The Lipid Composition and Permeability to the Triazole Antifungal Antibiotic ICI 153066 of Serum-grown Mycelial Cultures of *Candida albicans*

By CHRISTOPHER A. HITCHCOCK,¹† KEITH J. BARRETT-BEE² AND NICHOLAS J. RUSSELL¹*

¹ Department of Biochemistry, University of Wales College of Cardiff, PO Box 903, Cardiff CF1 1ST, UK

² Pharmaceuticals Division, ICI plc, Alderley Park, Macclesfield SK10 4TG, Cheshire, UK

(Received 5 January 1989; revised 10 April 1989; accepted 12 April 1989)

The total lipid content of *Candida albicans* (serotype A; NCPF 3153) exponential-phase mycelial cultures grown in tissue-culture medium 199 (containing 10%, v/v, foetal calf serum) was 29.8 ± 8 mg (g dry weight)⁻¹ (mean $\pm$ SD). The weight ratios of phospholipid to neutral lipid and phospholipid to non-esterified sterol were 2.6 ± 0.4 and 24.9 ± 0.5 , respectively. The major phospholipid was phosphatidylcholine with smaller amounts of phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol and diphosphatidylglycerol; the most abundant fatty acids were palmitic, palmitoleic, oleic and linoleic acids. The major neutral lipids comprised esterified sterol, triacylglycerol and non-esterified fatty acid with a smaller amount of non-esterified sterol. The fatty acid compositions of the three fatty-acid-containing neutral lipids were distinct from each other and the phospholipids. Comparison with previous data on yeast cultures of *C. albicans* A grown in glucose broth shows that mycelial cultures have a larger lipid content, lower phospholipid to neutral lipid ratio and higher phospholipid to non-esterified sterol ratio. We now show that mycelial cultures were more permeable to a [¹⁴C]triazole antifungal antibiotic compared with exponentially growing yeast cultures of several azole-sensitive strains. Taken together these data are consistent with there being a relationship between the phospholipid/non-esterified sterol ratio of a culture and its ability to accumulate a triazole.

INTRODUCTION

Candida albicans is a widespread opportunistic fungal pathogen responsible for superficial and systemic candidoses (Odds, 1988). The fungus is dimorphic, growing as either a budding yeast or filamentous mycelium, and both forms are invariably present in lesions. The mycelial form appears not to be essential for virulence *in vivo*, but the weight of evidence suggests that it is important in the pathogenesis of candidoses (for a review see Odds, 1988). The morphological transformation is promoted by a large number of factors *in vitro* (Odds, 1985), but the most important factor *in vivo* is probably serum.

The azole (*N*-substituted imidazole and triazole) class of antifungal antibiotics is used in the treatment of both superficial and deep-seated candidoses (Fromtling, 1988). One characteristic of all the azoles is that they inhibit mycelial growth, which is an important mode of action in view of the suggested pathogenicity of this morphological form (Borgers, 1985). The MIC values

† Present address: Department of Microbiology, University of Leeds, Leeds LS2 9JT, UK.

Abbreviations: TCM, tissue culture medium 199 containing 10% (v/v) foetal calf serum; YMA, yeast morphology agar; YNB, yeast nitrogen base; MIC, minimum inhibitory concentration.

of the azoles *in vitro* vary considerably with the conditions used in the sensitivity tests and this phenomenon is associated especially with the yeast-to-mycelial transformation (Odds, 1980; Borgers, 1985). However, in sensitivity tests containing serum the order of potency among azoles corresponds more closely to their efficacy *in vivo* (Borgers, 1985). This situation may reflect the presence of the mycelial form in infections.

The azoles probably work by inhibiting ergosterol biosynthesis in *C. albicans* by binding to 14 α -sterol demethylase, an important enzyme in the ergosterol biosynthetic pathway (Van den Bossche *et al.*, 1983). Compared with yeast cultures, ergosterol biosynthesis is more sensitive to azoles in mycelial cultures, and this observation has been used to explain the efficacy of azoles *in vivo* (Van den Bossche *et al.*, 1980).

