Influences of Cellular Susceptibility to Amphotericin B
and of Post-irradiation Growth Conditions on Inactivation of
Candida albicans by Ultraviolet Radiation.

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INTRODUCTION

Non-budding cells of *Candida albicans* show enhanced survival following ultraviolet (uv) irradiation if grown at low temperature, in the presence of sterols or long chain fatty acids, or on minimal rather than enriched medium (Busbee and Sarachek, 1969; Sarachek and Higgins, 1972). Sarachek and Higgins (1972) have summarized evidence suggesting that each of these effects related to recovery from uv induced disturbance of organizational features of the cell membrane which are critical for the initiation of cell division. Recently, Molzahn and Woods (1972) have shown that acquisition by yeasts of resistance to polyene antibiotics, such as amphotericin B, is associated with qualitative and quantitative shifts in cellular contents of membrane associated sterols. We report here observations on the uv responses of amphotericin B-resistant and amphotericin B-sensitive strains of *C. albicans* which provide further indication that the post-irradiation effects of temperature, lipids and nutritional enrichment depend on the constitution of the cell membrane.

MASTER

MATERIALS AND METHODS

The amphotericin B-sensitive test organism, WD-18-4, is a methionine

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and lysine auxotroph derived from the wild type stock, 526, originally provided by H.F. Hasenclever, National Institutes of Health, Bethesda, Maryland, U.S.A. To secure amphotericin B-resistant mutants of WD-18-4, washed cells were exposed to non-lethal doses of uv (30 ergs/mm^2), plated at a density of $ca 10^7$ cells per plate on enriched medium containing the antibiotic and incubated at 25^0 C or 37^0 C . Repetition of this procedure with clonal populations isolated successively from plates containing $5 \mu\text{g}$, $20 \mu\text{g}$, and $50 \mu\text{g}$ amphotericin B per ml allowed final selection of stable, high level drug resistant mutants from plates containing $100 \mu\text{g}$ amphotericin B per ml. Twenty randomly chosen mutants obtained through independent lines of selection (10 selected at 25^0 C and 10 selected at 37^0 C) were observed to differ in growth rates on antibiotic-free medium but to be very similar in their degrees of antibiotic resistance and their radiobiological properties. Data given in Tables 1 and 2 compare the growth rates and susceptibilities to inactivation by amphotericin B of WD-18-4 and the representative group of its antibiotic-resistant derivatives described in this report; the resistant strains WD-18-4(5A), WD-18-4(7A) and WD-18-4(11A) were obtained by selection at 25^0 C whereas strains WD-18-4(20B) and WD-18-4(25B) were obtained by selection at 37^0 C . Procedures for measuring growth rates and cellular inactivation by amphotericin B were those of Bish and Sarachek (1967) and of Sarachek and Higgins (1972). For both kinds of determinations, experimental flasks were incubated on reciprocal shakers operating at 80, 1.5 inch strokes per minute.

The minimal and yeast extract-peptone enriched media of Busbee and Sarachek (1969) were used; the minimal medium was routinely supplemented with $25 \mu\text{g/ml}$ of lysine and methionine to support growth of the doubly auxotrophic test organisms. Where indicated media were supplemented with ergosterol or

amphotericin B (Fungizone, E.T. Squibb and Sons, Inc.) as described by Sarachek and Higgins (1972). Cells were grown for irradiation on enriched medium at 25⁰ C; washed cell suspensions were irradiated by exposure to a 8 watt General Electric germicidal lamp having peak emission at 2534 Å at a dose rate of 15 ergs/mm²/sec as measured by an International Light Inc. ultraviolet dosimeter, model UV 254. The specific protocols of Busbee and Sarachek (1969) were followed in preparing stationary phase, non-budding cell populations for irradiation, for uv treatments and for scoring post-irradiation survivals; log phase populations were obtained for irradiation as described by Sarachek and Higgins (1972) and frequencies of mutation from auxotrophy to prototrophy were determined by the procedure of Sarachek *et al.* (1969).

