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# Lipids of Yeasts

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## INTRODUCTION

The earlier literature on yeast lipids has been reviewed by Hunter and Rose (127). More recently much research has been undertaken in this area of yeast biochemistry particularly in the fields of membranology of mitochondria (174, 175), the possible commercial production of fat (246), and the control of opportunistic organisms (104). The major purpose of this review has been to describe and evaluate the present state of knowledge of yeast lipid biochemistry. In particular the importance of various factors influencing the cellular composition and distribution has been discussed.

## CELLULAR LIPIDS

### Extraction

Yeast lipids have been defined in two operational forms dependent upon the ease of extraction. The "readily extracted" lipids are generally best recovered (218, 232, 298) using a neutral two-solvent extractant of chloroform-methanol (35, 81); "bound" lipids require an acidified extractant to affect their release (5). A "bound" fatty acid fraction can be recovered by methanolic potassium hydroxide treatment of cells previously extracted with chloroform-methanol (255). Acid (2) and base (3, 254) hydrolysis may be required to permit subsequent total extraction of the various bound forms of yeast sterols. Incomplete recovery of polar phospholipids may result unless there is sufficient water in the extracting medium to produce a two-phase system (224). Generally polyphosphoinositides are best extracted with an acidified solvent system (251), although Steiner and Lester (282) have employed a basic system of ethanol-water-diethyl ether-pyridine (15:15:15:1) in the isolation of di- and triphosphoinositides from yeast.

Lipid is most efficiently recovered from freeze-dried or freeze-thawed yeast cells (275) and this procedure is commonly employed. Mechanical disintegration of the cell (170) and drying yeast at moderate temperatures (171) also enhance lipid extraction. Particular care must be taken to avoid possible enzymic decomposition of the phospholipid components during the operative steps (109, 316). Activation of membrane-bound phospholipases may also arise on treatment of cells with acetone or aqueous ethanol, particularly at room temperature (171). A convenient procedure for lipid extraction has been proposed by Letters (171) involving the heating of yeast cells with 80% ethanol at 80 C for 15 min to deactivate enzymes and split lipid-protein linkages. The yeast residue is then extracted with chloroform-methanol (2:1, vol/vol), the extract is concentrated, and the lipids are solubilized in chloroform. Failure to purify the initial extract by washing with water and re-extracting can result in falsely high lipid values (126). Thus variations in reported total lipid values may be related to the conditions of extraction. The most reproducible results have been obtained after hydrolysis and recovery of the total fatty acid component which can then be used as an index of total lipid (248).

The total lipid content of yeast cells is subject to a variety of influences. However, it is possible to group the various yeasts, based on a low, medium, or high lipid content (Table 1). This loose classification cannot be accorded taxonomic significance as there are obvious overlaps; thus members of the genus *Candida* occur in all three groups.

### Subcellular Distribution

Various membranous structures have been recognized (184) in yeast and have been assumed by Hunter and Rose (127) to contain the bulk of the cellular lipids. In general, unfractionated membranous systems possess a lipid:protein ratio of approximately 1 (88, 163, 176). However, more detailed studies require the availability of procedures for the isolation and separation of these systems in a high degree of purity.

**Cytoplasmic (plasma) membrane.** Methods for the isolation and identification of yeast cytoplasmic membrane have been discussed by Schibeci et al. (262, 263). Two general approaches have been employed (Fig. 1). One method involves the conversion of cells to protoplasts by enzymic digestion of the cell wall and recovery of a total membrane fraction after osmotic lysis. This procedure has been employed with *Saccharomyces cerevisiae* (17, 37, 176, 262) and *Candida utilis* (88). Isolation of the plasma membrane can then be achieved by isopycnic centrifugation (262). Treatment of protoplasts with lipase and various phospholipases (211) produced no observable morphological changes. Thus consideration that the enzymic action may be detrimental to the membrane integrity has not yet been substantiated. The second procedure for plasma membrane preparation involves the mechanical disintegration of the cell and the recovery of the cell envelope (187, 291, 292), i.e., wall plus plasma membrane. A plasma membrane-enriched fraction can then be obtained by differential centrifugation either before (187) or after removal of the cell wall fraction by enzymic digestion (291). However, the second procedure has been found (68)

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TABLE 1. *Total cell lipid of various strains of yeasts*[a]

| Lipid (% cell dry wt) | Strain | Reference |
|---|---|---|
| Low (<5%) | *Candida albicans* | 214 |
| | *C. lipolytica* | 201 |
| | *C. utilis* | 61, 311 |
| | *Lipomyces starkeyi* | 58 |
| | *Rhodotorula glutinis* | 311 |
| | *Saccharomyces fragilis* | 216 |
| Medium (5 to 15%) | *Blastomyces dermatitidis* | 65 |
| | *C. lipolytica* | 51, 147, 148, 218, 311 |
| | *C. scottii* | 147 |
| | *C. tropicalis* | 201, 311, 312 |
| | *Debaromyces hansenii* | 195 |
| | *Endomycopsis vernalis* | 343 |
| | *Hanseniasporo valbyensis* | 112 |
| | *Hansenula anomala* | 311 |
| | *Histoplasma capsulatum* | 66, 213 |
| | *L. starkeyi* | 58 |
| | *Mucor rouxii* | 255 |
| | *Pullularia pullulans* | 194 |
| | *R. glutinis* | 147 |
| | *R. graminis* | 311 |
| | *S. carlsbergensis* | 225, 267 |
| | *S. cerevisiae* | 44, 128, 140, 170, 176 |
| High (>15%) | *B. dermatitidis* | 5 |
| | *Candida 107* | 311 |
| | *Cryptococcus terricolus* | 232 |
| | *E. vernalis* | 343 |
| | *H. capsulatum* | 5 |
| | *H. duboisii* | 5 |
| | *L. lipofer* | 189 |
| | *L. starkeyi* | 58, 278, 295 |
| | *R. glutinis* | 352 |
| | *R. gracilis* | 78 |
| | *R. graminis* | 110 |
| | *S. cerevisiae* | 48 |
| | *Trigonopsis variabilis* | 266 |

[a] Cell cultivation under different conditions; lipid extraction generally involved the treatment of freeze-dried cells with a neutral chloroform:methanol system.

to produce a high degree of contamination due to entrapment of intracellular components.

The importance of lipids in the yeast plasma membrane structure has been evaluated by Kopp (161) in the light of ultrastructure studies.

**Intracytoplasmic elements.** Ultracentrifugal methods, applied to yeast, have permitted the isolation of nuclei (252), vacuoles (121, 131, 188, 317), "promitochondria" (57, 260), mitochondria (1, 15, 26, 49), and the inner and outer mitochondrial membranes (1, 15, 26). Particles which are almost wholly lipid in composition, containing 90 to 95% of the total weight as lipid, have been isolated from *S. cerevisiae* (52).

**Cell wall.** A cell wall preparation is generally obtained (67, 143, 256) after rupture of the cell and removal of the intracytoplasmic components and plasma membrane by centrifugation and frequent washings. The possibility of contamination by particles of lipid-rich plasma membrane should always be considered. A lipid content of 3 to 10% of the cell wall dry weight has been reported (180). A significant quantity of the cell wall lipid occurs in a bound form except in the notable cases of *Candida albicans* and *S. cerevisiae* (Table 2). Kidby and Davies (155) have discussed the biological activity of the cell wall in terms of a proposed structure, but the role of the lipid component has not yet been resolved.

## LIPID COMPONENTS

### Fatty Acids

**Chemical nature.** Significant amounts (approximately 5% of total lipid) of free fatty acid have been found in *S. cerevisiae* (44, 128), *Lipomyces starkeyi* (295), *Candida tropicalis* (102) and various other members of the genus *Can-*

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*dida* (311). However, free fatty acid can also arise by the enzymic hydrolysis of cellular phospholipids during the extraction procedure (316).

A typical lipid composition for yeast (Fig. 2) would be anticipated to have a total fatty acid content of 70 to 90%. In certain cases, however, the total fatty acid content of *S. cerevisiae* (140; unpublished findings from our laboratory), *Candida lipolytica* (218), and *Hansenula valbyensis* (112) has been found to account for approximately 40% of the total lipid extract. Several factors have been examined to account for these apparent discrepancies in fatty acid composition. Investigations of possible nonlipid contamination of the extract (112), the presence of high levels of acid-labile plasmalogens (140) or short-chain acids (unpublished findings from our laboratory) have not provided a satisfactory explanation.

FIG. 1. *Yeast plasma membrane preparation from protoplasts (Method A, see reference 37) and spheroplasts (Method B, see reference 291).*

FIG. 2. *General lipid composition determined for S. cerevisiae (44, 128, 138), S. carlsbergensis (225, 267), C. lipolytica (51, 311), and C. tropicalis (102, 311). Vertical bars represent range of observed values.*

In general, the fatty acid composition of yeast lipids shows a preponderance of $C_{16}$ and $C_{18}$ acids although a variety of other acids has been observed. A total of 33 acids ranging from $C_8$ to $C_{26}$ has been detected in *S. cerevisiae*, including significant quantities of isoprenoid-type acids (17). The contribution of $C_{20}$ to $C_{30}$ acids, however, has been found (337) to account for only 1 to 2% of the total acid component. A very minor polyethenoid acid component and $C_8$ to $C_{12}$ acids

TABLE 2. *Cell wall lipid of various strains of yeasts*

| Strain | Lipid (as % dry wt) | | Reference |
|---|---|---|---|
| | Readily extractable | Bound | |
| *Blastomyces dermatitidis* | 0.9 | 4.6 | 143 |
| *Candida albicans* | 0.5–1.1 | 0.03–0.6 | 154 |
| | 1.8 | | 32 |
| *Histoplasma capsulatum* | 1.5–2.0 | | 66, 67 |
| *Mucor rouxii* | 0.8–2.8 | 4.3–4.9 | 22; S. Safe, personal communication |
| | 8.5[a] | 1.8[a] | S. Safe, personal communication |
| *Nadsonia elongata* | 11 | | 72 |
| *Paracoccidioides brasiliensis* | 1.1–1.6 | 6.7–9.5 | 143 |
| *Saccharomyces cerevisiae* | 9.4 | 0.8 | 154 |
| | 2.9–9.0 | | 48, 191 |
| *Trigonopsis variabilis* | | | |
| Ellipsoidal | 0.7 | 4.7 | 266 |
| Triangular | 2.0 | 6.7 | 266 |

[a] Filamentous, non-yeast-like form of organism obtained on growth under aerobic conditions.

