

Effect of miconazole on growth and aflatoxin production by *Aspergillus parasiticus*

Abstract

At 5 μ M, miconazole prevented the growth of *Aspergillus parasiticus* Speare in a number of media. Sensitivity to miconazole was increased approximately 10-fold in a medium containing glycerol. At sub-inhibitory concentrations, miconazole stimulated aflatoxin synthesis on media which normally support toxin formation. Miconazole inhibited respiration and altered mitochondrial ultrastructure, suggesting that miconazole inhibits growth and stimulates aflatoxin production by depressing mitochondrial activity.

Introduction

In recent years, a series of imidazole derivatives has been developed for the clinical treatment of mycoses. Miconazole (1-(2,4-dichlorophenyl)-2-(2,4-dichlorophenyl) methoxy ethyl-1 H-imidazole) is one of the most widely employed and has been shown to be effective against a variety of yeasts and fungi [15, 17]. Inhibition of cytochrome P-450 leading to a disruption of ergosterol synthesis has been proposed as the mechanism of action underlying the antifungal activity of imidazole and triazole derivatives [21]; however, miconazole has been also reported to be an active inhibitor of both plasma membrane and mitochondrial ATPases in yeast [11, 13, 17, 18]. Portillo & Gancedo [17, 18] recently suggested that inhibition of mitochondrial ATPase is the compound's primary site of action against *Saccharomyces cerevisiae* Hansen and *Candida albicans* (Robin) Berkhouk. Guinet & Mazoyer [14] reported that *Aspergillus flavus* Link is among members of that genus sensitive to miconazole. *Aspergillus flavus* and the closely related species, *A. parasiticus* Speare, are noted for their ability to synthesize various polyketide mycotoxins including aflatoxins, and mitochondrial activity has been suggested as one of

the factors regulating the production of this class of carcinogens [7, 12, 15, 19]. No research has been reported concerning the effects of miconazole on mycotoxin production. The objective of the current study was to characterize the effects of miconazole on growth and aflatoxin production by *A. parasiticus*, particularly in regard to understanding the bioregulation of polyketide mycotoxin synthesis.

Materials and methods

Microorganism

Aspergillus parasiticus NRRL 2999 was used throughout the study. It was maintained on potato dextrose agar (Difco) slants, and spore suspensions prepared as described previously [20].

Media

Yeast extract sucrose medium (YES) was prepared as described previously [10]. Peptone-mineral salts (PMS), glucose-mineral salts (GMS), and glycerol-mineral salts (GyMS) media were prepared by com-

binning 60 g of the carbon source with 10 g KH_2PO_4 , 4 g $(\text{NH}_4)_2\text{SO}_4$, 2 g MgSO_4 , 1 ml metal mix, and 1000 ml H_2O . The metal mix was a modification of that of Mateles & Adye [16] and has been described previously [6]. Peptone-glucose-mineral salts medium (PGMS) was prepared in a similar manner except both carbon sources were added at a level of 60 g/l. The pH of the various media was adjusted to desired levels using 10 N HCl or NaOH. The media were then dispensed in appropriate volumes and, unless otherwise specified, sterilized by autoclaving for 15 min at 15 psi.

Culture techniques

The effects of miconazole on cultures initiated from conidia were studied using 50-ml Erlenmeyer flasks containing 14 ml of medium (YES, PMS, GMS, or GyMS). Unless otherwise specified, miconazole was added by diluting a stock solution of 300 μM miconazole in water/ethanol (1:1) with water/ethanol (1:1), and transferring 1.0 ml to each flask to yield desired final concentrations. Control flasks received 1 ml of water/ethanol without miconazole. Unless otherwise specified, the ethanol concentration of all cultures was 2.8% (w/v). Each flask then received 0.2 ml of *A. parasiticus* spore suspension to produce an inoculum of approximately 1.3×10^4 conidia/ml. All flasks were incubated without agitation at 28 °C for 7 days, and then analyzed for pH, aflatoxin production, and mycelial dry weight.

