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Death Resulting from Fatty Acid Starvation in Yeast

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Mutants of *Saccharomyces cerevisiae* having the genotypes *fas*1 (fatty acid synthetase minus) and *fas*1, *ole*1 (fatty acid synthetase and fatty acid desaturase minus) were found to undergo logarithmic death when deprived of required fatty acids, whereas *ole*1 strains did not. During the first 2 to 3 h of fatty acid starvation, macromolecular synthesis occurred at apparently normal rates, although cell division stopped by the end of the 1st h. Cell death commenced at approximately the 2nd to the 3rd h, and within 24 h, depending upon conditions, 2 to 4 log orders of death had occurred. The loss of viability was accelerated by the addition of detergent, but could be largely prevented by the interruption of protein synthesis, either by amino acid starvation or by the use of cycloheximide. The possible significance of this phenomenon in terms of membrane biosynthesis is discussed.

The conidia of inositol requiring *Neurospora* mutants (9) and biotin auxotrophs of *Aspergillus* (13) die if they are permitted to germinate in the absence of inositol or biotin, respectively. This phenomenon has been termed unbalanced growth and has been used as a means of selecting other types of auxotrophs (9, 13). A second mutation for an additional requirement, such as an amino acid, confers protection against death of the conidia, giving double mutants a selective advantage. Because the biotin and inositol mutations both affect lipid synthesis, it has been suggested that death may result from defective cell membranes (2).

Death due to unbalanced growth has similarly been observed in inositol-requiring yeast of the species *Kloeckera apiculata* and *Saccharomyces carlsbergensis* (16). In the yeast *Saccharomyces cerevisiae*, it has likewise been reported that starvation for the vitamins biotin (8) or pantothenate (19) results in a high percentage of dead cells. Both biotin and pantothenate are required for fatty acid synthesis (12, 20).

Fatty acid mutants of *S. cerevisiae* provide an opportunity for a direct study of the basis of this phenomenon. Two types of yeast fatty acid mutants are available: fatty acid desaturase mutants (*ole*), which are unable to produce unsaturated fatty acids (15), and fatty acid synthetase mutants (*fas*), which are unable to synthesize saturated fatty acids (6, 17). The *fas* mutants can, however, desaturate exogenously supplied long-chain saturated fatty acids (6), and *ole* mutants synthesize saturated fatty acids (7). A strain of the *ole*1, *fas*1 genotype is, therefore, unable to produce either saturated or unsaturated fatty acids and is completely dependent upon an exogenous supply. In the present study, both single mutants (*ole*1, *Fas*$^+$ and *fas*1, *Ole*$^+$) and double mutant strains (*ole*1, *fas*1) have been employed to study the viability of cells starved for fatty acid. The circumstances under which death is either accelerated or prevented are explored.

MATERIALS AND METHODS

Yeast strains. The isolation and characterization of saturated fatty acid-requiring mutants (*fas*) have been previously described (6, 17). The mutant strain SH-1 containing an allele at the *fas*1 locus (M. R. Culbertson and S. A. Henry, Genetics, in press) was employed in this study. This allele is ochre suppressible (4).

The desaturase mutant KD-115 (*ole*1) was kindly provided by Bernadine Wisnieski. Strain 4335-7-3α (*lys*2-1, *ade*2-1, *trp*1-1) was obtained from the Berkeley collection.

Double mutant strains (*fas*1, *ole*1) were constructed by a standard genetic cross followed by sporulation and dissection. The double auxotrophs were identified by their failure to complement both parent types and their inability to grow in the absence of both saturated and unsaturated fatty acid (each parent requires only one fatty acid). Subsequently, the segregation of both markers was confirmed in crosses involving the double mutant strains. The two loci (*ole*1 and *fas*1) are unlinked; the *ole*1 marker is located on chromosome

VII (18), and *fas*1 is located on chromosome XI, fragment 5 (1; M. R. Culbertson and S. A. Henry, Genetics, in press). In double mutant strains, both phenotypes revert simultaneously. This is due to suppression of both markers, presumably by an ochre suppressor. Because the frequency of such revertants was not great in the double mutant, reversion did not prove to be a problem.

Several such double mutant strains were employed, giving similar results. All of the results reported here, however, are derived from two such strains, BW1-1C (*ole*1, *fas*1, *a*) and BW2-5C (*ole*1, *fas*1 *lys*2-1, *a*). The latter was constructed by a cross of BW1-1C to 4335-7-3Bα (*ade*2-1, *trp*1-1 *lys*2-1). Because *lys*2-1 is also ochre suppressible, it reverted with the two fatty acid mutations.

Chemicals. Myristic and oleic acid >99% pure were obtained from the Hormel Institute. Cycloheximide and the detergents Tergitol NP40, Tween 80, and Tween 40 were obtained from Sigma Chemical Co., St. Louis, Mo.

Isotopes. L-Arginine-^{14}C (U), specific activity 313 mCi/mmol, was obtained from New England Nuclear Corp., Boston, Mass. Uracil-5-^{3}H, specific activity 26 C/mmol, was obtained from Amersham/Searle.

Yeast media. Fatty acid mutants were maintained on 1% yeast extract, 2% peptone, and 2% glucose (YEPD) medium containing 1% Tween 80 and 1% Tween 40 (YEPD + TW) as a fatty acid supplement. This mixture of Tween will support growth of all three types of mutants: *Ole*$^+$, *fas*1 and *ole*1, *Fas*$^+$, and *ole*1, *fas*1.

The composition of complete defined medium has been described elsewhere (3). This medium was also supplemented with Tween as described above or with purified fatty acids. Fatty acids were supplied at 10^{-3} M with 3% Tergitol NP40 as a detergent unless otherwise specified. The saturated fatty acid used in all cases was myristic acid, and the unsaturated fatty acid was oleic acid. Myristic acid was chosen because it supports optimal growth of *fas* mutants (6), and oleic acid supports *ole* mutants (21).

Growth conditions for starvation experiments. The mutants were cloned, and the clones were streaked onto YEPD + TW plates and allowed to grow for 1 or 2 days at 30 C. From these freshly cloned cultures, an inoculum was transferred to liquid YEPD + TW and allowed to grow at 30 C until it reached log phase. Log-phase cells were washed twice with the starvation medium (containing no fatty acid or only one fatty acid) and then were resuspended in the starvation medium. For labeling experiments, an isotope was given at the time of transfer at 2 μCi of ^{3}H-uracil per ml or 0.1 μCi of ^{14}C-arginine per ml. Samples of 0.5 ml from cultures containing a starting number of 1.5×10^{6} cells/ml were mixed at intervals with an equal volume of 20% cold trichloroacetic acid and allowed to stand for at least 20 min at 0 C. Samples were then filtered with membrane filters (HAWP 02500) and washed with 20 vol of cold 5% trichloroacetic acid. The filters were dried and counted by liquid scintillation.

