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CHANGES IN CELL PERMEABILITY FOLLOWING A MARKED REDUCTION OF SATURATED FATTY ACID CONTENT OF *ESCHERICHIA COLI* K-12

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SUMMARY

The present study examines the extent to which the fatty acid composition of the membrane lipid can be altered by nutritional means in mutants of *Escherichia coli* defective in total fatty acid synthesis. These changes are compared to those observed in wild type cells subjected to the same conditions of fatty acid supplementation. Abnormalities in physiological behavior of whole cells and membranes are related to extremes in fatty acid composition that can be produced in the mutant but not the wild type cells. In particular, when the saturated fatty acid content of the membrane lipid is reduced below approx. 15 %, the barrier properties of the membrane toward small molecules such as K^+ and a lactose analog decreases abruptly. This change is also reflected in the diminished temperature dependence of passive permeability and of NADH oxidase activity associated with the cytoplasmic membrane. Detailed studies on the properties of specific membrane functions in relation to the physical behavior of membrane lipids should be possible with this biological system possessing a relatively simple membrane lipid structure in which the mole percentage of specific lipid components can be systematically varied.

INTRODUCTION

Most natural membranes possess a complex lipid composition. However, it is not known how this heterogeneity relates to membrane structure and biological function. Observations that many kinds of cells can utilize exogenous fatty acids for phospholipid synthesis and at the same time reduce their own fatty acid synthesis has led to attempts to simplify the fatty acid composition of biological membranes by nutritional means. The microbial systems most fully utilized in these studies are *Mycoplasma* (including *Acholeplasma*), *Saccharomyces*, and *Escherichia coli*. Of these, the latter has several advantages: for example, it grows readily on simple defined media under aerobic or anaerobic conditions, requires and produces no sterols or

Abbreviations: ONPG, *o*-nitrophenyl-thiogalactoside; IPTG, isopropyl-thiogalactoside.

triglycerides, and possesses a very simple phospholipid and fatty acid composition. In each of these systems, there are examples of extensive replacement in the membrane lipid of fatty acyl groups normally produced by the cell with fatty acids derived from the medium [1–5]. The availability of fatty acid biosynthetic mutants in bacteria [6–12], yeast [13–15], and fungi [16] has greatly expanded these studies and made it possible to determine the ability of different fatty acids to be incorporated into phospholipid and to sustain physiological processes [2, 8, 10, 17–25]. Furthermore, these biological systems in which the lipid composition can be simplified and systematically adjusted are valuable for defining the physical behavior of membrane lipids and how their physical state affects membrane structure and function [2, 23, 26–31].

In the present study, we have determined how the fatty acid composition of membrane phospholipid changes under various conditions of fatty acid supplementation utilizing *E. coli* mutants defective in total fatty acid synthesis. Secondly, we have focused on one type of change in composition and have taken a first step toward characterizing the role of saturated fatty acids in biological membranes that contain almost exclusively phospholipids but no sterols as the lipid components.

MATERIALS AND METHODS

Materials

Fatty acids were obtained from NuCheck Prep (Elysian, Minn.) or Hormel (Austin, Minn.); labeled compounds were from New England Nuclear; and Brij 58 was the gift of Atlas Chemical Co.

Strains and media

All bacterial strains used in this study are K-12 strains of *E. coli*. LA1-6 and LA2-22 are thermosensitive mutants for fatty acid biosynthesis derived from AB1623 [12]. AB1623 *fad*E cells and LA2-22 *fad*E cells were derived by conjugation of AB1623 and LA2-22, respectively, with HfrC *fad*E cells as described earlier [5]. The *fad*E cell defect prevents degradation of fatty acids derived from the medium [32].

Minimal growth media were Medium 63 [33] or Medium 56-LP [9] containing 0.4 % glycerol, 5 mM potassium glutamate and 0.5 μg/ml yeast extract. In Medium 56-LP, 10 mM phosphate was used instead of 0.3 mM phosphate and the potassium concentration was adjusted to the indicated levels. The potassium concentration of Medium 63 was 115 mM. These two media are designated 63-GBGY and 56LP-GBGY. In experiments employing radioisotopes, leucine (0.4 mM), isoleucine (1.25 mM), and valine (1 mM) were also present in Medium 56LP-GBGY. Brij 58 (400–1600 μg/ml) was added to solubilize the fatty acid supplement (100 μg/ml). Cell innocula were obtained by growing the appropriate strain overnight at 30 °C in the absence of fatty acid supplement unless stated otherwise. All cultures were grown subsequently at 37 ± 0.3 °C in a New Brunswick gyratory water bath shaker under designated conditions of fatty acid supplementation. Growth was followed turbidometrically with a Klett–Summerson colorimeter at 660 nm or with a Gilford spectrophotometer at 600 nm.

Viability was measured by diluting the cells appropriately in minimal growth medium at room temperature and plating them in top agar (pour plate method).

Lipid analysis

Unless otherwise stated, cells were sampled from the culture, collected by centrifugation, and washed one time with Medium 63. Lipids were extracted from the cells, phospholipids were isolated by thin-layer chromatography, and fatty acid methyl esters were prepared from the total phospholipid fraction and analyzed by gas–liquid chromatography employing methods described earlier [25].

Net synthesis of DNA, RNA, protein, and phospholipids

Cell innocula were grown in Medium 56LP-GBGY overnight at 30 °C in the presence of a radioisotopically labeled precursor to achieve steady-state conditions for labeling and then subcultured at 37 °C in fresh medium containing the same labeled compound. In general, procedures were similar to those described by Glaser et al. [34]. Net accumulation of DNA and phospholipids was measured with $^{32}PO_4^{3-}$ (12.4 μCi/ml culture medium to give a specific radioactivity of 1.24 Ci/mole in the medium). Aliquots of the cultures (0.8 ml) were sampled periodically during growth and added to 3 ml of methanol–chloroform (2 : 1, v/v). The phospholipids were extracted from the supernatant and counted in Bray's solution. The pellets were suspended in 1 ml of 0.5 M NaOH, incubated overnight at 37 °C to hydrolyze the RNA and then added to 1 ml of 20 % trichloroacetic acid. The samples were filtered on HA 0.45 μm Millipore filters, and then the latter were washed with 5 % trichloroacetic acid and counted in RPI 3a70 liquid scintillation fluid containing 0.005 % sodium dodecylsulfate. For measurement of net accumulation of RNA and protein, [^{14}C]uracil (0.3 μCi/ml culture medium, 1.35 Ci/mole) and [^{3}H]leucine (0.2 μCi/ml culture medium, 0.5 Ci/mole), respectively, were added to separate cultures. Unlabeled thymidine (50 μg/ml culture medium) was added to the culture medium containing radioactive uracil. At different times during growth of the cultures, cell aliquots (0.5 ml) were sampled and added to 1 ml 10 % trichloroacetic acid. The samples were collected on filters and these were washed and counted as described above for the DNA samples. Blanks were determined by addition of aliquots of the culture medium to a tube containing the radioactive precursor and either chloroform–methanol in the case of the phospholipid measurement or trichloroacetic acid for the DNA, RNA, and protein determinations.

