THE BIOGENESIS OF MITOCHONDRIA

III. The Lipid Composition of Aerobically and Anaerobically Grown *Saccharomyces cerevisiae* as Related to the Membrane Systems of the Cells

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ABSTRACT

The growth conditions known to influence the occurrence of mitochondrial profiles and other cell membrane systems in anaerobic cells of *S. cerevisiae* have been examined, and the effect of the several growth media on the lipid composition of the organism has been determined. The anaerobic cell type containing neither detectable mitochondrial profiles nor the large cell vacuole may be obtained by the culture of the organism on growth-limiting levels of the lipids, ergosterol, and unsaturated fatty acids. Under these conditions, the organism has a high content of short-chain saturated fatty acids (10:0, 12:0), phosphatidyl choline, and squalene, compared with aerobically grown cells, and it is especially low in phosphatidyl ethanolamine and the glycerol phosphatides (phosphatidyl glycerol + cardiolipin). The high levels of unsaturated fatty acids normally found in the phospholipids of the aerobic cells are largely replaced by the short-chain saturated acids, even though the phospholipid fraction contains virtually all of the small amounts of unsaturated fatty acid present in the anaerobic cells. Such anaerobic cells may contain as little as 0.12 mg of ergosterol per g dry weight of cells while the aerobic cells contain about 6 mg of ergosterol per g dry weight. Anaerobic cell types containing mitochondrial profiles can be obtained by the culture of the organism in the presence of excess quantities of ergosterol and unsaturated fatty acids. Such cells have increased levels of total phospholipid, ergosterol, and unsaturated fatty acids, although these compounds do not reach the levels found in aerobic cells. The level of ergosterol in anaerobic cells is markedly influenced by the nature of the carbohydrate in the medium; those cells grown on galactose media supplemented with ergosterol and unsaturated fatty acids have well defined mitochondrial profiles and an ergosterol content (2 mg per g dry weight of cells) three times that of equivalent glucose-grown cells which have poorly defined organelle profiles. Anaerobic cells which are low in ergosterol synthesize increased amounts of squalene.

INTRODUCTION

The cytological characteristics of *Saccharomyces cerevisiae*, grown under both aerobic and anaerobic conditions, are largely determined by the composition of the growth medium (Yotsuyanagi, 1962; Wallace and Linnane, 1964; Morpurgo et al., 1964; Polakis et al., 1964, 1965; Linnane, 1965; Wallace et al., 1968). The mitochondria of the aerobic cell are under the control of a glucose

repression, which determines both their composition and their number per cell; however, cells grown aerobically on a glucose-yeast extract medium always contain four well defined membrane systems: nuclear, plasma, vacuolar, and mitochondrial. In contrast, when the organism is grown anaerobically on the same glucose-yeast extract medium, only nuclear and plasma membranes are readily recognizable, the vacuolar membrane is decreased markedly in extent, while mitochondrial membrane cannot be positively identified (Wallace and Linnane, 1964; Wallace et al., 1968). The supplementation of the medium with ergosterol and unsaturated fatty acids enables the organism to form its large cell vacuole and rather poorly defined mitochondrial membranes (Morpurgo et al., 1964; Wallace et al., 1968). Replacement of glucose, the major carbon source of the medium, by galactose in the presence of ergosterol and unsaturated fatty acid supplements facilitates the elaboration by the anaerobic cells of well defined nuclear, plasma, vacuolar, and mitochondrial membrane systems (Polakis et al., 1965; Wallace et al., 1968). The apparent inability of the anaerobic cell to form mitochondrial membranes in the absence of supplements of ergosterol and unsaturated fatty acids and the marked decrease in amount of vacuolar membrane serve to emphasize that the nature of the lipid matrix plays an important part in the formation and function of membrane systems.

In the present paper, the lipid composition of whole cells of *S. cerevisiae*, grown anaerobically on different media which Wallace et al. (1968) have shown to influence the morphological characteristics of the cell, is described and correlated with the occurrence of the various membrane systems of the cell. These media contain different levels of ergosterol and unsaturated fatty acids and have either glucose or galactose as major carbon sources. The lipid composition of aerobically grown cells is also reported.

