JOURNAL OF BACTERIOLOGY, June 1978, p. 808-820
0021-9193/78/0134-0808$02.00/0

Vol. 134, No. 3
Printed in U.S.A.

Dependence of *Escherichia coli* Hyperbaric Oxygen Toxicity on the Lipid Acyl Chain Composition

JOHN B. HARLEY,† GEORGE M. SANTANGELO, HOWARD RASMUSSEN,† AND HOWARD GOLDFINE*

Departments of Microbiology and of Biochemistry and Biophysics, School of Medicine, the University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received for publication 10 November 1977

This study examines certain membrane-related aspects of oxygen poisoning in *Escherichia coli* K1060 (*fabB fadE lacI*) and its parent strain, K-12 Ymel. Cells were grown to exponential or stationary phase in a minimal medium and exposed to air plus 300 lb/in^2 of O_2 as a suspension in minimal salts. After an initial lag, both strains lost viability with apparent first-order kinetics. Hyperbaric oxygen was more toxic to cells harvested during the exponential phase of growth than to cells harvested from the stationary phase of growth for both strains K-12 Ymel and K1060. Control suspensions exposed to air plus 300 lb/in^2 of N_2 did not lose viability during a 96-h exposure. The sensitivity of the unsaturated fatty acid auxotroph, strain K1060, to hyperbaric oxygen increased as the degree of unsaturation of the fatty acid supplement increased. Cells grown with a cyclopropane fatty acid (9,10-methyleneoctadecanoate) were the most resistant; cells grown with a monounsaturated fatty acid (oleate) were intermediate; and those grown with polyunsaturated fatty acids (linoleate and linolenate) were most sensitive to hyperbaric oxygen. The parent strain, K-12 Ymel, lost viability in hyperbaric oxygen most similarly to strain K1060 supplemented with oleate. To determine the relative effect of hyperbaric oxygen on the survival of *E. coli* with saturated membranes, substrains of K1060 were selected for growth on 12-methyltetradecanoate or on 9 or 10-monobromostearate. Substrains grown with a saturated fatty acid supplement were equally or more sensitive to hyperbaric oxygen than when the same substrains were grown with a cyclopropane fatty acid supplement. The lipid acyl chain composition was determined in *E. coli* K1060 before and after exposure to hyperbaric oxygen or hyperbaric nitrogen. The proportion of nonsaturated acyl chain lipid of either the oleate- or the 9,10-methyleneoctadecanoate-supplemented K1060 remained unchanged after hyperbaric gas exposure. In strain K1060 supplemented with linoleate and grown to stationary phase, however, the relative unsaturated acyl chain content after hyperbaric exposure decreased in both gases. This finding prompted an investigation of the role of lipid oxidation in hyperbaric oxygen toxicity. Assays of potential lipid oxidation products were performed with linoleate-grown cells. The lipid hydroperoxide and peroxide content of the lipid extract increased by 6.9 times after 48 h of air plus 300 lb/in^2 of O_2; malondialdehyde and fluorescent complex lipid oxidation products showed much smaller or no changes. Lipid extracts from hyperbaric oxygen-exposed cells were not toxic to viable *E. coli* K1060, nor did they increase the rate of loss of viability in cells simultaneously exposed to hyperbaric oxygen. Linoleic acid hydroperoxide at 1.0 mM had no effect on the viability of *E. coli* K-12 Ymel and only marginally decreased the viability of *E. coli* K1060 supplemented with linoleate. We conclude that the kinetics of oxygen toxicity in *E. coli* are dependent upon the particular unsaturated fatty acyl chain composition of the membrane, that the membrane composition influences the rate-limiting step in hyperbaric oxygen toxicity, but that a direct structural effect of hyperbaric oxygen on the membrane lipid, such as oxidation of the unsaturated acyl chain moiety, is unlikely in these cells.

Although hyperbaric oxygen is universally toxic to free-living organisms (3), the mechanism of oxygen poisoning remains undefined. The discovery of superoxide dismutase (28), however, has provided strong support for the theory of Gerschman et al. that oxygen toxicity is me-

† Present address: Department of Medicine, School of Medicine, Yale University, New Haven, CT 06510.

diated by the free radical derivatives of oxygen (17). On the other hand, the particular site(s) and detailed mechanism(s) of oxygen toxicity are more unclear. Since the discovery that hyperoxia oxidizes sulfhydryl groups of soluble proteins, thereby lowering specific enzyme activities in cell homogenates (6, 20), much work on oxygen poisoning has been concerned with characterizing the oxygen-induced inhibition of a rather large number of sulfhydryl-containing enzymes from a variety of preparations (21). In none of these cases has an enzyme inhibition observed in cell homogenates been demonstrated to account for the physiological alterations observed in intact cells. In fact, in *Escherichia coli* extracts the sulfhydryl group concentration falls rapidly under hyperoxia, but in intact cells the intracellular sulfhydryl concentration does not change even after 6 h of a growth-inhibitory level of oxygen (6.2 atm [35]).

On the other hand, some data suggest that hyperbaric oxygen may act at the membrane or other surface-associated site. In contrast to the intracellular sulfhydryl concentration, the surface-associated sulfhydryl groups in intact viable *E. coli* decrease by 38% after 5 h of 6.2 atm of O_2 (35). In yeast the uptake of glycine, leucine, and uracil is inhibited, and intracellular potassium is lost in cells exposed to hyperbaric oxygen (37, 43). Erythrocytes exposed to hyperoxia or other oxidants in vitro hemolyze, and the extent of hemolysis has been correlated with increases in presumed lipid oxidation products (30, 42). Hyperoxia and lipid hydroperoxides both obliterate the aldosterone-induced short-circuit response of the toad bladder (1).

This study was designed to determined whether the acyl component of the bacterial envelope lipids is involved in the mechanism of oxygen poisoning in *E. coli*. Various unsaturated fatty acids or their structural analogs were provided to an unsaturated fatty acid auxotroph of *E. coli* K-12. The consequent alterations in the kinetics of oxygen-induced viability loss are characterized and discussed in relation to the membrane composition. The results show that a membrane-associated process(es) must be an important element of oxygen toxicity in this experimental system. Additional experiments demonstrate that a direct effect of hyperbaric oxygen on the compositional integrity of membrane lipid, particularly through lipid oxidation, is unlikely.

MATERIALS AND METHODS

Bacterial strains and culture media. *E. coli* K-12 Ymel, K19 (*fadE*), and K1060 (*fabB fadE lacI*) were obtained from Peter Overath. These bacteria were grown at 30°C in a reciprocal shaking water bath. Growth was monitored with a Klett-Summerson colorimeter. The minimal medium used was salts E (44) supplemented with 0.4% glucose and 1.0 mg of thiamine hydrochloride per liter. L broth or agar (26) was used as the complex medium. For the fatty acid auxotrophs and the accompanying control experiments, these media were supplemented with 0.04% polyethylene-23-lauryl ether (Brij 35) and 0.01% free fatty acid, which was added from a 5% stock solution in 95% ethanol. A Yellow Springs oxygen electrode was used to determine that the aerobically grown cultures were 95 to 100% saturated with atmospheric oxygen throughout growth. The phenotype of the *fabB* marker was demonstrated in all relevant experiments by showing that growth on L agar required an unsaturated fatty acid supplement. Inability to grow with 0.1% oleate as the carbon source in minimal agar was the phenotypic test used for *fadE*.

Hyperbaric exposure and viability. Bacterial cultures were grown to exponential phase (120 Klett units, approximately 4×10^8 cells/ml) or stationary phase (500 to 700 Klett units, approximately 2×10^9 cells/ml). The latter were incubated in stationary phase for 9 to 12 h; the beginning of stationary phase was taken as the intersection point of the extrapolated mid-exponential- and stationary-phase growth plots. Cultures were harvested by centrifugation (6,000 × *g*, 10 to 15 min) at room temperatures, washed twice, and suspended in salts E containing 1.0 mg of thiamine hydrochloride per liter at approximately 2.0×10^9 bacteria/ml. The cell suspensions were exposed to ambient air plus 300 lb/in^2 of O_2 or N_2 at 30°C and eccentrically agitated in a Bethlehem Corp. hyperbaric chamber (model 615-HPSP) or in a chamber designed and constructed at the Institute for Environmental Medicine of the University of Pennsylvania. With these agitation conditions, the cell suspensions were 95% saturated with 300 lb/in^2 of O_2 within 15 s as determined with the oxygen electrode.

