FORMATION OF THE COLONY IN THE FUNGUS *CHAETOMIUM**

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Summary

Growth in the fungus *Chaetomium globosum*, on agar medium, is decribed in its following aspects: growth of the hypha, increase in size of the colony, and change of density of the hyphae within the colony.

Individual hyphae increase in length at constant rate but branching produces exponential growth in a mass of hyphae.

The colony has been studied from germination of the spore until a constant rate of marginal expansion is set up. On germination there is a lag phase of growth; this is followed by acceleration of growth to an exponential phase; and finally a steady state is set up in the margin so that it expands at constant rate.

It is concluded that the formation of the colony can be described on the basis of growth of the hyphae, by postulating a *growth unit*. This is defined as a free-growing hyphal tip associated with a growing mass of constant size in constant environment.

I. INTRODUCTION

Although fungal colonies have been the subject of innumerable physiological studies, the way in which growth proceeds in the colony and the question whether growth concerns the mass of mycelium as a whole or becomes restricted to some part of it have hardly received attention.

It is the purpose of this paper to describe how growth proceeds in a fungal colony grown on a solid medium. This involves descriptions of growth in the colony and in the individual hyphae, and the correlation of these to describe the formation of the colony.

The fungus studied was the ascomycete *Chaetomium globosum*.

II. EXPERIMENTAL METHODS

(*a*) *Culture Medium*

The standard medium upon which the fungus was grown was 2 per cent. malt agar. When the effect of starvation was studied, plain agar was used as the culture medium. In the general experiments, the medium was contained in petri dishes 10 cm in diameter which had been selected for uniformity, particularly for an even thickness of glass in the bottom. The dishes were filled with medium to a depth of 6–8 mm. The colonies were grown at a constant temperature of 28°C.

*The work was carried out partly at the University of Tasmania and partly at the University of Sydney.

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(*b*) *Methods of Culture*

Except for an experiment in which hyphal-tip inoculation was used, all colonies were grown from single spores. Such inoculations were made by picking up a single spore from a group of spores on agar, using a fine platinum point and high-power binocular magnification, and transferring it to a marked site on an agar plate. Such inoculations were inspected to ensure that only single spores had been transferred.

Hyphal tips were inoculated by transferring a hyphal tip from the margin of a colony by the above method.

(*c*) *Measurements*

(i) *Rate of Increase in Length of Individual Hyphae.*—This was studied by photographing hyphae from time to time. Enlargements were made of these photographs, from which hyphal lengths were determined.

(ii) *Rate of Enlargement of Surface of Colony.*—Each colony was measured along two axes at right angles and an average of the measurements used. This method gave rise to some error in very young colonies which, consisting of only a few hyphae, were usually not circular.

Colonies having a radius less than 1–2 mm were measured under the microscope, using ocular micrometers. For measurements up to about 100 μ, the micrometer unit was equal to about 17·5 μ and readings could be estimated to about one-third of a unit, i.e. to about 6 μ; in the range 100–1000 μ the micrometer unit was 87·5 μ and readings could be estimated to about one-quarter of a unit, i.e. to about 22 μ.

(iii) *Rate of Increase in Length of Mycelium after Germination.*—This was studied during the first few hours of growth after germination of the spore, from a photographic record. Length of mycelium was determined from suitable enlargements. Phase-contrast microscopy was used.

(iv) *Effect of Depth of Medium on Surface Growth.*—To determine whether depth of medium affected rate of growth of the surface mycelium, a technique was required which would minimize all variables other than depth of medium. For example, it was found that the rates of radial enlargement of colonies grown on shallow (1–2 mm) and deep (8–10 mm) media were usually, though not always, faster on the shallow medium in young colonies, but faster on the deep medium in older colonies (5–30 mm radius). An experiment was therefore set up in which the one plate was divided into shallow (0·5 mm) and deep (4·5 mm) regions by placing a thick slab of glass in it. Spores were then plated on the surface of the medium along the line of the edge of the slab.

(v) *Growth of Colony below Surface of Medium.*—Growth of mycelium into the medium was measured in vertical sections through the colony. The scales used were the same as for measurements of surface growth. A series of measurements was made on colonies grown in petri dishes.