RESULTS

Cellular Inactivation by uv: Previous studies have shown that post-irradiation growth temperatures, levels of nutritional enrichment and contact with lipids affect recovery of *C. albicans* from uv induced, non-genetic forms of damage which are critical only to the survival of cells which are not dividing when irradiated (Busbee and Sarachek, 1969; Sarachek and Higgins, 1972; Sarachek *et al.* 1969, 1970). Curves depicted in Fig. 1 compare survivals of actively dividing cell populations of the amphotericin B-sensitive strain, WD-18-4, and its antibiotic-resistant derivatives, strains WD-18-4(5A) and WD-18-4(25B), when grown after irradiation at 25⁰ C or 37⁰ C, on minimal or enriched medium, in the presence or absence of 5X10⁻⁵ M ergosterol. Comparable curves for non-budding populations of WD-18-4, WD-18-4(5A), WD-18-4(25B), and three additional resistant variants are presented in Fig. 2. It is seen that dividing cells of antibiotic-sensitive and antibiotic-resistant strains are very

much alike in radiosensitivities; the post-irradiation recoveries are slightly higher at 25⁰ C than 37⁰ C, are totally unaffected by either ergosterol or the nutritional quality of their growth medium. In contrast, survival of non-budding cells is more influenced by the post-irradiation conditions tested than by cellular sensitivities to amphotericin B. In all cases, non-budding cells yield survival curves with shoulders prior to the onset of the exponential phase of inactivation; curves obtained at 37⁰ C also show characteristic tailing at high uv dosages due to the radioresistance expressed only at that temperature by the small fraction of budded (2% to 5%) unavoidably present in otherwise unicellular populations prepared according to our procedures (Busbee and Sarachek, 1969). The effects of the post-irradiation treatments and of amphotericin B resistance on cellular recovery are reflected in the following quantitative differences between the curves.

(i) Temperature: Low growth temperature enhances post-uv recovery of both antibiotic-resistant and sensitive cells; their 37⁰ C survival curves have shorter shoulders and greater rates of exponential inactivation than corresponding 25⁰ C curves. On minimal medium, the two types of cells show equivalent overall inactivational kinetics at 37⁰ C and their exponential rates of inactivation at 25⁰ C are also alike. However, 25⁰ C survival curves produced by WD-18-4 have much broader shoulders than those of its antibiotic-resistant derivatives. Evidently, cellular changes resulting from mutation to amphotericin B resistance lessen the effectiveness of low growth temperature in enabling irradiated cells to resist fixation of inactivational damage.

(ii) Nutritional enrichment: Irradiated cells of WD-18-4 survive equally well on minimal or enriched medium at 25⁰ C. At 37⁰ C, the organism's

rate of exponential inactivation is higher on enriched than on minimal medium. All of the antibiotic-resistant strains show equal recovery on minimal or enriched medium at either temperature; at 37⁰ C, their exponential rates of inactivation are equivalent to that for WD-18-4 grown on minimal medium.

(iii) Ergosterol: Post-irradiation growth on ergosterol extends the shoulders of the 25⁰ C and 37⁰ C survival curves for WD-18-4 but does not affect the organism's exponential rate of inactivation at either temperature. Consequently, at either temperature, survival of WD-18-4 throughout the exponential phase of inactivation is *ca* 3X greater in the presence of the ergosterol than in its absence. Ergosterol is much less effective in promoting survival of antibiotic-resistant strains at 25⁰ C and has no detectable effect on their survival at 37⁰ C.

The responses of non-budding cells of strain WD-18-4 to the three post-irradiation treatments tested are typical of those which have been reported for wild type strains of *C. albicans* (Busbee and Sarachek, 1969; Sarachek and Higgins, 1972). The interrelated changes in these responses effected by rendering cells genetically resistant to amphotericin B indicate that each of the treatments affects a different aspect of some central mechanism involved in post-irradiation recovery by the non-budding cell and that the biochemical properties of cells which determine their relative susceptibilities to amphotericin B are basic features of that mechanism.

UV-Induced Reversion from Auxotrophy to Prototrophy: Pyrimidine dimers (and related pyrimidine adducts) are the principal uv-induced lesions in DNA contributing to cellular inactivation and mutation, and are the only forms of immediate uv damage to DNA known positively to be reparable *in vivo* by enzymatic action (Setlow, 1968). Both light-dependent and light-independent