The fractional survival was determined by diluting the cellular suspensions in salts E and plating samples of the various dilutions onto L agar (supplemented with 0.01% oleate and 0.04% Brij 35 where necessary). To determine that the apparent loss of viability was not due to the formation of bacterial aggregates, the number of cells per colony-forming unit was determined in a Petroff-Hausser bacterial counting chamber for each strain and growth condition. In every case the percentage of single-cell colony-forming units exceeded 85% and did not change after exposure to hyperbaric oxygen or nitrogen.

Saturated fatty acid experiments. Colonies were selected that could use 12-methyltetradecanoate or 9- or 10-monobromostearate (13, 34) to satisfy the fatty acid requirement of strain K1060 by plating bacteria onto L agar supplemented with 0.04% Brij 35 and 0.01% fatty acid. The plating efficiency was 10^{-5}. Selected colonies were grown in L broth supplemented with 0.04% Brij 35 and 0.01% fatty acid at 37°C. A sample of these cultures was also grown in a similar medium supplemented with 0.01% 9,10-methyleneoctadecanoate. Exponential- and stationary-phase cultures were harvested, washed, suspended in salts E plus 1.0 mg of thiamine hydrochloride per liter, and exposed to ambient air plus 300 lb/in^2 of O_2 or N_2 at 37°C. Unfortunately, these strains slowly reverted to *fab*$^+$ after continued subculturing with the saturated

fatty acid supplements; therefore, reversion was tested as described above and by fatty acid analysis of samples of the bacterial suspensions taken immediately before exposure to hyperbaric conditions.

Fatty acid analysis. Bacteria were harvested by centrifugation at 6,000 × *g* for 10 min at room temperature. Lipids were extracted separately from both the supernatant and the cellular pellet by the method of Bligh and Dyer (4) after titrating to pH 2.0 with HCl. The cellular lipid was separated into neutral and polar fractions on a 1.0-g silicic acid column (200/400 mesh, Unisil; Clarkson Chemical Co., Williamsport, Pa.) by elution with chloroform followed by methanol. Lipid was generally transesterified in methanolic HCl as adapted from Ferguson et al. (10). The resulting fatty acid methyl esters were quantitated on a Perkin-Elmer 990 gas chromatograph equipped with a Columbia Scientific Industries Infotronics model CRS-208 automatic digital integrator and a column of 10% EGSS-X on Gas-Chrom P (⅛ in. by 6 ft. [ca. 0.21 cm by 1.83 m], 100/200 mesh; Applied Science Laboratories, State College, Pa.) at 180°C. Heptadecanoate or its methyl ester was used as an internal standard. Fatty acid methyl esters were identified by comparison of retention times with those of known standards. Transesterification with methanolic HCl resulted in some degradation of cyclopropane fatty acids. In the results reported here, less than 2% was degraded; for these cases the retention times of the degradation products were characterized, and the 9,10-methyleneoctadecanoate values given are corrected for losses. Extended incubations in methanolic HCl, however, resulted in complete destruction of the cyclopropane methyl esters. When degradation exceeded 2%, the analysis was repeated by a different method. For these cases the lipids were hydrolyzed in alkali (2-h reflux at 85°C in 50% methanol containing 10% KOH), and the resulting fatty acids were extracted and methylated with diazomethane.

The lipids from strains K1060-112S and K1060-2B were not separated into supernatant, cellular polar, and cellular neutral fractions before analysis. The retention times of the octadecenoates, oleate and *cis*-vaccenate, were similar but not identical to that of 9- or 10-monobromostearate. To improve the resolution, the saturated and unsaturated methyl esters were separated on 20% $AgNO_3$-impregnated Silica Gel G thin-layer chromatographic plates with hexane-chloroform-methanol (30:68:2, vol/vol/vol) and separately analyzed by gas chromatography.

Lipid oxidation products. Lipid oxidation products were directly determined by three assays. To avoid further oxidative degradation during analysis, samples were manipulated under N_2, solvent was never completely evaporated, and samples were stored at −15°C in the presence of ethanol. The lipid peroxide and hydroperoxide content was determined in samples of approximately 4×10^{11} bacteria and their respective supernatants (200 ml) with the iodometric procedure of Swoboda and Lea (38). After extraction by the Bligh and Dyer method (4), the lipid was dissolved in acetic acid-chloroform (3:2, vol/vol) and purged with solvent-saturated nitrogen for 15 min. An excess of saturated aqueous KI was added, and the reaction mixture was incubated for 1 h in the dark. Then 7.0 ml of 0.5% cadmium acetate was added, and the absorbance of the aqueous phase taken at 350 nm was compared with that of KIO_4 standards.

Malondialydehyde was assayed by a procedure based upon the thiobarbituric assay for β-formylpyruvic acid (45). One part aqueous 1% thiobarbituric acid in 0.05 N NaOH was added to 4 parts bacterial suspension, and immediately the mixture was placed in boiling water for 20 min. Sodium sulfate was then added to a final concentration of 1.0 M. The chromophore was extracted into cyclohexanone and centrifuged at 2,000 × *g* for 10 min to clear the cyclohexanone phase. Over 95% of the chromophore was extracted into cyclohexanone. The optical density difference of the cyclohexanone phase between 234 and 600 nm was used to monitor the malondialdehyde concentration. Malondialdehyde standards were generated by treating malonaldehyde bis-(dimethyl acetal) (Aldrich Chemical Co., Milwaukee, Wis.) with 0.1 N HCl.

The complex secondary products of lipid oxidation were assayed by a fluorescence method (7). A portion of the lipid extract was dissolved in chloroform-methanol (2:1, vol/vol), and the relative magnitude of the fluorescence emission maximum at 420 to 425 nm (excitation at 350 to 365 nm) was determined in a Perkin-Elmer double-beam fluorescence spectrophotometer (model 512).

Lipid dispersions. Strain K1060, which had been grown to stationary phase on a linoleate supplement, was prepared for hyperbaric oxygen exposure as described above. The cellular lipid was extracted by the Bligh and Dyer procedure (4) either immediately before or after the bacterial suspension was exposed to air plus 300 lb/in^2 of O_2 for 48 h. Lipid (7.0 mg/ml) was sonically treated in E salts under nitrogen at 5°C with three 1-min, 70-W bursts, using an Ultrasonic Inc. Sonicator cell disrupter with the microtip. Dispersions were immediately warmed to 30°C and added to suspensions of stationary-phase cells of strain K-12 Ymel or K1060 that had previously been harvested, twice washed, and suspended in salts E plus 1.0 mg of thiamine per liter. The resulting cell suspensions were then incubated either in air or in air plus 300 lb/in^2 of O_2. Viability was determined periodically as described above. The final concentration of added lipid was 1, 10, or 100 times the amount of lipid per unit volume recovered from a 2×10^9-cells/ml suspension.

Linoleic acid hydroperoxide preparation. Linoleic acid hydroperoxide was prepared from linoleic acid with soybean lipoxygenase by a modification of the method described by Egmond et al. (9). Linoleic acid (1.0 to 5.0 mM) was incubated in 0.1 M sodium borate at pH 9.0 with 1.0 to 20.0 mg of lipoxygenase per ml (Sigma Chemical Co., St. Louis, Mo.) in an oxygen-saturated atmosphere at 0°C. Accumulation of the hydroperoxide was monitored at 234 nm and calculated by using an absorption coefficient of 25,000 M^{-1} cm^{-1}, which was within 2% of the hydroperoxide content as determined by the iodometric assay. When either 100% or maximal conversion was reached, the linoleic acid hydroperoxide was extracted into diethyl ether and concentrated by flash evaporation at 30 to 35°C. Caution was exercised to avoid evaporating all of the solvent at any purification step. When this occurred, most of the hydroperoxide was lost. Over

85% of the starting material was recovered as the hydroperoxide derivative and could be stored for months without significant decay in ethanolic solutions at −15°C.

