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Two experimental approaches have been used to investigate the interaction of sterols with respiration in yeast. Cells were tested with an antifungal sterol, azasterol, and a mutant was obtained with an altered sterol composition. Polarographic measurements were done on mitochondria for both experiments.

The effects of azasterol on mitochondrial respiration were tested in two ways. In the first experiment, azasterol was added directly to mitochondria. The second method involved the isolation of mitochondria from azasterol grown cells. In the former, polarographic measurements with ethanol as substrate showed no coupling of mitochondria in the range of 25-50 µg azasterol/ml. Mitochondria from cells grown in the presence of azasterol were inhibited at much lower concentrations. No respiration was observed at 25 ng azasterol/ml, and respiration with no coupling occurred at 10 ng azasterol/ml. Coupling was present at 1.25 ng azasterol/ml. All three azasterol concentrations showed similar ignosterol/ergosterol ratios by U.V. absorption, suggesting ignosterol may not be the only factor affecting respiration.

Polarographic measurements were made with a sterol mutant and wild-type mitochondria at varying temperatures. Coupling was observed with the mutant above the permissive growth temperature. The R/C and ATP/O ratios were similar to the wild-type. The respiratory control ratios were measured by dividing the polarographic slope of state 3 respiration (mitochondria in the presence of substrate and ADP) by the polarographic slope of state 4 respiration (mitochondria in the presence of substrate and the ADP is utilized). However, the mutant was more susceptible to Na^+ ions, suggesting altered permeability, and had higher QO_2 values at the elevated temperatures. The QO_2 values suggest the sterol is not as effective in forming the intermediate gel state at the elevated temperatures as ergosterol. Results from the two experiments suggest there is an interaction between sterols and respiration.

Factors Affecting Mitochondrial Respiration in Yeast

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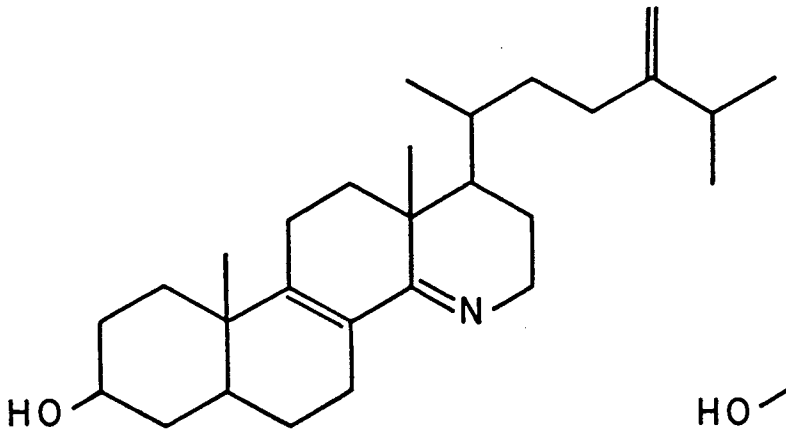
INTRODUCTION

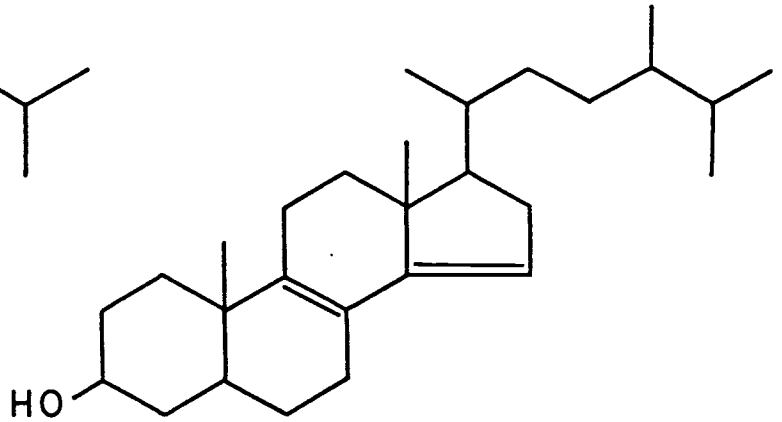
The present communication involves two experimental approaches investigating the interaction of sterols with respiration. Cells were tested with an antifungal sterol, azasterol, and a sterol mutant was obtained with an altered sterol composition.