METHODS

Culture Conditions

The strain of *S. cerevisiae* and the details of the aerobic and anaerobic growth conditions used in this study are described in an accompanying communication (Wallace et al., 1968).

Two types of media have been used, a synthetic medium and a complex medium. The complex medium contained in each liter of distilled water:

Difco yeast extract	5 g	$CaCl_2$	0.1 g
NaCl	0.5 g	KH_2PO_4	1.0 g
$MgCl_2 \cdot 6H_2O$	0.7 g	$FeCl_3$	5 mg
$(NH_4)_2SO_4$	1.2 g	Glucose or galactose	50 g.

This medium is denoted as YE-G or YE-GAL depending upon whether glucose or galactose, respectively, was used as the carbohydrate.

The synthetic medium contained in each liter of distilled water: glucose, 50 g; tripotassium citrate, 5 g; $(NH_4)_2SO_4$, 3.8 g; KH_2PO_4, 2.0 g; citric acid, 1.0 g; $CaCl_2$, 400 mg; NaCl, 250 mg; $MgSO_4 \cdot 7H_2O$, 250 mg; $ZnSO_4 \cdot 7H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 17.5 mg; $FeCl_3$, 2.5 mg; H_3BO_3, 1 mg; $CuSO_4 \cdot 5H_2O$, 0.1 mg; KI, 0.1 mg; aspartic acid, 100 mg; adenine, 11 mg; inositol, 50 mg; calcium pantothenate, 5 mg; *para* aminobenzoic acid, 2 mg; thiamine, pyridoxine hydrochloride, and nicotinic acid, each 1 mg; riboflavine, 200 μg; biotin, 10 μg; and folic acid, 2 μg.

In lipid-supplemented media, ergosterol and Tween 80 were added at final concentrations of 0.002% (w/v) and 0.5% (w/v), respectively, except as otherwise stated. Tween 80 (fatty acid composition; oleic 71 moles %, palmitoleic 13 moles %, myristoleic 3 moles %, and saturated fatty acid 13 moles %) served as a water-soluble source of unsaturated fatty acid.

Analytical Methods

Lipid-soluble phosphorus in the various extracts was determined by the method of Allen (1940). For quantitation, the phospholipid types were first separated by thin-layer chromatography, eluted as described by Skipski et al. (1964), and the phosphorus content of each eluted component was determined by the method of Chen et al. (1956). Recovery of lipid-soluble phosphorus applied to the thin layer chromatogram was 96 ± 3%.

Sterol was extracted after alkaline hydrolysis and estimated as ergosterol from the absorption spectrum at wavelengths between 260 and 310 mμ (Shaw and Jeffries, 1953) and by the Liebermann-Burchard reaction carried out at 0°C (Cook, 1958).

Plasmalogen was estimated by the iodine absorption method of Williams et al. (1962).

For determination of the fatty acid content and composition of the cells, a known weight of pentadecanoic acid was added to a known weight of cells, the mixture was saponified, and the fatty acids were extracted after acidification. The fatty acids were methylated with diazo methane as described by

Roper and Ma (1957), and separated by vapor phase chromatography with Apiezon L (20%) or polyethylene glycol adipate (30%) as the stationary phase (James, 1960). The fatty acids were identified from their relative retention times on the two stationary phases (James, 1960). The fatty acid content was calculated with the use of the pentadecanoic acid as an internal standard. The standard fatty acid mixture (mixture KE) of Applied Science Laboratories, State College, Pa., was used to calibrate the columns. Quantitative results with the standard fatty acid mixture agreed with the stated composition data, with a relative error less than 10% for minor components (<10% of total mixture) and less than 5% for major components (>10% of total mixture), in keeping with the criteria of Horning et al. (1964).

Chromatographic Standards

All chromatographic standards were the best commercially available grades. The purity of the neutral lipid and phospholipid standards was checked by silicic acid chromatography with a hexane: ether: acetic acid (80:20:2) solvent system for neutral lipids (Malins and Mangold, 1960), and a chloroform: methanol: acetic acid: water (25:15:4:2) solvent system for phospholipids (Skipski et al., 1964). Where necessary, impurities were removed by preparative thin-layer silicic acid chromatography.