In another series the effect was studied of restriction of the surface of the medium to a small area. In these experiments colonies were grown in tubes of 7 mm and 12 mm radius, and the depths to which hyphae grew were measured in vertical

sections through the colony. In long experiments there was shrinkage in the agar column: this was allowed for in determining the depth of growth.

(vi) *Density of Hyphae over Surface of Colony.*—Regional densities of surface hyphae were determined from photographs of the surfaces of colonies. Tracings of the surface hyphae were made from these photographs—being in sharpest focus they could usually be distinguished from other hyphae. On the tracings concentric rings were described about the centre of the colony. Hyphal lengths were measured in each ring and unit densities calculated; in some of the larger colonies the measurements were made on a sector or sectors, or upon other areas, when the whole colony could not be dealt with satisfactorily. From such measurements also the whole length of hyphae in the surface of colonies could be determined.

TABLE 1

LENGTHS (μ) OF THREE HYPHAE* AT SUCCESSIVE TIMES OF MEASUREMENT

Hypha No.	Part Measured	Time of Measurement (hr min): 0.00	1.15	2.50	4.30	6.30	9.50	12.40
I	Main hypha	44	67	115	168	235	342	437
	Branch hyphae	—	—	—	10	24	288	778
	All hyphae	44	67	115	178	259	630	1215
II	Main hypha	72	119	147	187	226	336	
	Branch hyphae	—	—	19	45	79	403	
	All hyphae	72	119	166	232	305	739	
III	Main hypha	62	101	154	211	269	349	
	Branch hyphae	—	—	—	61	255	837	
	All hyphae	62	101	154	272	524	1186	

*The correlation coefficients for the three sets of data (omitting the first point of each (see second footnote, p. 56)) are all highly significant—arithmetic scale: I, 0·998; II, 0·990; III, 0·997; logarithmic scale: I, 0·996; II, 0·927; III, 0·998.

III. MORPHOLOGY

The spores of *Chaetomium globosum* were thick-walled and ellipsoidal, the axes measuring about 8 and 9 μ. Germination occurred 2–2½ hr after plating, the germ tube being protruded from a pore at one end of the spore.

The young colony consisted of one, perhaps two, hyphae, usually unbranched at first, so that their elongation produced asymmetry. As branching proceeded, more and more of the free space around the spore became occupied, so that the colony gradually became circular, this form being attained fully in a colony of about 1 mm radius. As the colony enlarged further, the arrangement of the marginal hyphae became stabilized, the hyphae mostly pointing radially outwards in the advancing

border. Such a stabilized arrangement of marginal hyphae was associated with a constant rate of marginal expansion.

The mycelium was septate, the "cells" being multinucleate and their length rather variable (13–48 μ). Septa were not found close to the tips of the hyphae, so that there was a long apical segment (46–120 μ). The diameters (2·8–4·2 μ) of hyphae formed in the margin of the colony were greater than the diameters (0·8–2·2 μ) of hyphae formed subsequently within the colony; the latter were progressively finer as branching continued. Hyphal diameters were smaller in starved colonies.

IV. Growth in the Colony

(*a*) *Growth of a Hypha*

The rate of growth of individual hyphae was studied by measuring the increase in length of hyphae and their branches. Measurements on three hyphae* are given in Table 1 and those on hypha I are plotted on arithmetic and logarithmic scales in Figure 1. They show that:

(i) Growth in length of an individual hypha proceeds at constant rate (Fig. 1, *EF*, (*AD*)).

(ii) Growth in a hypha and its branches is exponential,† the amount of new hyphal material added being proportional to the mass present (Fig. 1, *BC*).

Linear growth of individual hyphae is associated with a continual increase in the number of hyphae, so that growth in the whole mass is proportional to the amount present and the growth curve is exponential. Branching occurs at *A* (Fig. 1) and this represents a length of hypha of the order of 130 μ (region of log 2·10 μ). In six other sets of measurements the point *A* varied between 110 and 180 μ (average of seven measurements 150 μ).

