Biochem. J. (1975) **146**, 409–416
Printed in Great Britain

Membrane-Lipid Unsaturation and Mitochondrial Function in *Saccharomyces cerevisiae*

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(*Received* 25 *August* 1974)

The lipid composition of yeast cells was manipulated by the use of an unsaturated fatty acid auxotroph of *Saccharomyces cerevisiae*. There was a 2–3-fold decrease in the concentration of cytochromes $a+a_3$ when the unsaturated fatty acid content of the cells was decreased from 60–70% of the total fatty acid to 20–30%. The amounts of cytochromes *b* and *c* were also decreased under these conditions, but to a lesser extent. Further lipid depletion, to proportions of less than 20% unsaturated fatty acid, led to a dramatic decrease in the content of all cytochromes, particularly cytochromes $a+a_3$. The ATPase (adenosine triphosphatase), succinate oxidase and NADH oxidase activities of the isolated mitochondria also varied with the degree of unsaturation of the membrane lipids. The lower the percentage of unsaturated fatty acid, the lower was the enzymic activity. Inhibition of mitochondrial ATPase by oligomycin, on the other hand, was not markedly influenced by the membrane-lipid unsaturation. Non-linear Arrenius plots of mitochondrial membrane-bound enzymes showed transition temperatures that were dependent on the degree of membrane-lipid unsaturation. The greater the degree of lipid unsaturation, the lower was the transition temperature. It was concluded that the degree of unsaturation of the membrane lipids plays an important role in determining the properties of mitochondrial membrane-bound enzymes.

It has been long recognized that lipids play an important role in determining the function and properties of mitochondrial enzymes (Fleischer *et al.*, 1962; Green & Tzagoloff, 1966), and several mitochondrial membrane-bound enzymes have been shown to have a requirement for, or are associated with, phospholipids (Jurtshuk *et al.*, 1963; Kagawa & Racker, 1966; Kopaczyk *et al.*, 1968; Tzagoloff, 1969; Zahler & Fleischer, 1971). Despite these extensive studies, the precise role of lipids in determining the biological activity of membrane-associated functions remains obscure.

One approach to this problem has been the use of membrane-lipid mutants of *Escherichia coli* (Silbert & Vagelos, 1967; Wilson *et al.*, 1970; Overath *et al.*, 1970; Esfahani *et al.*, 1971; Harder *et al.*, 1972) and *Acholeplasma laidlawii* (McElhaney & Tourtellotte, 1969; Steim *et al.*, 1969; Engelman, 1971; De Kruyff *et al.*, 1973; McElhaney *et al.*, 1973). The yeast *Saccharomyces cerevisiae* offers the additional advantage of studies on mitochondrial membranes. Extensive studies have been made on the effects of unsaturated fatty acid on the energy metabolism of yeast mitochondria (Linnane & Haslam, 1970; Proudlock *et al.*, 1971; Haslam *et al.*, 1971, 1973). The oxidative ability and cytochrome content of unsaturated fatty acid-depleted cells and mitochondria were reported to be essentially the same as those of unsaturated fatty acid-supplemented cells and mitochondria. It was concluded from these studies that unsaturated fatty acid depletion leads to a progressive and specific loss of oxidative phosphorylation.

In the present paper the unsaturated fatty acid composition of yeast cells and mitochondria was manipulated by the use of a mutant defective in fatty acid desaturase activity (Resnick & Mortimer, 1966; Keith *et al.*, 1969). Contrary to previous findings, the respiratory properties and cytochrome content of cells and mitochondria were found to be markedly influenced by the degree of unsaturation of the membrane lipids. The temperatures at which discontinuities in Arrhenius plots were observed were also found to be dependent on lipid unsaturation.

Methods

Growth of organism

The fatty acid desaturase mutant of *S. cerevisiae* was obtained from Dr. M. Baird (Department of Genetics, University of Sheffield, Sheffield, U.K.)