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have a greater occurrence in brewer's as compared to baker's yeast (289). Long-chain 2-hydroxy acids, particularly 2-OH-$C_{26}$, are primarily associated with the neutral glycolipid of the cell envelope (217). The fatty acid composition of the lipid of cell wall has been noted to be similar to that of cell envelopes in *S. cerevisiae* (217, 290) and that of cell sap in *Blastomyces dermatitidis* and *Histoplasma capsulatum* (67). In the case of *Nadsonia elongata* the cell wall is characterized by a high content of saturated fatty acids (72).

Yeasts generally abound in unsaturated fatty acids, 18:1 being a major component. The polyunsaturated acids, 18:2 and 18:3, are usually associated with specific yeast strains (136). Characterization of these acids can be achieved by gas chromatography of the products of oxidative degradation (e.g., 201), but gas chromatography-mass spectrometry (200, 208) is to be preferred. Thus, oleic acid (*cis*-octadec-9-enoic acid) has been determined to be the 18:1 component in *C. tropicalis* (201), *L. starkeyi* (297), and *Mucor rouxii* (257). Only very low quantities of vaccenic acid (*cis*-octadec-11-enoic acid) (189, 274) and traces of cyclopropane fatty acids (148), both of which are major fatty acid components of many bacterial lipids (146), have been observed in yeast. A major acid present in *C. lipolytica* and *C. tropicalis* grown on certain *n*-alkanes has been identified as 17:1 (201, 311).

**Mutant strains.** Two general classes of *S. cerevisiae* mutants are available in which the capacity to synthesize either saturated or unsaturated fatty acid is impaired. Mutant strains deficient in $\Delta^9$-desaturase activity (152, 153) have proved to be particularly useful in studying the unsaturated fatty acid requirements of yeasts and their function in the structure of membranous systems. Several saturated fatty acid-requiring mutants (118, 264) possess an apparently inactive fatty acid synthetase system. The temperature-sensitive mutant LK181 (197) has an intact fatty acid synthetase but requires the additions of $C_{12}$ to $C_{14}$ acids for growth at 37 C. This requirement was attributed to a possible allosteric effect of these particular acids on an altered cellular protein. Certain mutants auxotrophic for saturated fatty acid (117) have been found to be capable of growth in the absence of the required acid when the synthesis of protein is depressed. This conditional relief of auxotrophy is perhaps attributable to the relief of the imbalance in the specific fatty acids and proteins required for normal membrane synthesis.

**Biosynthesis.** The pathways for fatty acid synthesis in yeast have been documented (127), but the mechanics of regulation are less well defined. The initial step of de novo fatty acid biosynthesis involving acetyl coenzyme A (CoA) carboxylase (EC 6.4.1.2) has been suggested (92, 93, 288) as being under negative feedback control by long-chain fatty acyl CoA. This influence is in turn governed by the extent of fatty acyl CoA incorporation into membranous systems (288). Reduction of the cellular content of acetyl CoA carboxylase has been noted (142) in the presence of long-chain fatty acids and may assume particular significance in the observed (201) inhibition of fatty acid biosynthesis by higher odd-chain fatty acid. Evidence has been obtained for the partial repression of the fatty acid synthetase complex in *Candida* 107 growing on *n*-alkanes (93). The capacity of acetyl CoA synthetase (EC 6.2.1.1) to form CoA esters of short-chain acids in *S. cerevisiae* grown under aerobic conditions is inhibited markedly by long-chain fatty acyl CoA (259). The conclusion was reached by Satyanarayana and Klein (259) that different proteins are involved in the synthetase activity in aerobic and anaerobic cells. Studies made on *C. tropicalis* grown on *n*-tetradecane have shown (168) the existence of four different types of acyl CoA synthetase having specific substrate requirement and intracellular location.

Fatty acid synthesis has been inferred to occur by either of two mechanisms in studies on yeast growing on various *n*-alkanes (201). Odd-chain fatty acids were observed to arise from elongation of odd-chain fatty acid precursors while even-chain fatty acids arose by de novo synthesis. The occurrence of a similar elongation system has also been recognized (221) in a mutant of *S. cerevisiae* that showed a capacity to synthesize higher acids from $C_{13}$ to $C_{17}$ acid supplements, but which was incapable of de novo fatty acid synthesis.

Two general processes for the formation of unsaturated fatty acids have been discussed by Erwin (79). Introduction of unsaturation into yeast fatty acid, however, would appear to be restricted to oxygen-dependent desaturase systems. Thus not only is the content of unsaturated fatty acids in *S. cerevisiae* (42, 140) decreased under anaerobic conditions, but the 18:1 acid component in a variety of yeasts (189, 201, 257, 299) has been established as being *cis*-octadec-9-enoic acid. The presence of the alternate anaerobic pathway for the introduction of unsaturation would be anticipated (79) under these conditions to produce *cis*-octadec-11-enoic acid as occurs in certain bacteria. Only limited occurrence of vaccenic acid has been noted in yeast (189, 274), although *Schizosaccharomyces*

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*japonicus* appears to utilize an anaerobic pathway for the formation of unsaturated acids (42). Polyunsaturation of $C_{18}$ acid seems to be species specific and is primarily associated with petite-negative yeasts (42, 136).

**Biological significance.** Fatty acid composition appears to be a most important variable in determining membrane morphology (120, 133). Considerable information on the importance of specific fatty acids has been derived by Keith and his co-workers (153) from studies on the growth requirements of desaturase mutants of *S. cerevisiae*. Saturated fatty acids $C_5$ to $C_{20}$ (19, 74, 241) and elaidic acid (74, 241) were determined to be ineffective, whereas *trans*-hexadec-9-enoic acid and *trans*-octadeca-9,12-dienoic acid supported some growth. Marked growth-promoting activity, however, has been found (341, 342) to be associated with fatty acid possessing a *cis*-$\Delta^9$-bond. The extent of growth obtained with various isomeric 20:3 acids (340) would appear to be enhanced by a $\Delta^8$-bond and depressed by a $\Delta^{17}$-bond. Increasing unsaturation of the acid supplement has been shown (19) to increase the cell yield with 22:6, 20:5, and 20:4 producing greatest activity. The biological significance of this finding is not clear since these particular acids are not normal components of *S. cerevisiae*. Decreasing the level of unsaturated fatty acid supplement resulted in a more rapid induction of respiratory incompetent cytoplasmic petite mutants (186). Eletr and Keith (74) concluded from spin-label studies that the occurrence of *cis* double bonds in membrane structures inhibits a relatively ordered packing of alkyl chains in the region between the unsaturated bond and the terminal methyl group.

Unsaturated fatty acid has been found to be required as a component of yeast mitochondrial lipid to permit the coupling of electron transport to phosphorylation (240, 241) and active cation transport (115). In addition the physical state of the lipid component has been deduced by Eletr and Keith (75) to be a factor influencing membrane capacity for oxygen uptake. Arrhenius plots of various membrane-bound enzymes have revealed (4, 113, 134, 331) a dependence of the phase transition point on the nature of the mitochondrial lipid. In particular, unsaturated fatty acid appears to induce a lowering of the energy of activation due to presumed conformational changes in membrane structure in the immediate vicinity of the enzyme. However, a simple correlation between fatty acid composition, lipid physical state, and membrane biological activity may not exist (31, 331). The immobilizing effect of protein (31) on the fluidity of membrane lipids requires further consideration.

**Taxonomy.** Fatty acid composition as a basis for taxonomy of yeast has been considered by Shaw (268) and found to be generally unsatisfactory. Thus, while many members of the *Deuteromycetes* (*Fungi Imperfecti*) including the genus *Candida* are characterized by high amounts of linoleic acid, other species possess a low content of polyethenoid acids. *Ascomycetes*, subclass *Hemiascomycetes* (*Protoascomycetes*) as exemplifed by *S. cerevisiae* are generally regarded as primitive or degenerate and appear to be unable to synthesize polyunsaturated fatty acids (136). The differences existing in fatty acid composition between petite-positive and petite-negative yeasts have been suggested by Bulder and Reinink (42) to be of some taxonomic value in the genus *Saccharomyces*. In all cases, however, variation in growth conditions (79) can markedly influence the nature of the cellular fatty acid component.

### Hydrocarbons and Sterols

The contribution of unsaponifiable matter to the total lipid of yeast ranges from 12% in *Candida scottii* (147) to over 80% in sporulating *S. cerevisiae* (50) and can account for 4 to 25% of the cell dry weight (76). Although quantitative data are not generally available, its composition has been determined to consist primarily of sterol or hydrocarbon depending upon growth conditions (157, 162). Minor quantities of other compounds have been described including carotenoid pigments (269), tocopherol (182), alkanediols, and 1-alkenyl ethers (28). The polyisoprenoid component of *S. cerevisiae* has been implicated (13, 223, 265) in the biosynthesis of the cell wall polysaccharide.

**Hydrocarbons.** The determination of the chemically unreactive hydrocarbons has been facilitated by the application of gas chromatography. Thus the occurrence of over 40 straight- and branched-chain alkanes, ranging from $C_{10}$ to $C_{31}$, has been observed (16) in *Saccharomyces oviformis* and *Saccharomyces ludwigii*. The minor long-chain ($C_{20}$ to $C_{31}$) component was characterized by the presence of 12 members of the *n*-alkane series plus small variable quantities of squalene. Confirmation of the presence of squalene in *S. cerevisiae* has been made by gas chromatography-mass spectrometry (unpublished observations from our laboratory). Squalene has been considered by Lanyi et al. (165) to influence the spacing of lipid molecules in membranous structures and hence membrane permeability characteristics.

**Sterols (i) Chemical nature.** Assay of sterol,

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either gravimetrically as the digitonide complex, or spectrophotometrically as ergosterol, can yield different values for the total sterol content of the yeast cell (176). Furthermore, the determination of specific sterols is a problem and is hampered by the lack of authentic standard compounds. Sole reliance on ultraviolet absorption spectroscopy as a means of identification has been criticized (346), but gas chromatography-mass spectrometry is a satisfactory procedure (334).

