PROCESSES AFFECTING THE GROWTH OF *PHYCOMYCES* SPORANGIOPHORES

Thesis by

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This thesis is dedicated to my

folks

and my

kid sister

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ABSTRACT

The stage IV sporangiophore (spph) of Phycomyces is a very large single cell. It is 0.1 mm in diameter and grows to a length of 10 cm. The sporangiophore bears asexual spores at its tip in a spherical sporangium. The sporangiophore elongates via a 2 mm long growing zone just beneath the sporangium. Most of this thesis deals with the interaction of the growing zone with the rest of the sporangiophore and with the sporangium.

The top of the sporangiophore appears to have the most active metabolism. The top 6 mm of the sporangiophore consumes 1/2 of the oxygen. The oxygen consumption of the whole spph and of this 6 mm long section does not change as the spph elongates from 25 mm to 50 mm. The oxygen consumption of the sporangium is very much less than that of the sporangiophore.

The whole sporangiophore can grow independently from the mycelium. Similiarly, the top 3 or 4 mm can elongate independently from the rest of the sporangiophore. It can grow for about 10 hours without the lower part of the sporangiophore. For the first 4 hours its rate of growth is nearly normal.

The elongation of the sporangiophore is much more dependent upon the sporangium. If the sporangium is removed,

the growth of the sporangiophore decreases to 1/10 of the normal rate within 2 hours. Six to sixteen hours after sporangium removal, the sporangiophore produces a branch sporangiophore from the former growing zone (figure 1). The spores in the sporangium appear to synthesize some compound (or compounds) which stimulates the sporangiophore's elongation and inhibits its branching.

FIGURE 1. Four sporangiophores in successive stages of branch formation. (photograph by Dr. David Dennison).

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GENERAL INTRODUCTION

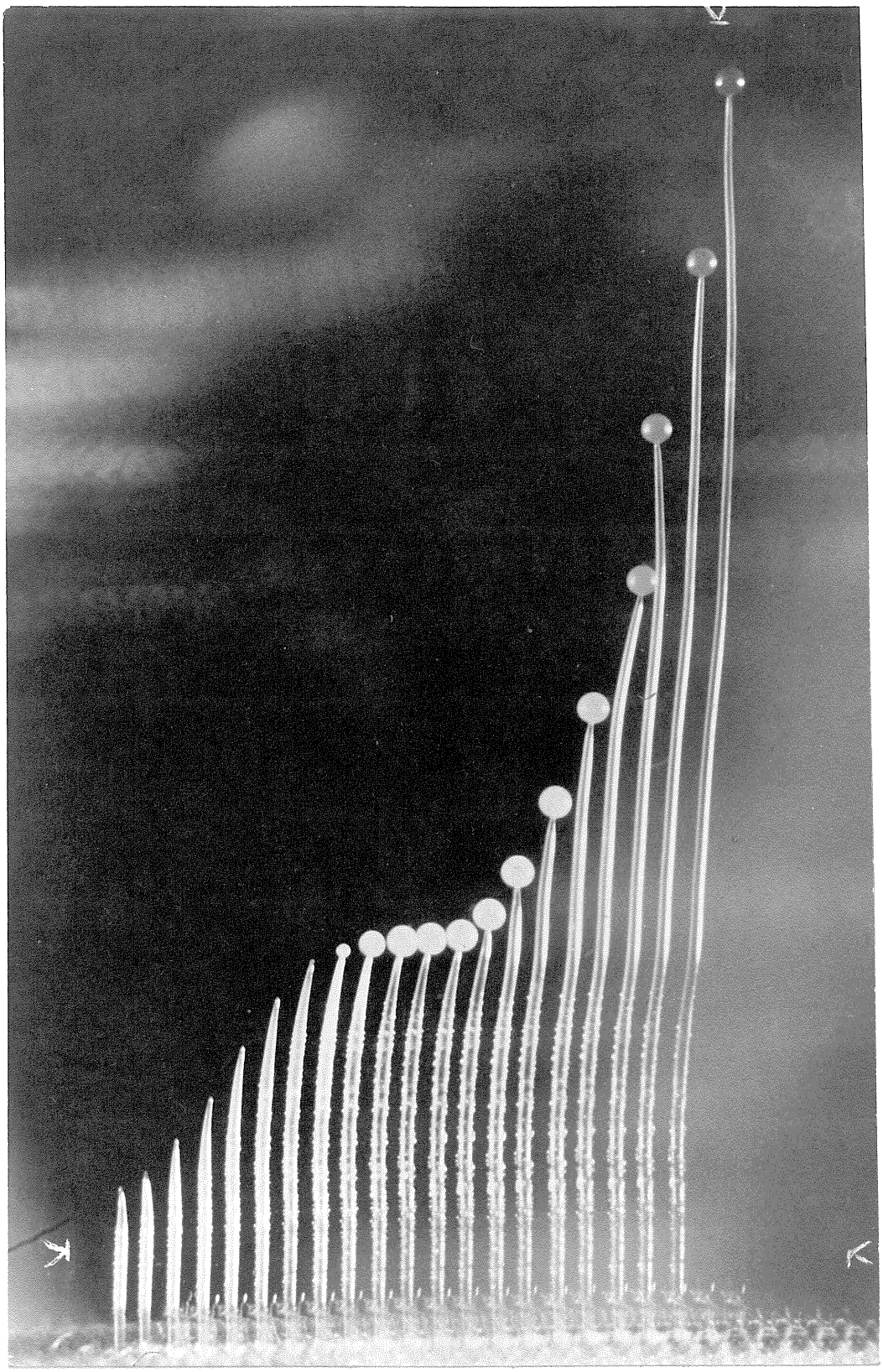
Phycomyces is a fungus and a genus in the family Mucoraceae. In the Mucoraceae, sporangiophores (spphs) produce and bear the vegetative spores. The spores are produced inside a sporangium which develops at the tip of the spph. In Phycomyces, the spph goes through three states of development (see Figure 1): an initial elongation phase (defined as stage I), a spore producing phase (stage II and III), and a second elongation phase (stage IV). The sporangium develops during the second phase. After the sporangium has finished enlarging a cell wall is formed which separates it from the spph. Spores are formed by enclosing a small piece of sporangium cytoplasm within a cell wall. Each spore acquires a random aliquot of cytoplasm containing 1 to 7 nuclei and many mitochondria. The usual agents of spore dispersal are animals which rupture the sporangium by brushing against it or ingesting it and then carry the sticky spores with them (Ingold, 1940; R. K. Benjamin, personal communication). After the spores mature, the spph starts elongating rapidly (3 mm hr^{-1}); apparently to lift the sporangium up and facilitate spore dispersal.

The hyphae in Mucoraceae are nonseptate. The spph of Phycomyces is a single cell. It has a large central vacuole. The cytoplasm is between the cell wall and the vacuole. It contains many nuclei, mitochondria, glycogen granules, lipid droplets, and ribosomes. The top portion of the stalk has

FIGURE 1. Maturation of Phycomyces Sporangiophores

Twenty photographs were taken at one hour intervals of a developing spph. Initially the spph has the appearance of a large hypha. During this phase (first 7 photographs) it is called a stage I spph. It elongates at a rate of 1 to 2 mm per hour and its growing zone is at the apex. In stage II the spph stops elongating and develops a spherical sporangium at its apex (photos 8 and 9). In stage III no outward change takes place, but spores develop within the sporangium (photos 10-12). In stage IV the spph commences growing again (13-20) and the sporangium turns black. In photographs 17 through 20, the spph has reached a steady growth rate of about 3 mm per hour. The diameter of the spph's stalk is 0.1 mm and the diameter of the sporangium is 0.5 mm.

(photograph by Lois Edgar)



more nuclei and mitochondria and fewer lipid droplets and glycogen granules than the lower part. The growing zone of stage I spphs is at the apex. In stage IV spphs it is just beneath the sporangium. It starts 0.1 mm beneath the sporangium and is 2 mm long. The spph extends approximately $\frac{1}{2}$ mm into the sporangium. This extension is called a columella. It is separated from the sporangium by the cell wall mentioned above. The term stalk, as used here, includes all of the spph except the columella. A more extensive review of the physiology of Phycomyces is given in Bergman, et al. (1969).

This thesis deals primarily with stage IV spphs. It describes the influence of the non-growing parts of the spph upon the spph's growth. It also compares the level of respiration of the growing zone with that of the rest of the spph. Chapter 1 discusses new findings concerning the influence of the spores on the spph's growth. If the sporangium is removed, growth stops and several hours later the former growing zone produces a branch spph. This branch matures and bears more spores. It is shown that the spores produce some material which allows apical growth and prevents branching.

Chapter 2 describes the extent of the growing zone's independence from the stalk below. Gruen (1959) has shown that the spph is largely independent from the mycelium since plucking it from the mycelium does not affect its growth rate, if its foot is kept in water. Similiarly, we find that

the growing zone can grow independently of the rest of the stalk for about 10 hours. Its growth rate for the first 3 or 4 hours is almost normal.

This independence from the lower stalk indicates that the processes supporting growth probably take place within the top 3 to 4 mm. Chapter 3 compares the respiration of the top of the spph with that of the lower part in order to estimate how much of the spph's metabolism supports growth. The top 6.5 mm of the spph consumes $\frac{1}{2}$ of the oxygen.

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CHAPTER 1

APICAL DOMINANCE OF *PHYCOMYCES* SPORANGIOPHORES

INTRODUCTION

The term apical dominance is used to explain the pattern of growth of vascular plants. It is best exemplified by conifers. In these plants, the shoot at the top of the plant is the fastest growing shoot. It suppresses the growth of the other shoots. If the apical shoot is removed or stops growing, the inhibition is removed and one or more of the subapical shoots start growing faster, becoming the new apical shoot (or shoots). In vascular plants, a hormone, produced in the growing tip of the apical shoot and transported downward, is used to control the pattern of growth. This hormone is indoleacetic acid (IAA). In fungi, the same pattern of growth exists in the mycelium and in many of the reproductive organs (Burnett, 1968; Robertson, 1968). This has led to the suggestion that hormonal control of growth exists in fungi too. The evidence at present is suggestive but not conclusive.

Hyphae

When a fungus spore germinates on nutrient agar, one or more hyphae emerge and start to grow and branch. The branching and the tendency of the hyphae to grow out radially from the center gradually create a circular mycelium. In the circular mycelium, the most actively growing tips are at the periphery and are closely spaced. It is between the time of

spore germination and the formation of a circular mycelium that the conifer-like or monopodial growth of hyphae is studied. By the time a circular mycelium becomes established the perturbing effects of nutrient depletion and production of inhibitory metabolites become too great.

During monopodial growth, the main hypha has the fastest growth rate and the largest crossection. There are usually no branches within 20 to 100 microns of the tip (Sussman et. al., 1964; Smith, 1923). Behind this region the primary branches form. They are smaller in diameter and have a slower growth rate than the main hypha. Secondary branches may later form from the primary branches. They are still smaller and grow still more slowly. In Coprinus disseminatus the ratio of the growth rates of the main, the primary and the secondary branch hyphae is 10:7:2 (Butler, 1961). In Phialophora atra the ratio between the main hypha and the primary branches is 10:4 (Larpent, 1961). The relative rate of growth of the main hypha and the primary branches is constant in Coprinus for at least 3 hours after the branch has formed and in Phialophora for at least 6 hours. How strongly the diameter affects the rate of growth, if at all, is uncertain. Main hyphae with the same diameter as primary branches have a higher growth rate than the branches (Butler, 1961).

Branching is also acropetal: the newest branches are formed nearest to the tip (Robertson, 1966). Some species

with septate hyphae produce a group of branches at the apical end of each cell (Butler, 1958). There is an order of formation within this group. The first branch forms when the cell is the second cell along the hypha (Figure 1). A second branch may form after the hypha has elongated and the cell has become the third cell. These first two branches are normal primary branches. One or two branches may form later when the cell is the fourth or fifth cell. These branches are smaller and wind around the larger hyphae as they grow. This branching sequence can be described as four different groups of branches each forming acropetally but at different distances from the apex. The interesting point is that the cells of the main hypha always form branches at their apical end and that the same sequence of branches forms at each cell. Perhaps the apex is still exerting some control over the cells. This branching pattern forms when the hyphae are growing over nonnutritive surfaces, such as a glass plate, and are trying to colonize new food sources. Thus the control of the sizes and the growth rates of the branches seems to be internal.

If the apex of the main hypha controls the growth of the side branches, removing the apex should result in an increase in the growth rate of the branches. This was done with Phialophora atra which has typical monopodial growth when grown on nutrient agar (see above; Larpent, 1961). If the

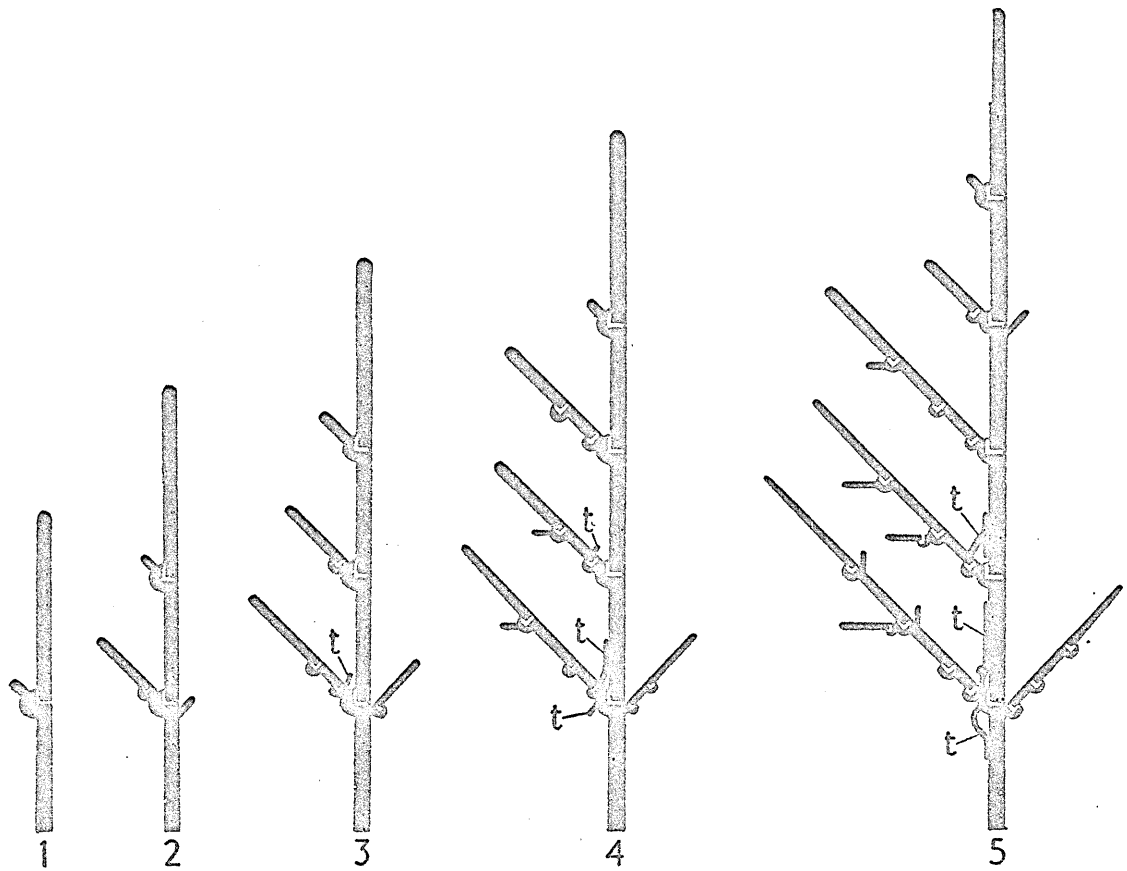


FIGURE 1. The branching pattern of the colonizing hyphae of Merulius lacrymans. The drawings numbered 1 through 5 show successive stages of growth. The branches marked with a "t" are small branches which wind around the main hypha and the larger branch hyphae (from Butler, 1958).

apical 400 microns of the main hypha are removed, at least some of the branches start growing faster. Some of them start growing faster within one hour (the net growth was measured each hour); others have an hour latency. Rhizoctonia solani has a similiar response (Larpent, 1962). These experiments indicate that the apex does affect the growth of the branches but give no indication of the mechanism. A significant fraction of the main hypha in front of the branches was removed and this could prevent a decrease in substrate concentration around the branches. In both species, the apex regenerates and after 2 hours is growing at ½ to 3/4 the normal rate. The branches, however, continue to grow at their new higher rate.

Similar experiments with other species have been reported. In Coprinus disseminatus the primary branches of the main hypha start growing faster if a nearby branch on the same hypha stops growing (Butler, 1961). There is no change in the growth rate of the main axis. In Neurospora crassa removing the apex of a hypha results in the formation of a large number of small branches behind the abscission. None of the branches becomes the dominant branch (Robertson, 1968).

Hyphal growth is not always monopodial. The apices of Fusarium hyphae occasionally branch dichotomously (Robertson, 1966), and Allomyces macrogynus usually branches this way (Machlis, 1957). Monopodial control in Schizophyllum commune

seems to be weak, if it exists (Volz and Niederpruem, 1968). Branch hyphae which have formed within 50 microns of the tip of main hyphae often grow as fast or faster than the main hyphae. Schizophyllum also has septate hyphae, but it does not branch predominantly from the apical end of these cells like most septate fungi.

Species which usually grow monopodially can be converted to other growth patterns by mutating them. The clock mutant of Neurospora crassa has a high percentage of dichotomous branching (Sussman et. al., 1964). Initially after germination its growth is mainly monopodial. However, as growth continues dichotomous branching becomes increasingly common. This is accompanied by the formation of a dense band of mycelium. Eventually growth stops. The process is repeated when a few subapical hyphae start growing again, initially monopodially, and then revert to dichotomous branching. There are similiar clock mutants in Ascobolus immersus (Berliner and Neurath, 1965) and a vague mutant in which the growth of the main hypha stops, a primary branch becomes the main hypha, and then it in turn stops and is replaced (Chevaugeon, 1959).

Thus it appears that most fungi have a conifer-like growth, and that in most septate fungi, the cells along the hyphae produce branches at their apical end. The species and mutants which do not can be described as lacking the proper

genetic complement. However, there is almost no evidence on how the conifer form of growth is maintained. In every case it can be argued that the growth of the branch hyphae is limited by the supply of nutrients. In the colonizing hyphae, the nutrients are transported along the hyphae to the growing tips and it may be that because the main hypha is largest in diameter it gets most of the nutrients.

It is very difficult to keep the composition of the nutrient medium invariant as the mycelium grows, and the morphology of the mycelium is very sensitive to changes in environment. It is possible to change the strongly monopodial growth form of Rhizoctonia solani into one showing virtually no monopodial control (like that of Schizophyllum commune) by changing the nutrients in the medium (Larpent, 1962). Some wild type strains of Neurospora crassa can be converted to the clock phenotype by adding 0.8% sorbose and 0.1% sucrose to the medium (Sussman, et al., 1964). Basidiobolus ranarum has septate hyphae and the cells show polarity in their branching (on nutrient media). However, the cells will reverse their polarity and produce new branches at their basal ends if the concentration of nutrients in that direction is increased (Raciborski, 1907).

Plunkett (1966) compared the growth rate of the main hyphae of Mucor hiemalis with the branching frequency at pH's 3.5 through 7.5. Both the growth rate and the branching

frequency were highest at the high pH's, but they had different response curves. The frequency of branching increased three fold from pH 5.5 to 7.5 even though the growth rate remained constant. Plunket attempted to keep the agar media uniform by continually perfusing them with nutrient solutions of the same pH. Several amino acids also increased the branching frequency (especially tryptophane) and others decreased the frequency (especially arginine and lysine). He found several other metabolites that changed the branching frequency and came to the conclusion that the branching frequency was dependent upon the general metabolism.

Thus it is not yet clear whether monopodial growth is maintained by the supply of nutrients or by transport of stimuli through the protoplasm. It is also possible that a growth hormone or a metabolic inhibitor diffuses through the growth medium. The hormones involved in mating are transported in this way (Barksdale, 1969) and, apparently, so are those causing anastomoses of vegetative hyphae (Langeron and Vanbreuseghem, 1952).