In vivo o-nitrophenylthiogalactoside (ONPG) hydrolysis and β-galactosidase activity

Lactose transport and β-galactosidase activity were induced with 0.5 mM isopropylthiogalactoside (IPTG) in an overnight innoculum growing at 30 °C in Medium 63-GBGY. Induction was maintained with IPTG when the cells were subcultured at 37 °C. Aliquots of the culture medium were harvested periodically during growth, and washed once with Medium 63-GBGY containing 50 μg of chloramphenicol per ml, but lacking inducer, Brij, and fatty acids. These manipulations were performed at room temperature. The cells were resuspended in Medium 63-GBGY at room temperature and immediately assayed for ONPG hydrolysis. The assay for in vivo ONPG hydrolysis was similar to that described by Fox and Kennedy [35]. It was conducted in a total volume of 0.4 ml Medium 63-GBGY containing 10 mM NaN_3, 2 mM ONPG, and cells equivalent to 10–100 μg of protein. After incubation at the desired temperature, the reaction was stopped with 0.6 ml of 1 M K_2CO_3. A blank was prepared by adding K_2CO_3 to the cell suspension before the

addition of ONPG. All assays were read at 420 nm and the micromoles of ONPG released computed from the millimolar extinction coefficient of 4.34. Passive permeability was measured when 6 mM thiodigalactoside was present in the cell suspension. Active transport was calculated as the total in vivo ONPG hydrolysis less the passive permeability. Total β-galactosidase activity was determined by sampling cells (1 ml) periodically during growth. Unwashed samples were treated with a drop of toluene and incubated at 37 °C for 20 min. The release of nitrophenol from ONPG at 37 °C was then determined.

Preparation of cytoplasmic (inner) membranes and assay of NADH oxidase

Cell membranes were prepared by isopycnic centrifugation of sonicated spheroplasts exactly as described by Osborn and coworkers [36, 37]. NADH oxidase activity in the cytoplasmic membrane fraction was assayed as described by Osborn et al. [37] using a Gilford 240 recording spectrophotometer. It was completely inhibited by 10 mM cyanide. Measurements were made at temperatures ranging from 20 to 43 °C and controlled within 0.2 °C by circulating ethylene glycol from a Forma Bath through the jacket of the cuvette chamber.

Protein analysis

Protein was determined by the modified microbiuret method of Mokrasch and McGilvery [38] unless stated otherwise.

RESULTS

Growth and fatty acid composition of fab^{ts} mutants under various conditions of fatty acid supplementation

When the temperature-sensitive mutants, LA1-6 and LA2-22 *fad*E cells, are grown at 30 °C and then subcultured at 37 °C, several patterns of growth are observed depending on the kinds of fatty acid supplements available (Fig. 1). As a step toward understanding the physiological behavior of the cells under these various culture

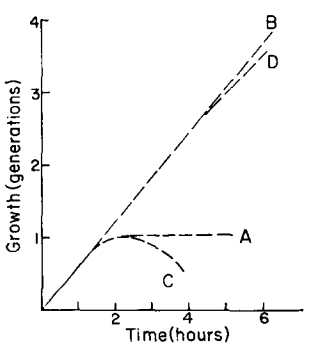

Fig. 1. Growth scheme of *fab*^{ts} mutants on various fatty acid supplements. Cell innocula were grown at 30 °C and subcultured at 37 °C at time zero as described in the text. Patterns, A, B, C, and D are characteristic of growth of the cells on various supplements which sustain growth for 1.0 to 2.0, unlimited, about 1.0, and 3.0 to 4.0 generations, respectively.

TABLE I

PATTERN OF GROWTH AND FATTY ACID COMPOSITION OF *fab*^{ts} MUTANTS UNDER VARIOUS CONDITIONS OF FATTY ACID SUPPLEMENTATION

Cells of the strains shown in the table were grown at 37 °C in Medium 63-GBGY with the fatty acid supplements indicated as described in the text. Cultures which grew as depicted in Patterns A and C were harvested when growth stopped; those which grew as shown in Patterns B and D were collected in late exponential phase. Lipid analysis was performed as described in the text.

Pattern of growth, strain and supplement[a]	Extent of growth[b] (generations)	Fatty acid composition											
		Saturated						Monounsaturated					
								cis				*trans*	
		14	15	16	17	18	T[c]	14	16	17	18	18	T[c]
A													
1. LA2-22 *fad*E none	1.0	14		30			44		36		18		54
2. LA1-6 none	1.0	17		38			55		15		28		43
3. LA1-6 14:0	1.3	53		19			72		19		8		27
4. LA1-6 *cis*-Δ^9-14:1	1.0	17		36			53	5	14		28		47
5. LA1-6 *cis*-Δ^9-18:1	1.9	8		15			23		20		54		74
6. LA1-6 14:0 + *cis*-Δ^9-16:1	1.0	22		28			50		28		20		48
7. LA1-6 15:0 + *cis*-Δ^9-14:1	1.3	10	48	15			73	7	73		6		26
8. LA1-6 15:0 + *cis*-Δ^9-16:1	1.7	8	29	11			48		39		11		50
9. LA1-6 16:0 + *cis*-Δ^9-14:1	2.0	9		64			73	11	11		4		26
10. LA1-6 17:0 + *cis*-Δ^9-14:1	1.0	6	2	12	46		66	14	10		9		33
11. LA1-6 17:0 + *cis*-Δ^9-16:1	1.8	8		8	29		45		48		6		54
12. LA1-6 18:0 + *cis*-Δ^9-16:1	1.0	10		36		4	50		32		18		50

