plot shown in figure 1. This energetics plot reveals that for a range of frequencies net work per cycle is being done by the muscle (i.e. W positive) and reaches a maximum. As frequency is increased, W begins to decrease and becomes zero approximately at the frequency where dynamic stiffness is a minimum (figure 1a). This frequency is denoted by f_d. At this frequency, E also reaches a minimum. Beyond f_d, viscous effects dominate and W becomes negative. In the vicinity of f_d therefore, a change occurs in the performance of the muscle, with net work being done before f_d, while viscous effects dominate at frequencies beyond f_d. As frequency is increased further, W goes through a minimum and then begins to increase again. This is paralleled by a steady increase in E. Therefore at higher frequencies another change appears in muscle behaviour - from one where viscous effects are at their peak to behaviour which becomes more like that of an elastic device. This tendency towards elastic behaviour at higher frequencies is in agreement with tension transient studies performed on living frog fibres[10].

Implications of these studies to the mechanical response of tetanized muscle when subjected to sinusoidal length changes was highlighted by a study performed by Steiger and Rüegg[11]. They found that activated insect fibrillar muscle, when driven with small-amplitude sinusoidal perturbations, not only performed oscillatory work (i.e. W positive) but also displayed what was termed an 'oscillation-induced extra ATPase'. This oscillation-induced extra ATPase (termed the biochemical equivalent of the Fenn effect) varied with frequency of oscillation in a similar manner to the work curve. No extra ATPase was induced for frequencies greater than the frequency where dynamic stiffness was minimum (i.e. f_d). They concluded that work per cycle and ATPase activity reflect a frequency dependence of actin-myosin interaction rate in a sliding filament system. For the limited frequency range investigated by Steiger and Rüegg[11] the oscillatory work curve obtained by them agreed with the work curve shown in figure 2.

When the temperature of the bathing solution was changed we observed the stiffness minimum, and hence the frequency where extra induced ATPase activity ceased, to move. Figure 3 shows this temperature dependence for the range 18–28 °C. The Arrhenius plot revealed an activation energy of the process reflected by the parameter f_d, to be 18 kcal/mole. This figure is in close agreement with values quoted for the activation energy of ATPase activity of myosin[12] which was proposed as the rate limiting step of the cross-bridge cycle.

To further investigate this temperature dependence a more efficient procedure for obtaining frequency responses must be employed. Reports on this are in preparation.

1 The authors wish to thank the Australian Research Grants Commission for provision of the laser, and L.I. Patterson for construction of the mechanical apparatus.
2 K.E. Machin and J.W.S. Pringle, Proc. R. Soc. Lond. B *152*, 311 (1960).
3 B.R. Jewell and J.C. Rüegg, Proc. R. Soc. B *164*, 428 (1966).
4 P. Heinl, Pflügers Arch. *333*, 213 (1972).
5 M. Kawai, P.W. Brandt and M. Orentlicher, Biophys. J. *18*, 161 (1977).
6 W. Halpern and N.R. Alpert, J. appl. Physiol. *31*, 913 (1971).
7 R.H. Abbott, Cold Spring Harb. Symp. quant. Biol. *37*, 647 (1973).
8 G.H. Rossmanith, thesis, Macquarie University, 1977.
9 M.E. Van Valkenberg, Prentice-Hall, Englewood Cliffs, N.J., 1964.
10 L.E. Ford, A.F. Huxley and R.M. Simmons, J. Physiol. *269*, 441 (1977).
11 G.J. Steiger and J.C. Rüegg, Pflügers Arch. *307*, 1 (1969).
12 M. Bárány, J. gen. Physiol. *213*, 455 (1967).

Induction of zygospores at high temperatures in the thermophilic species *Mucor miehei* with aspartic acid and phenylalanine

B.S. Mehrotra[1] and B.M. Mehrotra

Botany Department, Allahabad University, Allahabad (India), 6 March 1979

Summary. The importance of amino acids in the induction of zygospores in mucors has been realized for the first time. It was found that in the thermophilic species *Mucor miehei* zygospores at high temperatures (40–50 °C) can be induced by addition of phenylalanine and aspartic acid.

During our studies of thermophilic fungi it was observed that the homothallic and thermophilic species *Mucor miehei* Cooney and Emerson produces zygospores at 35 °C but fails to produce them at 50 ± 1 °C. Earlier, Lasure and Ingle[2] had also observed that this otherwise homothallic species failed to produce zygospores and a pigment at higher temperatures (45 °C). It was thought that such fungi are possibly able to produce certain essential amino acids for the induction of zygospores at low temperatures but fail to do so at high temperatures. This expectation has been justified by subsequent studies. They are briefly reported here.