It should be possible to determine whether the predominant control is communicated through the cytoplasm: (1) The apical cell (approximately 50 microns long) of at least two fungi, Aspergillus niger (Nishi et. al., 1968) and Fusarium oxysporum (Robertson, 1961), grows normally if the rest of the hypha is removed. It should be possible to

dissect out a short section of the hypha behind the apical cell in both main hyphae and branch hyphae and measure the growth rate of the apices. Communication through the cytoplasm should be prevented in this case. (2) Butler (1961) noted that if one branch hypha stopped growing the other branches nearby grew faster. After a branch is removed, one could measure the growth rates of other branches on the same side and on the opposite side of the main axis. Competition for substrates should be much less between branches on opposites sides than between those on the same side, but communication through the cytoplasm should be the same. (3) It should be possible to prevent diffusion between the main hypha and a branch by placing a solid barrier between them. Using similiar methods Chevaugeon and van Houng (1969) were able to show that a substance causing metabolic imbalance in the vague mutant of Ascobolus immersus was transported within the hyphae.

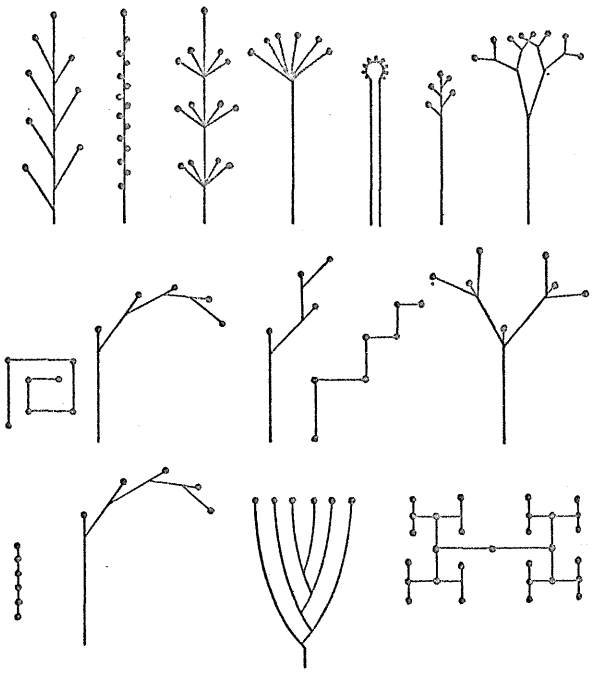
Reproductive Organs

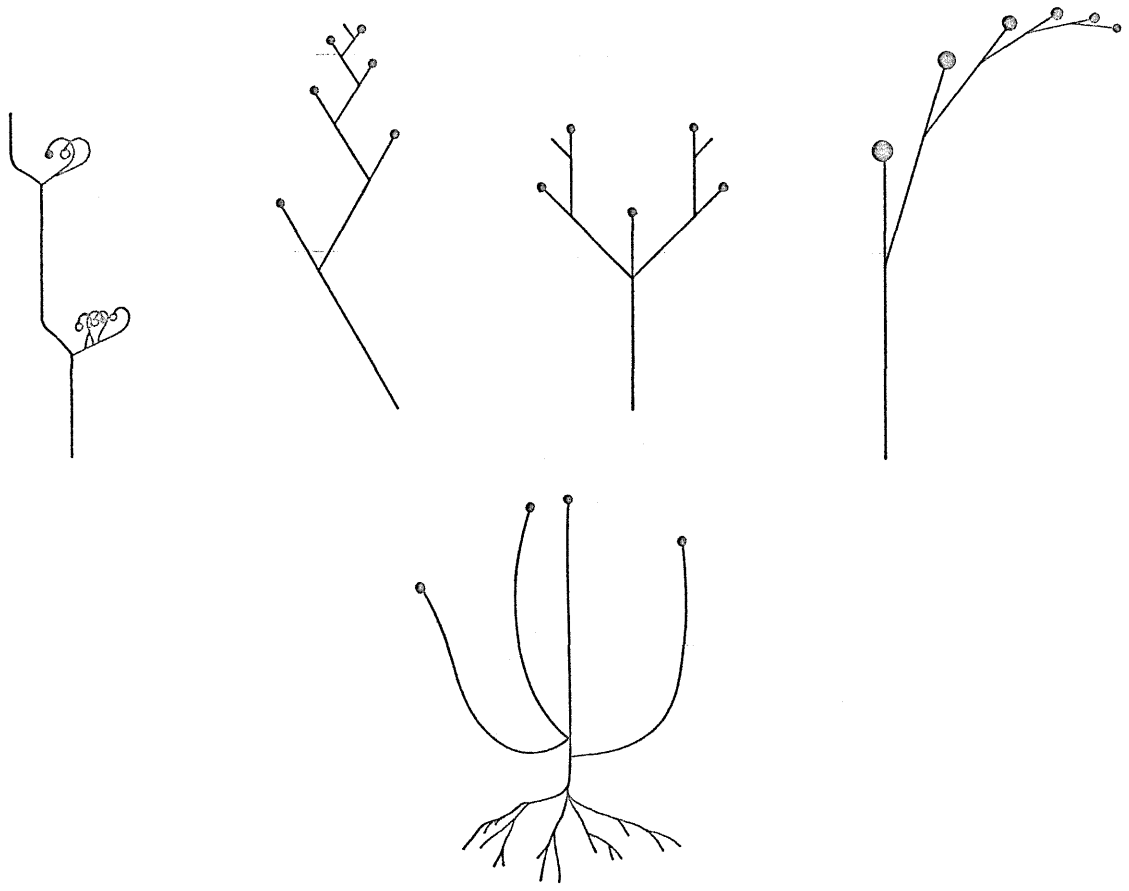
The reproductive organs of fungi have intriguing morphologies, suggesting some kind of growth control (Figure 2). They are aerial and thus should be much less affected by the external environment. One can argue that the control of growth must be internal, like that of the colonizing hyphae.

There is one type of reproductive organ which does appear

FIGURE 2.

Schematic drawings of typical asexual reproductive structures. Above are conidiophores from Ascomycetes (from Burnett, 1968) and below are sporangiophores from Phycomycetes (from Grehn, 1932; Burnett, 1968).





to control its elongation from within. These are the carpophores or mushrooms. Unlike those shown in Figure 2, they consist of many strands of hyphae. After the carpophore has differentiated into its basic mushroom shape, the cap (pileus) enlarges and the stem (stipe) elongates. The growing zone in the stipe is just beneath the cap (Burnett, 1968). In it the cells elongate but don't divide. On the underside of the cap are gills which bear the spores. In Agaricus bispores and Flammulina velutipes, if the gills are removed the stipe stops elongating and the cap stops enlarging (Gruen, 1963 and 1969). In Agaricus bispores the cap and the stipe will continue to grow after the gills have been removed if some of the gills are placed on an agar block and this agar block is stuck on the underside of the cap (Urayama, 1956; Hagimoto and Konishi, 1960). It appears that the gills produce a growth promoting substance necessary for stipe elongation and cap expansion.

Elongation of the stipe does not have an absolute dependence upon the gills. The longer the stipe gets the less its growth is affected by removal of the gills. Agaricus carpophores usually grow to a height of about 9 cm (Gruen, 1963). If all of the cap is removed, except the part directly above the stipe (this operation removes all of the gills), when the carpophore is 2 cm long, the stipe will grow only another 5 mm. If the same operation is performed when the

carpophore is 5 cm long, the stipe will grow to its normal height (Gruen, 1963; Hagimoto, 1963). If the entire cap is removed, the stipe will grow 1.5 to 2 cm (Gruen, 1963). The stipe and trama (the trama and gills compose the cap) do contain some growth stimulating substance because pieces of them will stimulate the growth of decapitated carpophores (Hagimoto and Konishi, 1960). Thus in older carpophores, the trama and stipe apparently have enough of the substance to support normal growth. However, it has not been determined whether the trama and the stipe start to produce the substance, whether they accumulate it from the gills or whether the stipe requires less of it in order to grow.

The degree of dependence varies among different mushroom species. The elongation of Flammulina stipes is never normal after removal of the gills (Gruen, 1969). The elongation of the stipes of several Coprinus species appears to be independent of the cap once they have started to elongate (Borriss, 1934; Hagimoto and Konishi, 1959).

Attempts have been made to characterize and isolate the active substance (or substances) using the growth of Agaricus stipes and caps as a bioassay. The activity of gill extracts was tested by diffusing them into agar blocks and putting the blocks on gill-less carpophores. The substance appears to be a small molecule: it is stable in boiling water, in 1 N NaOH and HCl (at least some activity remains after 1 hour), and

diffuses through cellophane membranes. It is soluble in ether, acetone, and ethanol (Hagimoto and Konishi, 1960).

Ethanol extracts from Coprinus, Hypholoma fasciculare and Armillaria matsutake were active on Agaricus. IAA (the growth hormone of higher plants) did not stimulate growth. It was applied in the agar block at concentrations of 5×10^{-3} to 5×10^{-7} M. (Hagimoto and Konishi, 1960). The upper limit of endogenous IAA in higher plants is about 5×10^{-4} M. (Leopold, 1955).

In a preliminary report, Konishi and Hagimoto (1962), stated that they found 12 amino acids in the ethanol extract of Agaricus gills. Ten of them (leucine, cysteine, glutaminc acid, glutamine, serine, asparagine, glycine, threonine, valine and proline) promoted growth if applied individually at 10^{-4} M. Ammonium sulfate and ammonium choloride also stimulated elongation. If this report is correct and if the amino acids stimulate nearly normal growth, then this raises the possibility that the gills are merely supplying nutrients to the stipe and trama. Chitin and chitosan are the major components of most hyphal cell walls (Aronson, 1966). They are, respectively, N-acetyl glucosamine and glucosamine polymers. One nitrogen atom is contained in each subunit. This may be the reason why the growing regions require nitrogen. However, it is important to note that the source of this growth promoting substance comes from the top and not from the

mycelium. Perhaps the fungi have adapted this requirement of nitrogen into a growth control mechanism which allows the spore bearing gills to control the growth of the sterile parts of the carpophore.

Phycomyces Sporangiophores

Stage IV spphs normally grow to a height of 10 to 15 cm. Usually the sporangiophore does not branch. However, if something causes the spph to stop growing prematurely (e.g. physical damage to the growing zone), a branch often forms in the former growing zone. This branch is initially a stage I spph. It grows 2 to 5 mm, matures into a stage IV spph, and then continues growing. This growth behavior has led to the postulate that the apex controls the growth of the spph. IAA has been applied to the side of the growing zone in lanoline pastes (Banbury, 1952) and in water drops (Thimann and Gruen, 1960) with no positive results.

Until recently, the only experiments supporting apical dominance in Phycomyces spphs have been those done by Helen Götze in 1918. She discovered that if sections of the spph stalk (1 cm long sections or longer) were dissected out and placed on wet filter paper, they would regenerate spphs, but only at the apical end. Last year Gruen (Gruen and Ootaki, 1969) briefly reported similar results. Together their results indicate that sections from stages I, II, III, and IV show

polarity in the regeneration of spphs.

Götze also reported that if a section of the stalk was grown with its base in a nutrient solution and its apex in air, the basal end would form mycelium and the apical end would form a spph. An inverted section formed mycelium at its physiological apex (now the bottom); however, it produced a branch spph at the air-water interface, not at the physiological base. This finding indicates that the polarity of the spph is not changed as readily as that of mycelium.

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MATERIALS AND METHODS

Culture Methods

Phycomyces blakesleeanus Burgeff (strain 1555 (-) of the Northern Regional Research Laboratory) was used. Normally it was cultured on potato dextroxe agar in shell vials or petri plates as described by Dennison (1961). Spphs whose sporangia were removed or replaced were immediately placed in a closed chamber with water on the bottom and wetted tissue paper on the sides.

If large quantities of sporangium suspension were desired, *Phycomyces* was grown on glucose asparagine agar (Zankel, et al., 1967) with yeast extract (Difco) added. One gram of yeast extract was added per liter of medium. It was added to the agar preparation, autoclaved, and then mixed with the asparagine and glucose solutions. This medium is called GA plus yeast medium. Approximately 50 spores were inoculated per petri plate. After the first stage I spphs appeared the plates were opened and placed in stainless steel trays (17 cm x 25 cm) and the trays covered with glass plates. The spphs were grown at 22° to 25° under fluorescent lights (intensity about 20 μ Watts cm^{-2}).

Sporangium Removal and Replacement

After a spph has matured into stage IV and then grown

1-2 cm, its sporangium wall has become fragile. By placing a forefinger and thumb on either side of the sporangium and then pulling the spph away with the other hand, the sporangium and the spph can be separated without damaging the spph.

To give the spph a second sporangium, the second sporangium was first embedded just beneath the surface of a drop of beef fat (Figure 3A). This beef fat is rendered over boiling water, filtered through a filter paper (Whatman #2), and stored at -10°. Small aliquots are then remelted in a 80° water bath. A drop of this melted beef fat (0.05 ml) was applied to a glass plate with a Pasteur pipette. Just before the drop solidified, the top of a spph was stuck into it (Figure 3A, #1). After it solidified the spph was removed, leaving the sporangium in place (3A, #2). The glass plate was kept on ice to speed the cooling of the fat droplet.

A spph was rapidly divested of its sporangium, turned upside down, and its columella stuck inside the sporangium which was in the beef fat (3A, # 3 & 4). This process takes 60 to 90 seconds. The spphs which were to receive a second sporangium were grown in shell vials. After the spphs were 3 to 4 cm tall, the vials were placed on their sides and left that way for 3 hours. The spph grows upward [spphs are phototropic and will grow towards a light placed above them (Bergman, et al., 1969)]. After 3 hours the spph has grown about one cm and the bend is about one cm below the sporangium.

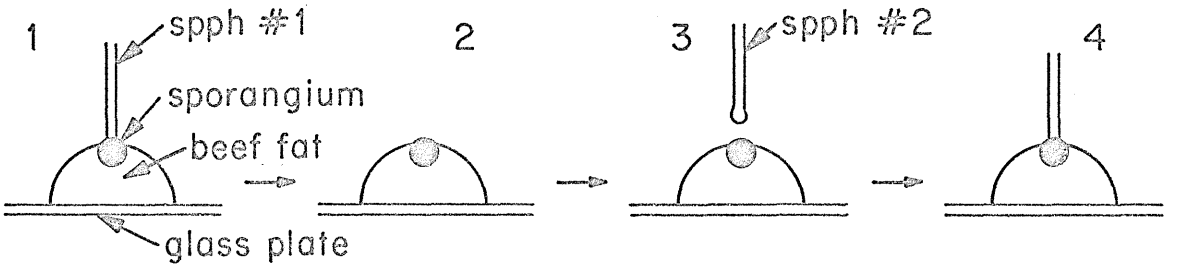
FIGURE 3. A_1 - A_4 show the procedure used to transfer a sporangium from one spph to another

The sporangium from the first spph was embedded in a drop of beef fat (#1). A small part of the sporangium near the spph was always left protruding above the surface of the fat drop. After the fat drop had solidified, the spph was removed, leaving the sporangium embedded in the beef fat (#2). Then the columella of a second spph (minus its own sporangium) was placed into this sporangium through the hole left by removing the first spph (#3 & 4).

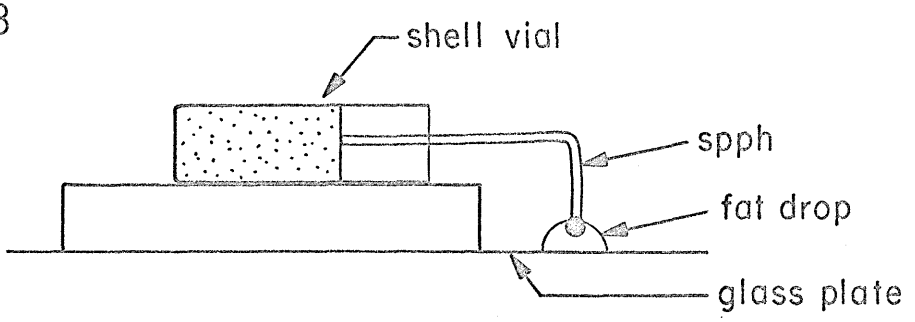
B shows the entire second spph with its new sporangium.

C illustrates how spphs were hung by their columellas so that large drops of sporangium supernanant could be placed over the columellas. The spphs were hung from the stand. The stand supported the large supernanant drops (1/20 ml). The spphs elongated downward. The top of the stand was a piece of plexiglas (Rohm & Haas) with V-shaped notches ($\frac{1}{2}$ cm wide at the base, 1 cm long) cut in it. A piece of parafilm was laid over the plexiglas. Slightly smaller notches were cut in it so that they corresponded to the notches in the plexiglas. However, these notches had convex sides so that the apex of these notches was long and narrow. The apex was wide enough to allow the stalk to fit into it, but narrow enough to prevent the columella from slipping through. The spphs had a block of mycelium and agar still attached to their feet which supplied the spphs with water. These drawings are not to scale.

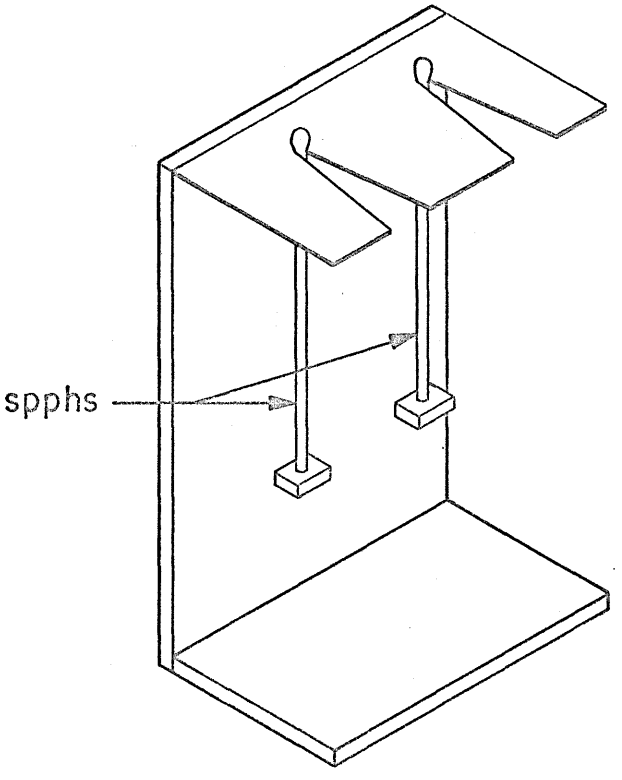
A


1
spph #1
sporangium
beef fat
glass plate
2
3
spph #2
4

B


shell vial
spph
fat drop
glass plate

C


spphs

Then the original sporangium was removed and the top of the spph inverted and placed in the sporangium embedded in the beef fat (Figure 3B). The glass plate with the spphs was placed over a light causing the spphs to try to grow downward. Instead of growing downward the growing zone pushed the rest of the stalk upward. Thirty hours later the net growth of the spphs was measured and the number of spphs which had branched was checked.

Preparation of the Sporangium Suspension

Spphs were grown up on GA + yeast in petri plates. When they were 3 to 5 cm tall, their sporangia were ruptured in a small amount of water. Sporangia can be ruptured by rubbing them against any solid surface. I ruptured these sporangia by turning the spphs upside down and rubbing the sporangia against the top half of a petri plate which contained 1 ml of distilled water.

While the sporangia were being ruptured, the plate was tipped so that the water stayed along one side. Afterwards the petri plate was leveled and the sporangium contents were suspended in the water with a spatula. This was repeated until the sporangium contents from 25 plates had been suspended in one ml of water. This gave about 3/4 ml of suspension (some of the water is lost because it sticks to the spphs). The suspension was stored on ice.

Fractionation of the Sporangium Suspension

The sporangium suspension was separated into a spore pellet and a supernatant fraction. To do this separation about 6 ml of the suspension were centrifuged in a clinal centrifuge for 40 seconds at top speed. This gave 3 ml of supernatant and 3 ml of spores.

