Effects of hyperoxia on composition and rate of synthesis of fatty acids in *Escherichia coli*

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ABSTRACT Growth of and fatty acid synthesis in *Escherichia coli* were inhibited by oxygen at partial pressures above 1 atm and were prevented by exposure to oxygen at 4.2 atm on membranes incubated on a minimal medium. Growth and fatty acid synthesis returned to control rates when cells were removed from hyperoxia to air. The spectrum of fatty acids produced was unchanged by oxygen at pressures which reduced the rate of synthesis. *In situ* fatty acids were stable to oxygen at pressures which prevented growth and synthesis. Reinitiation of synthesis after complete inhibition in hyperoxia occurred without production of aberrant fatty acids. Fatty acid synthetase specific activity was virtually unchanged, compared with air controls, in cells exposed either to 3.2 or to 15.2 atm of oxygen. The spectrum of fatty acids synthesized by cell-free extracts during incubation in 4.2 atm of oxygen was not different from air-incubated controls. Synthetase assays included added NADPH, acyl carrier protein, mercaptoethanol, and malonyl coenzyme A; hence, damage, other than reversible sulfhydryl oxidation, to the apoenzymes of synthetase was ruled out.

SUPPLEMENTARY KEY WORDS biosynthesis · lipids · gas–liquid chromatography · oxygen toxicity · hyperbaric oxygen · fatty acid synthetase

Oxygen at elevated pressures is known to be toxic for a variety of life forms, from single cells through higher mammals (see Ref. 1 for a review). Studies of potential sites of metabolic damage have shown that a variety of enzymes are sensitive to hyperoxia. In general, sulfhydryl-containing enzymes and coenzymes are relatively more sensitive to oxygen (2). Relatively little is known about the effects of hyperoxia on the metabolism of lipids (1). We have initiated a study in this area because of the importance of lipids as components of cell membranes and because of their significance in membrane-related functions of oxidative phosphorylation and transport.

Fatty acids are synthesized in *Escherichia coli* by the same mechanism as the extramitochondrial pathway of mammals (3). Therefore, studies with this microorganism should have relevance at the biochemical level to the general question of the effects of hyperoxia on lipid metabolism in other life forms.

MATERIALS AND METHODS

Unless otherwise specified, bacteria were exposed to hyperoxia by the membrane culture technique (4), which is briefly described here. Bacteria from exponentially growing cultures were diluted in sterile potassium phosphate buffer, 0.05 M, pH 7.0, at 4°C to obtain the desired cell concentration (a cultural optical density of 1.00 at 500 nm is equivalent to 6.7×10^8 bacteria/ml). Bacteria were uniformly deposited onto the surface of 47-mm diameter, cellulose acetate membranes (0.45-μ pore size; Millipore Corp., Bedford, Mass.) by vacuum filtration of 10 ml of suspension per membrane. Inoculated membranes were aseptically transferred to the surface of solid medium which was prewarmed to 37°C. The desired number of control and experimental membrane cultures were prepared from identical volumes of the same cell suspension.

E. coli strain E-26 was cultured using the minimal salts medium of Winkler and de Haan (5), with 0.5% sodium acetate instead of glucose for all cultures. Membrane cultures were exposed to gases at elevated pressures in a model 614 hyperbaric chamber (Bethlehem Corp., Bethlehem, Pa.). Air temperature in the chamber was monitored with a probe thermometer sealed into the chamber and externally connected to a Tele-Thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Pressurizations were done at rates so that temperatures did not exceed 43°C, even transiently. Humid-

ity was provided by an open container of water in the chamber.

Rate of synthesis of fatty acids was measured using membrane cultures containing approximately 10^7 bacteria per membrane and incubated on 6 ml of acetate–minimal salts–agar medium containing sodium acetate-^{14}C (10^6 dpm/membrane culture). Cultures were incubated in air at 37°C for 8–10 hr before transfer into hyperbaric oxygen. For analysis, membranes containing the bacteria were placed into 4 ml of 2 N HCl in scintillation vials. The amount of acetate-^{14}C which was incorporated into fatty acids was measured by liquid scintillation spectroscopy using the method of Cronan (6), except that heating to 121°C was done in an autoclave. Uninoculated membranes were treated identically to obtain control levels of radioactivity, which were subtracted. Similar experiments were performed using cultures incubated in stirred liquid medium in a pressure cell (model 602, Amicon Corp., Lexington, Mass.). Samples (2–6 ml) were filtered (0.45-μ cellulose acetate membranes; Millipore Corp.), washed with potassium phosphate buffer, 0.05 M, pH 7.0, and cells and membranes were treated as described for membrane cultures. Optical density measurements of cells were made at 500 nm. All cultures were incubated at 37°C.