However, another possible factor in determining the sensitivity of mycelial cells could be their ability to accumulate azole antifungal antibiotics. There is evidence that ketoconazole is taken up by *C. albicans* via a saturable, active-transport process (Boiron *et al.*, 1987). Although it is not known how the triazole ICI 153066 enters *C. albicans*, we have shown that yeast cultures of different strains (including azole-resistant strains) have quite different permeabilities to this antifungal antibiotic (Hitchcock *et al.*, 1986, 1987*a, b*). Furthermore, on the basis of a comparison of the membrane lipid composition of the strains, it was suggested that there was a relationship between the phospholipid to non-esterified sterol ratio of a strain and its ability to take up triazole. Shimokawa *et al.* (1986) suggested that an altered membrane sterol composition of some mutants of *C. albicans* was responsible for their increased sensitivity to a range of drugs.

In this paper, we report the detailed lipid composition and permeability to triazole of mycelial cultures of *C. albicans* (serotype A), grown in tissue-culture medium 199, supplemented with foetal calf serum. This medium was used as a source of serum because it resembles the situation *in vivo*, with the expectation of changes in fungal membrane lipid composition that could clarify the efficacy of azoles *in vivo*. Compared with yeast cultures, mycelial cultures grown in tissue-culture medium have a larger phospholipid to non-esterified sterol ratio and are more permeable to a triazole antifungal.

METHODS

Materials. Phospholipid and neutral lipid standards were purchased from Sigma. Solvents and other chemicals were of analytical grade and were purchased from BDH. The radioactive triazole [¹⁴C]ICI 153066 was synthesized at ICI Pharmaceuticals Division, Alderley Park, Macclesfield, UK.

Organism and culture conditions. Stock cultures of *Candida albicans* (serotype A; NCPF 3153) were maintained on slopes of yeast morphology agar (YMA, Difco) as described by Hitchcock *et al.* (1986). Late-exponential-phase mycelial cultures were grown in 1 litre of tissue culture medium 199 (Gibco) supplemented with 10% (v/v) foetal calf serum (Sigma), in 2 litre Erlenmeyer flasks at 37 °C without shaking for 16 h; the medium was inoculated with 3×10^4 yeasts washed from an agar slope with sterile distilled water. After incubation for 3 h, $92 \pm 2\%$ (mean $\pm$ SD) of the inoculum had formed germ-tubes, as measured by haemocytometer counts. Mycelia grew as a thin white mat on the bottom of the flask and were harvested by filtration through filter paper under negative pressure. Filtered mycelia were washed thoroughly with 2×1 litre of deionized water before transfer to a round-bottom flask for shell-freeze-drying. Freeze-dried mycelia from 1 litre cultures weighed 60 ± 10 mg.

Late-exponential-phase yeast cultures were grown in yeast nitrogen base containing 2% (v/v) glucose (YNB) as described by Hitchcock *et al.* (1986).

Protein and DNA estimations. The protein and DNA contents were measured in homogenates of mycelia broken by three passages through a French pressure cell (American Instrument Co.), at 1.3×10^5 kPa. Protein was estimated by the method of Lowry using a bovine serum albumin standard. DNA was estimated by the method of Leyva & Kelly (1974) using a calf thymus DNA standard.

Lipid extraction and analysis. Lipids were extracted from freeze-dried cultures by the method of Bligh & Dyer (1959) after phospholipase activities had been inactivated with hot 2-isopropanol (Hitchcock *et al.*, 1986). Non-lipid contaminants were removed from total lipid extracts with Sephadex G-25 (Wells & Dittmer, 1963). The purified lipid was fractionated into individual phospholipids and neutral lipids by thin-layer chromatography. Phospholipids were quantified by estimation of their phosphorus and fatty acid content, and acyl lipids by their fatty acid content. Esterified and non-esterified sterols were identified and quantified by GLC and GC-MS. The details of these methods are given in Hitchcock *et al.* (1986).

Measurement of azole uptake. Freshly harvested mycelia were washed with 0.1 M-potassium phosphate buffer, pH 7.4, containing 0.1% (v/v) Tween 80 and 0.1 M-potassium phosphate, pH 5.5. The uptake of

Table 1. *Fatty acid composition of the total phospholipid and the major individual phospholipids in C. albicans mycelial cultures*

The total lipid was fractionated into total phospholipid or individual phospholipids by TLC, and fatty acid methyl esters prepared by transmethylation, as described in Methods. Values are means $\pm$ SD of at least two GLC analyses of fatty acids in phospholipids from three separate cultures. PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; ND, not detected; UFA, unsaturated fatty acid; DBI, double bond index.