Application of Drops to Spphs

Small drops of sporangium suspension, the spore pellet, the supernatant and distilled water were applied to the columellas of spphs with a glass hair. The diameter of the hair was the same as that of spph's stalk. It was dipped into a large drop ($\frac{1}{2}$ ml) of one of the four substances and then jerked upward out of the drop. This left a few small drops on the hair. A second smaller hair was used to move one of the drops down to the end of the larger hair. The drop was then touched to the columella. Care was taken not to touch the glass hair itself to the columella. If the drops on the larger hair were too small, some of them were pooled by running the small hair along the larger hair.

Often the spore pellet was too viscous to form small drops. If so several drops of water were added to the 3 ml pellet.

To apply large drops of supernatant to the columellas of spphs, the spphs were hung by their columellas (Figure 3C).

Spphs grown on potato dextose agar in petri plates were used. A small piece of agar (about 2 mm cubed) was cut out of the agar and left attached to the foot of the spph. This supplied the spph with water and nutrients. If the spphs were kept in a moist, closed chamber, the agar did not dry out. After the spph had been removed from the agar, it was hung from the stand as shown in Figure 3C. A drop of supernatant was then placed over the columella.

Heat Inactivation of Single Sporangia

If the tip of a spph is dipped into water at 80°C the sporangium almost always ruptures after 10 to 20 seconds. However, if the spph is dipped into liquid beef fat at 80°C, the sporangium does not rupture nearly as often.

Culturing Sections from Spph Stalks

Spphs were grown on potato dextrose agar in petri plates. They were plucked and placed on a narrow strip of wet filter paper (1 cm wide); their stalks perpendicular to the long axis of the strip. Three to four cm long spphs were used and both ends of the spphs extended beyond the filter paper. To keep the ends suspended above the ground the filter paper had previously been placed on a long narrow block of plasticine (1 cm wide and 1 cm thick). After about 20 spphs had been placed on the filter paper, both ends were cut off, leaving

1.5 to 2.0 cm of stalk. The stalk sections were then placed in a closed chamber which had water on the bottom.

Mutagenesis and Selection of Mutants with Short Spphs

Spores were mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine as described in Heisenberg and Cerda-Olmedo (1968). The mutagenized spores were plated on GA plus yeast plates; about 20 viable spores on each plate. The pH of the GA plus yeast medium was adjusted to 3.2 with HCl. This low pH causes the mycelium to assume a colonial growth form (Bergman, et al., 1969). The slow colonial growth of the mycelium allows inoculation of many spores per plate. When the first stage I spphs appeared the plates were opened and placed in room lights. Mutants whose mycelium grew like wild type but whose spphs did not grow higher than 3 cm were selected. A single sporangium was picked from each of these mutants and the spores grown up on potato dextrose agar. Those mutants which produced short spphs on this medium also were saved.

RESULTS

Characterization of the Phenomenon

After a spph has formed a sporangium and has commenced elongating, the cell wall around the sporangium becomes increasingly fragile. The spores and the other sporangium

contents are then easily removed from the spph. Extending into the sporangium from the spph's stalk is the columella. It has a cell wall which is continuous with the stalk's cell wall. Thus when the sporangium is removed the spph does not loose its turgor pressure.

However, when the sporangium is removed the growth gradually slows down and eventually stops (Figure 4). Figure 4 shows the growth for only the first two hours after the sporangium is removed. The total growth after removal of the sporangium is about 3 mm (Table I). The growth stops even under humid conditions. These spphs were in sealed chambers with water on the bottom and wet tissue paper on the sides. If a small drop of water is placed on the columella, the spphs still grows only 3 mm (Table I).

Six hours after the sporangium has been removed, branch spphs start to form from the spphs' old growing zones (Figure 5). Ninety-nine per cent of the spphs eventually (within 30 hours) form branches (Table I). The branching frequency of spphs with water drops on their columellas is 97 per cent. Nearly all of the spphs produce only one branch, a few produce two. Sometimes the branch grows from the columella, especially if the spph is lying on agar. The branch spphs go through the four stages of development and are phototropic.

The decline in the growth rate and the branch formation can be prevented if a drop of a suspension of sporangium

FIGURE 4. Decline in the Growth Rate after Sporangium Removal

The growth rate of the spphs was measured from 15 minutes before the sporangium was removed (given in the upper left hand corner of the figure) until two hours after sporangium removal. The rate of growth was determined by measuring the net growth during each five minute interval. The curve is the average of ten experiments. The relatively slow growth during the first ten minutes is probably due to damage caused by sporangium removal. The average net growth during the two hours after sporangium removal was 1.8 mm.

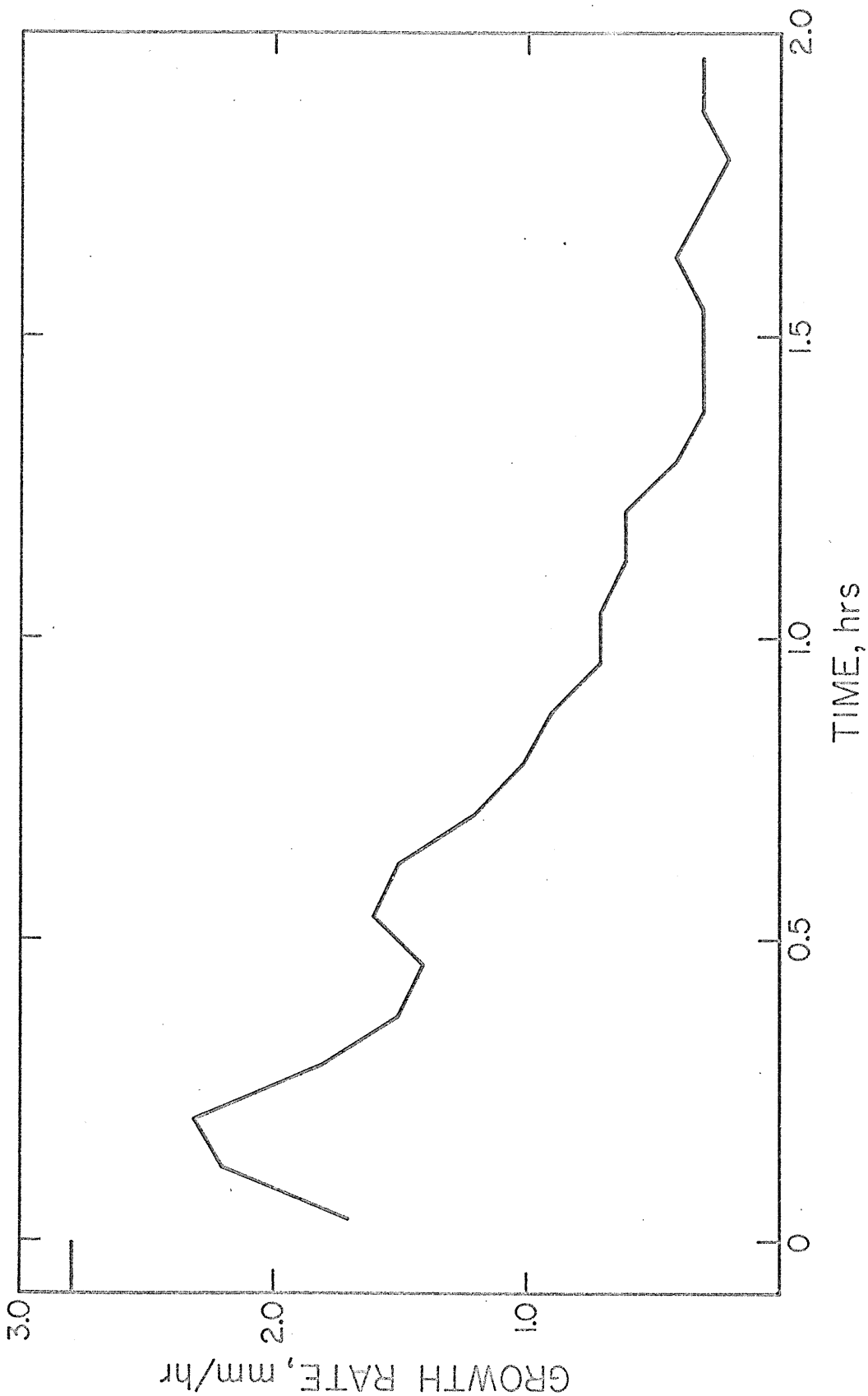

GROWTH RATE, mm/hr
3.0
2.0
1.0
0
0.5
1.0
1.5
2.0
TIME, hrs

FIGURE 5. Branch Formation after Removal of the Sporangium

The figure gives the fraction of spphs which formed branches during successive two hour intervals after the sporangium was removed. The fractions are plotted as a per cent of the total number of spphs used (289). The time of formation of the branch spph was defined as the time when the branch changed from a bump on the side of the growing zone to an identifiable stage I spph. Eighty-three per cent of the spphs formed branches within the 30 hours.

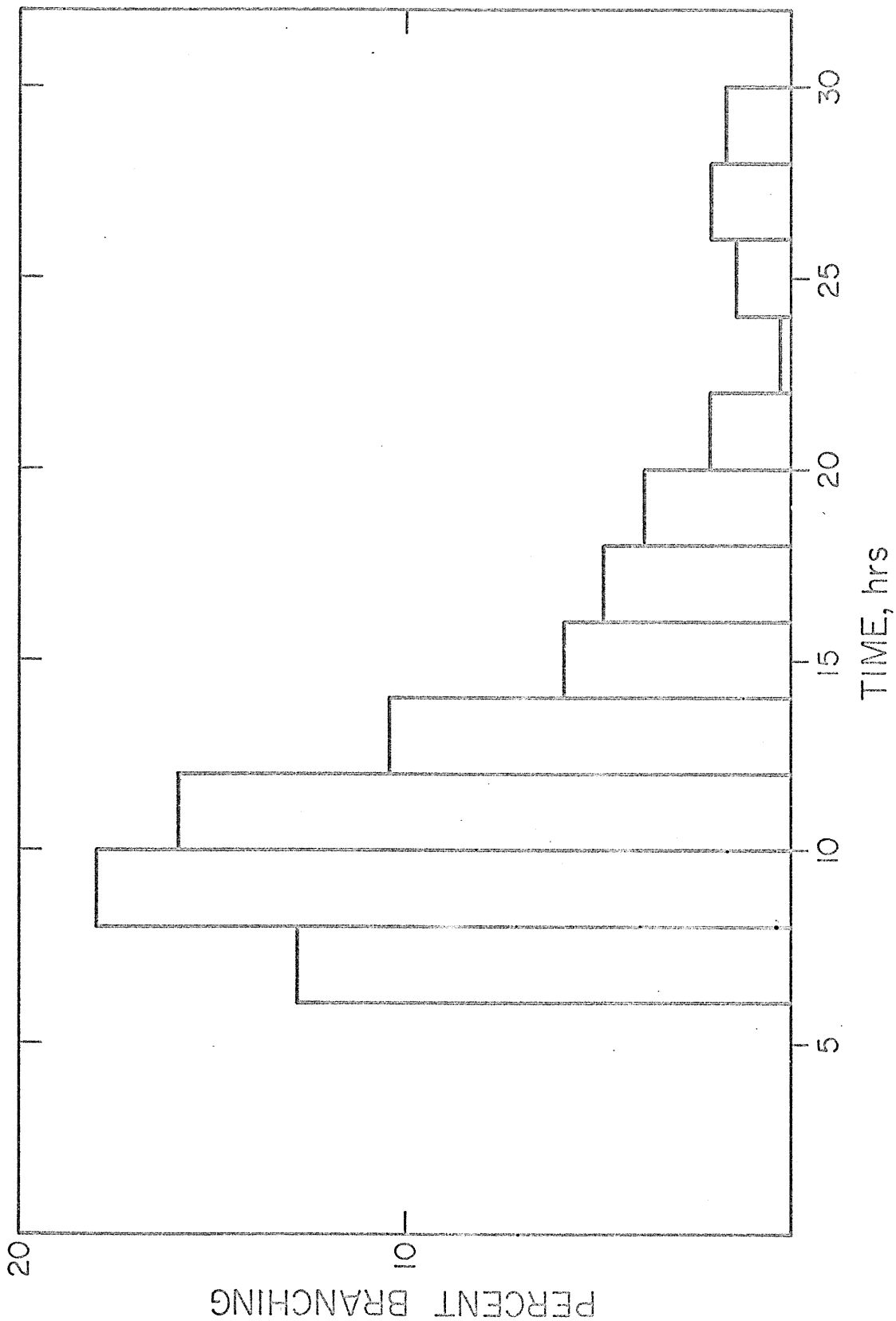

PERCENT BRANCHING
20
0
TIME, hrs
5
10
15
20
25
30

TABLE I

Effect of New Sporangia and Drops of Sporangium Suspension

After the spph's sporangium has been removed, the spph stops growing and later it usually branches. The table gives the per cent of spphs which branched and those which did not (1,a and b) and lists the net growth of both classes. The net growth of these spphs was never more than 9 mm.

If a new sporangium or a drop of sporangium suspension is placed over the columella of a spph, the spph is often stimulated to grow more than 9 mm (2 and 3). Spphs which grew more than 9 mm were defined as spphs whose growth had been stimulated. A spph can either grow more than 9 mm, or branch, or do both or do neither. The table lists the percentages of spphs in each of these catagories (not in that order) and gives their averaged growth.

The total number of spphs measured in 1a was 78; in 1b was 72; in 2 was 161; and in 3 was 55. The net growth and branching percentages were measured after 30 hours. Spphs which did not grow at least 1 mm and did not branch were considered damaged and were not included in the table. They comprised about 1 per cent of the total.

	A		B		C		D	
	Growth < 1 cm No Branching		Growth < 1 cm Branching		Growth ≥ 1 cm No Branching		Growth ≥ 1 cm Branching	
	%	Net Growth	%	Net Growth	%	Net Growth	%	Net Growth
1. Spphs without sporangia								
a. with water drops on columellas	3	0.30 cm	97	0.26 cm	0	---	0	---
b. bare columellas	1	0.30 cm	99	0.34 cm	0	---	0	---
2. Spphs with drops of sporangium suspension	35	0.45 cm	29	0.31 cm	33	3.0 cm	2	1.1 cm
3. Spphs with new sporangia	18	0.38 cm	27	0.26 cm	53	2.0 cm	2	1.1 cm

contents is placed on the columella (Table I). The suspension was prepared by rupturing about 5 x 10^4 sporangia in one milliliter of distilled water (see methods section). A drop of the suspension was placed on the columella with a glass hair. The drop was the same size as the original sporangium (about 0.04 µl). It was placed on the columella within a minute after removal of the sporangium.

After the original sporangium has been removed, growth continues also if the sporangium is replaced by another sporangium (see methods section) (Table I).

After a spph's sporangium has been removed, it will grow another 1-8 mm. The variation of the growth of spphs with bare columellas (Table I; 1b) is 3.4 $\pm$ 0.15 mm, with a tail extending not beyond 8 mm. Any spph whose growth has been stimulated by a new sporangium or drop of sporangium suspension should grow more than 8 mm. Thus spphs which grew 1 cm or more were defined as spphs whose growth was stimulated. In Table I the spphs are grouped as to whether they grew 1 cm or more and whether they branched.

If the growth of the spphs is stimulated, then their branching is strongly inhibited. There are practically no spphs which grew more than 1 cm and which had branched after 30 hours. Those that did, grew only slightly more than 1 cm. Even if the growth of the spphs is not stimulated, the branching is often inhibited. The branching frequency of

spphs which were given a drop of sporangium suspension but which did not grow is only 45%. That of spphs with a water drop on their columellas is 97%.

Fractionation of the Sporangium Suspension

The sporangium suspension was separated into a spore fraction and a supernatant fraction by differential centrifugation (see methods section). The volume of the spore pellet and the supernatant were about equal. The spores were washed two times and a few drops of water added to the final pellet to make it less viscous. The supernatant fraction contains a large number of particles which look like vesicles in the light microscope. This fraction contains proteins, lipids and free fatty acids (Meissner, personal communication), and fragments of the sporangium cell wall. The spore fraction is composed almost solely of spores. It contains some cell wall fragments.

The spore fraction stimulates the growth of sporangium-less spphs as effectively as the whole sporangium suspension (Table II). The same size drops were used as with the sporangium suspension. Supernatant drops of the same size stimulate very little if any growth (Table II, 2a). This result indicates that most of the growth stimulating material is in (or is produced by) the spores.

If the supernatant drops are replaced every hour, a higher percentage of spphs grow than with single drops and they grow

TABLE II. Growth Promoting Activity of the Spore and Supernatant Fractions

	A		B		C		D	
	Growth < 1 cm No Branching		Growth < 1 cm Branching		Growth ≥ 1 cm No Branching		Growth ≥ 1 cm Branching	
	%	Net Growth	%	Net Growth	%	Net Growth	%	Net Growth
1. Spore fraction	22	0.48 cm	40	0.35 cm	37	3.8 cm	1	1.2 cm
2. Supernatant fraction								
a. single drop	30	0.44 cm	66	0.35 cm	0	---	4	1.2 cm
b. series of drops	16	0.54 cm	65	0.48 cm	13	2.6 cm	6	1.2 cm
c. single large drop	8	0.48 cm	30	0.21 cm	62	3.0 cm	0	---

The same convention was used in this table as that in Table I.

The total number of spphs used in number 1 was 290; in part 2a., 107; in part 2b., 191; in part 2c., 60.

more (Table II, #2b). The new drops were applied every hour for six to eight hours.

For these experiments three or four milliliters of supernatant were made at the beginning of the experiment and stored on ice.

Single large drops of supernatant stimulate growth as well as the small spore-fraction drops do (#2c). The large drops have a volume of 0.05 ml; equal to about 1000 of the small drops. These large drops require a different procedure. Each spph was hung by its columella at the apex of a V-shaped slot cut in parafilm (see methods section, Figure 3). Drops of supernatant were put on the parafilm over the columellas. As a control, spphs were hung from the parafilm and drops of water or drops of phosphate buffer (0.01 M phosphate (pH 6.5) and 0.1 M Sucrose) were placed over the columellas. None of the spphs with phosphate buffer drops grew more than 7 mm and 30 out of 31 branched. None of the spphs with a water drop grew more than 3 mm and 4 out of 22 branched.

Perhaps the spphs use or destroy the stimulating material and the small drops of supernatant have only enough material to support growth for a short time. If the supernatant is stored at room temperature in air for 15 hours its activity does not appear to decrease. This indicates that the spphs are probably inactivating the material.

If the supernatant is heated to 80°C for 15 minutes its

activity does not appear to be affected (Table III). However, if the spores are heated to 80°C for 15 minutes they are no longer active. Similarly inactive are sporangia which have been heated for 30 seconds. A temperature of 80°C inactivates many proteins (Joly, 1965). Perhaps enzymes within the spores normally synthesize the growth stimulating substance. Freezing the sporangia may partially inactivate them; however, insufficient spphs were measured to determine the degree of this inactivation.

Effect of Leaving Spphs without Sporangia for Various Times

After the sporangium is removed the spph's growth slows down and the spph probably begins to modify the growing zone in order to produce a branch spph. There should be a time after which the processes involved with branch formation can no longer be reversed. Presumeably by this time the growing zone cannot reinitiate growth and the spph cannot respond to the stimulus from a new sporangium.

To determine when this "point of no return" occurs, sporangia were removed from the spphs, the spphs were left without sporangia for 15, 30, 45, and 60 minutes, and then small drops of spores were put over the columellas. In some cases the columella was left bare between the times of sporangium removal and spore application, in other cases a small water drop was put on. Figure 6 shows the percentage

TABLE III. The Effect of Heat and Cold on the Activity

	A		B		C		D	
	Growth < 1 cm No Branching		Growth < 1 cm Branching		Growth ≥ 1 cm No Branching		Growth ≥ 1 cm Branching	
	%	Net Growth	%	Net Growth	%	Net Growth	%	Net Growth
1. Heated supernatant								
a. series of drops	24	0.50 cm	63	0.44 cm	8	2.6 cm	5	1.5 cm
b. single large drop	28	0.46 cm	17	0.43 cm	52	2.9 cm	2	1.0 cm
2. Heated spore fraction	28	0.49 cm	72	0.38 cm	2	1.0 cm	0	---
3. New heated sporangium	9	0.25 cm	81	0.32 cm	0	---	0	---
4. New frozen* sporangium	64%		0.29 cm		36%		1.5 cm	

The same convention was used in this table as was used in Table I. The total number of spphs measured in part 1a was 119; in part 1b was 63; in part 2 was 51; in part 3 was 23; and in part 4 was 39. The heated sporangia were heated to 80°C for 30 seconds and then, after cooling, were put on the spphs. The heated spore and supernantant fractions were heated to 80°C for 15 minutes. The frozen sporangia were kept at -10°C for 15 minutes.