(*b*) *Increase in Size of Colony*

Rate of growth of the colony was measured in the first place as rate of expansion of the mycelium over the surface of the medium. The relationship between growth over the surface and growth into the medium was then investigated. The effect of depth of medium upon growth of the colony was studied (1) when the area of the surface of the medium was unlimited but its depth was restricted, and (2) when the area of the surface was restricted but its depth was unlimited.

Rate of change of mean colony radius (Fig. 2) was exponential until the radius was about 0·1 mm. This phase was followed by a period of declining logarithmic

*These measurements were made on hyphae growing in a hyphal-tip inoculation; similar results were obtained for hyphae in young, single-spore colonies. The hyphae measured, and their branches, were of the same diameter.

†The part *BA* of the graph *BC* (Fig. 1) deviates from the straight line because the whole of the growing mass is not being included in the measurements. This can be shown by adding an equal amount to all values, making the first value about 150 μ, when the initial deviation disappears. Over-compensation produces an opposite deviation.

rate but increasing linear rate until, at a radius of 3–5 mm, the rate of radial expansion became constant. Thereafter, it continued at constant rate until obstructed, the largest colonies measured still enlarging in this way at a radius of about 70 mm.

Starvation had the effect of reducing the rate of growth. The reduction in the exponential rate was not apparent when measured in terms of colony radius, but was shown by measurements of the total length of hyphae in the surface.

The measurements of colony radius plotted in Figure 2 do not give any information about the earliest growth of the colony, during the first few hours after germination. Rate of increase in length of the mycelium during this period was

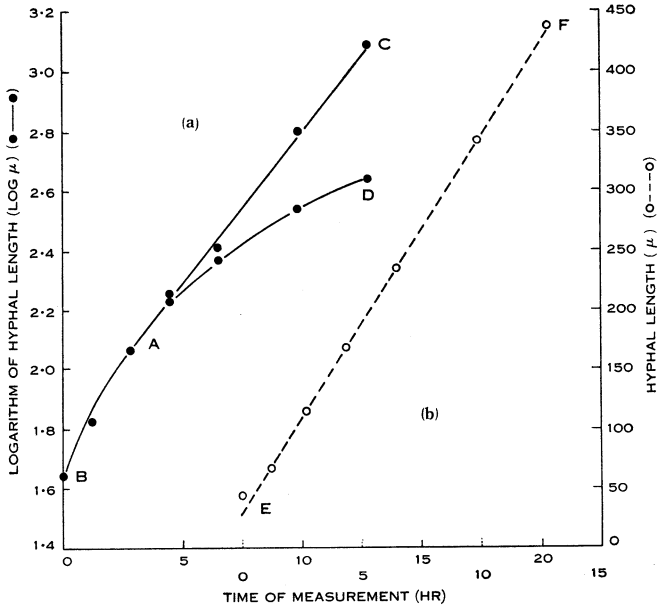

Fig. 1.—Growth of hypha I. (*a*) Logarithm of hyphal length (log μ) plotted against time of measurement: *BA*, unbranched main hypha; *AC*, main hypha with its branches; *AD*, main hypha alone. (*b*) Rate of elongation of main hypha alone.

linear, with equal increments of length in unit time (Fig. 3). This constant rate does not appear to continue for long and there is some evidence that the rate is accelerating in mycelium 50 μ long.

Growth downwards into the agar medium kept pace with growth over its surface, making the colony hemispherical. The least squares equation for data on a series of colonies in size between 0·1 and 8·5 mm radius was

$$y = 1{\cdot}0032\,x + 0{\cdot}048,$$

which is very close to the straight line $y = x$ passing through the origin.

Growth over the surface of the medium was not affected by the depth of the medium—the surfaces of colonies in plates in which the medium was partly deep and partly shallow were circular.

When colonies were grown in tubes, restricting the surface of the medium, growth downwards kept pace with that over the surface for a time, but slowed markedly when the surface hyphae reached the wall of the tube. It is suggested tentatively that this slowing was associated with inefficient gaseous diffusion, due to occlusion of the surface.