The sterol component has been generally determined to range from 0.03 to 4.6% of the yeast cell dry weight (76) accounting for <1 to 10% of the total cell lipid. Yeasts of the genus *Saccharomyces* are particularly rich in sterols (69). Ergosterol has been identified as the major sterol in *S. cerevisiae* (6, 25, 76, 101), *Kluyveromyces fragilis* (234), and *C. albicans* (43), and can account for over 90% of the total sterol. Strain differences can, however, exist as shown by the major occurrence of ergosta-5,7,22,24(28)-tetraen-3β-ol in *S. cerevisiae* N.C.Y.C. 366 (128, 176) and a variety of sterols in several mutant strains Table 3).

Sterols occur both unesterified or esterified to fatty acids characterized by a high content of 18:1, 16:1 and, in certain genera, 18:2 acids (17, 67, 128, 176, 181, 225). Esterified derivatives of various sterol biosynthetic intermediates have been observed in *S. cerevisiae* (228). The bulk of the sterol component in *S. cerevisiae* has been determined to be esterified (128). A significant quantity of sterol occurs in a tightly bound form of unknown nature in *S. cerevisiae* (2, 3) and accounts for 75% of the total sterol in the yeast-

TABLE 3. *Sterol components and presumed enzyme lesion in selected mutant strains of S. cerevisiae*

| Mutant | Sterol occurrence | Metabolic lesion | Reference |
|---|---|---|---|
| *olerg* (growth requirements for fatty acid + ergosterol) | ? | ? | 144 |
| *erg* (growth requirement for ergosterol) | | Conversion of squalene to lanosterol | 145 |
| Ergosterol-deficient | Lanosterol<br>4,14-Dimethylcholesta-8,24-dien-3β-ol<br>4,14-Dimethylergosta-8,24 (28)-dien-3β-ol<br>14-Methylergosta-8,24 (28)-dien-3β-ol | C14 demethylase | 314 |
| *pol* 1 (*nys* 1) (polyene [nystatin]-resistant) | Zymosterol<br>Cholesta-5,7,24-trien-3β-ol<br>Cholesta-5,7,22,24-tetraen-3β-ol | C24 methyl transferase | 24 |
| *pol* 2 (polyene resistant) | Ergost-8-en-3β-ol<br>Ergosta-8,22-dien-3β-ol<br>Ergosta-5,8,22-trien-3β-ol<br>Fecosterol | $\Delta^8$:$\Delta^7$ Isomerase | 24 |
| *pol* 3 (*nys* 3) | Ergosta-7,22-dien-3β-ol<br>Ergosta-8,22-dien-3β-ol<br>Ergosta-7,22,24 (28)-trien-3β-ol<br>Ergosta-8,22,24 (28)-trien-3β-ol<br>Episterol<br>Fecosterol | 5,6-Dehydrogenase | 24, 229 |
| *pol* 5 | Episterol<br>Ergosta-5,7-dien-3β-ol<br>Ergosta-5,7,24 (28)-trien-3β-ol<br>Ergosta-8,14,24 (28)-trien-3β-ol | 22,23-Dehydrogenase | 24 |

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like cells of *M. rouxii* (254). Acid-labile steryl derivatives, presumably including steryl glycosides, have been observed in *S. cerevisiae* (2, 17).

**(ii) Mutant strains.** Various mutant strains of *S. cerevisiae* have been employed (Table 3) in the elucidation of the pathways of sterol biosynthesis. Particular use has been made of mutants resistant to polyene antibiotics. Details on the particular genetic lesions are generally lacking but the information available on the presumed affected enzyme systems indicates close metabolic relatedness (85, 203). The nature of the sterol component resulting from a defect in a particular synthetic enzyme system is summarized in Table 3.

**(iii) Biosynthesis.** All the sterols named in Fig. 3 have been isolated from various *S. cerevisiae* strains and have been considered as possible biosynthetic intermediates by Barton and his co-workers (23–25). The efficiency with which cell-free extracts have been able to con-

FIG. 3. *Possible pathways of metabolic conversion of sterols in yeast; named compounds have been isolated from S. cerevisiae (25, 84, 333).*

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vert these postulated intermediates has been examined and the available details on some of the proposed enzyme systems have been reviewed by Weete (333). Information, however, on the sequence of action of these enzymes is generally lacking, but evidence for a multiplicity of sterol biosynthetic pathways in yeast has been presented (84).

The initial step of lanosterol conversion involves the removal of the two methyl groups at position 4 and the methyl group at position 14. Inability to carry out the demethylation at position 14 has been found (314) to be accompanied by the accumulation of 4,14-dimethyl sterol derivatives. This finding suggests that the other 4-methyl group is subject to earlier removal. Sterols carrying a methyl group at position 4 undergo little methylation at position 24 (204, 228). *S*-adenosylmethionine:$\Delta^{24}$ sterol methyl transferase (C24 methyl transferase), involved in the methylenation process, apparently shows greatest substrate specificity for cholesta-7,24-dien-3$\beta$-ol (116) although zymosterol (204) is also used. This enzyme activity has been found to be primarily located in the promitochondria or mitochondria of *S. cerevisiae* (304) and to be enhanced by the presence of a fermentable carbon source and molecular oxygen (279). Formation of cholesta-7,24-dien-3$\beta$-ol from zymosterol would require the activity of $\Delta^8$:$\Delta^7$ isomerase (24).

Several pathways possibly exist for the enzymic conversion of the two C24 methylene sterols, fecosterol and episterol. The molecular events presumably involve a 5,6-dehydrogenase for the introduction of additional unsaturation in ring B, a 22,23-dehydrogenase for the dehydrogenation of the side chain and a methylene reductase for the reduction of the methylene group at position 24. The absence of any of these three systems would be anticipated to result in the accumulation of different sterols, whereas the presence of all three systems would permit the formation of ergosterol. The relationship between the availability of a particular reaction mechanism in vivo and sterol product is given in Table 4. A major biosynthetic pathway from episterol to ergosterol has been deduced (83, 84) as involving the sequential introduction of unsaturation at position 22,23 and then at position 5,6 followed by the reduction of the methylene group at position 24.

Additional knowledge on the characterisitics of the enzyme systems involved in sterol biosynthesis in yeast should provide a better understanding of the mechanisms controlling the

TABLE 4. *Relationship between the occurrence of anticipated enzyme systems and products of reaction during sterol biosynthesis in yeast*

| Reaction system | | Sterol product |
|---|---|---|
| Present | Absent | |
| | 5,6-Dehydrogenase<br>22,23-Dehydrogenase<br>Methylene reductase | Ergost-7,24(28)dien-3$\beta$-ol (episterol) |
| 5,6-Dehydrogenase | 22,23-Dehydrogenase<br>Methylene reductase | Ergosta-5,7,24(28)trien-3$\beta$-ol |
| 22,23-Dehydrogenase | 5,6-Dehydrogenase<br>Methylene reductase | Ergosta-7,22,24(28)trien-3$\beta$-ol |
| Methylene reductase | 5,6-Dehydrogenase<br>22,23-Dehydrogenase | Ergost-7-en-3$\beta$-ol |
| 5,6-Dehydrogenase<br>22,23-Dehydrogenase | Methylene reductase | Ergosta-5,7,22,24(28)tetraen-3$\beta$-ol |
| 5,6-Dehydrogenase<br>Methylene reductase | 22,23-Dehydrogenase | Ergosta-5,7-dien-3$\beta$-ol |
| 22,23-Dehydrogenase<br>Methylene reductase | 5,6-Dehydrogenase | Ergosta-7,22-dien-3$\beta$-ol |
| 5,6-Dehydrogenase<br>22,23-Dehydrogenase<br>Methylene reductase | | Ergosterol |

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process. The activity of hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34) participating in the initial biosynthetic step, of mevalonate formation, has been considered (150, 151) to be under feedback control of ergosterol or some acidic products of ergosterol metabolism. Control of sterol biosynthesis may occur (204) at the reaction step catalyzed by *S*-adenosylmethionine:$\Delta^{24}$ sterol methyl transferase which has been suggested (307) to be competitively inhibited and possibly repressed by ergosterol. Studies (345) made on three strains of *S. cerevisiae* have revealed that the conversion of ergosta-5,7,22,24(28)-tetraen-3$\beta$-ol to ergosterol is subject to differing metabolic controls. The existence of several biosynthetic routes for ergosterol suggests the possibility of several regulatory mechanisms.

Various factors influencing the synthesis of sterol by yeast have been discussed by Hamilton-Miller (106). In particular, the composition of the growth medium (77, 128) and stage of the growth cycle (44, 76) have been noted to have an effect on the amount of sterol produced. The availability of oxygen requires special attention as it governs both the type of sterol synthesized (59, 60, 256) and also the quantity of sterol. The lower levels of ergosterol occurring under anaerobic conditions in *S. cerevisiae* (84, 140, 309) and the yeast-like form of *Mucor genevenis* (99) have been reported to be accompanied by increased amounts of squalene. The apparent accumulation of this hydrocarbon reflects, in part at least, the observed sensitivity to lack of oxygen of several reaction steps including oxidative cyclization of squalene (compare reference 206), oxidative demethylation (199, 206), oxidative desaturation of ring B (313), and methylation at position 24 (204). In certain mutants of *S. cerevisiae* the inability to form ergosterol has been traced (21) to lesions in the biosynthesis of porphyrins rather than sterols and indicates the probable importance of an active respiratory chain in the conversion of squalene to ergosterol.

**(iv) Biological significance.** Both free and esterified sterol have been detected in various membranous systems of *S. cerevisiae* (249), although the suggestion has been made by Rose and his co-workers (125, 128) that steryl esters may be preferentially concentrated in certain intracellular structures rather than the plasma membrane. Significant quantities of free and esterified sterol occur in the cell wall of *C. albicans* (32), *B. dermatitidis* (67), and *H. capsulatum* (67). Although the cell wall of *S. cerevisiae* has been found (17, 217) to be deficient in sterol, its mannan component possesses a general capacity to bind sterol in vitro (305).

The major function of sterol in yeast has been regarded by Proudlock et al. (242) to be one of structural influence upon the dynamic state of membranes. Comparison may therefore be made with the observed biological roles of cholesterol in influencing phospholipid-protein interactions, membrane permeability and membrane-bound enzyme activity in animal systems (227). The specificity for $C_{28}$ sterols in yeast does not appear to be absolute, since certain mutants of *S. cerevisiae* (94, 144, 145) or wild-type *S. cerevisiae* growing anaerobically (97, 242) can have the growth requirement for ergosterol met by supplementation with various $C_{27}$ or $C_{29}$ sterols. The chemical characteristics associated with functional membrane sterols have been reviewed by Nes (212), but a major requirement is that the molecule possesses a highly planar nature (242). Absence of compounds with the required steroid conformation would be expected to produce a functionally impaired membrane (212).