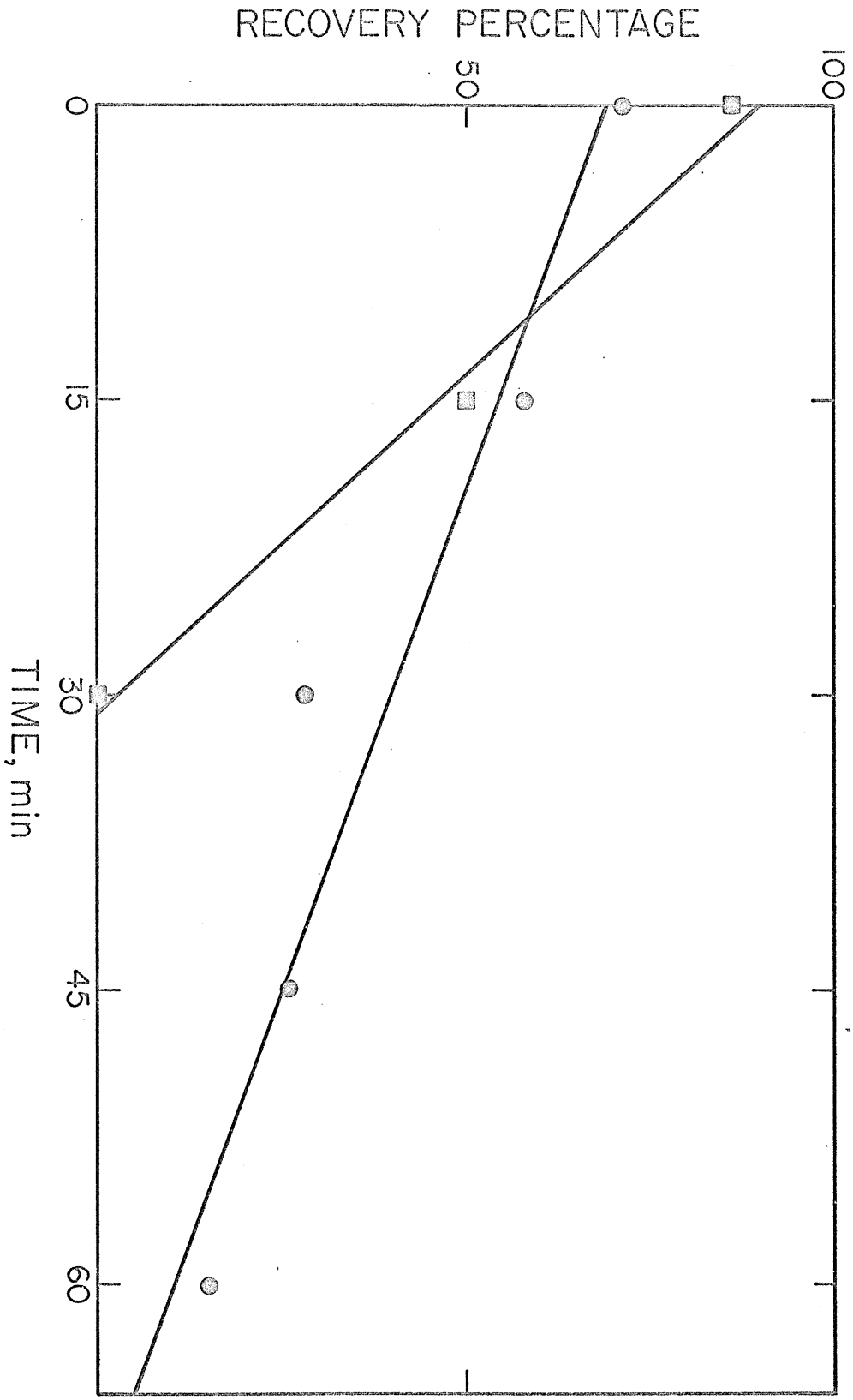
*The branching of the spphs used in part 4 was not measured.

FIGURE 6

Effect of leaving spphs without sporangia for 15 minutes to an hour. Sporangia were removed from spphs and the spphs left for 15, 30, 45 or 60 minutes. In some cases the columellas were left bare (□), in others a water drop was placed on each of them (◎). After the prescribed time a drop of spore suspension was placed on the columellas. For each time span, the fraction of spphs which grew at least 1 cm was plotted as a "recovery percentage." None of the spphs with bare columellas recovered if the columellas were left bare for 30 minutes or longer.

The points at time zero are controls. In the case of the water-drop spphs the sporangium was removed, a water drop was placed on the columella and immediately removed, and finally a drop of spore suspension was placed over the columella. The percentage of growing spphs in both controls is higher than that listed in Table II. This is primarily because the techniques used in applying the spores improved: a better spore concentration was used and the glass hair used to apply the spores was never allowed to touch the columellas.

Each point on the curves was obtained from 40 to 60 spphs. Growth was measured after 30 hours.


RECOVERY PERCENTAGE
0
50
100
TIME, min
15
30
45
60

of spphs which grew after being without sporangia for various times. It appears that even before the spphs stop growing they loose the capacity to respond to the substance produced by the spores. However, no tests were made to determine whether the substance actually got into the spphs. It is possible that the columella's wall becomes impermeable. The difference in duration of sensitivity of spphs with and without water drops may indicate that the cell wall becomes less permeable after it dries out slightly.

In comparison to the growing zone, the spores appear to be unaffected by the separation. They retain the capacity to produce the growth promoting substance for at least 24 hours after they have been removed from the spph. If they are stored in a pellet at 0^{o} for 24 hours their activity does not decline measureably.

Effect of the Higher Plant Hormone, Indoleacetic Acid

Indoleacetic acid (IAA) is a growth hormone in vascular plants. The plants' endogenous concentration is optimal and applying more does not stimulate growth (Troxler & Hamilton, 1965). However, the growth can be inhibited by application of a competitive inhibitor of IAA, and then IAA can be given to the plants to overcome the inhibition. This method is used to test if IAA is active as a hormone when there is no way to eliminate the endogenous supply of IAA.

P-chlorophenoxybutyric acid is such a competitive inhibitor (McRae & Bonner, 1953). Gruen (1965) has found IAA in Phycomyces spphs at about the same concentration as it is found in higher plants.

I repeated this experiment on spphs which had sporangia. The spphs were grown upside down with their tops (1 to 2 cm) in various solutions of IAA and p-chlorophenoxybutyric acid. The pH of the solutions was 6.5. The net growth of the spphs was measured and compared to that of spphs in 0.01 M phosphate buffer (pH 6.5). Concentrations of p-chlorophenoxybutyric acid higher than 5×10^{-4} M were inhibitory. If IAA was added to these solutions it did not overcome the inhibition and at high enough concentrations had an additional inhibitory effect. A solution of 5×10^{-4} M p-chlorophenoxybutyric acid and 5×10^{-4} M IAA was inhibitory. Neither of the compounds by themselves at this concentration were inhibitory.

A simplier approach is to remove the spph's endogenous supply of "hormone" by removing the sporangium and then apply IAA to the columella. Small drops of various concentrations of IAA were applied (Table IV). None of the solutions appear to have any growth promoting effect. Concentrations of 3×10^{-3} M and 3×10^{-2} M inhibited both growth and branching.

Regeneration Polarity along the Spph's Stalk

Götze (1918) and Gruen & Ootaki (1969) have shown that segments cut out of the spph's stalk regenerate spphs from

TABLE IV

The Effect of Indoleacetic Acid

Concentration of Indoleacetic Acid	% of Spphs which grew 1 cm or more	Growth (cm)	% of Spphs which Branched
3×10^{-2} M	0 (34)*	0.06	14
3×10^{-3} M	0 (23)	0.12	52
3×10^{-4} M	3 (27)*	0.49	100
3×10^{-5} M	0 (31)*	0.29	93
3×10^{-6} M	0 (40)	0.34	90

The numbers given in the parenthesis are the number of spphs measured in each experiment. The indoleacetic acid solutions had a pH of 5.

*One spph in each of these groups grew 2 to 3 cm. The reason for this may be that they had a few spores left on their columellas. At least one of them did. These 3 spphs were not included in the tabulations. In addition one of the spphs which had 3×10^{-4} M indoleacetic acid on its columella grew 1.2 cm. It was included in the tabulations.

their cut ends. They have also shown that the apical end of the segment produces spphs with a much greater frequency than the basal end. Stages I-IV show this polarity. Table VI gives similar data for stage IV spphs.

Presumably there is some substance which regulates this polarity. I wanted to determine whether this substance came only from the growing tips. It seemed likely that the apical end of isolated sections would form a spph first, and that this spph might inhibit the basal end from developing one. What was needed was a technique which eliminated the top (or its influence) and prevented the segment's apical end from forming a spph.

If a spph is placed inside a capillary (inside diameter 0.6 mm) which contains water, it becomes asphyxiated because the diffusion of oxygen down the capillary is not rapid enough. If the top of the spph is placed inside the capillary, that part becomes asphxiated and its role in the metabolism of the rest of the spph is eliminated (see Chapter 2). This is analogous to cutting off the top section.

With this technique 1 cm sections of stage IV spphs were isolated. The spphs were placed partially inside capillaries: the top cm inside the capillary and the bottom 2 or 3 cm outside. Then the bottom of the spph was cut off, leaving only 1 cm of the stalk outside the capillary. The apical end of these sections does not produce a spph. However, the

TABLE V

Regeneration of Spphs by Sections Isolated from the Stalk

	% Regeneration by the apical end of the section	% Regeneration by the basal end of the section
1. Section from the middle of the spph	62 (104)	18
2. Base section of* the stalk	66 (26)	0
3. Spphs with apex in capillary and base cut off	0 (52)	4
4. Spphs with base in capillary and apex cut off	60 (68)	0

The numbers given in the parenthesis are the number of spphs measured in each experiment. Spphs were stage IV spphs and were 3-5 cm long. The sections were 1-2 cm long. The number of branches formed was measured 30-40 hours after the sections were isolated.

*Part 2 is a control. If a section is cut from the middle of the stalk, the lower end might not produce a branch because the lower part of the stalk lacks the proper cytoplasmic components. If this were the case, then sections from the base of the stalk should not be able to form spphs either. However, the basal sections form spphs at a normal rate. These sections did not form spphs at their basal ends because the foot of the spphs was left intact. Below the growing zone, the stalk can form a spph only if the cell wall has been cut or damaged.

bottom end is still prevented from forming one (Table V). Spphs which had their basal ends in capillaries and their tops cut off were used as controls. They developed spphs at their apices normally.

Mutants

It should be possible to find mutants whose spores do not produce the growth stimulating material. I attempted to do this by mutagenizing wild type Phycomyces with nitrosoguanidine (see methods section) and selecting for mutants which had short spphs. Two types of mutants were found: those that did not start growing again after they matured into stage IV and those that did but usually did not grow more than 1 or 2 cm.

The mutants were further selected for those with two additional characteristics: (1) Stage I spphs should grow like wild type. Wild type stage I spphs can be prevented from maturing and forced to continue elongating by placing them in an enclosed space (e.g. a closed petri plate) (Bergman, et al., 1969). Only mutants which behaved in the same way were kept. If the wild type stage I spphs are taken out of the enclosed space they mature into stage IV and commence elongating again. However, the mutants soon stopped elongating after they had been allowed to mature into stage IV, just as they do normally. (2) Stage IV spphs should produce branches after they stop elongating. This selection was made to eliminate

mutants whose spphs stop growing prematurely because they die. Mutants whose spphs had these two characteristics and were short were called nanus (nan) mutants (Table VI). Two wild strains were found which also had this growth habit. They are included in Table VI.

These mutants could still be defective in a large number of things, none of them of interest in these experiments. I tried to further characterize the mutants by determining whether their growth could be restored to normal by wild type spores. The four mutants whose stage IV spphs did not elongate (nan 1 and 2, UBC 1 and CBS 284.35) were tried. None of them could be restored to normal growth. However, our approach may be at fault. Wild type spphs can respond to the growth stimulating substance for only $\frac{1}{2}$ to 1 hour after their sporangia have been removed, (i.e. before growth has ceased.) If this is the case with these mutants, it would appear rather hard to apply the wild type spores at the right time.

It would have been better to try to restore the growth of nan 3 and 4 by applying wild type spores before they had stopped growing, to make them grow as tall as wild type spphs. It should also be possible to get temperature sensitive mutants which elongate like wild type at 16° and stop if transferred to 26°. Similar temperature sensitive developmental mutants have been found (Bergman, et al., 1969). These two techniques should allow one to find mutants whose spphs stop

TABLE VI

Nanus Mutants

Name	Sex	Characteristics
1. nan 1	-	This mutant has very thick stage I spphs. Usually they mature into stage II when they are 2-3 mm tall. When spph matures into stage IV growth does not recommence.
2. nan 2	-	Stage I spphs appear normal. Stage IV spphs do not elongate.
3. nan 3	-	Most of the stage IV spphs do not elongage; some do (as much as 3 cm).
4. nan 4	-	Most of the stage IV spphs do not elongate; some do (an average of 2 cm).
5. UBC 1*	+	Stage IV spphs do not elongate.
6. CBS 284.35**	+	Stage IV spphs do not elongate.

The spphs from the short *nanus* strains (nan 1, nan 2, UBC 1, and CBS 284,35) often go through 3 cycles of growth stoppage and branch formation.

*This strain was isolated near Vancouver, British Columbia by R. J. Bandoni of the Botany Department of the University of British Columbia.

**This strain is from the culture collection of the Centraalbureau voor Schimmelcultures, Baarn, Nederland.

growing because their spores do not produce the growth stimulating substance. If the reason the mutants spphs stop growing is not the spores, but something inside the spph, then the problem becomes much less approachable. There are probably a large number of reasons why the spph may stop none of which have anything to do with apical dominance. This type of mutant could be detected by testing whether its spores stimulate the growth of wild type spphs.

DISCUSSION

The elongation of stage IV spphs requires the sporangium. If the sporangium is removed elongation slows down and stops. Later, 6-16 hours after the sporangium has been removed, the spph grows a branch and starts elongating again. The great majority of the growth promoting activity of the sporangium is in the spores. A small amount of the activity is in the supernatant fraction (vesicles and sporangium cell sap). If small drops of the spore or supernatant fraction are applied to the columellas of spphs, only the spores stimulate the spph's growth. If large drops (1000 times larger) of supernatant are applied, the supernatant will stimulate growth. The activity of the spores is heat sensitive, but that in the supernatant is not.

If small drops of supernatant are replaced every hour with new small drops, the growth of the spphs is stimulated. This indicates that there is either a spontaneous decay of the activity or that the spph uses up all of the substance in the small drop. The activity in the supernatant does not appear to decrease if the supernatant is stored at room temperature for 15 hours.

These results may mean that the spores are synthesizing some substance (or substances). The substance itself is not heat sensitive but the enzymes synthesizing it are. This

substance then travels by diffusion or is transported into the spph. The spph uses or destroys this substance at about the same rate that the spores produce it.

The carpophores of several Basidiomycetes also regulate their growth internally (see introduction). The gills contain some substance which promotes the elongation of the stalk and the expansion of the cap. It is interesting that the gills are the spore bearing part of the carpophore just as the sporangium is the spore containing part of the spph. Perhaps the spore regulate the growth of the carpophore. Given that the purpose of the spph and the carpophore is to disperse spores, the control of their growth by the spores seems very functional.

The growing zone appears to be very sensitive to the presence of the spores. Two hours after sporangium removal, the growth rate of the growing zone is only 1/10th of the normal rate. The growing zone appears to be unable to respond to the stimulus from the spores if it has been without a sporangium for an hour. Normally the growing zone is sensitive to light (Bergman, et al., 1969). It will grow towards the light's source and will temporarily grow faster (give a growth response) if the intensity of the light is increased (e.g. Figure 4; Chapter 3). If the spph's sporangium is removed, the growing zone's response to light is rapidly altered. Forty-five minutes after sporangium removal the

size and duration response is very variable. After one hour and 45 minutes the growth response is very small or non-existent. These results are from only a few experiments. It would be interesting to study in greater detail the interaction of these two stimuli (the light and the growth "hormone") which affect growth.

In flowering plants a single compound, indoleacetic acid (IAA) controls both the growth of the apex and the branching (Bonner & Galston, 1952). It is necessary for the division and elongation of cells and thus affects the tip's growth. In some unknown manner it inhibits the growth of lateral branches. The same situation may exist in Phycomyces spphs. The spores control both the elongation of the spph and its branching. In this regard it would have been nice if IAA had stimulated the spph's growth. However, IAA has no effect and there may be more than a single compound involved; in fact branching can be inhibited even though growth is not stimulated. The growth of carpophores appears to be stimulated by at least 10 different amino acids and even by NH_4^+ (Konishi & Hagimoto, 1962). The branching pattern of Mucor hiemalis is affected by 15 amino acids (Plunkett, 1966). It may be that a similar number of physiologically equivalent compounds regulate the growth of stage IV spphs.

The fact that the growth of stage IV spphs is regulated by the spores makes it a specialized process and may mean that it

is unique to stage IV spphs. It may not function in the mycelium or even in stage I spphs.

One of the most immediate problems is to isolate the compound or compounds involved in the growth regulation. The application of large drops to hanging spphs appears to be a good bioassay. Then the effect of the compounds or compounds on the branching, the elongation, the polarity of regeneration, and the growth of stage I spphs and the mycelium could be tested.

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CHAPTER 2

LOCAL METABOLIC ANATOMY IN *PHYCOMYCES* SPORANGIOPHORES

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Local Metabolic Autonomy in Phycomyces Sporangiophores[1]

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Abstract. The degree of dependence of the growing zone of the *Phycomyces* sporangiophore upon other parts of the stalk was tested by inhibiting glycolysis and oxidative phosphorylation in the stalk below the growing zone. Initially the growing zone is capable of nearly normal growth when the metabolism in the rest of the stalk is inhibited in this way. However, the growing zone appears to become depleted of something normally supplied from below, because after 3 to 4 hr its growth rate slows down and the growth stops much sooner than in normal sporangiophores. The rest of the sporangiophore appears to have a similar degree of local autonomy because isolated sections from below the growing zone can support protoplasmic streaming for 10 to 20 hr.

The sporangiophore of *Phycomyces blakesleeanus* is an asexual fruiting body composed of a single celled stalk which supports a sporangium containing numerous spores. The stalk has a 2 to 3 mm growing zone just beneath the sporangium and, at the other end, a "foot" which connects the sporangiophore to the mycelium and allows uptake of nutrients and water. Substrates and subcellular particles are moved up and down the stalk in channels at a rate of about 3 μ/sec (11). The stalk has a diameter of about 0.1 mm and can grow to a length of about 10 cm. After the sporangium has matured, the steady state growth rate is about 3 mm/hr. This rate can be temporarily increased by permanent or temporary increases in the intensity of illumination of the growing zone, and can be decreased by decreases in the light intensity (12).