repair processes have been demonstrated in bacteria and certain eucaryotic cells. Light-dependent repair (photoreactivation) involves the monomerization of dimers by means of a specific, light-activated enzyme (Setlow *et al*, 1965). Two systems for light-independent (dark) repair have been identified; one removes dimers and restitutes normal DNA structure before post-irradiation replication and the other corrects structural anomalies introduced into newly synthesized DNA following replication of dimer containing DNA (Smith, 1971). *C. albicans* cannot photoreactivate (Busbee and Sarachek, 1969). Efforts to evaluate slight changes in its dark repair activities by the direct chemical procedures applicable to other organisms are not feasible because of the small quantity of DNA per yeast cell and the difficulties in specifically labeling the bases of yeast DNA. However, fluctuations in uv mutagenesis can serve as a sensitive, indirect means for assaying such activities. Though both pre- and post-replicative repair processes promote post-irradiation survival of cells by reconstructing functional DNA molecules; pre-replicative repair does so accurately whereas post-replicative repair is error prone and constitutes the step by which mutations are established in survivors (Witkin, 1969). Thus, uv mutagenesis is enhanced by conditions which suppress pre-replicative repair and diminished by those which interfere with post-replicative repair.

To detect a possible connection between genetic resistance to amphotericin B and the efficacy of DNA dark repair, non-dividing cells of WD-18-4 and of three of its antibiotic-resistant variants were compared at three uv dosages for mutation to loss of their growth requirements for lysine and methionine. Table 3 shows that, at each dosage level, post-irradiation survivals of antibiotic-resistant variants are significantly lower than that of WD-18-4 at 25⁰ C but not at 37⁰ C. Frequencies of prototrophic revertants, however,

are alike for all strains at 25⁰ C as well as 37⁰ C. These observations indicate that relative susceptibilities of cells to amphotericin B do not affect dark repair of DNA and support the view that the post-irradiation effects of temperature, nutritional enrichment and lipids which are modified by amphotericin B resistance relate to cellular recovery from non-genetic forms of inactivational, uv damage.

DISCUSSION

Polyene antibiotics inactivate yeasts by combining with the principal sterols of the cell membrane and altering the membrane's structure so as to destroy its ability to function as a differential permeability barrier (Lampen *et al*, 1962; Andreoli *et al*, 1969). Ergosterol and 24,(28)-dehydroergosterol are the predominant but not exclusive sterols found in wild type yeasts. Five unlinked genes, pol 1 through pol 5, have been identified in *Saccharomyces cerevisiae* which control sterol biosynthesis and constitute the genetic determinants of cellular resistance to polyenes (Molzahn and Wood, 1972). Mutation at any one of these loci elevates the total amount of sterol present in cells but renders them completely deficient for 24,(28)-dehydroergosterol. In addition, pol 1 and pol 3 mutants produce new, as yet unidentified, uv absorbing sterols, pol 2 mutants contain no detectable uv absorbing sterol, pol 3 mutants are severely deficient for ergosterol and the sole uv absorbing sterol in pol 4 and pol 5 mutants is ergosterol. Mutation at each locus, except pol 4, confers polyene resistance on cells. The proposal has been advanced that the polyene resistances of pol mutants probably reflect the diminution of polyene binding sites within their cell membranes due to deficiencies in ergosterol and 24,(28)-dehydroergosterol and/or the presence of new sterols having

little affinity for the antibiotics (Woods, 1971; Bard, 1972; Molzahn and Woods, 1972). Though a comparably comprehensive picture of the biochemical genetic basis of polyene resistance in *C. albicans* does not exist, the reasonable presumption that it, too, involves changes in cellular sterols is supported by Athar and Winner's (1971) finding that amphotericin B-resistant variants of *C. albicans* are grossly deficient in ergosterol.

Explanation of polyene resistance solely in terms of sterol binding sites, however, must be reconciled with the observations that (i) the ergosterol contents and the levels of cellular resistance to the polyene, nystatin, in pol 1 and pol 3 mutants are inversely related (Woods, 1971), (ii) both pol 4 and pol 5 mutants contain roughly equivalent amounts of ergosterol but differ significantly in susceptibility to polyenes (Molzahn and Woods, 1972), (iii) the extent of reduction of the binding of nystatin by resistant strains of *C. tropicalis* and *C. stellatoidea* is not adequate to explain the degree of cellular resistance to the drug (Lampen and Arnow, 1959) and (iv) the detrimental effects of membrane bound polyenes depend upon the ratio of phospholipid to sterol within a membrane rather than the absolute amount of sterol present (Kinskey *et al*, 1965; Demel, *et al*, 1968). These observations do not exclude polyene: sterol binding as a significant factor in the antibiotic resistance of pol mutants, but they do imply that other modifications in the cell membrane attending changes in its sterol composition may also contribute to such resistance. Though the nature of cell membrane structure is still largely speculative, it is generally conceded that sterols are critical in mediating associations between the phospholipids and proteins of the eucaryotic membrane. Studies with model systems indicate that the highly hydrophobic, rigid planar structure of the sterol