Extraction of Lipids

For total fatty acid and sterol analyses, whole cells were saponified by the method of Shaw and Jeffries (1953), and the nonsaponifiable material and fatty acids were extracted by standard procedures.

In order to extract the total lipid, we completely disrupted the cells by three or four passages at about 5°C through a French pressure cell at 7–8 tons per sq. in., and then we extracted with chloroform: methanol (2:1) (v/v) by the method of Folch et al. (1957). The residue was further extracted with 10 volumes of a mixture of chloroform: methanol: 10 N hydrochloric acid (66:33:2) (v/v). The extracts were separately washed as described by Folch et al. (1957), dried, redissolved in chloroform: methanol (19:1) (v/v), and then pooled. For determination of the efficiency of the lipid extractions, the cell residues were saponified and their sterol and fatty acid contents determined. By this criterion, complete lipid extraction was obtained in all cell types except for those grown anaerobically with both ergosterol and Tween 80 supplements. In these latter cells, extraction was 85% complete on the basis of fatty acid recovery and 75% on the basis of sterol; it has not been possible to improve on these values.

Fractionation of Lipids

The extracted lipids were applied to a silicic acid column prepared by the method of Haahti (1961) and the lipids eluted by hexane (fraction A); chloroform (fraction B); chloroform-methanol (1:1) and methanol (these two eluates were combined to make up fraction C).

Identification of the Major Components in the Lipid Fractions

FRACTION A: The lipid eluted by hexane was identified as squalene by its mobility relative to authentic squalene on silicic acid thin-layer chromatography with a hexane: ether: acetic acid (80:20:2) solvent system (Malins and Mangold, 1960), and also by its retention time relative to squalene and cholestane standards on vapor-phase chromatography with a silicone oil (SE-30, 1%) stationary phase at 215°C (Haahti, 1961; Brooks and Hanaineh, 1963).

FRACTION B: The lipid eluted from the silicic acid column by chloroform was further fractionated by thin-layer silicic acid chromatography with a hexane: ether: acetic acid (80:20:2) solvent system. The major components, viz. sterol ester, triglyceride, free sterol, and free fatty acid, were identified on the basis of their mobility relative to the appropriate standards before and after alkaline hydrolysis (Malins and Mangold, 1960). The sterol was identified as ergosterol on the basis of its UV spectrum and its retention time on vapor-phase chromatography with SE-30 (1%) as stationary phase at 225°C, relative to ergosterol, cholestane, and cholestan-3β-ol standards (Brooks and Hanaineh, 1963). Fraction B is denoted in this paper as the "glyceride fraction," as this lipid class represented the major component.

FRACTION C: The lipid eluted from the silicic acid column by chloroform-methanol and methanol solvents was separated by thin-layer chromatography with the chloroform: methanol: acetic acid: water (25:15:4:2) solvent system of Skipski et al. (1964). The chromatograms were charred with concentrated sulfuric acid (Wagner et al., 1961) for detection of all organic material, stained with iodine vapor (Mangold, 1961) and rhodamine 6G (Marinetti et al., 1958) for location of all lipid material, ninhydrin (Skidmore and Entenman, 1962) for amino lipids, the modified Dragendorff's reagent (Wagner et al., 1961) for choline-containing lipids, and acid molybdate (Skipski et al., 1964) for phosphorus-containing lipids. The phospholipid components, viz. phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl glycerol, and cardiolipin, were identified on the basis of their R_f values relative to appropriate phospholipid standards (chromatographed simultaneously), and on the basis of their staining behavior. The occur-

rence of serine, inositol, and ethanolamine phosphatides was further confirmed by acid hydrolysis, followed by alkaline phosphatase hydrolysis and subsequent chromatography of the water-soluble products as described by Wheeldon et al. (1962). Fraction C is denoted as the "phospholipid" fraction in this paper.