The aim of this paper is primarily to determine whether the growing zone is dependent upon the rest of the sporangiophore for the substrates and energy necessary for the processes of growth such as cell wall and membrane synthesis. The part of the stalk below the growing zone contains significant quantities of mitochondria, nuclei, and other subcellular particles and might supply the growing zone with substrates. If the growing zone is dependent upon substrates from below, it should have control over the rates of this supply so that they can be adjusted to coincide with the varying growth rates. In cells smaller than the sporangiophore of *Phycomyces,* protoplasmic streaming and diffusion are rapid enough to move substrates throughout the cell in periods comparable to their turnover periods, and thus allow the adjustment of the rates of synthesis of substrates to their rates of use even though the sites of synthesis and use are separated. However, comparison of the rates of synthesis with the rates of streaming and diffusion within the sporangiophore, indicates that the length of the sporangiophore limits such communication to short sections within the stalk. The turnover periods of ATP and oxygen, both indicative of the level of metabolism (8) within the sporangiophore, are respectively 1 sec (13) and 5 sec. [The turnover period for oxygen was calculated from the rate of consumption per sporangiophore which is about 1.0×10^{-9} moles/min (Goodell, in preparation) and assuming that the concentration of dissolved oxygen in the sporangiophore is the same as in distilled water at 20°: 0.26 μmoles/ml (7)]. The time which diffusion takes to move small molecules from one end of a 3 centimeter long sporangiophore to the other can be calculated to be several days on the average, and the time required for streaming to be several hr. [The time required for small molecules to diffuse along the sporangiophore is based on the average diffusion coefficient of small molecules in water at 20° which is about 10^{-5} cm^2/sec (2)]. This difference between the rates of transport and metabolism indicates that the growing zone might have its own supply of substrates and the machinery required for their metabolism.

It has been shown that the sporangiophore as a whole can grow independently of the mycelium (6). If the sporangiophore is plucked and its base put into water, it will continue to grow at a normal rate for about 15 hr. The experiments reported in this paper indicate that growing zones which have been isolated from the rest of the sporangiophore are capable of nearly normal growth for 3 to 4 hr afterwards.

Materials and Methods

Phycomyces blakesleeanus Burgeff [strain 1555 (−) of the Northern Regional Research Laboratory]

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was cultured on potato dextrose agar in plates or shell vials as described by Dennison (5). The experiments were done on stage IV sporangiophores (4) because they grow at a constant rate of 2 to 3 mm per hr for many hr and have the most easily observable streaming. All sporangiophores were plucked when in stage IV and initially 2 to 4 cm long. They were either completely immersed in aqueous solution (14) or a portion of the top of the stalk was left out of the aqueous solution. When the tops of sporangiophores were in air, they were kept in humidity close to 100 % by placing them in closed vessels containing water or a dilute aqueous solution.

Anoxia was used as the inhibitor of oxidative phosphorylation, and NaF and iodoacetate to inhibit glycolysis. Anoxia was produced by placing a plucked sporangiophore in a microchamber perfused with a stream of nitrogen, or by putting the sporangiophore into a capillary tube (inside diameter 0.6 to 0.9 mm) containing water. (This procedure takes advantage of the slow rate of diffusion of oxygen down the capillary compared to the rate of consumption of oxygen by the sporangiophore. See the Results section.) The effect of chemical inhibitors of glycolysis was checked by immersing plucked sporangiophores in 0.01 M and 0.1 M (pH 7) solutions of the inhibitors. Sporangiophores were immersed in phosphate buffer solution (pH 7, 0.01 M) as a control. To inhibit both glycolysis and respiration, sporangiophores were first immersed in 0.01 M NaF solution for 10 min and then put into the nitrogen chamber or into capillaries filled with 0.01 M NaF solution.

The effect of inhibition of glycolysis and respiration in nearby regions upon growth was determined by putting various portions of the lower part of the sporangiophores into capillary tubes filled with 0.01 M NaF solution. The bases of the capillaries were stuck into plasticine (spread on the bottom of a pan) in order to stand the capillaries up and to prevent movement of the NaF solution through the capillary. The pan was then filled with the same NaF solution until the upper lips of the capillaries were just covered. This arrangement prevented evaporation from the top of the capillaries while assuring concurrent anoxia. The growth rate was determined by measuring every 2 hr the net increase in the length of the sporangiophore. To minimize the physical damage to sporangiophores with only 0.2 mm or less of their stalk out of the capillary, a hair was used to push the sporangiophore down into the capillary. The hair was tied to the base of the sporangiophore and both the sporangiophore and the hair were inserted into the capillary. After positioning the sporangiophore inside the capillary the free end of the hair was cut off.

As a check of the effectiveness of this method, varying fractions of the sporangiophore were physically isolated by pinching a short section shut with a small strip of lead (3 mm × one-half mm × one-fourth mm). The stalk below the pinched section was removed to be certain that the clamping was tight enough to maintain turgor pressure in the upper section of the sporangiophore. The sporangiophore was then stood up by embedding the lead strip in plasticine, and 1 to 4 mm of the new "foot" were immersed in 0.01 M phosphate buffer (pH 7).

Growth was measured with a ruler if the interval between measurements was an hr or more. When the average growth rate over shorter intervals was desired, a 17 power microscope was used. In these experiments, any sporangiophore which had not grown after the first 2 measurements was considered physically damaged and was discarded.

If the effect upon streaming of isolating a section of the sporangiophore was to be observed, the stalk on both sides of a short segment was put into a capillary filled with a 0.01 M NaF solution. Segments were also isolated by pinching off and removing the stalk on both sides. In this case the sections were immersed in distilled water.

Results

Inhibition of Oxidative Phosphorylation and Glycolysis. Methods of inhibition of glycolysis, oxidative phosphorylation or both were tested for their ability to inhibit streaming or growth in regions where they were applied without affecting nearby regions.

If sporangiophores are put into nitrogen streaming stops within 1.5 min in the growing zone and in a varying fraction of the sporangiophore below the growing zone (where there is rapid cessation of streaming, the times required for the inhibition are given in table I). In sporangiophores shorter than 2.5 cm, streaming usually stops throughout the sporangiophores. In longer sporangiophores the proportion of the stalk in which streaming in nitrogen continues for at least 10 min tends to increase as the length of the sporangiophores increases, as does the rate of this residual streaming, although it is never more than about half the normal rate. The inhibition of streaming can be readily reversed by replacing the nitrogen with air. Streaming can be stopped by putting the sporangiophore in nitrogen and then restarted in air many times with no apparent permanent effects. Inhibition of streaming in nitrogen is not due to dehydration of the sporangiophore because water saturated N_2 gas has the same effect and dry air has no inhibiting effect.

Streaming in nitrogen in the lower regions of most sporangiophores longer than 2.5 cm continues for 30 to 40 min. When the sporangiophores are immersed in inhibitors of glycolysis (0.1 and 0.01 M NaF) for 10 min and then put into nitrogen, streaming stops throughout the entire length of the sporangiophores within 90 sec even though the inhibitors of glycolysis alone have no effect upon streaming for at least 2 hr (see table I).

It can be calculated that the sporangiophores inside capillaries (inside diameter 0.6–0.9 mm) filled

Table I. *Effects on Cytoplasmic Streaming of the Inhibition of Oxidative Phosphorylation and Glycolysis*

The times required to stop streaming in parts a and b are those for stopping it in the upper part of the sporangiophore, except in the case of 0.1 M NaF where streaming stopped throughout the sporangiophore at approximately the same time. In part c the times given are for stoppage throughout the sporangiophore.

Inhibitors and the conc used	Time required to stop streaming
a) Of oxidative phosphorylation[1]	
Nitrogen gas	30–90 sec
Capillary[2]	10–15 min
b) Of Glycolysis	
0.1 M NaF (pH 7)	2 hr
0.01 M NaF (pH 7)	more than 24 hr
0.1 M iodoacetate	about 2 hr
c) Of glycolysis and oxidative phosphorylation	
0.1 M NaF (pH7) and nitrogen gas	30–90 sec
0.1 M or 0.01 M NaF (pH 7) and capillary	10–15 min

[1] Chemical inhibitors of oxidative phosphorylation also stop streaming rapidly. KCN (0.001 M, 0.01 M both pH 7), dinitrophenol (0.005 M, pH 7), and sodium azide (0.01 M) stopped streaming in the upper portion of the sporangiophore within 5 min. Streaming stopped throughout the length of the sporangiophore within 30 min. In combination with 0.01 M NaF, streaming stopped within 10 to 30 min. However, the mode of action of these inhibitors is uncertain because oxygen uptake continues normally for at least 2 hr when these inhibitors are added to extracellular lysates of sporangiophores.

[2] Anoxia is produced inside a capillary because the sporangiophores use oxygen faster than it is replaced by movement of oxygen down the capillary *via* diffusion. The time required for streaming to stop is essentially that required for the sporangiophore to use up all the O_2.

with water quickly produce local anoxia because they use up the oxygen dissolved in the water within a few min and longitudinal diffusion of oxygen inside the capillary is too slow to replace it. [The diffusion coefficient of oxygen in water at 18° is 1.98×10^{-5} cm²/sec (2).] Streaming in short sporangiophores and in the tops of long sporangiophores inside capillaries again shows greater dependence upon oxidative phosphorylation than does streaming in the bases of long sporangiophores (see table I). Streaming also stops sooner in the bases of long sporangiophores when the capillaries are filled with NaF solution rather than with distilled water. With NaF streaming always stops within 15 min in that part of the sporangiophore inside the capillary (except the top 1.0 to 1.5 mm section just below the upper lip of the capillary), and continues normally outside of the capillary for at least 24 hr. In the top 1.0 to 1.5 mm section of the sporangiophore inside the capillary, streaming continues for the duration of the experiment because of the availability of oxygen *via* diffusion.

Streaming often stops temporarily for about 1 min after the sporangiophores have been put into nitrogen and resumes 1 or 2 min later. This reinitiation can sometimes also be seen in sporangiophores placed in capillaries. This phenomenon probably can be attributed to the Pasteur effect.

Anoxia also inhibits growth. When the sporangiophores are placed in nitrogen, the growth rates drop to 10 % of the normal value within 3 or 4 min. However, this slow rate of growth continues for at least 15 min. If the sporangiophores are returned to aerobic conditions after 10 min, the growth rate increases slowly and is less than one-half normal 30 min later (K. Bergman, personal communication). Thus the streaming appears to be more rapidly and more completely inhibited by anaerobic conditions than is growth.

NaF has no inhibitory effect upon the growth of sporangiophores. Sporangiophores grown with their bases in NaF and with the top 1.5, 4.5, 9.5, and 14.5 mm of the stalk out of the solution show no significant differences in either the rate or duration of growth. They grow as fast and for as long a time as sporangiophores grown in 0.01 M phosphate buffer (pH 7) (fig 1). Sporangiophores also continued to grow for at least 12 hr when their tops are immersed in 0.01 M NaF.

Effects of the Inhibition or Removal of Part of the Sporangiophore on the Metabolism of the Rest of the Sporangiophore. Inhibition of both streaming and growth can be localized within a part of the sporangiophore through the use of capillaries filled with NaF solution. Using this method (see Methods

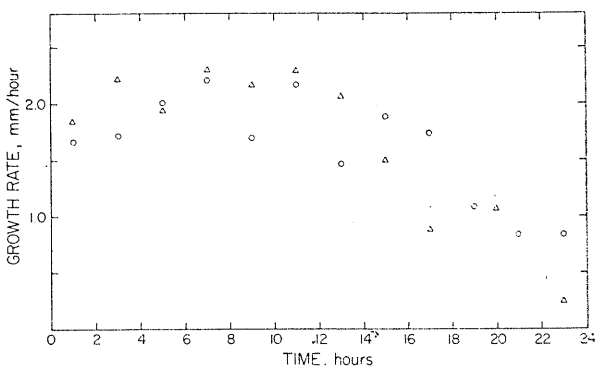

FIG. 1. Dispensibility of glycolysis for growth. Growth rates of sporangiophores with their bases in 0.01 M NaF (pH 7) (○) and in 0.01 M phosphate buffer (pH 7) (△) were measured. The points given for sporangiophores grown in NaF solution represent the grand averages of sporangiophores which initially had the top 1.5, 4.5, 9.5, and 14.5 mm of their stalk above the NaF solution. A total of 40 sporangiophores was used. The points given for sporangiophores grown in phosphate buffer represent the average growth of 10 sporangiophores which initially had 9.5 mm of their stalk out of the solution. Each point represents the average growth rate during a 2 hr period.

section) the extent to which part of the sporangiophore is dependent upon the metabolism of the rest was tested. As a check on the completeness of the inhibition of metabolism produced by anoxia and NaF, part of the stalk was removed (see Methods section).

Cytoplasmic streaming continues for 10 to 20 hr when a one millimeter section is physically isolated by pinching off the stalk on both sides. The same result is obtained with sections isolated from the rest of the sporangiophore using capillaries filled with NaF solution. A one millimeter section was left outside the capillaries and streaming continued in this section and in the two 1.0 to 1.5 sections on either side inside the capillaries.

We measured the growth of sporangiophores which initially had the top 0.5 mm, 1.5 mm, 2.5 mm, 9.5 mm or 14.5 mm portions of their stalks outside of the capillaries. As can be seen in figures 2 and 5, both the maximal growth rates and the total growth are much reduced when the top 1.5 mm or less of the sporangiophore stalk is left outside of the capillary. The growth rates of sporangiophores having the top 9.5 and 14.5 mm outside are initially as fast as sporangiophores grown in aerobic NaF or in phosphate buffer; however, after 7 hr the growth rate declines more rapidly and stops sooner than in the controls (figs 1 and 2). The sporangiophores with 2.5 and 4.5 mm outside initially have nearly normal maximum growth rates (respectively 80 and 90 % that of the controls), but the growth rates decline more rapidly than is the case with longer sections outside. Thus there is a sharp increase in both the maximal growth rate and the total growth as a greater fraction of the stalk is allowed to function until 2.5 millimeters are outside of the capillary (figs 2 and 5). As the uninhibited fraction is increased beyond this point the maximum growth rate increases only slightly and the total growth changes much less rapidly than before. However, the total growth never reaches that of uninhibited plucked sporangiophores.

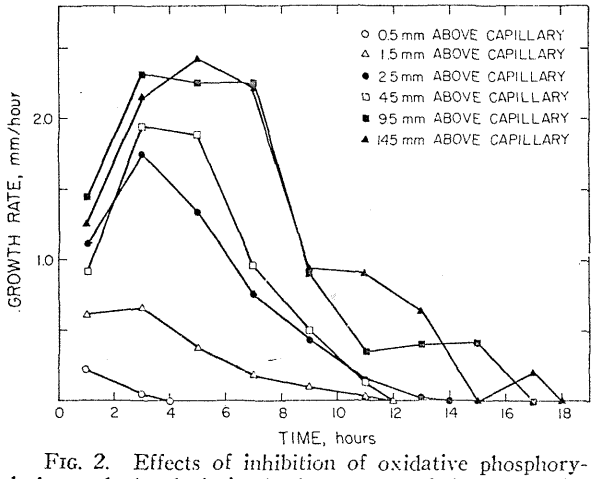

FIG. 2. Effects of inhibition of oxidative phosphorylation and glycolysis in the lower part of the sporangiophore on growth rates. The bases of the sporangiophores were placed in capillaries (to produce anoxia) filled with 0.01 M NaF solution (to stop glycolysis). Each curve is the average growth rate of 20 sporangiophores. Each point represents the average growth rate during a 2 hr period. The sporangiophores initially had the top 0.5, 1.5, 2.5, 4.5, 9.5, or 14.5 mm outside of the capillaries.

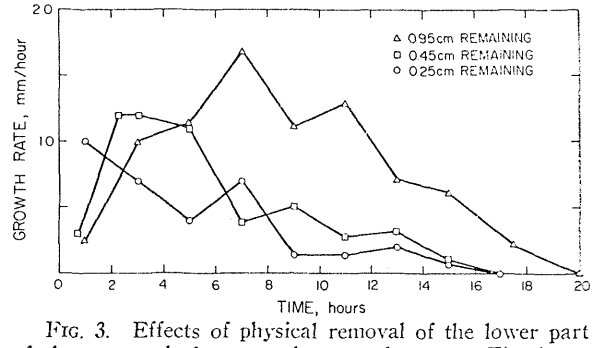

FIG. 3. Effects of physical removal of the lower part of the sporangiophore on the growth rates. The bases of the sporangiophores were removed after the stalk was pinched shut with a lead strip. The sporangiophores initially had the top 2.5, 4.5, and 9.5 mm remaining. Each curve is the average of 10 sporangiophores.

Sporangiophores whose lower portions have been physically removed show about the same net growth as sporangiophores put into capillaries with the same portion outside of the capillary. The growth rates are slower but the growth continues for a longer time (fig 3). The best correspondence in total growth between these sporangiophores and those in the capillaries is obtained if it is assumed that the top 1.0 mm of the stalk inside the capillary metabolizes normally and the rest of the stalk inside the capillary has no active effect upon growth (fig 5).

The growth rates described above are averages for 2 hr of growth. To determine whether sporangiophores with 1.5 mm of the stalk above the capillary could initially grow at normal rates, the growth rate was measured at 10 min intervals with a microscope. The rate of growth of these sporangiophores is reduced to 0.7 mm/hr within the first 10 min (fig 4).

For the first 2 hr the growth rate of any sporangiophore partially inside a capillary is slow. This appears to be caused by inserting the sporangiophores into the capillaries, because sporangiophores which are stuck into empty capillaries so that 9.5 mm remain outside also show this slower initial growth rate. However, the growth rates of these sporangiophores later return to normal. Also the growth rates of sporangiophores which initially have part of their growing zones inside empty capillaries (0.5 and 1.5 mm of the stalk above the capillary) return to normal levels within 2 or 3 hr. Figure 4 shows that sporangiophores with 1.5 mm outside grow at 80 % of the normal rate of plucked sporangiophores within 30 min.

The sporangiophores used in these experiments

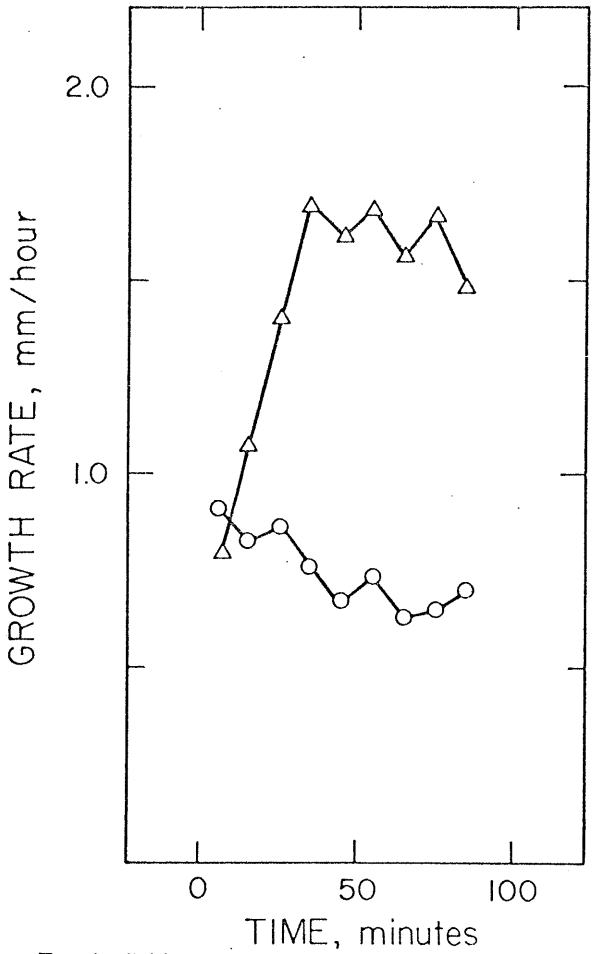

FIG. 4. Initial effects of inhibition of the lower part of the sporangiophore on growth rates. The sporangiophores were plucked and put into capillaries so that the top 5 mm of the stalk was initially above the capillaries. Three hr later the sporangiophores were adjusted so that 1.5 mm remained above the capillary. The capillaries of the test sporangiophores (— ○ —) were then filled with NaF solution. The capillaries of the control sporangiophores (— △ —) were left empty. The ○ points represent the average of 15 sporangiophores and the △ represents the average of 7 sporangiophores. Each point gives the average growth rate during a 10 min period.