provides a defined center for ordering the flexible, hydrocarbon chains of phospholipids which in turn influence the disposition of the membrane protein (Wallach, 1969). The distributions of double bonds and substituent groups within both sterols and phospholipids markedly condition interactions between the two classes of lipids (Demel *et al.*, 1972; Ghosh and Tinoco, 1972; Hsia and Boggs, 1972), and the ratio of sterol to phospholipid within a membrane can affect the transitions between lamellar and micellar organizations which are important to membrane biosynthesis and membrane associated metabolic activities (Glauert and Lucy, 1968). Thus, the qualitative and quantitative changes in cellular sterols accompanying acquisition of resistance to polyenes might well be expected to cause significant alterations in the organizational and functional properties of the cell membrane. That changes of this sort do occur in membranes of polyene resistant cells of *C. albicans* is indicated by reports of their special tendencies for aggregation and rapid settling in saline solution and their diminished capabilities for morphogenesis of germ tubes, pseudohyphae or chlamydospores (Athar and Winner, 1971).

Experiments reported here show that mutation of *C. albicans* to resistance to amphotericin B causes interrelated modifications in the special effects of post-irradiation growth temperatures, levels of nutritional enrichment and contact with lipids on survival of uv irradiated non-dividing cells. The modifications are probably not due to changes in the abilities of cells to repair uv induced pyrimidine dimers in DNA since antibiotic-sensitive and resistant cells are equally mutable by uv. Earlier studies have indicated that the three post-irradiation conditions affect cellular recovery from non-genetic forms of uv induced damages and have provided

evidence that protein synthesis is the essential element in temperature and nutrition dependent recoveries whereas lipids ameliorate uv-induced disturbance of the lipoidal organization of the cell membrane (Busbee and Sarachek, 1969; Sarachek and Higgins, 1972; Sarachek *et al*, 1969, 1970). Sarachek and Higgins (1972) proposed that the uv vulnerable cellular functions subject to these recoveries may be involved in (i) formation of a membrane associated protein required to initiate cell division and (ii) the maintenance of a lipoidal membrane structure necessary for proper incorporation or operation of the protein within the membrane. This suggested functional interconnection of the three recovery processes through dependence on the organization of the cell membrane accords well with the present observation of differences in expression of those processes by amphotericin B-sensitive and amphotericin B-resistant cells.

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ABSTRACT

Earlier investigations have shown that inactivation of non-budding cells of *C. albicans* by ultraviolet radiation is determined in part by (i) their post-irradiation incubation temperature, (ii) opportunity for exposure to long chain fatty acids or sterols and (iii) the nutritional quality of their post-irradiation growth medium. These cultural conditions do not affect the viability of cells which are budding when irradiated nor the frequency of mutants among the survivors of irradiated, unbudded populations. Several lines of evidence have indicated that the post-irradiation growth conditions influence recoveries from non-genetic forms of uv-induced damage which are potentially lethal only for cells which are not in the process of division when irradiated. Studies reported here show that mutation to resistance to the polyene antibiotic, amphotericin B, changes the composite pattern of survival responses of uv treated unbudded cells to the three post-irradiation conditions. Acquisition of amphotericin B resistance does not alter the uv mutability of such cells nor the vulnerability of budding cells to uv inactivation. Differences in susceptibilities of cells to polyene antibiotics are known to be determined by differences in the composition and structural arrangement of their membranes. The present observations are consonant, therefore, with a previous proposal that post-irradiation temperature, nutrition and contact with lipid affect cellular recoveries from damages to processes which (i) are essential for initiation of cell division and (ii) are related to the organization of the cell membrane.