The effects of miconazole on aflatoxin production by pregrown mycelia were studied using the replacement culture technique of Buchanan & Lewis [4]. After sequential culturing in YES and PMS, the disrupted mycelia were transferred in 2.0 g portions to 50-ml flasks containing 14 ml of medium (PMS, GMS, and PGMS) and 1 ml of water/ethanol containing the appropriate amount of miconazole. All flasks were subsequently incubated without agitation at 28 °C for 48 h, and then analyzed for pH, aflatoxin production, and mycelial dry weight.

Analyses

After determining the pH of the culture medium, each culture (medium + mycelium) was extracted thrice with 20 ml portions of CHCl_3 . The extracts were concentrated on a rotary evaporator, and the aflatoxins separated by thin layer chromatography (silica gel, 20 × 20 cm) [8] using chloroform-acetone-water (93:7:1) as the eluting solvent. Separation of extracts from peptone-based media was modified such that the TLC plates were pre-developed with anhydrous diethyl ether in order to eliminate interfering fluorescent pyrazines [2]. The aflatoxins (B_1 , B_2 , G_1 , G_2) were then quantitated with a fluorodensitometer (365 nm excitation filter, 436 nm emission filter) (model CS-930, Shimadzu Co.). The lower limit of detection was approximately 10 ng aflatoxin/culture. After extraction, the mycelia were collected on pre-dried and weighed filter paper, rinsed thoroughly with distilled water, and dried for 24 h at 85 °C. Mycelial dry weights were then determined gravimetrically.

Respirometry

The effect of miconazole on respiration in *A. parasiticus* was determined using a modification of the replacement culture protocol [7]. Replacement media were GMS, PMS, and GyMS, with miconazole being added as a water/ethanol (1:1) solution.

Electron microscopy

The effect of miconazole on the ultrastructure of *A. parasiticus* was studied using the replacement culture technique described above. After sequential culturing in YES and PMS, portions of the mycelia were transferred to 50-ml flasks containing 15 ml of PMS (initial pH = 4.5) with or without 1 μM miconazole. Miconazole was introduced either by direct addition to the media which was subsequently sterilized by filtration, or by adding 1.0 ml of the appropriate water/ethanol solution. One set of miconazole-free control cultures received 1.0 ml of water/ethanol, while a second set had no additions. The replace-

ment cultures were then incubated for 24 h without agitation. Control cultures of PGMS without ethanol or miconazole were prepared in a similar manner.

After 24 h incubation, samples of the mycelia were fixed for 2–3 h at room temperature with 2% glutaraldehyde in 0.08 M sodium cacodylate buffer (pH 7.0). The samples were then rinsed three times with the same buffer in conjunction with harvesting by lightly centrifuging with a bench top clinical centrifuge. The loose cell pellet, measuring approxi-

mately 0.3 ml, was mixed with 2–3 drops of molten 2% agar in the same buffer at 42 °C. The mixture was pipetted onto a clean glass slide, allowed to harden for 3–4 min, and cut into 2 mm³ blocks. The blocks were subsequently post-fixed with 1% osmium tetroxide in the same buffer for 3 h, dehydrated with a graded water/acetone series and embedded in Spurr's low viscosity resin. Ultrathin (60–70 nm) sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and observed in a Zeiss EM 10 transmission electron microscope.

Table 1. Effect of miconazole on growth and aflatoxin production by *A. parasiticus* cultured in various media.

Medium	Miconazole (μ M) ^c	Mycelium dry weight (mg)	pH ^d	Aflatoxin culture (μ g)	Aflatoxin mycelium (ng/mg)
YES	0.00	275 (1) ^a	6.4 ^a	602.9(26.1) ^a	2190
	0.01	284 (3)	6.1	625.8(46.8)	2200
	0.05	295 (2)	6.2	639.5(77.7)	2170
	0.10	293 (1)	5.6	755.1(15.4)	2580
	0.50	236(16)	3.9	554.0(42.6)	2350
	1.00	123(11)	4.4	222.4(30.2)	1810
	5.00	NG ^e	5.8	0.0	-
GMS	0.00	94 (5) ^a	2.1 ^a	115.1(11.3) ^a	1220
	0.01	96(16)	2.1	137.1 (5.2)	1430
	0.05	117(10)	2.0	166.3 (4.5)	1420
	0.10	127 (5)	2.0	161.0 (4.0)	1270
	0.50	149 (3)	2.0	183.4 (5.0)	1230
	1.00	28 (7)	2.9	13.2 (1.3)	470
	5.00	NG	4.9	0.0	-
PMS	0.00	145 (9) ^b	7.2 ^b	2.8 (0.9) ^b	20
	0.01	159 (9)	7.2	3.2 (0.6)	20
	0.05	153 (6)	7.2	5.8 (1.1)	38
	0.10	159 (6)	7.1	13.1 (1.2)	82
	0.50	96(12)	6.3	6.9 (0.5)	72
	1.00	57 (9)	5.5	0.6 (0.1)	11
	5.00	NG	5.5	0.0	-
GyMS	0.00	146 (2) ^a	2.3 ^a	165.2(13.4) ^a	1132
	0.01	140(10)	2.3	177.0 (5.6)	1264
	0.05	123 (4)	2.4	135.7(15.3)	1103
	0.10	93 (8)	2.9	55.1 (5.0)	592
	0.50	NG	5.3	0.0	-
	1.00	NG	5.4	0.0	-
	5.00	NG	5.4	0.0	-