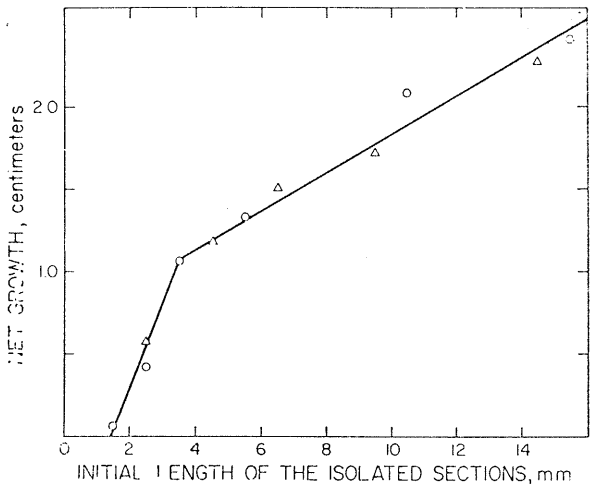

FIG. 5. Effects of the isolation of the top of the sporangiophores on the net growth of the sporangiophores. Sporangiophores had a bottom fraction of their stalks inhibited by anoxia and sodium fluoride (— ○ —) or physically removed (—△—). From 25 to 50 sporangiophores were measured for each of the points (○) and from 15 to 25 for each of the points (△). To obtain the best correlation between the 2 kinds of experiments it was assumed that the upper 1 mm of the sporangiophores inside the capillary was not inhibited. The initial length of the sections isolated in this way includes this 1 mm section.

varied in their initial length from 2 to 4 cm. However, no dependence on initial length was found in the net growth among sporangiophores whose top 9.5 or 14.5 mm were outside the capillaries in spite of the fact that the longer sporangiophores had a significantly greater fraction of their length inside the capillaries.

Discussion

Streaming continues for 10 to 20 hr in isolated 1 mm sections of the sporangiophore stalk. This indicates that the energy sources and other substrates necessary for streaming are contained within each such section in sufficient quantities to permit such streaming to persist over long periods of time.

The metabolic processes directly associated with growth also appear to be almost completely localized within the growing zone. Sporangiophores with the upper 2.5 mm or more of their stalks outside of the capillary have maximal growth rates at least 80 % that of uninhibited plucked sporangiophores. However, the growth rate of sporangiophores with 1.5 mm outside the capillary, is reduced to one-third of normal immediately after the sporangiophores are put into the capillary. Sporangiophores with 0.5 mm outside are capable of growth rates only 10 % of normal. This indicates that the whole growing zone must be active to enable the sporangiophore to grow at normal rates.

Long-term normal growth depends also upon the active metabolism of the lower part of the sporangiophore. The total growth and the duration of normal growth increase as the uninhibited portion of the stalk is increased even after all of the growing zone is left out of the capillary. Growth for extended periods may depend upon basic substrates which are translocated to the growing zone and used for repair or for cell well or membrane synthesis.

The conclusions reached above are somewhat ill defined because not all of the metabolism of the portion of the sporangiophore inside the capillary is inhibited. Streaming in the top 1.0 to 1.5 mm of

the stalk inside the capillary indicates a significant level of metabolism in this region and there may be some metabolic processes taking place in the lower regions. However, the best correspondence in net growth between sporangiophores which are put into capillaries and those with their bases physically removed is obtained if it is assumed that the top 1.0 mm of the stalk inside the capillary metabolizes normally and the portion below has no active effect upon growth. This indicates that the capillary technique inhibits all of the metabolism of the stalk inside the capillary except the upper 1.0 mm. This inference is supported by the observation that after streaming stops (10–15 min in the region below the top 1.0–1.5 mm section), there is obvious degeneration of the cytoplasm, and streaming does not reinitiate if the sporangiophore is removed from the capillary. These observations indicate that the top 2.5 to 4.0 mm of the stalk are necessary for normal initial growth rates.

The growth rates of sporangiophores with their bases removed after the stalk was pinched shut are less than those of corresponding sporangiophores in capillaries. The growth rate appears to be decreased because the stalk cannot take up water as effectively as the "foot" and not because of permanent damage to the growing zone. A waxy layer coats the cell wall except in the growing zone (3), and would probably limit the water uptake rate. Even though sporangiophores, which initially have only 4.5 mm of their stalk remaining, are pinched off close to the growing zone, their initial growth rate (for the first 5 hr) is the same as that of sporangiophores with 9.5 mm remaining. The net growth of all of these sporangiophores is also the same as corresponding sporangiophores in capillaries.

The rapid cessation of growth and streaming when the sporangiophores are in nitrogen or inside capillaries indicates that they are highly dependent upon oxidative phosphorylation, as has previously been shown for both the whole thallus (1) and for the spores (16). In spite of this dependence, the sporangiophores can temporarily change from aerobic to anaerobic metabolism (glycolysis), as indicated by the reinitiation of streaming 2 or 3 min after the sporangiophores have been put into nitrogen and by the longer duration of streaming in the lower region of long sporangiophores of inhibitors of glycolysis are not used. The reinitiation of streaming can probably be attributed to the Pasteur effect because it indicates that the rate of glycolysis is limited by respiration under aerobic conditions and that this limitation is lifted when the sporangiophore is placed into anaerobic conditions. This phenomenon was named by Warburg (15) for Pasteur, who first described it in yeast (9) and, later in *Mucor* (10), a genus of fungi closely related to *Phycomyces*.

Acknowledgments

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CHAPTER 3

OXYGEN CONSUMPTION OF *PHYCOMYCES* SPORANGIOPHORES

INTRODUCTION

The sporangiophore (spph) of Phycomyces goes through four stages of development. In order to determine something about the energy requirements of the spph during maturation, the respiration rates of stages I, III, and IV were measured. When a spph matures from stage I to stage III its oxygen consumption increases markedly. After a spph has matured into stage IV its respiration rate does not increase. Stage III spphs have the highest rate of oxygen consumption per unit dry weight.

The only conspicuous change in the spph after it matures into stage IV is its increase in length. Stage IV spphs usually grow to a height of about 10 cm with little increase in dry weight (Gruen, 1959). However, they grow very rapidly, much faster than the mycelium and nearly as fast as any of the fast growing Ascomycetes mycelia. Phycomyces hyphae grow 0.6 mm hr^{-1} (Bergman, personal communication). Neurospora hyphae grow about 4 mm hr^{-1} (Burnett, 1968). The spph's growing zone is relatively independent of the rest of the stalk beneath it (Chapter 2). The growing zone's rapid growth rate and the apparent unimportance of the rest of the stalk for growth may indicate that the metabolism of the growing zone is more active than that of the rest of the spph. In order to determine something about the relative metabolic activity of the growing zone, the rate of oxygen consumption along the spph

was measured. The effect of a growth response (a temporary increase in the growth rate) on the rate of oxygen consumption was also measured. We found that the top 6.5 mm of the spph have the highest rate of oxygen consumption and that there was no measureable change in the respiration during a growth response.

MATERIALS AND METHODS

Culture Methods

Phycomyces blakesleeanus Burgeff (strain 1555 (-) of the Northern Regional Research Laboratory) was cultured on potato dextrose agar in petri plates as described by Dennison (1961). Only the first and second crops of spphs were used. If stage IV spphs were to be used, the plates were opened when the first stage I spphs appeared. This ensures that the spphs will mature into stage II spphs when they are $\frac{1}{2}$ to 1 cm long. If stage I spphs longer than 1 cm were desired, the open petri plates were placed in beakers and the vessels sealed. This created the trapped air effect (Rudolph, 1958) which prevents stage I spphs from maturing into stage II but allows them to grow. The spphs were also kept in the dark which enhances the effect. If long stage III spphs were desired, the petri plates were taken out of the beakers and the spphs allowed to mature. Care was taken to prevent contamination by bacteria,

since some bacterial strains are capable of spreading over the mycelium and up the stalk of the spph.

Manometric Apparatus

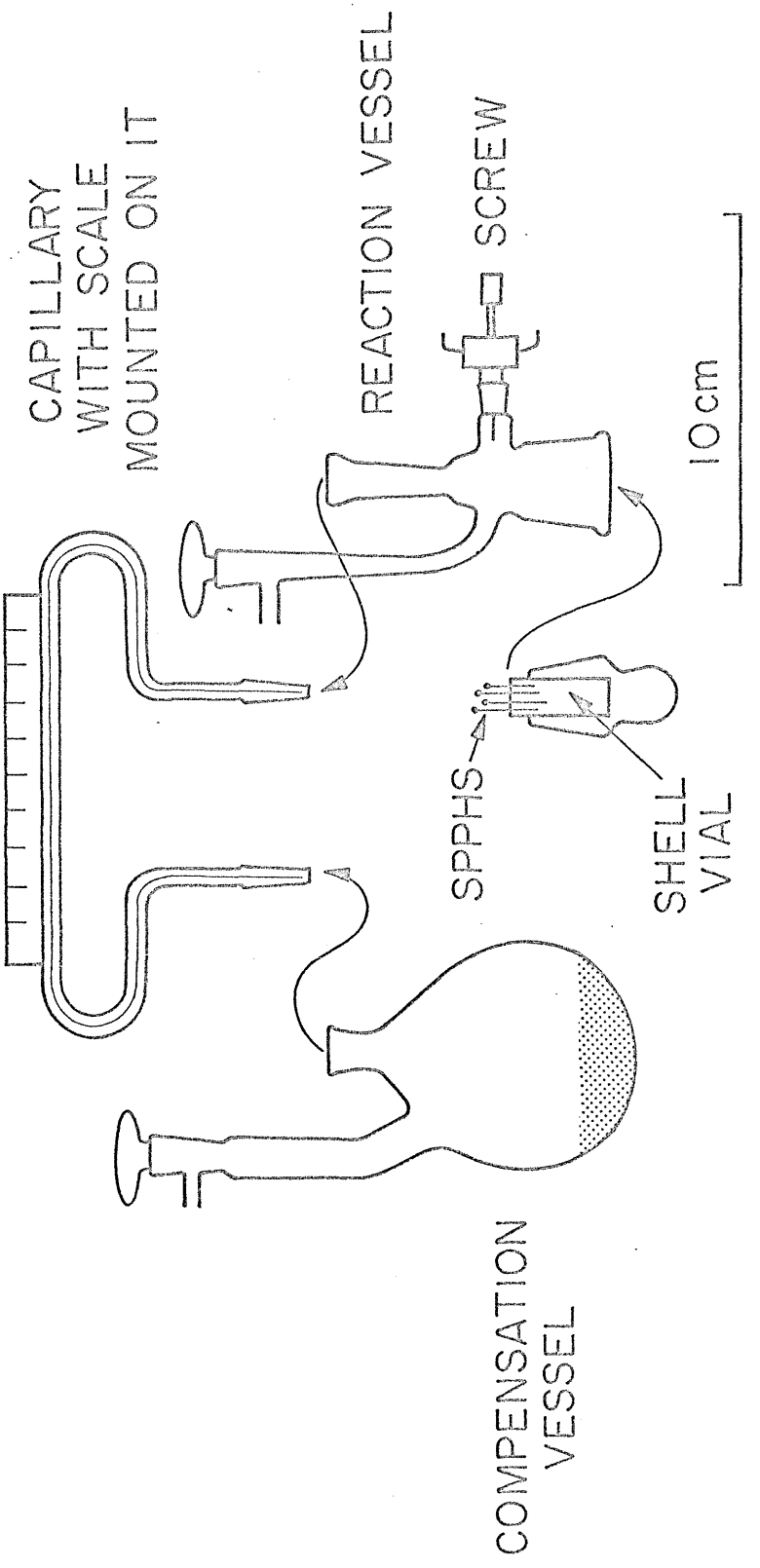
A Fenn differential respirometer was used (Figure 1). The reaction vessel was constructed with a ground glass joint so that the bottom could be removed. Ten to thirty spphs were plucked and placed on a piece of wet filter paper and this was rolled to fit inside a shell vial (1 cm x 3.5 cm). Distilled water was added to the shell vial. No part of any of the spphs was immersed. A second filter paper was rolled around the outside of the shell vial and saturated with 0.2 N KOH. The shell vial was then placed inside the bottom of the reaction vessel and the vessel closed. Fifteen milliliters of distilled water were placed inside the compensation vessel.

The manometer containing the spphs was placed in a constant temperature water bath at 25° (maintained to $\pm$ 0.1° by a Bronwill #20 constant temperature circulator) and allowed to equilibrate to ambient temperature and pressure for an hour. The two vessels were then closed to the outside and readings made for 2 to 6 hours. Often the observed rate of oxygen consumption did not become constant until after the first half hour. The rate of growth of stage IV spphs inside the manometer was periodically measured and found to be normal for plucked spphs (Gruen, 1959).

The flask constant for the respirometer was calculated

FIGURE 1, Fenn Respirometer

The reaction vessel, which contains the sporangiophores (spphs), has a volume of 10 ml and the compensation vessel a volume of 94 ml. The capillary connecting the two vessels has a cross sectional area of 0.35 mm^2. To put spphs inside the reaction vessel, the lower part of the vessel was removed and the spphs, in a shell vial, were placed inside the lower half. The reaction vessel was then closed. The rate of oxygen consumption was determined by measuring the movement of a drop of manometric fluid inside the capillary. The scale indicated in the drawing is only approximate.


CAPILLARY
WITH SCALE
MOUNTED ON IT
REACTION VESSEL
SCREW
10 cm
SPPHS
SHELL
VIAL
COMPENSATION
VESSEL

from the equation given by Umbreit et al. (1964). Krebs' manometric fluid was used with triton X-100 (Sigma) and without dye (Krebs, 1951a). The size of the capillary was calibrated with mercury.

The response time of the respirometer was determined with the technique described by Kok (1960). Hedera canariensis (ivy) leaves were used in the assay. The experimental conditions used to measure spph oxygen consumption were duplicated as closely as possible. A piece of the leaf (1 cm x 2.5 cm) was removed from the leaf's edge. It was rolled inside a larger piece of filter paper (3 cm x 2.5 cm) and 2/3 of the filter was placed inside a shell vial. The leaf segment was in the top third of the filter paper so it was not inside the vial. In this way gas could diffuse away from both surfaces of the leaf. The filter was wetted and the vial filled with distilled water.

The oxygen consumption of the leaf in the dark was measured and then a bright light turned on and the oxygen production measured. The time required for the manometer to make the transition between the steady states of oxygen consumption and production is a measure of its response time. The stimulating light caused a transient heating effect. This was measured by repeating the experiment with completely dehydrated leaf segments. These measurements were then subtracted from these obtained with normal leaves.

Determination of Dry Weights

Thirty to fifty spphs were placed on a cover slip and the cover slip was put in a drying oven at 80° for 12 hours (Gruen, 1959). Their weight was measured with a Mettler balance.

Determination of the Rate of Oxygen Consumption along Spphs

The oxygen consumption of the top sections of stage IV spphs was measured by putting the rest of the spph inside water filled capillaries (10 µl Microcaps, inner diameter 0.6 mm). See the methods section of Chapter 2 for details. The capillaries were placed into a holder (a brass rod, 1 mm x 2.5 cm, with 14 holes drilled in its end for the capillaries). The brass holder was placed in a shell vial and water added to the vial until it was just beneath the upper lips of the capillaries.

Measuring the Effect of Increased Growth Rate on Oxygen Consumption

The growth rate of stage IV spphs inside the reaction vessel was temporarily increased by giving them a single saturating step-up in light intensity. The saturating stimulus was sometimes followed by a "sunrise" (Bergman, et al., 1969). The stimulating light source was an incandescent light filtered with a blue filter (Corning 5-61). For

the single step-up stimulus, the spphs were adapted for an hour (inside the reaction vessel) to an intensity of 2.5 x 10^{-3} μ Watts/cm^2 and then the light was increased to 2.5 μ Watts/cm^2. For the "sunrise" program, the spphs were dark adapted for an hour. Then the light was turned on (intensity, 1 x 10^{-2} μ Watts/cm^2) and every 1.5 minutes thereafter the intensity of the light was doubled. After the maximum intensity (20 μ Watts/cm^2) was reached the light was left on. The light intensities were measured with an RCA 935 phototube.

The rate of oxygen consumption was measured every minute by measuring the net movement of the manometric fluid with a microscope (Gaertner, 23 X). To make the readings with the microscope, a red light was used which did not stimulate any response in dark adapted spphs. The heating element in the constant temperature circulator causes short-term volume fluctuations within the manometer. The heating element was turned off during these experiments.

The size of plucked spphs' growth response to these light stimuli was measured. The stimulating light was the same one used in the oxygen measurements but was adapted with a beam splitter as described by Delbrück and Reichardt (1956). The same microscope and red light were used to measure the growth rates of the spphs.

To test whether the growth response was dependent upon oxygen consumption, the air around a spph was replaced by N_2.

This replacement was done either 4 or 7 minutes after a saturating step-up. At the beginning of the experiment a glass chamber (5.5 mm in diameter and 30 mm long) was placed over the top 15 to 20 mm of the spph. A hole in the top of the chamber connected it to a cylinder of compressed N_2 gas. At the prescribed time the air in the chamber was replaced by a stream of nitrogen. Spphs which had not been plucked were used.

RESULTS

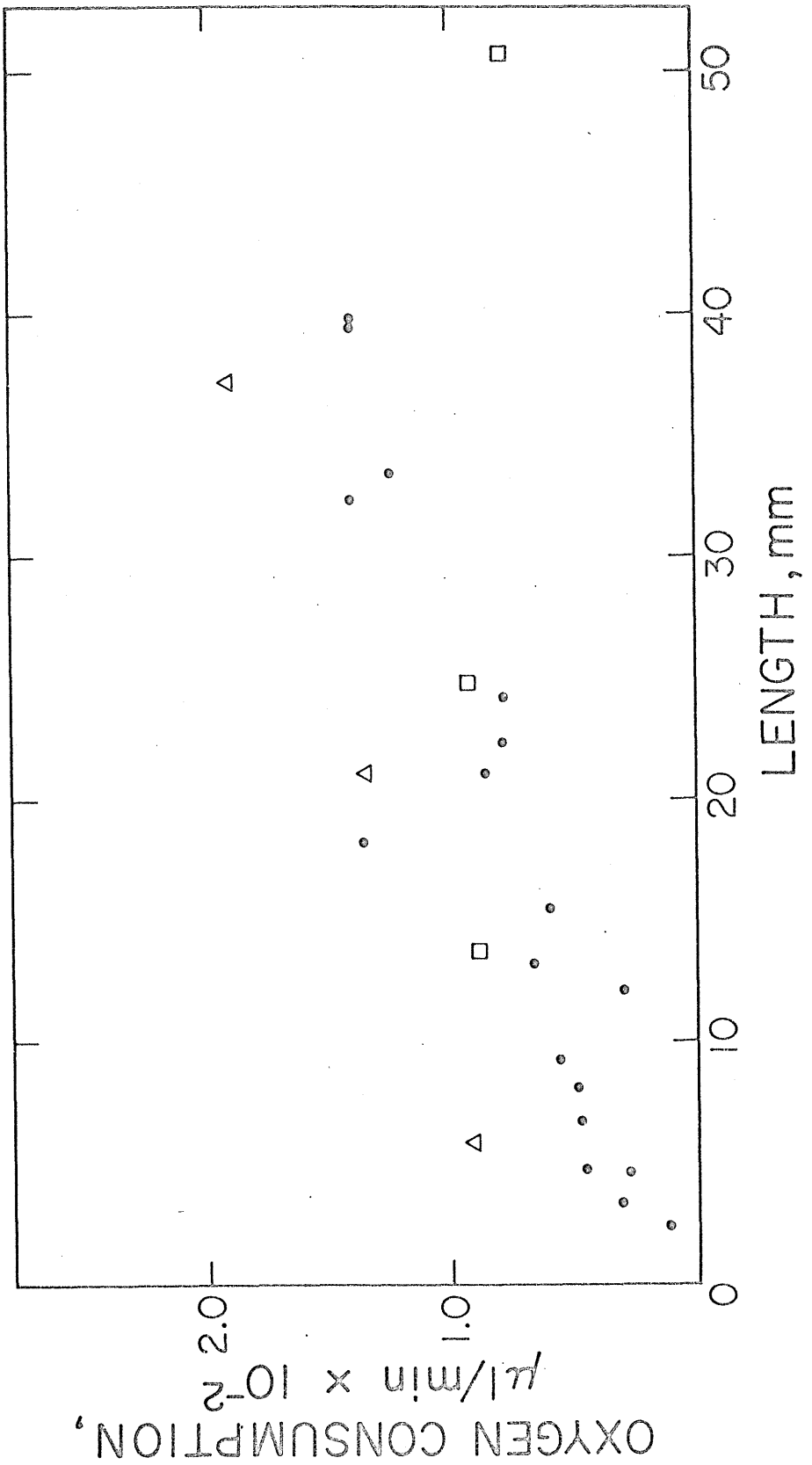
Respiration of stage I, III, and IV Spphs

The spph can follow two courses of development. Normally when it is about 5 mm long it matures from stage I into stage III and then into stage IV. However, it is prevented from maturing if it is in an enclosed space. Apparently a gas is produced by the thallus which prevents maturation (Bergman et al., 1969). In this case the spph continues to grow in stage I. When it grows out of the enclosed space (or if the enclosing vessel is removed) the spphs matures. Figure 2 shows the oxygen consumption of spphs in both paths of development.