Ergosterol has been implicated (258) as a component of a particular membranous system concerned with the initiation of cell division in *C. albicans*. The capacity for the sterol-induced recovery from the damaging effects of ultraviolet irradiation is considered (258) to be species specific. Decrease in the total sterol content of yeast mitochondria has been associated with a depression in oligomycin sensitivity (299) and an increase in the temperature of phase transition (54) of adenosine 5'-triphosphatase activity. Replacement of ergosterol by the less planar ergosta-8(9),22-dien-3$\beta$-ol in certain mutant strains of *S. cerevisae* resulted in a lowered transition temperature for mitochondrial *S*-adenosylmethionine-$\Delta^{24}$ sterol methyl transferase and cytochrome oxidase (308). This effect was attibuted to a less efficient packing of membrane phospholipids. Although ergosterol has been found (306) to be intimately associated with yeast cytochrome oxidase, it is considered nonessential for enzyme activity. Thus Thompson and Parks (306) have suggested that various lipid combinations may provide suitable environments for enzyme activity.

Interaction of membrane-bound sterol with the polyene macrolide antibiotics (104, 105, 106, 164) has stimulated much interest both as a possible means of controlling opportunistic yeast (104) and as an experimental system for studying the function of sterol in membranes (106). Yeasts containing ergosterol appear to be especially sensitive to the action of polyene antibiotics, whereas a low sterol content is associated with resistance (20, 41). Variable yeast

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sensitivity to polyenes has been correlated with differences in the sterol composition of several strains of *Candida* (12, 103, 105, 346) and *S. cerevisiae* (20, 85, 203, 309, 344). Fryberg et al. (85) have concluded that polyene affinity is greatest with $\Delta^{5,7}$-sterols, e.g., ergosterol, and lowest with the less planar $\Delta^{8,24}$-sterol, namely, fecosterol. Impaired access of polyene antibiotic to sterol located in the cell surface membrane has been considered (231) in explanation of resistance, but the chemical nature of the sterol component is believed (105) to be more important. A major consequence of nystatin action is the leakage of the bulk of the intracellular $K^+$ from *C. albicans* (107) and *S. cerevisiae* (319). These findings endorse the concept of membrane sterol being associated with permeability factors.

## Glycerophospholipids

**Chemical nature.** Most yeast species have a phospholipid content of 3.0 to 7.0% of the cell dry weight (171). The various phospholipids and their abbreviated names are shown in Fig. 4. A representative distribution (as the percentage of total phospholipid) of 35 to 55% phosphophatidyl choline (PC), 20 to 32% phosphatidyl ethanolamine (PE), 9 to 22% phosphatidyl inositol (PI), 4 to 18% phosphatidyl serine (PS), and <10% minor components has been described by Letters (171). The minor phospholipids include various lysoderivatives, phosphatidyl glycerol (PG), phosphatidyl glycerol phosphate (PGP), diphosphatidyl glycerol (DPG), phosphatidic acid (PA), phosphatidyl monomethylaminoethanol, phosphatidyl dimethylaminoethanol, diphosphoinositol (DPI), and triphosphoinositol (TPI) (90, 132, 281). The relatively high values of 11% and 8% reported (132) for lysophosphatidyl choline (LPC) and lysophosphatidyl ethanolamine (LPE), respectively, in *S. cerevisiae* may represent artifact formation during isolation. Polyphosphoinositides are not frequently encountered in yeast, but the occurrence of PI-DPI-TPI in molar ratios of 140:4:1 has been observed in *S. cerevisiae* (169, 282). Comparable very low levels of DPI have been noted (239) in *Kloeckera brevis*. Specific examination for the presence of plasmalogens in *S. cerevisiae* (140) and *Lipomyces lipofer* (189) have revealed only trace quantities.

Most phospholipids are characterized by a high level of unsaturated fatty acid which is replaced to a significant extent by 16:0 acid in the PS-PI component of *C. tropicalis* (64), *S. cerevisiae* (17, 176, 290), *Schizosaccharomyces pombe* (339), *B. dermatitidis* (67), and *H. capsulatum* (67). Whereas the PS component of *L. starkeyi* has been determined (295) to be almost entirely 1-palmitoyl, 2-oleoyl-*sn*-glycero-3-phosphoryl-L-serine, the PC and PE components are composed of several molecular species. The nature of the molecular species of PC, however,

FIG. 4. *Possible pathways of metabolic conversion of glycerophospholipids in yeast. All species have been found in S. cerevisiae (see reference 281). ADP, Adenosine 5'-diphosphate; CTP, cytidine 5'-triphosphate; and PP, pyrophosphate.*

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can vary depending on the particular biosynthetic pathway involved (325).

**Biosynthesis.** The possible pathways of biosynthesis and metabolic interrelationships of the various phospholipids in the eukaryotic cell have been discussed by Mangnall and Getz (183) and are summarized in Fig. 4. The formation of PA in *S. pombe* (339) and *S. cerevisiae* (281, 339) has been determined to involve the specific acylation of *sn*-glycero-3-phosphoric acid. Cytidine diphosphate (CDP)-diglyceride, which can arise from PA (129, 281), appears to be the major precursor of most phospholipids. Evidence has, however, been obtained for the occurrence of a CDP-nitrogenous base reaction with 1,2-diacyl-glycerol in the formation of PE (281) and PC (53, 324, 325). An alternate mechanism for the formation of PC in *S. cerevisiae* involves the sequential methylation of PE, phosphatidyl monomethylaminoethanol, and phosphatidyl dimethylaminoethanol, but the process is depressed in the presence of choline (325). Synthesis of PE can also involve the decarboxylation of PS (281). Reaction of CDP-diglyceride with serine and inositol has been implicated (281) in the synthesis of PS and PI, respectively, although an alternate but undefined mechanism has been considered (339) to be of major importance for PI formation in *S. pombe*. DPG synthesis in *S. cerevisiae* has been found (53) to involve reaction of CDP-diglyceride with PG, although other possible routes for polyglycerophospholipids have been discussed (276). An exchange of the alcohol unit between the various phosphatidyl derivatives would allow a rapid interchange of phospholipid types. Steiner and Lester (281) have shown that there is interconversion (via CDP-diglyceride) between endogenous PI and PS (and thence PE as well).

A detailed study by Cobon et al. (53) has shown that yeast microsomal fractions contain the two general pathways for PC synthesis (Fig. 4), as well as the systems for the formation of PA, PS, and PI. The synthesis of DPG is restricted to the mitochondria which also contain enzyme systems for the formation of PE and PG. Mitochondria of yeast apparently differ from those of animal cells in possessing a limited ability to synthesize the acidic phospholipids PA, PS, and PI. Phosphatidylinositol kinase activity, responsible for the conversion of PI to DPI, has been suggested by Wheeler et al. (338) to be primarily associated with the plasma membrane in *S. cerevisiae*. Ready solubilization of this enzyme, however, may occur (302).

The mechanisms for modification of the fatty acid composition of glycerophospholipids apparently involve two general processes. Direct desaturation of intact PC has been observed in *Torulopsis utilis* (300) and *C. lipolytica* (243). The deacylation-reacylation process is believed (296) to be involved in the metabolic turnover of PE but not PS in *L. starkeyi*. The metabolic significance of the specific deacylation of PI occurring at the cell surface accompanied by the secretion of glyceryl phosphoryl inositol (11) awaits further explanation.

**Cellular distribution.** The glycerophospholipid content (as percentage of total lipid) of the cell wall has been determined to range from zero in *S. cerevisiae* (17, 217) to 15% in *Trigonopsis variabilis* (266) and 39% in *C. albicans* (32). Although slight differences have been noted (132, 249) in the intracellular distribution of the major phospholipids in *S. cerevisiae*, specific localization of certain minor components may exist. Thus DPG is primarily associated with yeast mitochondria although this occurrence is not absolute (53). Increasing quantities of DPG have been taken (132) as an index of the development state of mitochondria. Fully developed mitochondria have also been found (226) to possess lower PI values than promitochondria. Mitochondriogenesis, in general, is accompanied by a high turnover of PE and PS (135).

**Biological significance.** The relative constancy of the total phospholipid component in yeast is suggestive of some primary function. Phospholipids have been considered (140) to be major structural components of yeast membranes in which a preferential concentration of unsaturated fatty acid is of importance. Mitochondria depleted of unsaturated fatty acid, and consequently depressed in oxidative phosphorylation capacity, have been noted (114) to have unchanged quantities of total phospholipid but a lower content of PI. The precise importance of specific phospholipids in yeast membranous systems has not been established, but is presumably comparable to that in membranes of high organisms (190). A cyclic turnover of polyphosphoinositides involving PI → [DPI and TPI] → PI, accompanied by an alteration in adenylate energy charge has been suggested (301) as possibly facilitating the diffusion of ions across membranes in *S. cerevisiae*. Hydrolysis of PGP to PG has been considered (63) to be important in the active transport of D-galactose and amino acids. Variations between phospholipid levels and enzyme activities in yeast are not believed (30) to be linked by any causal relationship. Evidence is available, however, for the protective action of phospholipid against oligomycin action on yeast mitochondrial adenosine 5′-triphosphatase (56, 273). Sub-

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stantiation has not been obtained for a possible correlation between the total cellular phospholipid component in *B. dermatitidis* (65) and *H. capsulatum* (213) and virulent properties toward mice.

### Sphingolipids

Free phytosphingosine bases containing 18 C atoms and 20 C atoms in the ratio of 9:1 have been found (156) to account for 2 to 3% of the total lipid of *Candida intermedia*, whereas fully and partially acetylated derivatives of phytosphingosine and dihydrosphingosine have been reported (100, 286, 336) in *Hansenula ciferrii*. More generally, however, sphingosines occur as integral units of several yeast lipids ranging in structure from the relatively simple cerebrins to the complex glyco- and inositol phosphoryl sphingolipids. A variety of sphingosines, dihydrosphingosines, and phytosphingosines have been determined as components of sphingolipids in *Torulopsis utilis* (277), *C. utilis* (327), and *S. cerevisiae* (315, 328). The mechanisms for possible metabolic interrelationships between the various sphingolipids have been discussed by Gatt and Barenholz (89).