RESULTS

Anaerobic Growth Requirements for Ergosterol and Unsaturated Fatty Acids

Table I presents a summary of the variation in cytological appearance of cells of *S. cerevisiae* grown anaerobically on complex media. The cytological characteristics of the cells are determined by the nature of the carbon source in the medium and whether or not the YE-G medium was supplemented with ergosterol and Tween 80 (source of unsaturated fatty acids). However, Andreasen and Stier (1953, 1954) have shown that ergosterol and unsaturated fatty acid supplements are both essential for the anaerobic growth of *S. cerevisiae* on chemically defined media. Initially, it appeared that our strain of the organism did not require these supplements, as it grew on the YE-G medium after repeated anaerobic subcultures (Table I); but, when grown on a chemically defined synthetic medium, the organism was, indeed, found to have an absolute requirement for both ergosterol and unsaturated fatty acid supplements. The growth response to trace amounts of these lipids was dramatic (Fig. 1); 120 μg of ergosterol and 400 μg of Tween 80 per liter of the synthetic medium resulted in the same cell density as that attained on the YE-G medium (about 1 mg/ml dry weight). However, maximal anaerobic growth (3.5 mg/ml dry weight) required supplements of at least 5 mg/liter of ergosterol and about 500 mg/liter of Tween 80. The initial very sensitive growth response to Tween 80 (half-maximal response requires about 0.5 mg/liter) is followed by a much slower increase in growth in the higher concentration range.

The anaerobic growth of the cells on the YE-G

TABLE I

Growth and Cytology of S. cerevisiae on a Variety of Media

Growth conditions	Cell yield	Main membrane systems present in cell
Medium	*mg dry weight per ml*	
Aerobic		
Synthetic	6.5	Not examined
YE-G	5.5	Plasma, nuclear, vacuolar, mitochondrial
Anaerobic		
Synthetic*	Trace‡	Not examined
Synthetic + Tween 80 + ergosterol	3.5‡	Not examined
YE-G	1.1	Plasma, nuclear
YE-G (repeated anaerobic growth)	0.9‡	Plasma, nuclear
YE-G + ergosterol	1.2	Plasma, nuclear
YE-G + Tween 80	1.3	Plasma, nuclear, vacuolar, free cytoplasmic
YE-G + Tween 80 + ergosterol	3.0	Plasma, nuclear, vacuolar, ill-defined mitochondrial profiles
YE-GAL + Tween 80 + ergosterol	3.0	Plasma, nuclear, vacuolar, mitochondrial profiles

The cell yield was measured after 18 hr of aerobic and 24 hr of anaerobic growth (early stationary phase of growth cycle). The values given are the means of at least six determinations; variation from the mean was within ±10%. Trace = <0.2 mg dry weight per ml. The composition of the various media is given in the Methods section. For details of the cytology, see Wallace et al. (1968).

* Addition of either Tween 80 or ergosterol alone to this medium did not allow growth to take place.

‡ These cultures were grown from inocula cultured anaerobically on YE-G medium. The anaerobic inocula were transferred anaerobically as described by Wallace et al. (1968). All other cultures were grown from aerobic inocula.

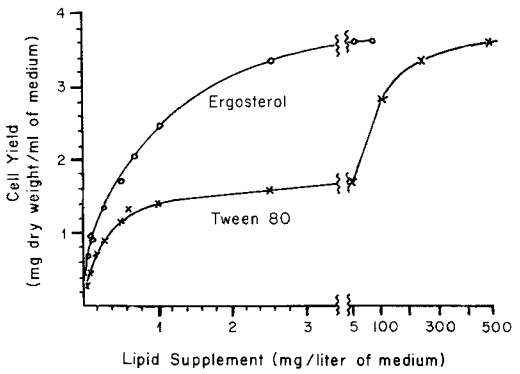

FIGURE 1 Growth-promoting ability of ergosterol and Tween 80 for the anaerobic growth of *S. cerevisiae*. ○——○ indicates the growth response to varying amounts of ergosterol added to the synthetic medium containing excess Tween 80 (5 g/liter), and ×——× indicates the growth response to varying amounts of Tween 80 added to the synthetic medium containing excess ergosterol (20 mg/liter). The cell yield was measured after 24 hr of anaerobic growth (early stationary phase of growth cycle) from inocula grown anaerobically on the YE-G medium. The inoculum (10 mg dry weight of yeast per liter of medium) was transferred anaerobically as described by Wallace et al (1968).

medium was shown to be limited by the trace amounts of ergosterol and unsaturated fatty acids in the Difco yeast extract. Addition of a large excess of ergosterol (20 mg/liter) and Tween 80 (5 g/liter) to the YE-G medium allowed a threefold increase in final cell yield; the single addition of either of these components did not promote additional growth. While analysis of the Difco yeast extract has revealed the presence of sterol and unsaturated fatty acids, the precise quantitation of these compounds has not been possible, owing to the small amounts of them in the yeast extract.