Optical density readings were taken in calibrated test tubes on a Coleman Jr., spectrophotometer, model 6D at 600 nm.

Cell viability was measured by plating cells onto YEPD + TW at known dilutions throughout the period of starvation. Plates were counted after 3 days of incubation at 30 C. Each point on the cell survival figures in the text represents the average value of two to ten plates. The percentage of survival was calculated from the number of cells present at the time of transfer. Alternatively, the number of dead cells was monitored by staining with methylene blue (10). For this test, a solution of 0.6% methylene blue was mixed 1:5 with the liquid yeast culture. After 5 min in the stain, the culture was examined with a microscope, and the percentage of cells that took up the stain was determined.

Cycloheximide, when used, was added at 100 μg/ml. This amount of antibiotic was found to stop the incorporation of radioactive amino acids into trichloroacetic acid precipitable materials in all of the strains utilized.

Cultures were assayed for revertants by plating onto unsupplemented medium at the beginning and end of all experiments.

RESULTS

Growth curves for fully supplemented cultures of the various strains used in this study are shown in Fig. 1. All fatty acid-requiring strains (*ole*1, *Fas*$^+$ or *Ole*$^+$, *fas*1 or *ole*1, *fas*1) failed to achieve the growth rate of nonrequiring strains such as 4335-7-3B, even when grown under optimal conditions.

When starved for either saturated or unsaturated fatty acid or both, cultures of double mutant strains (*ole*1, *fas*1) increase in optical density for 4 to 6 h (Fig. 2). During this time, the optical density doubles at least once, and the increase is indistinguishable from fully supplemented cultures for 2 to 4 h, depending upon which fatty acid(s) was omitted (Fig. 2). Cell counts taken by hemocytometer during this period failed to show an equivalent increase in cell number (Fig. 2). At 35 C, the increase in cell number stops after the 1st h of starvation and never approaches even a single doubling.

Incorporation of radioactive uracil or amino acid into trichloroacetic acid-precipitable material under the various starvation conditions at 35 C continues at a rate indistinguishable from fully supplemented cultures for 2 to 4 h (Fig. 3, 4). After this period, there is additional reduced incorporation for several more hours, particularly in the cultures supplemented with myristic acid only and in cultures containing no fatty acid and no detergent.

Plating of cells of genotype *ole*1, *fas*1 onto supplemented medium (YEPD + TW) throughout the starvation period reveals that cell death occurs, commencing at about the 2nd to the 3rd h of fatty acid starvation, when either or both types of fatty acid are omitted from the medium

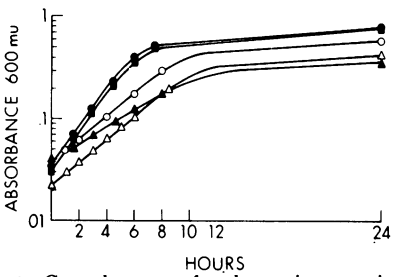

FIG. 1. *Growth curves for the various strains used in this study in complete defined medium at 35 C.* ●, *Strain 4335-7-3Bα ade2, lys2, trp1; no fatty acid, no detergent; doubling time, 90 min.* ■, *Strain 4335-7-3Bα; no fatty acid; 3% Tergitol NP40.* ○, *Strain ole1, Fas⁺; 10^{-3} M myristic acid; 10^{-3} M oleic acid; 3% Tergitol NP40.* ▲, *Strain ole1, fas1; 10^{-3} M myristic acid; 10^{-3}M oleic acid; 3% Tergitol NP40.* △, *Strain Ole⁺, fas1; 10^{-3} M myristic acid; 10^{-3} M oleic acid; 3% Tergitol NP40.*

(Fig. 5). The timing, extent, and kinetics of this death are dependent upon a number of conditions, including temperature (Fig. 6), the fatty acid(s) omitted from the medium (Fig. 5), the type of growth medium (YEPD or defined) (Fig. 7), and the amount of detergent present (Fig. 7). Cell death is more rapid at higher temperatures and is also accelerated by increasing amounts of detergent. The maximum rate of cell death is achieved when 1% or more Tergitol NP40 is present in the growth medium. Increasing amounts of detergent above this level have little additional effect. When detergent concentration is 0.01% or less, there is little effect compared with cultures in which there is no detergent. Under the most extreme conditions (35 C, 1 to 3% Tergitol NP40 in YEPD medium containing no fatty acid), 3 to 4 log orders of cell death occur in a 24-h period, with the most rapid decline in viability starting between the 2nd and 3rd h of starvation and continuing for about 8 h.

Examination by microscope of cells of BW1-1C (*ole1*, *fas1*) starved for 24 h at 35 C in YEPD media for either saturated or unsaturated fatty acid or both, and stained with methylene blue, revealed a proportion of dead cells that is in close agreement with the percentage of surviving cells shown in Fig. 5. In all of the cultures, without regard to the particular fatty acid(s) omitted, the few unstained and, therefore, presumably surviving cells consisted primarily of large unbudded cells and cells with a large completed bud, Virtually no cells with buds at an intermediate stage of development were observed among the survivors. Cells maintained in YEPD plus oleic acid (10^{-3} M) were the most abnormal in appearance. Phase microscopy of unstained cells from these cultures revealed that many were enlarged and highly vacuolated. At 24 h, about 80% of the cells in such cultures were no longer refractile, and the cytoplasm could be seen to have contracted and