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and was the original strain, denoted KD 115, of Resnick & Mortimer (1966). Cells were grown in 10-litre New Brunswick Fermenters (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.) at 30°C in media containing 1% yeast extract, 0.2% peptone and mineral salts (Wickerham, 1946). Unsaturated fatty acid supplements were added in the form of Tween 80. The composition of the fatty acids of the Tween 80 used in all the experiments was determined by g.l.c. analysis of the methyl esters (Watson *et al.*, 1973) and was 74% oleic acid, 12% palmitoleic acid and the rest a mixture of saturated fatty acids of chain length C_{10}–C_{14}. The carbon source was ethanol (0.5%) or glucose (0.4–1%). A 1% inoculum from a starter culture grown for 24h on 0.5% ethanol or 0.5% glucose was used. The cells were harvested at early stationary growth phase. For cells grown on 0.5% ethanol, the growth corresponded to 0.8mg dry wt. of cells/ml of medium for cells grown on low concentrations of Tween 80 (50–100μg/ml of medium) and 3.5mg dry wt. of cells/ml of medium for cells grown on high concentrations of Tween 80 (2–4mg/ml of medium). For cells grown on 0.5% glucose, the growth corresponded to 1.2 and 4mg dry wt. of cells/ml of medium respectively for cells grown on low and high amounts of Tween 80.

Preparation of mitochondria and determination of fatty acids

Methods for the preparation of mitochondria and determination of fatty acids were as described in the preceding paper (Watson *et al.*, 1975).

Enzyme assays

Succinate oxidase and NADH oxidase activities were measured as described in the preceding paper (Watson *et al.*, 1975).

ATPase* was assayed in a reaction mixture containing 5mM-ATP, 2mM-$MgCl_2$ and 50mM-Tris–HCl (pH7.5–10.0) or 50mM-Tris–maleate (pH6.0–7.0), and 100–200μg of mitochondrial protein, all in a final volume of 1.0ml. The reaction was started by the addition of ATP and terminated by the addition of 10% (v/v) trichloroacetic acid. The precipitate was sedimented by centrifugation and 0.5ml of the supernatant was used for the assay of phosphate (King, 1932).

O_2 uptake of cells

Cells were harvested and washed twice in 50mM-potassium phosphate buffer, pH7.4. The washed cells were suspended at a concentration of 20–40mg dry wt./ml of buffer. O_2 uptake was measured with a Rank oxygen Electrode (Rank, Cambridge, U.K.) connected to a Churchill Thermocirculator (Churchill Instruments Co., Perivale, Middx., U.K.). The assay medium contained, in a final volume of 2.5ml, 50mM-potassium phosphate buffer, pH7.4, and 20mM-glucose.

Cytochrome spectra

Cells. Cells were washed two or three times in 50mM-potassium phosphate buffer, pH7.4, and then suspended in 50% (w/v) sorbitol–50mM-potassium phosphate buffer, pH7.4, at 25–30mg dry wt./ml. Cytochrome spectra were recorded in a Unicam SP.1800 spectrophotometer in 1cm-light-path cells (final volume 2.5–3.0ml) by using the turbid-solution cell position and a slit width of 0.6mm. The contents of the sample cell were reduced with a few grains of dithionite and the reference was oxidized with 10μl of H_2O_2.

Mitochondria. Mitochondria were suspended in 0.25M-sorbitol–20mM-Tris–HCl (pH7.4)–1mM-EDTA to a concentration of 4–5mg of protein/ml. The sample and reference cells were treated as described for whole-cell cytochrome spectra. The difference spectra were analysed by using the following wavelength pairs and extinction coefficients: cytochrome *c*, 550–540nm, $E_{mM} = 19$ (Wilson & Epel, 1968); cytochrome *b*, 550–540nm, $E_{mM} = 22$ (Wilson & Epel, 1968); cytochromes $a+a_3$, 605–630nm, $E_{mM} = 24$ (van Gelder, 1966).

Dry-weight and protein determinations

These were as described by Watson *et al.* (1975).

Materials

Tween 80 was obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. All other chemicals were obtained as described in the preceding paper (Watson *et al.*, 1975).

Results

Preliminary experiments on the fatty acid desaturase mutant were conducted to establish the relationship between growth conditions and unsaturated fatty acid content of the cells. Table 1 summarizes the results obtained by growing cells on glucose and ethanol. Growth of cells on ethanol in media low in unsaturated fatty acid (50–100μg of Tween 80/ml) gave cells which contained between 25 and 40% of the total fatty acid as unsaturated acid. It was not possible to lipid-deplete cells below about 25% unsaturated fatty acid by growth on ethanol, since a loss in cell viability was observed. However, cells containing less than 20% of the total fatty acid as unsaturated fatty acid could be obtained by growing cells on glucose as carbon source. Cells containing high proportions of unsaturated fatty acid (60–70% of the total fatty acid as unsaturated fatty acid) were obtained by growth in media containing 2–4mg of

* Abbreviation: ATPase, adenosine triphosphatase.