Azasterol (Figure 1) was used for several reasons. The antimycotic agent has been shown to inhibit growth and to affect sterol synthesis in Saccharomyces cerevisiae (4,12). In the presence of azasterol, the cells accumulate an unusual sterol that has been identified as $\Delta^{8,14}$ergostadiene-3β-ol (ignosterol) (Figure 1) (12). Cells cultured on respiratory substrates are more sensitive to the antimycotic agent than cells grown on fermentative substrates (4). The physiological effects on these cells may be an interaction of azasterol on the respiratory apparatus, or altered sterol synthesis could be affecting respiration. Both possibilities were examined. Since cells grown in the presence of low azasterol concentrations may show ignosterol and the presence of ergosterol, the mitochondrial sterols were extracted and examined by U.V. absorption.

The second experiment involved the nystatin-resistant mutant that has $\Delta^{8(9),22}$ergostadiene-3β-ol as the major sterol instead of ergosterol (Figure 2) (29). Besides the altered sterol, the mutant shows a lower permissive growth temperature than the isogenic wild-type. To determine if the altered sterol affects respiration, the temperatures were varied on mitochondria from the mutant and the wild-type.

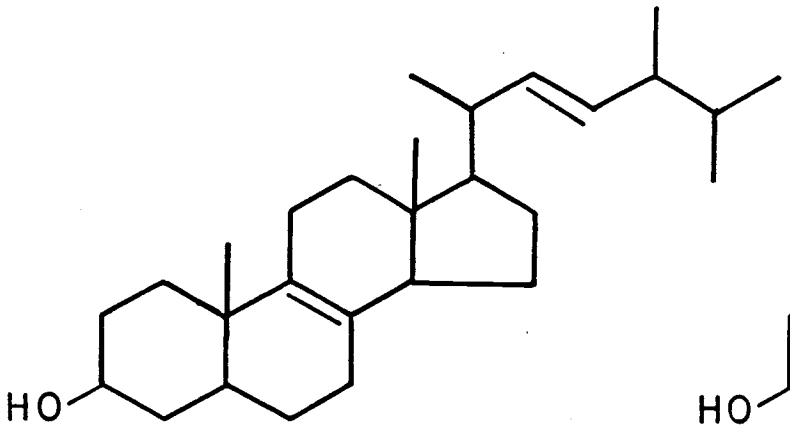


AZASTEROL

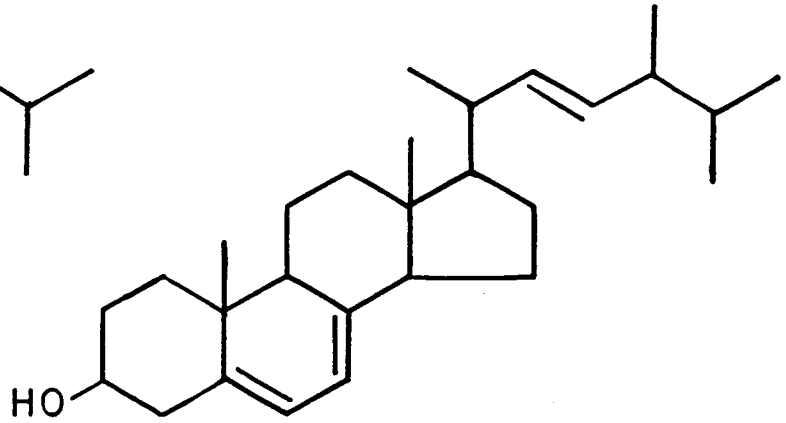


IGNOSTEROL

Figure 1



ERGOSTADIENE



ERGOSTEROL

Figure 2

LITERATURE REVIEW

A relationship between ergosterol and respiration has been proposed in yeast cells. Past experiments have been at the cellular and mitochondrial level. An important aspect in many of these studies was to be able to control the sterol in the cell. Controlling the type or quantity of sterol in growing yeast has been done by growing the cells anaerobically and adding the desired sterol, or through sterol mutants, or by antifungal sterols such as azasterol. Since the relationship between sterols and respiration would be through a membrane interaction, studies have been done on the affects of sterols on membrane characteristics. Also, azasteroids are of interest since past experiments with these drugs have shown inhibitory affects on oxidative phosphorylation and sterol synthesis.

Although the relationship between ergosterol and respiration is still being studied, Andreason and Steir (2) have demonstrated that yeast require ergosterol for growth under anaerobic conditions in defined media. In the absence of ergosterol, there was only a one to two-fold increase in cells. When sterol was added, the cell yield was only one-third that of cells grown aerobically. It was also shown that ergosterol could be replaced by cholesterol to promote anaerobic growth in yeast cultures, although to a lesser degree. In addition to ergosterol, oleic acid was required (3).