Fatty acid	Composition (% w/w) $\pm$ SD				
	Total PL	PC	PE	PI	PS
16:0	17.8 $\pm$ 0.6	11.4 $\pm$ 0.6	26.8 $\pm$ 0.2	28.7 $\pm$ 0.3	20.1 $\pm$ 0.3
16:1	14.9 $\pm$ 0.1	16.6 $\pm$ 1.3	12.2 $\pm$ 0.6	4.7 $\pm$ 0.7	18.4 $\pm$ 0.5
16:2	0.9 $\pm$ 0.1	2.2 $\pm$ 0.9	1.7 $\pm$ 0.5	5.1 $\pm$ 0.3	2.7 $\pm$ 0.7
18:0	7.5 $\pm$ 0.4	13.9 $\pm$ 0.8	14.4 $\pm$ 0.8	13.9 $\pm$ 1.1	3.0 $\pm$ 0.1
18:1	35.6 $\pm$ 1.6	32.1 $\pm$ 1.5	36.0 $\pm$ 0.9	36.9 $\pm$ 1.0	31.5 $\pm$ 0.5
18:2	20.9 $\pm$ 0.2	22.0 $\pm$ 0.2	8.9 $\pm$ 0.2	11.5 $\pm$ 0.6	20.2 $\pm$ 1.8
18:3	2.2 $\pm$ 0.3	3.0 $\pm$ 0.5	ND	ND	ND
% UFA	75.5	75.9	58.8	58.2	72.8
18/16 ratio	2.0	2.35	1.46	1.62	1.33
DBI	1.04	1.06	0.69	0.75	0.96

[U- 14 C]ICI 153066 (specific radioactivity 19.4 Ci mg $^{-1}$, 718 GBq mg $^{-1}$) by mycelial cell suspensions (50–70 mg wet weight ml $^{-1}$) was measured by the method of Hitchcock *et al.* (1986), except that assays were terminated by filtering, as described by Ryley *et al.* (1984).

The uptake of radiolabel by yeast cell suspensions (10⁹ cells ml $^{-1}$) was measured using the method described in Hitchcock *et al.* (1986). Routinely, parallel control experiments with autoclave-killed cells were done to establish the amount of radiolabel binding to cells. That amount was reproducibly 30% of the radioactivity incorporated.

RESULTS

Lipid content

The amount of total lipid extracted from late-exponential mycelial cultures was 29.8 $\pm$ 8 mg lipid (g dry weight) $^{-1}$, or 290 $\pm$ 20 μ g lipid (mg total cellular protein) $^{-1}$, or 13.6 $\pm$ 1.3 mg (mg total cellular DNA) $^{-1}$ (means $\pm$ SD; n = 3), respectively. The weight ratios of phospholipid to neutral lipid and phospholipid to non-esterified sterol were 2.6 $\pm$ 0.4 and 24.9 $\pm$ 0.5 (n = 3), respectively.

Polar lipid composition

Polar lipids comprised 72.2 $\pm$ 0.5% (n = 3) of the total lipid and consisted entirely of phospholipid. The phospholipid composition was characterized by a large content of phosphatidylcholine, which accounted for 54.9 $\pm$ 1.4% of the total phospholipid. There were smaller amounts of phosphatidylethanolamine (12.3 $\pm$ 0.8%), phosphatidylinositol (15.0 $\pm$ 0.5%), phosphatidylserine (6.7 $\pm$ 0.4%), phosphatidylglycerol (2.8 $\pm$ 0.2%) and di-phosphatidylglycerol (9.5 $\pm$ 0.6%), and a trace (<1%) of sphingomyelin (values are means $\pm$ SD of experiments on three separate cultures). The fatty acid compositions of individual phospholipids differed, and data for the major phospholipids and total phospholipid are shown in Table 1.