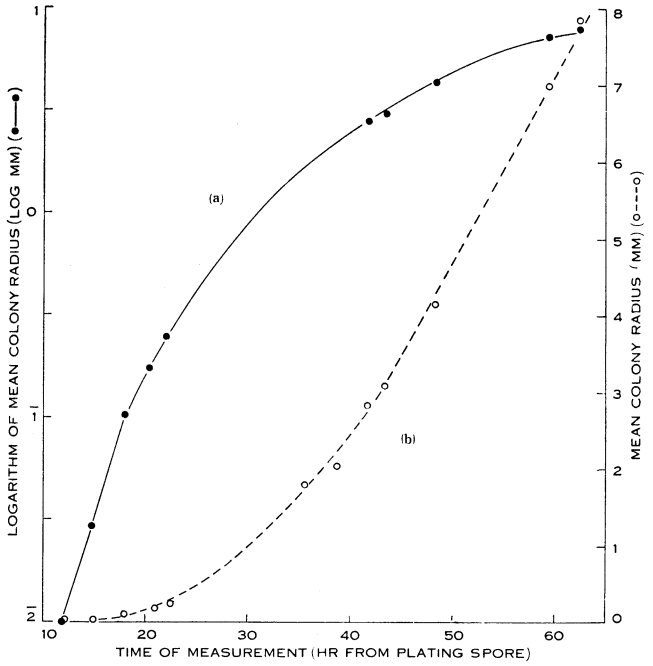

Fig. 2.—Increase in size of surface of colony. (*a*) Logarithm of mean colony radius plotted against time of measurement. (*b*) Rate of increase of mean colony radius.

(*c*) *Density of Hyphae within the Colony*

The distribution of hyphae in a colony was studied by measuring change of hyphal density over its surface. Hyphal lengths were measured in concentric rings of surface and unit densities calculated by the formula:

$$d_1 = l_1/(r_1^2 - r_0^2),$$

where d_1 = density over the ring,

l_1 = length of hyphae in ring,

r_1 = outer radius of ring, and

r_0 = inner radius of ring.

The curves of density variation over the surface of the colony are of two types (Fig. 4). In colony (*a*) (radius $<0{\cdot}216$ mm) change in density is exponential from the centre outwards, but in larger colonies ((*b*)–(*g*)) change in density is exponential only in the margin and inside this changes more and more slowly.

The curves also bring out three other features of growth in the colony. Firstly, the decline from the marginal exponential phase began at about the same density in all the colonies, at a value of 10–20 units (1·0–1·3 on log scale). In other words,