B													
13. LA2-22* 16:0+*cis*-Δ^9-16:1	3.5	4		34			39		53		8		61
14. LA2-22 *fad*E 17:0+*cis*-Δ^{10}-17:1	3.5	2		3	29		34		7	57	2		66
15. LA1-6 16:0+*cis*-Δ^9-18:1	>10	4		46			50		14		35		49
16. LA1-6 17:0+*cis*-Δ^9-18:1	>10	3	2	3	41		49		11		40		51
17. AB1623*fad*E* none	3.0	2		32			34		41		24		65
18. AB1623*fad*E* 16:0	3.0	1		60			61		33		6		39
19. AB1623*fad*E* *cis*-Δ^{11}-18:1	3.0	2		22			24		34		42		76
20. AB1623*fad*E* *trans*-Δ^{11}-18:1	3.0	1		18			19		32		32	17	81
C													
21. LA2-22*fad*E 16:0	1.2	2		72			74	1	19		6		26
22. LA2022*fad*E 18:0	1.0	3		11		60	74		19		7		26
23. LA2-22*fad*E** 16:0	1.0			65		3	68		6		23		29
24. LA2-22*fad*E** 18:0	0.8	1		6		58	65		7		28		35
D													
25. LA2-22*fad*E *cis*-Δ^{11}-18:1	3.2			2			2		10		88		98
26. LA2-22*fad*E *trans*-Δ^{11}-18:1	3.7	1		3		1	5		10		27	58	95

[a]A, B, C. and D patterns of growth refer to those depicted in Fig. 1.
[b]Refers to number of generations of growth at 37 °C before harvest.
[c]T is total saturated or monounsaturated fatty acid percentages.

* Data for these cultures were taken from earlier publications [5, 12].

** Cultures in which cell innocula were grown at 30 °C with *cis*-Δ^{11}-18:1 prior to subculturing at 37 °C with the indicated supplement.

conditions, we sought first to determine how growth related to changes in the quality and in the amount of phospholipid synthesis.

When LA1-6 or LA2-22 *fad*E cells are cultured without supplement at 37 °C, the cells grow for one to one and one-half generations (Fig. 1, Curve A). They remain viable for at least a few hours after growth stops, as can be shown by the restoration of normal growth within 1 h following the addition of saturated and unsaturated fatty acids to the culture medium (data not shown). Fatty acid compositions of the phospholipid from the two mutants harvested at the time growth ceased are given in Table I (Lines 1 and 2). The fatty acid content of phospholipid from wild type (AB1623 *fad*E) cells growing exponentially in the absence of supplement is shown for comparison (Line 17). Although the phospholipid from LA1-6 and LA2-22 *fad*E cells contains relatively more saturated fatty acids, especially 14 : 0, than that from AB1623 *fad*E cells, it is apparent that growth stopped without any extreme shift in composition such as that observed following growth on long chain saturated or unsaturated fatty acids as described below.

Many conditions of supplementation lead to a relatively brief period of growth resembling that of the unsupplemented cultures (Fig. 1, Curve A). We have included here culture conditions in which the growth interval ranged from one to two generations. This inability to support growth is not due to the failure to incorporate the supplement(s) (Table I, Lines 3–12). In each instance, the fatty acid content of the phospholipid is enriched with respect to the supplement(s) although not always extensively (for example, *cis*-Δ^9-14 : 1, Lines 4,7,9,10). Only certain combinations of saturated and unsaturated fatty acids maintain growth indefinitely (Fig. 1, Curve B). The fatty acid composition of phospholipid from cells cultured on some effective pairs are given in Table I (Lines 13–16). Although the relative amounts of saturated (34–50 %) and unsaturated (50–66 %) fatty acid groups vary somewhat in these examples, the proportions are well within the range (20–60 % saturated and 40–80 % unsaturated fatty acids) accommodated by wild type cells when supplemented with either saturated or unsaturated fatty acid (Table I, Lines 18 and 19). The presence of fatty acids other than those provided in the culture medium arises from residual fatty acid synthesis in the mutants at 37 °C [12] and, in the case of LA1-6, also from oxidation of the supplement prior to its incorporation into the phospholipid. In order to readily appreciate the contribution of synthesis and supplementation to the fatty acid composition at 37 °C, the LA2-22 *fad*E culture whose composition is given here (Line 14) was grown with odd-chain saturated and unsaturated fatty acids not normally found in *E. coli*.

A third pattern of growth (Fig. 1, Curve C) is evident when mutant cells are provided with a long chain saturated fatty acid (16 : 0 and 18 : 0). After an initial brief period of growth, the cell density decreased abruptly due to cell lysis as demonstrated below by the release into the medium of the cytoplasmic enzyme, β-galactosidase. Thus, this pattern is distinct from that depicted in Fig. 1 by Curve A in which growth is followed by a stable stationary phase and the cells do not die. The fatty acid compositions of the phospholipids isolated from cells grown with long chain saturated fatty acids are shown in Table I (Lines 21–24). The compositions can be influenced by several variables. Studies with LA2-22 *fad*E cells, which cannot oxidize fatty acids and which were harvested before the cell density began to drop, provide an approximate estimate of the saturated fatty acid content associated with cessation of growth (74 %,

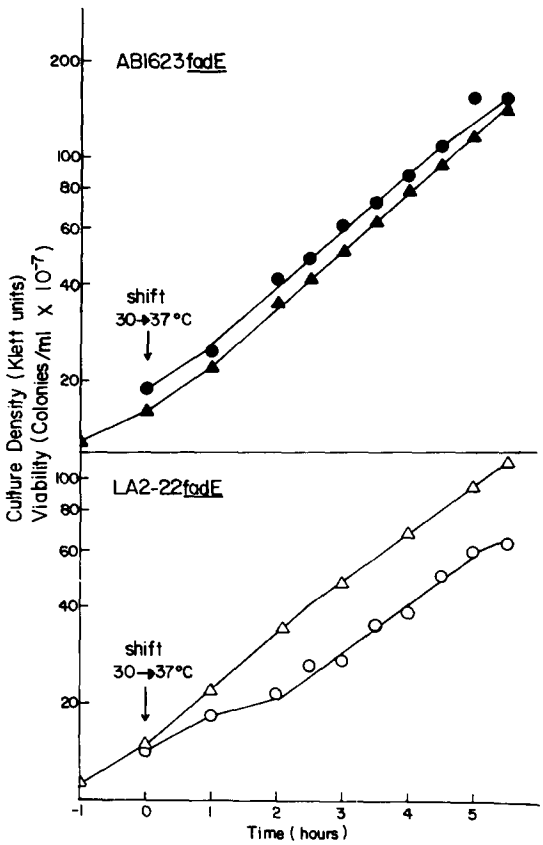

Fig. 2. Growth of AB1623 *fad*E and LA2-22 *fad*E cells at 37 °C with *cis*-vaccenate as measured by cell turbidity and viability. Experimental conditions are described in the text. Triangles and circles are optical densities and viabilities, respectively.