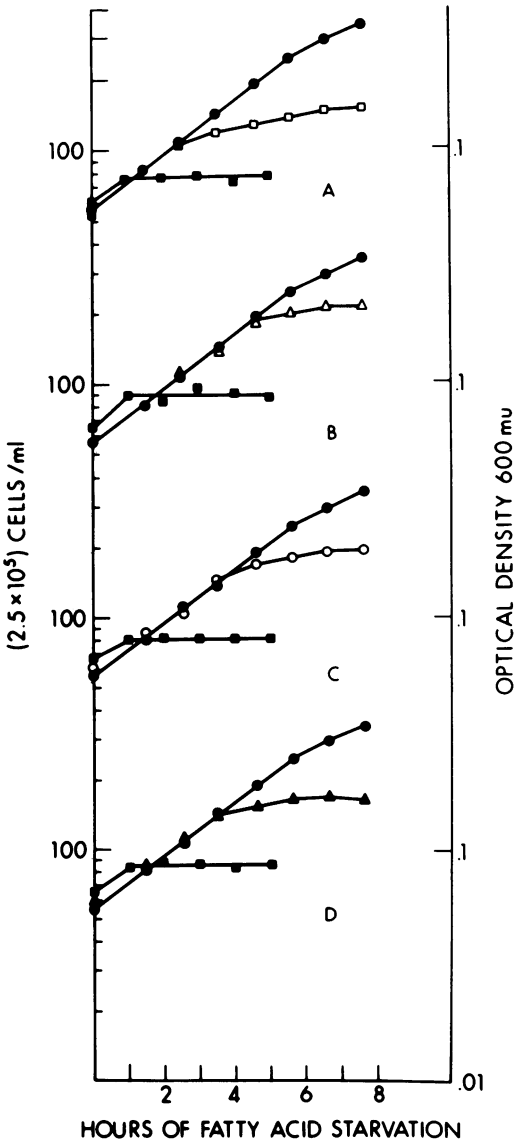

FIG. 2. *Growth curves and cell counts of strain BW1-1C (ole1, fas1) during fatty acid deprivation in defined medium at 35 C. Graphs A to D illustrate growth under four different starvation conditions. Cell counts obtained by hemocytometer* (■) *are shown in each case. A growth curve for a fully supplemented culture* (●) *is given for comparison. (A)* □, *10^{-3} M myristic acid, no oleic acid, 3% Tergitol NP40. (B)* △, *No myristic acid, 10^{-3} M oleic acid, 3% Tergitol NP40. (C)* ○, *No fatty acid, no detergent. (D)* ▲, *No fatty acid, 3% Tergitol NP40.*

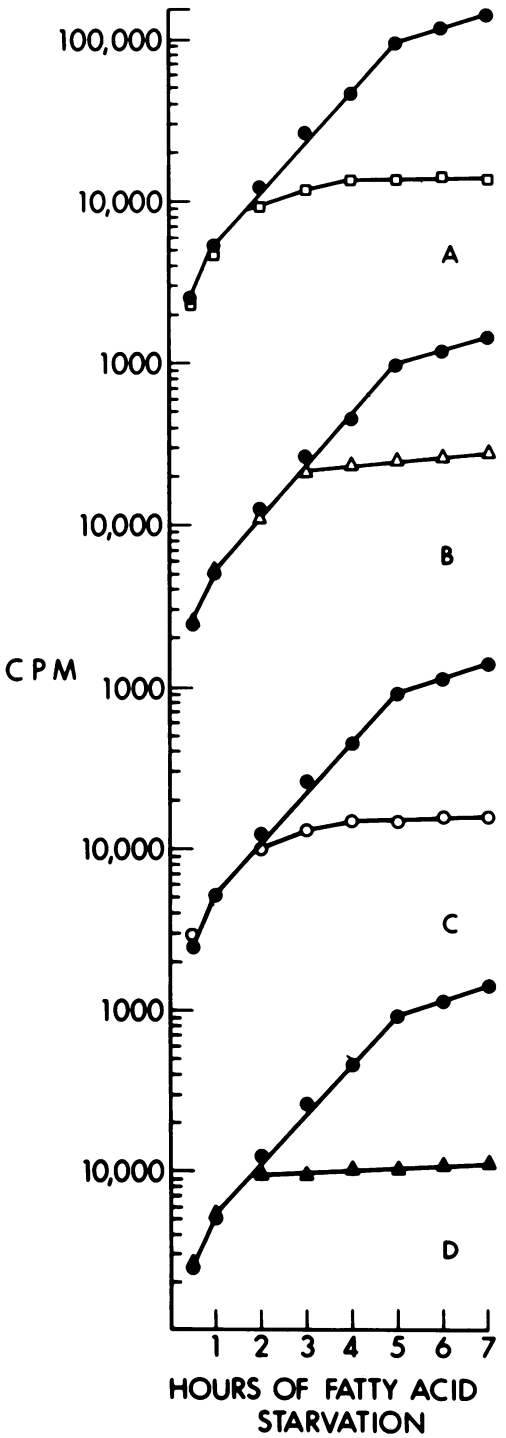

FIG. 3. *Incorporation of ^{3}H-uracil into trichloroacetic acid-precipitable material during fatty acid deprivation. Conditions and symbols are identical to those given in Fig. 2. Labeling conditions are given in Materials and Methods. Incorporation by a fully supplemented culture (●) is shown for comparison.*

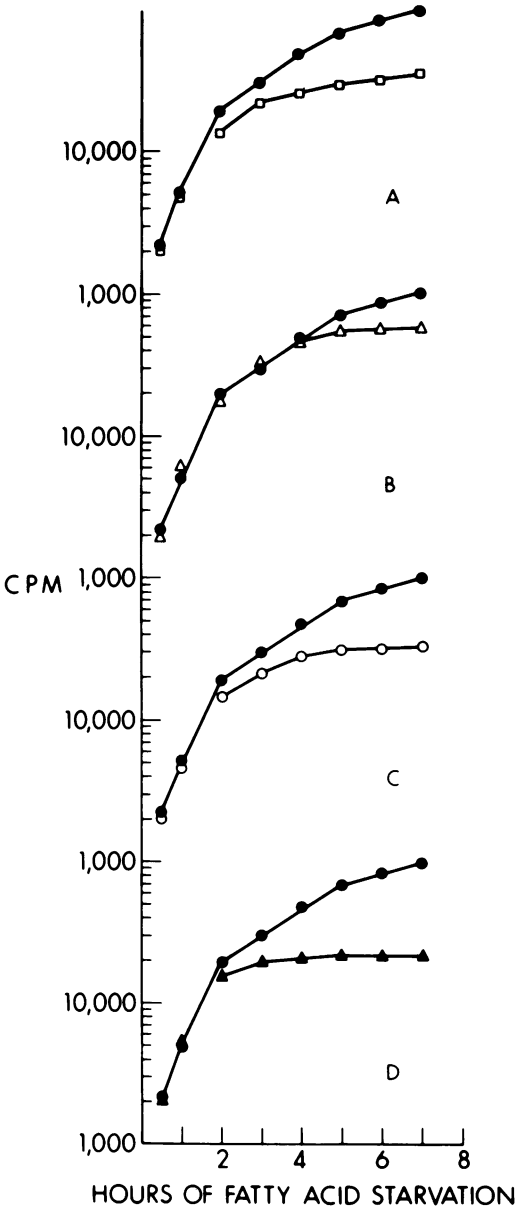

FIG. 4. *Incorporation of ^{14}C-arginine into trichloroacetic acid-precipitable materials during fatty acid deprivation. Conditions and symbols are as shown in Fig. 2, and 3.*

separated from the cell wall. The optical density of such cultures had decreased at 24 h from the reading obtained at 8 h.