To obtain bacterial fatty acids for gas–liquid chromatography, membrane cultures were inoculated with approximately 10^8 bacteria per membrane. The membrane cultures were incubated as described for the determination of rates of synthesis of fatty acids. Bacteria were resuspended from membranes in potassium phosphate buffer, pH 7.0, 0.05 M. The cells from 10 membrane cultures were pooled and centrifuged at 12,000 *g* for 10 min at 4°C. The pellet was resuspended in 20 ml of 2.5 N KOH in 85% (v/v) methanol; approximately 1 mg of hydroquinone was added as antioxidant and the solution was left overnight at 55°C. Fatty acids were extracted (7), dried over Na_2SO_4, filtered, and evaporated. They were then methylated (8) and diluted with heptane to a concentration of approximately 0.1 mg/μl for injection into a gas chromatograph (Barber-Colman, Series 5000, Nuclear-Chicago, Des Plaines, Ill.) equipped with a hydrogen flame detector, 1:10 column effluent splitter, and gas flow proportional counter. The columns were packed with 20% ethylene glycol succinate on Gas-Pack WAB, 60–80 mesh (Chemical Research Services, Inc., Addison, Ill.). Analyses were done isothermally with helium as the carrier gas at a flow rate of 80 ml/min. Column temperatures of 158°C and 180°C were used. A different column material and a different temperature were used for one experiment as shown in the footnote to Table 5. Fatty acids were identified by comparison of relative retention times with known standards (Applied Science Laboratories, State College, Pa.) except for 14:0 (3-OH), 17:0 cyc, and 19:0 cyc, which were identified by comparison with published relative retention times (9, 10). Fatty acid compositions were calculated by expressing the areas under individual peaks as percentages of the total area under all peaks. All peaks were reported; those with areas of less than 1% were reported to be present in trace amounts.

Fatty acid synthetase activity was measured in cell-free extracts prepared from early stationary phase cultures grown at 37°C in minimal salts–acetate broth and aerated by shaking. Cells were harvested and washed twice with imidazole–HCl buffer, pH 7.4, 0.01 M, by centrifugation at 4°C. A 10% suspension (w/v) of cells in the same buffer was exposed to ultrasonic energy using the Biosonik system (Bronwill Scientific, Inc., Rochester, N.Y.). The vessel containing the cell suspension was immersed in a stirred salt water and ice bath, and ultrasonic energy at 60% of maximum power was supplied for a total of 5 min; power on for 30 sec alternated with power off for 30 sec so that the temperature of the extract did not rise above 4°C. The extract was centrifuged at 39,000 *g* for 10 min at 4°C and the supernatant was recentrifuged at 100,000 *g* for 30 min at 2°C. Mercaptoethanol, final concentration 0.01 M, was added to the supernatant; this fraction will be referred to as the 100,000 *g* crude extract. The same procedure was used to prepare extracts of bacteria which had been exposed to 3.2 atm of oxygen using membrane cultures inoculated with 10^9 cells per membrane and incubated in hyperoxia as previously described. Fatty acid synthetase specific activity was measured by radioactive assay (11).

Synthetase was partially purified as described by Lennarz, Light, and Bloch (11) from air-grown cells and from such cells after exposure for 5 hr to a mixture of 1 atm of air and 15 atm of oxygen. The cells were exposed in a pressure chamber in 200 ml of minimal salts–acetate medium (6.4×10^9 cells/ml) in a 500-ml flask; agitation was with a magnetic stirrer. The specific activities from air-grown and oxygen-exposed cells were compared by an optical assay (12). The composition of the fatty acids synthesized in air and in 4.2 atm of oxygen by synthetase derived from air-grown cells was measured (Table 5).