Chemicals. *cis*-9-Octadecenoate (oleate), *cis,cis*-9,12-octadecadienoate (linoleate), and all *cis*-9,12,15-octadecatrienoate (linolenate) were purchased from Nu Check Prep, Inc. (Elysian, Minn.). 9,10-Methyleneoctadecanoate and 12-methyltetradecanoate were purchased from Analabs, Inc. (North Haven, Conn.). Serdary Research Laboratories, Inc. (London, Ontario), supplied 9- and 10-monobromostearate. Brij 35 was purchased from Sigma Chemical Co. (St. Louis, Mo.). Difco Laboratories (Detroit, Mich.) supplied the constituents of L agar. All other chemicals were reagent grade.

RESULTS

Manipulation of the fatty acid composition. When the unsaturated fatty acid auxotroph, *E. coli* K1060, was grown to exponential or stationary phase with various 18-carbon fatty acid supplements, the unsaturated fatty acyl chains or their cyclopropane derivatives in the cell lipids were essentially derived from the unsaturated fatty acid supplied in the medium (Tables 1-3). The generation times of strain K1060 with four unsaturated or cyclopropane fatty acid supplements were identical (2.0 h).

Viability of *E. coli* K1060 in ambient air plus 300 lb/in² of O_2 or N_2. After incorporation of 9,10-methyleneoctadecanoate (19:cyclopropane), oleate, linoleate, or linolenate, the bacteria were harvested, washed, and exposed to ambient air plus 300 lb/in² of O_2 or N_2. Since the potential interactions of free fatty acid in the medium with oxygen would complicate the interpretation of any survival data, the bacteria were exposed to the hyperbaric gases as resting-cell suspensions in minimal salts. Neither a carbon source nor a fatty acid supplement (plus detergent) was present during hyperbaric oxygen exposure. Samples were removed periodically, and viability was determined by plating dilutions of the cell suspensions onto L agar supplemented with oleate.

The kinetics of hyperbaric oxygen-induced viability loss in K1060 varied with the unsaturated fatty acid lipid component in bacteria harvested from both the exponential and stationary phases of growth (Fig. 1 and 2). The bacteria in hyperbaric nitrogen showed little or no loss of viability over 96 h.

As the extent of unsaturation of the fatty acid supplement increased, the duration of the lag before bacteria began to lose viability shortened, and the rate of viability loss in cells harvested from the exponential phase increased in hyperbaric oxygen (Fig. 1). The kinetics of viability loss of stationary *E. coli* K1060 were somewhat different (Fig. 2). For these cells the duration of the lag before viability loss began in hyperbaric oxygen was independent of the particular unsaturated fatty acid supplement with the exception of the linolenate-supplemented cells, which had a slightly shorter lag. The rate of viability loss again increased as the extent of unsaturation of the fatty acid supplement increased.

Exponential-phase *E. coli* K-12 and K1060 were generally more sensitive to 300 lb/in² of O_2 than were stationary-phase *E. coli* (Fig. 3). For each fatty acid supplement in strain K1060, the lag in exponential-phase cells was the same or shorter and the rate of viability loss was consistently greater.

Viability of wild-type *E. coli* K-12 Ymel in ambient air plus 300 lb/in² of O_2. The parent strain of K1060, strain K-12 Ymel, was also sensitive to hyperbaric oxygen (Fig. 3). K-12 Ymel from both phases of growth showed a lag of about 24 h before beginning to perish in air plus 300 lb/in² of O_2. Once begun, the bacteria harvested from the exponential growth phase lost viability more quickly (−0.082 logs/h) than did the stationary-phase cells (−0.050 logs/h). The kinetics of hyperbaric oxygen killing of the exponential- and stationary-phase wild-type bacteria were most similar to those seen with oleate-supplemented strain K1060. As in strain K1060, air plus 300 lb/in² of N_2 was not toxic to K-12 Ymel harvested from either exponential or stationary phase (data not shown).

In similar experiments, the survival kinetics of two other wild-type K-12 strains and K19 (*fadE*, a parent of K1060) in ambient air plus 300 lb/in² of O_2 or N_2 were essentially identical to those presented in Fig. 3 for K-12 Ymel (unpublished data). We have presented, therefore, the general kinetics of air plus 300 lb/in² of O_2 or N_2 survival for salts E suspensions of wild-type *E. coli* K-12 strains grown aerobically in a minimal medium. These data also lead to a second inference: neither unsaturated fatty acid synthesis (*fabB*) nor fatty acid β-oxidation (*fadE*) is an important function for *E. coli* survival during, or recovery after, hyperbaric oxygen exposure under these experimental conditions.

The addition of detergent (0.04% Brij 35) or a fatty acid supplement (0.01%) to the minimal salts growth medium or to the L agar did not alter the survival kinetics of K-12 Ymel in air plus 300 lb/in² of O_2. In addition, changing the L-agar fatty acid supplement from oleate to a polyunsaturated fatty acid (linoleate or linolenate) did not alter the survival kinetics of K1060 (data not presented). Therefore, neither the presence of nonionic detergent and fatty acid in the growth medium or recovery agar nor the particular fatty acid used in the recovery agar

TABLE 1. *Lipid acyl chain composition of E. coli K1060 supplemented with 9,10-methylene-octadecanoate*[a]

Fraction	Composition																		
	Initial							After 72 h in air + 300 lb/in^2 of N_2							After 72 h in air + 300 lb/in^2 of O_2						
	Sat.[b]	16:1	17:cyc	18:1	19:cyc	Unknown	Total	Sat.	16:1	17:cyc	18:1	19:cyc	Unknown	Total	Sat.	16:1	17:cyc	18:1	19:cyc	Unknown	Total
Exponential phase																					
Cellular pellet																					
Polar lipid	44.4[c]			1.2	26.5		72.1	38.4		1.1	1.5	27.1		68.1	38.9			1.2	26.5		66.6
Neutral lipid	8.1	2.7	0.3	2.7	4.7		18.5	8.8	1.0		1.2	7.9		18.9	9.7	0.7		1.0	10.0		21.4
Supernatant	4.5	1.3	0.2	1.0	2.5		9.5	6.7	0.6		0.7	4.8		12.8	5.3	0.8		0.8	5.1		12.0
Total[d]	57.0	4.0	0.5	4.9	33.7			53.9	1.6	1.1	3.4	39.8			53.9	1.5		3.0	41.6		
Stationary phase																					
Cellular pellet																					
Polar lipid	49.7	0.3	1.0	0.7	13.9		65.6	40.2	0.2	0.3	0.7	12.5	0.3	54.2	39.2	0.3	0.4	0.5	12.1		52.5
Neutral lipid	6.5	0.4		0.5	20.1	0.3	27.8	14.1	0.3	0.1	0.3	20.2	0.6	36.0	15.0	0.2	0.1	0.6	18.4	0.3	34.6
Supernatant	2.5	0.5		0.8	2.3	0.5	6.6	8.3	0.1		0.3	0.9	0.2	9.8	7.3	0.3		0.7	4.1	0.4	12.8
Total	58.7	1.2	1.0	2.0	36.3	0.8		62.6	0.6	0.4	1.7	33.6	1.1		61.5	0.8	0.5	1.8	34.6	0.7	

[a] Cultures of strain K1060 were grown to the stated phase of growth on a fatty acid supplement (0.01%) in salts E, glucose (0.4%), and Brij 35 (0.04%). They were harvested, washed twice, and exposed to air plus 300 lb/in^2 of oxygen or air plus 300 lb/in^2 of nitrogen. 25-ml samples of 2×10^9 cells/ml were harvested by centrifugation at 6,000 × *g* for 10 min at 20 to 25°C, and the acyl chain composition of the supernatant and the polar and neutral lipid of the cellular pellet was analyzed by gas chromatography (see text).

[b] Fatty acid methyl esters are designated by the total number of carbons in the acyl chain followed either by cyc, indicating a cyclopropane derivative, or by the number of double bonds in the acyl chain. Sat. designates the saturated acyl chain content of each fraction. Fatty acyl methyl esters with a retention time of 19:cyc include both the cyclopropane derivative of oleate and *cis*-vaccinate, and 18:1 include the contributions from both oleate and *cis*-vaccinate.

[c] Percentage by weight.

[d] The weight percentage each acyl chain length contributes to the total.