Table 1. *Fatty acid composition of cells grown on low and high supplements of unsaturated fatty acid*

The fatty acid composition was determined as outlined in the Methods section. The fatty acids are denoted by the convention C and subscript number of carbon atoms: number of unsaturated linkages. The results presented are those from a typical experiment and the fatty acids are expressed as a percentage of the total fatty acid.

Growth conditions	Unsaturated fatty acid supplement (mg/ml)	Fatty acid content							Unsaturated fatty acid (%)
		C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{16:1}$	$C_{18:1}$	
0.5% Ethanol	0.10	5.5	5.4	6.4	46.7	7.4	4.2	24.4	28.6
	3.0	0.8	1.4	2.7	27.0	14.5	2.6	51.0	63.6
0.5% Glucose	0.10	5.5	5.6	10.8	54.8	4.8	3.5	15.0	18.5
	3.0	1.0	2.3	3.1	22.4	2.2	15.7	53.3	69.0
1% Glucose	0.10	5.7	5.9	9.2	60.3	5.9	1.1	11.9	13.0
	3.0	0.7	0.6	1.8	22.6	6.2	16.0	52.1	68.1

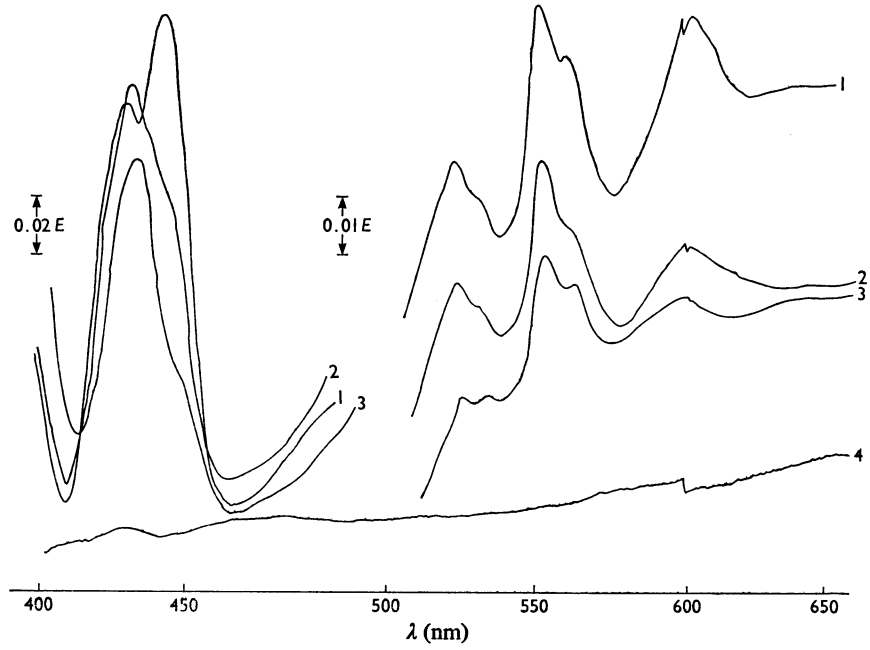

Fig. 1. *Cytochrome spectra of cells containing different proportions of unsaturated fatty acid*

The cytochrome reduced-minus-oxidized difference spectra were recorded as outlined in the Methods section. Trace 1: cells containing 62% unsaturated fatty acid; cell concn. 24.4mg dry wt./ml; growth on 0.5% ethanol. Trace 2: cells containing 32% unsaturated fatty acid; cell concn. 26.3mg dry wt./ml; growth on 0.5% ethanol. Trace 3: cells containing 18% unsaturated fatty acid: cell concn. 28.3mg dry wt./ml; growth on 0.4% glucose. Trace 4: baseline.

Tween 80/ml on ethanol (0.5%) or glucose (0.4–1%) as substrates. These results were essentially in agreement with those of Proudlock *et al.* (1971).