Early experiments on the interaction between sterol synthesis and respiratory growth were on a cellular level. Maguigan and Walker (17) determined ergosterol synthesis under aerobic and anaerobic

conditions, and found that ergosterol formation coincides with respiration. Sterol synthesis occurred early in the course of aeration and was accompanied with an increase in total lipid. Also, the rate of sterol formation was much greater than cellular growth. Klein (13) demonstrated yeast synthesize little, if any, ergosterol under anaerobic conditions. However, upon aeration with a suitable carbon source, the increase in sterol production was three- to eight-fold in a 24 hour period. Tchenn and Bloch (25) demonstrated the accumulation of squalene under anaerobic conditions, and its rapid conversion to ergosterol upon aeration. Molecular oxygen is necessary in forming the 3β-hydroxy group, the removal of the extra methyl groups of lanosterol, and for shifting of the double bonds in the late steps of synthesis. Molecular oxygen is also necessary for the induction of respiratory enzymes (19). The magnitude of the induction increases when the enzyme along the respiratory chain is closer to oxygen.

Elevated temperatures have been shown to induce a high percentage of respiratory deficient petite strains in yeast. Sherman (21) and Ycas (30) observed that growth at 40 C increased cytoplasmic petite mutations. Sherman (22) noted the cells at 40 C entered a death phase similar to thymine-less death, which could be averted by the addition of yeast extract or oleic acid. Loginova et al. (15) found increased growth at 39 C to 40 C when ergosterol, tween-80, or oleic acid was added to the medium. Starr and Parks (23) measured the effect of incubation temperature on yeast sterol yield. Cells were previously grown anaerobically in a rich medium, then resuspended in aerated

nonproliferating suspensions. The maximal rate of sterol formation correlated with the optimal growth temperature at 30 C. It was also noted that sterol synthesis decreased rapidly with increasing temperatures. At 40 C and above, synthesis was initiated, but declined on prolonged incubation. Starr and Parks (23) showed the presence of ergosterol reduces by one-half the number of petite colonies at 40 C. This was true whether ergosterol was suspended in albumin or tween-80. Yeast were grown in Wickerham's medium, where growth is normally limited at 40 C. Supplementation of oleic acid and ergosterol permitted cell growth and prevented the death phase described by Sherman. When tween-80 was used alone or with oleic acid, an initial increase occurred followed by cell death and a decline in the optical density of the culture.

Reports have shown the presence of sterol in mitochondria structures. Thompson and Parks (27) found ergosterol copurifies with cytochrome oxidase. However, the sterol is not essential for in vitro cytochrome oxidase activity. Another experiment by Thompson and Parks (26) showed the presence of a sterol-synthesizing enzyme within the mitochondria. The enzyme was S-adenosyl-L-methionine: Δ^{24} sterol methyltransferase. For the enzyme assay, the transfer of the ^{14}C methyl group from S-adenosyl (Me-^{14}C) methionine to the substrate zymosterol was measured. For mitochondria, cells were homogenized and subjected to differential centrifugation. Mitochondrial separation of the outer, inter, inner membranes and the matrix was through digitonin treatment, differential and ultracentrifugation, and sonication. The

enzyme from ethanol grown cells was assayed with the markers cytochrome oxidase and malic dehydrogenase for comparative purposes. Approximately 86% of the cytochrome oxidase activity is found in the inner membrane, while malic dehydrogenase is found mainly in the matrix. Sterol methyltransferase was shown to associate with the inner membrane and the matrix. In contrast, cells grown in 10% glucose had methyltransferase activity in the microsomal fraction (105,000 x g pellet from the 25,000 x g supernatant). However, large concentrations of glucose are known to inhibit mitochondrial maturation, and there is an accumulation of promitochondria. By using a sucrose density profile of the promitochondrial fraction, the sterol methyltransferase correlated with the promitochondrial protein absorption at 280 nm. Cytochrome oxidase activity was completely repressed (the enzyme is not present in promitochondria). The experiment demonstrated that glucose grown cells contain sterol methyltransferase in the promitochondria. This experiment also showed that synthesis of the enzyme was not prevented by high levels of glucose.