^a \bar{x} (\pm SEM), n = 3 replicate cultures

^b \bar{x} (\pm SEM), n = 6 replicate cultures

^c Miconazole added as water/ethanol solutions (1 ml/culture).

^d Initial pH = 5.5

^e No growth.

Results and discussion

Conidia-initiated cultures

The activity of miconazole against *A. parasiticus* was initially determined in various media (YES, GMS, PMS, GyMS) inoculated with conidia (Table 1). The effect on growth was similar in YES, GMS, and PMS; growth was partially inhibited by 0.5, 1.0, and 0.5 μM , respectively, and completely inhibited by 5.0 μM . A small, dose-related increase in mycelium dry weights was observed with sub-inhibitory concentrations of miconazole. This is in general agreement with the results Guinet & Mazoner [14] who observed that 26 of 29 *A. flavus* isolates were inhibited by $\leq 13 \mu\text{M}$ miconazole. When *A. parasiticus* was cultured in medium containing glycerol as sole carbon source (GyMS), miconazole was approximately 10-fold more active, with growth partially inhibited by 0.05 μM and completely inhibited by 0.5 μM . No stimulation of growth was observed with sub-inhibitory concentrations. Portillo & Grancedo [17] similarly observed that *Saccharomyces cerevisiae* and *Candida albicans* were more sensitive to miconazole when cultured in a glycerol-based medium as compared to a glucose-containing medium. They suggested that this differential response reflected the effects of fermentable versus non-fermentable substrates in conjunction with a mitochondrial site of action. The present results do not support this hypothesis since PMS, which contains a non-fermentative carbon source (i.e., amino acids), gave a response similar to that observed with media containing fermentable carbohydrates (YES and GMS). Further, the decline in pH values observed in the GyMS cultures indicate that glycerol was catabolized to an organic acid(s), implying that *A. parasiticus* at least partially metabolizes glycerol via a fermentative mode. This suggests there is a specific attribute of glycerol metabolism that enhances miconazole activity.

No reduction in aflatoxin accumulation occurred until miconazole concentrations were sufficient to produce at least partial inhibition of growth. In fact, a small dose-related increase in aflatoxin accumulation was observed in the YES and GMS receiving miconazole concentrations subinhibitory to growth,

and in PMS cultures with 0.1 μM miconazole there was a 4-fold increase over the low level of toxin detected in control cultures.

It has been previously reported that PMS does not support aflatoxin production [1, 4, 5, 7, 8]. Thus, the low level of toxin production observed in the PMS control cultures and its subsequent stimulation by miconazole suggested an atypical response. Since all cultures including the controls had received an addition of 1.0 ml of ethanol/water, it was considered that toxin production in the PMS cultures was due to the use of ethanol as a solubilizing agent. To test this possibility, the effect of miconazole on growth and aflatoxin production in PMS was re-evaluated using media to which miconazole was added directly to a large volume of media to avoid the use of ethanol-containing stock solutions (Table 2). The dry weights of the mycelia of the ethanol-free PMS cultures were substantially less than those observed with the equivalent ethanol-containing cultures (Table 1), which may reflect differences in the level of growth or accumulation of secondary metabolites [4]. However, the effect of miconazole on growth was generally equivalent to that observed in the ethanol-containing PMS cultures, while aflatoxin production was not detected in ethanol-free control or miconazole-treated cultures. This confirmed that the aflatoxin production detected in the ethanol-

Table 2. Effect of miconazole on growth and aflatoxin production by *A. parasiticus* in conidia-initiated PMS cultures without ethanol.

