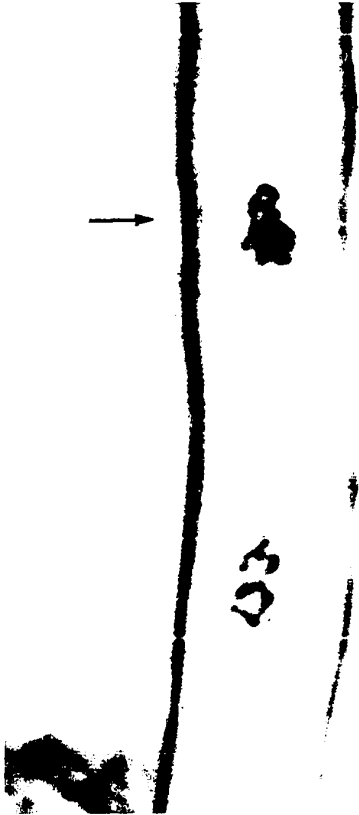
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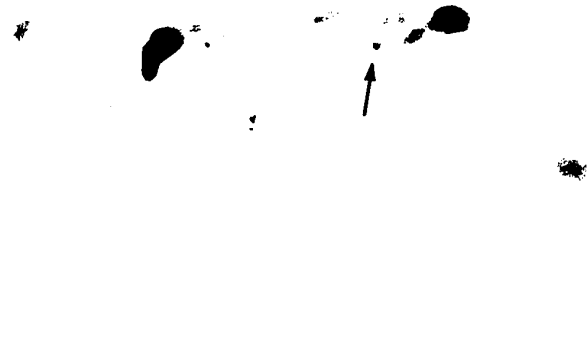
36 **37**

Fig. 38   C. setosus homokaryon. Filamentous nuclei and condensed
           nuclei in adjacent hyphal compartments. The double spherical
           nuclear structure (arrow) may be indicative of nuclear
           division according to the Bakerspigel theory or it may be a
           result of overlapping of two adjacent nuclei. Feulgen
           staining.

38

Fig. 39   C. setosus homokaryon. A horseshoe or incomplete ring
           nucleus and an adjacent condensed spherical nucleus. The
           Feulgen negative area in the horseshoe nucleus is thought to
           be occupied by a nucleolus. Feulgen staining.

Fig. 40   C. setosus homokaryon. A condensed nucleus with a centriole-
           like body. Feulgen staining.

Fig. 41   C. setosus homokaryon. A nucleus at the early filament stage.
           The Feulgen negative or less densely stained subterminal area
           in the filament is thought to be occupied by a nucleolus.
           Feulgen staining.

Fig. 42   C. setosus homokaryon. The lower nucleus is condensed and in
           the process of unfolding or elongating. The upper nucleus is
           not as condensed and is at an early filament stage or a late
           horseshoe stage. The Feulgen negative area in the nucleus is
           thought to be occupied by a nucleolus. Feulgen staining.

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Fig. 43   C. setosus homokaryon. The lower nucleus is a horseshoe
           nucleus with a triangular shaped centriole-like body attached
           by a thin thread. Feulgen staining.

Fig. 44   C. setosus homokaryon. A ring nucleus (center). The Feulgen
           negative or less dense area in the center of the ring is
           thought to be occupied by a nucleolus. Feulgen staining.

Fig. 45   C. setosus homokaryon. A ring nucleus with a centriole-like
           body located at a distance. The Feulgen negative area in the
           center of the ring is thought to be occupied by a nucleolus.
           Feulgen staining.

Fig. 46   C. setosus homokaryon. The lower nucleus is partially
           condensed and has a Feulgen negative area (arrow) that is
           thought to be occupied by a nucleolus. A centriole-like
           body is attached at the top of this nucleus. Feulgen
           staining.

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Fig. 47   C. setosus homokaryon.   Ring nuclei occurring adjacent to
short particulate filamentous nuclei.   Feulgen staining.

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Fig. 48 C. setosus homokaryon. Ring nuclei (arrows and inserts).
The Feulgen negative or less dense areas in the center of the
rings are thought to be occupied by nucleoli. Feulgen
staining.

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Fig. 49   <u>C. setosus</u> homokaryon. The lower nucleus is pictured twice and is in the form of a ring. The Feulgen negative or less dense area in the center of the ring is thought to be occupied by a nucleolus. Feulgen staining.

Fig. 50   <u>C. setosus</u> homokaryon. A ring nucleus (upper center). The Feulgen negative or less dense area in the center of the ring is thought to be occupied by a nucleolus. Feulgen staining.

Fig. 51   <u>C. setosus</u> homokaryon. An obstructed filamentous nucleus. Feulgen staining.

Fig. 52   <u>C. setosus</u> homokaryon. An obstructed filamentous nucleus. Feulgen staining.

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Fig. 53   C. setosus homokaryon. Filamentous nuclei each with a
          terminally located centriole-like body (arrows). Feulgen
          staining.

Fig. 54   C. setosus homokaryon. A late stage of karyokinesis (lower
          nucleus) in which individual chromatin bodies are evident.
          Feulgen staining.

Fig. 55   C. setosus homokaryon. A late stage of karyokinesis with
          faint individual chromatin bodies. Feulgen staining.

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Fig. 56   C. setosus homokaryon. A late stage of karyokinesis with
          faint individual chromatin bodies (arrow). Feulgen staining.

Fig. 57   C. setosus homokaryon. A stage of karyokinesis that is
          earlier than that depicted in Fig. 56. The chromatin bodies
          are larger (arrow). Feulgen staining.

Fig. 58   C. setosus homokaryon. A late division stage. Feulgen
          staining.

Fig. 59   C. setosus homokaryon. A late division stage (arrow). The
          large ring structure is not due to nucleolar formation but is
          due to medial separation of two daughter filaments. Feulgen
          staining.

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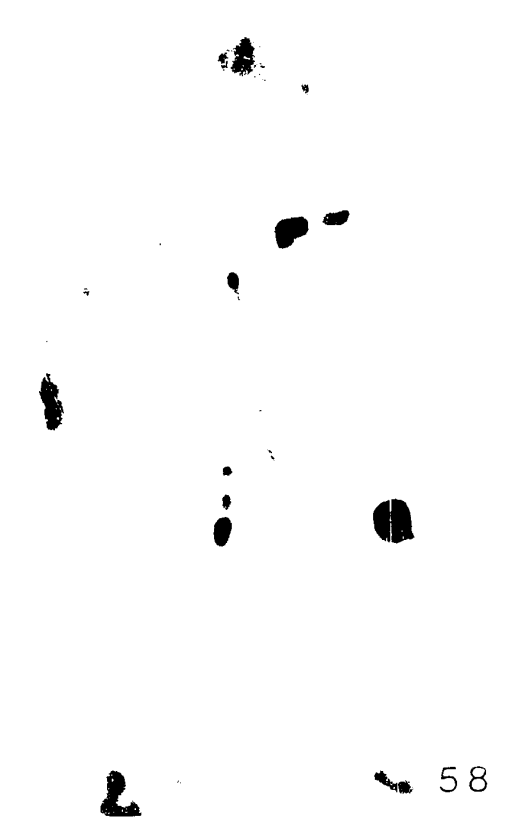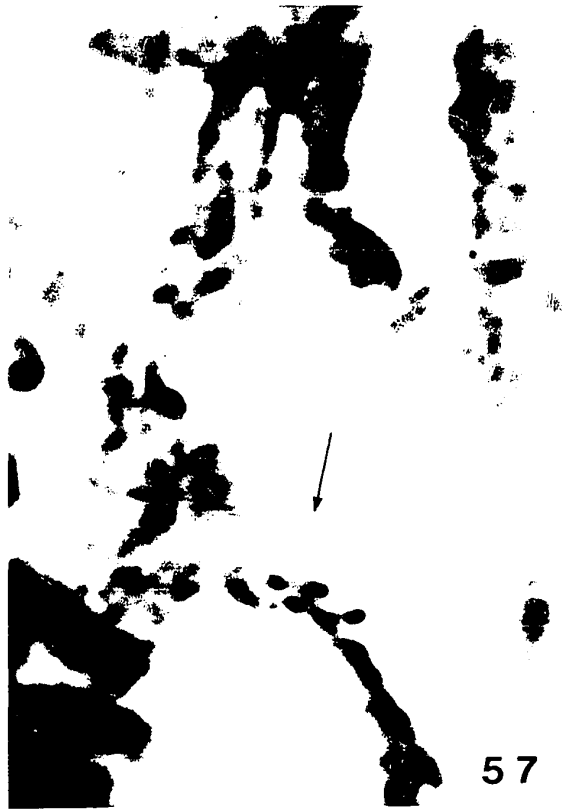
Fig. 60   C. setosus homokaryon.   Early and late phases of nuclear
           division.   The upper two arrows point to nuclear filaments.
           The lower three arrows point to nuclear filaments undergoing
           longitudinal division or strand separation.   The lower
           picture is a less exposed portion of the upper picture.   It
           shows one nucleus (center) to be a ring and another (left) to
           to be in a Y configuration.   Feulgen staining.

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Fig. 61   C. setosus homokaryon. The upper nucleus is filamentous and appears double while its centriole-like body does not appear double. Feulgen staining.

Fig. 62   C. setosus homokaryon. A conidia-like structure. Feulgen staining.