The relationship between ergosterol and respiratory competency would be primarily through membrane interaction. Ergosterol has been shown to affect membrane characteristics. Cobon and Haslam (10) found that the phase transition temperature of mitochondrial ATPase could be altered by varying the concentration of ergosterol. Cells were grown anaerobically in a medium containing excess unsaturated fatty acids and a range of supplemented ergosterol. Isolated promitochondria had the same fatty acid composition, but the sterol content ranged from 7 to 105 mg/g mitochondrial protein. Although the amount of sterol varied,

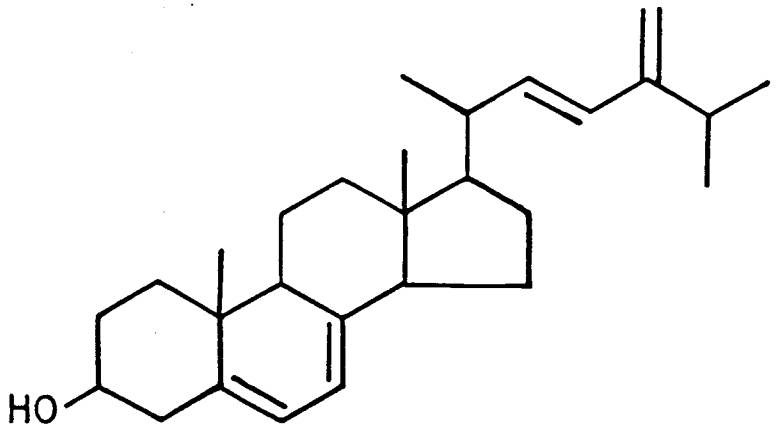
all Arrhenius plots of the mitochondrial ATPase demonstrated a discontinuity. The Arrhenius activation energies were +40 K/mole above the transition temperature and +80 K/mole below. However, with increasing amounts of ergosterol the transition temperature decreased. With the range of 105-7mg ergosterol/g protein, the former decreased the transition temperature 17 C.

Altered sterols also have been shown to affect membrane characteristics. Thompson and Parks (28) showed a 4-6 C decrease in phase transition temperatures with the mitochondrial enzymes cytochrome oxidase and S-adenosylmethionine: Δ^{24}-sterol methyltransferase with nys-3 and 3701B-n3 as compared to 3701B. The two nystatin resistant mutants contain primarily $\Delta^{8(9),22}$ergostadiene-3β-ol as their major sterol, while 3701B contains ergosterol. With cytochrome oxidase, the transition temperatures of the two mutants were identical, 4 C. Since 3701B-n3 is a mutant isogenic to 3701B, the fact that both mutants have the same transition temperatures precludes the possibility of a protein difference between 3701B and nys-3. Since the difference observed in the Arrhenius kinetics could be due to altered lipid composition, a quantitative assay was done on the major lipids oleic and palmitoleic acid. Very little lipid variation was found between the strains. In contrast, Arrhenius kinetics of methyltransferase from promitochondria from all three strains demonstrated a single transition temperature at 6.5 C. Lipid analysis was the same as those in mitochondria, while the sterol was only 1/5 as much.

In a later paper, Thompson and Parks (29) demonstrated that a nystatin-resistant mutant containing the sterol ergostatetraene (Figure 3), had the same phase transition for methyltransferase as 3701B. Both strains had a sterol containing a $\Delta^{5,7}$ unsaturation in the B ring. Strains with the $\Delta^{5,7}$ unsaturation were compared to the strains having a $\Delta^{8(9)}$ unsaturation. Results show sterols with the $\Delta^{5,7}$ unsaturation have higher transition temperatures than sterols with the 8(9) position. An experiment was done to determine if the mitochondrial enzymes from nystatin-resistant clones have more protection in the mitochondria. Temperature inactivation studies were done on cytochrome oxidase and methyltransferase. Four strains were used. Two had the sterol $\Delta^{8(9),22}$ergostadiene-3β-ol, one contained $\Delta^{5,7,22,24(28)}$-ergostatetraene-3β-ol, and one had ergosterol. The optimal temperatures were the same for all four strains, with methyltransferase at 30-34 C, while cytochrome oxidase was at 26-28 C. All the strains demonstrated 80% loss of activity at 50 C for 3 minutes with methyltransferase, and 80% loss of activity of cytochrome oxidase within 2 minutes of incubation at 40 C.

Besides 3701B-n3 having the altered sterol which demonstrated the lower transition temperatures, Thompson and Parks (29) showed the mutant has a lower permissive growth temperature. Gonzales (unpublished data) has shown 3701B-n3-67 is unable to grow at 37 C, while the wild-type does grow at 38 C.

Past experiments with azasteroids have shown affects on oxidative phosphorylation, sterol metabolism, active transport of amino acids, and growth inhibition (8,4,12). Chesnut *et al.* (8) demonstrated the



ERGOSTATETRAENE

Figure 3

15-azasteroid 1,10, 11a-tetrahydro-11a-methyl-2H naphth (1,2-g) indol-7-ol (known as hydroxyimine) inhibits uptake of ^{14}C-alanine, ^{14}C-leucine, and other amino acids in mouse lymphocyte cells. The concentration of the azasteroid added was 20 µg/ml.