The double mutant (*ole*1, *fas*1) was deprived of fatty acids for varying lengths of time (Fig. 8) and then resupplied with fatty acid. Even when deprived for only 2 h, a period during which macromolecular synthesis is apparently unaffected, optical density increases are indistin-

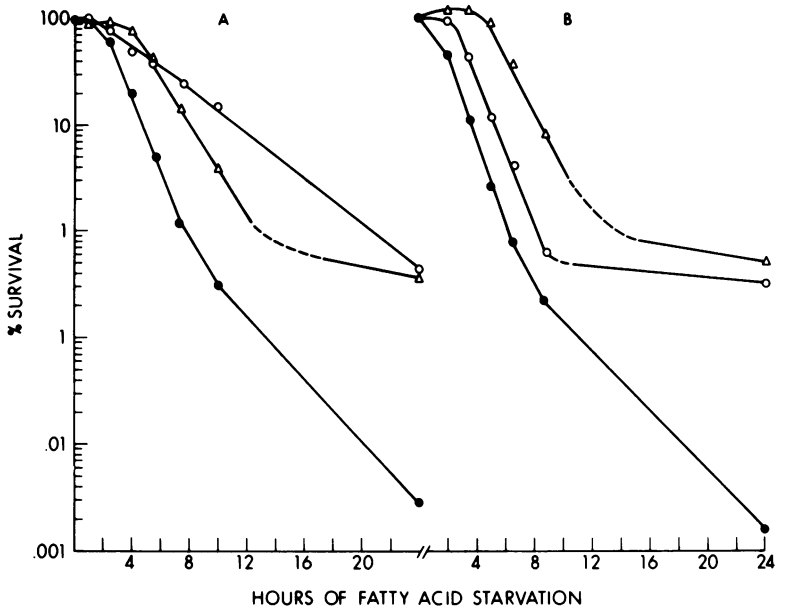

FIG. 5. *Cell survival of BW1-1C (ole1, fas1) was measured by plating onto supplemented medium during fatty acid deprivation at 35 C. (A) Defined medium: O, 10^{-3} M myristic acid, no oleic acid, 3% Tergitol NP40; Δ, no myristic acid, 10^{-3} M oleic acid, 3% Tergitol NP40; ●, no fatty acid, 3% Tergitol NP40. (B) YEPD medium, supplementation and symbols as in A.*

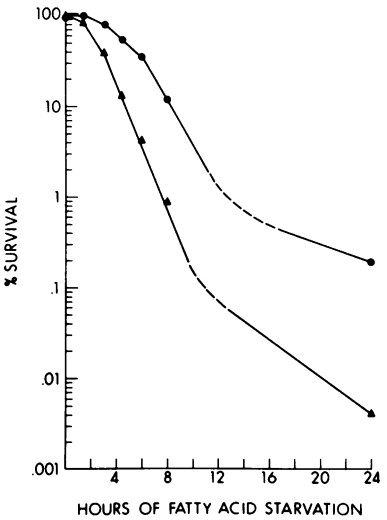

FIG. 6. *Cell survival at 35 and 25 C. BW1-1C (ole1, fas1) in defined medium with no fatty acid, 3% Tergitol NP40. ●, 25 C; ▲, 35 C.*

guishable from supplemented cultures, and no cell death has occurred (Fig. 2-4, 9); there is a considerable lag before growth is resumed, and the rate achieved is not fully comparable to the unstarved condition. For longer periods of starvation, the lag is longer and the effect upon the growth rate is proportionately greater. During longer periods of starvation, it is expected that loss of viability may contribute to this effect, causing optical density to become an inaccurate measure of growth. Consequently, cultures were also plated during recovery (Fig. 9). Although death is immediately halted by the addition of fatty acid, there is a lag in excess of 8 h before growth is resumed. As the culture recovers, it grows slowly at first, gradually accelerating (Fig. 9).

Because it was unclear from the experiments with *ole1*, *fas1* strains whether both mutations or only one was responsible for cell death, the single mutants (*ole1*, *Fas*$^+$ and *Ole*$^+$, *fas1*) were examined for cell viability under fatty acid starvation conditions. Growth of *ole1*, *Fas*$^+$ mutants is supported by unsaturated fatty acids such as oleic acid (21), but not by saturated fatty acids. The converse is true in *Ole*$^+$, *fas1* mutants (6). Both mutants underwent increases in optical density during starvation comparable to that reported for the double mutant. All *ole1* strains became "clumpy" (that is, cells formed clumps and would not remain suspended) during fatty acid starvation. This effect first became appreciably noticeable after about 6 h of starvation, the clumps then consisting of hundreds of cells. The unstarved *ole1* strains are only minimally clumpy, forming clumps of no more than five or six cells in YEPD + TW. The "clumpy" condition was not observed in double mutants (*ole1*, *fas1*) or *fas1* strains. This clump-

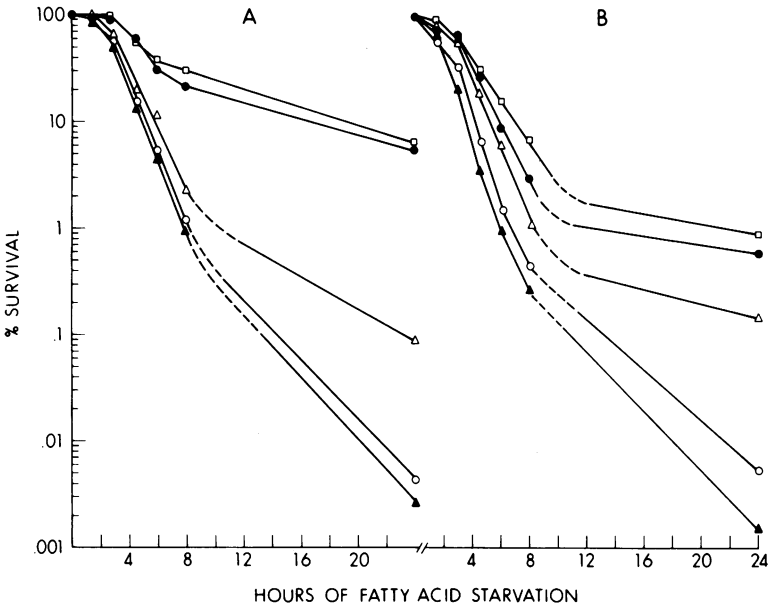

FIG. 7. *Effect of Tergitol NP40 concentration on cell survival. BW1-1C (ole1, fas1) at 35 C. (A) Defined medium, no fatty acid;* □, *no Tergitol NP40;* ●, *0.01%;* △, *0.1%;* ○, *1%;* ▲, *3%. (B) YEPD medium, no fatty acid, detergent concentrations and symbols as in A.*

ing makes growth estimates based on either plating or optical density somewhat inacurrate.