Fig. 63   C. setosus homokaryon. Filamentous nuclei. The upper nuclear filament is undergoing longitudinal division (strand separation). Feulgen staining.
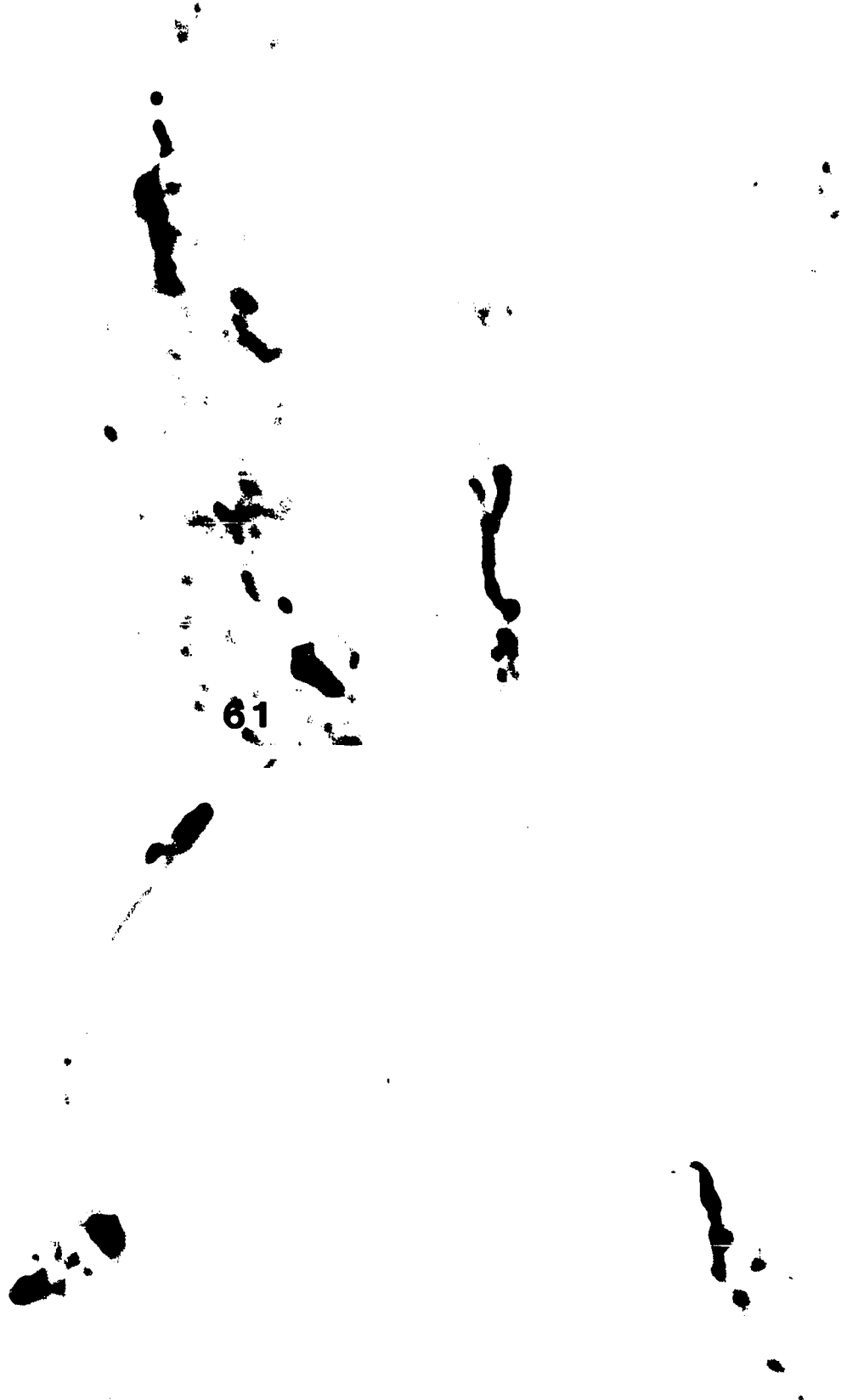
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Fig. 64   C. setosus homokaryon.   Conidia-like structures at hyphal
tips.   Cotton blue lacto-phenol staining.
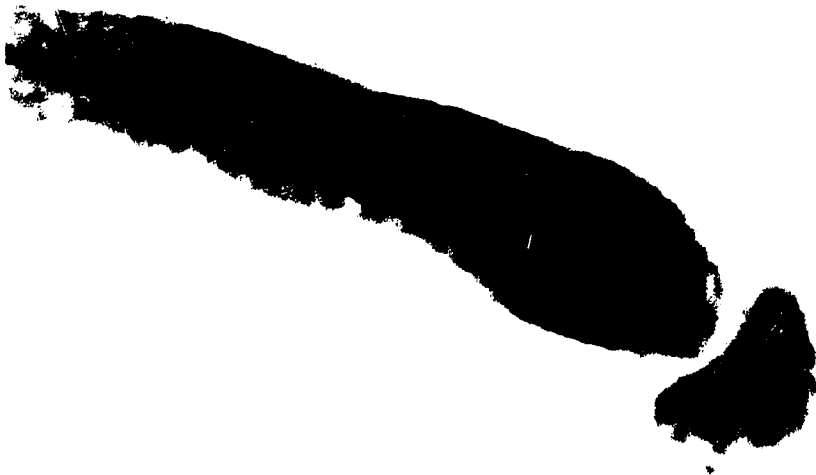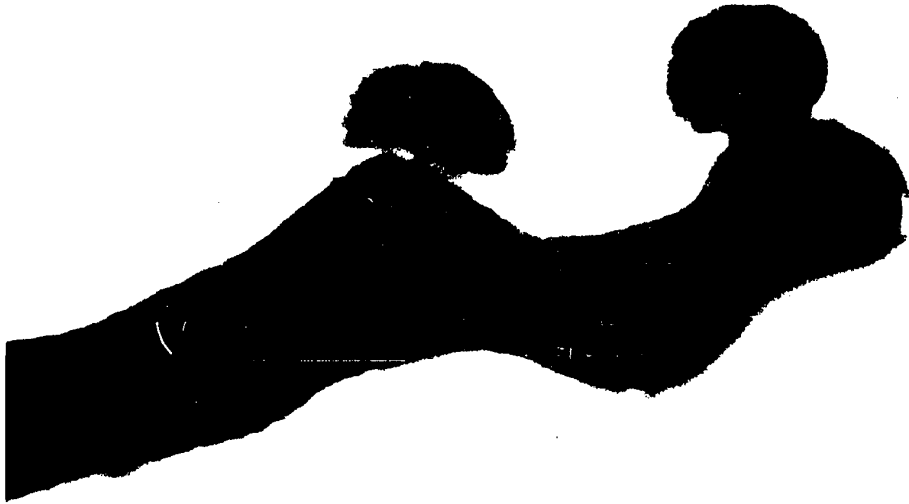
Fig. 65   C. setosus homokaryon.   Conidia-like structures, one of which
           is being formed by constriction from the hypha.   Cotton blue
           lacto-phenol staining.

Fig. 66   C. setosus homokaryon.   Conidia-like structures.   Cotton blue
           lacto-phenol staining.

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Fig. 67   C. setosus homokaryon.   Conidia-like structures.   Cotton blue lacto-phenol staining.

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Fig. 68   C. setosus homokaryon. Spore-like structures. Cotton blue lacto-phenol staining.

Fig. 69   C. *setosus* homokaryon.   Spore-like structures.   Cotton blue lacto-phenol staining.

69

Fig. 70   C. setosus dikaryon. A condensed nucleus with a centriole-
          like body. Feulgen staining.

Fig. 71   C. setosus dikaryon. A condensed nucleus with a centriole-
          like body connected by a faintly stained thread. Feulgen
          staining.

Fig. 72   C. setosus dikaryon. Two condensed nuclei. The upper nucleus
          is in the process of unfolding or elongating. The less dense
          area in this nucleus is thought to be occupied by a nucleolus.
          Feulgen staining.

Fig. 73   C. setosus dikaryon. An unfolding or elongating nucleus. The
          less dense area in this nucleus is thought to be occupied by a
          nucleolus. Feulgen staining.

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Fig. 74 C. setosus dikaryon. Condensed and filamentous nuclei.
        Feulgen staining.

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Fig. 75   C. setosus dikaryon. A partially condensed nucleus in the
           process of unfolding or elongating. The chromatin has
           encircled a Feulgen negative area that is thought to be
           occupied by a nucleolus. Feulgen staining.

Fig. 76   C. setosus dikaryon. An unfolding or elongating nucleus.
           The chromatin has encircled a Feulgen negative area that is
           thought to be occupied by a nucleolus. Feulgen staining.

Fig. 77   C. setosus dikaryon. A filamentous nucleus. Feulgen
           staining.

Fig. 78   C. setosus dikaryon. An undivided condensed nuclear filament
           with two post-division centriole-like bodies. Feulgen
           staining.

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Fig. 79   C. setosus dikaryon.   Condensed and filamentous nuclei.
          Feulgen staining.

Fig. 80   C. setosus dikaryon.   Ring and horseshoe nuclei.   The
          Feulgen negative or less dense area in the center of ring
          and horseshoe nuclei is thought to be occupied by a
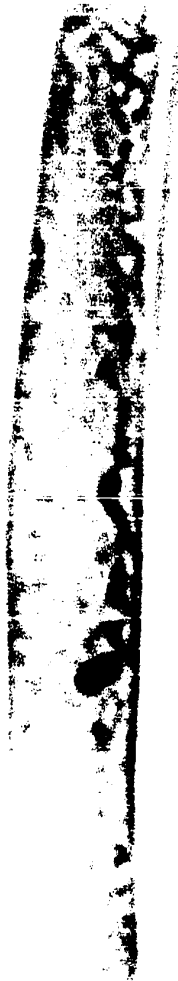   nucleolus.   Feulgen staining.

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Fig. 81   C. setosus dikaryon. Ring and horseshoe nuclei (arrows).
The Feulgen negative or less dense area in the center of ring
and horseshoe nuclei is thought to be occupied by a nucleolus.
Feulgen staining.

Fig. 82   C. setosus dikaryon. Filamentous nuclei. Feulgen staining.

Fig. 83   C. setosus dikaryon. Filamentous nuclei. Feulgen staining.

82   83

Fig. 84   C. setosus dikaryon.   In the terminal hyphal segment, the
          uppermost nucleus appears to be double.   In the hypha to the
          right of this terminal segment, the lower nucleus also appears
          to be double.   Feulgen staining.

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Fig. 85   C. setosus dikaryon.   The nucleus appears to be double.
           Feulgen staining.

Fig. 86   C. setosus dikaryon.   The Y nuclear configuration indicates
           longitudinal division or strand separation of the nuclear
           filament.   Whether the short filament above the Y configuration
           is part of the dividing nucleus is not known.   Feulgen
           staining.