With rat mitochondria, Chesnut et al. (8) observed coupling with the substrate succinate at 50 µg of hydroxyimine, but no coupling when the substrates glutamate or malate were present with 1 µg of azasteroid. The inhibition site of the azasteroid was suggested as an interaction with NADH and thereby blocking the transfer of the protons to the flavoprotein, in a similar manner to amytal. However, when glutamate, azasteroid and ADP are present and succinate is added, the succinate demonstrated uncoupling. If amytal is added to the glutamate, azasteroid, and ADP prior to the succinate, coupling is demonstrated.

Another azasteroid has shown strong antifungal properties. The antimycotic agent has been isolated from the mold Geotrichum flavo-brunneum (5). The main component is 15-aza-24-methylene-D-homo-cholestadiene (18). Bailey, et al. (4) demonstrated low concentrations of azasterol inhibit growth and affect sterol synthesis in Saccharomyces cerevisiae. Yeast cells in the presence of azasterol accumulate an unusual sterol identified as $\Delta^{8,14}$ergostadiene-3β-ol (ignosterol) (12). Azasterol has also been shown to increase total sterol accumulation. Hays, et al. (12) demonstrated the presence of ignosterol at 1 ng azasterol/ml. Azasterol has been shown to inhibit transmethylation of zymosterol in vitro and in vivo (4). Inhibition was competitive. The antimycotic agent also causes competitive inhibition of sterol 24(28) methylene reductase (12). Cells

cultured on respiratory substrates were found to be more sensitive to azasterol inhibition than cells cultured on fermentative substrates (4). With 2% ethanol as substrate, inhibition of colony growth occurred at azasterol concentrations greater than 6 ng/ml, while with 2% glucose-containing agar plates, growth occurred with azasterol concentrations as high as 16 to 18 ng/ml.

MATERIALS AND METHODS

Organisms and Cultural Conditions

Saccharomyces cerevisiae strain 3701B a haploid uracil auxotroph and strain 3701B-n3-67 a nystatin-resistant mutant clone isogenic to 3701B were used in this study. Stock cultures were maintained on agar slants containing 1.0% tryptone, 0.5% yeast extract, 2.0% ethanol and 2.0% agar. Cultures were stored at 4 C. Organisms were grown routinely in broth cultures of the same medium as the above, without the agar. Aerobic growth was carried out in 2-liter Erlenmeyer flasks containing 1 liter of broth. The flasks were inoculated with 10 mls of late log phase cells for 3701B, and 20 mls for 3701B-n3-67. The cultures were shaken for 20-24 hours at 27 C. Cells were centrifuged at 5,000 x g while in the logarithmic growth phase, and washed once with distilled water.

Preparation of Mitochondria

Cells were treated with 0.5M β-mercaptoethanol, 0.1M tris pH 9.3 at 2 mls/g wet weight of cells. The suspension was incubated at 28-30 C for 5 minutes in a water bath shaker, then centrifuged at 3,020 x g for 3 minutes and the supernatant decanted. Cells were washed twice with 0.7M sorbitol, 0.3M mannitol, 1.0 mM EDTA, 10mM citrate phosphate buffer pH 5.8. Centrifugation was at 3,020 x g for 3 minutes. For digestion of the cell wall, 2 mls of the above buffer and 0.5 mls of glusulase were added per gram of wet weight cells. The suspension was shaken in a 28-30 C water bath at slow speed for 1 hour, and then centrifuged for 10 minutes at 3,020 x g. Glusulase was centrifuged at

12,100 x g for 10 minutes to remove debris, and was stored at 0 C for reuse. Protoplasts were washed twice with 1.3 M sorbitol, 0.1mM EDTA, 10 mM potassium phosphate buffer and adjusted to pH 6.6 with tris. The protoplasts were resuspended in 0.6M mannitol, 0.1mM EDTA (K^+), 0.2% BSA pH 7.2 adjusted with tris. Protoplasts were in a 10 ml volume with buffer and ruptured in a French press at 1,000 p.s.i. The suspension was centrifuged at 1,935 x g for 5 minutes, and then at 750 x g at 10 minute intervals to remove debris. Mitochondria were recovered by centrifuging the supernatant at 8,000 x g for 10 minutes. The reddish-brown mitochondrial pellet was resuspended in the same buffer, centrifuged once more at 750 x g for 10 minutes to remove debris, and the 8,000 x g and 750 x g centrifugations were repeated. Mitochondria were stored at 4 C.

