487

The Bursting Tendency of Hyphal Tips of Fungi: Presumptive Evidence for a Delicate Balance between Wall Synthesis and Wall Lysis in Apical Growth

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SUMMARY

A wide diversity of treatments can cause rapid and extensive bursting of hyphal tips of *Mucor rouxii*. Most hyphal tips from colonies grown on full-strength agar medium burst readily when flooded with distilled water. In contrast, hyphal tips from colonies grown on diluted medium survived flooding with distilled water but succumbed to dilute aqueous solutions (particularly acids, but also neutral salts, EDTA, alcohols, acetone, detergents). Apex bursting was generally inhibited by alkaline solutions but took place at certain concentrations of ethanolamine or NH_4OH. Mg^{2+}, Mn^{2+} or Ca^{2+} caused pronounced swelling of the hyphal apex followed by an occasional burst. Sharp temperature increases also brought about apical disintegration regardless of nutrient concentration.

Colonies of *Aspergillus fumigatus*, *Fusarium* sp., *Neurospora crassa* (wild-type and two morphological mutants), *Penicillium claviforme*, *Rhizoctonia solani* and *Rhizopus arrhizus* also underwent apical bursting when flooded with water or rapidly heated. *Schizophyllum commune*, several *Phytophthora* spp., and some morphological mutants of *N. crassa* were refractory.

The bursting of hyphal tips by flooding colonies with water is not simply an osmotic phenomenon; its temperature coefficient (Q_{10} 1·3 to 2·0) suggests the additional participation of biochemical reaction(s) as a rate-limiting step in the bursting process. Probably, enzymatic degradation and hence weakening of the wall is a prerequisite for bursting. These observations are offered as circumstantial evidence supporting the following conclusions: (i) the growing tips of fungi have a large wall lytic potential; (ii) the release of this activity during growth must be gradual and delicately co-ordinated with wall synthesis; (iii) this balance can be easily disturbed by a wide variety of external stimuli and the ensuing surge of lytic activity results in the violent disintegration of the hyphal apex.

INTRODUCTION

This investigation is part of a project on the biochemical bases of hyphal morphogenesis in fungi. Since apical growth is the mechanism by which a hyphal tube is generated (Reinhardt, 1892; Smith, 1923), one of the main objectives of our study is the elucidation of the biochemical events involved in wall construction at a hyphal tip. A general consideration of the process of wall growth suggests that two different types of biochemical activities are probably essential for apical wall extension: those responsible for adding new polymers (wall synthesis) and those responsible for plasticizing the existing wall fabric (wall lysis) (see Discussion).

Direct quantitative evidence for wall synthesis in the hyphal apex can be obtained by autoradiography (Bartnicki-Garcia & Lippman, 1969; Gooday, 1971; Katz & Rosenberger,
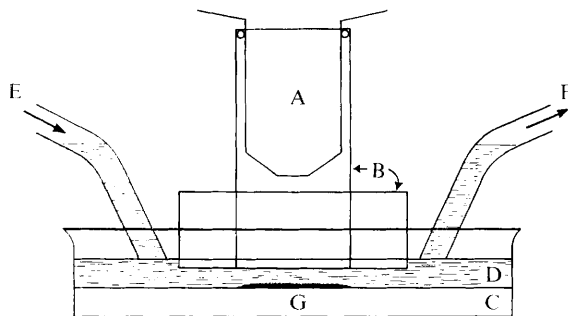
Fig. 1. Device used to study hyphal tip bursting at different temperatures. Objective lens (A) fitted with plastic housing (B). Agar layer (C) flooded with running water (D) from a constant-temperature bath entering through inlet tube (E) and removed by suction (F). Fungal colony (G).

1971 a); however, direct evidence for the participation of lytic enzymes in apical wall extension is unavailable.

The present findings, which amplify those made earlier by Robertson (1958, 1959) and by Park & Robinson (1966a) are offered as circumstantial evidence pointing to the existence of a large potential of wall lytic activity in the hyphal apexes and its possible participation in the process of apical wall growth.

METHODS

Organisms. The principal fungus employed was *Mucor rouxii* IM-80. *Neurospora crassa* (wild-type RL3-8A and eight morphological mutants thereof) were supplied by S. Brody of the University of California, San Diego; *Rhizopus arrhizus* QM-1032 by E. T.Reese from the U.S. Army Laboratories, Natick, Massachusetts, U.S.A.; *Phytophthora palmivora* (P113) by M. Aragaki from the University of Hawaii, Honolulu. All other fungi were obtained from the fungal collections of this Department.

Culture media. Ordinary YPG agar (1X.YPG) contained (g/l): Difco yeast extract, 3; Difco peptone, 10; glucose, 20; and Difco Bacto agar, 25. The medium was adjusted to pH 5·5 with 2 N-H_2SO_4. YPG of other concentrations was prepared by increasing or decreasing the concentration of nutrients as indicated. V-8 agar was prepared from V-8 juice (Campbell Soup Co., Camden, New Jersey); $CaCO_3$ (15 g) was added to 1000 ml V-8 juice, mixed and centrifuged at 1000 g for 15 min. The final medium contained 200 ml of this clear supernatant and 15 g agar per litre of deionized water. Cultures were grown in 100 × 15 mm clear polystyrene Petri dishes containing 15 ml of agar medium. *Mucor rouxii* and *Neurospora crassa* RL3-8A (wild) were inoculated from a washed spore suspension by a platinum loop. The inoculum was deposited in the centre of the plate. Cultures were incubated until colonies achieved a diameter of 1 to 1·5 cm.