Fig. 87   C. setosus dikaryon.   The nuclear filament (arrow) appears to
           consist of 10, or maybe even 11, chromatin bodies.   To the
           right of the arrow is a partially visible Y configuration
           indicating longitudinal division of a nuclear filament.
           Feulgen staining.
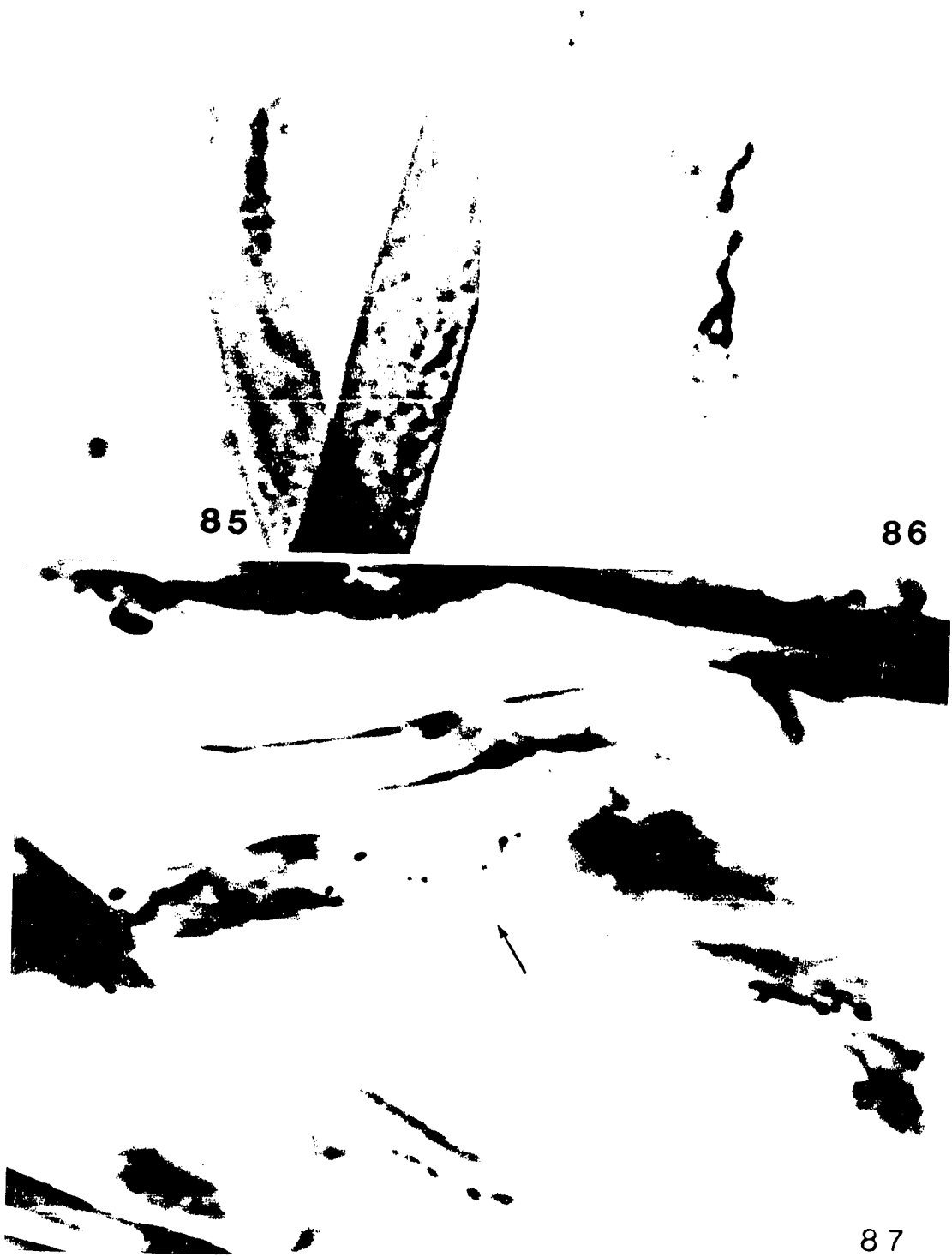
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Fig. 88   C. bulleri homokaryon. Condensed nuclei, one of which is
accompanied by a centriole-like body. Feulgen staining.

Fig. 89   C. bulleri homokaryon. Condensed and filamentous nuclei.
Feulgen staining.

Fig. 90   C. bulleri homokaryon. Condensed and filamentous nuclei.
The lowermost condensed nucleus (which is also pictured in
Fig. 108) is accompanied by two small bodies. The body
furthest from this nucleus is fainter and may be at a
different focal level. The small body closest to the
nucleus is thought to be the centriole-like body and is
adjacent to a small protrusion of chromatin from the
condensed nucleus. This protrusion may be indicative of a
connection between the nucleus and the centriole-like body.
Feulgen staining.

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Fig. 91   <u>C. bulleri</u> homokaryon. Condensed and filamentous nuclei.
           The condensed nucleus is in the process of unfolding or
           elongation. The filamentous nucleus (arrow) is in the
           process of longitudinal division or strand separation.
           Feulgen staining.

Fig. 92   <u>C. bulleri</u> homokaryon. A nuclear filament with a subterminal
           Feulgen negative or less dense area that is thought to be
           occupied by a nucleolus. Feulgen staining.

Fig. 93   <u>C. bulleri</u> homokaryon. A nuclear filament with a subterminal
           Feulgen negative or less dense area that is thought to be
           occupied by a nucleolus. Feulgen staining.
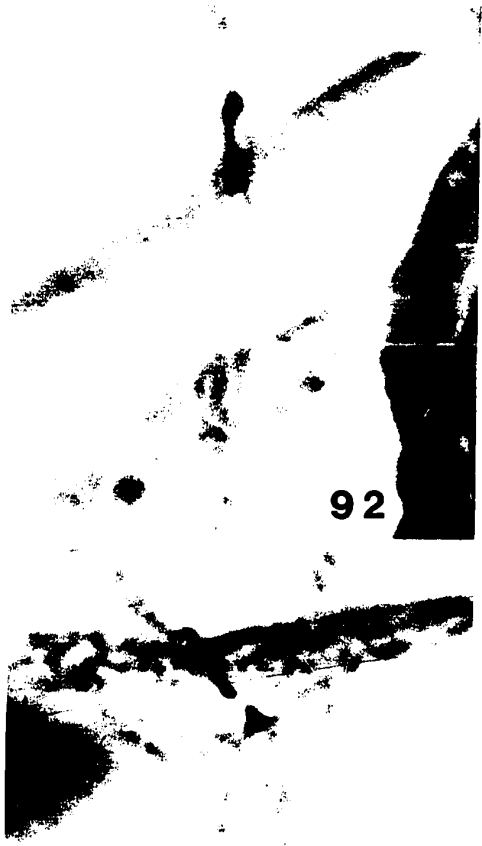
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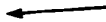
91   93

Fig. 94   C. bulleri homokaryon. This structure is thought to be two
           nuclei (arrows). The upper nucleus, which is in the
           condensed state, appears to be unfolding or elongating. The
           lower nucleus is in the form of a ring with a tail and a
           centriole-like body is located below the ring. The Feulgen
           negative interior of the ring is thought to be occupied by
           nucleolar material. Feulgen staining.

Fig. 95   C. bulleri homokaryon. The upper nucleus is in the form of
           a ring. The Feulgen negative or less dense area in the
           center of the ring is thought to be occupied by a nucleolus.
           Feulgen staining.

Fig. 96   C. bulleri homokaryon. A side view of a horseshoe nucleus
           (arrow). The center of the horseshoe is thought to be
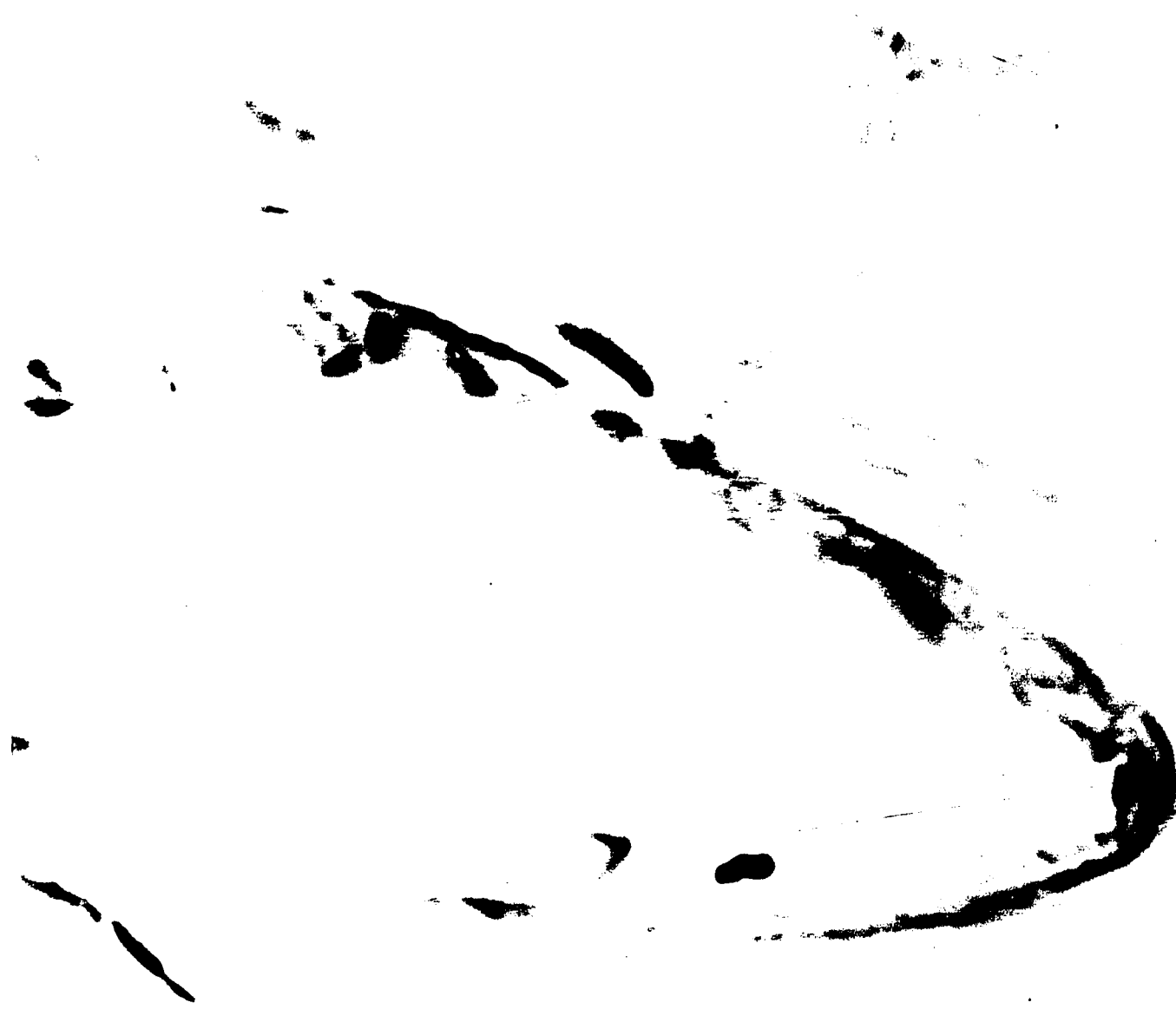   occupied by a nucleolus. Feulgen staining.
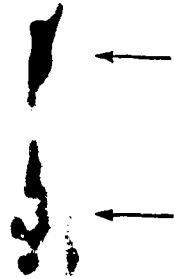
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Fig. 97　C. *bulleri* homokaryon. Condensed, ring and filamentous
nuclei. The Feulgen negative or less dense area in the ring
nucleus is thought to be occupied by a nucleolus. Feulgen
staining.

Fig. 98   C. bulleri homokaryon. A filamentous nucleus with a
           terminally located centriole-like body (arrow). Feulgen
           staining.

Fig. 99   C. bulleri homokaryon. A filamentous nucleus with a
           terminally located centriole-like body (arrow). The
           filament may actually be two nuclear filaments in tandem
           and the center darkly stained body may be the second
           centriole-like body. Feulgen staining.

Fig. 100   C. bulleri homokaryon. A filamentous nucleus with a
           terminally located centriole-like body (arrow). Feulgen
           staining.