Effect of Growth Conditions on Lipid Composition

Marked differences were found in the lipid composition of the cytologically distinct cells resulting from aerobic growth on the YE-G medium and anaerobic growth on the YE-G, YE-G + ergosterol, YE-G + Tween 80, YE-G + ergosterol + Tween 80, and YE-GAL + ergosterol + Tween 80 media.

TOTAL LIPID CONTENT

The lipid content of the cell types is given in Table II in terms of their total fatty acid, squalene, sterol, glycerides, and phospholipid.

TABLE II

Lipid Composition of Cytologically Distinct Forms of S. cerevisiae

Growth conditions*	Total lipid	Total fatty acid	Ergosterol	Squalene	Glycerides‡	Phospholipid§
Medium						
Aerobic						
YE-G	100 ± 4	68 ± 4	6.2 ± 0.5	2 ± 1	62 ± 4	35 ± 4 (30)
Anaerobic						
YE-G	62 ± 3	19 ± 2	0.12 ± 0.04	19 ± 3	27 ± 3	16 ± 3 (14)
YE-G + ergosterol	33 ± 1	18 ± 2	0.70 ± 0.2	8 ± 1	9 ± 2	16 ± 2 (12)
YE-G + Tween 80	102 ± 7	46 ± 5	0.12 ± 0.04	16 ± 2	55 ± 4	31 ± 3 (28)
YE-G + Tween 80 + ergosterol	62 ± 2	19 ± 2	0.73 ± 0.2	6 ± 2	35 ± 3	21 ± 3 (18)
YE-GAL + Tween 80 + ergosterol	50 ± 2	34 ± 4	2.20 ± 0.4	3 ± 1	17 ± 3	30 ± 4 (25)

All values are in mg per gram dry weight of yeast, and are the means of at least four replicates, ± standard deviations.

* Growth conditions are as indicated in the Methods section.

‡ This fraction in addition to the glycerides includes the free fatty acids of the cells, the sterol and some unidentified material.

§ Values in brackets indicate the weight of phospholipid calculated from the lipid-soluble phosphorus, assuming a molecular weight of 800.

Aerobically grown cells contained about 100 mg of lipid/g dry weight and anaerobically grown cells only about 60 mg/g or less, except for those supplemented with Tween 80 alone, where the lipid content was also about 100 mg/g. The cells grown on YE-G + ergosterol medium had only 33 mg total of lipid/g dry weight of cells, containing comparatively little of the glyceride fraction.

Although the total fatty acid and glyceride content of the different cell types varied approximately with the total amount of lipid, there was no strict proportional relationship among these three entities. In the cells grown anaerobically on YE-G, YE-G + Tween 80, and YE-G + Tween 80 + ergosterol media the total amounts of fatty acid were less than might be expected on the basis of the total amounts of phospholipid and glyceride fractions. To account for this apparent deficit of fatty acid we considered the possibility of large amounts of plasmalogen in these cells, but we detected only trace amounts. This aspect of the work has not been pursued further.

Cells grown anaerobically on YE-G and YE-G + Tween 80 media contained about 0.12 mg of ergosterol/g dry weight of cells, which was only about 2% of the level found in aerobically grown cells. Cells grown anaerobically on media supplemented with ergosterol incorporated additional amounts of this sterol. It is of interest that the anaerobic cells grown on YE-GAL + Tween 80 + ergosterol contained about 2.2 mg of ergosterol/g dry weight of cells, about three times as much ergosterol as the equivalent cells grown on glucose. On the other hand, the levels of squalene found in the cells were inversely related to the cells' ergosterol content, varying from about 2 mg/g dry weight in aerobic cells, to 20 mg/g dry weight in the anaerobic cells grown without added ergosterol.

The quantity of phospholipid in the different cell types ranged from 16 to 35 mg/g dry weight of cells. The highest levels of phospholipid occurred in the aerobically grown cells and varying levels were present in the anaerobically grown cells, which can be correlated with the extent of the membrane formation in the cells (compare Tables I and II). This correlation will be discussed in another section of the paper.