TABLE 2. *Lipid acyl chain composition of E. coli K1060 supplemented with oleate*[a]

Fraction	Composition																	
	Initial						After 72 h in air + 300 lb/in^2 of N_2						After 72 h in air + 300 lb/in^2 of O_2					
	Sat.[b]	16:1	17:cyc	18:1	19:cyc	Total	Sat.	16:1	17:cyc	18:1	19:cyc	Total	Sat.	16:1	17:cyc	18:1	19:cyc	Total
Exponential phase																		
Cellular pellet																		
Polar lipid	33.0[c]	0.4	0.4	53.4	7.4	94.6	27.3	0.1	0.3	40.1	13.6	81.4	22.5	0.3	0.4	36.8	4.1	64.1
Neutral lipid	1.5		0.1	1.8		3.4	3.7		0.1	8.7	0.6	13.1	7.5	0.1	0.2	17.1	1.4	26.3
Supernatant	1.3		0.1	0.5		1.9	2.6		0.1	2.7	0.2	5.6	3.0	0.1	0.2	5.6	0.5	9.4
Total[d]	35.8	0.4	0.6	55.7	7.4		33.6	0.1	0.5	51.5	14.4		33.0	0.5	0.8	59.5	6.0	
Stationary phase																		
Cellular pellet																		
Polar lipid	47.9	0.6	0.9	18.9	9.1	77.4	40.6	1.1	1.0	13.8	13.9	70.4	32.7	0.5	1.3	12.5	7.5	54.5
Neutral lipid	7.1	0.2		10.9	0.2	18.4	7.5	0.4	0.2	8.0	1.4	17.5	6.3	0.1	0.1	7.4	0.9	18.4
Supernatant	2.7	0.3	0.3	0.6		3.9	6.9	0.9	0.3	3.4	0.7	12.2	17.8	1.7	0.8	8.6	1.8	30.7
Total	57.7	1.1	1.2	30.4	9.3		55.0	2.4	1.5	25.2	16.0		56.8	2.3	2.2	28.5	10.2	

[a-d] Refer to Table 1.

TABLE 3. *Lipid acyl chain composition of E. coli K1060 supplemented with linoleate*[a]

Fraction	Composition																		
	Initial							After 72 h in air + 300 lb/in^2 of N_2							After 72 h in air + 300 lb/in^2 of O_2						
	Sat.[b]	16:1	17:cyc	18:1	18:2	Unknown	Total	Sat.	16:1	17:cyc	18:1	18:2	Unknown	Total	Sat.	16:1	17:cyc	18:1	18:2	Unknown	Total
Exponential phase																					
Cellular pellet																					
Polar lipid	51.7[c]	0.2	0.8	0.4	43.4		96.9	46.1		0.8	0.5	31.8		79.2	40.0	0.1	0.7	0.3	27.7		68.8
Neutral lipid	1.0			0.1	1.1		2.2	3.7			0.2	9.5		13.4	9.4	0.1	0.1	0.1	13.1		22.8
Supernatant	0.9			0.1			1.0	3.1			0.1	4.5		7.7	4.6	0.5	0.1	0.7	2.4		8.3
Total[d]	53.6	0.2	0.8	0.6	44.9			52.9		0.8	0.8	45.8			54.0	0.7	0.9	1.1	43.2		
Stationary phase																					
Cellular pellet																					
Polar lipid	68.0			0.4	19.8	1.0	89.2	52.7	6.1	2.0	3.2	15.3	0.4	79.7	64.9		1.9	0.3	18.0	0.4	85.5
Neutral lipid	2.0	0.2		0.3	3.2		5.7	2.3	0.5		0.5	0.6		3.9	6.2	0.1		0.1	0.4	0.2	7.0
Supernatant	2.5	0.3		0.3	1.9	0.1	5.1	9.9	1.5	0.4	1.5	1.9	1.2	16.4	5.5	0.1	0.1	0.2	1.4	0.2	7.5
Total	72.5	0.5		1.0	24.9	1.1		64.9	8.1	2.4	5.2	17.8	1.6		76.6	0.2	2.0	0.6	19.8	0.8	

[a-d] Refer to Table 1.

added artifacts to the K-12 Ymel or K1060 survival kinetics in hyperbaric oxygen.

Lipid acyl chain analyses. The lipid acyl chain compositions of strain K1060 supplemented with 19-cyclopropane, oleate, or linoleate were determined immediately before and after exposure to the hyperbaric gases (Tables 1-3). Certain observations can be made by using this data. The unsaturated (plus cyclopropane) acyl chain content of the cellular neutral lipid increased in exponential-phase cells exposed to hyperbaric oxygen or nitrogen, whereas it decreased in stationary-phase cells. On the other hand, changes in the unsaturated-plus-cyclopropane lipid acyl chain content of the polar and supernatant fractions did not follow any obvious pattern.

The polar cellular lipid contribution to the total lipid decreased in both hyperbaric oxygen and nitrogen (Tables 1-3), with corresponding

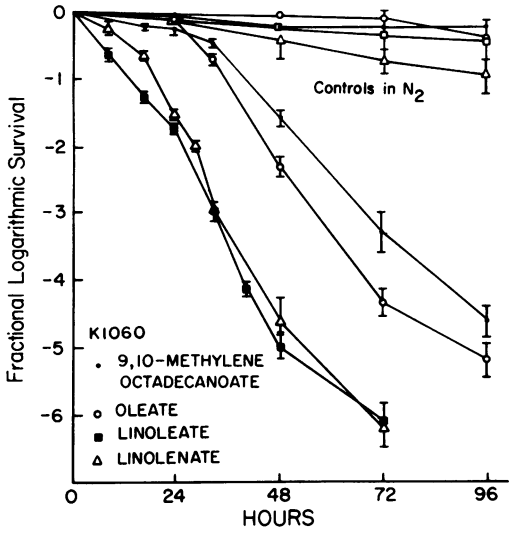

FIG. 1. *Hyperbaric oxygen toxicity in E. coli K1060 after growth to exponential phase. Bacteria were grown in minimal medium supplemented with various fatty acids and then exposed to air plus 300 lb/in² of O_2 or N_2 in minimal salts. Each data point represents the logarithmic average (± standard error of the mean) of 8 to 20 determinations of the viable count.*

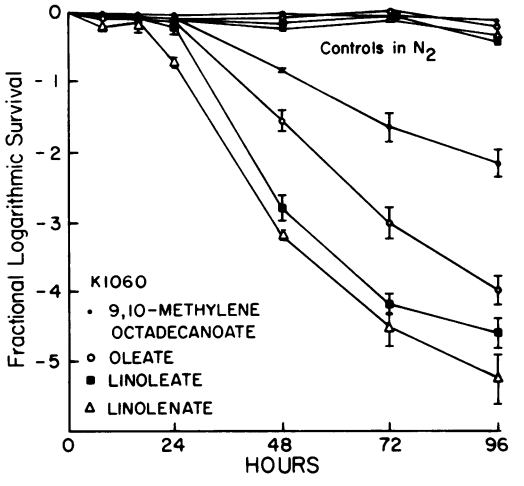

FIG. 2. *Hyperbaric oxygen toxicity in E. coli K1060 after growth to stationary phase. Refer to Fig. 1 and the text for other details.*