Lines 21 and 22). When the mutant cells contain more *cis*-Δ^{11}-18 : 1 relative to *cis*-Δ^{9}-16 : 1 (produced by growth with *cis*-Δ^{11}-18 : 1 at 30 °C prior to subculturing at 37 °C in a medium which contains a saturated fatty acid), they stop growing sooner and at somewhat lower total saturated fatty acid contents (68 and 65 %, Lines 23 and 24) than cells which possess less *cis*-Δ^{11}-18 : 1 (Lines 21 and 22). In all instances where growth followed the pattern shown in Fig. 1, Curve C, the saturated fatty acid content obtained when growth stopped (for example, 74 % Line 21) was greater than that attained by wild type cells cultured with the same supplement (61 % Line 18). A better estimate of the maximum content of saturated fatty acid compatible with normal physiological processes would be that obtained from mutant cells harvested just before the growth rate slowed.

When mutant cells are provided certain long chain unsaturated fatty acids, growth continues normally for at least two to three generations and then begins to slow down. Since the cell growth is clearly more protracted here than that represented by Fig. 1, Curve A, it is depicted by Curve D. This pattern was seen with *cis*- or *trans*-vaccenic acid as supplement. The cells remain viable as shown in Fig. 2 for LA2-22

*fad*E cells grown with *cis*-Δ^{11}-18 : 1. The viable cell count relative to the cell density is lower for LA2-22 *fad*E cells compared to AB1623 *fad*E cells. This difference is due to the formation of cell chains by the mutant (as seen under the phase contrast microscope) on raising the culture temperature from 30 to 37 °C. These chains are reduced by vortexing to cell pairs for the most part and, consequently, each clone which arises after plating is generally derived from two rather than one cell(s). This behavior of LA2-22 *fad*E cells is found for cells supplemented with 16 : 0 plus *cis*-Δ^{9}-16 : 1 as well as with *cis*-Δ^{11}-18 : 1 alone. Long chain unsaturated fatty acids can be incorporated equally well into both positions of the phospholipid [5] and, hence, these supplements alone might be expected to sustain phospholipid synthesis. That this is the case is shown by the very high unsaturated fatty acid content (98 and 95 %) of the phospholipid from these cultures after about three generations of growth (Table I, Lines 25 and 26). The unsaturated fatty acid composition of the mutant cells grown for several generations with *cis*- or *trans*-Δ^{11}-18 : 1 is far in excess of that found in AB1623 *fad*E cells grown under identical conditions (compare Lines 25 and 26 with Lines 19 and 20, respectively).

TABLE II

RELATIVE AMOUNTS OF LIPID, PROTEIN, AND DEOXYNUCLEIC ACID IN CELLS OF STRAINS AB1623 AND LA2-22

AB1623 and LA2-22 cells which had been grown overnight at 30 °C in Medium 63-GBGY containing Brij 58 and *cis*-Δ^{9}-18:1 were subcultured at 30 or 37 °C with indicated fatty acid supplements. Two generations later aliquots were removed for lipid and protein analysis while the cells were in exponential growth. Cell pellets were washed with 0.01 M Tris buffer, pH 7.2, to remove traces of inorganic phosphate from the medium and then extracted for lipid according to the method of Bligh and Dyer [39]. Lipid extracts were analyzed for organic phosphate using lecithin and glucose 6-phosphate as standards [40]. Protein in washed cell pellets was determined by the method of Lowry et al. [41]. AB1623 *fad*E and LA2-22 *fad*E cells were grown in Medium 56LP-GBGY containing 125 mM K^+ and $^{32}PO_4^{3-}$ as described in the text under Net synthesis of DNA, RNA, protein and phospholipids. After three generations of experimental growth at 37 °C, aliquots were removed from the cultures to determine the radioactivity in DNA and phospholipid. The amounts (nmoles) of deoxynucleic acid phosphate and lipid phosphate were computed from the radioactivity in the two fractions and the known specific radioactivity of the precursor phosphate.

Strain	Growth temperature (°C)	Fatty acid supplement	nmoles lipid phosphate per mg protein	nmoles lipid phosphate per nmole DNA phosphate
AB1623	30	none	0.143	
AB1623	37	*trans*-Δ^{9}-16:1	0.138	
AB1623	37	*cis*-Δ^{9}-18:1	0.144	
AB1623*fad*E	37	*cis*-Δ^{11}-18:1	0.142	0.89
LA2-22	30	none	0.100	
LA2-22	37	*trans*-Δ^{9}-16:1	0.104	
LA2-22	37	*cis*-Δ^{9}-18:1	0.084	
LA2-22*fad*E	37	*cis*-Δ^{11}-18:1	0.097	0.35

Macromolecular synthesis in LA2-22 fadE cells under various conditions of fatty acid supplementation

The observations on fatty acid composition presented above reveal specific qualitative changes in the phospholipid of the mutant in response to various conditions of fatty acid supplementation. Preliminary studies with AB1623 (or AB1623 *fad*E) and LA2-22 (or LA2-22 *fad*E) cells revealed that the mutant cells grown at 30 °C without supplement or at 37 °C with various fatty acids contained less phos-