Assay of Oxidative Phosphorylation

Oxygen consumption was recorded by a Gilson Oxygraph containing a Clark-type electrode. Procedures for respiratory control ratios, ADP/O ratios and polarograph buffer have previously been described (14,20). Protein was determined by the method of Lowry et al. (16). Bovine serum albumin was used as the standard.

ATP Assay by Luciferin-Luciferase Enzyme System

A 50 µl sample was removed from the polarograph during state 4 respiration and diluted 200-fold in boiling buffer consisting of: 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid pH 7.5, and 25 mM magnesium acetate. This is a modification of the boiling water extract procedure (9,24). The extract was placed in an ice bath.

ATP and ADP were measured for each sample by the luciferin-luciferase enzyme method, and another aliquot containing a final concentration of 0.595 μM ATP was used as an internal standard. Light emission was determined in 0.2 ml samples in an Aminco Chem-Glow photometer. Reactions were started with 0.1 ml of firefly lantern extract containing 50 mM potassium arsenate and 20 mM magnesium sulfate, pH 7.4. ATP concentrations were determined by a standard curve.

Sterol Identification

Sterols were recovered from the yeast by saponification (17% KOH, 0.14% pyrogallol in methanol: water 5:2) and extraction with n-hexane. Ultraviolet absorption (U.V) spectroscopy was performed on a Cary model 11 recording spectrophotometer.

Materials

Azasterol used in these experiments, designated A25822B, was a generous gift from the Eli Lilly & Co.

Firefly lantern extract was purchased from Sigma Chemical Co. Glusulase was purchased from Endo laboratories. All other compounds were analytical reagent grade.

RESULTS

To determine the inhibitory level of azasterol on coupled mitochondria, the concentration was varied when added to mitochondria in a polarograph vessel. The concentration of mitochondrial protein was similar for all experiments. Table I shows the gradual decrease of respiratory control with increasing concentrations of azasterol. The ADP/O ratios were lower than the control starting at the range of 51-58 µg azasterol/mg of mitochondrial protein (15 µg azasterol/ml). Depending on the mitochondrial preparation, no coupling occurred from 95-193 µg azasterol/mg of mitochondrial protein (25-50 µg azasterol/ml).

Table I.

µg Azasterol/ml in vessel	ADP/O Ratios	R/C Ratios	µg Azasterol/mg mitochondrial protein	ATP/O Ratios
0	1.4-1.9	2.1-4.6	0	1.1-1.5
5	1.4-1.9	2.1-2.6	17-19	0.8-1.2
10	1.4-1.8	1.9-2.3	34-38	1.1-1.2
15	1.2-1.7	1.4-2.9	51-58	0.9-1.3
25	N-1.6	N-1.8	85-95	N-0.9
40	N-1.9	N-1.4	136-154	N-0.8
50	N	N	170-193	N

N = no coupling

In our laboratory, a broth culture containing 2% ethanol was inoculated with 3701B at 15-20 klett units. Azasterol suspended in ethanol was added to the culture with the final concentration of 25 ng azasterol/ml. When used as an inoculum for fresh media with the same amount of ethanol and azasterol, there was no growth.

To determine the physiological effects of cells grown in the presence of azasterol on mitochondria, the concentrations of azasterol

were varied. The broth cultures were inoculated to 15 klett units. Wild-type aerobically grown cells with 25 ng azasterol/ml were harvested at 180-225 klett units. Polarographic measurements of the mitochondria showed no respiration. The proportion of ignosterol (ε18,000,λ_{max} 250 nm) to ergosterol (ε11,900,λ_{max} normally 294 nm, 282 nm and 271 nm) was 16.69 (ignosterol = 94%) from U.V. absorption spectra data. Mitochondria from cells grown in the presence of 10 ng azasterol/ml showed respiration but no coupling. The ignosterol/ergosterol ratio was 25.1 and 15.1 (ignosterol = 96%, 93.4%). At 1.25 ng azasterol/ml, coupling occurred at 25 C and 37 C with all three substrates. These mitochondria have similar polarographic measurements to 3701B (not shown). The ignosterol/ergosterol ratio was 17.58 (ignosterol = 96.2%). A previous report showed more ergosterol present at 1 ng azasterol/ml (12). The sterols were assayed at stationary phase, as compared to log phase in this report.