The most abundant fatty acids in each phospholipid were 16:0, 16:1, 18:1 and 18:2. Phosphatidylcholine was the most unsaturated phospholipid which was reflected in a high percentage unsaturated fatty acid and double bond index. It was characterized by a high content of 16:1 relative to 16:0 and was the only phospholipid to contain 18:3. Phosphatidylethanolamine and phosphatidylinositol were the most saturated phospholipids due mainly to their relatively large content of 16:0, giving them a low percentage unsaturated fatty acid and double bond index.

Table 2. *Fatty acid composition of non-esterified fatty acid and acyl neutral lipids in C. albicans mycelial cultures*

Non-esterified fatty acid and acyl neutral lipid were fractionated from total lipid and fatty acids analysed as described in Methods. Values are means $\pm$ SD of at least two GLC analyses of neutral lipids from three separate cultures. NEFA, non-esterified fatty acid; TAG, triacylglycerol; ES, esterified sterol; ND, not detected; UFA, unsaturated fatty acid; DBI, double bond index.

Fatty acid	Composition (% w/w) $\pm$ SD		
	NEFA	TAG	ES
16:0	27.6 $\pm$ 1.6	18.6 $\pm$ 0.4	14.6 $\pm$ 0.4
16:1	10.5 $\pm$ 2.5	5.7 $\pm$ 0.8	15.5 $\pm$ 0.4
16:2	3.4 $\pm$ 1.4	2.3 $\pm$ 0.6	8.2 $\pm$ 0.6
18:0	12.6 $\pm$ 1.5	29.3 $\pm$ 1.5	14.8 $\pm$ 0.3
18:1	32.3 $\pm$ 0.8	37.2 $\pm$ 0.6	41.5 $\pm$ 0.5
18:2	13.2 $\pm$ 2.3	8.6 $\pm$ 0.4	6.3 $\pm$ 0.4
18:3	2.0 $\pm$ 1.5	ND	ND
%UFA	61.5	53.8	71.5
18/16 ratio	1.45	2.82	1.63
DBI	0.82	0.65	0.86

Table 3. *Non-esterified and esterified sterol compositions of C. albicans mycelial cultures*

Sterols were fractionated from the total lipid by TLC and analysed by GLC and GC-MS as described in Methods. Values are the means of two analyses of sterols from two separate cultures (values varied by <2%). NES, non-esterified sterol; ES, esterified sterol.

Sterol	Composition (% w/w)	
	NES	ES
Ergosterol	57.0	55.3
Ergosta-7-ene	24.5	25.7
Ergosta-5,7-dien-3 β -ol	10.0	12.7
Ergosta-7,22-dien-3 β -ol	4.0	3.2
24-Methylene-24,25-dihydrolanosterol	3.0	2.5
Zymosterol	1.5	0.9

Neutral lipid composition

The neutral lipid comprised 27.8 $\pm$ 0.8% ($n = 3$) of the total lipid and consisted mainly of triacylglycerol (39.1 $\pm$ 1.0%), esterified sterol (27.7 $\pm$ 0.8) and non-esterified fatty (20.4 $\pm$ 0.8%) acid with smaller amounts of non-esterified sterol (8.8 $\pm$ 0.4%) and squalene (4.2 $\pm$ 1.8%) (values are means $\pm$ SD of experiments on three separate cultures). The fatty acid compositions of the three acyl neutral lipids were different from each other (Table 2) and the phospholipids (cf. Table 1).

The esterified sterol was the most unsaturated of the acyl lipids (71.5% unsaturated fatty acid) due to the relatively large proportion of 18:1. In contrast, the triacylglycerol was the most saturated of the acyl lipids and contained a large proportion of 18:0, giving it a relatively high C18/C16 ratio and small percentage unsaturated fatty acid and double bond index.

The sterol compositions of the esterified and non-esterified sterols were similar (Table 3). Both compositions were notable for the low content of ergosterol, which accounted for approximately half of the total sterols, the remainder of which were metabolic precursors of ergosterol.