The results of cell viability tests for *ole*1, *Fas*$^+$ and *Ole*$^+$, *fas*1 single mutant strains are shown in Fig. 10. Although the *fas*1 strain shows a decline in viability comparable to the double mutant strains, the *ole*1 strain shows nowhere near so dramatic a loss of viability. If the clumping that occurs with the *ole*1 strain after several hours of starvation affects these data, it would be expected to cause the number of colonies to decrease, making the loss of viability appear greater than actuality. Thus, the cell death may not even be as great as it appears in Fig. 10. Furthermore, the plating of the *ole*1 strain reveals a substantial increase in cell number subsequent to the removal of fatty acid which is not observed in *ole*1, *fas*1 or *fas*1 strains. Also in contrast to double mutant and *Ole*$^+$, *fas*1 strains, the addition of Tergitol NP40 has little effect upon *ole*1, *Fas*$^+$ mutants.

It is important to ascertain whether the viability loss reported here is unique or whether it is typical of all auxotrophic mutants deprived of their required growth factor under otherwise growth-supporting conditions. For this reason, the strain 4335-7-3Bα *lys*2, *ade*2 *trp*1 was tested for cell death in the absence singly of tryptophan, lysine, or adenine. When deprived of any one of these requirements, cell number increases slightly and viability is stable for about 8 h (Fig. 11). Thereafter, there is gradual cell death at a rate much slower than either *fas*1 or *fas*1, *ole*1 strains. The addition of 3% Tergitol NP40 has only a minimal effect on the outcome. These results appear quite similar to those obtained with the *ole*1 strains starved for unsaturated fatty acid.

In the *Neurospora* (9) and *Aspergillus* (13) lipid mutants previously discussed, a second mutation, such as amino acid auxotrophy, was found to provide substantial protection against cell death. To determine whether this is also true in the yeast fatty acid mutants, the strain BW 2-5C (*ole*1, *fas*1, and *lys*2) was employed. This strain was first starved for lysine in the presence of fatty acid. During subsequent starvation for fatty acid, "fatty-acidless" death was almost completely prevented for the first 8 h (Fig. 12), followed by gradual death thereafter.

Likewise, when cycloheximide (100 μg/ml) is given 1 h before transfer to medium lacking fatty acid (but containing cycloheximide), cell death is largely prevented (Fig. 13). The amount of cell death actually observed in starved cells after a period of 8 h in cycloheximide is comparable to that seen in fully supplemented cells treated with cycloheximide. Virtually the same result can be otained by adding cycloheximide at the time of transfer or up to 30 min after transfer. If the cycloheximide is added at 1 h after transfer, the rate of cell death is considerably diminished but is greater than in the fully supplemented control. At 2 h, there is

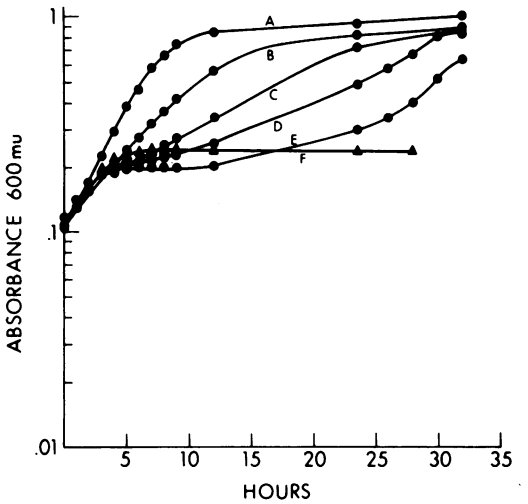

FIG. 8. *Recovery from fatty acid deprivation. Strain BW1-1C (ole1, fas1) was starved for varying lengths of time at 35 C in YEPD (no fatty acid, no detergent) and then resupplied with YEPD + TW. Curves show optical density increase during the period of starvation followed by the period of recovery; the shift occurring at the time point is indicated as follows. (A) Continuous supplementation in YEPD + TW; (B) shifted at 2 h to YEPD + TW; (C) shifted at 3 h; (D) shifted at 4 h; (E) shifted at 5 h; (F) continuous starvation.*

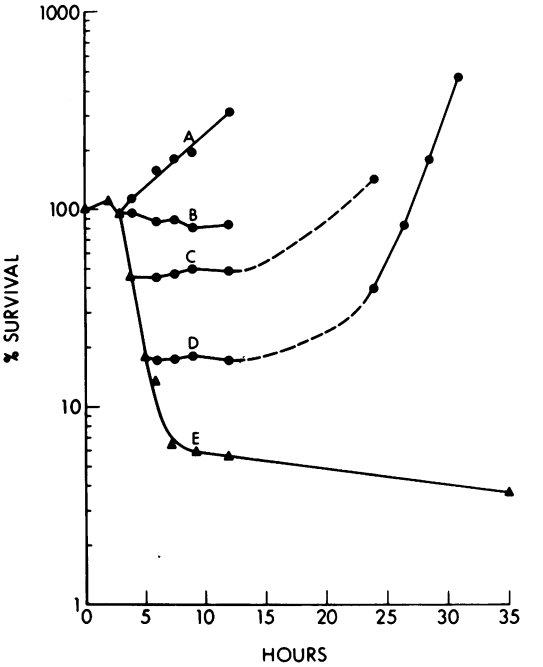

FIG. 9. *Recovery from fatty acid deprivation was measured by plating of cells. Conditions are given in the legend of Fig. 8. Final points are not shown for A, B, and C because the cultures had reached stationary phase. (A) Shifted at 2 h to YEPD + TW; (B) shifted at 3 h; (C) shifted at 4 h; (D) shifted at 5 h; (E) continuous starvation.*