Phospholipid Composition

The analysis of the phospholipid fractions is given in Table III; each component is expressed as a percentage of the total phospholipid of the cell type. Incomplete resolution was obtained between phosphatidyl serine and phosphatidyl inositol and between phosphatidyl glycerol and cardiolipin, and so combined figures are given for these two pairs.

The composition of the phospholipid fractions from all the anaerobic cell types was very similar, but differed markedly from that of the phospholipid fractions from the aerobic cells.

TABLE III

Phospholipid Composition of Cytologically Distinct Forms of S. cerevisiae

Growth conditions	Phospholipid fractions (Percentage of Total Phospholipid)			
Medium	*PC*	*PI + PS*	*PE*	*GP*
Aerobic				
YE-G	25.8 ± 0.8	17.0 ± 1.9	38.3 ± 1.2	11.7 ± 0.6
Anaerobic				
YE-G	48.8 ± 2.0	22.3 ± 1.6	20.3 ± 1.4	4.3 ± 1.3
YE-G + ergosterol	43.3 ± 2.2	26.1 ± 1.0	22.4 ± 1.5	5.2 ± 0.8
YE-G + Tween 80 + ergosterol	48.0 ± 2.3	24.3 ± 0.9	22.4 ± 2.0	4.8 ± 1.0
YE-GAL + Tween 80 + ergosterol	43.4 ± 2.4	24.8 ± 0.8	22.2 ± 1.7	5.6 ± 0.7

The results given are the means of four replicate experiments, ± the standard deviations. Abbreviations: *PC* = phosphatidyl choline, *PI* = phosphatidyl inositol, *PS* = phosphatidyl serine, *PE* = phosphatidyl ethanolamine, *GP* = glycerol phosphatides (includes cardiolipin and phosphatidyl glycerol).

In the aerobic cell, phosphatidyl choline accounted for about 25% of the total phospholipid, phosphatidylethanolamine for about 40%, and the glycerol phosphatides (phosphatidylglycerol and cardiolipin) for about 12%. These results are qualitatively similar to those reported by Letters (1966) for aerobic cells. In contrast, in all anaerobic cell types, phosphatidylcholine represented almost 50% of the total phospholipid, phosphatidylethanolamine only 20%, and the glycerol phosphatides about 5%.

Calculation of the amounts of the individual phospholipids per g dry weight of cells (Table IV) further emphasizes the differences between the aerobic and anaerobic cells and also reveals differences between the various anaerobic cell types. Especially notable are the levels of phosphatidylethanolamine and glycerol phosphatides. The anaerobic cells grown on YE-GAL + Tween 80 + ergosterol medium contained only about half as much of these compounds as the aerobic cells, but twice as much as the anaerobic cells cultured on YE-G and YE-G + ergosterol media.

Fatty Acid Composition

The fatty acids in all cell types included only straight-chain saturated and monoenoic acids with chain length of 18 carbon atoms or less. Hydroxy acids, branched chain acids, and polyunsaturated acids were not found.

Cells grown aerobically on YE-G medium presented a predominantly unsaturated fatty acid picture, the monoenoic acids (16:1, 18:1) accounting for about 85% of the total acids. The short-chain

TABLE IV

Cellular Content of Phospholipids in Cytologically Distinct Forms of S. cerevisiae

Growth conditions	Phospholipid components			
Medium	*PC*	*PI + PS*	*PE*	*GP*
	mg per g dry weight of yeast			
Aerobic				
YE-G	7.7	5.1	11.5	3.5
Anaerobic				
YE-G	6.8	3.1	2.8	0.6
YE-G + ergosterol	5.2	3.1	2.7	0.6
YE-G + Tween 80 + ergosterol	8.6	4.4	4.0	0.9
YE-GAL + Tween 80 + ergosterol	10.8	6.2	5.6	1.4

The results given were calculated from the total phospholipid content of the cells (Table II) and the percentage phospholipid composition (Table III). The phospholipid abbreviations are as given in Table III.