Triazole uptake

The results of a typical experiment comparing the uptake of [14 C]ICI 153066 by yeast and mycelial cultures of *C. albicans* A are shown in Fig. 1. The amount of radioactivity incorporated

Fig. 1. Uptake of [^{14}C]ICI 153066 by exponential-phase mycelial (●) and yeast (■) cultures of *C. albicans*. Values for each time point are the mean of triplicate determinations on cells from one culture (values varied by <10%).

by both cultures was linear for 8 min, after which the uptake of triazole plateaued. The rate of uptake of ICI 153066 by mycelial cultures was $0.43 \pm 0.03 \text{ nmol min}^{-1} (\text{g dry weight})^{-1}$ compared with $0.15 \pm 0.02 \text{ nmol min}^{-1} (\text{g dry weight})^{-1}$ for yeast cultures (values are the means $\pm$ SD of experiments on three separate batches of exponential-phase cultures). The value for yeast cultures corresponds to $2.46 \text{ pmol min}^{-1} (10^9 \text{ yeasts})^{-1}$ (calculated on the basis that $10^9 \text{ yeasts} = 16 \pm 1 \text{ mg dry weight}$) which is in good agreement with $2.52 \text{ pmol min}^{-1} (10^9 \text{ yeasts})^{-1}$ given by Hitchcock *et al.* (1986).

DISCUSSION

The lipid content of mycelial cultures grown in serum-containing tissue-culture medium is approximately 11 times that of yeast cultures grown in YNB (Hitchcock *et al.*, 1986). This compares closely to the value of 14 times obtained by Davies & Denning (1972) using mycelial and yeast cultures grown in a similar way to that in the present study. It is apparent that serum-grown mycelial cultures contain more lipid than those grown in serum-free media (Yamaguchi, 1974; Ghannoum *et al.*, 1986), which may reflect the incorporation of serum lipids into fungal cells in addition to *de novo* synthesis. In addition, the total lipid contains proportionately more neutral lipid relative to polar lipid: the ratio of polar lipid to neutral lipid in mycelial cultures is 2.6 compared with 3.6 in yeast cultures grown in YNB (Hitchcock *et al.*, 1986).

Ghannoum *et al.* (1986), working with *C. albicans* ATCC 10231, reported morphology-related differences in phospholipid and neutral lipid compositions between yeast and mycelial forms grown together in mixed culture. However, in the present study with *C. albicans* A it is not known whether the differences in lipid composition between mycelial and yeast (Hitchcock *et al.*, 1986) cultures are related directly to the morphological change or different media or both. The same argument applies to other published changes in phospholipid (Marriott, 1975; Sundaram *et al.*, 1981; Yano *et al.*, 1982) and neutral lipid (Marriott, 1975; Sundaram *et al.*, 1981) compositions that accompany mycelial growth under a variety of growth conditions. Furthermore, there are no data on mycelial cultures grown in serum-supplemented tissue-culture media for comparison.

Mycelial cultures of *C. albicans* A grown in tissue-culture medium contained the same phospholipids as yeast cultures grown in glucose broth (Hitchcock *et al.*, 1986) but the composition was quite different, due mainly to the large amount of phosphatidylcholine in the mycelial form (54.9 compared with 25.4%, w/w). However, there are similarities in the phospholipid compositions of both morphological forms. For example, phosphatidylcholine and

phosphatidylinositol were the most unsaturated and saturated (expressed in terms of percentage unsaturated fatty acid) phospholipids, respectively. The large amount of phosphatidylcholine in mycelial cells contributes greatly to the large percentage unsaturated fatty acid value of the total phospholipid. Mycelial phospholipids in other strains are also more unsaturated than those of the yeast form (Yano *et al.*, 1982; Ghannoum *et al.*, 1986), and it has been suggested that the increased fluidity of membranes which this causes might be important for mycelial growth (Yano *et al.*, 1982). The activities of several membrane-bound enzymes depend on fluidity (for a review see Sanderman, 1978), and although it is not known if chitin synthase shows such a dependence, this enzyme is twice as active in the plasma membrane of mycelial cells (Braun & Calderone, 1978).

In common with the polar lipids, the mycelial neutral lipid composition was distinct from that of the yeast form (Hitchcock *et al.*, 1986). Mycelial cultures contained more esterified sterol and less non-esterified sterol. The significance of the relatively large amount of esterified sterol (27.7 compared with 8.1%, w/w in yeast cultures) is not known: it may be a storage lipid and/or a membrane component (Grover & Cushley, 1979). In addition, the esterified sterol fatty acyl composition was more unsaturated in mycelial cells (71.5 compared with 47.2%, w/w unsaturated fatty acid in yeast cultures).