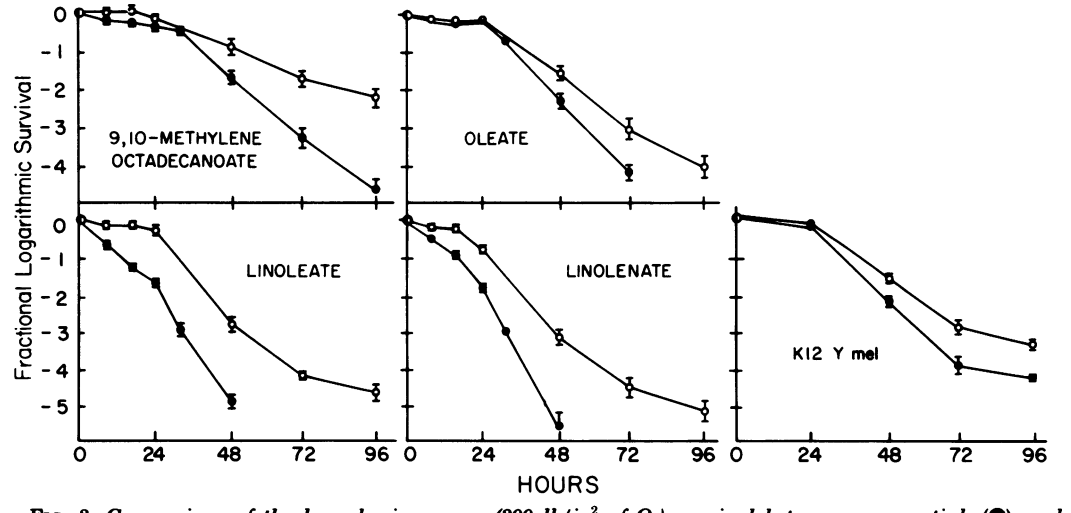

FIG. 3. *Comparison of the hyperbaric oxygen (300 lb/in² of O_2) survival between exponential- (●) and stationary- (○) phase E. coli K-12 Ymel and E. coli K1060 supplemented with four different fatty acids (designated). Refer to Fig. 1 and the text for other details.*

increases in the supernatant lipid contribution to the total. Only in the case of oleate-supplemented K1060 did the loss of polar lipids in hyperbaric oxygen exceed the loss in hyperbaric nitrogen. The changes in the neutral lipid contribution to the total lipid were variable in hyperbaric nitrogen, whereas in hyperbaric oxygen the neutral lipid increased in all cases except in the oleate-supplemented stationary-phase K1060 cells (Table 2). There was no consistent change in the lipid composition that could be correlated with sensitivity to hyperbaric oxygen.

Since K1060 can neither synthesize nor β-oxidize unsaturated fatty acids, any alteration in the unsaturated contribution to the total lipid is of particular interest. The only example of such a change was found in stationary-phase linoleate-supplemented cells (Table 3). The total linoleate in the sample decreased after exposure to hyperbaric oxygen, but an even greater decrease was seen after exposure to hyperbaric nitrogen. The lipid acyl chains of three separate sets of samples from K1060 were analyzed with similar results. The median analysis is presented in Table 3. There is no obvious explanation for the mechanism of linoleate removal in hyperbaric nitrogen; however, its decrease in hyperbaric oxygen raises the possibility that lipid oxidation may be at least partially responsible. This possibility is considered below.

Table 4 shows the lipid acyl chain composition in exponential- and stationary-phase K-12 Ymel cells and the effect of the nonionic detergent-plus-fatty acid growth supplement on exponential-phase cells. The latter additions resulted in a shift in the distribution of the total lipid 16-carbon and 18-carbon unsaturated acyl chain components; 16:1 plus 17:cyclopropane decreased by 7.0% and 18:1 plus 19:cyclopropane increased by 5.4% after oleate and Brij 35 were added to the medium. The unsaturated and cyclopropane lipid acyl chains are considered together, since the monounsaturated acyl chains are the immediate precursors of the cyclopropane acyl chains. These differences, however, did not affect survival in hyperbaric oxygen (data not presented). Indeed, even the rather radical differences in the particular fatty acid composition between the exponential-phase K1060 supplemented with oleate and K-12 Ymel had little effect on the kinetics of hyperbaric oxygen survival (Fig. 3 and Tables 2 and 4).

Saturated fatty acid analogs of unsaturated fatty acids. Colonies of K1060 were selected that could utilize fatty acid analogs in addition to unsaturated fatty acid supplements for growth. Substrains that grew on 9- or 10-monobromostearate or 12-methyltetradecanoate were selected, cultured in fatty acid-supplemented L broth, and exposed to air plus 300 lb/in^2 of O_2 or N_2 as cell suspensions in salts E. The doubling time with the saturated fatty acid supplements (0.9 h for K1060-2B on 12-methyltetradecanoate and 1.1 h for K1060-112S on 9- or 10-monobromostearate) was longer than when these strains were grown on a 19:cyclopropane supplement (0.5 h).

Figure 4 shows the survival of representative substrains of K1060 adapted to each saturated fatty acid analog compared with a parallel suspension of the same substrain that had been supplemented with 19:cyclopropane. The 19:cyclopropane-supplemented cultures were more resistant to 300 lb/in^2 of O_2 in all cases but one (Fig. 4C), where resistance was approximately the same.

Table 5 presents the fatty acid analyses of samples of these bacterial suspensions taken before exposure to hyperbaric gases. These data show that the saturated fatty acids were indeed incorporated into the membranes, though to varying extents. The leakage of the *fabB* marker in these strains was comparable to that of strain K1060.

Lipid oxidation products. There has been no previous measurement of the accumulation

TABLE 4. *Lipid acyl chain compositions of E. coli K-12 Ymel before hyperbaric oxygen treatment*[a]

Strain (supplement)	Composition									
	12:0[b]	14:0	15:0	16:0	16:1	17:cyc	18:0	18:1	19:cyc	Unknown
Exponential phase										
K-12 Ymel (oleate)		4.2	0.7	37.1	11.6	17.6	0.8	24.6	3.5	
K-12 Ymel (none)		2.1	0.3	35.9	20.8	15.4	0.6	20.7	2.0	2.1
Stationary phase										
K-12 Ymel (none)	0.2	2.6	0.1	41.8	0.8	36.0	0.6	0.9	16.3	0.8

[a] Fatty acyl chain composition of K-12 Ymel before exposure to hyperbaric oxygen or nitrogen. Results are reported as the weight percentage of the total fatty acids present in each case and represents duplicate determinations from a single sample.

[b] As in Table 1.

of lipid oxidation products or assessment of the toxicity of lipid acyl chain hydroperoxides in similar experimental circumstances; therefore, a direct structural injury to the membrane by lipid oxidation must be more carefully considered as a possible mechanism of hyperbaric oxygen toxicity. The relative resistance of oleate-supplemented cells to hyperbaric oxygen when compared with linoleate-supplemented cells and the selective decrease in linoleate seen in stationary-phase cells exposed to hyperbaric oxygen are observations consistent with a lipid oxidation mechanism of oxygen toxicity.

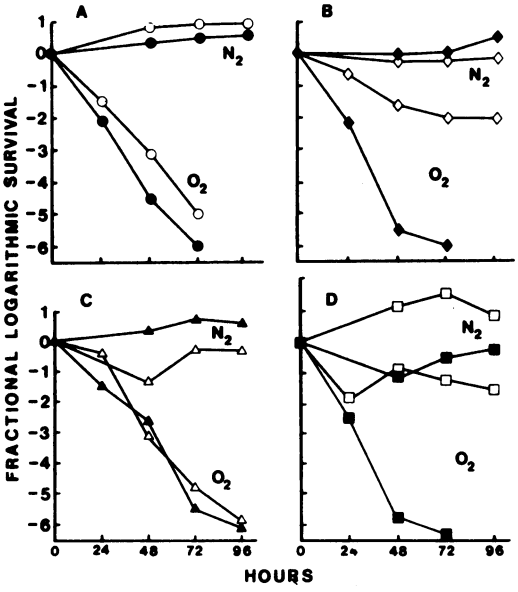

FIG. 4. *Hyperbaric oxygen toxicity in representative substrains of E. coli K1060 that are capable of incorporating saturated analogs of unsaturated fatty acids. Viability after exposure to air plus 300 lb/in² of O_2 (O_2) or air plus 300 lb/in² of n_2 (N_2). (A and B). Substrain K1060-2B after growth to exponential (A) or stationary (B) phase, supplemented with 12-methyltetradecanoate (●, ◆) or 9,10-methyleneoctadecanoate (○, ◇). (C and D) Substrain K1060-112S after growth to exponential (C) or stationary (D) phase, supplemented with 9- or 10-monobromostearate (▲, ■) or 9,10-methyleneoctadecanoate (△, □).*

To assess the entire pathway of peroxidative lipid degradation, three different assays of possible autoxidation products were performed. The first recoverable autoxidation intermediate is the hydroperoxide, which was assayed by an iodometric method (38). Since these hydroperoxides decompose and generate products reactive with thiobarbituric acid, such as malondialdehyde, thiobarbituric acid assays were done to monitor these products. Malondialdehyde, as well as other carbonyls and aldehydes that may be generated, react with primary amines to form conjugated Schiff-base products. The assay for these fluorescent products should constitute a relative measure of the total accumulation of lipid oxidation products (39).