Flooding with water and other dilute solutions. To observe the behaviour of hyphal tips upon addition of distilled water or other solutions, a Reichert Zetopan microscope was fitted with a protective plastic housing attached to the low power (× 10) objective lens. Two procedures were employed: for routine observation, the colonies were simply flooded with 20 ml of liquid; for experiments on the effect of temperature of the flooding water, a flow system was devised to rapidly equilibrate the temperature of the fungal colony (Fig. 1). A large volume of distilled water was maintained at the desired temperature using a refrigerated and heated bath and circulator (Forma Temp Jr.; Model 2096, Forma Sci. Inc., Marietta, Ohio, U.S.A.). The water was pumped at a rate of 200 ml/min and delivered as a jet near the
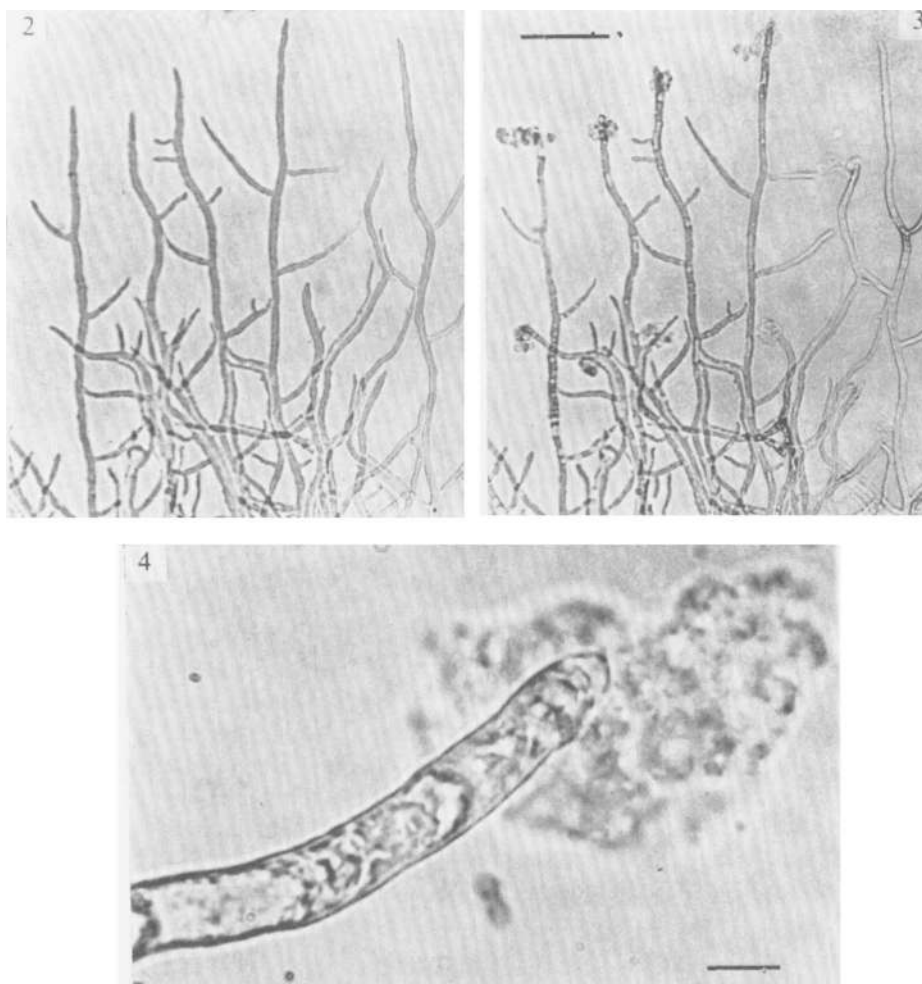
Fig. 2 to 4. Bursting of hyphal tips of *Mucor rouxii* grown on 1X. YPG by flooding colony with distilled water. Fig. 2 taken immediately after addition of water. Fig. 3, 1 min later. (Scale marker = 100 μm.) Fig. 4, detail of burst tip. (Scale marker = 10 μm.)

periphery of the colony. Excess water was removed by suction at the opposite side of the colony to permit a rapid stream of water to travel across the fungal colony. Bursting events were scored by hand with a microswitch connected to the event marker of a linear recorder at a chart speed of 6 in/min.

Heat shock. Agar plates with colonies 1 to 1·5 cm in diameter were heat shocked by placing the covered dishes in a forced-air oven (Precision Sci. Co. Model 18) at the temperatures and times indicated.