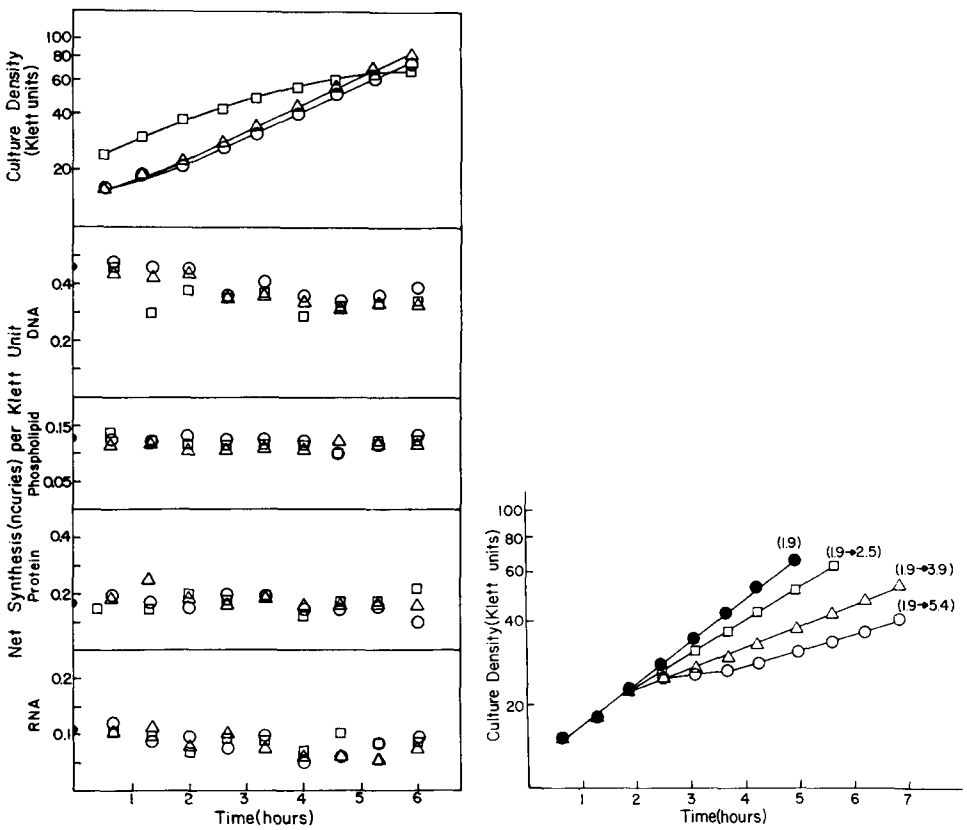

Fig. 3. Net synthesis of DNA, phospholipid, protein, and RNA in LA2-22 *fad*E cells grown under various conditions of fatty acid supplementation. LA2-22 *fad*E cells were grown in Medium 56LP-GBGY containing 150 mM K^+ and the incorporation of different radioisotopes in separate cultures were determined as described in the text. Cultures were shifted from 30 to 37 °C at zero time (●). ○, △, □, cells grown with 16:0 plus *cis*-Δ^9-16:1, with *cis*-Δ^{11}-18:1, and without supplement, respectively.

Fig. 4. Effect of fatty acid supplement and K^+ concentration on growth of LA2-22 *fad*E cells at 37 °C. The cell innoculum was grown in Medium 56LP-GBGY containing 24 mM K^+ as described in the text and then washed with Medium 56LP containing 5 mM K^+. At time zero, the cells were resuspended in Medium 56LP-GBGY containing various concentration of K^+ and cultured at 37 °C with the fatty acid supplement indicated. ●—●, LA2-22 *fad*E cells with 16:0 plus *cis*-Δ^9-16:1 at 16, 24, 44, 84 or 204 mM K^+ or with *cis*-Δ^{11}-18:1 at 84, 104 or 204 mM K^+; ○—○, △—△, □—□, LA2-22 *fad*E cells with *cis*-Δ^{11}-18:1 at 16, 24 and 44 mM K^+, respectively. Numbers in parentheses are doubling times in hours; where generation times increase, two numbers are given.

pholipid relative to protein or deoxynucleic acid than wild type cells (Table II). In addition to this difference, LA2-22 cells were found to be approximately twice as long as AB1623 cells when viewed by phase contrast microscopy. Using the dimensions of 1.0 μm $\times$ 2.0 μm for the average *E. coli* cell, the altered lipid content can be explained only in part by a reduced surface to volume ratio associated with the formation of elongated rods. Hence, it seems likely that the amount of envelope per cell dry weight might be reduced in LA2-22 as it is in elongated cells growing on a favorable carbon source or in an enriched medium [42, 43]. In the present study we wished to know if the lipid content would be affected in LA2-22 *fad*E cells by the availability and type of acyl groups in the culture medium at 37 °C. Using appropriate radioisotopes to monitor synthesis, we observed that net synthesis of phospholipid and protein continued unchanged for a few generations after the shift from 30 to 37 °C (Fig. 3), while nucleic acid synthesis declined slightly. Since growth of LA2-22 *fad*E cells at 37 °C with *cis*-vaccenate led to a membrane fatty acid composition dramatically different from that normally found in *E. coli*, we sought to determine what effect this change had on the functional properties of the membrane.

Altered permeability of LA2-22 fadE cells grown on cis-vaccenic acid

In the course of the studies on macromolecular synthesis described above, it was observed that the growth rate of LA2-22 *fad*E cells cultured at 37 °C on *cis*-Δ^{11}-18 : 1 became dependent (after half a generation) on external potassium levels at concentration below 80 mM (Fig. 4). On the other hand, when the mutant was provided with both 16 : 0 and *cis*-Δ^{9}-16 : 1 as supplement, growth was not affected by potassium concentrations in the medium as low as 14 mM. These findings suggested that a change in cell permeability occurred in response to an altered membrane lipid composition produced by growth on *cis*-Δ^{11}-18 : 1. To establish this correlation, we examined passive permeability and active transport of the lactose analog, ONPG in whole cells as a function of the changing fatty acid composition of the membrane phospholipids (Fig. 5). When LA2-22 *fad*E cells were grown at 37 °C for approximately half a generation on *cis*-Δ^{11}-18 : 1, passive permeability of the sugar analog abruptly increased (16-fold) and active transport declined concurrently. The increased hydrolysis of ONPG was not due to the release of β-galactosidase from the cells under the assay conditions. In addition, there was no cell lysis in the culture as shown by the absence of β-galactosidase activity in the medium (Fig. 5). These changes occurred after the saturated fatty acid content had fallen below approx. 15 % (Fig. 5). If this experiment was performed in media containing low potassium concentrations, the growth rate decreased at this same composition (data not shown). When wild type cells (AB1623 *fad*E) were grown under identical conditions, the saturated fatty acid composition decreased to a steady-state level of 19 % and there was no change in permeability or transport (Fig. 5b). Furthermore, although active transport fell to about 60 % that of AB1623 *fad*E cells, there was no change in permeability if the mutant was cultured with both saturated and unsaturated fatty acids as supplements (Fig. 5c; steady-state saturated fatty acid content was 38 %) [12]. To further characterize the change in passive permeability, we determined the activation energy for permeability from Arrhenius plots made at different time points during growth on 16 : 0 and *cis*-Δ^{9}-16 : 1 or on *cis*-Δ^{11}-18 : 1 alone (Fig. 6). In LA2-22 *fad*E cells cultured on 16 : 0 and *cis*-Δ^{9}-16 : 1 (Fig. 6a) and AB1623 *fad*E cells cultured on