Tables 6 through 8 present assays for lipid oxidation products in strain K1060 grown to stationary phase on a linoleate supplement. The iodide-reducible peroxide in the cellular lipid extract increased by a factor of 6.9 during the course of a 48-h exposure to air plus 300 lb/in² of O_2 and by a factor of 1.75 after 48 h of air plus 300 lb/in² of N_2. This change was not seen in the supernatant lipid fraction; the specific iodide-reducible peroxide per milligram of lipid in this fraction dropped during hyperbaric exposure to either gas (Table 6). The thiobarbituric acid-reactive products increased to almost the same

TABLE 5. *Lipid acyl chain compositions before hyperbaric oxygen treatment*[a]

Strain (supplement)	Composition											
	14:0	15: methyl	15:0	16:0	16:1	17:0	17:cyc	18:0	18:br	18:1	19:cyc	Unknown
E. coli K1060-2B												
Exponential phase												
(15:methyl)	1.0	17.0		52.8	0.7		1.7	15.3		5.3	3.5	2.7
(19:cyc)	0.6		0.2	15.1			15.7	3.2		5.3	64.2	
Stationary phase												
(15:methyl)	6.4	59.9		29.3	0.3		0.5	2.3		0.8	0.4	
(19:cyc)	0.6		0.2	15.1			15.7	3.2		1.1	64.1	
E. coli K1060-112S												
Exponential phase												
(18:Br)	2.6			15.5	7.5			21.1	53.4			
(19:cyc)	0.2		0.3	29.3			24.7	5.6		1.9	35.6	2.3
Stationary phase												
(18:Br)	9.9			32.5	5.2		8.2	3.0	33.3		3.4	4.4
(19:cyc)	3.5		0.2	29.8		0.4	23.7			0.4	39.2	2.4

[a] Fatty acyl chain compositions of substrains of *E. coli* K1060 capable of growth with a saturated fatty acid analog of monounsaturated fatty acids. 12-methyltetradecanoate and 9- or 10-monobromostearate are designated by 15:methyl and 18:Br, respectively. Refer to Table 1 and text for other details.

TABLE 6. *Iodide-reducible hydroperoxides and peroxides in lipid extracts*[a]

Fraction	Concn	
	nmol/100 ml of suspension	nmol/mg of acyl chain
Cellular pellet		
0 h	13.0	2.4
48 h of air + 300 lb/in^2 of N_2	21.5	4.2
48 h of air + 300 lb/in^2 of O_2	76.6	17.0
Supernatant		
0 h	4.6	21.8
48 h of air + 300 lb/in^2 of N_2	5.1	16.1
48 h of air + 300 lb/in^2 of O_2	5.7	15.6

[a] Iodometric determination of the hydroperoxide and peroxide content of the lipid extract of suspensions of *E. coli* K1060 that had been supplemented with linoleate and grown to stationary phase.

TABLE 7. *Thiobarbituric acid-reactive material in cell suspensions*[a]

Condition	Concn	
	nmol/100 ml of suspension	nmol/mg of acyl chain
0 h	8.5 ± 0.08	1.5 ± 0.01
48 h of air + 300 lb/in^2 of N_2	12.6 ± 1.6	2.3 ± 0.03
48 h of air + 300 lb/in^2 of O_2	13.0 ± 0.9	2.7 ± 0.2

[a] Thiobarbituric acid assays of malondialdehyde equivalents in linoleate-supplemented K1060 suspensions that had previously been grown to stationary phase (± standard deviation). Refer to text.

extent in both gases (Table 7). We also observed that the fluorescent lipid oxidation products in the cell pellet did not increase, whereas their content in the supernatant fraction did rise slightly (Table 8).

If *E. coli* K-12 strains are able to repair acyl chain hydroperoxides, significant changes in the lipid acyl chain composition may be observed and no accumulations of the lipid oxidation products beyond the hydroperoxide will occur. We have been unable to demonstrate an enzymatic lipid hydroperoxide-reducing activity in lysed cell preparations and in particular have found no glutathione peroxidase activity in strains K1060 and K-12 Ymel (C. Bello, G. Santangelo, and J. Harley, unpublished data).

Toxicity of the lipid extract. The toxicity of the lipid from cells that had perished in hyperbaric oxygen was determined. The lipid from K1060 supplemented with linoleate and grown to stationary phase was extracted after the bacterial suspension was exposed to air plus 300 lb/in^2 of O_2 for 48 h. Linoleate-supplemented K1060 were then treated with dispersions prepared from this lipid extract. Recent work has shown that phospholipids prepared in a similar manner are incorporated into the membrane lipid (23). The lipid dispersions were not toxic to resting K1060 that had been previously grown to stationary phase with linoleate, even at levels that were 10 times higher than the lipid-per-unit volume found in the original bacterial suspensions (data not presented).

To discover whether the lipid dispersions altered the kinetics of hyperbaric oxygen toxicity, the dispersions were added to bacterial suspensions, and the mixture was exposed to air plus 300 lb/in^2 of O_2. Surprisingly, the lipid dispersions delayed the loss of bacterial viability in hyperbaric oxygen. In addition, the degree of protection increased with increasing additions of exogenous lipid. Lipid dispersions both from hyperbaric oxygen-treated bacterial suspensions and from suspensions that had not been exposed to hyperbaric oxygen showed this protective effect (data not presented).

These results argue that the lipid extract, at least in the presence of the entire lipid complement, does not contain concentrations of toxic substances sufficient to affect bacterial viability. On the other hand, the protective effect of exogenous lipid is another result consistent with a membrane-related function influencing the survival of *E. coli* in hyperbaric oxygen.

Toxicity of linoleic acid hydroperoxide. Linoleic acid hydroperoxide or other iodide-reducible substance increased substantially in hyperbaric oxygen-exposed suspensions of *E. coli* K1060 grown on linoleate (Table 6). The hydroperoxide, of course, represents the first recover-

TABLE 8. *Complex fluorescent products of lipid oxidation*[a]

Fraction	Fluorescent units	
	Per 100 ml of suspension	Per mg of acyl chain
Cellular pellet		
0 h	11.8	2.2
48 h of air + 300 lb/in^2 of N_2	6.9	1.4
48 h of air + 300 lb/in^2 of O_2	10.2	2.4
Supernatant		
0 h	11.9	56.5
48 h of air + 300 lb/in^2 of N_2	19.4	61.6
48 h of air + 300 lb/in^2 of O_2	20.1	65.3

[a] Relative fluorescence (excitation at 350 to 365 nm and emission at 420 to 425 nm) of the lipid extract from a suspension of linoleate-supplemented K1060 grown to stationary phase. Fluorescent unit is defined as 2% of the fluorescence of 1.0 μg of quinine sulfate per ml in 0.1 N sulfuric acid (excitation at 348 nm, emission at 437 nm).

able product in oxygen-mediated lipid oxidation. The toxicity of linoleic acid hydroperoxide was assessed to determine whether it mimicked the effects of hyperbaric oxygen on *E. coli* viability.

Synthesis of linoleic acid hydroperoxide with soybean lipoxygenase generates mainly the 13-hydroperoxide (70 to 100%), with a smaller proportion of the 11-hydroperoxide of linoleic acid (0 to 30%) (8). The effect of very high levels of these hydroperoxides was evaluated in *E. coli* K-12 Ymel and linoleate-supplemented strain K1060, from both phases of growth. As much as 1.0 mM linoleic acid hydroperoxide was not toxic to *E. coli* K-12 Ymel. Linoleic acid hydroperoxide at 0.1 mM was without effect in resting K1060 that had been grown with linoleate, and 1.0 mM was not significantly toxic relative to controls after 96 h ($P = 0.09$). Linoleic acid hydroperoxide at 0.1 mM was about 500 times the concentration of iodide-reducible material found in the cell suspension (Table 6). Since approximately 25% of the added 1.0 mM linoleic acid hydroperoxide was recovered in the washed cellular pellet as determined by the iodide reduction method, a large fraction of the added hydroperoxide was associated with the cell. In addition, the concentration of linoleic acid hydroperoxide in the bacterial suspensions did not change after 8 h and decreased only slightly after a 24-h exposure to 1.0 mM linoleic acid hydroperoxide (G. Santangelo, C. Bello, and J. Harley, unpublished data).