Although both morphological forms contained the same proportion of triacylglycerol, the fatty acyl compositions were different, the mycelial triacylglycerol being more saturated (53.8 compared with 80.7% unsaturated fatty acid).

Mycelial lipid contained a significant amount of non-esterified fatty acid, which is thought to be the product of (lyso)phospholipase digestion (Christie, 1982). However, the fatty acid composition was unlike that of any phospholipid (or the total phospholipid), indicating that the enzyme(s) lacked specificity for a particular phospholipid. The same is true for (lyso)phospholipases in yeast cultures of a variety of *C. albicans* strains (Hitchcock *et al.*, 1986, 1987a).

We have shown recently that different strains of *C. albicans* yeasts take up the triazole ICI 153066 at a rate that is proportional to their phospholipid/non-esterified-sterol ratio (Hitchcock *et al.*, 1986). The small amount of non-esterified sterol in mycelial cultures (8.9 compared with 23.8%, w/w in the yeast form), together with the large amount of phospholipid, accounts for the high phospholipid/non-esterified sterol ratio, which is 29.7 in the mycelial form and 15.4 in the yeast form. Moreover, comparison of the phospholipid/ergosterol ratios reveals an even larger difference between the two morphological forms (51.3 in the mycelial form and 15 in yeast form), because ergosterol comprises only 57% of the total non-esterified sterol in mycelial cells. If it is assumed that this ratio for whole cells reflects that of membranes, then together with the more unsaturated phospholipids, mycelial membranes are expected to be considerably more fluid. Such a change in fluidity probably would alter passive diffusion or active transport of the triazole, depending on its mechanism of uptake (Russell, 1989) and, therefore, could explain the increased permeability of mycelial cells to ICI 153066, which may be an important factor in determining the potency of this triazole against the mycelial growth phase (Ryley *et al.*, 1984). The data for mycelial cultures are consistent with there being a relationship between triazole permeability and the ratio of phospholipid/non-esterified sterol. However, it is not known if the ratio determined for total cellular lipid reflects that of the plasma membrane and/or other membranes, and currently we are investigating the mechanism of uptake of triazole in cells from yeast and mycelial cultures. It would be interesting to know if the proposed relationship between lipid composition and uptake of ICI 153066 extended to other triazole antifungal antibiotics.

We should like to thank Mrs Anne Oldfield for performing the GC-MS analysis of sterols. The financial support of the Science and Engineering Research Council is acknowledged.

REFERENCES

BLIGH, E. G. & DYER, W. J. (1959). A rapid method of total lipid extraction and purification. <i>Canadian Journal of Biochemistry and Physiology</i> 37 , 911-917.	BOIRON, P., DROUHET, E., DUPONT, B. & IMPROVISI, L. (1987). Entry of ketoconazole into <i>Candida albicans</i> . <i>Antimicrobial Agents and Chemotherapy</i> 31 , 244-248.