RESULTS

Apical disintegration by 'osmotic shock'

Mucor rouxii. If a colony of *Mucor rouxii*, growing on 1X.YPG agar, was flooded with distilled water, there was extensive bursting of the hyphal tips around the entire colony margin (Fig. 2 to 4). After addition of the water, there was a short delay, the duration

Table 1. *Effect of concentration of growth medium on apical bursting*
of Mucor rouxii

YPG medium concentration	Bursting*
4×	+ + + + + (4 s)
2×	+ + + + + (4 s)
1×	+ + + + (4 s)
½×	+ + + (8 s)
¼×	+ + (14 s)
⅛×	−
1/16×	−

* Relative intensity and approximate time of initiation of bursting after flooding the colony with distilled water at 22 °C.

of which depended on temperature, before hyphal tips began to explode; within a few seconds, a barrage of bursting occurred with hyphal tips continuing to burst, with diminishing frequency, for 1 or 2 min longer.

In most cases, the cytoplasm was violently discharged through a small invisible opening at the apical dome, and often extruded as a long narrow twisting mass. Tips of main hyphae as well as those of side branches were susceptible to bursting but since the fungus is co-enocytic only the primary tip or one side branch (rarely both) disintegrated.

Cultivation conditions greatly affected the ability of the tips to burst upon flooding with distilled water. For example, nutrient concentration was critical. When the fungus was grown on concentrated YPG media, it burst more rapidly and extensively than on dilute media (Table 1). At dilutions of X/8.YPG or X/16.YPG medium good colony growth was obtained but the hyphal tips no longer burst upon addition of distilled water. We made use of this divergent response (see subsequent sections) to test the effect of solutes on apex bursting: inhibition of bursting was tested on colonies grown on 1X.YPG medium; promotion of bursting was determined on colonies grown on X/16.YPG medium.

Colony age was also important. Young colonies, grown on 1X.YPG for less than 9 h, and older colonies (18 h or longer) did not readily burst. Also the incidence of bursting was higher in crowded areas of the colony. To minimize this variability of response, tests were made with colonies inoculated and incubated under similar conditions.

Other fungi. A number of fungi were tested and most underwent apical bursting when their colonies, grown on 1X.YPG agar, were flooded with distilled water. Extensive bursting similar to that of *Mucor rouxii* was exhibited by *Rhizopus arrhizus*, *Rhizoctonia solani* and *Aspergillus fumigatus*; colonies of *Penicillium claviforme* and *Fusarium* sp. yielded fewer burst tips.

In wild-type *Neurospora crassa*, bursting was extensive but it took place much more slowly than in *Mucor rouxii* (see below). Several morphological mutants of *N. crassa*, affected in their pattern of hyphal development (Brody, 1972), were also examined. Only the *pile* and *ropy* mutants showed a clear tendency to burst though they did it less extensively than the wild type. With the other mutants, few (*peak, ragged*) or no bursts (*colonial-2, crisp, frost, balloon*) were observed. Seemingly, the bursting tendency is correlated with the spreading ability of the mycelium (i.e. hyphal elongation rate) for only the wild-type and the two mutants, *ropy* and *pile*, with smaller but clearly spreading colonies were prone to bursting.

Schizophyllum commune and four species of *Phytophthora* (*Phytophthora palmivora, P. cinnamomi, P. megasperma* and *P. parasitica*; grown on 1X.YPG or V-8 agar showed no evidence of apical bursting when flooded with distilled water.

Table 2. *Rate and temperature coefficient of hyphal tip bursting upon flooding with distilled water*

	Mucor rouxii			Neurospora crassa		
Temperature	Bursting time (s)*	Q_{10}		Bursting time (s)*	Q_{10}	
5 °C	30·2	2·03		133·5	1·72	
15 °C	14·7			77·6		
25 °C	10·7	1·37		44·5	1·78	

* The most frequent time of bursting (mode) was obtained by interpolation from a frequency distribution graph of bursting times; data were grouped at 3 s intervals for *Mucor rouxii*, and 20 s intervals for *Neurospora crassa*. Colonies were grown for 13·5 to 17·5 h on 1X.YPG agar for *M. rouxii* and 17 to 23 h for *N. crassa*.

In *Mucor rouxii* (Fig. 3, 4), *Rhizopus arrhizus* and *Aspergillus fumigatus*, bursting occurred mainly through the apical dome and rarely behind it; in *Neurospora crassa* (Fig. 5) or *Penicillum claviforme*, the cytoplasm was also frequently extruded immediately behind the apical dome and in *N. crassa*, sometimes through the lateral walls. Occasionally, bursting was observed in an intercalary hyphal segment. Rizvi & Robertson (1965) also found that hyphal disintegration in *Neurospora crassa* was slightly subapical.

Temperature coefficient of bursting

The effect of temperature on apical bursting was assessed by flooding the plates of *Mucor rouxii* or *Neurospora crassa* with running distilled water at 5, 15 and 25 °C. A single microscopic field (× 10 objective) focused on the periphery of a colony, containing an average of 40 hyphal tips of *M. rouxii* (fewer for *N. crassa*), was maintained under continuous observation and the rupture time of each hyphal tip was scored. Twelve to fifteen plates were examined at each temperature. Bursting rates were compared by determining the most frequent time of bursting (mode) at each temperature (Table 2). At any given temperature the hyphal tips of *M. rouxii* burst much more rapidly than those of *N. crassa* but in either fungus the speed of bursting was highly dependent on the temperature of the flooding water. Modal time of bursting decreased with increasing temperature. Temperature coefficient values were higher than 1·0 for both fungi (Table 2).