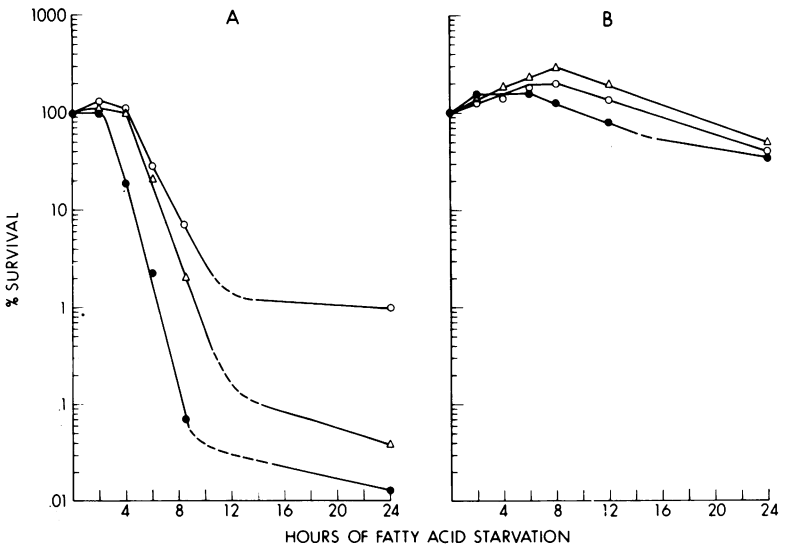

FIG. 10. *(A) Cell survival during fatty acid deprivation in the fas1, Ole⁺ strain. (This strain grows when supplemented with myristic acid only). YEPD medium is at 35 C. ○, No myristic acid, 10^{-3} M oleic acid, 3% Tergitol NP40; △, no fatty acid, no detergent; ●, no fatty acid, 3% Tergitol NP40. (B) Cell survival during fatty acid deprivation in the ole1, Fas⁺ strain. (This strain will grow when supplemented with oleic acid only.) YEPD medium is at 35 C. ○, 10^{-3} M myristic acid, no oleic acid; 3% Tergitol; NP40. ●, no fatty acid, no detergent; △, no fatty acid, 3% Tergitol NP40.*

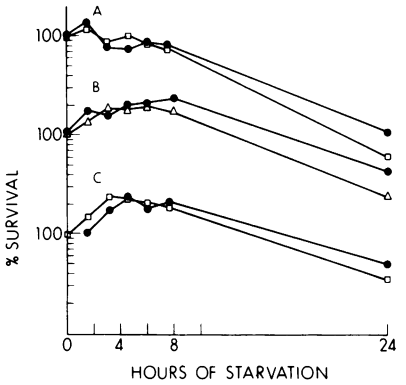

FIG. 11. *Cell survival during starvation for lysine (A), adenine (B), and tryptophan (C); strain 4335-7-3Bα (lys2, ade2, trp1) in defined medium with one requirement omitted in each case at 35 C. (A) Minus lysine: ●, no detergent, no fatty acid; □, 3% Tergitol NP40, no fatty acid. (B) Minus adenine: ●, no detergent, no fatty acid; △, 3% Tergitol NP40, no fatty acid. (C) Minus tryptophan: ●, no detergent, no fatty acid; □, 3% Tergitol NP40, no fatty acid.*

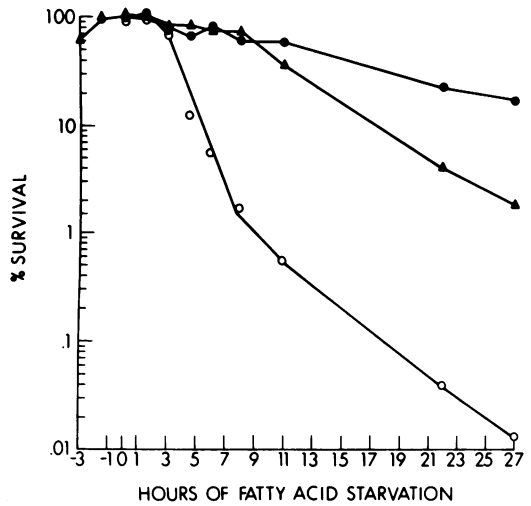

FIG. 12. *Effect on cell survival of prior starvation for lysine in strain BW2-5C (ole1, fas1, lys2). Defined medium is at 35 C. ●, Deprived of lysine from −3 h, continuously supplemented with 10^{-3} M myristic acid and 10^{-3} M oleic acid, 3% Tergitol NP40. ▲, Deprived of lysine from −3 h, supplemented with 10^{-3} M oleic acid and 10^{-3} M myristic acid, 3% Tergitol NP40. Shifted at 0 h to defined medium with no lysine and no fatty acid, 3% Tergitol NP40. ○, Supplied with lysine and fatty acid supplements until 0 h. Shifted at that time to defined medium plus lysine, no fatty acid, 3% Tergitol NP40.*

even less protection. At 3 h or later, the rate of cell death is unaffected by the addition of antibiotic. Once cell death has begun, it cannot be stopped by the addition of cycloheximide.

The rate of death is also decreased in petite cells of a given strain. This is illustrated in Fig. 14. This results in substantial selection for petites during fatty acid starvation, and frequently the final population after starvation is almost 100% petite, whereas the starting population contained only a few percent. There is no evidence that the cells are converted to petites during this process; rather, petites survive preferentially.

DISCUSSION

Both unsaturated and saturated fatty acids are essential not only for normal growth, but also for the maintenance of cell viability in yeast strains of the genotype *fas1*, *ole1*. In the absence of either or both types of fatty acid, cellular processes such as protein and ribonucleic acid synthesis, as measured by incorporation of precursors into trichloroacetic acid-precipitable material, continue for several hours (Fig. 3, 4). Under these conditions, however, cell division appears to be halted within 1 h (Fig. 2), cell viability is affected within 2 or 3 h (Fig. 5), and the ability of cells to resume normal growth when resupplied with fatty acid is affected by the 2nd h (Fig. 8).

Optical density increase of the cultures seems

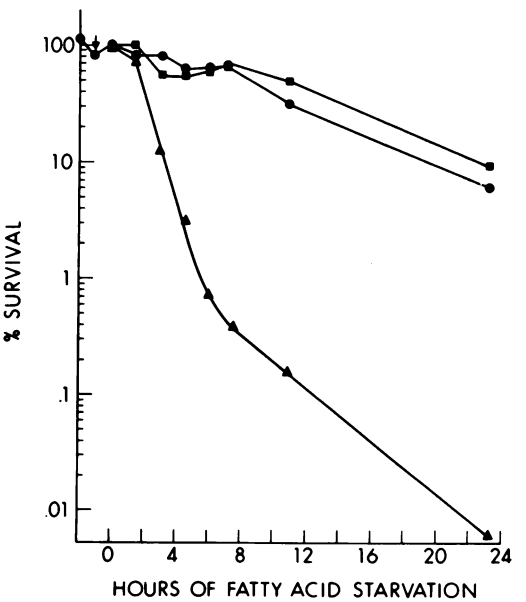

FIG. 13. *Effect of cycloheximide on cell survival of strain BW1-1C (ole1, fas1). ■, Cycloheximide (100 μg/ml) was given at −1 h in YEPD + TW. Culture was not subsequently shifted. ●, Cycloheximide (100 μg/ml) was given at −1 h in YEPD + TW: shifted at 0 h to YEPD; no fatty acid, 3% Tergitol NP40; cycloheximide (100 μg/ml). ▲, No cycloheximide was given; shifted at 0 h to YEPD; 3% Tergitol NP40, no fatty acid.*

to parallel macromolecular synthesis rather than cell number. The fact that cell counts taken by hemocytometer and optical density do not agree as measurements of cell growth points out the potential hazard of using optical density to measure cell proliferation under abnormal culture conditions. For example, when cells are transferred to medium containing only potassium acetate and no nitrogen, optical density increases substantially in haploid strains in which no cell division occurs (5). Optical density may be expected to increase with cell enlargement as well as increasing cell numbers. Although enlargement of the starved cells has not been quantified, examination by microscope suggests that this may be at least a partial explanation for the optical density increase.