Miconazole (μM) ^a	Mycelium dry weight (mg)	pH ^b	Aflatoxin culture (μg)
0.00	80(4) ^c	7.6 ^c	0.00 ^c
0.01	76(4)	7.7	0.00
0.05	79(2)	7.6	0.00
0.10	91(9)	7.6	0.00
0.50	83(3)	7.6	0.00
1.00	21(7)	6.7	0.00
5.00	NG ^d	5.6	0.00

^a Miconazole was added directly to medium, which was then sterilized by filtration.

^b Initial pH = 5.5.

^c \bar{x} (\pm SEM), n = 4 replicate cultures.

^d No growth.

Table 3. Effect of initial pH on the activity of miconazole in conidia-initiated cultures of *A. parasiticus*.

Medium	Miconazole (μM) ^a	Initial pH	Mycelium dry weight (mg)	Final pH	Aflatoxin culture (μg)	Aflatoxin mycelium (ng/mg)
YES	0.0	4.5	217(10) ^b	7.2 ^b	304.5(39.1) ^b	1372 ^b
		5.5	195 (5)	7.3	186.9(30.3)	974
		6.5	185 (6)	7.4	161.1(22.7)	853
		7.5	164 (4)	7.8	86.6(12.5)	723
	0.1	4.5	216 (7)	7.1	344.2(29.9)	1581
		5.5	205 (7)	6.8	243.4(27.3)	1198
		6.5	202 (6)	7.1	205.5(10.9)	1023
		7.5	174 (4)	7.4	153.8(16.6)	875
	1.0	4.5	241 (8)	4.2	443.3(43.5)	1581
		5.5	152(18)	4.7	176.4(51.4)	997
		6.5	202 (6)	4.8	255.3(40.6)	1370
		7.5	196 (8)	5.4	302.3(12.7)	1555
PMS	0.0	4.5	202(25) ^c	6.8 ^c	26.5 (2.8) ^c	146 ^c
		5.5	253 (5)	7.4	11.4 (2.7)	45
		6.5	253(15)	7.6	13.8 (5.4)	51
		7.5	230(13)	7.5	0.2 (0.1)	<1
	0.1	4.5	216 (8)	6.5	26.1 (8.0)	118
		5.5	249 (6)	7.2	24.7 (3.2)	100
		6.5	218(18)	7.1	83.5(19.0)	365
		7.5	183(12)	7.3	12.9 (6.7)	67
	1.0	4.5	91(13)	4.7	0.4 (0.1)	4
		5.5	99 (2)	5.7	2.1 (0.9)	22
		6.5	108 (9)	6.5	<0.1	<1
		7.5	125(11)	7.3	<0.01	<1

^a Added as a water/ethanol solution.

^b \bar{x} (\pm SEM), n = 9 replicate cultures.

^c \bar{x} (\pm SEM), n = 6 replicate cultures.

containing PMS cultures was a consequence of the use of ethanol, and suggested that miconazole was increasing its incorporation into aflatoxins.

When similar experiments (data not shown) were performed using media containing ethanol, acetate, citrate, or malate as sole carbon sources and supplemented with 0.0 or 0.1 μM miconazole, only ethanol supported aflatoxin production, although acetate, citrate and malate supported some growth. This indicates that miconazole can increase aflatoxin production on substrates that normally support toxin synthesis, but cannot stimulate the mould to initiate toxin synthesis on normally non-supportive substrates [5].

The effect of initial pH on the activity of miconazole

was characterized by examining conidia-initiated YES and PMS cultures containing 0.0, 0.1, and 1.0 μM miconazole (Table 3). The effect of miconazole on growth of *A. parasiticus* did not appear to be influenced by initial pH with the exception of a small stimulation of growth in the pH 7.5/YES cultures. However, differences were noted with regard to aflatoxin production. The stimulation of aflatoxin formation by miconazole was distinctly greater on a relative basis when the initial pH was near neutrality. This differential stimulatory effect appeared to be largely due to miconazole overcoming the decreased production of aflatoxins in cultures having a more neutral initial pH.