Apex bursting of Mucor rouxii by flooding with various solutions

Acids. Dilute solutions of inorganic and organic acids were effective promoters of apical bursting. Application of 0·001 M solutions of sulphuric acid, phosphoric acid, acetic acid and citric acid, or carbonated water caused a rapid, massive disintegration of the growing tips of *Mucor rouxii* in colonies grown on X/16.YPG medium (Fig. 6, 7). The acids were effective in the range 0·001 to 0·1 M. At higher concentrations, bursting was probably avoided because of osmotic protection.

The pH range conducive to apical disintegration was determined by using a 0·01 M-acetic acid solution adjusted to various pH values with NaOH or NH₄OH (Table 3). Bursting decreased significantly at pH values above 4·5 to 5·0 and ceased at 5·5 to 6·0 under the conditions of the experiment. The response varied slightly from experiment to experiment probably reflecting minor changes in conditions.

Alkalis. In most instances alkaline solutions inhibited or failed to elicit apex bursting (Table 4). Thus, the hyphal tips of colonies of *Mucor rouxii* grown on 1X.YPG agar burst
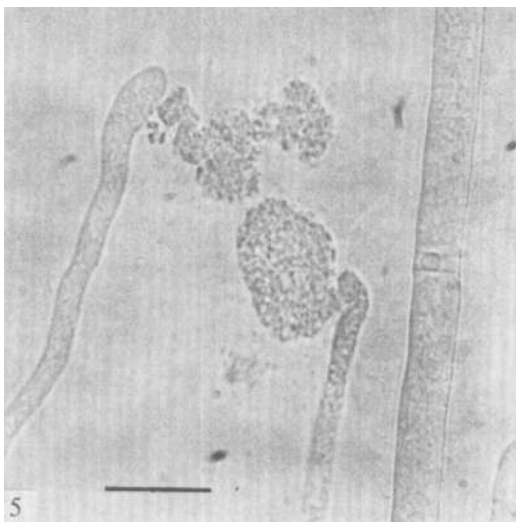
Fig. 5. Burst hyphal tips of *Neurospora crassa* (wild-type). Colony grown on 1X.YPG and flooded with drops of distilled water. (Scale marker = 20 μm.)
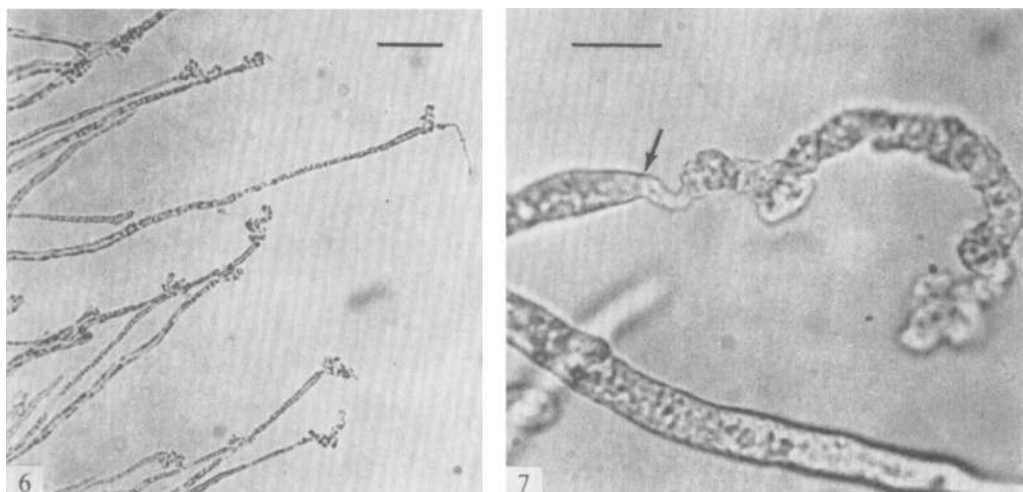

Fig. 6, 7. Bursting of hyphal tips of *Mucor rouxii* with 0·1 M-acetic acid. Arrow points at site of bursting. Colony grown on X/16.YPG. (Scale markers = 30 μm, Fig. 6; and 10 μm, Fig. 7.)

more slowly and less extensively when flooded with dilute solutions of NaOH or KOH (0·001 to 0·1 M) than with water. NH$_4$OH also inhibited bursting at low concentrations (0·001 to 0·01 M) but, surprisingly, not at higher concentrations (0·05 to 1·0 M). With 2-aminoethanol, slow but extensive apex bursting occurred at 0·1 M but at other concentrations there was complete inhibition.