When the stage I spph is 5 mm long its oxygen consumption is about 0.4×10^{-2} µl/min. If it continues to grow in stage I its rate of oxygen consumption increases but not as rapidly as before. It increases by 0.3×10^{-2}µl min^{-1} cm^{-1}.

FIGURE 2

The respiration rate of stage I (·), III (△), and IV (□) spphs as a function of their length. Each value given for the respiration of stage I spphs is the result of one measurement using 10 to 30 spphs. The values given for stage III and IV spphs are the average of 9 to 10 experiments each with 10 to 30 spphs. The lengths of the spphs indicated by each point varied by ± 15 per cent. The stage IV spphs used in these experiments had changed into stage III when 6 mm long.


OXYGEN CONSUMPTION,
μl/min x 10⁻²
2.0
1.0
0
10
20
30
40
50
LENGTH, mm

If a spph matures into stage III when it is 5 mm long, its respiration rate doubles to 0.9×10^{-2} µl min^{-1}. Its dry weight increases also, but its rate of respiration per unit dry weight is higher than stage I spphs (Table I). When the spph matures from stage III into stage IV its oxygen consumption stays the same. The respiration of stage IV does not increase as the spph elongates. By the time a spph has become 50 mm tall its respiration rate has dropped slightly. With the elongation there is an increase in dry weight, though not as much as in stage I; so that the respiration per unit dry weight decreases (Table I).

When stage I spphs mature into stage III their respiration rate increases by 0.5×10^{-2} µl min^{-1}. This is true of 6 mm, 21 mm and 37 mm long stage III spphs (Figure 1). When stage I spphs are about 20 mm long their dry weight does not increase when they mature into stage III (Table I). Thus the increase in respiration seems to be due to an increased level of synthesis and not to additional cytoplasm received from the mycelium. Stage III spphs have a higher respiration rate per unit dry weight than either stage I or stage IV spphs.

The respiration of stage IV spphs which have matured from these longer stage III spphs is higher than that of the stage IV spphs shown in Figure 2.

The respiratory quotient (RQ or volume of CO_2 produced

TABLE I

The Dry Weight of Spphs and the Oxygen Consumption per Unit Dry Weight

Stage	Length (mm)	Dry Weight (μg)	Q_{O_2} (O_2 Consumption per Unit Dry Weight) ($\mu l\ hr^{-1} mg^{-1}$)
I	6	28 (200)*	10.2
I	18	94 (167)	5.4
III	6	36 (300)	15.6
III	19	96 (172)	7.8
IV	15	66 (150)	7.8
IV	25	77 (200)	7.2
IV	50	97 (145)	4.8

*The number in parenthesis is the number of spphs weighed.

divided by the volume of O_2 consumed) of stage IV spphs is 0.6. This value is the average of 5 experiments with a total of 86 spphs. The average length of the spphs was 2.4 cm. The RQ for the oxidation of carbohydrates is 1 and that for lipids is about 0.7 (Fruton and Simmonds, 1961). The low RQ probably indicates that the spphs are oxidizing more lipids than carbohydrates and are probably also oxidizing some compounds partially [e.g. the conversion of amino acids to gallic acid (Bergman, et al., 1969)]. This low RQ has also been observed in other fungi (Van Etten, et al., 1966; Sussman et al., 1956).

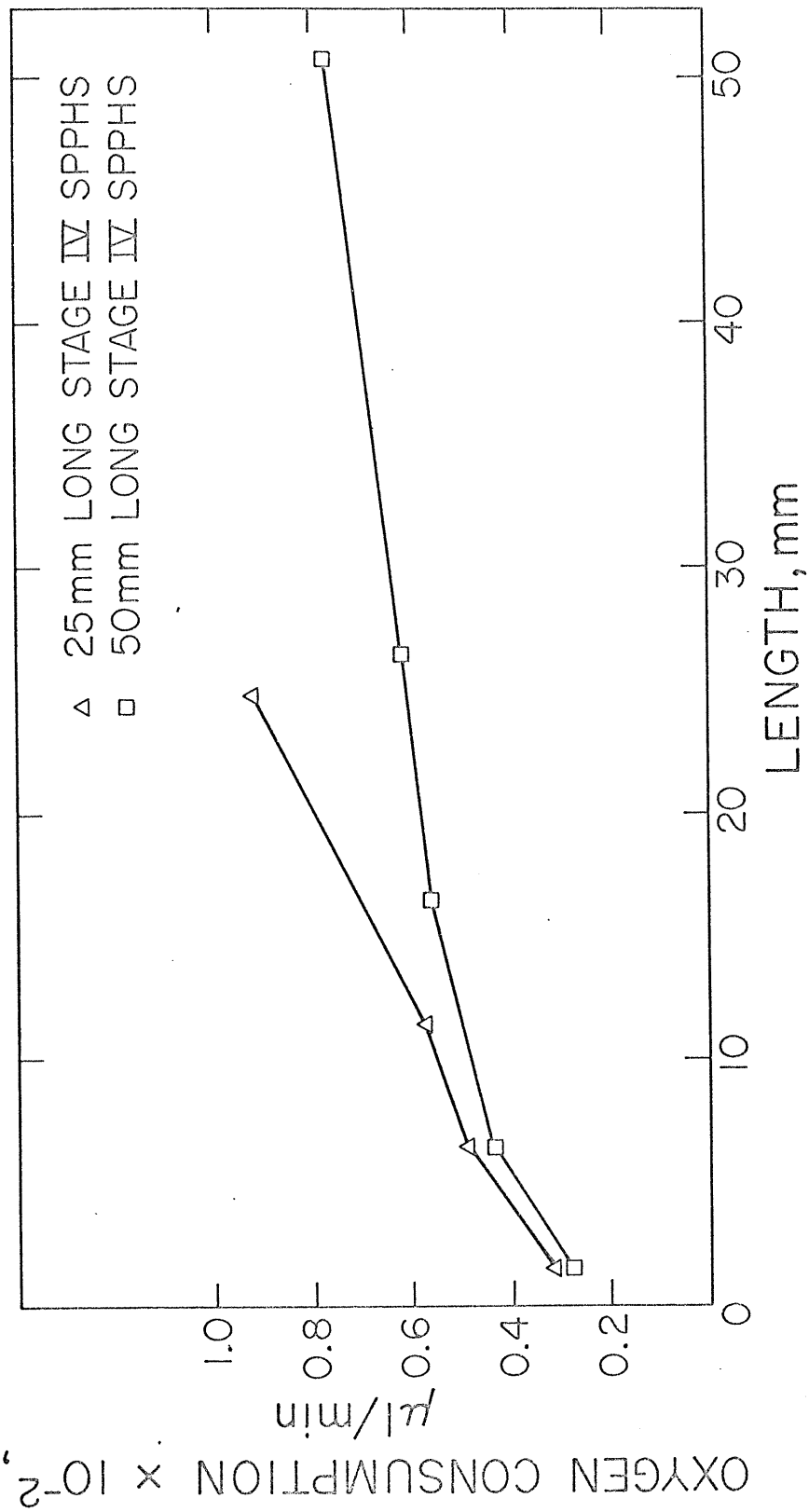
To measure the CO_2 evolved, the KOH had to be removed from the reaction vessel. This allows the concentration of CO_2 to increase during the experiment which may have an effect on the rate of respiration. However, no measurements were made to ensure that this was not the case with spphs (Krebs, 1951b).

Oxygen Consumption along the Length of Stage IV Spphs

The respiration rates of different top portions (the top 1.5 mm, 6.5 mm, and 11.5 mm) of 25 mm long stage IV spphs are given in Figure 3. The respiration of similiar lengths of 50 mm stage IV spphs is also given. These values are plotted so that the oxygen consumption along the spph can be estimated. The lengths given for the segments include the columella.

FIGURE 3. Oxygen Consumption along the Length of Stage IV Sporangiophores

The graph shows the oxygen consumption of the top 1.5, 6.5, and 11.5 mm and of the whole (spph) for 25 mm long spphs, and corresponding lengths for 50 mm spphs. From these values, the rate of oxygen consumption along the spphs can be estimated. The value given for the top 1.5 mm was measured by putting all of the spph inside a water filled capillary except the sporangium (diameter 0.5 mm). It is assumed that the top 1 mm of the stalk inside the capillary consumes oxygen normally. The other values were obtained in the same way. Each point on the curve is the average of nine measurements each using 13 or 14 spphs.


OXYGEN CONSUMPTION × 10⁻², μl/min
1.0
0.8
0.6
0.4
0.2
0
10
20
30
40
50
LENGTH, mm
25mm LONG STAGE IV SPPHS
50mm LONG STAGE IV SPPHS

The oxygen consumption by part of the spph was measured by placing the rest inside a water filled capillary (see methods sections). It is assumed that the part of the spph outside the capillary and the top 1 mm inside the capillary consume oxygen at a normal rate and that the rest of the spph does not consume any oxygen. This can be checked by measuring the oxygen consumption of 25 mm spphs whose bottom 13 mm are outside the capillaries. The addition of this rate of consumption to that of 25 mm spphs whose top 10 mm are outside the capillaries should give a rate of oxygen consumption equal to that of whole spphs. The averaged rate for the bottom sections of 126 spphs is 0.42×10^{-2} µl min^{-1}. The respiration rate of the top sections is 0.60×10^{-2} µl min^{-1}. The two values together give 1.02×10^{-2} µl min^{-1} compared to 0.97×10^{-2} µl min^{-1} for whole spphs. The value is high but within experimental error.

The amount of oxygen in the capillary can be estimated in another way. Phycomyces spphs have a phenol oxidase which gradually oxidizes the phenolic compounds in the cell wall and causes them to polymerize and turn dark green. It is dependent upon molecular oxygen (Mahler and Cordes, 1966). The cell wall inside the capillary remains colorless except for the $\frac{1}{2}$ to 1 mm section nearest the end of the capillary. This section, however, turns green after about 24 hours as does the part of the stalk outside the capillary. The results

of Chapter 2 indicate that only the top 1.0 to 1.5 mm receives oxygen by diffusion, the rest becomes asphyxiated.

About 1/3 of the oxygen consumed by the spph is consumed by the top 1.5 mm. This section includes the sporangium, the columella and 1 mm of the growing zone. If the sporangium is removed (the sporangium wall is fragile), the rate of oxygen consumption is not changed. The oxygen consumption of the top 6.5 mm sections of spphs with sporangia is 0.51×10^{-2} $\mu l\ min^{-1}$. If the sporangium is removed, the spphs consume 0.53×10^{-2} $\mu l\ min^{-1}$.

Rudolph (1960) has measured the respiration rate of dormant spores. There are about 10^5 spores in a sporangium and, assuming they are dormant, they would consume 1.5×10^{-4} μl of O_2 per minute. This is 3 per cent as much as the top 1.5 mm consumes. Thus the sporangium appears to consume very little oxygen.

The columella and the top mm of the growing zone appear to account for nearly all of the oxygen consumed by the top 1.5 mm. It seems likely that the columella consumes most of this, and that the growing zone does not consume more oxygen than do equal sized sections from the rest of the stalk. When the oxygen consumption of the top 1.5 mm is measured, only the top 0.5 mm is outside of the capillary. Probably only the respiration rate of the top 0.5 mm is normal. The oxygen consumption of the stalk of 25 mm long spphs is nearly constant

from top to bottom. If the growing zone consumed a large fraction of the total oxygen, one would expect that the oxygen consumption of the top 5 mm of the stalk would be higher than that of other 5 mm long sections (Figure 3).

The Rate of Oxygen Consumption during a Growth Response to a Light Stimulus

Stage IV spphs will give a growth response to an increase in the intensity of light impinging on the growing zone (Bergman, et al., 1969; see Figure 4). When the spphs were in the manometer, the light intensity was increased sufficiently so that the spphs would give a maximium growth response (see methods section). The same step-ups were given to spphs whose top 5.5 mm were outside of capillaries. Step-ups followed by "sunrises" were given to whole spphs. The growth responses to these stimuli are given in Figure 4. In no case could I measure a change in the rate of oxygen consumption. The rate of oxygen consumption was monitored with a microscope from fifteen minutes before the light stimulus until fifteen minutes after the end of the response.

In order to determine whether the growth response requires oxygen, the ambient air was replaced by nitrogen at the start of and during a growth response (Figure 5). In both cases the growth declined rapidly, nearly to zero.

The response time of the manometer was measured with

FIGURE 4. Growth Responses to Light Stimuli

The curves show the increased growth rate of stage IV sporangiophores (spphs) in response to an increase in the light intensity. In curves A, B, and D the light was increased in a single step-up (a 1000 fold increase) at time zero. In C the step-up was followed by a "sunrise" (a doubling of the light intensity every 1.5 minutes).

Curve A is the control: the response of unplucked spphs. Their growth response is larger than that of the plucked spphs shown in the other curves. Curves B and C show the response of normal, plucked spphs; D shows plucked spphs with only the top 6.5 mm functioning. The results of all three experiments with plucked spphs are nearly the same. Thus it appears that spphs with just their top 6.5 mm functioning give a normal growth response. The response of plucked spphs to "sunrises," given alone or after a step-up, was much smaller than that of attached spphs.

Curve D is the average of ten experiments, the rest of five experiments.

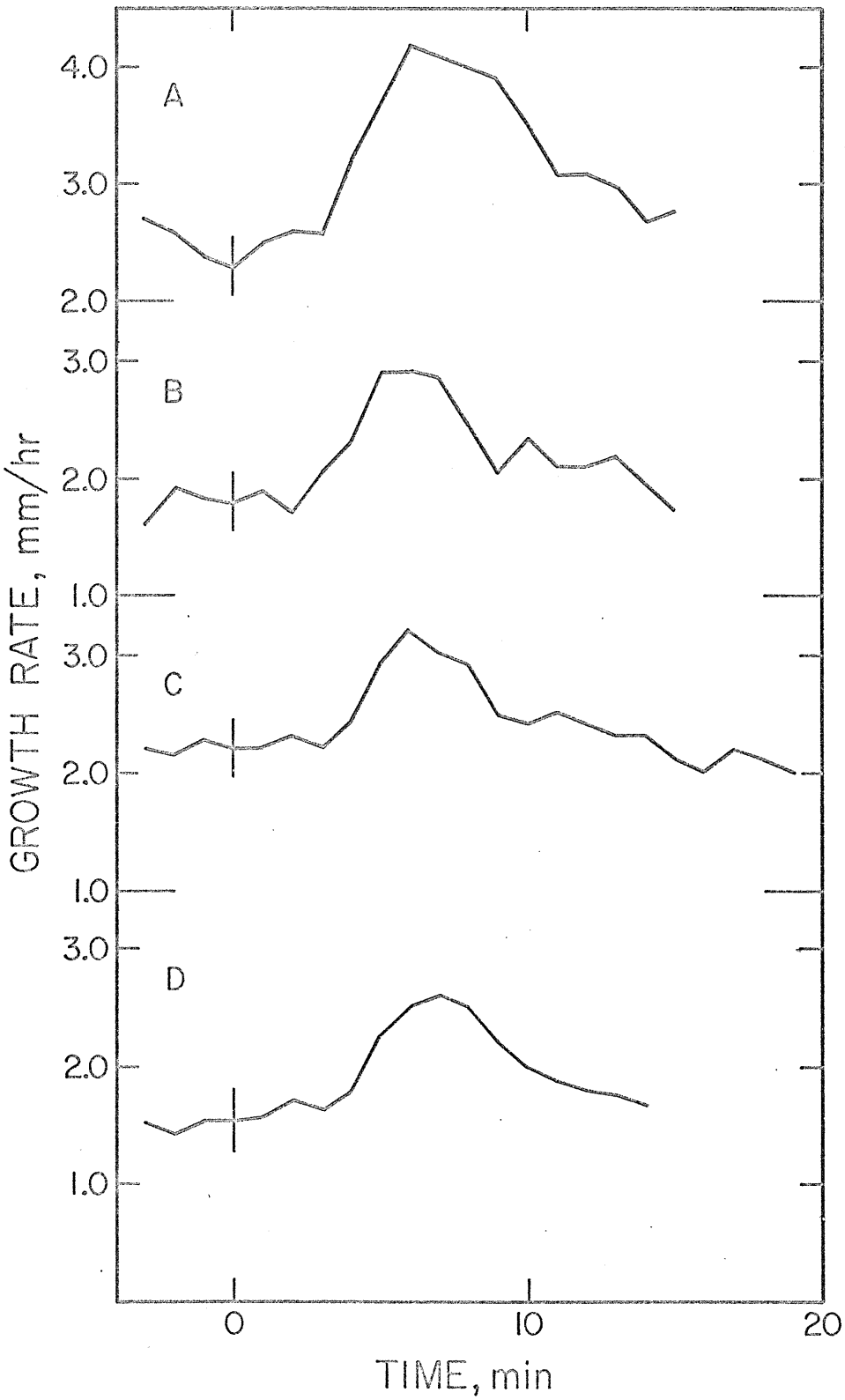
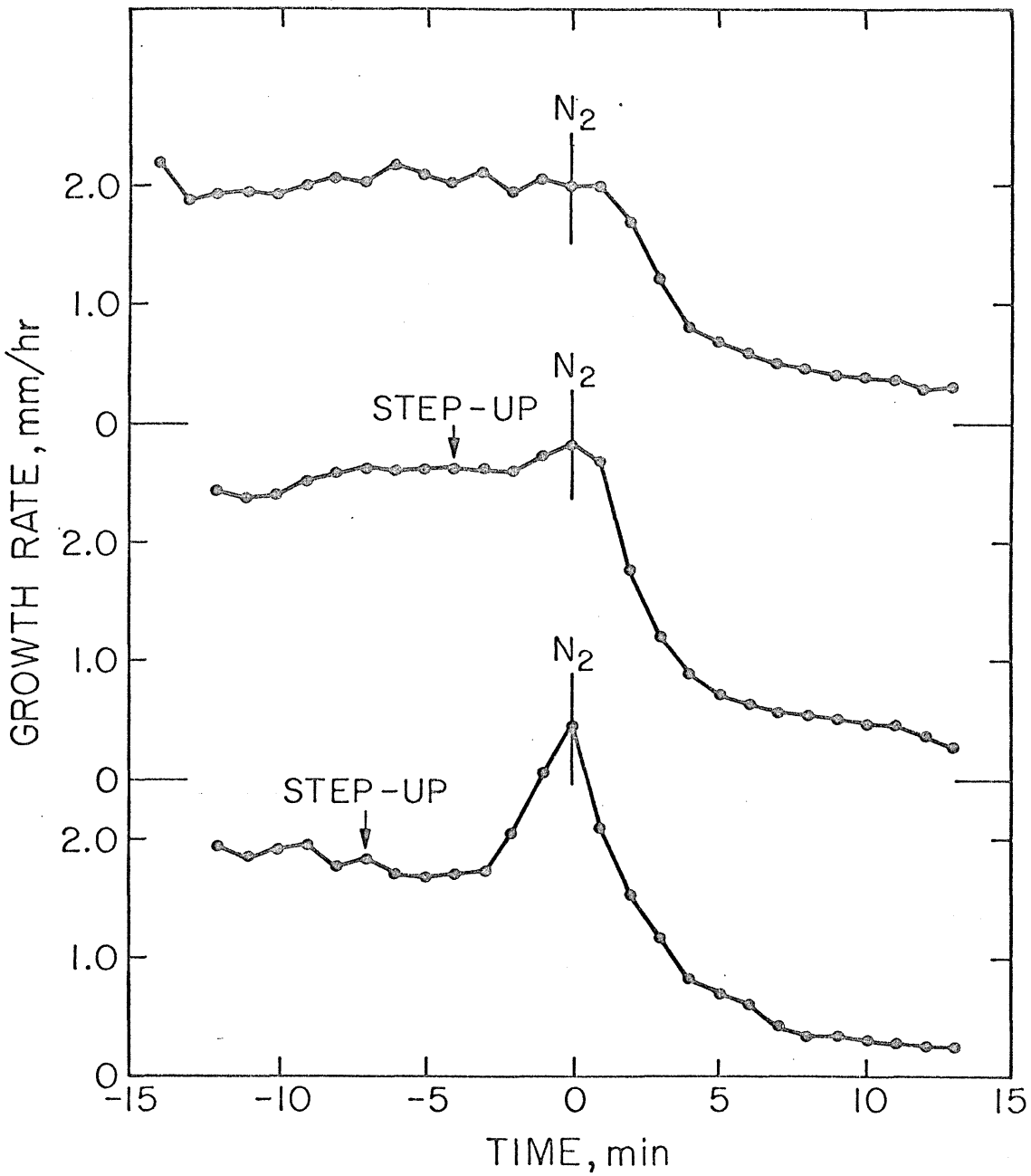

4.0
3.0
2.0
A
3.0
2.0
1.0
B
3.0
2.0
1.0
C
3.0
2.0
1.0
D
GROWTH RATE, mm/hr
0
10
20
TIME, min

FIGURE 5. The Effect of Anoxia on the Growth and Growth Response of Stage IV Spphs

To determine if the growth response had an immediate need for oxygen, the air around a spph was replaced by nitrogen gas (at time zero). The upper curve gives the effect of nitrogen on spphs in steady state growth. The middle curve is of spphs just starting their growth response when the air was replaced by N_2. The air was replaced four minutes after the light stimulus. The bottom curve is of spphs in the middle of their growth response. The light stimulus was given 7 minutes before the N_2. Each curve is the average of 4 experiments.