Miconazole also affected the final pH of the YES

and PMS cultures. In YES, which contains both carbohydrate and amino acids, *A. parasiticus* typically produces a decline in pH until the carbohydrate source becomes depleted, followed by a rise in pH as amino acids and/or organic acids are used as secondary carbon sources [3]. In PMS, which contains amino acids as its sole carbon source, only the rise in pH is observed [2]. In the present experiment, miconazole at 1.0 μM prevented the characteristic pH rise in both YES and PMS. Since the mould grew in miconazole-containing PMS, the catabolism of amino acids was not blocked. It is possible that miconazole may inhibit the utilization of organic acids or the generation of NH_4^+ ions from deamination reactions. Evaluation of these possibilities will require further research.

Replacement cultures

In order to examine the effect of miconazole on

aflatoxin synthesis while minimizing fungal growth, the replacement culture technique of Buchanan & Lewis [4] was employed. The mould was grown initially in YES, the mycelial pellets, disrupted, and then reincubated in PMS which does not support aflatoxin synthesis. The mould was then transferred to PMS, GMS, and PGMS (glucose + peptone) containing various concentrations of miconazole added as water/ethanol solutions. These results are presented in Table 4.

In the PMS cultures, miconazole caused a dose-related increase in aflatoxin accumulation similar to that observed with conidia-initiated PMS cultures (Table 1) except that there was continued increases in toxin production by the pregrown mycelia in the presence of 10 mM miconazole. Stimulation of aflatoxin production was also observed in the GMS replacement cultures in conjunction with increasing miconazole concentrations up to 1.0 μM . No increase in aflatoxin accumulation was observed in replacement medium containing both glucose and

Table 4. Effect of miconazole on aflatoxin production by replacement cultures of *A. parasiticus*.

Replacement medium	Miconazole (μM) ^a	Mycelium dry weight (mg)	pH ^b	Aflatoxin culture (μM)	Aflatoxin mycelium (ng/mg)
PMS	*	187(20) ^c	–	0.9 (0.1) ^c	5 ^c
	0.00	399(14)	6.9 ^c	2.9 (0.5)	7
	0.01	428(10)	6.8	5.2 (1.0)	12
	0.10	426 (7)	6.8	11.8 (3.3)	28
	1.00	375 (8)	6.5	15.1 (8.3)	40
	10.00	315(12)	5.9	19.4 (4.6)	62
GMS	*	141(16)	–	1.0 (0.2)	7
	0.00	346(12)	2.4	85.4 (8.6)	247
	0.01	338 (8)	2.4	93.0(12.0)	275
	0.10	353 (7)	2.4	117.0(25.2)	331
	1.00	395(13)	2.4	182.6(18.0)	462
	10.00	296(22)	2.8	104.6(19.0)	353
PGMS	*	188 (3)	–	1.8 (0.2)	9
	0.00	372 (5)	2.7	408.0(20.7)	1097
	0.01	355 (4)	2.7	370.3 (9.2)	1043
	0.10	342(14)	2.7	355.5(26.6)	1040
	1.00	369 (8)	2.7	399.6(11.2)	1083
	10.00	303(14)	3.2	183.0(27.1)	604

* Values at time of transfer.

^a Miconazole added as a water/ethanol solution.

^b Initial pH = 5.5.

^c \bar{x} (\pm SEM), n = 4 replicate cultures.

peptone (PGMS), possibly indicating that the combination of carbon sources eliminated the stimulatory effect of miconazole or that aflatoxin production was already maximal in this medium, obviating any further increase.

Respirometry

Since the primary site of action for miconazole in yeasts has been reported to be mitochondria [17, 18], the effect of miconazole on respiration in *A. parasiticus* was evaluated using GMS, PMS, and GyMS as replacement media (Figs. 1–3). The pattern of post-transfer respiration observed with control cultures was similar to that observed in previous investigations for replacement cultures without ethanol [4, 7]. At 0.1 μM , miconazole did not affect respiration in any of the replacement media. However, 10 μM miconazole delayed the elevation in respiratory activity observed in control cultures after approximately 24 h of post-transfer incubation. This is in contrast to the results of Portillo & Gancedo [17] who found that 10 μM miconazole did not affect respiration in *S. cerevisiae* or *C. albicans*.