**Chemical nature.** Mixtures of ceramides (cerebrins) consisting of various phytosphingosine and dihydrosphingosine derivatives have been identified in *T. utilis* (277). The material was characterized by the presence of $C_{18}$ to $C_{26}$ saturated and $\alpha$-hydroxy acids with 2-hydroxyhexacosanoic acid predominating. The various cerebrins isolated from *S. cerevisiae* contained *n*-hexacosanoic acid, 2-hydroxyhexacosanoic acid and (+)erthyro-2,3-dihydroxyhexacosanoic acid accompanied by minor quantities of $C_{24}$ to $C_{27}$ saturated unbranched 2,3-dihydroxy acids (123, 238, 335).

Simple cerebroside-type lipids (*O*-glycosyl ceramide) are generally present in only trace quantities but consist primarily of a sphingosine base, 2-hydroxyoctadecanoic acid, and D-galactose as isolated from *S. cerevisiae* (328) and *C. utilis* (327). High levels of hexacosanoic and 2-hydroxyhexacosanoic acids characterized a glycosphingolipid present in the cell envelope of baker's yeast (217). Some species of *Torulopsis, Candida, Cryptococcus,* and *Rhodotorula* are highly efficient in the production of glycolipids which are subsequently excreted into the medium (285).

Several inositol phosphoryl sphingolipids are present (272, 283) in *S. cerevisiae* including mannosyl di-(inositol phosphoryl) ceramide which has been determined (284) to account for approximately 20% of lipid-soluble inositol. A complex sphingolipid containing inositol, phosphorus, mannose, and high levels of 2-hydroxyhexacosanoic and hexacosanoic acids was determined (217) to be present in the cell wall and plasma membrane of baker's yeast. $C_{18}$-phytosphingosine was determined (315) to be the major and dehydrophytosphingosine and dihydrosphingosine were determined to be the minor long-chain base components. The possible occurrence of other inositol sphingolipids, which are stable to mild alkaline methanolysis, has been considered (283). Four monoinositol phosphorylceramide components have been identified (272) in *S. cerevisiae*. Preliminary studies (10) have shown that phosphosphingolipids have a metabolic origin in PI.

**Biological significance.** The role of sphingolipids in yeast is unknown. It has been suggested (235) that the presence of acetylated phytosphingosine on the cell surface of *H. ciferrii* may be responsible for the tendency of the yeast to form pellicles in liquid media.

### Glycolipids

In addition to the complex glycosphingolipids small quantities of other glycolipids have been determined in *S. cerevisiae* (17, 28, 217, 315). At least four different acyl glucose derivatives have been noted (38). Glycolipids are characterized by a relative abundance of odd-chain and $>C_{18}$ fatty acids (17, 217). The presence of steryl glycosides, sulfolipids and acyl glucoses has been observed (315) in the cell envelope of *S. cerevisiae*. Steryl glycosides appear to be restricted to the plasma membrane whereas monogalactosyl diglycerides and sulfolipids are distributed throughout the cell (17).

Acyl glucose, which has been noted to occur intracellularly when glucose is present in the growth medium, has been suggested as being involved in the storage or transport of glucose in yeast (38).

## FACTORS INFLUENCING CELLULAR LIPID COMPOSITION

### Growth Cycle

A variety of conditions influence the growth of yeast and must be taken into account in any meaningful assessment of the cell lipid composition. In closed systems, such as batch cultures, the composition of the medium is modified by each successive generation of cells and must ultimately result in unbalanced growth. Steady states, however, can be obtained when the yeast is grown in highly controlled open systems, e.g., the computer-controlled chemostat. The considerable advantages associated with

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the use of synchronous cultures have recently been reviewed by Hartwell (111).

Initial stages of growth of *S. cerevisiae* in batch culture have been found (44) to involve a progressive decrease in total lipid, mainly at the expense of the triglyceride component. Thus the energy requirements of the developing cell would appear to be met, in part at least, by fatty acid oxidation. Increase in lipid production has been noted during the late exponential phase of growth of *S. cerevisiae* (44) and *C. lipolytica* (218, 311). Entrance to the stationary phase, however, results in a rapid decrease in total lipid content due to depletion of the carbon source (61). Cellular senescence arising from carbon source deprivation has been associated (202) with the induction of lipid peroxidase activity. Subsequent peroxidation might be anticipated to produce a lowering of membrane polyethenoid acid content and hence biological activity. The unsaturated fatty acid component of microsomal and supernatant fractions varies with the state of cell growth, ultimately reaching a value of 70% of the total acid in the late exponential phase (45). Increase in cell wall unsaturated fatty acid accompanies the growth of *C. tropicalis* on *n*-alkanes but not glucose (253). A high content of polyunsaturated fatty acid has been determined (311) to be associated with the increased quantity of triglyceride present in *C. lipolytica* entering the stationary phase of growth.

Decreased growth rates, as determined with chemostat cultures of *C. utilis*, have been observed (61, 192) to result in increased quantities of polyunsaturated acids, particularly at the latter stages of growth. In *S. cerevisiae*, growth rate, at least as influenced by temperature, has been found (128) to have no effect on fatty acid composition. Increased synthesis of PC and steryl ester, however, results from lowered growth rate. These general alterations in lipid patterns may reflect differences in the development of intracellular membranous systems. Further studies in this area would be profitable.

## Sporulation

Sporulation in the diploid yeast *S. cerevisiae* is markedly dependent upon the state of cell growth and the composition of the medium (82). The process is accompanied by an increase in cellular lipid (50, 130). Unsaponifiable matter constitutes the major lipid components at the initiation of sporulation, particularly with cells in the exponential phase of growth (50). Sterol can comprise up to 81% of the total lipid and has been ascribed (50) a special role in the induction of sporulation. A correlation has been noted (130) between increasing sterol level and the formation of membranous systems in developing asci. Two main periods of particular lipid synthesis have been observed (119, 130) during sporulation. The initial phase is predominantly associated with the formation of phospholipid and triglyceride which coincides with membrane development. The relative proportions of the various phospholipids remain unaltered (130). Synthesis of primarily neutral lipid occurs in the second phase and coincides with the appearance of mature asci. Abundant lipid granules are apparent in the germinating ascospores but diminish during several cell division cycles in the transition from spore to vegetative cell (280).

Certain hydrocarbon-utilizing ascosporogenous yeasts, e.g., *Endomycopsis lipolytica*, may sporulate as a result of contact between the cells and growth medium alkanes. This process may be a special characterization associated with hydrocarbon utilization (177).

## Carbon Source

In addition to various environmental factors, the nature of the carbon source has a pronounced effect on the quantity and composition of yeast lipid.

**Glucose.** Yeasts growing on glucose as carbon source show various responses which reflect differences in metabolic behavior. A broad categorization into Crabtree-positive (respiratory-deficient) and Crabtree-negative (respiratory-sufficient) yeasts has been suggested by De Deken (62). The Crabtree-positive yeasts, exemplified by many members of the genus *Saccharomyces*, metabolize glucose primarily via glycolysis. On the other hand the Crabtree-negative yeasts generally lack glycolytic enzymes (205) and oxidize glucose via aerobic metabolism in which the pentose phosphate pathway is of major importance (198). The two yeast categories, therefore, show different capacities in the utilization of increasing concentrations of glucose in the growth medium. Thus Crabtree-positive yeasts exhibit diauxic growth in which glucose fermentation and ethanol production occur initially followed by a second phase involving oxidation of the accumulated ethanol (323). Catabolite repression can occur in the Crabtree-positive yeast but is of little significance in Crabtree-negative yeasts growing on media containing less that 10% glucose (73, 271).

The quantities of total lipid, steryl ester, and phospholipid have been found (138) to be decreased in *S. cerevisiae*, a Crabtree-positive

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yeast, as the glucose concentration of the incubation medium was increased from 2 to 10 g/liter. These lipid changes were accompanied by a slight increase in unsaturation of the fatty acid component. The fatty acid pattern of the glucose-repressed cell would appear to be intermediate between those described by Jollow et al. (140) for aerobically and anaerobically grown *S. cerevisiae*. An active synthesis of phospholipid has been noted (135) during glucose repression in *S. cerevisiae*. The process of derepression was accompanied by a drastic reduction in phospholipid biosynthesis. The quantity of total phospholipid, however, remained unaffected, although the PE component was partially replaced by PS. A decrease in the amount of DPG has been noted (39) at very high glucose concentration (50 g/liter), but this effect has been attributed rather to a simultaneous state of anaerobiosis (178).

The Crabtree-negative yeasts, including many members of the genus *Candida*, show a tendency to accumulate lipid when the glucose concentration of the medium is increased (14, 138). High quantities of triglyceride, in excess of 80% of the total lipid, have been observed in glucose-grown *L. starkeyi* (296), and *C. lipolytica*, *C. tropicalis*, *C. utilis*, *Candida* 107, *Hansenula anomala*, *Rhodotorula glutinis*, and *Rhodotorula graminis* (311). The major accumulating triglyceride species in *L. starkeyi*, *Candida* 107, and *R. graminis* has been determined (295, 296, 311) to be saturated-unsaturated-saturated. A slight increase in the quantity of total phospholipid occurs in glucose-grown *L. starkeyi* and involves increased proportions of PC at the expense of PS. The major molecular species of PC and PE have been found to be 1-$C_{16:1}$, 2-$C_{18:1}$ and 1-$C_{18:1}$, 2-$C_{18:2}$ and to be considerably different from those present in cells grown in the absence of glucose. The PS component has been identified as almost entirely 1-palmitoyl - 2 - oleoyl - *sn* - glycero - 3 - phosphoryl - L - serine (295, 296). Crabtree-negative yeasts possess the general capacity to synthesize polyunsaturated fatty acids, especially at high concentrations of glucose and oxygen (14, 138, 296). These acids are primarily associated with the phospholipid component of *L. starkeyi* (295).