Tests on colonies grown on X/16.YPG plates failed to show bursting in response to flooding with KOH or NaOH solutions (molarity range: 0·001 to 1·0; pH range: about 9 to 13·5). Yet, at certain concentrations, NH$_4$OH or 2-aminoethanol caused apical bursting (Table 4). It seems unlikely that these apical disintegrations were caused by the alkalinity of

Table 3. *Effect of pH on apex bursting in Mucor rouxii*

Colonies (11 to 12 h old) grown on X/16.YPG agar were flooded with 20 ml of acetic acid solutions adjusted to each pH value with either NaOH or NH₄OH.

	Flooding solutions			
	0·01 M-acetic acid + 1 M-NaOH		0·01 M-acetic acid + 1 M-NH₄OH	
pH	Intensity*	Rapidity†	Intensity*	Rapidity†
3·4	+ + + +	2 s	+ + + +	2 to 3 s
4·0	+ + + +	2 to 3 s	+ + + +	2 to 3 s
4·6	+ + + +	2 to 3 s	+ + + +	3 to 4 s
5·0	+ + +	2 to 3 s	+ + +	5 s
5·5	+ +	5 s	±	
6·0	—		—	

* The intensity of the reponse was scored as follows: (+ + + +) most of the tips burst; (+ + +) over one-half of the tips burst; (+ +) about one-third burst; (+) only a few tips burst; (±) a rare burst on the entire colony; (—) none.
† Rapidity refers to the approximate time for initiation of bursting.

Table 4. *Apex bursting of Mucor rouxii flooded with alkaline solutions*

		Culture medium						
		1X.YPG				X/16.YPG		
Base	Conc.	Initial pH*	Time† (s)	Intensity‡	Final pH*	Time†	Intensity‡	Final pH*
Water		7·0	7	+ + + +	7·4	.	—	7·6
NaOH	0·001 N	11·2	15	+ +	c. 9	.	—	9·8
NaOH	0·01	12·0	40	+ +	11·2	.	—	11·5
NaOH	0·05	12·6	35	+ + +	12·2	.	—	11·9
NaOH	0·1	12·9	60	+ +	12·6	.	—	12·1
NaOH	0·5	13·5		—	13·3	.	—	12·4
NaOH	1·0	13·5		—	13·5	.	—	13·5
KOH	0·001 N	10·8	17	+ +	9·1	.	—	9·2
KOH	0·01	11·8	11	+ +	10·9	.	—	
KOH	0·05	12·5	25	+ + +	12·3	.	—	11·7
KOH	0·1	12·9	30	+ +	12·6	.	—	12·6
NH₄OH	0·001 N	10·6	31	+	9·5	.	—	10·1
NH₄OH	0·01	10·6	21	+	9·9	.	—	10·3
NH₄OH	0·05	11·0	7	+ + + +	10·4	.	±	10·6
NH₄OH	0·1	11·2	9	+ + + +	10·7	45	+	10·7
NH₄OH	0·5	11·5	7	+ + + +	11·2	16	+ + +	11·4
NH₄OH	1·0	11·6	7	+ + + +	11·5	11	+ +	11·6
2-Aminoethanol	0·001 N	10·3		±	8·4		—	
2-Aminoethanol	0·01	10·8		—	9·1		—	10·2
2-Aminoethanol	0·1	11·3	55	+ + + +	10·3	180	+ + +	10·8
2-Aminoethanol	1·0	11·8		—				

* Initial pH of flooding solution was determined with a Corning triple-purpose glass electrode. Final pH was determined after the flooding solution was in contact with the agar for 5 to 10 min.
† Approximate time of initiation of the bursting response.
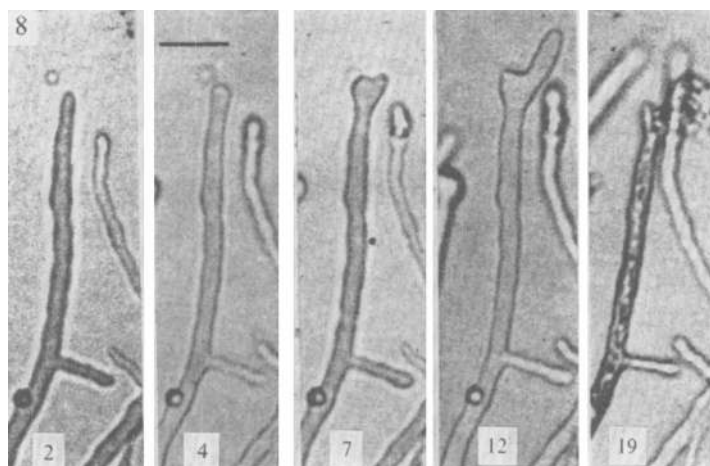‡ Intensity score as in Table 3.

Fig. 8. Apical swelling and bursting of a hyphal tip of *Mucor rouxii* flooded with 0·01 M-MnCl₂. Colony grown on X/16.YPG. Numbers indicate incubation time (min) after flooding. The apical burst shown at 19 min is a rare event in colonies thus treated. (Scale marker = 25 μm.)

the external medium, since KOH or NaOH was ineffective at the same pH range. Possibly, the action of NH₄OH and 2-aminoethanol was related to their ability to diffuse across the permeability barrier of the fungus.