N2
STEP-UP
N2
STEP-UP
N2
2.0
1.0
0
2.0
1.0
0
2.0
1.0
0
GROWTH RATE, mm/hr
-15
-10
-5
0
5
10
15
TIME, min

Hedera leaf sections (see methods section) to test whether the manometer could respond to the five minute growth response. The rate of oxygen consumption by the leaves in the dark was measured and then a bright light was turned on causing the leaves to suddenly start producing oxygen. The manometer requires four minutes to respond fully to this change (Figure 6). This result indicates that the manometer can respond rapidly enough to detect a five minute transient in the respiration rate.

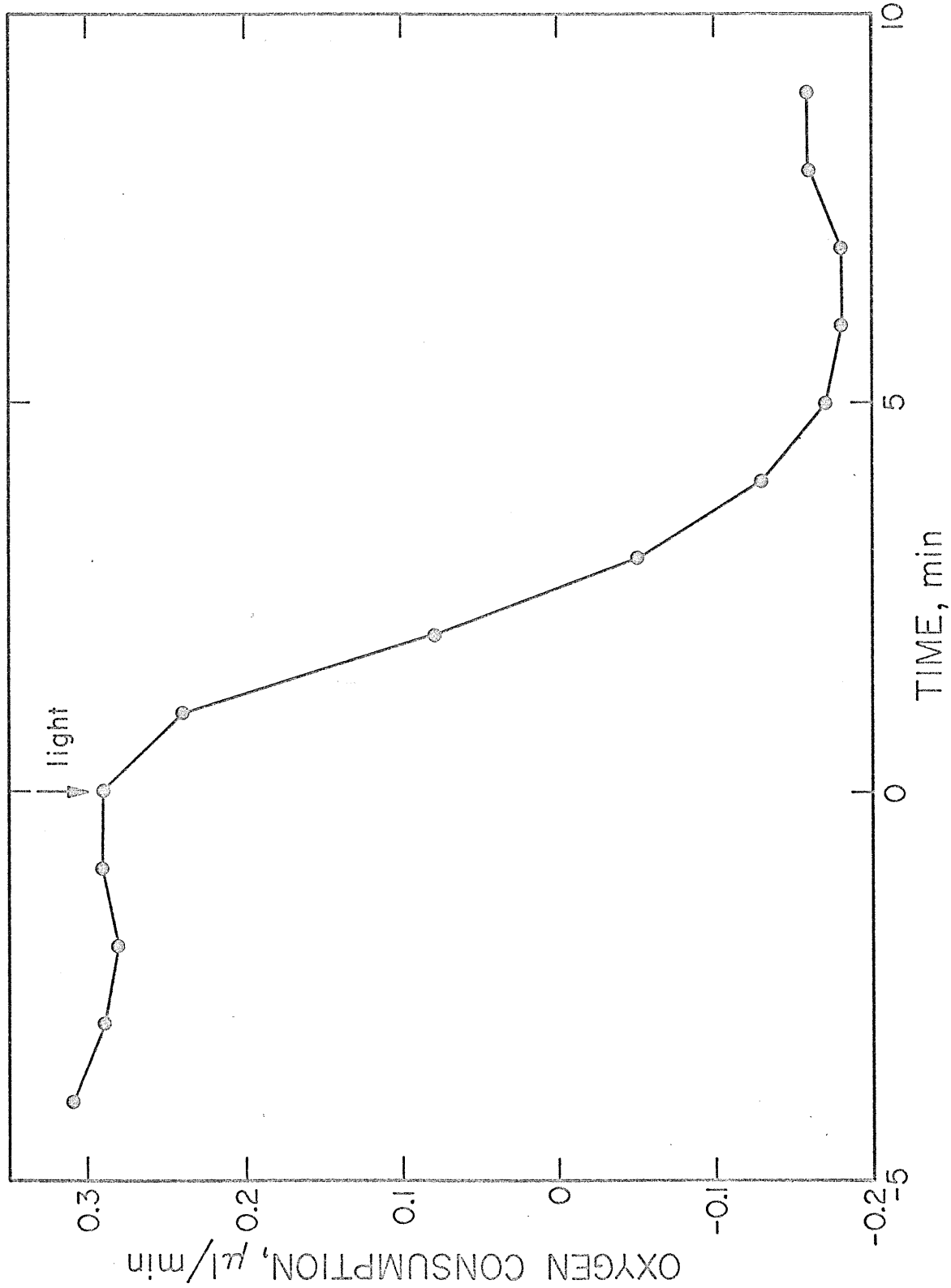
DISCUSSION

When spphs mature from stage I into stage III their respiration rate increases markedly (by 0.5×10^{-2} $\mu l\ min^{-1}$). Thereafter it appears to remain nearly the same. The respiration of stage IV spphs which developed a sporangium when they were 5 mm long decreases somewhat by the time they are 50 mm long. Stage III spphs have the highest rate of oxygen consumption per unit dry weight. It is in stage II and III that the spores are produced; therefore it appears that this process requires a significant amount of the spph's energy.

In contrast to stage IV spphs, the rate of oxygen consumption of stage I spphs increases as they elongate. Their dry weight also increases faster than stage IV's does. Thus it appears that the stage I spphs gets more cytoplasm

FIGURE 6. Response Time of the Manometer

Pieces of Hedera leaf (1 cm x 2.5 cm) were used for the assay. The leaf sections were first kept in the dark, where they consumed oxygen, and then a bright light was turned on causing them to produce oxygen. The time it takes the manometer to go from one steady state to the next is a measure of the response time of the manometer. The curve is the average of four experiments.


OXYGEN CONSUMPTION, μl/min
0.3
0.2
0.1
0
-0.1
-0.2
-5
0
5
10
TIME, min
light

from the mycelium than stage IV does as it elongates.

Both stage I and stage IV spphs grow faster than the mycelium does. However, their respiration per unit dry weight is less than that of the mycelium (Table II). Table II also lists some Q_{O_2} values of other fungi.

In 25 and 50 mm long stage IV spphs the top 6.5 mm consumes one-half of the oxygen, and the columella appears to consume nearly 2/3 of this. As the spphs elongate from 25 to 50 mm, the rate of respiration of the growing zone and the columella remains the same while the oxygen consumption along most of the rest of the stalk is diluted by the elongation. This may indicate that a large fraction of the spph's metabolism is required for the maintainance of the various processes associated with growth. If so it probably implicates the involvement of the columella in growth. This involvement may include processes associated with apical dominance. In vascular plants, the growing root tips also have a higher respiration rate than the older parts of the root (Lundegardh, 1966).

However, it is possible that the high rate of oxygen consumption in the top of the spph has no direct connection to growth. There is no detectable increase in the rate of oxygen consumption associated with the light stimulated growth response. A calculation based on the energy required to synthesize cell wall predicts that a fifty per cent

TABLE II

Oxygen Consumption per Unit Dry Weight of Several Fungi

Organism	Q_{O_2} ($\mu l\ hr^{-1} mg^{-1}$)	Reference
1. Yeast	40-80	(Fruton and Simmonds, 1961)
2. Neurospora (germinating spores)	16-24	(Sussman, et al., 1956)
3. Rhizoctonia soloni (mycelium)	8-16	(Van Etten, et al., 1966)
4. Sclerotium bataticola (mycelium)	8-20	(Van Etten, et al., 1966)
5. Schizophyllum commune (thallus, including carpophores)	20-80	(Wessels, 1965)
6. Phycomyces blakesleeanus (thallus, including spphs)	15-55	(Wassink, 1934)
7. Phycomyces blakesleeanus (germinating spores)	11	(Rudolph, 1960)
8. Phycomyces spphs	5-15	(Table I)

increase in growth rate would require only a 1-3 per cent increase in the rate of respiration of the spph (see Appendix). It appears that the synthesis of the cell wall itself requires relatively little energy and that perhaps this is all that is required during a growth response.

There are two other examples of poor correlation between growth rates and respiration rates. The respiration of the top 6.5 mm of stage IV spphs was measured by placing the rest of the spph inside a capillary. Figure 2 of Chapter 2 shows that 7 to 8 hours after doing this the growth rate of these spphs has declined to $\frac{1}{2}$ its maximum value. However, the oxygen consumption of these top sections does not decline during this period. Spphs with and without sporangia have the same rate of oxygen consumption. Spphs which have been without sporangia for two hours grow only 1/10th the normal rate (Chapter 1, Figure 4). However, it is possible that such processes as branch formation have a compensating effect on the rate of oxygen consumption.

Using the rate of oxygen consumption of the spphs as an index of the level of metabolic activity requires the assumption that oxygen consumption is tightly coupled to phosphorylation in the mitochondria. In Phycomyces spphs this assumption is open to question. The mitochondria isolated from stage IV spphs have very low P/O ratios (0.1) and almost no respiratory control (Meissner, personal communication).

The spphs have an extraordinary high content of free fatty acids, 36 per cent of the total lipid (Teranishi, 1969). Some long chain fatty acids have been reported to uncouple oxidative phosphorylation (Borst et al., 1962). The free fatty acids do not appear to be degradation artifacts of isolation because the spphs were immersed into boiling isopropanol immediately after plucking. This procedure should inactivate all of the enzymes that might liberate free fatty acids from more complex lipids.

Mitochrondia isolated from spphs contain free fatty acids (Teranishi, 1969); whether there are any in mitochrondia in vivo is unknown. Two arguments can be made for normal coupled oxidative phosphorylation in vivo. If most of the oxygen consumption is not coupled to phosphorylation, then the rate of oxygen consumption of spphs might be expected to be higher than that of other fungi (on a dry weight basis). However, the Q_{O_2} of other fungi is the same or higher (Table II). The mycelium of Phycomyces does not have the high levels of fatty acids (Teranishi, 1969).

Isolated Neurospora mitochondria have a P/O ratio of 0.4 (Eakin, 1968); whereas the P/O ratio of yeast mitochondria in vivo is a more normal 1.0 (Chance, 1959). Low P/O ratios of isolated mitochondria from fungi and vascular plants is usually attributed to the harsh conditions required to break their cell walls. This may be a factor contributing to the

low P/O values found in Phycomyces mitochondria. None the less the unusual free fatty acid level suggests the intriguing possibility that a large part of the mitochondria respiratory activity might be coupled to a function different than ATP production.

APPENDIX

The increase in cell wall volume (A) due to growth is given by

$$A = (r_1^2 - r_2^2)\pi v = 9.4 \times 10^{-9} \text{ cm}^3 \text{ min}^{-1}$$

where r_1 is the radius of the spph (50 μ)

r_2 equals r_1 minus the cell wall thickness (50 μ - 0.6 μ)

v is the spph's growth rate (50 μ min^{-1})

The cell wall is 60 % chitin and 15% chitosan (Meissner, personal communication). Chitin is an N-acetyl glucosamine polymer and chitosan is a glucosamine polymer. The molecular weights of the two monomers are 211 and 179. Assuming that the cell wall is all chitin and chitosan (at a 4:1 ratio), and that it has a density of 1.5; about 0.7×10^{-10} moles of N-acetyl glucosamine and glucosamine are added to the cell wall per minute.

One ATP equilvalent is required to add one monomer to the polymer chains (Mahler and Cordes, 1966). A 50% increase in the growth rate would require an increase of 0.6×10^{-11} moles of O_2 per minute (assuming a P/O ratio of 3). This is equal to 1.3×10^{-4} μl min.^{-1}, which is 1.4% of the amount of oxygen consumed by stage IV spphs.

Glycogen is the main storage carbohydrate in the spphs

(Bergman, et al., 1969). It can be degraded to glucose-1-phosphate, and then converted to glucosamine and N-acetyl glucosamine. This process requires another ATP equivalent (Mahler and Cordes, 1966), and would double the oxygen requirement.

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DISCUSSION OF CHAPTERS 2 and 3

Sporangiophores are commonly regarded as aerial hyphae, implying that there is very little difference between hyphae and spphs. In this discussion I would like to compare the growth and related physiology of the hyphae of several fungi and the spphs of Phycomyces. Most of the information about hyphae is limited to a few species.

Physiologically stage I and IV spphs appear to be similar to hyphae. Elongation of the hyphal cell wall apparently takes place within the apical 10 microns (Girbardt, 1955; Burnett, 1968). In Rhizoctonia solani and Sclerotium bataticola the oxygen consumption by the hyphae is highest (per unit dry weight) in the apical centimeter (Van Etten, et al., 1966). In Neurospora crassa (Zalokar, 1959b), Rhizopus sexualis and Mucor hiemalis (Hawker, et al., 1968) the concentration of protein, RNA, mitochondria, endoplasmic reticulium and nuclei is highest in the 150 microns nearest the tip. Nuclei are, however, excluded from the 10 μ growing zone. Behind the apical 150 μ. the concentration of storage compounds is higher, and the hyphae become vacuolated, so that the protoplasm content decreases. The anatomy of Phycomyces hyphae is similar (Thornton, 1966).

Electronmicroscopy shows a similar distribution of particles in the spphs of Phycomyces (Thornton, 1966 and 1968).

The only major difference is that stage IV spphs have a subapical growing zone. Nonetheless the morphology and metabolism of the growing zone is very similar to that of subapical regions in hyphae. The growing zone has nuclei and a central vacuole (Bergman, et al., 1969). It is not the growing zone but the apex, the columella, which has the highest metabolic activity.

The growth of hyphae in some species appears to depend upon the supply of substances from the subapical regions. In Neurospora crassa, if all but the apical millimeter of the hypha is removed, the growth rate is reduced by forty per cent (Ryan, et al., 1943). The net growth was measured 2.5 hours after abscission. Growth is not normal unless at least 10 mm is present. If the subapical region is on a complete medium it can support the growth of the apex across incomplete media. There are a number of species which can do this (Schütte, 1956; Thrower and Thrower, 1968; Butler, 1958). They are called colonizing species. In these species carbohydrates and nitrogen compounds can be transported at least 5 cm to the apex and concentrated there. Phosphorous is not moved as readily. Neurospora and the other species will move material to the tip even if the tip is on a complete medium (Ryan, et al., 1943; Thrower and Thrower, 1968).

In isolating the apical region of Neurospora, the hyphae were cut. Neurospora has septate hyphae. A septum usually

does not completely isolate one cell from the next because it has a central pore (Burnett, 1968). The pores are small; usually about 1/20 of a micron in diameter. Sometimes cell organelles move through them but the pores are too small to allow the cytoplasm to rush out of the cell when the justaposed cell is cut. It is possible that the abscission could cause a decrease in the turgor pressure of the cell. No studies were made in Neurospora of the time required to seal the pore but it seems likely that the cytoplasm is viscous enough to seal it rapidly.

There are also species which cannot colonize (Schütte 1956; Thrower and Thrower, 1968). They do not move nutrients along the hyphae nearly as well. In one of these species, Aspergillus niger, the apical cell (approximately 50 μ long) can grow normally if the rest of the hypha is removed (Nishi, et al., 1968). The apex of this fungus does not depend upon the subapical part of the hypha nearly as much as Neurospora, but it must have a complete medium in order to grow. Aspergillus grows only one per cent as rapidly as Neurospora (Smith, 1923; Burnett, 1968). Aspergillus' slow growth may be related to its noncolonizing growth habit.

Apparently for hyphae to grow, they must get substrates from the environment or from the subapical region of the hypha, or both. If the growing zone of a stage IV spph is isolated from the lower part of the spph, it has neither of these

sources yet it can grow nearly normally for 3 or 4 hours. Spphs which are 1 cm long when plucked will grow another 5 or 6 cm if their bases are in distilled water (Gruen, 1959). However, the term growth is vague. In stage IV spph's growth means elongation without any increase in the level of metabolism. The rate of oxygen consumption does not increase as the spph elongates and the dry weight of attached spphs increases much less than it does in stage I spphs. The amount of neutral lipid (G. Meissner, personal communication), glycogen (Laurent, 1885) and cell wall increase as the spph elongates, probably accounting for most of the increase in dry weight. The production of cell wall and plasmolemma do not appear to require much of the spph's energy.

In mycelium growth involves an elaboration of more protein, DNA, RNA (Gottlieb and Van Etten, 1964; Zalokar, 1959a) and an increase in the rate of oxygen consumption (Van Etten, et al., 1966).

The growing zone does have a long term dependence upon the lower part of the stalk. The net growth of the spph increases as more of the stalk is left uninhibited. Each additional 5 millimeters of uninhibited stalk supports an additional 7 mm of growth. However, the nature of the dependence is unclear. When a stage IV spph becomes 10 to 15 cm long its growth slows down and eventually stops (Gruen, 1959). The protoplasm in these spphs, even in the growing zone, has

become very much diluted by the elongation. It is possible that the growth stops because the protoplasm has become too dilute to support growth, not because the spph has used up something essential for growth. This same dilution occurs in the isolated apical sections.

The growth of stage I spphs has more of the characteristics of hyphal growth. It elongates only $\frac{1}{2}$ as rapidly as stage IV spphs (Bergman, et al., 1969), but its growth involves acquisition of more material from the mycelium. Its respiration increases with the increase in dry weight. In some elegant experiments, Zalokar (1969) estimated the increase in the cell components of the stage I spph as it elongated from 5 to 15 mm. Nuclei increase 4 fold, mitochondria 6 fold, ribosomes and membranes 2 fold, and glycogen 12 fold. When the spph is in stages I, II, and III, it rapidly takes up P^{32} from the mycelium; however, in stage IV the amount of P^{32} remains constant (Bergman, et al., 1969). Stage IV spphs do, however, become labeled with C^{14} if labeled acetate and amino acids are applied to the mycelium (Meissner and Goodell, unpublished). The growth of stage I spphs appears to be analogous to that of colonizing hyphae. It is interesting that if a stage I spph is plucked, it immediately stops elongating, forms a sporangium and then continues growing (Gruen, 1959).

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