TABLE V

Fatty Acid Composition of Cytologically Distinct Forms of S. cerevisiae

Growth conditions	Fatty acid composition*							
Medium	10:0	12:0	14:0	16:0	18:0	14:1	16:1	18:1
	(moles/100 moles of fatty acid mixture)							
Aerobic								
YE-G	Tr	1	1	9	3	Tr	53	32
Anaerobic								
YE-G	29	21	10	30	8	Nil	1	1
YE-G + ergosterol	26	17	9	36	10	Nil	2	1
YE-G + Tween 80	Tr	2	9	22	4	2	14	46
YE-G + Tween 80 + ergosterol	Tr	Tr	5	20	5	2	20	48
YE-GAL + Tween 80 + ergosterol	Nil	Tr	4	21	5	2	19	49

* The fatty acids are denoted by the convention; number of carbon atoms: number of unsaturated linkages.

Tr = Trace (<1%). The results given are the means of at least four replicate experiments, approximated to the nearest whole number.

TABLE VI

Fatty Acid Composition of Glyceride and Phospholipid Fractions Derived from Cytologically Distinct Forms of S. cerevisiae

Growth conditions	Fraction	Fatty acid composition*						
Medium		10:0	12:0	14:0	16:0	18:0	16:1	18:1
		(moles/100 moles of fatty acid mixture)						
Aerobic								
YE-G	Glycerides	Nil	1	2	8	3	53	33
	Phospholipids	Tr	Tr	1	13	4	51	30
Anaerobic								
YE-G	Glycerides	33	25	10	25	7	Tr	Tr
	Phospholipids	14	14	11	42	10	4	5
YE-G + ergosterol	Glycerides	27	24	11	29	8	Tr	Tr
	Phospholipids	27	13	7	38	10	3	2
YE-G + Tween 80 + ergosterol	Glycerides	2	3	9	21	6	11	48
	Phospholipids	Tr	2	2	20	3	18	55

* The fatty acids are denoted by the convention; number of carbon atoms: number of unsaturated linkages.

Tr = Trace (<1%). The results given are those of typical experiments; the values have been approximated to the nearest whole number.

saturated acids (10:0, 12:0) occurred in negligible amounts, and the longer-chain saturated acids made up the remaining 15% of the total (Table V).

By contrast, cells grown anaerobically on YE-G or YE-G + ergosterol media, which supply only traces of unsaturated fatty acid, had a predominantly saturated fatty acid composition, with less than 3% monoenoic acids. In these anaerobic cell types, the short-chain saturated acids, decanoic and dodecanoic (10:0, 12:0), made up 50% of the total fatty acids.

Yet another fatty acid composition was apparent for cells grown anaerobically in the presence of Tween 80. These cells no longer formed large amounts of decanoic and dodecanoic acids, but utilized in part the monoenoic acids present in the Tween. The fatty acids of these cell types were made up of about 65% monoenoic and 35% saturated acids, which still represents a much greater degree of saturation than that normally found in aerobic cells (about 15% saturated acids).

The distribution of the unsaturated fatty acids between the phospholipid and glyceride fractions of the cells was examined (Table VI). In the aerobically cultured cells, the fatty acid composition of the glyceride and phospholipid fractions was similar. However, in those anaerobically grown cells which contained only small amounts of unsaturated fatty acids, (cells grown on YE-G, YE-G + ergosterol), these acids were preferentially concentrated in the phospholipid fraction rather than in the glycerides.

DISCUSSION

The present results on the lipid composition of anaerobically grown *S. cerevisiae* taken together with those of our previous paper (Wallace et al., 1968) serve to emphasize that the formation of mitochondria by yeast is under a complex series of controls, one of which is the availability of ergosterol and unsaturated fatty acids to the cell. It has also become apparent that a catabolite repression and oxygen determine the extent and enzyme composition of mitochondrial membrane. Oxygen is required for the synthesis of the classical cytochromes of the mitochondrial membrane (Slonimski, 1953; Somlo and Fukahara, 1965; Wallace et al., 1968), while the catabolite repression influences the amounts of the cytochromes formed aerobically and the amount of mitochondrial membrane formed both aerobically and anaerobically

(Yotsuyanagi 1962; Polakis et al., 1964, 1965; Linnane, 1965; Wallace et al., 1968). Logical discussion of our lipid results, therefore, requires not only consideration of the individual roles of the unsaturated fatty acids and ergosterol and their interactions, but also an attempt at the integration of the lipid effects into a concept of the control of membrane formation by the cells.