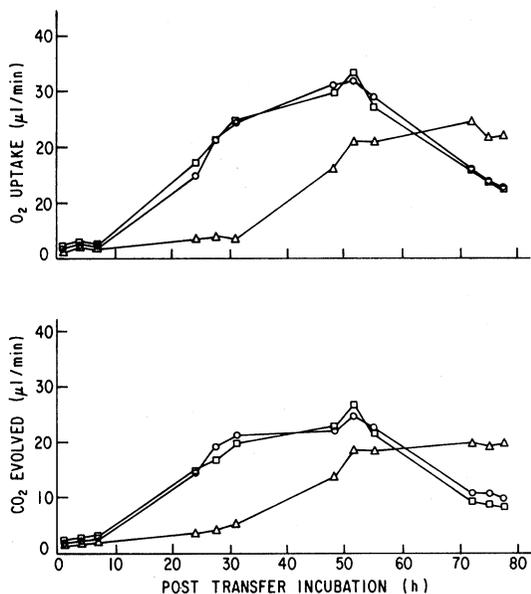


Fig. 1. Oxygen utilization and carbon dioxide production by replacement cultures of *Aspergillus parasiticus* after transfer to GMS containing 0.0 (\square), 0.1 (\circ), and 10.0 (Δ) μM miconazole.

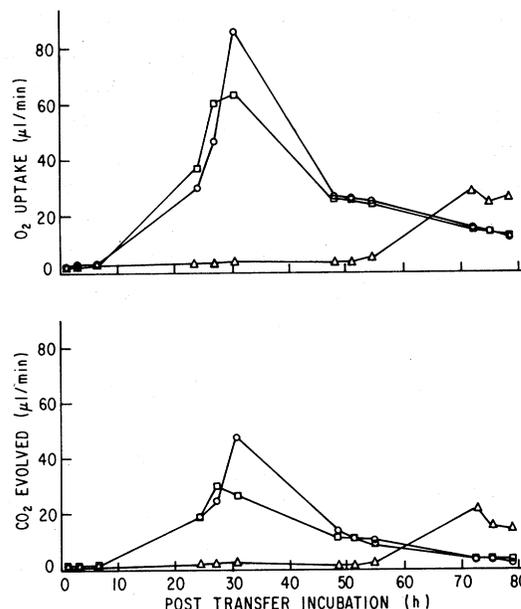


Fig. 2. Oxygen utilization and carbon dioxide production by replacement cultures of *Aspergillus parasiticus* after transfer to PMS containing 0.0 (\square), 0.1 (\circ), and 10.0 (Δ) μM miconazole.

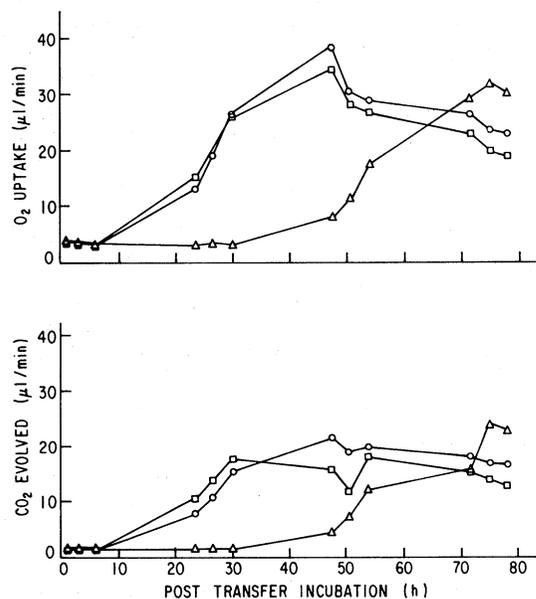


Fig. 3. Oxygen utilization and carbon dioxide production by replacement cultures of *Aspergillus parasiticus* after transfer to GyMS containing 0.0 (\square), 0.1 (\circ), and 10.0 (Δ) μM miconazole.