REFERENCES

- Andreoli, T. E., Dennis, V. W., and Weigl, A. M. (1969) The effect of amphotericin B on the water and nonelectrolyte permeability of thin lipid membranes. *J. Gen. Physiol.*, 53:133.
- Athar, M. A. and Winner, H. I. (1971) The development of resistance by *Candida* species to polyene antibiotics *in vitro*. *J. Med. Microbiol.* 4:505-517.
- Bard, M. (1972) Biochemical and genetic aspects of nystatin resistance in *Saccharomyces cerevisiae*. *J. Bacteriol.* 111:649-657.
- Bish, J. R. and Sarachek, A. (1967) Influences of temperature and adenine concentration upon the cultural instability of a red adenine auxotroph of *Candida albicans*. *Mycologia* 59:671-688.
- Busbee, D. L., Sarachek, A. (1969) Inactivation of *Candida albicans* by ultraviolet radiation. *Arch. Mikrobiol.* 64:289-314.
- Demel, R. A., Crombag, F. J. L., Van Deenen, L. L. M. and Kinsky, S. C. (1968) Interaction of polyene antibiotics with single and mixed lipid monolayers. *Biochim. Biophys. Acta* 150:1-14.
- Demel, R. A., Geurts Van Kessel, W. S. M., and Van Deenen, L. L. M. (1972) The properties of polyunsaturated lecithins in monolayers and liposomes and the interactions of these lecithins with cholesterol. *Biochim. Biophys. Acta* 266:26-40.
- Ghosh, D. and Tinoco, J. (1972) Monolayer interactions of individual lecithins with natural sterols. *Biochim. Biophys. Acta* 266:41-49.
- Glauert, A. M. and Lucy, J. A. (1968) Globular micelles and the organization of membrane lipids. *in* The Membranes, vol. 4. (A. J. Dalton and F. Haguenau, Eds.) Academic Press:New York, pp 1-32.

- Higgins, N. P. and Sarachek, A. (1970) Absence of interaction between photoreactivation and temperature-dependent dark recovery of ultraviolet irradiated yeasts. *Mycopathologia* 40:357-361.
- Hsia, J. C. and Boggs, J. M. (1972) Influence of pH and cholesterol on the structure of phosphatidylethanolamine multibilayers. *Biochim. Biophys. Acta* 266:18-25.
- Kinsky, S. C., Gronau, G. R. and Weber, M. M. (1965) Interaction of polyene antibiotics with subcellular membrane systems. *Mol. Pharmacol.* 1:190-201.
- Lampen, J. O. and Arnow, P. (1959) Significance of nystatin uptake for its antifungal action. *Proc. Soc. Exptl. Biol. Med.* 101:792-797.
- Lampen, J. O., Arnow, P. M., Borowska, Z., Laskin, A. I. (1962) Location and role of sterol at nystatin-binding sites. *J. Bact.* 84:1152-1160.
- Molzahn, S. W. and Woods, R. A. (1972) Polyene resistance and the isolation of sterol mutants in *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* 72:339-348.
- Sarachek, A. and Higgins, N. P. (1972) Effects of ergosterol, palmitic acid and related simple lipids on the recovery of *Candida albicans* from ultraviolet irradiation. *Arch. Mikrobiol.* 82:38-54.
- Sarachek, A., Bish, J. T., Ireland, R. (1970) Relative susceptibilities of caffeine-sensitive and caffeine-resistant strains of *Candida albicans* to inactivation and mutation by ultraviolet radiation. *Arch. Mikrobiol.* 74:244-257.
- Sarachek, A., Goering, R. B., Bish, J. T. (1969) Differential effects of growth temperatures on inactivation and mutation of *Candida albicans* by ultraviolet radiation. *Arch. Mikrobiol.* 67:189-198.

- Setlow, J. K., Boling, M. E., Bollum, F. J. (1965) The chemical nature of photoreactivable lesions in DNA. *Proc. Nat. Acad. Sci.* 53:1430-1436.
- Setlow, R. G. (1968) The photochemistry, photobiology and repair of polynucleotides. *in* *Progress in Nucleic Acid Research and Molecular Biology*, vol. 8. (J. N. Davidson and W. E. Cohn, Eds.) Academic Press:New York. pp 257-295.
- Smith, K. C. (1971) The roles of genetic recombination and DNA polymerase in the repair of damaged DNA. *in* *Photophysiology*, vol. 6. (A. C. Giese, Ed.) Academic Press:New York, pp 210-278.
- Wallach, D. F. H. (1969) Membrane lipids and the conformations of membrane proteins. *in* *Membrane Proteins* (Proc. Symposium, New York Heart Association, Nachmansohn, Symp. Chairman) Little, Brown and Co., Boston pp 3-26.
- Witkin, E. M. (1969) Ultraviolet-induced mutation and DNA repair. *Ann. Rev. Genet.* 3:515-522.
- Woods, R. A. (1971) Nystatin-resistant mutants of yeast:alterations in sterol content. *J. Bacteriol.* 108:69-73.