Perhaps the most obvious effect of the growth conditions is seen in the fatty acid composition of the cell lipids. In the aerobic cells and also in the anaerobic cells grown in the presence of excess unsaturated fatty acid, monounsaturated fatty acids greatly predominate. In contrast, in cells cultured anaerobically on growth-limiting amounts of unsaturated fatty acid (cells grown on YE-G, YE-G + ergosterol), only small amounts of the total fatty acids are monounsaturated, and there is a dramatic increase in the decanoic and dodecanoic acid contents which now make up half the total. In these cells, the small amounts of unsaturated fatty acids are concentrated in the phospholipid fraction. It is probably valid to consider that the phospholipids are major structural components of membranes and that the preferential concentration of the unsaturated fatty acids in them has some functional significance. These cells present the simplest membrane pattern of any of the cells, which consists mainly of nuclear and plasma membranes. It is apparent that this minimal membrane system can function with a major proportion of its fatty acids replaced by the abnormal short-chain saturated acids. Bloch and coworkers (Meyer and Bloch, 1963; Meyer, Light, and Bloch, 1963) have earlier reported a marked increase in the amounts of twelve and fourteen carbon atom-saturated fatty acids in anaerobically grown yeast, starved of unsaturated fatty acid. However, their organism required, in addition to small amounts of unsaturated fatty acids, a large supplement of hydroxystearic or stearolic acid for anaerobic growth; hydroxystearic acid accounted for about 15% of the total fatty acid found in their organism (Light et al., 1962).

A good correlation between the lipid composition, the development of vacuolar and mitochondrial membranes, and the sterol and unsaturated fatty acid contents of the cells is apparent. As previously stated, the cells grown on the lipid-deficient YE-G medium have mainly nuclear and plasma membrane systems. The inclusion of additional ergosterol in the medium (YE-G + ergosterol medium) does not appear to affect the cytological characteristics of the cells. Cells cultured on the YE-G + Tween 80 medium form vacuolar membrane and considerable amounts of cytoplasmic membrane. However, cells grown on the YE-G + Tween 80 + ergosterol medium form both a large cell vacuole and primitive mitochondrial profiles and no longer elaborate the large amounts of cytoplasmic membrane which, therefore, appears to result from an imbalance in the amount of available unsaturated fatty acid and ergosterol. Differences in the lipid composition of cells grown on the YE-G + Tween 80 and the YE-G + Tween 80 + ergosterol media are observed. The cells grown on the YE-G + Tween 80 medium have a high lipid content which is characterized by a comparatively high concentration of phospholipids and large amounts of glyceride and squalene; the inclusion of additional ergosterol in the medium leads to a substantial reduction in the total lipid, phospholipids, glyceride, and squalene and a rise in the ergosterol content of the cells. It is also of interest that, in general, the squalene content of the yeast has been found to vary inversely with the ergosterol content of the cells, and so it seems that ergosterol may act by some feedback mechanism to control the amount of squalene formed by the cells.

It appears that recognizable mitochondrial profiles are formed only by anaerobically grown cells provided that ergosterol and unsaturated fatty acids are freely available to the cell, and, in this regard, the effect of catabolite repression on the lipids of the cells is informative. The cells cultured anaerobically on the YE-G + Tween 80 + ergosterol medium compared with the corresponding galactose-grown cells have a high glyceride content, but are low in phospholipids and ergosterol. The higher ergosterol levels of the galactose-grown cells appear to relate well to the presence of numerous well defined mitochondrial profiles in these cells compared with the occurrence of a few poorly defined profiles in the glucose-grown cells. When it is realized that the ergosterol content of isolated yeast mitochondria is about 50 μg per mg of mitochondrial protein (Jollow, unpublished observations), it is perhaps not surprising that mitochondrial profiles have not been positively identified in cells grown anaerobically on YE-G medium. Such cells contain only a fiftieth of the levels of ergos-

terol found in aerobic cells and about one-twentieth of the levels detected in the anaerobic galactose-grown cells; so the amount of ergosterol in the YE-G cells allows little scope for the formation of mitochondrial profiles.

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