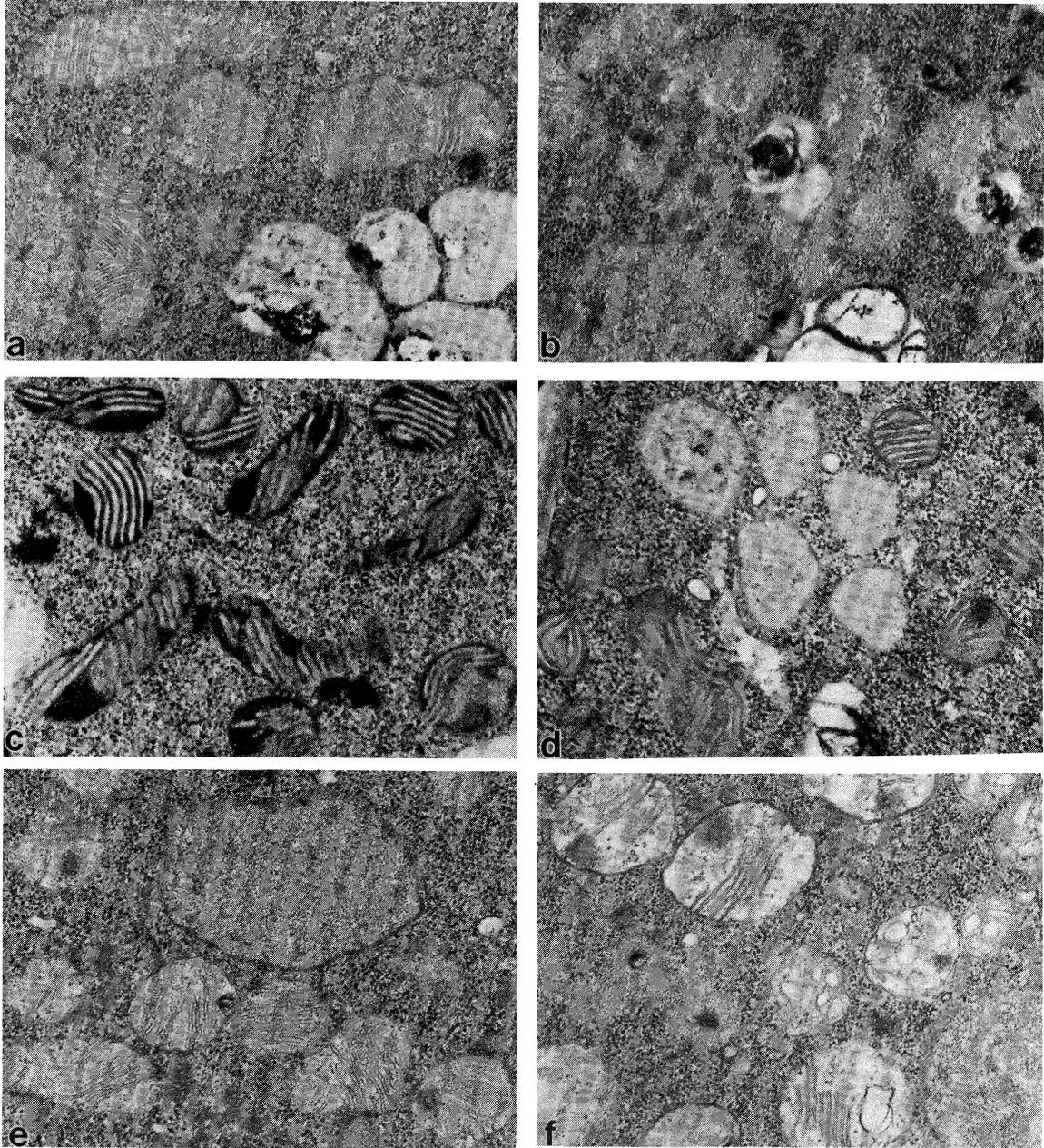


Fig. 4. Morphology of *Aspergillus parasiticus* mitochondria after 24 h incubation in peptone-mineral salts (PMS) replacement media (28 C, no agitation) with the following additions: a, none; b, 2.8% (w/v) ethanol; c, 6% glucose (=PGMS medium); d, 2.8% ethanol + 1 μ M miconazole; e and f, 1 μ M miconazole (\times 35000).