Fig. 101   C. bulleri homokaryon. A filamentous nucleus with a
           terminally located centriole-like body (arrow). Feulgen
           staining.
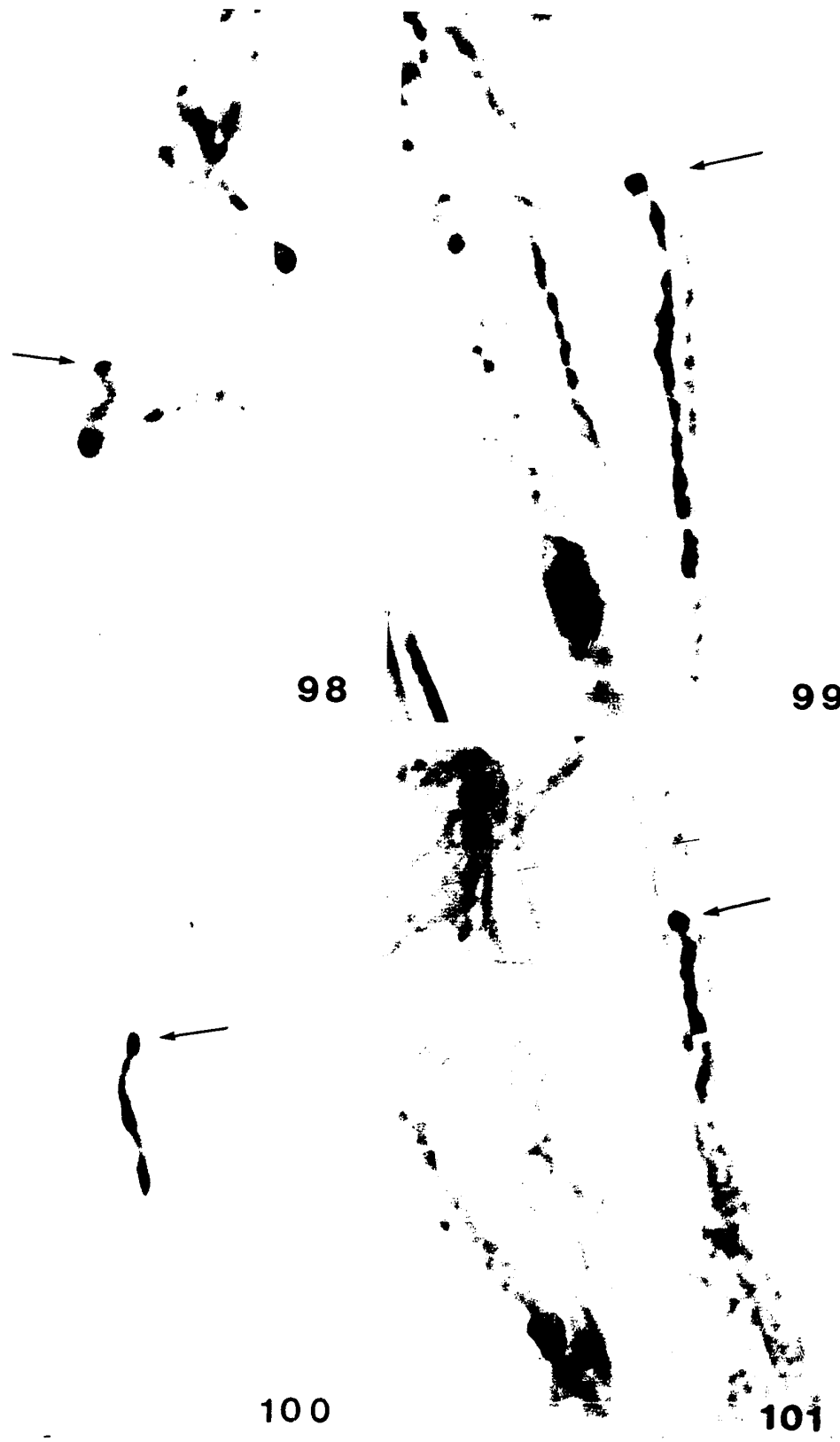
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Fig. 102   C. bulleri homokaryon. A filamentous nucleus with a
           terminally located centriole-like body (arrow). Feulgen
           staining.

Fig. 103   C. bulleri homokaryon. A filamentous nucleus consisting of
           seven chromatin bodies. Feulgen staining.

Fig. 104   C. bulleri homokaryon. A filamentous nucleus consisting of
           at least seven main chromatin bodies. Feulgen staining.

Fig. 105   C. bulleri homokaryon. A filamentous nucleus. Feulgen
           staining.

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Fig. 106   C. bulleri homokaryon. A filamentous nucleus consisting of over nine chromatin bodies (arrow). Feulgen staining.

Fig. 107   C. bulleri homokaryon. Early division of the centriole-like body and the subterminal chromatin body. Feulgen staining.

Fig. 108   C. bulleri homokaryon. Condensed and filamentous nuclei. (The condensed nucleus with the centriole-like body at the top of the picture is the same nucleus that is pictured in Fig. 90.) Approximately 15 chromatin bodies can be counted in the upper nuclear filament; however, this structure may consist of two filaments in tandem. Feulgen staining.

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Fig. 109 C. bulleri homokaryon. A nuclear filament undergoing
          longitudinal division or strand separation. Feulgen
          staining.

Fig. 110 C. setosus homokaryon. A nuclear filament undergoing
          longitudinal division or strand separation. Feulgen
          staining.

Fig. 111 C. bulleri homokaryon. A nuclear filament undergoing
          longitudinal division or strand separation. Just below
          and overlapping one of the strands is a condensed nucleus
          in the process of elongation or unfolding. To this nucleus
          is attached a centriole-like body by a faintly stained thread.
          Feulgen staining.

Fig. 112 C. bulleri homokaryon. A nuclear filament at the end state of
          division. The two strands which are held together at a
          terminal point appear to be twisted around each other.
          Feulgen staining.

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Fig. 113   C. bulleri homokaryon. A nuclear filament at the end stage
           of division. The two strands are held together at a terminal
           point. Feulgen staining.

Fig. 114   C. bulleri homokaryon. This nuclear configuration (arrow)
           may be indicative of longitudinal division (strand separation).
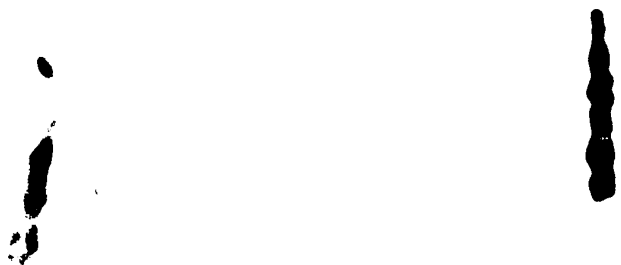   Feulgen staining.

Fig. 115   C. bulleri homokaryon. A nuclear filament undegcing
           longitudinal division (strand separation). The two strands
           appear to be homologous. Feulgen staining.

Fig. 116   C. bulleri homokaryon. A nuclear filament that is double
           and homologous. Feulgen staining.

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Fig. 117   C. bulleri homokaryon.   A nuclear filament undergoing
           longitudinal division (strand separation).   The centriole-
           like body (arrow) has also divided.   Feulgen staining.

Fig. 118   C. bulleri homokaryon.   A nuclear filament undergoing
           longitudinal division (strand separation) (arrow).
           Feulgen staining.

Fig. 119   C. bulleri homokaryon.   A condensed but duplicated nuclear
           filament.   Feulgen staining.

Fig. 120   C. bulleri homokaryon.   A nuclear filament undergoing
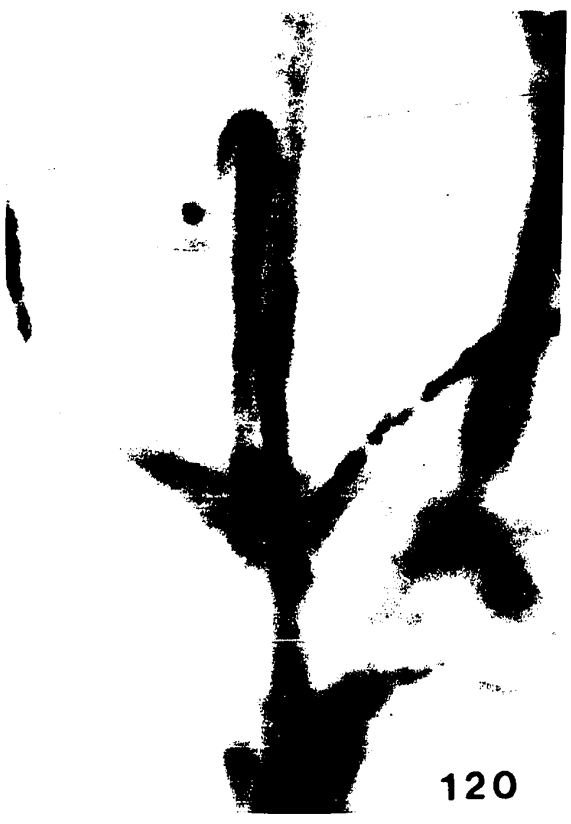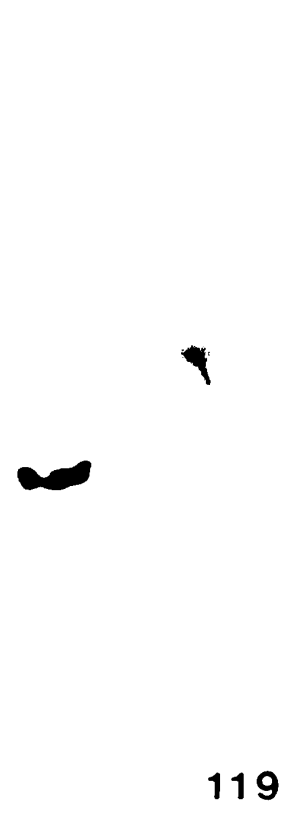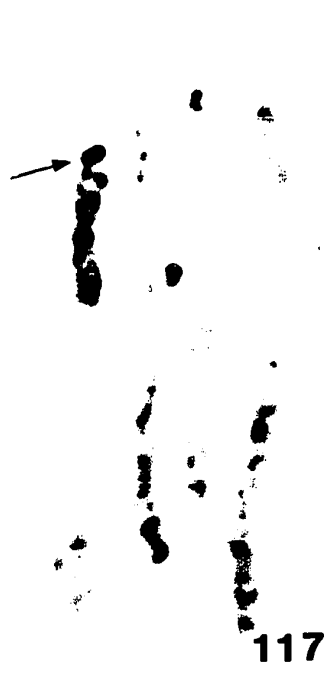   longitudinal division (strand separation).   Feulgen
           staining.

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Fig. 121   C. bulleri homokaryon. A nuclear filament in which longitud-
           inal division or strand separation is occuring. However, the
           centriole-like body (arrow) at the end of this filament has
           not yet divided. Feulgen staining.