Our own isolate of *M. miehei*, obtained from soil, was grown in flasks with a medium containing: dextrose 40 g; KH_2PO_4 0.5 g; $MgSO_4.7\ H_2O$ 0.25 g; thiamine chloride 0.5 mg; asparagine 2 g, and 1000 ml distilled water. For each experiment 1 set of 9 replicates was incubated at 35 °C and another set at 50 °C. At the end of the 4th, 8th and 12th day of incubation a set of 3 flasks were taken out from each of the 2 incubators and the contents of each set was separately passed through a filter. The mycelial extract of each set was obtained in ethanol and then analysed chromatographically for its amino acid content following Consdon et al.[3]. Phenol, saturated with ammonia solution, and a mixture of n-butanol-acetic acid and water (4:1:5) were used as 1st and 2nd running solvents, respectively. Ninhydrin at a concentration of 0.1% (w/v) in n-butanol was sprayed to locate the amino acid and amide spots. The spots were developed on heating the chromatograms for 20 min at 80 °C. 13 amino acids were detected (table 1) in the colony grown at 35 °C but only 8 were found in the mycelium growing at 50 °C. The 5 amino acids missing at 50 °C were aspartic acid, γ-amino n-butyric acid, asparagine, leucine and phenylalanine. From this it was concluded that 1 or more of these 5 amino acids are possibly essential for the initiation of sexual activity in the fungus and failure of the fungus to synthesize them at high temperature is responsible for the absence of zygospores at that temperature. This conclusion was substantiated by the experiments that followed.

Table 1. Table showing the presence of different amino acids at 35 and 50 °C in the mycelium of *Mucor miehei*

Amino acids	4th day		8th day		12th day	
	35 °C	50 °C	35 °C	50 °C	35 °C	50 °C
1 Asparagine	+	−	+	−	+	−
2 Aspartic acid	+	−	+	−	+	−
3 α-Alanine	+	+	+	+	+	+
4 γ-Amino-n-butyric acid	+	−	+	−	+	−
5 Glutamine	+	+	+	+	+	+
6 Glutamic acid	+	+	+	+	+	+
7 Glycine	+	+	+	+	+	+
8 Histidine	+	+	+	+	+	+
9 Leucine	+	−	+	−	+	−
10 Lysine	+	+	+	+	+	+
11 Phenylalanine	+	−	+	−	+	−
12 Serine	+	+	+	+	+	+
13 Valine	+	+	+	+	+	+

+, Present; −, absent.

Table 2. Effect of aspartic acid and phenylalanine on zygospore formation at 50 °C

Amino acid	Dextrose	Nitrogen concentration (g/l)		
		1.5	1	0.5
Aspartic acid	10 g	−	++	−
	5 g	+	+++	+
	4 g	+	−	−
	2 g	−	−	−
Phenylalanine	10 g	+	++	+
	5 g	+	+++	+++
	4 g	+	−	−
	2 g	−	−	−

−, No zygospore; + few zygospores; ++, many zygospores; +++, abundant zygospores.

Excluding asparagine, which had already been tested, being a constituent of the initial medium, the remaining 4 amino acids were supplied to the fungus grown at 50 °C by incorporating them singly in the basal medium. The basal medium used was the same as for the previous experiments except that instead of 40 g of dextrose, 5 g was used and asparagine was replaced by 1 of the remaining 4 amino acids singly in quantities so as to supply the same amount of nitrogen as in asparagine. It was observed (table 2) that out of the 4 amino acids tested aspartic acid and phenylalanine were able to induce abundant zygospore formation at 50 °C in *M. miehei*. Further studies on the role of amino acids in zygospore formation at high temperatures in thermophilic fungi are in progress.

1 Present address: Botany Department, Kumaun University, Naini Tal 263001 (India).
2 L.L. Lasure and M.B. Ingle, Mycologia *68*, 1145 (1976).
3 R. Consdon, A.H. Gordon and A.J.P. Martin, Biochem. J. *38*, 224 (1944).

Preformed azulene pigments of *Lactarius indigo* (Schw.) Fries (Russulaceae, Basidiomycetes)[1]

A.D. Harmon, K.H. Weisgraber[2] and U. Weiss[3]

Laboratory of Chemical Physics, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda (Maryland 20205, USA), 4 April 1979

Summary. The structure of a new naturally-occurring azulene, 1-stearoyloxymethylene-4-methyl-7-isopropenylazulene, obtained from an acetone extract of the blue mushroom *Lactarius indigo,* has been determined by chemical and spectral methods.

Azulenes have been obtained from a wide variety of plant sources[4], but in almost all cases, these pigments are not present in the living plant but are formed during isolation from suitable precursors[5]. We wish to report the occurrence of evidently pre-existing, exceedingly sensitive azulenes in the mushroom *Lactarius indigo,* and the determination of the structure of the main blue azulene from this species.

Fresh sporophores of fungi from this large genus contain a true latex[6] which, in a number of species, is highly colored. The only such species studied chemically so far, the orange *L. deliciosus* (Fr.) S.F. Gray, has yielded 2 azulenes, the blue lactarazulene (**1**)[7] and the reddish-violet lactaroviolin (**2**)[8]. However, neither of these occurs as such[9]; the orange color of the fungus is due, in European specimens, to the extremely sensitive dihydroazulenes (**3**) and (**4**)[10], while fungi from California have yielded lactarofulvene (**5**)[11]. The isolation of these pigments from a *Lactarius* species suggested the intriguing possibility that the color of the deep blue latex of young specimens[12] of *L. indigo* might be due to pre-existing azulenes. We have now shown that this is indeed true.

Preliminary chromatographic studies on 1 young specimen of *L. indigo* found in Potomac, Maryland, several years ago seemed to show the presence of 1 blue pigment, extractable with acetone and soluble in hexane. The absorption spectrum of the crude pigment suggested an azulene related to **1**