The delayed respiration observed with 10 μ M miconazole was of particular interest since that level of miconazole did not inhibit aflatoxin production by PMS and GMS replacement cultures of *A.*

parasiticus when compared to untreated controls (Table 4). Previous work in our laboratory with caffeine [3] and thioglycerol [9] indicated that those compounds inhibit toxin production and also

delayed or depressed respiration. This earlier work suggested an apparent relationship between the rise in respiratory activity observed with the mold after a period of post-transfer incubation and the synthesis of aflatoxins. However, the present study with miconazole suggests that the observed effect may have been coincidental and that the two physiological processes are not directly interrelated.

Electron microscopy

The stimulation of aflatoxin production by miconazole in ethanol-supplemented PMS cultures was evaluated further by observing the effects of miconazole on fungal ultrastructure (Fig. 4). Portions of pregrown, disrupted PMS-conditioned mycelium were suspended in PMS replacement media containing ethanol or miconazole, or both, and mitochondrial structure was assessed after 24 h incubation at 28 °C. Since aflatoxin-supporting cultures produce more toxin when not shaken, and because the replacement cultures had a physical consistency of mush, no attempt was made to aerate these cultures. Some of the mitochondrial changes observed at 24 h were thus due to partial anoxia. However, media-specific changes were also observed.

After 24 h in peptone replacement culture without miconazole or ethanol (Fig. 4a), mitochondria were expanded in volume and had well-defined membranes. The mitochondrial matrix was lighter than the surrounding cytoplasm. When 2.8% ethanol was present in a similar culture, mitochondrial membranes had varying degrees of definition. An example is shown in Fig. 4b. At one extreme, the mitochondria were slightly condensed, but indistinct. At the other extreme, mitochondria were totally indistinguishable except as areas which excluded ribosomes. This ultrastructure may reflect partial derepression in response to ethanol as a slowly-fermentable carbon source, modulated by local availability of oxygen. When a similar experiment was performed with PMS medium containing 6% glucose, mitochondria underwent extreme condensation of the matrix by 24 h (Fig. 4c).

When miconazole was added as a water/ethanol

solution to PMS replacement medium, mitochondria exhibited moderate condensation (Fig. 4d), suggesting that miconazole may have increased the utilization rate of ethanol as a carbon source. It is possible that a miconazole-induced mitochondrial lesion may cause increased carbon flux through extramitochondrial pathways. Miconazole did not cause mitochondrial condensation in the absence of the fermentable substrate. When miconazole was added directly (without ethanol) to PMS culture, mitochondrial volume was maintained (Fig. 4e). However, there was a tendency toward irreversible mitochondrial swelling (Fig. 4f), indicative of loss of inner membrane integrity. Additional studies (not shown) indicated that the damage observed was not equivalent to changes occurring because of decreased respiratory activity associated with extended post-transfer incubation (≥ 48 h).

If we assume that a mitochondrial lesion caused by miconazole is the same with or without ethanol, we can speculate that fermentation, and the accompanying mitochondrial condensation, protects mitochondria from the destabilizing effect of miconazole. Increased physical stability of mitochondria during a repressed or comparatively inactive physiological state would have survival value for the organism. This agrees with the observations of Portillo & Gancedo [17] that the organism is more resistant to the fungicidal properties of miconazole when cultured in the presence of a fermentable substrate. However, this does not explain the observation that the same level of miconazole inhibited growth of *A. parasiticus* in PMS with (Table 1) and without (Table 2) ethanol. It also does not account for the increased susceptibility of the mould when cultured in a glycerol-containing medium. In this case, an alternate possibility is that metabolism of glycerol is further stressing the mitochondria via increased activity of anaplerotic pathways such as the glycerol phosphate/dihydroxyacetone phosphate shuttle.

The reason underlying the increased production of aflatoxins in the presence of miconazole could not be specifically identified as a result of the present experimentation. One possibility is that miconazole affects the mitochondrial ATPase, depressing the transport of metabolites into the organelle. This

could result in an elevation of the cytoplasmic level of two-carbon precursors for aflatoxin synthesis, and accordingly increase production of the toxins. Evaluation of these hypotheses will require additional research.

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