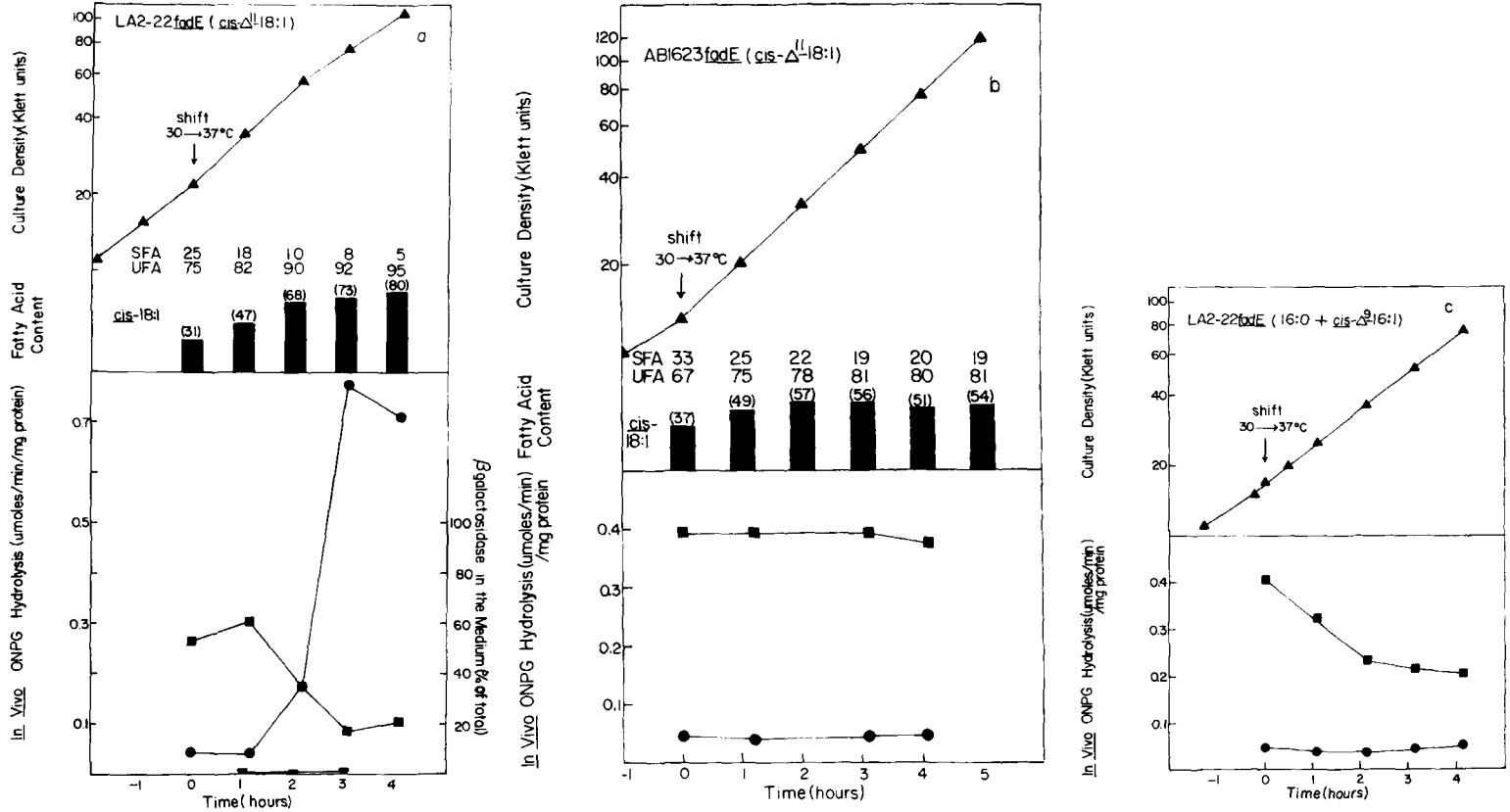

Fig. 5. Correlation of changes in vivo ONPG hydrolysis and fatty acid composition in AB1623 *fad*E and LA2-22 *fad*E cells. Cells were grown in Medium 63-GBGY with the fatty acid supplements indicated and aliquots were removed from the cultures for lipid analysis and measurement at 37 °C of in vivo ONPG hydrolysis (passive permeability and active transport) as described in the text. SFA and UFA, saturated and unsaturated fatty acid weight percentages, respectively. The height of the bar graphs corresponds to the relative amount of *cis*-vaccenate and the numbers in parentheses above the bars give the weight percentage of *cis*-vaccenate. The percentage of β-galactosidase activity in the culture medium was computed from measurements at 37 °C of total β-galactosidase activity (see text) in an aliquot of the culture and of the culture fluid after removal of the cells by centrifugation. ● — ●, ■ — ■, and ▼ — ▼, passive permeability, active transport, and β-galactosidase activity in the medium (as percent of total), respectively. a, b, and c, experiments with LA2-22 *fad*E cells on *cis*-1^{11}-18:1; AB1623 *fad*E cells on *cis*-1^{11}-18:1, and LA2-22 *fad*E cells on 16:0 plus *cis*-1^{9}-16:1, respectively.