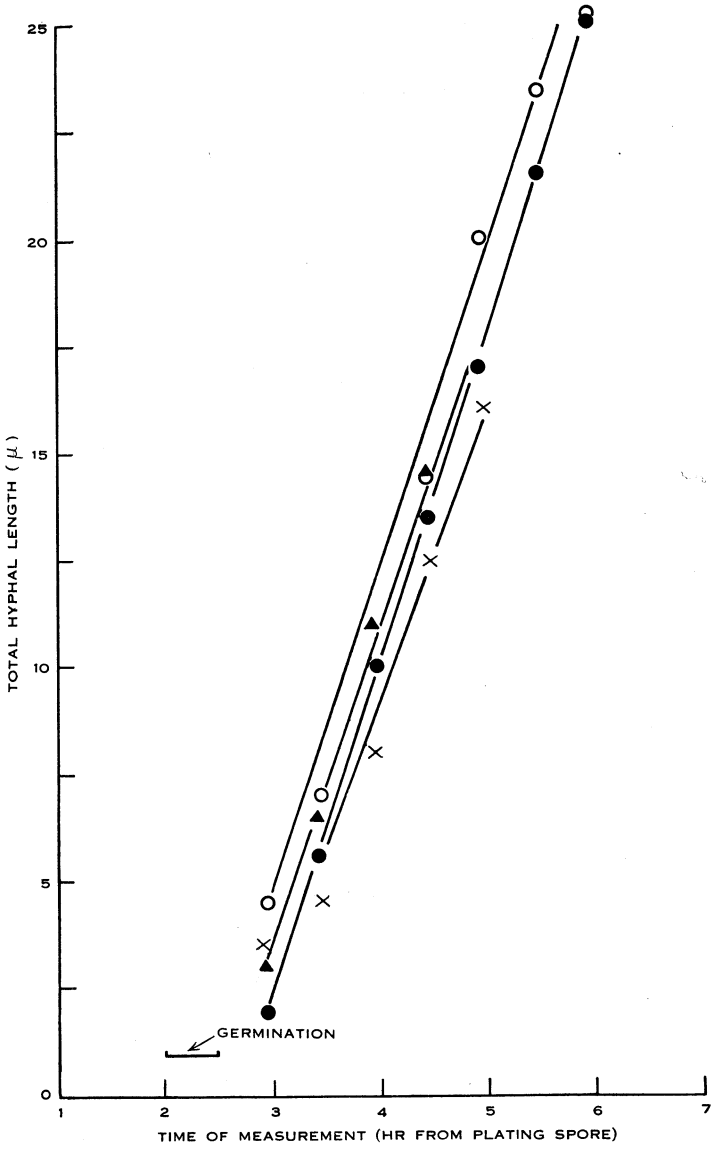

Fig. 3.—Rate of increase in length of mycelium in first few hours after germination. Individual measurements for four colonies (●, ○, ▲, and ×).

under the conditions of experiment the medium supported exponential growth only so long as the density of hyphae did not exceed 10–20 units. This is also shown in Figure 5 in which change of density is followed in some region, growth rate there

increasing exponentially at first but declining after a time. Secondly, density tended towards a maximum value, hyphal saturation extending outwards progressively as the colony enlarged. Thirdly, the extent of the outer fringe of exponential growth was of the order of 0·1 mm (100 μ).

The decline in growth rate within the central mass is actually more pronounced than the density determinations show. The latter are based on measurements of the *length* of hyphae and take no account of their diameter: hyphae formed within the colony are progressively finer as branching proceeds.

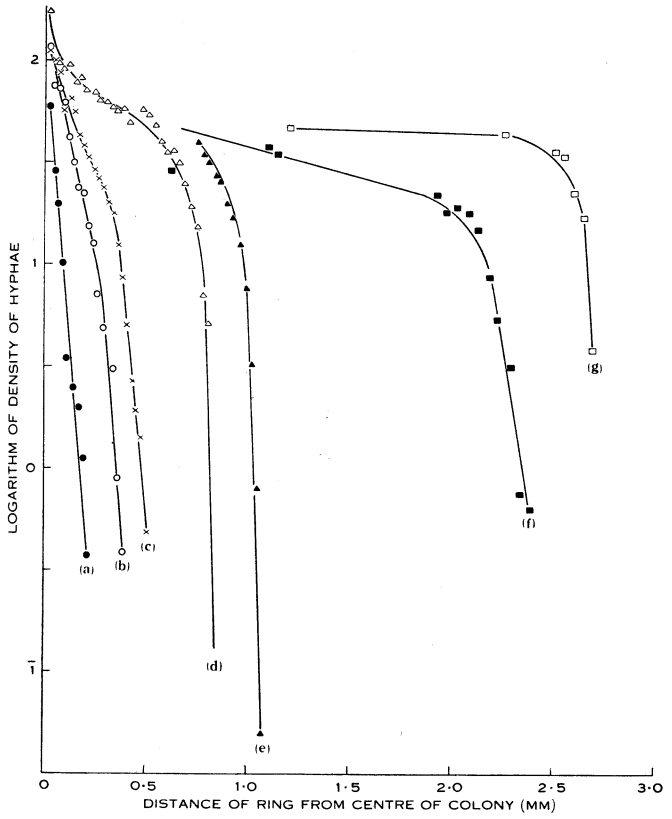

Fig. 4.—Variation in hyphal density over the surface of colonies of various sizes. Logarithmic scale.

In the larger colonies regional counts showed that branching followed a similar pattern to hyphal density. The counts did not permit accurate determination of this feature, but the difference between the marginal and central regions was clear.

Change of density in colonies grown on plain agar, and therefore starved, showed the same features as in colonies grown on the standard medium, but the density values were less.

V. Discussion

The picture built up by the measurements and observations reported above shows that growth in *Chaetomium* has its basis in the characteristics of hyphal growth: hyphae increase in length at constant rate, but because they branch from time to time, increase in length of the branching mass of hyphae is exponential.