Fig. 122   C. bulleri homokaryon. Two spherical nuclei with a slightly
           Feulgen positive connecting strand. Such a configuration
           may represent the Bakerspigel phenomenon of indirect division
           or may be a persistent telophase. The insert in the lower
           left hand corner is a less exposed picture of the same
           configuration. Feulgen staining.

Fig. 123   C. bulleri homokaryon. Spore-like structures. Feulgen
           staining.

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Fig. 124 C. bulleri homokaryon. Spore-like structures. Some of the
nuclei appear to be double. Feulgen staining.
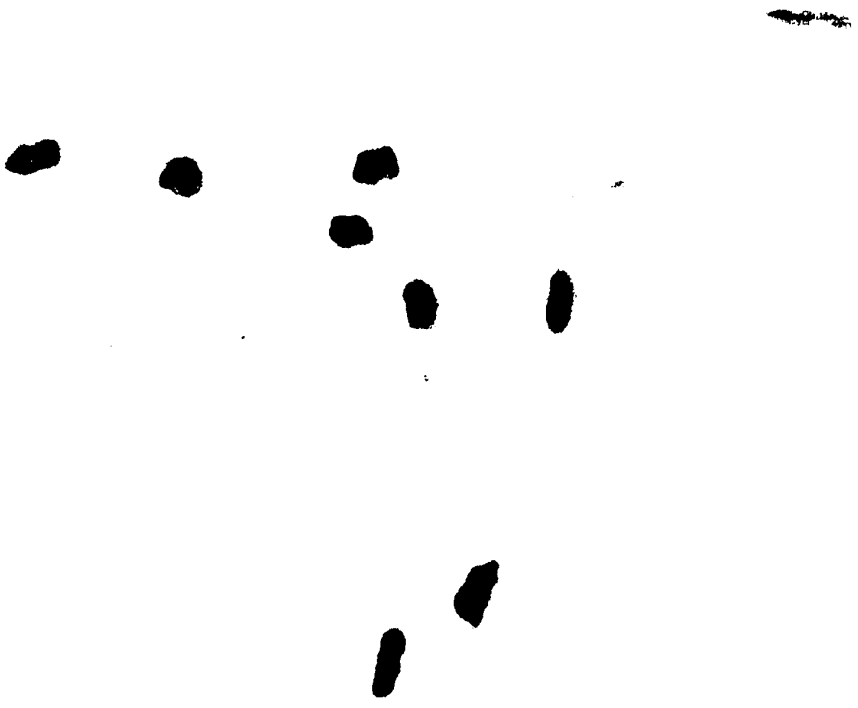
124

Fig. 125 C. bulleri homokaryon. A filamentous nucleus (arrow). Live
          material. Phase contrast microscopy.

Fig. 126 C. bulleri homokaryon. A filamentous nucleus (arrow). Live
          material. Phase contrast microscopy.

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Fig. 127   C. bulleri homokaryon.   Spore-like structures which are
           joined together by thick septa.   Cotton blue lacto-phenol
           staining.

Fig. 128   C. bulleri homokaryon.   Spore-like structures which are
           joined together by thick septa.   Cotton blue lacto-phenol
           staining.

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Fig. 129   C. bulleri homokaryon.   Spore-like structures which are
   joined together by thick septa.   Cotton blue lacto-phenol
           staining.

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Fig. 130 C. bulleri dikaryon. Condensed nuclei. Feulgen staining.

Fig. 131 C. bulleri dikaryon. A condensed nucleus in the process of
          unfolding or elongation. A centriole-like body, on the axis
          of elongation, is attached to the nucleus by a faintly stained
          thread or material. Feulgen staining.

Fig. 132 C. bulleri dikaryon. A condensed nucleus with a centriole-
          like body attached by a faintly stained thread or material.
          Feulgen staining.

Fig. 133 C. bulleri dikaryon. A condensed nucleus in the process of
          unfolding or elongation. A centriole-like body, on the axis
          of elongation, appears to be attached to the nucleus by a
          faintly material which may be a double thread. Feulgen
          staining.

130   131
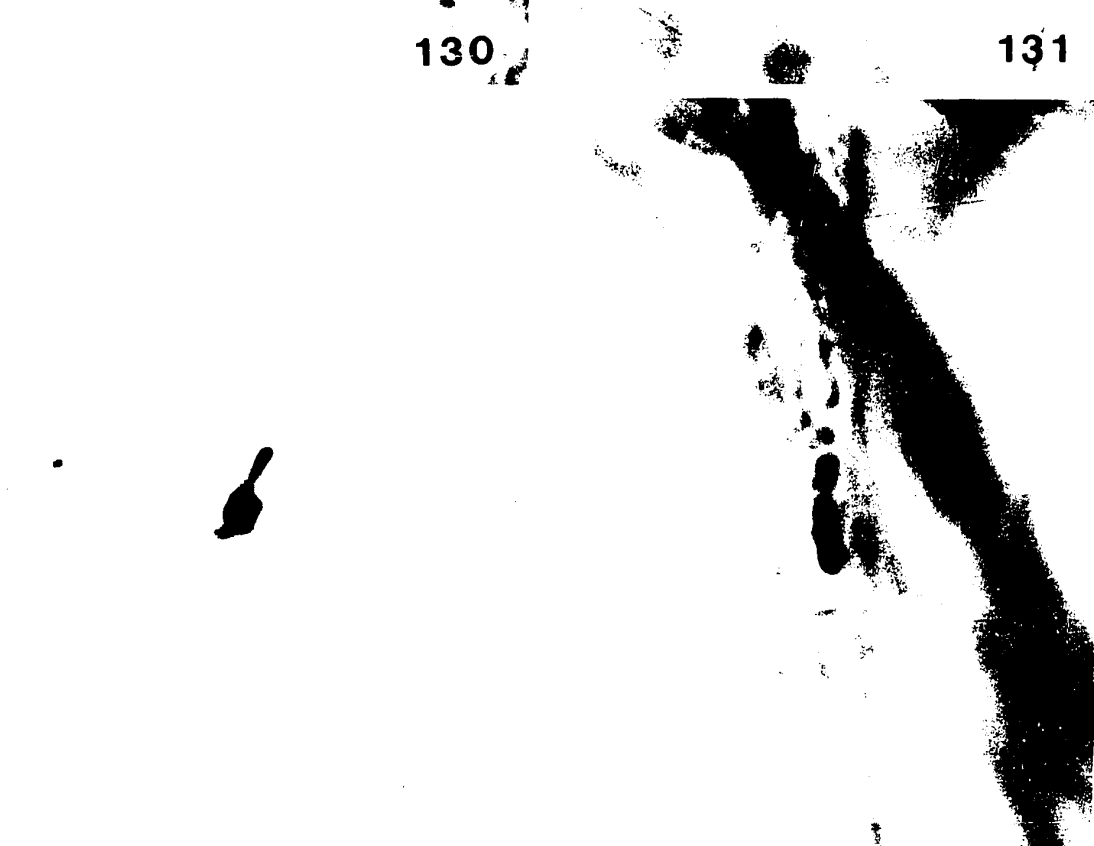
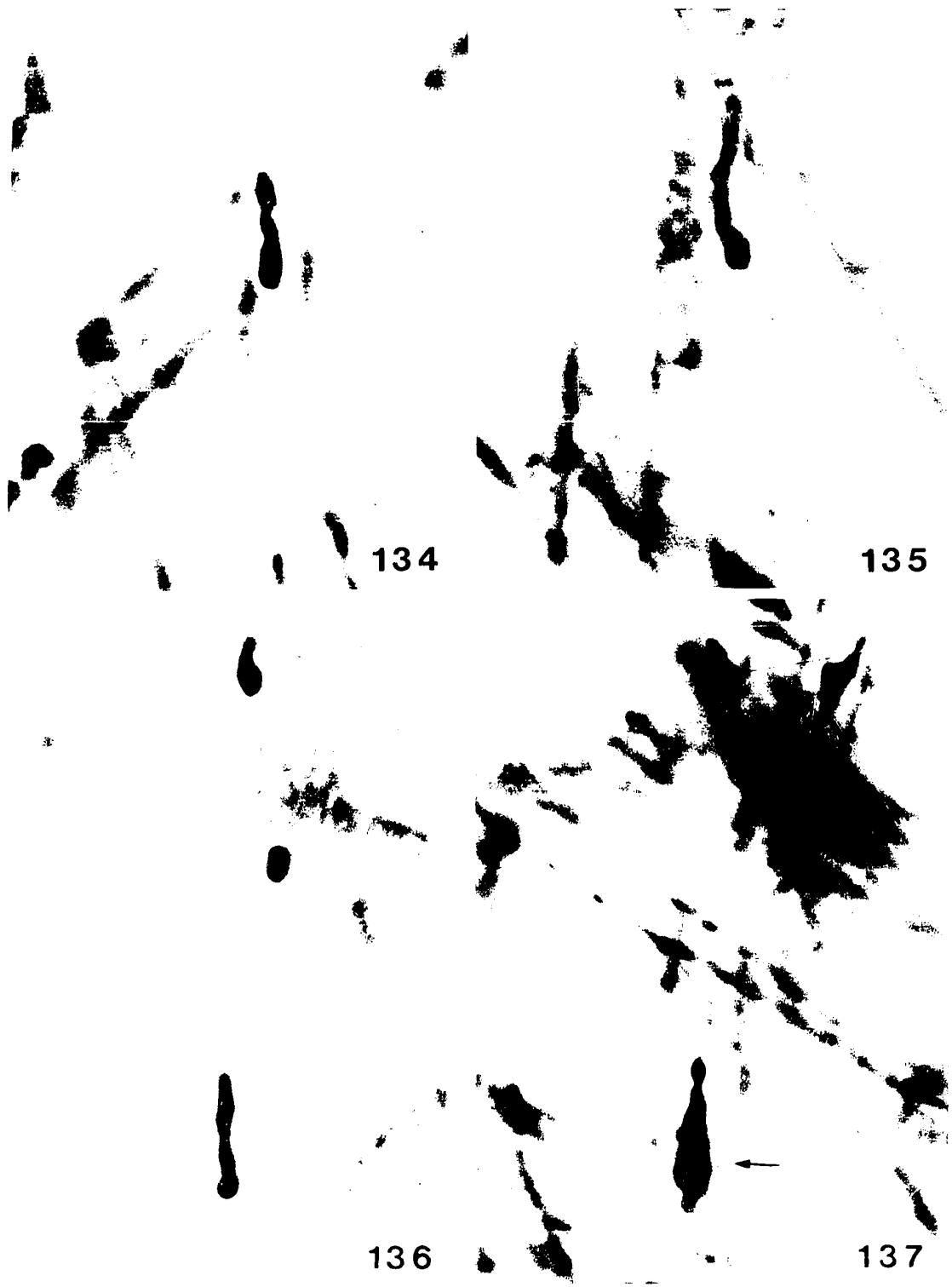132   133

Fig. 134 C. bulleri dikaryon. A condensed nuclear filament. Feulgen
         staining.