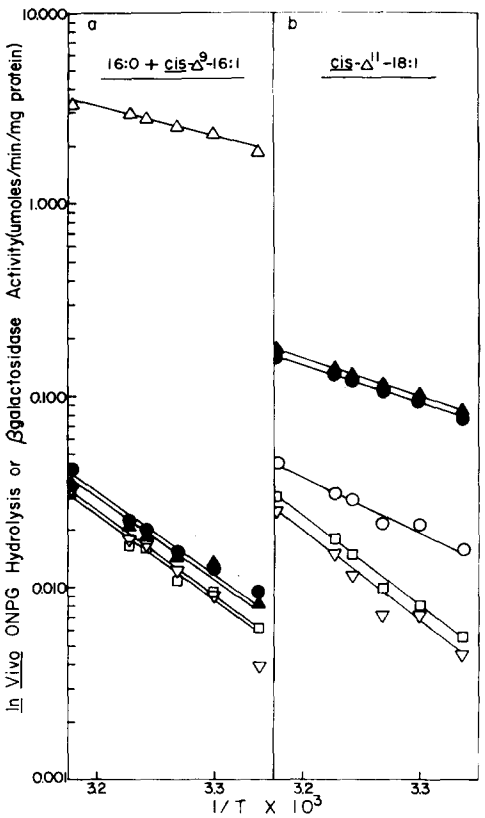

Fig. 6. Arrhenius plots of passive permeability toward ONPG and total β-galactosidase activity of LA2-22 *fad*E cells. Passive permeability toward ONPG was determined over the temperature interval shown for LA2-22 *fad*E cells cultured under the conditions described in the legend to Fig. 5. Measurements of passive permeability were made in a water bath in which the temperature was controlled within 0.2 °C. ▽, □, ○, ●, and ▲, measurements on aliquots taken at 1.0, 1.5, 2.0, 2.5, and 3.0 h after shifting culture medium from 30 to 37 °C, respectively. △, total β-galactosidase activity measured in aliquots taken from a culture 1.5 h after the temperature shift. a and b, experiments performed with cells grown on 16:0 plus *cis*-Δ^9-16:1 and on *cis*-Δ^{11}-18:1, respectively. For unknown reasons, the initial and final passive permeability levels in this experiment (a and b) were 4–5-fold below those measured in other experiments (for example, 5a–5c). Otherwise, the general pattern showing an initial activation energy of 21 kcal/mole shifting to a value of 9 kcal/mole was completely reproducible. The experiment shown here was chosen to illustrate the transition of the activation energy for passive permeability through an intermediate value (13 kcal/mole) between 21 and 9 kcal/mole (see b).

cis-Δ^{11}-18 : 1 (data not shown), the activation energy remained at 20 kcal/mole. However, in the mutant grown on *cis*-Δ^{11}-18 : 1, the activation energy decreased abruptly from an initial 21 to 9 kcal/mole after slightly less than one generation (1.5 h) This change occurred at the same time that passive permeability increased. That the activation energy of 9 kcal/mole approaches that for the β-galactosidase activity (7 kcal/mole) is consistent with the substantial reduction in the barrier property of the membrane toward ONPG. It is noteworthy that the Arrhenius plots for passive permeability are linear without any discontinuities. This finding indicates that altered

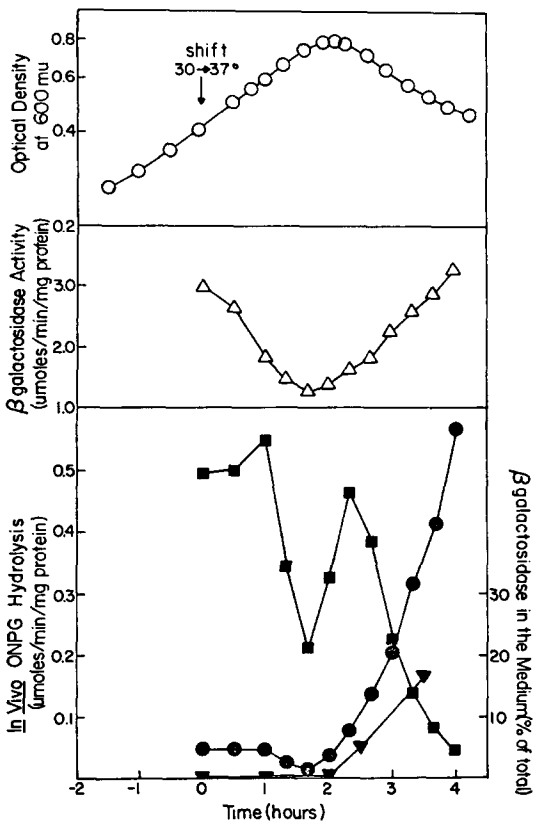

Fig. 7. In vivo ONPG hydrolysis in LA2-22 *fad*E cells grown on 18:0 at 37 °C. Cells were grown and measurements at 37 °C of in vivo ONPG hydrolysis (passive permeability and active transport) and of β-galactosidase activity and distribution were made as described in the legend to Fig. 5. ● — ●, ■ — ■, and ▼ — ▼, defined in the legend to Fig. 5. △ — △, defined in the legend to Fig. 6.

permeability could not be readily induced in LA2-22 *fad*E cells possessing normal barrier properties at 37 °C (that is, the temperature of growth) by assaying at 42 °C; and conversely, it could not be readily reversed in cells which already had reduced barrier properties at 37 °C by assaying at temperatures below 37 °C.

ONPG hydrolysis by LA2-22 *fad*E cells grown on a long chain unsaturated fatty acid can be contrasted with that of the mutant furnished a long chain saturated fatty acid. In the latter case, passive permeability decreases prior to the cessation of growth and then abruptly rises concomitant with a decline in cell turbidity and the release of β-galactosidase into the medium, indicating cell lysis (Fig. 7). The changes in active transport are complex but are accompanied by similar fluctuations in β-galactosidase activity until the time of cell lysis. The change in permeability and transport resemble those observed with the unsaturated fatty acid auxotrophs containing *trans*-unsaturated fatty acids in response to lowering the assay temperature [30]. The functional effects have been attributed to lateral phase separations in the membrane lipid undergoing a phase change from a relatively liquid to a relatively crystalline state [30, 44].