- BORGERS, M. (1985). Antifungal Azole Derivatives. In *The Scientific Basis of Antimicrobial Chemotherapy*, pp. 133–153. Edited by D. Greenwood & F. O'Grady. Cambridge: Cambridge University Press.
- BRAUN, P. C. & CALDERONE, R. A. (1978). Chitin synthesis in *Candida albicans*: comparison of yeast and hyphal forms. *Journal of Bacteriology* **133**, 1472–1477.
- CHRISTIE, W. W. (1982). *Lipid Analysis*, 2nd edn. Oxford: Pergamon Press.
- DAVIES, R. R. & DENNING, T. J. V. (1972). Growth and form in *Candida albicans*. *Sabouraudia* **10**, 180–188.
- FROMTLING, R. A. (1988). Overview of medically important antifungal azole derivatives. *Clinical Microbiology Reviews* **1**, 187–217.
- GHANNOUM, M. A., JANINI, G., KHAMIS, L. & RADWAN, S. S. (1986). Dimorphism-associated variations in the lipid composition of *Candida albicans*. *Journal of General Microbiology* **132**, 2367–2375.
- GROVER, A. K. & CUSHLEY, R. J. (1979). Cholesterol ester distribution in lecithin bilayer membranes. *Atherosclerosis* **32**, 87–91.
- HITCHCOCK, C. A., BARRETT-BEE, K. J. & RUSSELL, N. J. (1986). The lipid composition of azole-sensitive and azole-resistant strains of *Candida albicans*. *Journal of General Microbiology* **132**, 2421–2431.
- HITCHCOCK, C. A., BARRETT-BEE, K. J. & RUSSELL, N. J. (1987a). The lipid composition and permeability to azole of an azole- and polyene-resistant mutant of *Candida albicans*. *Journal of Medical and Veterinary Mycology* **25**, 29–37.
- HITCHCOCK, C. A., RUSSELL, N. J. & BARRETT-BEE, K. J. (1987b). Sterols in *Candida albicans* mutants resistant to polyene or azole antifungals, and of a double mutant *C. albicans* 6.4. *CRC Critical Reviews in Microbiology* **15**, 111–115.
- LEYVA, A. & KELLEY, W. N. (1974). Measurement of DNA in cultured human cells. *Analytical Biochemistry* **62**, 173–179.
- MARRIOTT, M. S. (1975). Isolation and chemical characterization of plasma membranes from the yeast and mycelial forms of *Candida albicans*. *Journal of General Microbiology* **86**, 115–132.
- ODDS, F. C. (1980). Laboratory evaluation of antifungal agents: a comparative study of five imidazole derivatives of clinical importance. *Journal of Antimicrobial Chemotherapy* **6**, 749–761.
- ODDS, F. C. (1985). Morphogenesis in *Candida albicans*. *CRC Critical Reviews in Microbiology* **12**, 45–93.
- ODDS, F. C. (1988). *Candida and Candidosis*, 2nd edn. London: Ballière Tindall.
- RUSSELL, N. J. (1989). Functions of lipids: structural roles and membrane functions. In *Microbial Lipids*, vol. 2, ch. 17. Edited by C. Ratledge & S. G. Wilkinson. London: Academic Press (in the Press).
- RYLEY, J. F., WILSON, R. G. & BARRETT-BEE, K. J. (1984). Azole resistance in *Candida albicans*. *Sabouraudia* **22**, 53–63.
- SANDERMANN, H. (1978). Regulation of membrane enzymes by lipids. *Biochimica et biophysica acta* **515**, 209–237.
- SHIMOKAWA, O., KATO, Y. & NAKAYAMA, H. (1986). Increased drug sensitivity in *Candida albicans* cells accumulating 14-methylated sterols. *Journal of Medical and Veterinary Mycology* **24**, 481–483.
- SUNDARAM, S., SULLIVAN, P. A. & SHEPHERD, M. G. (1981). Changes in lipid composition during starvation and germ-tube formation in *Candida albicans*. *Experimental Mycology* **5**, 140–147.
- VAN DEN BOSSCHE, H. V., WILLEMSSENS, G., COOLS, W., CORNELISSEN, F., LAUWERS, W. F. & VAN CUTSEM, J. M. (1980). *In vitro* and *in vivo* effects of the antimycotic drug ketoconazole on sterol biosynthesis. *Antimicrobial Agents and Chemotherapy* **17**, 922–928.
- VAN DEN BOSSCHE, H. V., WILLEMSSENS, G., COOLS, W., MARICHAL, P. & LAUWERS, W. (1983). Hypothesis on the molecular basis of the antifungal activity of *N*-substituted imidazoles and triazoles. *Biochemical Society Transactions* **11**, 665–666.
- WELLS, M. A. & DITTMER, J. C. (1963). The use of Sephadex for the removal of nonlipid contaminants from lipid extracts. *Biochemistry* **2**, 1259–1263.
- YAMAGUCHI, H. (1974). Mycelial development and chemical alteration of *Candida albicans* from biotin insufficiency. *Sabouraudia* **12**, 320–328.
- YANO, K., YAMADA, T., BANNO, Y., SEKIYA, T. & NOZAWA, Y. (1982). Modification of lipid composition in a dimorphic fungus, *Candida albicans* during the yeast to hypha transformation. *Japanese Journal of Medical Mycology* **23**, 159–165.