**Hydrocarbons.** Considerable attention has been focused on the growth of yeast on byproducts of the petroleum industry. Although the capacity of utilize hydrocarbons is generally nonexistent in *Saccharomyces* and many other yeasts (185, 261), it is an efficient process in certain genera, especially *Candida* and *Torulopsis* (33, 160, 261). Growth preferentially occurs on *n*-alkanes with chain length $C_9$ to $C_{18}$ while *n*-alk-1-enes are used less efficiently and by fewer yeasts (160, 246). Variations in growth response have been noted (236) between two strains of *C. lipolytica* utilizing specific hydrocarbons. In general $C_{10}$ to $C_{18}$ *n*-alkanes show little toxicity toward yeast due to low water solubility (91). The dispersed state, however, of the substrate hydrocarbon influences yeast growth, and is best achieved by use of a Vortex fermenter. Hydrocarbon assimilation appears to require intimate association between yeast cells and efficiently emulsified hydrocarbon and air droplets (34, 36, 96, 330). The growth rate of a *Candida* sp. has been shown (245) to be markedly increased in the presence of high concentrations of *n*-alkanes. Compared to glucose as carbon source, growth of *Candida* 107 on various *n*-alkanes gives a lower yield of cells (311). A lower specific growth rate has also been observed (73) for *C. tropicalis* incubated with *n*-hexadecane compared to glucose. In particular the utilization of hydrocarbons by yeast appears to be more heavily dependent upon an active respiratory system and the availability of oxygen than does the metabolism of glucose (73). The main factor influencing the yield of yeast incubated with hydrocarbon is probably the capacity to utilize the various initial products of metabolism (207).

Utilization of *n*-alkanes by yeast has been determined (95) to be a two-stage process. Alkane metabolism is initially associated with growth and then follows a zero order reaction. The precise mechanism of *n*-alkane uptake has not been established, but alkane-grown cells show a greater capacity to assimilate hydrocarbon than glucose-grown cells (92). Metabolic conversion of up to 25% of the assimilated hydrocarbon can occur (246). Fatty acid is the major product of metabolism although small quantities of *n*-alkanols may also accumulate (33, 158, 160). Pathways of alkane degradation have been observed to occur both in the cytoplasm and the mitochondria (166) and may involve several types of mechanism (160, 166). Alkane oxidation is an adaptive process in *C. lipolytica* (141) but is apparently dependent on both adaptive and constitutive enzyme systems (87, 167, 168). Yeast possesses a limited capacity for the utilization of intracellular fatty acid and may excrete excess free acid into the medium (321). A competition has been observed (27) between hydrocarbon and short-chain ($C_{12}$) fatty acid in the growth of *C. tropicalis*.

Quantitative rather than qualitative differences have been found in the cell lipid of several yeast strains grown on *n*-alkanes (233). Growth of various members of *Candida* on *n*-alkanes

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compared to glucose has been variously reported to result in greater (201, 218, 245) or lower (51, 201, 311) quantities of total lipid. These differences may reflect varying metabolic capacities of different strains, variations in environmental conditions or a combination of these effects. In addition an accurate quantitation of the lipid must be made by ensuring the complete chromatographic removal of any contaminating hydrocarbon (311).

The nature of the fatty acid component of cellular lipid reflects (Fig. 5) the composition of the alkane substrate (122, 126, 201, 233, 245, 321, 326) as well as the growth phase of the cells (201, 321). Shorter-chain ($<C_{13}$) *n*-alkanes are converted to various fatty acids apparently through the simultaneous operation of a chain elongation pathway and de novo synthesis after $\beta$-oxidation of the substrate. On the other hand the longer chain ($>C_{13}$) *n*-alkanes yield primarily fatty acid having the same chain length as the hydrocarbon substrate (201). Unsaturated fatty acid is more efficiently derived from even chain rather than odd-chain *n*-alkane (126). A particular accumulation of 17:1 acid has been observed in *C. lipolytica* (201) and *C. tropicalis* (201, 312) grown on $C_{11}$ to $C_{17}$ odd-chain *n*-alkanes. Significant conversion of odd-chain *n*-alkane to even-chain fatty acid can occur (126; 201). Details on the mechanism of this conversion are not available. In general the occurrence of $C_{16}$ and $C_{18}$ acids has been found (122, 126, 245) to be much lower for yeast grown on hydrocarbon ($<C_{16}$) compared to glucose or acetate.

FIG. 5. *Relationship between cellular fatty acid composition obtained after growth of C. tropicalis on individual n-alkanes and glucose (201). Cells were harvested during the late exponential growth phase.*

The composition of lipid obtained after growth of *C. tropicalis* on individual *n*-alkanes is shown in Fig. 6. Apart from some presently inexplicable deviations, increasing alkane chain length generally results in increased amounts of triglyceride but decreased quantities of (sterol + partial glyceride) and (steryl ester + wax). The phospholipid content appears to be independent of substrate hydrocarbon except for the very high value observed with growth on *n*-pentadecane. The quantity of triglyceride in *Candida* 107 grown on various *n*-alkanes has been shown (108, 311) to be considerably lower than the occurring in glucose-grown yeast. Triglycerides of the saturated-diunsaturated, triunsaturated, and saturated-unsaturated-saturated types been determined by Ratledge and co-workers (108, 246, 311) to predominate in alkane-grown *Candida* 107. This finding contrasts with the major occurrence of the saturated-unsaturated-saturated type in glucose-grown yeast. Growth on *n*-tetradecane or *n*-pentadecane produced a relatively high (>60% of the total acid) saturated acid content and resulted in the anticipated presence of significant quantities of trisaturated and disaturated-unsaturated types (246, 311). Specific physiological functions may be served by particular triglyceride types (311). The commercial production of lipid by alkane-grown yeast has been considered. The main potential advantage would appear to lie in the biosynthesis of specific fatty acid, e.g., odd-chain acid (247). At the present time the production of plant-like triglyceride by yeast is not considered (311) to be economically sound. An alkane-grown strain of *C. tropicalis* has been suggested (303) as a good source of ergosterol for the manufacture of vitamin D.

FIG. 6. *Lipid composition obtained after growth of C. tropicalis on individual n-alkanes (311) or a mixture (M) of ($C_{14}$ to $C_{20}$)n-alkanes (102).*

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An increase in cellular phospholipid is achieved with yeast grown on *n*-alkanes rather than on glucose (311). The composition of the phospholipid derived from *C. tropicalis* grown on $C_{14}$ to $C_{20}$ *n*-alkanes has been determined (64) to be (as percentage of the total) 41% PC, 37% PE, 8% PS, 5% LPC, 10% fully acylated DPG, and 5% fully acylated PG. A higher level of unsaturated and short-chain fatty acids is associated with phospholipid than with triglyceride (64, 312). Heptadeca-9,12-dienoic acid occurs primarily at position 2 of the phospholipid molecule (64). A consequence of the increased phospholipid level in yeast grown on *n*-alkanes is the observed proliferation of the cell plasma membrane and intracytoplasmic elements (179, 207, 222). It has not been established whether the observed (179) thickening of the plasma membrane and the development of deep invaginations are a requirement for, or a consequence of, hydrocarbon being the sole carbon source. Extension of the cell surface area could result in more efficient assimilation of the substrate (330). In addition the hydrophobic nature of the plasma membrane may govern the transport of hydrocarbon into the cell and could constitute the basis for the varying growth responses of *Candida* and *S. cerevisiae* (27). The several advantages of examining membranous systems derived from alkane-grown yeasts have been stressed (220, 270, 271). Variations in the fatty acid composition of mitochondria obtained from yeast grown on different *n*-alkanes have been found (270, 271) to influence membrane fluidity and enzyme activities. Further exploitation of alkane growth will be of value in studies on membrane structure-activity relationships.

## Nutrients

The general aspects of yeast nutrition have been discussed by Suomalainen and Oura (293). Certain nutritional factors have been recognized to have a particular influence on cell lipid composition.

**Nitrogen.** All yeasts are capable of utilizing ammonium salts, especially ammonium sulfate, as a source of nitrogen (293). Members of the genus *Saccharomyces*, however, differ from *C. utilis* in being incapable of assimilating nitrates (293). Comparison of the influence of $NO_3^-$ and $NH_4^+$ revealed (102) that the more highly oxidized nitrogen source resulted in a lower growth yield of *C. tropicalis*. The lipid content was characterized by increased levels of triglyceride and a partial replacement of 18:2 acid by saturated fatty acid. The highest growth yield and production of fatty acid for a *Candida* sp., grown on *n*-alkanes, has been observed (245) to occur in a nitrogen-deficient medium. Similar general findings have been obtained (343) on decreasing the level of the nitrogen source for *Endomycopsis vernalis*.

**Phosphorus.** Phosphorus is an essential growth factor for yeast (293). Increasing the phosphate concentration of the medium has been found (339) to have little effect on the phospholipid composition of *S. pombe*. Comparison of phosphate-limited with carbon-limited cultures of *S. cerevisiae* has shown (137) increased amounts of total lipid mainly as triglyceride in the phosphate-limited culture. Only small alterations were apparent in the composition of the polar lipids and fatty acid. In the case of phosphate-limited *C. utilis*, however, the increased content of lipid had an altered fatty acid pattern and the major phospholipid components were largely replaced by three unidentified phosphate-free polar lipids (137).

**Growth factors.** Different yeast species have highly specific vitamin requirements (293). Deficiency in certain of these growth factors can lead to pronounced alterations in the cell lipid.

**(i) Inositol.** Deficiency in inositol results in increased quantities of lipid, primarily as triglyceride, in *Saccharomyces carlsbergensis* (139, 172, 225, 267). The phospholipid component remains essentially unaltered except for a decrease in PI. The accumulated lipid has been observed (172) to occur as globules in the cell cytoplasm and to be accompanied by an apparent alteration in the nuclear membrane. Inositol deficiency influences the composition but not the quantity of cell wall lipid in *S. cerevisiae* (237). Johnston and Paltauf (139) have considered the consequence of inositol deficiency to be due to a more active fatty acid synthetase system.

**(ii) Pantothenic acid.** Omission of pantothenic acid from the culture medium results in a marked decrease in the total lipid content of *S. cerevisiae* (86, 124) and *H. valbyensis* (112). The reduction in the quantity of the phospholipid component has been reported (86, 124) to be accompanied by various changes in the unsaturated fatty acid content. These lipid disturbances have been associated with impaired mitochondrial development as determined from electron microscopic examination (86) and decreased quantities of mitochondrial DPG and PE (124). Pantothenic acid deficiency has been suggested (86) to be very similar to an anaerobic state with respect to mitochondriogenesis.

**(iii) Vitamin $B_6$.** Decreased total lipid and a marked reduction of 16:1 acid has been observed (112) with *H. valbyensis* grown in the absence of vitamin $B_6$. Although the total phos-

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pholipid level was unaltered, PI and phytosphingolipid were partially replaced by PC and PE. An intimate relationship between vitamin $B_6$ and the biosynthesis of unsaturated fatty acid and certain phospholipids has been considered (112). Studies (215) made on *S. carlsbergensis* suggest that the effect of thiamine in producing lowered quantities of cellular unsaturated fatty acid is a consequence of induced vitamin $B_6$ deficiency.