Cell spectra and respiration

Fig. 1 shows the cytochrome spectra of cells grown on ethanol in media containing low (50μg of Tween 80/ml) and high (3mg of Tween 80/ml) amounts of unsaturated fatty acid. Absorption maxima corresponding to cytochromes $a+a_3$ (605nm), cytochrome *b* (564nm) and cytochrome *c* (550nm) were observed in both cell types. However, the absorption peak for cytochromes $a+a_3$ was distinctly larger for lipid-supplemented cells than that for lipid-depleted cells. The difference in the content of cytochromes $a+a_3$ in the two cell types was also clearly evident when the absorption bands in the Soret region were examined. The Soret band of cytochromes $a+a_3$ (445nm) in the

Table 2. *Effects of unsaturated fatty acid on O_2 uptake and cytochrome content of intact cells*

Unsaturated fatty acids, O_2 uptake and cytochromes were determined as outlined in the Methods section. Unsaturated fatty acid was added in the form of Tween 80. The lipid-depleted and lipid-supplemented cells were grown in the presence of 50μg and 3mg of Tween 80/ml of medium respectively. The values are means±S.E.M. with the number of determinations in parentheses.

Growth conditions	Unsaturated fatty acid (%)	O_2 uptake (ng-atoms of O/min per mg dry wt.)	Cytochromes (nmol/mg dry wt.)		
			$a+a_3$	b	c
Ethanol (0.5%)					
1. Lipid-depleted	31±6 (6)	116±40 (6)	0.012±0.002 (6)	0.032±0.003 (6)	0.051±0.005 (6)
2. Lipid-supplemented	64±7 (6)	259±21 (10)	0.027±0.004 (10)	0.036±0.004 (10)	0.060±0.004 (10)
Glucose (0.4%)					
3. Lipid-depleted	17±5 (4)	82±25 (4)	0.0086±0.002 (4)	0.027±0.002 (4)	0.041±0.004 (4)
4. Lipid-supplemented	68±4 (4)	189±30 (4)	0.031±0.003 (4)	0.032±0.004 (4)	0.073±0.006 (4)

lipid-depleted cells was only observed as a shoulder to the larger cytochrome *b* peak (430nm). Cells which were more extensively depleted of unsaturated fatty acid by growth on glucose (0.4%) showed a much lower content of cytochromes $a+a_3$. Measurement of O_2 uptake by intact cells confirmed the lower cytochrome content of the lipid-depleted cells. A summary of the results obtained on whole-cell spectra and O_2 uptake is presented in Table 2.

Mitochondria

Quantitative analysis of the cytochrome content of isolated mitochondria was complicated by a variable loss of cytochrome *c* during the isolation procedures. Nevertheless, it was clearly evident that lipid-depleted mitochondria have a lower cytochrome content, particularly of cytochromes $a+a_3$, than lipid-supplemented mitochondria. The properties of the isolated mitochondria were further investigated by studying the effects of temperature on the succinate oxidase and NADH oxidase activities. Arrhenius plots for these two enzymes in mitochondria containing various proportions of unsaturated fatty acid are presented in Figs. 2 and 3. In all experiments, a non-linear Arrhenius plot, with an increase in activation energy at lower temperatures, was observed. The temperature at which a discontinuity was observed was dependent on the degree of unsaturation of the fatty acids of the mitochondrial membranes. The greater the percentage of unsaturated fatty acid, the lower the transition temperature. The transition temperatures varied from 28–30°C in mitochondria containing 15–25% unsaturated fatty acid to 15–18°C in mitochondria containing 60–70% unsaturated fatty acid. It was further noted that the specific activities of both the succinate oxidase and NADH oxidase were also dependent on the degree of unsaturation of the membrane lipids. The succinate oxidase activity in mitochondria containing 26% unsaturated fatty acids was approximately twofold

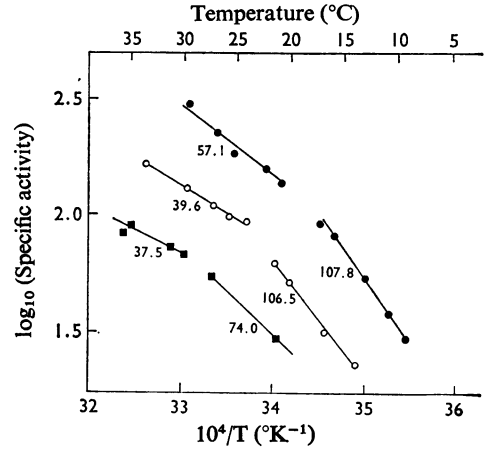

Fig. 2. *Arrhenius plots of succinate oxidase in mitochondria isolated from cells with different unsaturated fatty acid contents*