Sulfhydryl concentration was determined using the 4,4′-dithiodipyridine method of Grassetti and Murray (13), standardized with cysteine. Protein was measured by the method of Lowry et al. (14), using bovine albumin as the standard.

RESULTS

Single cells of *E. coli* did not grow into macroscopically visible colonies during approximately 1 day of incubation on the surface of nutrient agar (Difco) at 37°C when the

gas phase was 1 atm of air plus 4 atm overpressure of oxygen (Table 1). Control cultures, incubated in air or in a mixture of 1 atm of air and 4 atm of nitrogen, produced visible colonies within 8–10 hr. When oxygen-exposed cultures were reincubated in air at 37°C, colonies appeared in numbers comparable to controls, indicating that a bacteriostatic, not lethal, effect resulted from hyperoxia.

The bacteriostatic effect of hyperoxia was investigated further, using a defined minimal salts medium with acetate as the sole carbon source. In liquid culture, growth inhibition occurred rapidly at 4.2 atm of oxygen (Fig. 1). Cells recovered to the normal growth rate for this medium (80 min/generation) upon transfer from the hyperoxic environment to air at 37°C. The time required for recovery was proportional to the period of hyperoxia, e.g., 20 min for cells exposed for 1 hr, 1 hr for cells exposed for 2 hr, and about 8 hr for cells after 20 hr of exposure under the same conditions (Fig. 1).

When cells were exposed to oxygen partial pressures that were sufficient to stop growth, the pattern shown in Fig. 1 was obtained. Optical density continued to rise for about 30 min and subsequently decreased rapidly to approximately the optical density observed at the time of entry of the culture into hyperoxia. A similar pattern was seen when the rate of incorporation of acetate-^{14}C into fatty acids was measured using the same liquid culture medium with added radioactive acetate (Fig. 2). There was a net synthesis of fatty acids which continued for about 30 min at 4.2 atm of oxygen, and then the amount of radioactivity in the cellular fatty acids decreased to near the amount present before exposure to hyperoxia. The cells were removed by filtration and washed before being prepared for analysis of the radioactivity; hence, only those fatty acids present in whole cells or in structural elements which were retained by a cellulose acetate membrane with a porosity of 0.45 μ were detected.

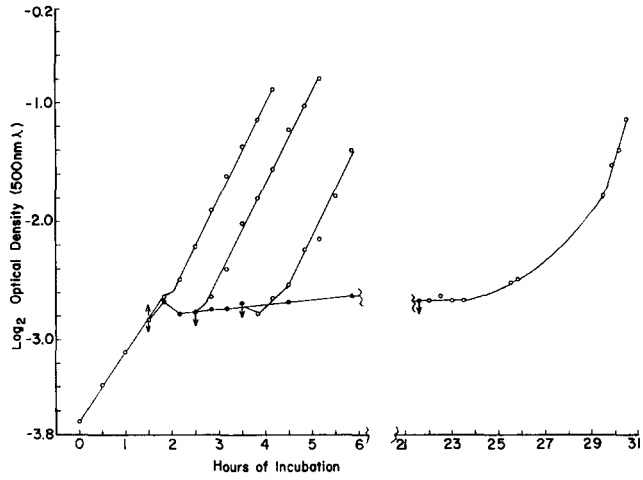

FIG. 1. Reversible growth inhibition of *E. coli* by hyperoxia. Cells were grown at 37°C in minimal medium, with acetate as the sole carbon source, in a sealed vessel, with stirring. Pressure was released to remove samples. An optical density change of 1.0 is equivalent to a doubling of cell number. The upward arrow indicates the point of pressurization to 4 atm overpressure with pure oxygen (4.2 atm total oxygen pressure); downward arrows indicate points of transfer to air atmospheres in flasks incubated in a reciprocating water bath at 37°C. Optical density of cultures in air, O—O; optical density of cultures in hyperoxia, ●—●. Pressure controls gave results as follows: 4 atm of helium plus 1 atm of air: 96 ± 5% of air control growth, and 4 atm of nitrogen plus 1 atm of air: 88 ± 6% of air control growth, when exposed for 1 hr (averages of three and seven determinations, respectively).