Neutral salts. Although the response was not as rapid or extensive as that attained with the acids, flooding with neutral salt solutions also caused disintegration of the hyphal tips in colonies grown for about 12 h on X/16.YPG. Bursting occurred only in a narrow concentration range of the salt. Thus while flooding with 0·05 M-NaCl produced many bursts per field, flooding with 0·01 or 0·1 M-NaCl had little or no effect. Solutions of KCl, potassium phosphate (K₂HPO₄ + KH₂PO₄; pH 6·8) or sodium phosphate (Na₂HPO₄ + NaH₂PO₄; pH 6·8) manifested essentially the same effects.

Miscellaneous. A number of other compounds were capable of eliciting bursting in colonies of *Mucor rouxii* grown on X/16.YPG. Aqueous solutions (10 %, v/v) of methanol, ethanol, and acetone and detergents, such as sodium dodecyl sulphate (0·1 to 0·5 mM at pH 6 or 8) and Tween 80 (0·5 % solution at pH 3·5, 4·5, 5·8 but not 6·9) were effective. Sulphydryl-containing compounds (0·01 or 0·1 M), e.g. glutathione and dithiothreitol, caused few bursts whereas mercaptoethanol was ineffective. EDTA (0·001 or 0·01 M; pH 5 to 7) was highly effective.

Apex swelling of Mucor rouxii by divalent metals

Contrary to the action of monovalent metals, chloride solutions of Mg²⁺, Mn²⁺ or Ca²⁺ caused little or no apical bursting on colonies of *Mucor rouxii* grown for about 12 h on X/16.YPG agar. Instead, there was pronounced apical 'swelling'. Almost all growing tips developed swellings of various shapes (spherical, ellipsoidal, pyriform) within a few minutes after flooding with 0·01 to 0·075 M solutions of the above salts (Fig. 8). On prolonged incubation one or sometimes two hyphal tubes emerged from the swelling. This behaviour resembled that described by Robertson (1958) for hyphal tips in colonies of *Fusarium oxysporum* flooded with distilled water. Occasionally a swollen tip burst (Fig. 8).
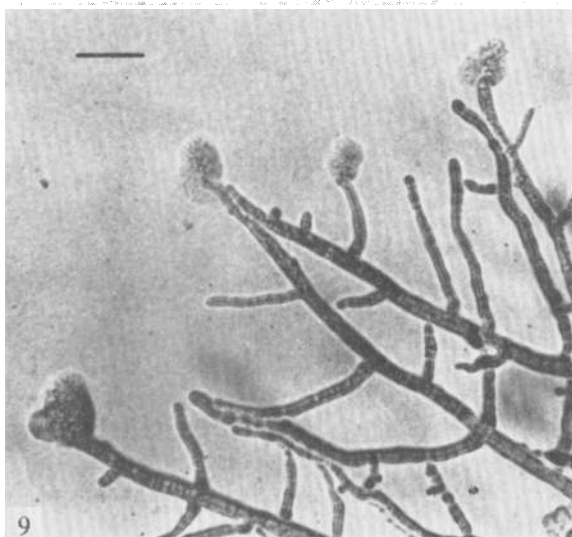
Fig. 9. Bursting of hyphal tips of *Mucor rouxii* by heat shock. Colony grown on 1X.YPG at 28 °C transferred to oven at 160 °C for 1 min. (Scale marker = 30 μm.)

Apex bursting by heat shock

Rapid heating of a colony of *Mucor rouxii* grown on 1X.YPG agar by placing plastic Petri dish cultures in a forced-circulation oven caused estensive bursting of the hyphal tips. Massive apical bursting occurred after 2 min heating at 95 °C or 1 min at 160 °C (Fig. 9). At lower oven temperatures (50 to 95 °C) the frequency of bursting diminished accordingly. At 50 °C only a rare burst was recorded after 15 min of heating. No attempt was made to measure the temperature attained by the agar surface in all these incubations but the temperature must have risen rapidly and continuously for bursting to have occurred. Thus, if a plate was mildly preheated (52 °C for 5 min), cooled at room temperature for 5 min and then heated at 95 °C for 2 min, essentially no bursting occurred.

Primary or secondary hyphal tips were susceptible to bursting by oven heating. Colonies grown on either X/16.YPG or 1X.YPG media were susceptible to bursting by heat shock but the extent of bursting was greater in the latter medium.

Heating also resulted in apex bursting in *Aspergillus fumigatus*, *Penicillium claviforme*, *Fusarium* sp., but not in *Phytophthora cinnamomi*, *P. palmivora* nor *P. parasitica*. Thus, fungi prone to bursting by osmotic shock, also burst by heat shock

A mild heat shock could also elicit bursting. Some of the peripheral hyphae from colonies of *Mucor rouxii* grown on 1X.YPG at 6 or 15 °C burst when the plates were transferred to either 25 or 36 °C. The reverse transfers (cold shock) did not cause any bursting.

The disintegration of hyphal tips by heat could be nullified by osmotic balance. Thus whereas pouring boiling water on to colonies of *Mucor rouxii* grown on either 1X.YPG or X/16.YPG caused extensive bursting, boiling solutions of 0·5 M-mannitol had no effect.