Fig. 135 C. bulleri dikaryon. A condensed nuclear filament. The
         upper end of the filament is particulate. Presumably the
         filament is unfolding from the lower end. Feulgen staining.

Fig. 136 C. bulleri dikaryon. Three condensed nuclei at different
         stages. The middle nucleus is almost spherical and has not
         yet begun to elongate. The upper nucleus is just beginning
         to unfold. The lower nucleus is a condensed filament.
         Feulgen staining.

Fig. 137 C. bulleri dikaryon. The upper nucleus is in the form of a
         ring with a tail. The Feulgen negative or less dense area in
         the center of the ring is thought to be occupied by a
         nucleolus. The lower nucleus is partially condensed. The
         lower part of this nucleus (arrow) is less dense where a
         nucleolus is thought to be inside. A centriole-like body is
         attached to the top end of this nucleus by a faintly stained
         thread. Feulgen staining.

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Fig. 138   C. bulleri dikaryon. The upper nucleus is in a ring
           configuration. The Feulgen negative area in the center of
           the ring is thought to be occupied by a nucleolus. The
           chromatin appears to be concentrated at two poles on the
           ring. Feulgen staining.

Fig. 139   C. bulleri dikaryon. A condensed particulate nuclear
           filament. Feulgen staining.

Fig. 140   C. bulleri dikaryon. A nuclear filament. Feulgen staining.

Fig. 141   C. bulleri dikaryon. A nuclear filament. Eight major
           chromatin bodies are visible. Feulgen staining.

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Fig. 142 C. bulleri dikaryon. A nuclear filament which has duplicated.
          Feulgen staining.

Fig. 143 C. bulleri dikaryon. A nuclear filament which is undergoing
          longitudinal division or strand separation. Feulgen staining.

Fig. 144 C. bulleri dikaryon. A nuclear filament which is undergoing
          longitudinal division or strand separation. Feulgen staining.

Fig. 145 C. bulleri dikaryon. A nuclear filament which is undergoing
          longitudinal division or strand separation. Feulgen staining.
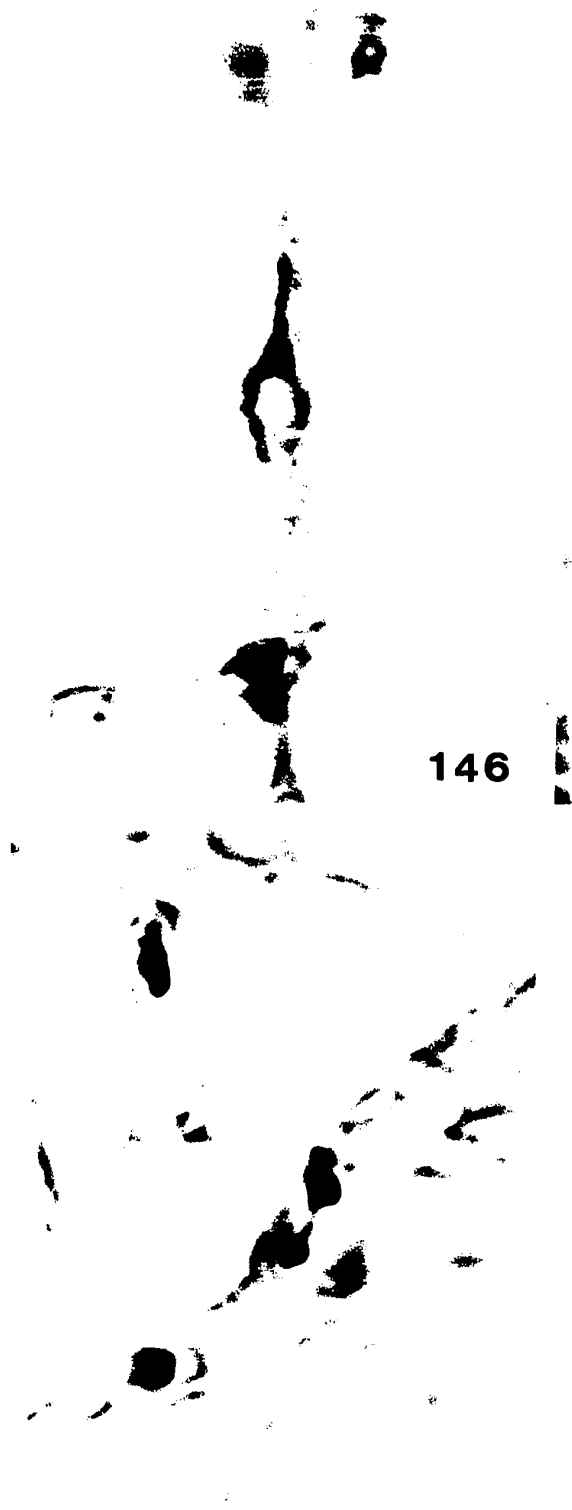
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Fig. 146   C. bulleri dikaryon. A nuclear filament which is undergoing
           longitudinal division or strand separation. Feulgen staining.
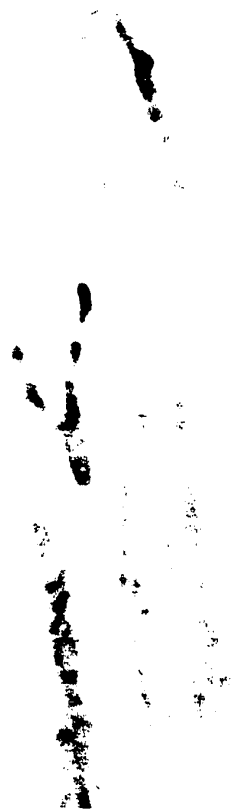

Fig. 147   C. bulleri dikaryon. A nuclear filament which is undergoing
           longitudinal division or strand separation. Feulgen staining.

Fig. 148   C. bulleri dikaryon. Two spherical condensed nuclei inter-
           connected by a faintly stained thread. This configuration
           may represent division in accordance with the Bakerspigel
           theory or it may be a result of a persistent telophase.
           Feulgen staining.

Fig. 149   C. bulleri dikaryon. Two condensed nuclei interconnected by
           a thin thread. This configuration may represent division in
           accordance with the Bakerspigel theory or it may be a result
           of a persistent telophase. Feulgen staining.

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Fig. 150   C. bulleri dikaryon. Clamp formation. The clamp nucleus is
           globular and the tube nucleus is filamentous and is undergoing
           longitudinal division (strand separation). Feulgen staining.

Fig. 151   C. bulleri dikaryon. Clamp formation. The clamp nucleus
           appears to be accommodating itself to the space available,
           i.e., to the curvature of the clamp tip, and does not seem to
           be a solid sphere. Feulgen staining.

Fig. 152 C. bulleri dikaryon. Clamp formation. The clamp nucleus is condensed and double (see insert in lower left hand corner) and the tube nucleus (upper part of picture) is filamentous and single. Feulgen staining.

Fig. 153 C. bulleri dikaryon. The four pictures are a sequence along
a length of the hypha and should be read one after another
starting with the top picture. The clamp connection is
complete. Nucleus "a" is in the process of replication and,
being the ultimate nucleus and barring nuclear passing, it
is probably one of the daughters of the tube nucleus of the
preceding division. Nucleus "b" may be in the process of
duplication and, being the penultimate nucleus and barring
nuclear passing, it is probably one of the daughters of the
clamp nucleus of the preceding division. In the clamp, the
other daughter nucleus (c) is unfolding into a filament,
destined to migrate into the penultimate hyphal compartment.
Feulgen staining.

a

b

c

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Fig. 154   C. bulleri dikaryon. The upper nucleus is filamentous and in
           a replicated condition and the lower nucleus is in the process
           of condensation, probably prior to clamp formation. This
           hyphal section is from a terminal compartment and the arrow
           at the top of the picture points to the direction of the
           hyphal tip. Feulgen staining.

Fig. 155   C. bulleri dikaryon. Clamp formation. All four of the
           post-division nuclei are in the condensed state. The insert
           in the upper right hand corner is the same picture but with
           the uppermost nucleus in focus. Feulgen staining.

154   155
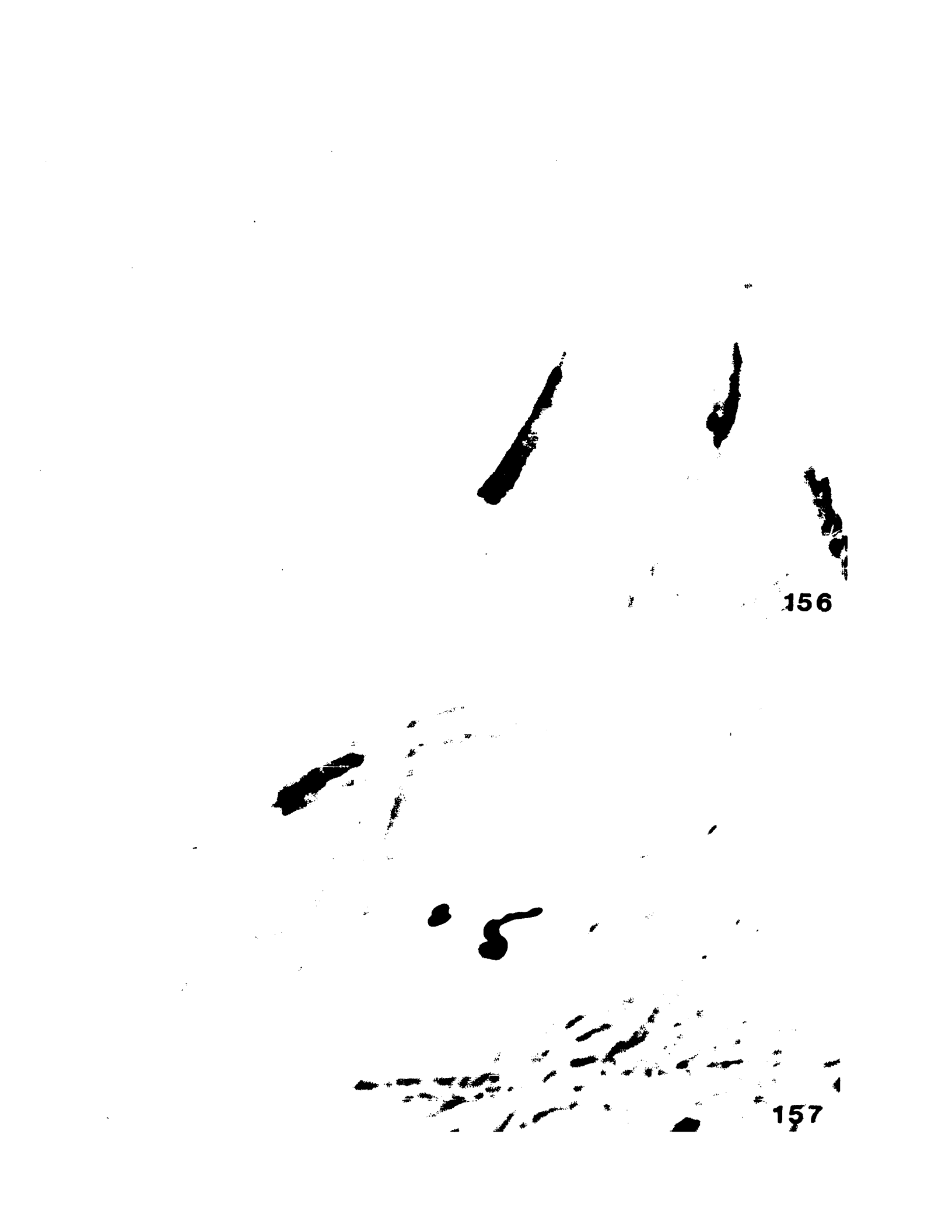
Fig. 156   C. bulleri dikaryon. The ultimate nucleus, which will
            probably function as the tube nucleus in the next division,
            has replicated while the penultimate nucleus, which will
            probably function as the clamp nucleus in the next division,
            is at an earlier stage of karyokinesis. The penultimate
            nucleus has a centriole-like body located to one side of it
            instead of being in line with the filament's axis. Feulgen
            staining.