**(iv) Biotin.** Impaired growth and lowered levels of $C_{16}$ and $C_{18}$ acids resulted from the growth of *S. cerevisiae* on a biotin-deficient medium (289). Unsaturated fatty acids have been considered to have a biotin-sparing effect since their addition to the incubation medium permitted normal growth.

### Miscellaneous Additives

**Sodium chloride.** Growth of *C. albicans* in the presence of increasing concentrations (0 to 10%) of NaCl has been noted (55) to inhibit cell growth and to produce considerable elevation of the lipid content (0.3 to 6.3% of cell weight). The associated decrease in unsaturated fatty acid involved mainly 18:1 acid.

**Choline and ethanolamine.** Supplementation of the growth medium of *S. cerevisiae* with choline has been shown (224) to increase the synthesis of phospholipid mainly as PC. The pathway of PC synthesis involving the methylation of PE (Fig. 4) appears to be repressed under these conditions (324). On the other hand only small increases in total phospholipid and PE occurred on medium supplemented with ethanolamine (224).

**Benzo(a)pyrene and dibenzanthracene.** Incubation of *S. cerevisiae* under aerobic or anaerobic conditions with small quantities (0.15 to 5 mg/liter) of the carcinogenic hydrocarbon benzo(a)pyrene has been found (18, 210) to reduce the cell lipid content. A particularly drastic reduction of the phospholipid component to 10% of the normal value occurred. Decreased levels of all phospholipid classes in both whole cells and protoplast membranes was partially compensated by an increase in the monoglyceride component. Similar, but less pronounced, effects were observed with the weakly carcinogenic agent dibenzanthracene(ac).

**Propanediol.** Good growth of *L. starkeyi* on propane-1,2-diol can be obtained after "training" (294). The presence of diol analogues of glycerophospholipids has been deduced, but no evidence was obtained for the formation of simple diol fatty acid ester. Minor quantities of these diesters have been noted in soil yeasts (28).

### Oxygen

Oxygen has a pronounced effect on the growth, general metabolism, and lipid composition of yeast. The more rigorously controlled system of chemostat culture is best employed to achieve oxygen-deficient conditions which can be then studied independently of other growth factors. Knowledge of the history of the inoculum is also critical to the proper interpretation of either microaerobic or anaerobic cultures. Yeasts have been broadly classified into "respiratory-sufficient" and "respiratory-deficient" strains when considering the predominant mechanism for energy derivation and requirement for molecular oxygen (62). Many studies on the development of a respiratory-deficient to a respiratory-sufficient state have been made on the facultative anaerobe *S. cerevisiae* and have been reviewed by Linnane and his coworkers (174, 175).

**Respiratory-deficient (Crabtree-positive) yeasts. (i) Lipid composition.** The general differences occurring in the lipid composition of *S. cerevisiae* as a consequence of growth under aerobic and anaerobic conditions are shown in Fig. 7. The lipid of anaerobically grown cells has a lower total level, a highly variable glyceride fraction, decreased phospholipid and sterol components, and increased hydrocarbon content. The major changes in the phospholipid composition of anaerobic cells are depicted in

FIG. 7. *Lipid composition obtained after growth of S. cerevisiae under aerobic (44, 128, 140, 162), anaerobic (140, 162) and lipid-supplemented anaerobic (140, 162) conditions. Cells were harvested during mid-exponential–early-stationary growth phase. Vertical bars represent range of observed values.*

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Fig. 8. Lowered DPG and PE and increased PC and PI levels occur and are particularly obvious on entering the stationary phase of growth (90, 140). The observed (132, 171) high content of lysoderivatives in anaerobically grown cells may be attributed to phospholipase activity. Whereas 80 to 90% of the fatty acid component associated with glyceride and phospholipid in aerobically grown *S. cerevisiae* has been found (140) to be 16:1 and 18:1 acids, the lipid of anaerobic cells is characterized by a high content (up to 50% of the total acid) of 8:0 to 14:0 acids and a low level of unsaturated fatty acid in the phospholipid fraction.

Differences in lipid composition of aerobically and anaerobically grown *S. cerevisiae* are reflected in the state of mitochondrial development. Specifically the decreased content of DPG in anaerobic cells can be taken as an indication of impaired mitochondrial synthesis (132, 171, 226). Promitochondria have been noted (226) to be characterized by decreased DPG and PE levels and elevated PI content. In addition a particularly low amount of ergosterol is present (226). The high levels of saturated and short-chain fatty acids associated with the lipids of promitochondria (97, 226, 331, 332) have been found (332) to be rapidly replaced by 16:1 and 18:1 acids after aerobic induction of mitochondria over a period of 30 to 120 min. A marked increase also occurs in the level of mitochondrial phospholipid after aeration of anaerobically grown cells (320). Change from anaerobic to aerobic conditions results in increased membrane fluidity (31) and the development of mitochondrial function (178).

**(ii) Lipid supplementation of growth medium.** Oxygen deficiency is generally recognized (8, 9) as the reason for sterol and unsaturated fatty acid auxotrophy in *S. cerevisiae*. The inhibition of growth obtained on unsupplemented media under anaerobic conditions is accompanied by a decrease in the quantities of cellular unsaturated fatty acid and a sterol to approximately 1/4 the values of aerobically growing cells (98). Supplementation of anaerobically growing *S. cerevisiae* with ergosterol dispersed in Tween 80, which also serves as a source of oleic acid, alters cellular lipid composition as shown in Fig. 7. Although the trend is toward increased glyceride content and apparent replacement of hydrocarbon by sterol, the amount and composition of phospholipid remains unaltered. Both the glyceride and phospholipid fractions are characterized by the replacement of the short-chain fatty acid component by high levels of 18:1 acid (140). Associated with these lipid changes is the appearance of mitochondria-like structures (140, 329). Promitochondria, in contrast to the whole cell, have been found (226) to possess a markedly decreased sterol component but a significantly increased phospholipid level on lipid supplementation of the incubation medium. The PI content was reduced but the values for DPG, PC, and PS approximated those observed with developed mitochondria (226).

FIG. 8. *Phospholipid composition obtained after growth of S. cerevisiae under aerobic (90, 128, 140) and anaerobic (90, 140) conditions. Vertical bars represent range of observed values.*

The lipid requirement of anaerobically growing cells can be satisfied, but a source of fatty acid by itself is not sufficient (8, 9, 59). Growth-promoting activity is most pronounced with oleic and linoleic acids (9) and less efficient with linolenic acid, octadec-9-ynoic acid, and 11,12-methylene stearic acid (9, 173). Increasing the degree of fatty acid unsaturation may extend the exponential phase of growth but produces no difference in growth rate or quantities of total lipid and phospholipid (7). A high proportion (54 to 65%) of the cell fatty acid has been shown (7) to be derived directly from the acid supplement. Comparison of cells grown on oleic or linoleic acid-supplemented media revealed that the cytoplasmic membrane resulting from the more unsaturated acid was more susceptible to osmotic lysis (7).

A definite requirement for sterol supplementation appears to exist (8). Addition of 10 ppm (10 $\mu$g/ml) of ergosterol has been determined (59) to replace the normal oxygen requirement of various strains of *S. cerevisiae*. Generally, any sterol possessing the hydroxyl group at position 3 with axial orientation is effective in permitting anaerobic growth (242). A further degree of specificity would, however, appear to exist as yeast grown under anaerobic conditions and cholesterol supplementation showed considerable replacement of the $C_{27}$ sterol by ergosterol after aeration (97). Studies (41) on *Schizosaccharomyces japonicus*, which is not auxotrophic for ergosterol, have revealed polyene sensitivity during aerobic growth and resist-

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ance under anaerobic growth. These findings can be interpreted as being suggestive of a change in the composition of the plasma membrane sterol component. Differences between strains of *S. cerevisiae* with regard to their oxygen requirement may reflect variations in sterol composition rather than the total sterol content (59, 60). A close correlation has been suggested (230) between loss of respiratory competency and suppression of sterol synthesis in yeast. Impaired sterol biosynthesis could be a primary consequence of oxygen deficiency (29, 59). The general assumption has been made (84, 157, 309) that the oxygen-sensitive process is in the conversion of squalene to ergosterol. Depressed 3-hydroxy, 3-methyl-glutaryl CoA reductase has also been observed (29) in anaerobic cells but would be expected to result in a lower quantity of squalene as well as of sterol. Exposure of anaerobic cells to oxygen appears to cause rapid enzyme induction for ergosterol biosynthesis (29). The rate of formation of both free and esterified sterol, however, proceeds at a slower rate than in aerobically grown cells (181).

**Respiratory-sufficient (Crabtree-negative) yeasts.** A general conclusion has been reached (73, 250) that the rates of synthesis of cellular components in strains of *Candida* are controlled by the capacity of the respiratory pathways. Thus the availability of oxygen governs the ability of *C. utilis* to synthesize polyunsaturated fatty acid (14, 40). At high oxygen tension the occurrence of linolenic acid was greatest (14), whereas at low oxygen the degree of unsaturation decreased and greater concentrations of $C_{16}$ accumulated at the expense of $C_{18}$ acids (40). Growth of *C. lipolytica* on hexadec-1-ene at low oxygen tension resulted in the incorporation of fatty acid derived directly from oxidation of the hydrocarbon. Formation of $C_{16}$ and $C_{18}$ acids predominated at higher aeration rates (159). It would, therefore, appear that low oxygen inhibits both the elongation and oxygen-dependent desaturase systems. The fatty acid patterns of total lipid and phospholipid of *C. utilis* grown under low oxygen have been found (14) to be similar. The mitochondrial content of oleic and linoleic acids in *Candida parapsilosis* grown under microaerobic conditions was unaltered, although the quantity of oleic acid in the whole cell was depressed compared to aerobic cultures (250).

## Temperature

The influence of temperature on the growth and metabolism of yeasts has been reviewed (287). In common with most living organisms, yeasts generally show a tendency to raise the lipid content and degree of unsaturation as the environmental temperature is dropped below that for optimal growth.