Spontaneous bursting of hyphal tips of Mucor rouxii

On careful examination, an occasional burst hyphal tip was seen on the margin of a colony of *Mucor rouxii* which had not been subjected to any exogenous disturbance. These plates had been incubated continuously under constant conditions and were examined im-

mediately after being removed from the incubator. Seemingly bursting could occur during the normal course of colony development. No effort was made to determine the frequency of spontaneous bursts nor the cultivation conditions affecting it.

DISCUSSION

Our findings confirm and expand previous reports on the proneness of hyphal tips of fungi to burst on addition of distilled water (Robertson, 1958, 1959) or dilute acetic acid (Park & Robinson, 1966a). At first sight, the bursting of hyphal tips upon exposure to water or hypotonic solutions might simply appear as an osmotic phenomenon – the hyphal walls, unable to withstand the sudden drop in external pressure, burst at their weakest zone, the growing tip. However, our findings indicate that the osmotic shock is not the key or sole cause of bursting. Active weakening of the apical wall seems also to be essential. If bursting upon addition of distilled water were purely an osmotic phenomenon, it would show a temperature coefficient near 1·0. The higher values observed (from 1·3 to 2·05) indicate the participation of a biochemical reaction(s), as the rate-limiting step in the bursting process. Presumably this biochemical reaction is one which weakens the wall to the point where it can no longer withstand the turgor of the cytoplasm.

The osmotic shock caused by addition of distilled water to a colony grown on X/16. YPG agar was insufficient to cause bursting of the apices. Yet, these hyphal tips were capable of bursting when flooded with dilute aqueous solutions of neutral salts or acids. Clearly, bursting did not occur simply because the wall failed to withstand the sudden decrease in external osmotic pressure; active weakening of the wall must have occurred, triggered by the various solutes present in the flooding water. Because of the diversity of agents triggering bursting (acids, neutral salts, alcohols, detergents, heat etc.) it seems improbable that this weakening effect is a direct chemical attack on the wall polymers by the agent in question (though this might also occur, as for example, in high-acidity treatments). More likely the effect is on wall metabolism.

The present observations constitute circumstantial evidence to support the following contentions: (i) the apices of fungal hyphae have a large potential of wall lytic activity; (ii) the release of this activity during growth must be a gradual process delicately co-ordinated with wall synthesis; (iii) the balance between synthesis and lysis of wall polymers can be easily disturbed and shifted in favour of lysis by a variety of external stimuli resulting in violent disintegration of the hyphal apex or, under certain conditions, the formation of large apical 'swellings'.

The need for at least two counterbalancing processes operating during apical wall construction has been recognized by others and formulated in similar or somewhat different terms (Robertson, 1965; Park & Robinson, 1966b; Green, 1969).

The equilibrium in wall metabolism, advocated herein, could be displaced in the direction of wall degradation by either an increase in wall lysis or a decrease in wall synthesis. Presumably such were the specific effects elicited by external application of a wall-splitting enzyme (lysozyme) or an inhibitor of wall synthesis (polyoxin D), respectively. Lysozyme, an enzyme with chitinase activity (Berger & Weiser, 1957), causes extensive and rapid bursting of hyphal tips of *Mucor rouxii* (Bartnicki-Garcia & Lippman, in preparation). Likewise, application of polyoxin D, an antibiotic which specifically inhibits chitin synthesis (Endo, Kakiki & Misato, 1970), results in bursting of hyphal apices of *M. rouxii* (Bartnicki-Garcia & Lippman, 1972). Similar conclusions can be made from other observations on fungal behaviour. For instance, digestion with hydrolytic enzymes causes apical disintegration of

hyphae of *Neurospora crassa* (Rizvi & Robertson, 1965). Likewise, protoplasts of different fungi, prepared by enzymatic digestion, are frequently seen emerging through the hyphal tips (Gascón, Ochoa & Villanueva, 1965; Bartnicki-Garcia & Lippman, 1966; Strunk, 1969). In yeast cells, 2-deoxyglucose, an inhibitor of the synthesis of wall hexosans (Farkaš, Svoboda & Bauer, 1969) causes cell lysis (Megnet, 1965). Significantly, the yeast cells lyse at the same sites where wall synthesis is believed to take place (Johnson, 1968). Similarly, the disintegration of hyphal apices of a mutant of *Neurospora crassa*, which occurs upon treatment with L-sorbose, is thought to be due to interference with the production of wall glucan (Rizvi & Robertson, 1965).

The facility with which hyphal tips undergo bursting suggests that the postulated balance between wall synthesis and wall lysis must be rather precarious and can readily drift in the direction of lysis. This may even occur during the normal growth of a hypha and would explain the occurrence of spontaneously burst hyphae in a seemingly undisturbed colony of *Mucor rouxii.*

Most of the fungi examined exhibited a marked propensity to undergo apical bursting by either 'osmotic' (flooding with water) or heat shock, but not *Phytophthora* sp. or *Schizophyllum commune*. Only treatment with 0·1 M-acetic acid caused an occasional burst in *Phythophthora* spp. The greater resistance of these fungi to bursting may reflect a greater metabolic stability of the apical wall.