O_2 uptake was measured polarographically as outlined in the Methods section. Specific activity was expressed as ng-atoms of O taken up/min per mg of protein. The numbers on the graphs are the activation energies in kJ/mol. ●, Mitochondria containing 67% unsaturated fatty acid; ○, mitochondria containing 31% unsaturated fatty acid; ■, mitochondria containing 15% unsaturated fatty acid.

higher than that of mitochondria containing 32% unsaturated fatty acid, and threefold higher than that of mitochondria containing 15% unsaturated fatty acid. The energy of activation for succinate oxidase and NADH oxidase was not greatly affected by the lipid unsaturation, although the activation energies for NADH oxidase, both above and below the transition temperature, were lower than that observed for succinate oxidase (Fig. 2 and 3).

The ATPase of the isolated mitochondria was investigated, since the effects of lipids on mitochon-

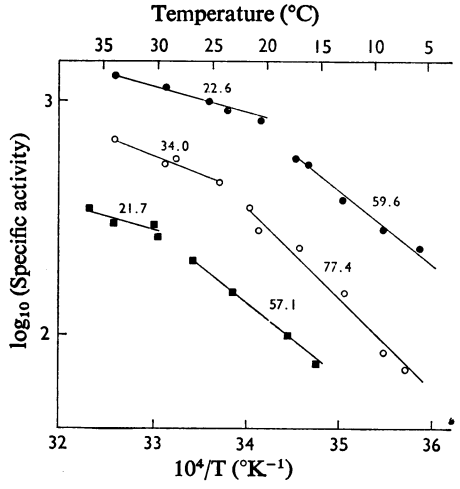

Fig. 3. *Arrhenius plots of NADH oxidase activity in mitochondria isolated from cells with different unsaturated fatty acid contents*

O_2 uptake was measured as outlined in the Methods section. Specific activity is expressed as ng-atoms of O taken up/min per mg of protein. The numbers on the graphs are the activation energies in kJ/mol. ●, Mitochondria containing 67% unsaturated fatty acid: ○, mitochondria containing 31% unsaturated fatty acid; ■, mitochondria containing 15% unsaturated fatty acid.

drial ATPase have been extensively studied (Kagawa & Racker, 1966; Kopaczyk *et al.*, 1968; Bulos & Racker, 1968; Pitotti *et al.*, 1972; Swanljung *et al.*, 1973). A broad maximum, centred at around pH9–9.5, was observed for the mitochondrial ATPase. The highest specific activity was obtained for mitochondria with the highest proportion of unsaturated fatty acid. No distinct maxima were observed in the pH range 6–8. However, inhibition by the antibiotic oligomycin was tested at both pH6.5 and 9.5, since it has been observed that yeast mitochondrial ATPase shows different sensitivities to oligomycin depending on the pH of the assay medium (Kovac *et al.*, 1968; Somlo, 1968; Houghton *et al.*, 1973). The results are summarized in Table 3. Mitochondria having the highest proportion of unsaturated fatty acid (62%) were slightly more resistant to inhibition by low concentrations of oligomycin when assayed at pH9.5 as compared with mitochondria containing lower proportions of unsaturated fatty acid. No marked differences were observed in the degree of inhibition by oligomycin of the ATPase of the lipid-supplemented and lipid-depleted mitochondria when activity was assayed at pH6.5. It was notable, however, that the mitochondrial ATPase was much more resistant to inhibition by oligomycin when activities were measured at pH6.5. This result is consistent with previous reports on the effect of pH on oligomycin inhibition of yeast mitochondrial ATPase (Kovac *et al.*, 1968; Somlo, 1968; Houghton *et al.*, 1973).