The isogenic nystatin-resistant mutant clone has $\Delta^{8(9),22}$-ergostadiene-3β-ol instead of ergosterol (29). The mutant was more susceptible to the glusulase preparation of mitochondria. Respiration with no coupling was more common in the mutant than the wild-type. Sodium ions from sodium succinate inhibited coupling in the mutant but not in the wild-type. Succinate as a substrate was adjusted with tris.

From our laboratory, 3701B-n3-67 is unable to grow at 37 C, while 3701B is known to grow at 38 C in ethanol (Gonzales, unpublished data). The mutant also grows at a lower temperature than 3701B. Polarographic measurements of the mutant and wild-type mitochondria were taken at different temperatures. Table II shows these measurements with three

Table II. Polarographic measurements of a nystatin resistant mutant vs. the wild-type.

Substrate	Temperature °C	ATP/O Ratios 3701B	ATP/O Ratios N3-67	R/C Ratios 3701B	R/C Ratios N3-67	QO_2[a] 3701B	QO_2[a] N3-67
αKG[b]	25	1.8-1.9	2.3-2.5	2.0-2.7	2.2-2.4	.54-.66	.66-.68
	30	1.7-2.0	1.8-2.2	1.9-2.5	2.0-2.2	.62-1.0	.87-.92
	37	1.9-2.2	1.6-1.7	2.0-2.5	1.9-2.0	.66-1.1	1.4-1.5
succinate	25	1.0-1.5	1.5-1.6	1.6-2.0	1.5-1.6	.70-.91	.84-.86
	30	1.0-1.3	1.1-1.2	1.3-1.7	1.4-1.5	.84-1.3	1.3-1.4
	37	0.9-1.3	1.1-1.7	1.3-1.5	1.3-1.4	1.0-1.3	1.8-1.9
ethanol	25	1.7-1.9	1.7-1.8	2.6-3.5	2.3-2.6	.80-.96	1.0-1.4
	30	1.6-1.8	1.5-1.6	2.7-3.3	2.2	1.1-1.5	1.4-1.5
	37	1.4-1.6	1.1-1.5	2.0-2.3	1.9	1.4-1.6	1.8-2.6
	38		1.0		1.7		3.2

[a] QO_2 = µg atoms of oxygen/mg protein/minute at state 3 respiration.

[b] = αKetoglutarate

different substrates. The mutant has similar R/C and ATP/O ratios as the wild-type at the varying temperatures. Coupling is present in the mutant at 38 C with ethanol. However, the QO_2 values of 3701B-n3-67 are higher than 3701B, particularly at the higher temperatures (Figure 4).