Before considering the mechanics of growth in *Chaetomium* further, relevant published work will be reviewed briefly. Only three papers, one by Castle (1940) and two by Smith (1923, 1924), have been seen which deal with the manner of growth of

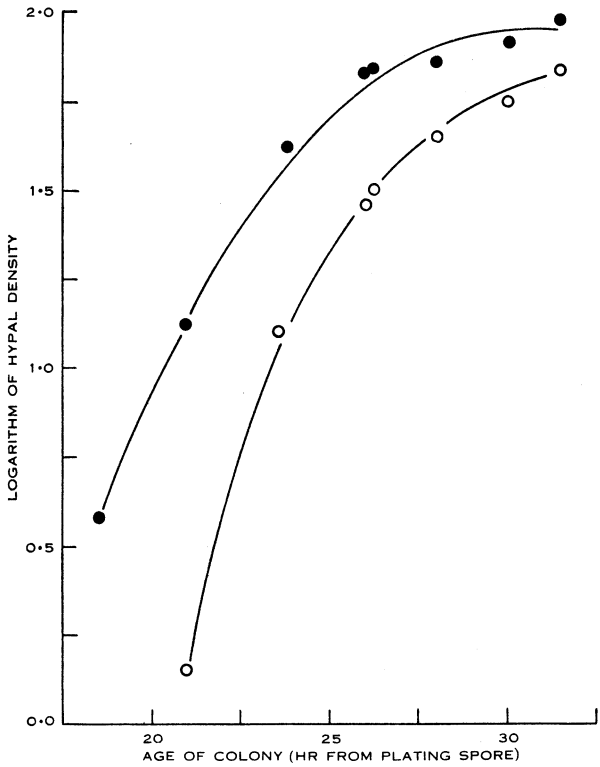

Fig. 5.—Variation in hyphal density at sites 0·12 mm (●) and 0·24 mm (○) from the centre of colonies of various sizes. Logarithmic scale.

fungal hyphae. Castle studied the growth of the sporangiophore of *Phycomyces*, a cell attached at one end to the mycelium from which it is nourished. More relevant to the present work, however, are Smith's studies of the growth of hyphae in several species of fungi.

Smith (1923, 1924) found in representative fungi of widely separated genera that growth in length of a hypha was purely apical, and observed that cross walls were not laid down in the tip of the hypha, i.e. the length of the apical segment was

greater than that of segments behind it, usually in the proportion of about 3 : 1. In the present work on *Chaetomium*, also, growth of the hypha was apical, and the apical segment was longer than those behind it, again in the proportion of about 3 : 1.

Smith (1924) showed that in *Botrytis* growth of a hypha with its branches was proportional to the total length of hyphae present, but that the growth rate of an individual hypha declined from the exponential rate—his measurements show that the rate of growth of the individual hypha was linear. Measurements given in his earlier paper (1923) show that growth of the hypha in *Fusarium* and *Pyronema* was linear also.

Smith (1924) also drew attention to differences in rate of growth between different hyphae and in the same hypha at different times. Similar observations were made on *Chaetomium* in the present work: the differences appear to be normal variation.

Leaving aside for the present any consideration of whether the growth rate of hyphae is intrinsically linear or intrinsically exponential, the formation of the colony in *Chaetomium* can be described on the basis of growth of the hyphae.

On germination of the spore, the mycelium, usually a single unbranched hypha, grows at constant rate for a time (Fig. 3), but later the growth rate increases until the colony is growing exponentially. Such exponential growth involves not only the individual hyphae, as shown by exponential increase in the radius of the colony (Fig. 2), but also the whole mass of mycelium, as shown by the exponential change in density of the hyphae (Fig. 4, colony (*a*)).

Thereafter, growth in the colony can be considered from two points of view, growth in the margin and growth within the colony. Growth takes place in the margin in such a way that the radius of the colony increases at constant rate (Fig. 2). Within the colony growth not only slows down progressively (Figs. 4 and 5), but the hyphae formed there are of smaller diameter, evidently a response to less favourable conditions for growth (*vide* starved growth), an instance of biological adaptation. A maximum hyphal density is attained gradually within the colony, there being a gradient between a central mass of maximum density and the margin (Fig. 4).