Two different but not mutually exclusive mechanisms may be invoked to explain how widely different bursting agents may interact with apical wall metabolism:

(1) *Effect on enzyme activity.* The treatments may drastically alter the milieu where wall synthetases and wall-splitting enzymes operate and thus upset their mutual balance. There is evidence that some polysaccharide synthetases (Wang & Bartnicki-Garcia, 1966; McMurrough, Flores-Carreón & Bartnicki-Garcia, 1971; Mishra & Tatum, 1972) as well as polysaccharide hydrolases (Mahadevan & Mahadkar, 1970) are intimately associated with the fungal wall and would thus be exposed to the environment. Changes in pH, ionic strength, and temperature are apt to inhibit polysaccharide synthetases *preferentially* over the more environmentally resistant hydrolases, thus allowing degradative processes to prevail. The high susceptibility of chitin synthetase of *Mucor rouxii* to changes in pH, ionic strength, and temperature (McMurrough & Bartnicki-Garcia, 1971) compared to the considerably more stable properties of chitin hydrolases isolated from other fungi (Tracey, 1955; Otakara, 1961) makes this explanation plausible.

(2) *Effect on enzyme release.* Conceivably, the treatments leading to bursting augment or provoke the discharge of vesicles containing autolytic enzymes. The observation that bursting is not always strictly apical but may occur subapically, or laterally, or that large apical swellings may be produced instead of, or before, bursting, suggest that the lytic activity is not necessarily restricted or fixed to the apical-dome wall. Conceivably, it resides initially in the cytoplasm, possibly in apical vesicles such as those seen by Brenner & Carroll (1968), McClure, Park & Robinson (1968), Girbardt (1969) and Grove & Bracker (1970). Although the contents of these vesicles are not known, some of them may harbour hydrolytic enzymes. Thus β-1,3-glucanase has been localized in vesicular elements of *Saccharomyces cerevisiae* comparable to the vesicles of hyphal tips (Matile, Cortat, Wiemken & Frey-Wyssling, 1971). During growth, apical vesicles are produced continuously, fuse with the plasma membrane and liberate their contents into the wall region (Grove, Bracker & Morré, 1970). We propose, then, that apical bursting may occur when an inordinately high proportion of lytic vesicles are forced to discharge their contents against the wall. This interpretation is in accord with cytological observations showing the pronounced lability of the vesicular apparatus in

hyphal tips (Girbardt, 1957, 1969; Bracker, 1971; Grove, 1971). For instance, mere washing of germinating cells of *Gilbertella persicaria* caused the disappearance of the apical vesicles (Bracker, 1971) and the formation of lomasome-like deposits under the cell wall. Since the highest concentration of vesicles occurs in the apical dome, this would be the zone most severely and rapidly degraded and, hence, the most frequent site of bursting, but the vesicles may also migrate to a subapical region causing a cytoplasmic discharge immediately below the apical dome or even further behind on the lateral walls. Some of the observed discharges of cytoplasm through lateral hyphal walls were probably at sites where branches were about to emerge and where the enzymatic machinery for apical growth was already congregated.

Flooding with divalent metal ions (Mg^{2+}, Mn^{2+}, Ca^{2+}) may cause only a partial increase in wall lytic activity – insufficient to weaken the wall to its bursting point but enough to temporarily increase the 'plastic' area of the growing apex thereby giving rise to a 'swollen' tip.

The participation of wall autolytic enzymes in morphogenetic processes of fungi seems indispensable in phenomena like hyphal anastomosis, clamp connection and sexual fusions which involve dissolution of walls. But it is probably true in less obvious ones. Nickerson & Falcone (1956) gathered experimental evidence for the operation of a protein disulphide reductase as a key step in plasticizing localized areas of the yeast wall where budding was to take place. Regarding apical growth, it is unlikely that the turgor pressure of the cytoplasm could account for the spatially restricted expansion of the growing apical wall (Green, 1969). The localized participation of wall-splitting enzymes in the growth zone is highly probable. Additional evidence implicating wall-splitting enzymes in apical growth lies in the correlation between the initiation of apical growth (e.g. branching, conidium germination) and an increase in wall-bound hydrolases (Mahadevan & Mahadkar, 1970; Mahadevan & Rao, 1970). Recently, Katz & Rosenberger (1971*b*), studying the lysis of a mutant of *Aspergillus nidulans* blocked in chitin synthesis, concluded that enzymes that open bonds in the wall and generate weak regions are an integral part of the normal growth process.

In a previous communication, we demonstrated the existence of a sharp gradient of wall synthesis in the hyphal tips of *Mucor rouxii* and speculated on the need for a parallel gradient of wall lysis (Bartnicki-Garcia & Lippman, 1969). Although it has not been possible to directly demonstrate the existence of such a gradient, the present findings suggesting the existence of concentrated wall-lytic activity in the hyphal apex, strengthen the belief that autolytic enzymes play a role in hyphal development.

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