The rapid cell death observed in the fatty acid starved strains is not a general characteristic of auxotrophic mutants that are starved for their requirements. This is demonstrated by the behavior of the adenine-, lysine-, and tryptophan-requiring strain 4335-7-3Bα (Fig. 11). Nor can rapid viability loss be attributed to the *ole*1 mutation, because strains having the genotype *ole*1, *Fas*$^+$ do not show this characteristic upon fatty acid starvation (Fig. 10). The characteristic loss of viability seems to be conferred upon the strain by the fatty acid synthetase mutation (*fas*1) (Fig. 10).

Mutations affecting lipid metabolism in other fungi (9, 13) and starvation in yeast for the vitamins biotin, pantothenate, or inositol (8, 16, 19) affect cell viability in a similar manner. If this is a phenomenon generally associated with lipid starvation in fungi, it is difficult to explain the comparatively high viability of the starved desaturase mutant (*ole*1). It cannot be argued that this mutation is "leaky" because it is an ochre-suppressible mutation and no residual ability to desaturate exogenous saturated fatty acids has been detected (7). However, although the *fas*1 mutant is totally unable to synthesize fatty acids, the desaturase mutant (*ole*1) can and does synthesize saturated fatty acids (7). In the absence of exogenous fatty acid, its lipids rapidly become deficient in unsaturated fatty acid (14). Therefore, the survival data from the *ole*1 mutant, when compared to those obtained from the other two types of fatty acid-requiring strains, imply that it is not an alteration in the ratio of saturated to unsaturated fatty acid that is lethal to the cell. Rather, it appears that the total failure to synthesize fatty acids or fatty acid containing lipids, or both, is involved in the death of *fas*1 and *ole*1, *fas*1 cells.

The desaturase mutant, when totally starved for fatty acid, should be formally equivalent to the double mutant supplied with saturated fatty acid only. However, under such conditions, the double mutant cells die rapidly (Fig. 5), in marked contrast to the continuing viability of the starved *ole*1 cells (Fig. 10). Under these conditions, the primary difference between the two strains is that the double mutant is supplied with exogenous fatty acid, whereas the desaturase mutant synthesizes its own. The difference in viability may, therefore, imply that exogenously supplied fatty acid is not fully equivalent to fatty acid that the organism synthesizes. Even the exogenous single fatty acids, however, provide some limited protection for the double mutant cells, because the rate and extent of cell death are reduced in the presence of either fatty acid compared with the results obtained in the total absence of fatty acid (Fig. 5).

"Clumping" of cells during fatty acid starva-

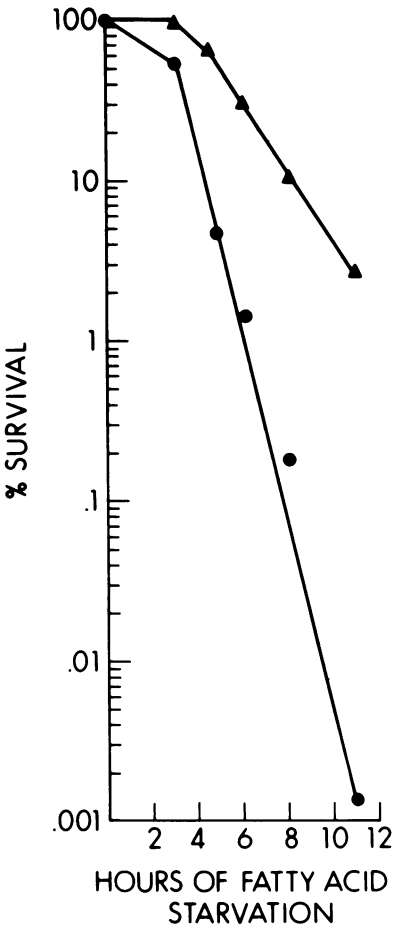

FIG. 14. *Survival of grande* (●) *and petite* (▲) *cells of strain BW2-5C* (*ole1, fas1, lys2*) *in complete defined medium, 3% Tergitol NP40, no fatty acid, 35 C.*

tion is also observed in *ole*1 strains and not in *ole*1, *fas*1 strains or in *fas*1 strains. Again, it may be argued that the primary difference between *ole*1 and *ole*1, *fas*1 strains during fatty acid starvation is the synthesis of saturated fatty acid by the former. It seems likely, therefore, that the clumping may be related to this difference. The synthesis of excess saturated fatty acid or its transport to the cell's exterior may alter the cell surface, perhaps causing it to become more hydrophobic.

Even after short periods of starvation not involving viability loss, the mutant strain *ole*1, *fas*1 fails to resume normal growth rates (Fig. 8, 9). There are two possible interpretations: one, that all of the cells have been altered in some way that causes them to have a lengthened cell cycle for a period after starvation or, and probably more likely, that some cells are dividing normally, but that some fraction has been stopped and requires a longer period of recovery. Cells at different stages of cell division may be affected differentially by fatty acid deprivation. The failure of cells that are actively budding to be proportionately represented among the survivors, as tested by methylene blue staining, lends support to this idea. This hypothesis could be tested directly by experiments with synchronous cultures.

The death observed in fatty acid-starved cells is dependent upon protein synthesis (or perhaps macromolecular synthesis in general), as illustrated by the protection provided by cycloheximide treatment (Fig. 13) or by prior starvation for amino acid (Fig. 12). These results are quite similar to those reported by Littlewood (11) for a temperature-sensitive mutant of *S. cerevisiae* that dies at the restrictive temperature unless protein synthesis is blocked. The specific metabolic defect in the Littlewood mutant has not been identified. However, the results reported here suggest that if temperature-sensitive mutants that are defective in various aspects of lipid metabolism were isolated, they might be expected to exhibit properties similar to the Littlewood mutant.