While the above remarks upon growth in the colony refer more particularly to growth in its surface, they are also generally applicable to the colony as a whole. The colony grown from a spore plated on the surface of the agar has the form of a hemisphere, growth proceeding radially into the medium at the same rate as growth in the surface.

The characteristics of hyphal growth appear to point to a functional unit of growth in *Chaetomium* rather than a morphological one; this will be referred to as a *growth unit*. This notion is based principally upon the observations on the growth of individual hyphae (Fig. 1), the hypha increasing in length at constant rate (linear growth), while the increase in length of the mass of hyphae is proportional to its amount (exponential growth) and is associated with branching. Branching occurred

when the hypha was of the order of 150 μ long (at A, Fig. 1), which is somewhat longer than the apical segment.*

Considered functionally, the growth unit will be a free-growing hyphal tip associated with a growing mass of constant size in constant environment. This is possible because of the tubular form of the hypha, the growing tip as it were continually leaving behind itself material in which growth is independent. In the growth unit, growth might be intrinsically exponential, i.e. proportional to the amount of growing material; or it might take place at constant rate, the occurrence of branching as the hypha becomes a certain length producing exponential growth in the mass of hyphae. Where growth involves the elongation of a tube there is little real difference between these two points of view. This tubular form also frees the hypha from the restriction of a limiting surface/volume ratio, and rate of growth is then subject to modification only by the environment.

It is, perhaps, easier to visualize growth in *Chaetomium* as being essentially exponential, and in these terms constant radial expansion of the margin will result from branching, and its rate will be the *value* of the exponential when the growth unit is at its full length (150 μ), this length depending upon the environment and upon the genotype. The initial linear phase on germination will represent a true lag phase of growth, to be explained in terms of some limiting factor in growth and metabolism, or of the necessary organization of the materials of the spore for growth (cf. Hinshelwood 1946). Recent work by Machlis (1957), showing that certain substances (e.g. glutamic acid) will reduce the lag phase, is relevant to this, although the "lag phase" of his studies is a composite period including all changes up to the rounding-off of the colony.

The principal difficulty in visualizing growth as being linear lies in there being a marked difference between the linear rate in the hyphae of the lag phase and the linear rate in the marginal fringe, the former being only about one-third of the latter. However, limiting factors can be brought forward in argument here.

Lastly, brief comment may be made upon the phenomenon of branching, the process which leads to the occupation of the medium. Presumably, branching occurs because the hypha cannot maintain exponential growth indefinitely—because extension of the mass is localized in the growing tip. Such localization will subject increase in length of the hypha to the limitation of the deposition of new wall material there, itself dependent upon many factors, including translocation of materials within the hypha. Such limitation will impose a constant rate of elongation upon the hypha, but the mass of hyphae can continue to grow exponentially because branching occurs. In fact a steady state is set up in the margin and this is expressed in a constant rate of marginal expansion. The independence of the growth units in the mass is seen clearly when the space available for growth is varied. Thus when an angular shape of actively growing mycelium is inserted into an agar

*The length of the growth unit in *Botrytis* can be derived from measurements given by Smith (1924), the point A being in the region of log $2 \cdot 5$–$2 \cdot 7$ μ, i.e. the length of the growth unit is 300–500 μ, which is three to four times its length in *Chaetomium*; some observations made in connexion with the experiments reported in this paper have shown that the exponential phase in *Botrytis* continues until the radius of the colony is about 500 μ.

plate and grows freely, rate of expansion over the surface of the agar is the same whether the hyphae are advancing along a line or are spreading out radially about an angle.

In this paper so far the term "colony" has been used without qualification. Here we are concerned with organization in the mycelium, not with specialization, and the evidence presented indicates a rather loose dependence between the individual hyphae through the growth unit.

In conclusion, it is suggested that growth by apical elongation of a tubular hypha associated with exponential increase of the mass by branching, might represent an evolutionary pathway by which a single-celled organism could become a larger multinucleate organism.

VI. Acknowledgments

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