The effect of temperature on the ATPase activities of mitochondria containing different amounts of unsaturated fatty acid is shown in Fig. 4. As with the oxidase activities, the temperature at which an increase in activation energy was observed was dependent on the degree of mitochondrial membrane-lipid unsaturation; the greater the degree of lipid unsaturation the lower was the transition temperature. There

Table 3. *Effect of unsaturated fatty acid content on the inhibition of mitochondrial ATPase by oligomycin*

Experimental details are given in the text. The results presented are those obtained in a typical experiment.

pH	Oligomycin (μg/mg of protein)	Unsaturated fatty acid (%) ...	Inhibition (%) 62	35	25	15
9.5	5		20	40	70	40
	10		35	60	80	60
	20		55	70	80	75
	50		80	75	85	85
6.5	5		8	10	10	5
	10		12	20	20	12
	20		25	35	35	25
	50		45	55	70	60

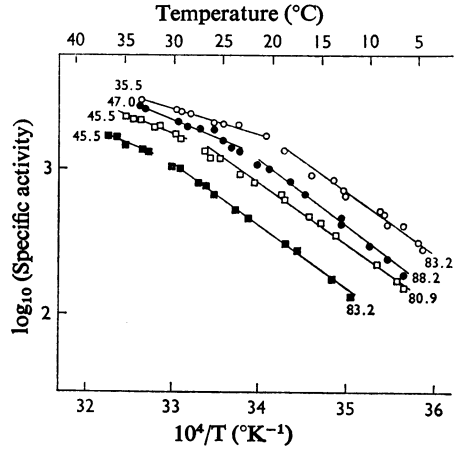

Fig. 4. *Arrhenius plots of mitochondrial ATPase of cells with different unsaturated fatty acid contents*

Enzyme activity was measured as outlined in the Methods section. Specific activity is expressed as nmol of phosphate released/min per mg of protein. The numbers on the graphs are activation energies in kJ/mol. ○, Mitochondria containing 68% unsaturated fatty acid; ●, mitochondria containing 45% unsaturated fatty acid; □, mitochondria containing 31% unsaturated fatty acid; ■, mitochondria containing 21% unsaturated fatty acid.

were no marked differences in the activation energies, above or below the transition temperature, of mitochondria having different membrane-lipid unsaturation.

Discussion

The only parameters that have been reported to be significantly influenced by a change in unsaturated fatty acid composition in liver mitochondria from essential fatty acid-deficient rats are those of a slower frequency of the oscillatory state of energy-linked ion transport and a decrease in the freedom of spin-label motion (Williams *et al.*, 1972). It must be pointed out, however, that although the unsaturated fatty acid composition is different in rats fed on diets, either deficient in or supplemented with essential fatty acids, the proportion of total unsaturated fatty acid remains remarkably constant (Guarnieri *et al.*, 1971; Stancliff *et al.*, 1969). This is primarily due to an increase in the synthesis of palmitoleic acid ($C_{16:1}$), oleic acid ($C_{18:1}$) and eicosatrienoic acid ($C_{20:3}$) in rats fed on diets deficient in essential fatty acids. The similarity in the proportion of unsaturated fatty acid is further reflected in spin-label studies, which have shown that phase transitions occur at approximately the same temperature in essential fatty acid-depleted and essential fatty acid-supplemented rat liver mitochondrial membranes (Williams *et al.*, 1972). This approach therefore is very limited in studies relating to mitochondrial function and lipid unsaturation.

On the other hand, the lipid composition of yeast can be readily manipulated by growing cells anaerobically (Andreason & Stier, 1954; Klein, 1955; Jollow *et al.*, 1968) and by the use of fatty acid auxotrophic mutants (Resnick & Mortimer, 1966; Keith *et al.*, 1969, 1973). The latter approach has been exploited by Proudlock *et al.*, (1971) and Haslam *et al.* (1971, 1973), who have reported that unsaturated fatty acid depletion leads to a specific loss in oxidative phosphorylation with no apparent effects on the respiratory competence of cytochromes of unsaturated fatty acid-depleted cells. By contrast, in the present studies, we report that unsaturated fatty acid depletion in a fatty acid desaturase mutant to values at which a decrease or loss of oxidative phosphorylation was observed by these authors (Haslam *et al.*, 1971) is also paralleled by a decrease in respiratory competence and cytochrome content. These effects were observed in the intact cell and in the isolated mitochondria.

In our hands it was not possible to lipid-deplete the desaturase mutant to values below about 25% unsaturated fatty acid by using ethanol as the carbon source, without a concomitant loss in the viability of the cells. However, further lipid depletion to values below 25% unsaturated fatty acid was achieved by growing cells on glucose. The question of glucose repression of cytochromes and respiratory activity is raised under these conditions, although these effects were minimized by harvesting cells in stationary growth phase and by the use of low concentrations of glucose. Growth of cells in continuous culture would be expected to eliminate these problems. In a preliminary communication, Linnane *et al.*, (1973) have reported that growth of the fatty acid desaturase mutant in a glucose-limited chemostat leads to low contents of cytochromes, particularly cytochromes $a+a_3$, when cells reached about 20% unsaturated fatty acid. These results are essentially in agreement with the present observations.