Table 1. Generation times of strain WD-18-4 and of several of its amphotericin B-resistant derivatives during logarithmic growth on enriched broth medium at 25° C or at 37° C.

<u>Strain</u>	Mean generation time in hours $\pm$ 1 S.E.)	
	<u>25° C</u>	<u>37° C</u>
WD-18-4	1.4 $\pm$ 0.1	1.1 $\pm$ 0.1
WD-18-4(5A)	1.7 $\pm$ 0.1	1.4 $\pm$ 0.1
WD-18-4(7A)	1.7 $\pm$ 0.1	1.5 $\pm$ 0.1
WD-18-4(11A)	1.8 $\pm$ 0.1	1.6 $\pm$ 0.2
WD-18-4(20B)	1.6 $\pm$ 0.1	1.3 $\pm$ 0.1
WD-18-4(25B)	2.1 $\pm$ 0.2	1.7 $\pm$ 0.1

Table 2. Cellular inactivation in cultures of strain WD-18-4 and of several amphotericin B-resistant derivatives of WD-18-4 containing 10 µg/ml of amphotericin B: percentages of surviving cells were assessed at intervals following addition of the antibiotic to log-phase populations growing in minimal broth at 25° C or at 37° C.

Strain	Cell survivals (%) after periods of incubation in the presence of amphotericin B									
	0.5 hr		1.0 hr		1.5 hr		3.0 hr		5.0 hr	
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
WD-18-4	4.0	29	2.2	0.08	0.37	<0.01	<0.01	-	-	-
WD-18-4(5A)	-	-	90	20	36	5.9	3.6	0.10	0.48	<0.01
WD-18-4(7A)	-	-	94	14	32	3.0	4.4	0.07	0.90	<0.01
WD-18-4(11A)	-	-	96	31	55	9.2	8.7	0.13	2.4	0.02
WD-18-4(20B)	-	-	91	22	38	5.0	8.0	0.10	2.3	<0.01
WD-18-4(25B)	-	-	97	66	53	22	18	1.1	6.0	0.25

Table 3. Percent survivals and frequencies of prototrophic revertants among survivors (± 1 S.E.) expressed at 25⁰ C and at 37⁰ C by uv irradiated, non-budding cell populations of strains WD-18-4, WD-18-4(11A) and WD-18-4(25B).

<u>Strain</u>	UV Dose (in seconds of exposure)			Methionine independent re- vertants per 10 ⁷ survivors		Lysine independent re- vertants per 10 ⁷ survivors	
		<u>25⁰C</u>	<u>37⁰C</u>	<u>25⁰C</u>	<u>37⁰C</u>	<u>25⁰C</u>	<u>37⁰C</u>
WD-18-4	30	94±2	78±1	21±3	16±3	5±1	4±2
	65	75±1	10±3	68±7	59±3	19±3	18±2
	80	51±2	1.1±0.3	73±6	81±6	48±6	39±3
WD-18-4 (11A)	30	82±1	80±3	22±2	18±4	6±1	5±1
	65	30±2	8.9±0.5	59±6	64±5	22±4	18±4
	80	9.1±1	0.9±0.4	79±4	69±7	55±7	42±5
WD-18-4 (25B)	30	88±1	83±3	15±5	23±4	7±1	5±1
	65	53±2	12±3	63±6	55±4	15±4	20±5
	80	21±1	1.2±0.2	71±8	79±4	42±6	51±7

Figure Legends

Fig. 1. Post-irradiation survivals of actively dividing cell populations of strain WD-18-4 and of two of its amphotericin B-resistant derivatives on enriched medium (squares) and on minimal medium with $5 \times 10^{-5} \text{M}$ ergosterol (triangles) or without ergosterol (circles) at 25°C (closed symbols) or 37°C (open symbols).

Fig. 2. Post-irradiation survivals of non-budding cell populations of strain WD-18-4 and of five amphotericin B-resistant strains derived from WD-18-4 on enriched medium (squares) and on minimal medium with $5 \times 10^{-5} \text{M}$ ergosterol (triangles) or without ergosterol (circles) at 25°C (closed symbols) or 37°C (open symbols).