Decrease in the growth temperature from 25 to 10 C for the mesophile *C. lipolytica* has been determined (147, 148) to result in increased lipid and a higher ratio of linoleic acid to oleic acid. The relative levels of linoleic and oleic acids dropped to the values of the inoculum at the end of active growth at 25 C. A similar readjustment was incomplete at 10 C (Fig. 9). Growth of *C. utilis* in either batch culture (80) or in the chemostat at fixed growth rate (40, 193) results in higher proportion of 16:1 and 18:3 acids at 10 C compared to 30 C. The specific alterations in 16:0, 18:1 and 18:2 acids are subject to limitation of glucose or nitrogen in the medium (193). A pronounced increase of 18:2 acid has been determined (102) in *C. tropicalis* grown at 28 C compared to 38 C. Cyclic variation of 18:1 and 18:3 acids has been observed (193) in a psychrophilic *Candida* sp., but is

FIG. 9. *Changes in fatty acid composition of (A) total lipids, (B) phosphatidyl choline, and (C) phosphatidylethanolamine at 25 C and 10 C. (Reproduced from reference 148 by kind permission of M. Kates and the Canadian Journal of Biochemistry.) Symbols: growth curves (□), linoleic acid (○), and oleic acid (△).*

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temperature independent. No marked alteration in fatty acid composition occurs on decreasing the growth temperature of *S. cerevisiae* (128), which, in any case, appears incapable of synthesizing polyunsaturated fatty acid (136). The desaturase system involved in unsaturated fatty acid formation has been found (196) to be stimulated in *C. utilis*, a Crabtree-negative yeast, at lower temperatures. Decrease in the hypothetical mean melting point of the cellular acids may also be achieved at lower temperatures through an increase in the content of $<C_{14}$ saturated acids. Such an effect has been observed in *C. tropicalis* growing on *n*-alkanes (312).

No major differences exist in the general lipid composition of a psychrophile (*C. scottii*) and a mesophile (*C. lipolytica*) grown at 10 C (147). Growth of *C. lipolytica* (147, 148) and *S. cerevisiae* (128) at temperatures higher than 10 to 15 C results in decreased lipid levels. In particular, the phospholipid component and the PE:PC ratio has been determined (148) to be decreased in *C. lipolytica* at higher temperatures. The tetraene molecular species of both PE and PC was elevated and maintained for a longer period of growth at lower temperatures. Lowering the growth temperature of batch cultures of *S. cerevisiae* from 30 C to 15 C resulted (128) in increased levels of triglyceride and phospholipid. The PC component was increased while PE remained virtually unchanged. The conclusion has been reached (128) that the increased synthesis of total phospholipid and triglyceride, and the depressed generation of sterol by cells grown in batch culture at lower temperatures, reflect a true temperature effect. The increased synthesis of PC, however, is associated with a lower growth rate.

### pH

The available information indicates that slight alteration in the pH of the medium influences the general composition rather than the quantity of cellular lipid. Response to a wide range of pH varies with the yeast strain. A similar total lipid content has been obtained (47) with *S. cerevisiae* grown at pH 5.5 and 6.0. Endogenous phospholipolytic activity has been noted (320) to be markedly increased at pH 5.5 and might be anticipated to result in increased levels of lyso-phospholipids. Growth of certain *Candida* yeasts in media ranging from pH 3.0 to 7.0 gave comparable lipid contents. Appreciable lipolysis of triglyceride, however, occurred at pH values not optimal for growth (71). The inhibitory effect of short-chain ($C_6$ to $C_{12}$) monocarboxylic acid on the growth of *C. tropicalis* has been found (70) to be most marked at pH 5.6. Examination of *C. lipolytica* showed (71) an increase in unsaturated fatty acid and polyglycerophospholipid and a decrease in PC and PE at lower pH values. The production of ergosterol in *C. tropicalis* growing on *n*-alkanes in pH dependent (303). Significantly lower quantities of total lipid occur in *L. lipoferus* (347) and *R. glutinis* (348) at pH values not supporting optimal growth.

## CONCLUDING COMMENTS

The bulk of lipids in yeast are phospholipid, unsaponifiable matter, and highly variable quantities of glycerides (Fig. 2). The sterol component differentiates bacteria (except for *Mycoplasmatales*). Yeasts, in turn, do not normally possess the relatively high levels of glycolipids and phosphonolipids characteristic of higher plants (146) and protozoa (310), respectively. As with any generalization, however, exceptions are to be expected. Yeast fatty acids are usually composed of $C_{12}$ to $C_{18}$ saturated and unsaturated acids. The small quantities of $C_{18}$ to $C_{26}$ saturated and 2-hydroxy acids are specifically associated with sphingolipids. Although unsaturated acids, particularly 16:1 and 18:1, are present in all yeast, species differences exist in the capacity to synthesize more highly unsaturated acids. The occurrence of polyunsaturated acids in certain yeast, and the replacement of the unsaturated component by short-chain ($<C_{14}$) acids under certain conditions in others, probably reflects their involvement in the required fluidity of membranes. More definite information on the role of fatty acids in membranes may be expected from further electron spin resonance studies on spin-labeled cells grown under various conditions (31, 75, 153, 209). The importance of the sterol component in influencing membrane structure is also recognized (220). Variation in membrane sterol composition appears to account for the varying effectiveness of polyene antibiotics. In this regard, the possible application of antilipogenic compounds such as cerulenin (219) for the inhibition of sterol biosynthesis has not been fully evaluated.

All yeasts contain nuclei, plasma membrane and a variable content of vacuolar and mitochondrial membranes (329). Many data have been accumulated on the development of yeast mitochondria. No simple association appears to exist between cell respiration and lipid, but respiratory activity seems to correlate with phospholipid content (46, 162). Glucose repression and anaerobic growth conditions influencing the biogenesis of mitochondria in Crabtree-

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positive yeast should provide valuable approaches to the study of cellular development and metabolic interrelationships. The yeast cytoplasmic membrane furnishes an important system for studies on the possible correlations between lipid composition and such physiological activities as cell viability, active transport, and enzyme action. A variety of membranous systems performing functions ranging from structures analogous to lysosomes in higher eukaryotic cells (188) to spherosomes or fat-accumulating bodies (179) and vesicles implicated in protein synthesis (121) are present in yeast cells. Studies on these various systems will give further insight into the importance of lipid in membranous systems as well as the interrelationships involved in membrane and cell development.

The exploitation of alkane-utilizing yeasts in the production of specific fats has been achieved (246), and may emerge as a commercially viable proposition. Methanol-assimilating yeasts, e.g., *Pichia methanolica* and *Candida boidinii* (149, 318, 322), may also prove to be useful. Considerable advantages should lie in the ready availability of the substrate and the nonrequirement for sophisticated fermentors as are required for yeast growth on the water-insoluble hydrocarbons. More detailed studies on yeasts other than *Saccharomyces* and *Candida* may be expected to be important for the commercial production of other lipids.

Any consideration of yeasts must recognize the existence of biochemically diverse genera which may show different emphases in lipid metabolism (compare reference 311). In addi-

TABLE 5. *General effects produced by culture factors on the lipid content and composition of yeasts*

| Factor | Condition | Reference | Total lipid | Phospholipid | Sterol | Unsaturation |
|---|---|---|---|---|---|---|
| Organism | Species; strain | | [a] | [a] | [a] | [a] |
| Growth rate | Decreased | 128, 192 | (+)[b] | (+) | (+) | (+), 0[b] |
| Development stage | (i) Lag and early exponential phases | 44, 45 | (−)[b] | (+) | 0 | (+) |
| | (ii) Later and post exponential phases | 44, 45, 192, 218, 311 | (+) | (−) | 0 | (−) |
| | (iii) Sporulation | 50, 119, 130 | (+) | (+) | (+) | (+) |
| Carbon source | (i) Increased glucose level | | | | | |
| | Crabtree positive | 39, 138 | (−) | (−), 0 | (−) | (−) |
| | Crabtree negative | 14, 39 | (+) | 0, (+) | (+) | (+) |
| | (ii) Hydrocarbon level (compared to glucose level) | | | | | |
| | Crabtree positive | 185, 246, 261 | [c] | [c] | [c] | [c] |
| | Crabtree negative | 201, 218, 245, 246 | (+) | | | (−) |
| | | 51, 108, 201, 311 | (−) | (+) | (+) | Reflects substrate chain length |
| Nitrogen source | Decreased level | 245 | (+) | | | |
| Phosphorus source | Increased level | 293 | (+) | 0 | | 0 |
| Growth factors | (i) Inositol or nicotinic acid deficiency | 112, 139, 172, 225, 267 | (+) | 0 | | |
| | (ii) Biotin, pantothenic acid or vitamin $B_6$ deficiency | 86, 112, 124, 289 | (−) | | | (−) |
| $pO_2$ | (i) High (aerobic) | 14, 44, 128, 140, 162 | (+) | (+) | (+) | (+) |
| | (ii) Low (anaerobic) | 40, 140, 162 | (−) | (−) | (−) | (−) |
| $pCO_2$ | High | 47 | (+) | | | (+) |
| pH | More acidic | 47, 71, 347, 348 | 0, (−) | 0 | 0 | (+) |
| Temperature | Decreased from 25 to 10 C | 40, 128, 147, 148, 193 | (+) | (+) | (−) | (+) |

[a] Considerable variations may occur.
[b] (+) Enhanced; (−) depressed; and (0) generally unaffected.
[c] Little or no growth.

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tion, yeasts readily lend themselves to genetic manipulation which may permit the development of specific lipid mutants. Such mutant strains, while of potential commercial significance, would also be very valuable in defining the biochemical importance of the various lipids in the eukaryotic cell. Evolutionary trends might also be examined in different genera and strains and related not only to cellular development of yeasts but also to higher organisms. Thus the cellular requirement and biosynthetic capacity for polyunsaturated fatty acids can be studied with fatty acid desaturase mutants or by comparison of the general basis for the occurrence and absence of these acids in Crabtree-negative and Crabtree-positive yeast, respectively. The inability of glucose-repressed anaerobic cells of *S. cerevisiae* to form monounsaturated acids (e.g., 140) would seem to be a "retrograde step" and appears to result in a primitive type of cell with lipid properties and membranous systems intermediate between those of bacteria (prokaryotes) and eukaryotic cells of animals and plants.

The considerable advantages of yeast as an experimental organism can only be fully realized when the conditions of experimentation are strictly defined. The use of the chemostat permits rigid control of growth factors including nutrients, degree of oxygenation, pH, etc., and is amenable to computer assistance. Particular consideration of the stage of the growth cycle at which the cells are harvested must be made in any comparative study. Ideally, synchronous growth should be sought to permit significant studies on cell development and senescence. The various factors discussed as influencing yeast lipid are summarized in Table 5.

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