The Littlewood mutant was used in the isolation of mutants sensitive to various inhibitors of protein synthesis (11). Likewise, inositol requirers of *Neurospora* (9) and biotin-requiring mutants of *Aspergillus* (13) were effectively used in the selection of other auxotrophic mutants. There is every reason to believe that the fatty acid-requiring strains will likewise prove to be valuable in the selection of various types of mutants. The types of mutations that might be selected if conditions were properly manipulated include temperature-sensitive mutations blocking protein synthesis and possibly macromolecular synthesis in general, mutants sensitive to inhibitors of macromolecular synthesis and, of course, certain types of auxotrophic mutants. Studies to determine the conditions necessary for effective selection are currently underway.

The selective advantage of petites (Fig. 14) gives an indication of the selective potential of this system. It is not clear why petites should die less rapidly than grandes. Possibly, their fatty acid requirements are less demanding due to the defective mitochondria, or perhaps it is simply a matter of slower growth during the period of starvation. Indeed, conditions promoting rapid metabolism, such as higher temperature (Fig. 6) and enriched medium (Fig. 5), tend to increase the rate of cell death. In any event, the preferential survival of petites may provide a partial explanation for the reported tendency of fatty acid requiring strains to generate petites at a high frequency (4).

Fatty acids are required for the synthesis of phospholipids, which are important structural elements of cell membranes. The cell death reported here may, therefore, result from membrane abnormalities caused by fatty acid starvation. The observation that the detergent Tergitol NP40 accelerates viability loss (Fig. 7) suggests that changes at the cell surface may be involved. This detergent has no effect upon the growth rate of fatty acid-independent cells (Fig. 1) and very little effect upon the viability of cells which have stopped growing due to treatment with cycloheximide (Fig. 13) or amino acid starvation (Fig. 11, 12). However, if weakening of the plasma membrane is involved in the death associated with fatty acid starvation, the membrane might become more susceptible to disruption by detergents.

The cell death described here is the result of some cellular "imbalance," quite probably involving cell membranes, that occurs when the synthesis of fatty acid-containing lipids is prevented in an otherwise functioning cell. The fact that the production of this imbalance can be prevented by halting protein synthesis suggests the following hypothesis. In the absence of fatty acids, the cell may continue to construct membranes that have an increasingly abnormal protein to lipid ratio, eventually resulting in nonfunctional membranes and consequently, death. If true, this hypothesis would imply a very important corollary—that the coupling of synthesis and assembly of the lipid and protein components of the cellular membranes is incomplete. This hypothesis is testable to the extent that the composition of the various

cellular membranes can be determined in starved and normal cells. However, even if substantial changes can be detected in membrane composition, it may be very difficult to establish the cause of cell death. Currently, the composition of these cells, their lipid metabolism during starvation, and their membrane physical properties and transport capacities are under investigation.

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LITERATURE CITED

1. Bürkl, G., H. Castroph, and E. Schweizer. 1972. Mapping a complex gene locus coding for part of the *Saccharomyces cerevisiae* fatty acid synthetase multienzyme complex. Mol. Gen. Genet. **119**:315-322.
2. Fincham, F. R. S., and P. R. Day. 1963. Fungal genetics. F. A. Davis, Philadelphia.
3. Hawthorne, D. C., and R. K. Mortimer. 1960. Chromosome mapping in *Saccharomyces* centromere-linked genes. Genetics **45**:1085-1110.
4. Henry, S. A., and S. Fogel. 1971. Saturated fatty acid mutants in yeast. Mol. Gen. Genet. **113**:1-19.
5. Henry, S. A., and H. O. Halvorson. 1973. Lipid synthesis during sporulation of yeast. J. Bacteriol. **114**:1158-1163.
6. Henry S. A., and A. D. Keith. 1971. Membrane properties of saturated fatty acid mutants of yeast revealed by spin labels. Chem. Phys. Lipids **7**:245-265.
7. Keith, A. D., M. R. Resnick, and A. B. Haley. 1969. Fatty acid desaturase mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **98**:415-420.
8. Kuraishi, H., Y. Takamura, T. Mizunaga, and T. Uemura. 1971. Factors influencing death of biotin deficient yeast cells. J. Gen. Appl. Microbiol. **17**:29-42.
9. Lester, H. E., and S. R. Gross. 1959. Efficient method for selection of auxotrophic mutants of *Neurospora*. Science **139**:572.
10. Lindegren, C. C. 1949. The yeast cell: its genetics and cytology. Educational Publishers Inc., St. Louis, Mo.
11. Littlewood, B. S. 1972. A method for obtaining antibiotic sensitive mutants in *Saccharomyces cerevisiae*. Genetics **71**:305-308.
12. Lynen, F. 1967. The role of biotin dependent carboxylations in biosynthetic reactions. Biochem. J. **102**:381-400.
13. Pontecorvo, G., J. A. Roper, L. M. Hemmons, K. D. MacDonald, and A. W. J. Bufton. 1953. The genetics of *Aspergillus nidulans*. Advan. Genet. **5**:141.
14. Proudlock, J. W., J. M. Haslam, and A. W. Linnane. 1971. Biogenesis of mitochondria 19: the effects of unsaturated fatty acid depletion on the lipid composition and energy metabolism of a fatty acid desaturase mutant of *Saccharomyces cerevisiae*. Bioenergetics **2**:327-349.
15. Resnick, M. A., and R. K. Mortimer. 1966. Unsaturated fatty acid mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **92**:597-600.
16. Ridgway, C. J., and H. C. Douglas. 1958. Unbalanced growth of yeast due to inositol deficiency. J. Bacteriol. **76**:163-166.
17. Schweizer, E., and H. Bolling. 1970. A *Saccharomyces cerevisiae* mutant defective in saturated fatty acid biosynthesis. Proc. Nat. Acad. Sci. U.S.A. **67**:660-666.
18. Sherman, F., and C. W. Lawrence. 1973. Genetics of the yeast *Saccaromyces*. *In* Robert King (ed.), Handbook of genetics. Van Nostrand Reinhold Co., New York.
19. Shimada, S., H. Kuraishi, and K. Aida. 1972. Unbalanced growth death of yeast due to pantothenate deficiency. J. Gen. Appl. Microbiol. **18**:383-397.
20. Vagelos, P. R., P. W. Majerus, A. W. Alberts, A. R. Larrabee, and B. P. Aihaud. 1966. Structure and function of acyl carrier protein. Fed. Proc. **25**:1485-1495.
21. Wisnieski, B. J., A. D. Keith, and M. R. Resnick. 1970. Double-bond requirement in a fatty acid desaturase mutant of *Saccharomyces cerevisiae*. J. Bacteriol. **101**:160-165.