Altered temperature characteristics of NADH oxidase in cytoplasmic membranes from LA2-22 fadE cells grown on cis-vaccenate

As one approach to exploring further the properties of membranes deficient in phospholipids containing saturated fatty acyl groups, we examined the activity of the membrane-bound cyanide-sensitive NADH oxidase. At all assay temperatures between 20 and 43 °C NADH oxidase activity was lower in the membranes prepared from the mutant than in those isolated from wild type cells (Fig. 8). More significantly, Arrhenius plots of these measurements revealed that this activity increased over the temperature range of 25–40 °C only about 1.5-fold in preparations from LA2-22 *fad*E cells grown on *cis*-vaccenate in contrast to 3–4-fold in those obtained from AB1623 *fad*E cells cultured in the same manner. As shown in Fig. 8 the dramatic reduction in the temperature dependence of NADH oxidation was associated with the virtual elimination of saturated fatty acids in the membrane lipid (see insert to Fig. 8).

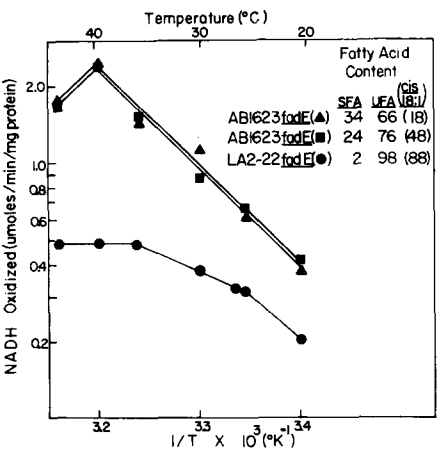

Fig. 8. Arrhenius plots of NADH oxidase activity in cytoplasmic membranes from cells grown with *cis*-vaccenate. AB1623 *fad*E and LA2-22 *fad*E cells were grown at 37 °C in Medium 63-GBGY to late exponential phase which amounted to three generations of growth following a shift in temperature from 30 to 37 °C. Cytoplasmic membranes were prepared and NADH oxidase activity was measured as described in the text. ▲, ■, and ●, measurements obtained with membranes from AB1623 *fad*E cells grown without fatty acid supplement, AB1623 *fad*E cells grown with *cis*-Δ^{11}-18:1, and LA2-22 *fad*E cells grown with *cis*-Δ^{11}-18:1, respectively. See legend to Fig. 5 for explanation of SFA, UFA, and the numbers given in parentheses.

DISCUSSION

Earlier studies with *E. coli* K 12 strains prototrophic for fatty acid synthesis demonstrated that normal growth was not affected by rather wide changes in membrane fatty acid composition produced by external fatty acid supplementation [5]. For example, unsaturated fatty acid content could be varied from about 40 to 80 % of the total. The molecular species of phospholipid present in the membrane normally includes molecules having one saturated and one unsaturated, two unsaturated, or two saturated fatty acyl groups [19, 45]. Changes in fatty acid composition conse-

quently must result in considerable variation in the relative amounts of these molecular species. The isolation of mutants affected in total fatty acid synthesis [12] has made it possible to greatly simplify the fatty acid composition of the membrane phospholipid and to explore the effects of controlling the type and amount of molecular species. This is illustrated dramatically when LA2-22 *fad*E cells are grown with *cis*-vaccenate until the fatty acid composition reaches 98 % unsaturated fatty acids; then, virtually the only molecular species which remain in the membrane lipid contain two unsaturated fatty acid chains (and largely *cis*-Δ^{11}-18 : 1). In the present study we demonstrated that various fatty acid supplements, identical or similar in structure to those synthesized by the wild type cell, supported normal growth of the mutant strain as long as the relative unsaturated and saturated fatty acid content generally remained within the range accommodated by the prototrophic strains. It may be inferred that the extremes determined for this range probably represent approximations of physiologically important limits. This generalization holds particularly for the maximum unsaturated fatty acid content (82 %) as shown in the present work. It is notable that wild type yeast can attain unsaturated fatty acid levels in the phospholipid of 94 % when cultured in the presence of oleic acid [2]; however, this organism also produces a sterol which influences the physical properties of the phospholipid in the membrane [46–48.] Estimates of the upper limit for saturated fatty acid obtained with *E. coli* unsaturated fatty acid auxotrophs have been as high as 80 % [19, 25, 49] although the present study demonstrated that growth of general fatty acid mutants stops at contents equal to or less than 74 %. The type and amount of specific molecular species are probably very important in determining the exact physiological limits with respect to fatty acid composition. Furthermore, the significance of such limits will be better understood when the physical behavior of various mixtures of membrane lipid molecules is known. The fatty acid biosynthetic mutants should provide a suitable biological system for generating membranes containing specific lipid mixtures and for exploring the physical state of the lipid by calorimetric [50] and paramagnetic resonance [30, 44] studies.

In an extensive series of studies utilizing liposomes made from synthetic or naturally occurring phospholipids, van Deenen and coworkers [46, 51] have shown that the permeability of these vesicles towards glycerol and erythritol, as reflected in measurements on the rate of permeation, was enhanced by the presence of ethylenic bonds in the fatty acyl groups of the phospholipids. K^+ permeability was affected in a similar manner [52]. Furthermore, this increase in permeability with increasing unsaturation was manifest by the membranes of intact *Acholeplasma* as well as by the bilayer of liposomes made from lipids extracted from these cells [53]. Fatty acid composition was not found to influence the activation energy for permeability of intact cells to glycerol and erythritol which measured 18 and 21 kcal/mole for the two molecules, respectively. Utilizing *E. coli* cells obtained at different times during growth on *cis*-vaccenate, we examined in this paper the effect on passive permeability of a progressive increase in the monoenoic fatty acid content of the membrane phospholipid. The permeability remained low with increasing unsaturated fatty acid levels in the membrane phospholipid until the composition exceeded 82 % in unsaturated fatty acid content. Thereafter, passive permeability increased sharply as the level rose from 82 to 92 % (Fig. 5a). Conversely, when the saturated fatty acid content was increased by growing the cells with stearate, passive permeability did not change initially and

then decreased prior to the onset of cell lysis (Fig. 7). Furthermore, the increased permeability observed in the present experiments with LA2-22 *fad*E cells grown on cis-Δ^{11}-18 : 1 was accompanied by a decrease in activation energy from 21 to 9 kcal/mole. These results strongly suggest highly cooperative changes in membrane structure occurring at compositions exceeding those tolerated by prototrophic cells. Considerable attention has been focused on the physical basis for the transition observed at high saturated fatty acid contents, especially when promoted by lowering the temperature [20, 29, 30]. The present work should generate interest in the nature of changes in membrane structure at very high unsaturated fatty acid compositions.

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