DISCUSSION

These experiments show that hyperbaric oxygen is, in general, toxic to wild-type *E. coli* K-12. After an initial lag, bacteria from both exponential and stationary phases of growth generally lose viability as an apparent first-order process. Viability loss in *E. coli* after hyperbaric exposure has been previously observed (18, 19, 22, 41, 48), but the kinetics of the process have not been similarly characterized.

Manipulation of the unsaturated fatty acyl chain component of the membrane changes the kinetics of *E. coli* K1060 viability loss in hyperbaric oxygen. Since the rate of viability loss increases with increasing unsaturation of the fatty acid supplement, as does the propensity to lipid oxidation, we examined the role that lipid oxidation plays in oxygen toxicity in this system.

Lipid acyl chain analyses show that the relative unsaturated acyl chain composition is usually not changed when suspensions of K1060 are exposed to hyperbaric oxygen (Tables 1-3). K1060 supplemented with linoleate and grown to stationary phase is an exception. Here there are significant relative losses of the polyunsaturated acyl chain species in either hyperbaric gas. We therefore examined linoleate-grown cells for accumulation of products of lipid oxidation during hyperbaric oxygen killing. Only the iodide-reducible material increases in lipid extracts. Neither lipid oxidation products reactive with thiobarbituric acid nor the complex fluorescent products of lipid peroxidation significantly increases after hyperbaric oxygen exposure (Tables 7 and 8). If lipid oxidation is an important aspect of hyperbaric oxygen poisoning, these data indicate that toxicity would be mediated by linoleate hydroperoxide and its phospholipid or glyceride derivatives.

We therefore tested the toxicity of linoleic acid hydroperoxides. They had little or no toxicity when added to bacterial suspensions at concentrations that were more than 500 times greater than that detected in the hyperbaric oxygen-exposed bacterial suspensions. Although 25% of the 1.0 mM linoleic acid hydroperoxide was recovered with the washed cellular pellet, it is remotely possible that the exogenous linoleic acid hydroperoxide was sequestered in such a way that a potential toxicity could not be expressed.

Lipid hydroperoxides have been toxic in most of the biological systems in which they have been tested. Administered orally to mammals, lipid hydroperoxides are toxic in some circumstances, but they are deadly poisons at low doses given intravenously or intraperitoneally (5, 11). The oxidation products of linoleate and methyl linoleate cause a profound decrease in the respiration and viability of Ehrlich ascites tumor cells at millimolar concentrations (33). These hydroperoxides are also toxic to yeast, other animal cells, and plants (14, 32, 46).

It has been claimed that hydroperoxides are also toxic to *E. coli*. Latterjet investigated the toxicity of cumene hydroperoxide and found that logarithmic-phase cells were much more sensitive than stationary-phase cells (25). His cumene hydroperoxide was only 40% pure, however, and he did not assess the toxicity of cumene alone. Gamage et al. have studied the toxicity of linoleic acid hydroperoxide in *E. coli* B (15, 16). Contrary to the findings reported here in *E. coli* K1060, they conclude that linoleic acid hydroperoxide causes viability loss. They determined viability by monitoring optical density and did not directly assess the viability as we have done. We found that 1.0 mM linoleic acid hydroperoxide had no effect on the growth of *E. coli* K-12 Ymel, but did partially inhibit the growth of an *E. coli* B strain. Linoleic acid hydroperoxide at 0.1 mM had no effect on the growth of either strain (G. M. Santangelo and J. B. Harley, unpublished data). The differences between these studies can be attributed to the *E. coli* strain

differences.

The data suggest that lipid oxidation is not the mechanism of hyperbaric oxygen poisoning in these experimental conditions. Nonsaturated acyl chains are not consumed in hyperbaric oxygen relative to hyperbaric nitrogen (Table 1-3). Although lipid extracts show a substantial increase in hydroperoxide content with hyperbaric treatment (Table 6), neither these lipid extracts nor synthesized linoleic acid hydroperoxide is toxic to these bacteria. Last, the secondary products of lipid oxidation, thiobarbituric acid-reactive material and the fluorescent complex products, do not accumulate in hyperbaric oxygen.

The difference in the survival kinetics of K1060 supplemented with 19:cyclopropane compared with those of K1060 supplemented with oleate is especially intriguing. 19:Cyclopropane-supplemented K1060 fare much better in hyperbaric oxygen. This suggests that cyclopropane acyl chains, which accumulate during stationary phage in *E. coli*, do have survival value under conditions that have not previously been tested (40). In *Streptococcus faecalis*, cyclopropane fatty acid-deficient, amethopterin-resistant mutants have been found to be more sensitive to low pH, high salt, and deoxycholate than their parent strains (24).

The lipid composition of strain K1060 can be altered to make the cells either more or less sensitive to hyperbaric oxygen than the parental strain, K-12 Ymel (Fig. 1-3). This strongly suggests that K1060 and K-12 Ymel perish in hyperbaric oxygen by the same mechanism. We conclude that the rate-limiting step in *E. coli* K-12 oxygen poisoning is affected by a membrane-associated process whose activity is altered by lipid acyl chain manipulations. There is clear precedence for this conclusion. The temperature dependence of a variety of transport proteins and membrane-bound enzymes, as well as their specific activities at given temperatures, vary when the unsaturated lipid composition of *E. coli* is altered (2, 27, 29).

Comparison of the survival kinetics of the substrains supplemented with 19:cyclopropane with those supplemented with 12-methyltetradecanoate or 9- or 10-monobromostearate in hyperbaric oxygen provides further evidence consistent with a membrane-associated model of oxygen toxicity. Manipulating the fatty acyl composition again results in changes in the kinetics of survival in hyperbaric oxygen. The particular mechanistic relevance of these survival differences in these particular substrains (Fig. 4) must be interpreted with caution, since changes in the growth rate indicate that a critical membrane-associated function for growth is hampered by forced growth on a saturated fatty acid supplement.

In a recent study, Yatvin has shown that the survival of *E. coli* K1060 subjected to γ-irradiation is affected by the fatty acid supplement when irradiation of oleate- and linolenate-grown cells is done at 4°C, but not at room temperature (47). These results suggest that a membrane-associated rate-limiting step is also involved in γ-irradiation killing of *E. coli*.

There are many structural and metabolic differences between stationary- and exponential-phase *E. coli* that may enhance the relative toxicity of oxygen for logarithmic-phase cells (Fig. 3). For example, the greater proportion of saturated acyl chain in the stationary-phase bacteria may alter the affected rate-determining membrane-related activity. In addition, changes in cellular defenses against reduced oxygen species are undoubtedly important. Although we have found no change in superoxide dismutase activity as wild-type *E. coli* pass into stationary phase (C. Bello, G. Santangelo, and J. B. Harley, unpublished data), catalase activity increases by fivefold in stationary-phase *E. coli* K-12 (12).

ACKNOWLEDGMENTS

We gratefully acknowledge the contribution of Cesar Bello, Joseph Lamb, Eric Clingen, Charles Hires, and Ann Kelley, and the advice of the late James Allen, Manfred Bayer, and David Goodman.

J.B.H. was a post-doctoral fellow of the National Institutes of Allergy and Infectious Disease (Public Health Service grant 1-F32 AI 05186-01). This study was supported by Public Health Service grants AI 08903 (from the National Institute of Allergy and Infectious Diseases) and AM 19813 (from the National Institute of Arthritis, Metabolism and Digestive Diseases).