The temperatures at which a discontinuity occurred in Arrhenius plots of mitochondrial membrane-bound enzymes in the fatty acid desaturase mutant were dependent on the degree of membrane-lipid unsaturation. The greater the degree of lipid unsaturation, the lower was the transition temperature. A similar phenomenon in yeast (Ainsworth *et al.*, 1972; Watson *et al.*, 1973) and mammalian mitochondria (McMurchie & Raison, 1973) has been observed. Spin-label studies on fatty acid mutants of *Neurospora crassa* and *S. cerevisiae* have also indicated the dependency of transition temperatures on membrane-lipid composition (Keith *et al.*, 1973). These observations indicate that it is the physical state of the membrane lipids that determines the transition temperatures of mitochondrial membrane-bound enzymes.

A number of recent studies support the idea that a liquid-crystalline or fluid state of membranes is necessary for the proper functioning of membrane-bound enzymes (Overath *et al.*, 1970; Kimelberg & Papahadjopoulos, 1972; Eletr *et al.*, 1973). The present report on yeast mitochondria suggests that a similar situation may also be operative in mitochondrial membranes. It is noteworthy, in this respect, that a characteristic of mitochondria from plants (Lyons *et al.*, 1964) and animals (Richardson & Tappel, 1962) is a large content of unsaturated fatty acids.

There are a number of other reports in the literature pertaining to the effects of lipid unsaturation on yeast mitochondrial activity and function. Studies on promitochondria from anaerobically grown yeast have shown that the degree of mitochondrial-lipid unsaturation has a marked effect on enzyme activity and morphology (Criddle & Schatz, 1969; Plattner & Schatz, 1969; Watson *et al.*, 1970). The mitochondrial protein-synthesizing system has also been reported to be influenced by the mitochondrial unsaturated fatty acid content (Forrester *et al.*, 1971; Watson *et al.*, 1971; Watson, 1972; Gordon & Stewart, 1972). Reconstitution studies by Kagawa *et al.* (1972) on energy-linked functions of ox heart mitochondria have shown that reconstitution was most effective with phospholipids containing un-

saturated fatty acid groups. Phospholipids containing fully saturated fatty acid groups were found to be actually inhibitory.

It should be noted, however, that a high degree of membrane-lipid unsaturation does not necessarily imply a fully functional mitochondrial membrane. Promitochondria, possessing an active mitochondrial protein-synthesizing system and having a similar degree of membrane-lipid unsaturation to normal mitochondria, have a low enzymic activity and lack respiratory competence (Criddle & Schatz, 1969; Paltauf & Schatz, 1969; Watson *et al.*, 1971). Further, mitochondria from a respiratory-deficient *petite* mutant of yeast have been reported to have an unsaturated fatty acid composition essentially identical with that of the respiratory-competent wild type (Packer *et al.*, 1973).

Previous studies have indicated that lipids play an important part in the mode of action of the antibiotics oligomycin and rutamycin on mitochondrial ATPase (Kagawa & Racker, 1966; Bulos & Racker, 1968; Kopaczyk *et al.*, 1968; Palatini & Bruni, 1970; Pitotti *et al.*, 1972). The present studies, utilizing an unsaturated fatty acid auxotroph of yeast, have shown that the mitochondrial membrane-lipid unsaturation may be extensively altered from 15 to 62% unsaturated fatty acid without significant differences in the degree of inhibition by oligomycin of the ATPase activity (Table 3). It therefore appears likely that the degree of membrane-lipid unsaturation is not a critical factor in determining the inhibitory effects of oligomycin on mitochondrial ATPase activity. This conclusion would suggest that, if phospholipids do play a role in conferring oligomycin sensitivity to mitochondrial ATPase, it would be the phospholipid head group or hydrophilic region rather than the hydrophobic alkyl chain which is the critical factor.

This work was supported by grants from the Science Research Council. E. B. acknowledges receipt of a Unilever Postdoctoral Fellowship.

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