Fig. 157   C. bulleri dikaryon. Clamp formation. The tube nucleus, in
            a state of elongation intermediate between the filamentous
            and spherical states, has divided. The clamp nucleus is in
            the process of dividing and the nuclear part which will not
            migrate through the clamp is already unfolding or elongating.
            Feulgen staining.

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Fig. 158   C. bulleri dikaryon.   Clamp formation.   The tube nucleus
           (right) is condensed and appears to have replicated but not
           separated.   The clamp nucleus is a double filament (the lower
           picture is the same but less exposed to show the double nature
           of the filamentous clamp nucleus). Feulgen staining.

Fig. 159   C. bulleri dikaryon.   Clamp formation.   The tube nucleus has
           replicated (see the lower picture which is less exposed and
           shows the double nature of the tube nucleus) but not separated,
           whereas the clamp nucleus has not replicated.   The clamp
           nucleus, in a filamentous condition, shows signs of condens-
           ation at the terminals.   This nucleus appears to be migrating
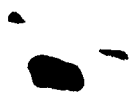   into the clamp.   Feulgen staining.

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Fig. 160   C. bulleri dikaryon.   Clamp formation.   This picture is of
the same clamp that is depicted in Fig. 161 except that this
picture was photographed through phase contrast optics.
Feulgen staining.

Fig. 161   C. bulleri dikaryon.   Clamp formation.   This picture is of
the same clamp that is depicted in Fig. 160 except that this
picture was photographed through bright field optics.   In
Figs. 160 and 161 the tube nucleus appears to have replicated
in the condensed spherical state (see lower Fig. 161 which is
the same as upper Fig. 161 except that it is less exposed).
The clamp nucleus is migrating into the clamp.   Fig. 160
indicates that the clamp nucleus is attached by a thin
Feulgen negative thread to a large globular Feulgen negative
body which is thought to be the nucleolus.   Feulgen staining.
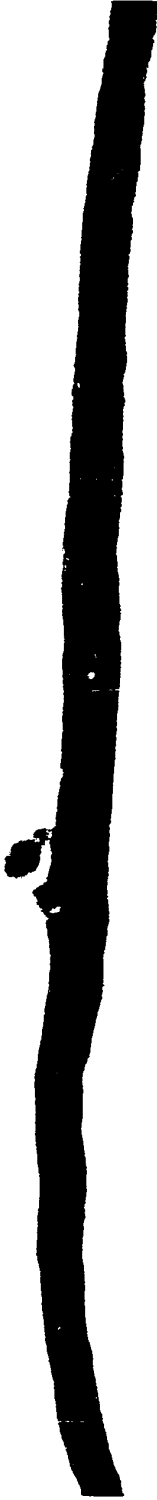
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Fig. 162   C. bulleri dikaryon. Two large spherical bodies in the
            upper part of the hypha are thought to be nucleoli. The
            picture was taken of a section of hypha not apparently
            undergoing new clamp connection formation. Small spheres
            which are thought to be fragments of filamentous mitochondria
            are present. Living material. Phase contrast microscopy.

Fig. 163   C. bulleri dikaryon. Long smooth filaments of uniform
            density which are thought to be filamentous mitochondria.
            One filament appears to be in a Y configuration. Living
            material. Phase contrast microscopy.

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Fig. 164   C. bulleri dikaryon. Two large spherical or oblong bodies
which are thought to be nucleoli are present in a hyphal
cell which is apparently not undergoing new clamp connection
formation. Living material. Phase contrast microscopy.

Fig. 165   C. bulleri dikaryon. One large spherical body which is
thought to be a nucleolus is present. Long smooth filaments
of uniform density and small spheres with diameters of
approximately the thickness of the filaments are also present.
The filaments are thought to be mitochondria and the small
spheres are thought to be fragments of the filamentous
mitochondria. Living material. Phase contrast microscopy.

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Fig. 166   C. bulleri dikaryon. A large spherical body is located on
            each side of an early clamp formation. These two spherical
            bodies are thought to be nucleoli. Living material. Phase
            contrast microscopy.
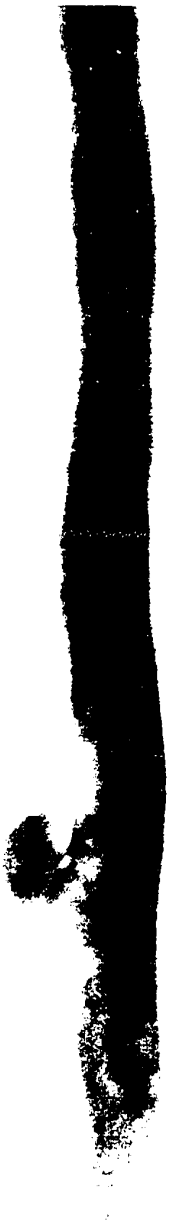

Figs. 167 to 171 inclusive.   C. bulleri dikaryon. These pictures are
                               of the same clamp connection formation
                               and are arranged sequentially according to
                               time.

Fig.	Time (minutes)
167	0
168	4
169	21
170	26
171	35

                               During the process of clamp formation the
                               two spherical bodies (nucleoli) draw close
                               together but do not touch (Figs.166 and
                               167) and then draw apart again (Fig. 168).
                               The two bodies disappear from view and a
                               septum is formed across the clamp (Fig.
                               169). . A septum is formed across the
                               hyphal tube and a small spherical body
                               (nucleolus) becomes visible in the clamp
                               (Figs. 170 and 171). Living material.
                               Phase contrast microscopy.

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Figs. 167 to 171 inclusive. <u>C</u>. <u>bulleri</u> dikaryon. These pictures are of the same clamp connection formation and are arranged sequentially according to time.

Fig.	Time (minutes)
167	0
168	4
169	21
170	26
171	35

During the process of clamp formation the two spherical bodies (nucleoli) draw close together but do not touch (Figs. 166 and 167) and then draw apart again (Fig. 168). The two bodies disappear from view and a septum is formed across the clamp (Fig. 169). A septum is formed across the hyphal tube and a small spherical body (nucleolus) becomes visible in the clamp (Figs. 170 and 171). Living material. Phase contrast microscopy.

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Fig. 172   C. olla dikaryon. Distinct chromatin bodies (arrow). These bodies are thought to be interconnected to constitute a late filament stage. Seven distinct chromatin bodies are visible. Two faintly stained bodies are also present and may be part of the nucleus. Feulgen staining.
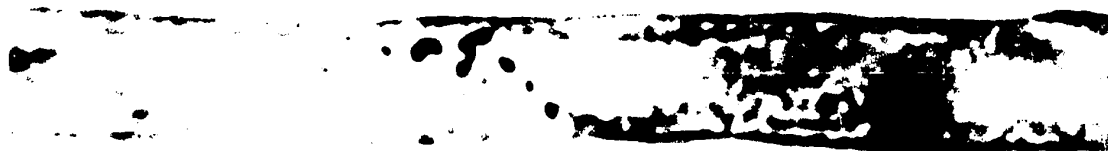
Fig. 173   C. olla dikaryon. Distinct chromatin bodies. These bodies are thought to be interconnected to constitute a late filament stage. Feulgen staining.

Fig. 174   C. olla dikaryon. Two nuclei, each consisting of chromatin bodies. The bodies of each nucleus are thought to be interconnected to constitute a late filament stage which is beginning to condense or fold up. Feulgen staining.

Fig. 175   C. olla dikaryon. Two nuclei, each consisting of chromatin bodies. The bodies of each nucleus are thought to be interconnected to constitute a late filament stage which is beginning to condense or fold up. At least 10 chromatin bodies and a centriole-like body (arrow) are present in one of these complements. The overall chromatin mass appears to exceed the amount present in a haploid nucleus and may be indicative of a polyneme chromosome stage. Feulgen staining.

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Fig. 176 C. olla dikaryon. Distinct chromatin bodies (arrow). These
          bodies are thought to be interconnected to constitute a late
          filament stage. The overall chromatin mass appears to
          exceed the amount present in a haploid nucleus and may be
          indicative of a polyneme chromosome stage. Feulgen staining.

Fig. 177 C. olla dikaryon. The nucleus on the left consists of
          chromatin bodies that are thought to be interconnected to
          constitute a late filament stage. Feulgen staining.

Fig. 178 C. olla dikaryon. The chromatin bodies (arrow) are thought
          to be interconnected to constitute a late filament stage.
          The overall chromatin mass appears to exceed the amount
          present in a haploid nucleus and may be indicative of a
          polyneme chromosome stage. Feulgen staining.