LITERATURE CITED

1. **Allen, J. E., and H. Rasmussen.** 1971. The effect of oxygen on cellular metabolism. Int. J. Clin. Pharmacol. **5**:26–33.
2. **Beacham, I. R., and D. F. Silbert.** 1973. Studies on the uridine diphosphate-galactose: lipopolysaccharide galactosyl transferase reaction using a fatty acid mutant of *Escherichia coli*. J. Biol. Chem. **248**:5310–5318.
3. **Bert, P.** 1878. Barometric pressure: researches in experimental physiology. (Translated by M. A. Hitchcock and F. A. Hitchcock.) College Book Co., Columbus, Ohio.
4. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
5. **Cortesi, R., and O. S. Privett.** 1973. Toxicity of fatty ozonides and peroxides. Lipids **7**:715–721.
6. **Dickens, F.** 1946. The toxic effects of oxygen on brain metabolism and on tissue enzymes. 2. Tissue enzymes. Biochem. J. **40**:171–187.
7. **Dillard, C. J., and A. L. Tappel.** 1971. Fluorescent products of lipid peroxidation of mitochondria and microsomes. Lipids **6**:715–721.
8. **Dolev, A., W. K. Rohwedder, and H. J. Dutton.** 1967. Mechanism of lipoxidase reaction. I. Specificity of hydroperoxidation of linoleic acid. Lipids **2**:28–32.
9. **Egmond, M. R., M. Brunori, and P. M. Fasella.** 1976. The steady-state kinetics of the oxygenation of linoleic acid catalyzed by soybean lipoxygenase. Eur. J. Bio-

chem. 61:93–100.
10. **Ferguson, K. A., M. Glaser, W. H. Bayer, and P. R. Vagelos.** 1975. Alteration of fatty acid composition of LM cells by lipid supplementation and temperature. Biochemistry 14:146–151.
11. **Findlay, G. M., H. H. Draper, and J. G. Bergan.** 1970. Metabolism of 1-^{14}C-methyl linoleate hydroperoxide in the rabbit. Lipids 5:970–975.
12. **Finn, G. J., and S. Condon.** 1975. Regulation of catalase synthesis in *Salmonella typhimurium*. J. Bacteriol. 123:570–579.
13. **Fox, C. F., J. H. Law, N. Tsukagoshi, and G. Wilson.** 1970. A density label for membranes. Proc. Natl. Acad. Sci. U.S.A. 67:598–605.
14. **Fränz, J., and T. B. Cole.** 1962. Effects of ultraviolet-irradiated methyl linoleate on cell division and respiration in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. 96:382–385.
15. **Gamage, P. T., T. Mori, and S. Matsushita.** 1971. Effects of linoleic acid hydroperoxides and their secondary products on the growth of *Escherichia coli*. Agr. Biol. Chem. 35:33–39.
16. **Gamage, P. T., T. Mori, and S. Matsushita.** 1971. Incorporation of ^{14}C linoleic acid hydroperoxides and their secondary products into the cells of *Escherichia coli*. Agr. Biol. Chem. 35:1254–1258.
17. **Gerschman, R., D. L. Gilbert, S. W. Nye, P. Dwyer, and W. O. Fenn.** 1954. Oxygen poisoning and X-irradiation: a mechanism in common. Science 119:623–626.
18. **Gregory, E. M., and I. Fridovich.** 1973. Oxygen toxicity and the superoxide dismutase. J. Bacteriol. 114: 1193–1197.
19. **Gregory, E. M., S. A. Goscin, and I. Fridovich.** 1974. Superoxide dismutase and oxygen toxicity in a eukaryote. J. Bacteriol. 117:456–460.
20. **Haugaard, N.** 1946. Oxygen poisoning. XI. The relation between inactivation of enzymes by oxygen and essential sulfhydryl groups. J. Biol. Chem. 164:265–270.
21. **Haugaard, N.** 1968. Cellular mechanisms of oxygen toxicity. Physiol. Rev. 48:311–373.
22. **Hopkinson, W. I., and A. G. Towers.** 1963. Effects of hyperbaric oxygen on some common pathogenic bacteria. Lancet ii:1361–1363.
23. **Jones, N. C., and M. J. Osborn.** 1977. Interaction of *Salmonella typhimurium* with phospholipid vesicles. Incorporation of exogenous lipids into intact cells. J. Biol. Chem. 252:7398–7404.
24. **Jungkind, D. L., and R. C. Wood.** 1974. Physiological differences between cyclopropane fatty acid-deficient mutants and the parent strain of *Streptococcus faecalis*. Biochim. Biophys. Acta 337:298–310.
25. **Latterjet, R.** 1956. Effects of radiation and peroxides on viral and bacterial functions linked to DNA specificity, p. 275–299. *In* G. E. W. Wolstenholme and G. M. O'Connor (ed.), CIBA Foundation Symposium on Ionizing Radiations and Cell Metabolism. Excerpta Medica, Amsterdam.
26. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190–206.
27. **Linden, C. D., K. L. Wright, H. M. McConnell, and C. F. Fox.** 1973. Lateral phase separations in membrane lipids and the mechanism of sugar transport in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 70:2271–2275.
28. **McCord, J. M., and I. Fridovich.** 1969. Superoxide dismutase: an enzymatic function for erythrocuprein. J. Biol. Chem. 244:6049–6055.
29. **Mavis, R. D., and P. R. Vagelos.** 1972. The effect of phospholipid composition on membranous enzymes in *Escherichia coli*. J. Biol. Chem. 247:652–659.
30. **Rose, C. S., and P. György.** 1952. Specificity of hemolytic reaction in viatmin E-deficient erythrocytes. Am. J. Physiol. 168:414–420.
31. **Schauenstein, E.** 1967. Autoxidation of polyunsaturated esters in water: chemical structure and biological activity of the products. J. Lipid Res. 8:417–428.
32. **Schauenstein, E., and G. Shatz.** 1959. Uber die biologische Bedeutung der reaktion mehrfach angesättigter Fettsäuren und deren Ester in Wasser. Fette Seifen Anstrichm. 61:1068–1076.
33. **Shuster, C. W.** 1955. Effects of oxidized fatty acids on ascites tumor metabolism. Proc. Soc. Exp. Biol. Med. 90:423–426.
34. **Silbert, D. F., R. C. Ladenson, and J. L. Honeggar.** 1973. The unsaturated fatty acid requirements in *Escherichia coli*: temperature dependence and total replacement by branched-chain fatty acids. Biochim. Biophys. Acta 311:349–361.
35. **Stees, J. L., and O. R. Brown.** 1973. Susceptibilities of intracellular and surface sulfhydryl groups of *Escherichia coli* to oxidation by hyperoxia. Microbios 7:257–266.
36. **Stirton, A. J., J. Turner, and R. W. Riemenschneider.** 1945. O absorption of Me esters of fatty acids and the effect of antioxidants. Oil Soap 22:81–83.
37. **Stuart, B., R. Gerschman, and J. N. Stannard.** 1962. Effect of high oxygen tension on potassium retentivity and colony formation of bakers' yeast. J. Gen. Physiol. 45:1019–1030.
38. **Swoboda, P. A. T., and C. H. Lea.** 1958. Determination of the peroxide value of edible fats by colorometric iodometric procedures. Chem. Ind. 1909–1910.
39. **Tappel, A. L.** 1975. Lipid peroxidation and the fluorescent molecular damage to membranes, p. 145–179. *In* B. F. Tramp and A. Arstila (ed.), Pathobiology of cell membranes, Vol. 1.
40. **Taylor, F., and J. E. Cronan, Jr.** 1976. Selection and properties of *Escherichia coli* mutants defective in the synthesis of cyclopropane fatty acids. J. Bacteriol. 125:518–523.
41. **Thaysen, A. C.** 1934. Preliminary note on the action of gases under pressure on the growth of micro-organisms. Biochem. J. 28:1330–1335.
42. **Tsen, C. C., and H. B. Collier.** 1960. The protective action of tocopherol against hemolysis of rat erythrocytes by dialuric acid. Can. J. Biochem. Physiol. 38:957–964.
43. **Vaughan, G. L.** 1971. Oxygen toxicity in a fusion yeast. J. Cell Physiol. 77:363–372.
44. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. 218:97–106.
45. **Warren, L.** 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234:1971–1975.
46. **Wilbur, K., N. Wolfson, C. Kenaston, A. Ottolenghi, M. Gaulden, and F. Bernheim.** 1957. Inhibition of cell division by ultraviolet irradiated unsaturated fatty acid. Exp. Cell Res. 13:503–509.
47. **Yatvin, M. B.** 1976. Evidence that survival of γ-irradiated *Escherichia coli* is influenced by membrane fluidity. Int. J. Radiat. Biol. 30:571–575.
48. **Yost, F. J., and I. Fridovich.** 1976. Superoxide and hydrogen peroxide in oxygen damage. Arch. Biochem. Biophys. 175:514–519.