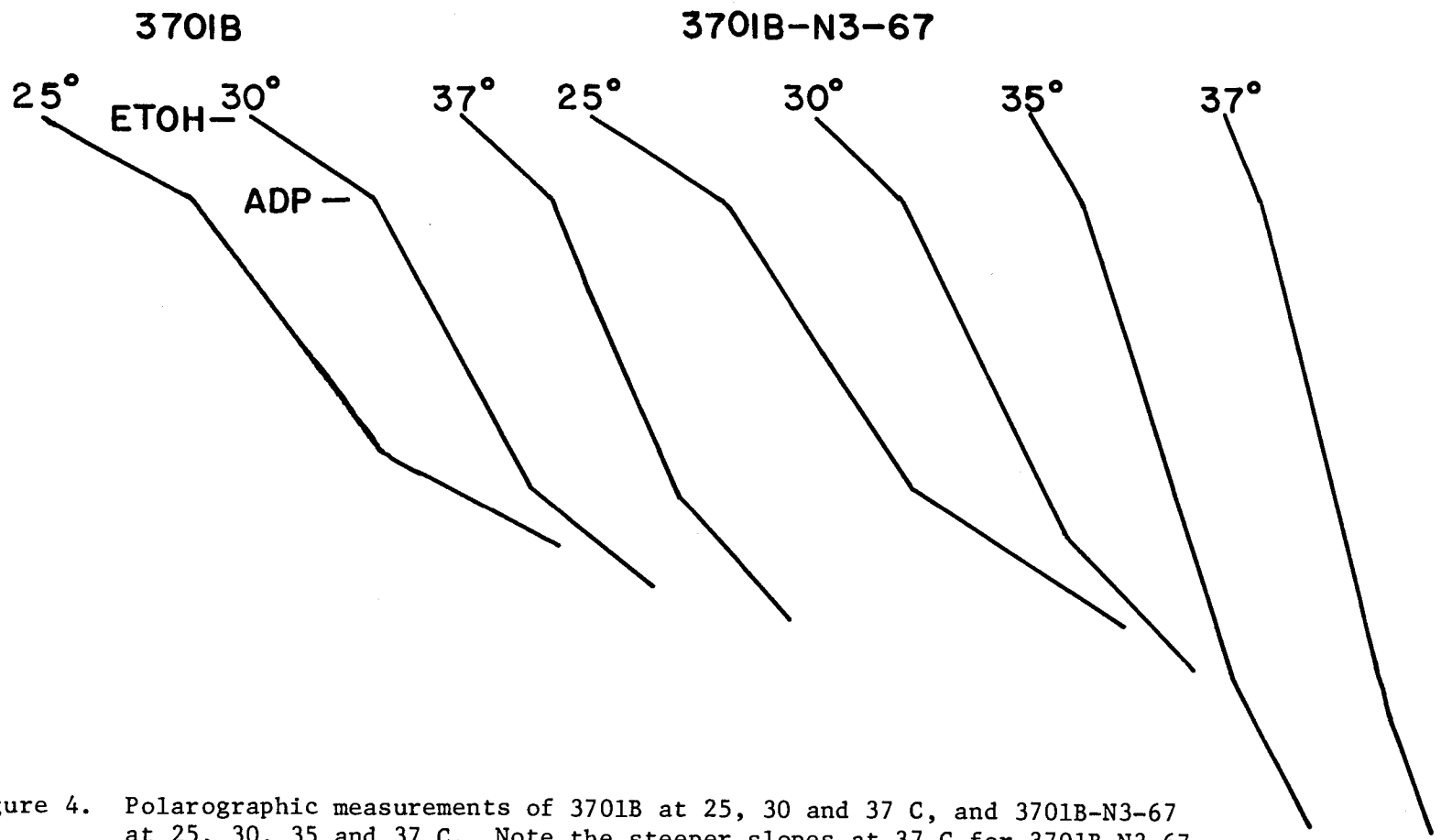


Figure 4. Polarographic measurements of 3701B at 25, 30 and 37 C, and 3701B-N3-67 at 25, 30, 35 and 37 C. Note the steeper slopes at 37 C for 3701B-N3-67 as compared to 3701B. The reaction medium contained .6 M mannitol, 10 mM potassium phosphate buffer (pH 6.6), 15 mM tris-maleate buffer (pH 6.6), 10 mM KCL, and 0.1 mM EDTA. Final protein concentration in the reaction mixture was 0.58 mg for 3701B-N3-67 and 0.55 mg for 3701B. Final concentration of ethanol as substrate was 0.5%, ADP concentration was 125 μM.

DISCUSSION

Direct addition of azasterol to mitochondria shows inhibition of coupling at 25-50 μg azasterol/ml. However, the concentration of azasterol necessary to inhibit respiration in growing cells is lower (25 ng azasterol/ml). The latter shows mitochondria containing the unusual sterol ignosterol. At 10 ng azasterol/ml, there was respiration with no coupling. Lack of coupling may be due to an increased sensitivity to ions in the buffers, or variability in the glusulase digestion. The ratios of ignosterol/ergosterol from U.V. absorption for the three varying ignosterol concentrations are similar. Only the two lower concentrations show respiration. Results suggest azasterol affects respiratory competency in the presence of ignosterol, but some other factor may be necessary for mitochondria to change from no respiration to the coupled state. Whether ergosterol is a factor could only be determined by quantitative experiments.

Evidence has shown high ergosterol levels decrease the enzyme activity of kynurenine hydroxylase (1). This suggests high levels of sterols may restrict motion of some lipid components and effect an enzyme conformation change resulting in decreased enzyme activity. This same effect may be true with high levels of ignosterol. Total sterol accumulation has been shown to be higher with certain azasterol concentrations (12).

Experiments with azasterol have shown multiple effects. The antimycotic agent may have another affect on mitochondria that is not known at the present time.

Previous reports have shown that sterols increase order and rigidity within the membrane (6,7,11). The geometry of the steroid nucleus is more effective in phospholipid packing when the structure is planar (7). The change in the B ring from a $\Delta^{5,7}$ unsaturation to a $\Delta^{8(9)}$ in 3701B-n3-67 removes the planar characteristic found in ergosterol. The higher QO_2 values with the mutant suggests the membrane is more fluid at the higher temperature than the wild-type. The increased sensitivity to sodium also suggests an increase in permeability. Since 3701B-n3-67 is still capable of coupling beyond the permissive growth temperature in ethanol, this would suggest the cessation of growth of 3701B-n3-67 at 37 C occurs during the formation of mitochondria. Results present here indicate that altered sterols have an effect on mitochondrial respiration.

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