Fig. 179 C. olla dikaryon. The chromatin bodies are thought to be
          interconnected to constitute a late filament stage. The
          overall chromatin mass appears to exceed the amount present
          in a haploid nucleus and may be indicative of a polyneme
          chromosome stage. Feulgen staining.
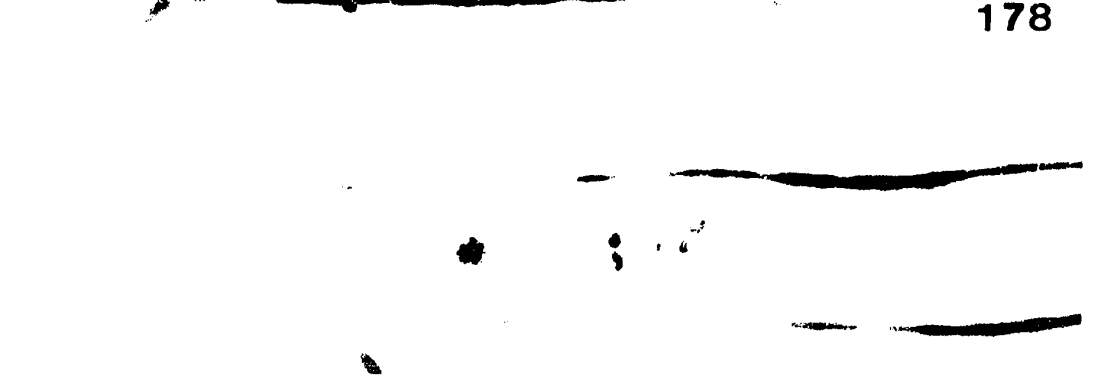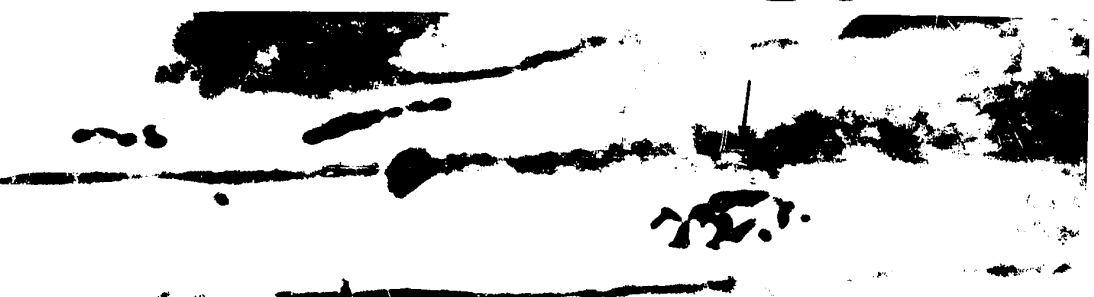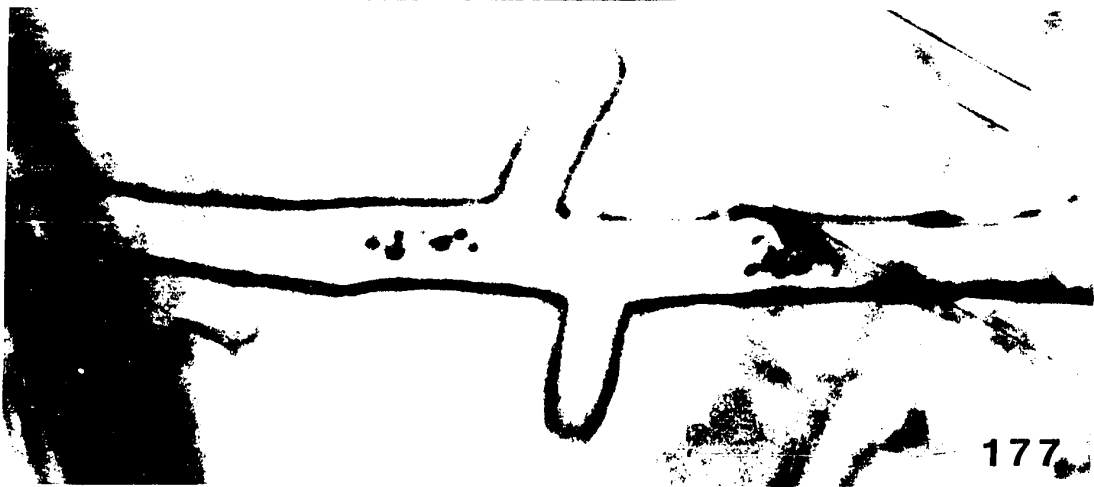
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Fig. 180 C. olla dikaryon. Nuclear filaments. Feulgen staining.

Fig. 181 C. olla dikaryon. Ring nuclei. The Feulgen negative center
          of the rings is thought to be occupied by a nucleolus.
          Feulgen staining.

Fig. 182 C. olla dikaryon. Ring nuclei. A centriole-like body (arrow)
          is attached to one of the rings. The Feulgen negative or less
          dense center of the rings is thought to be occupied by a
   nucleolus. Feulgen staining.

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Fig. 183   C. olla dikaryon. Ring nuclei. A centriole-like body (arrow) is attached by a faintly stained thread to one of the rings. Feulgen staining.

Fig. 184   C. olla dikaryon. A ring nucleus (center). A centriole-like body is located to the right of the nucleus. Feulgen staining.

Fig. 185   C. olla dikaryon. A ring nucleus undergoing division or strand separation. The Feulgen negative area in the ring is thought to be occupied by a nucleolus. Feulgen staining.

Fig. 186   C. olla dikaryon. An enlarged ring nucleus (arrow). Feulgen staining.

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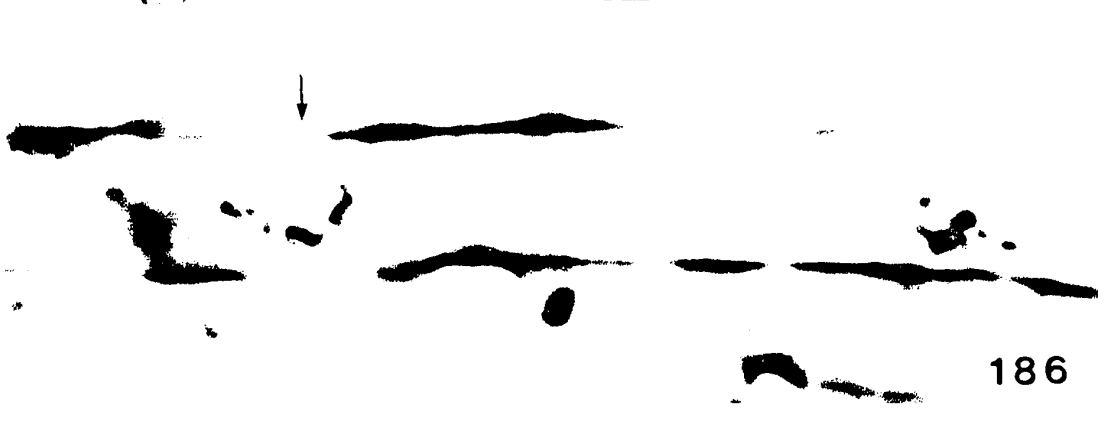
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Fig. 187   C. olla dikaryon. Condensed nuclei. The nucleus on the left
           is in the process of unfolding or elongation. Crystal violet
           staining.

Fig. 188   C. olla dikaryon. An elongated nucleus (early filament).
           Two Woronin bodies are visible (arrows). Crystal violet
           staining.

Fig. 189   C. olla dikaryon. A nuclear filament (center) and a
           condensed nucleus (right). The condensed nucleus is in the
           process of elongation or unfolding. Crystal violet staining.

Fig. 190   C. olla dikaryon. A Woronin body is present on each side of
           the tube septum. The clamp connection (arrow) is out of
           focus. Crystal violet staining.

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Fig. 191   C. olla dikaryon. Woronin bodies (arrows). Crystal violet staining.

Fig. 192   C. olla dikaryon. A nuclear filament in the process of longitudinal division or strand separation. The centriole-like body has divided as well. Crystal violet staining.

Fig. 193   C. olla dikaryon. A nuclear filament in the process of longitudinal division or strand separation. Crystal violet staining.

Fig. 194   C. olla dikaryon. A condensed nucleus (left) and a ring nucleus (arrow). The unstained or faintly stained area in the center of the ring is thought to be occupied by a nucleolus. Crystal violet staining.

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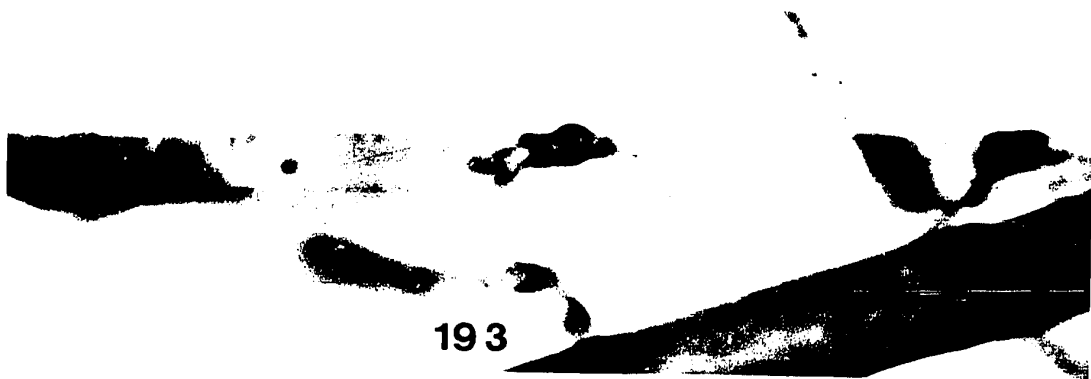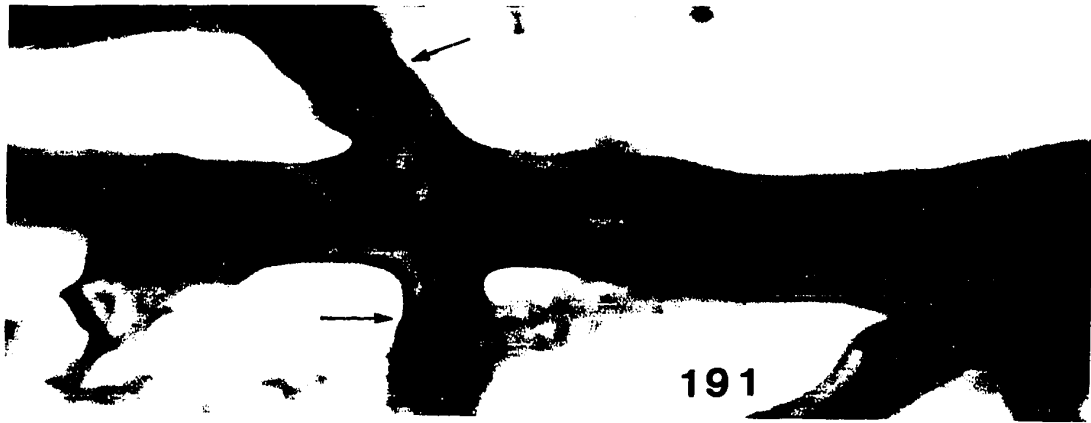
Fig. 195   C. olla dikaryon.   A ring nucleus with two centriole-like
           bodies (arrows).   Crystal violet staining.

Fig. 196   C. olla dikaryon.   An elongated or filamentous nucleus.   A
           Woronin body (arrow) is present next to a tube septum.
           Crystal violet staining.

Fig. 197   C. olla dikaryon.   A large ring nucleus.   The unstained
           center area of the ring is thought to be occupied by a
           nucleolus.   Giemsa staining.

Fig. 198   C. olla dikaryon.   Ring-like configurations which appear to
           consist of coiled up filamentous nuclei. Feulgen staining.
           Phase contrast microscopy.

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