TABLE 1 BACTERIOSTATIC EFFECT OF HYPEROXIA ON MEMBRANE CULTURES OF *E. coli* INCUBATED ON NUTRIENT AGAR AT 37°C

Conditions of First Incubation		Number of Colonies*		
Gaseous Environment	Duration	Control†	First Incubation	Second Incubation‡
	hr			
1 atm air + 4 atm N_2	9.5	132 ± 11.6	127 ± 5.5	127 ± 5.5
1 atm air + 4 atm N_2	30	132 ± 11.6	134 ± 7.1	134 ± 7.1
1 atm air + 4 atm O_2	8	124 ± 11.7	0	128 ± 12.3
1 atm air + 4 atm O_2	24	124 ± 11.7	0	108 ± 15.4

* Averages ± SD for five membrane cultures; only the zeros are significant at the 5% level, compared with air controls.
† Membrane cultures incubated in air only.
‡ In air for 18 hr.

Figs. 3 and 4 show the rates of incorporation of acetate-^{14}C into fatty acids when cultures on the surfaces of membranes were exposed to air, to 1–4 atm of oxygen, or to a mixture of 1 atm of air and 4 atm of nitrogen. Incorporation of acetate into fatty acids was inhibited at pressures above 1 atm of oxygen, and was prevented by oxygen at 4.2 atm. Pressure per se (nitrogen) did not inhibit incorporation.

The fatty acid composition of cells grown in oxygen at 1 atm under the conditions shown in Fig. 3 was examined by gas–liquid chromatography, and the results are shown in Table 2. Table 3 shows the fatty acid compositions of cells exposed as shown in Fig. 3 to oxygen at 4.2 atm, and after intervals of recovery in air. Palmitoleic (*cis*-9-hexadecenoic) acid was used as the standard for 16:1, and oleic (*cis*-9-octadecenoic) acid was the standard for 18:1 (Tables 2, 3). Presumably, the microbial fatty acids identified as 16:1 and 18:1 may have contained unseparated *cis*-7-hexadecenoic and *cis*-11-octadecenoic acids, respectively (15).

Crude 100,000 *g* cell-free extracts prepared from cells whose growth on membranes was stopped by 3 hr of exposure to oxygen at 3.2 atm contained fatty acid synthetase at the same specific activity as was found in air-

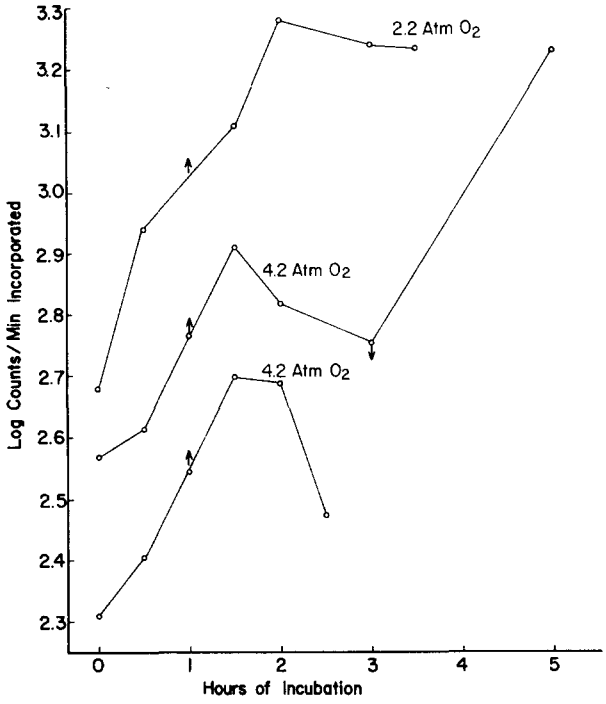

FIG. 2. Inhibition of growth and fatty acid synthesis in *E. coli* by hyperoxia. Cells were exposed as described in Fig. 1, except that acetate-^{14}C was present (2.3×10^6 dpm/ml) in the medium. The radioactivity in the fatty acids was measured by liquid scintillation spectroscopy (6). Upward arrows indicate times of transfer from air into hyperoxia, and the downward arrow shows the time of transfer back into air incubation.

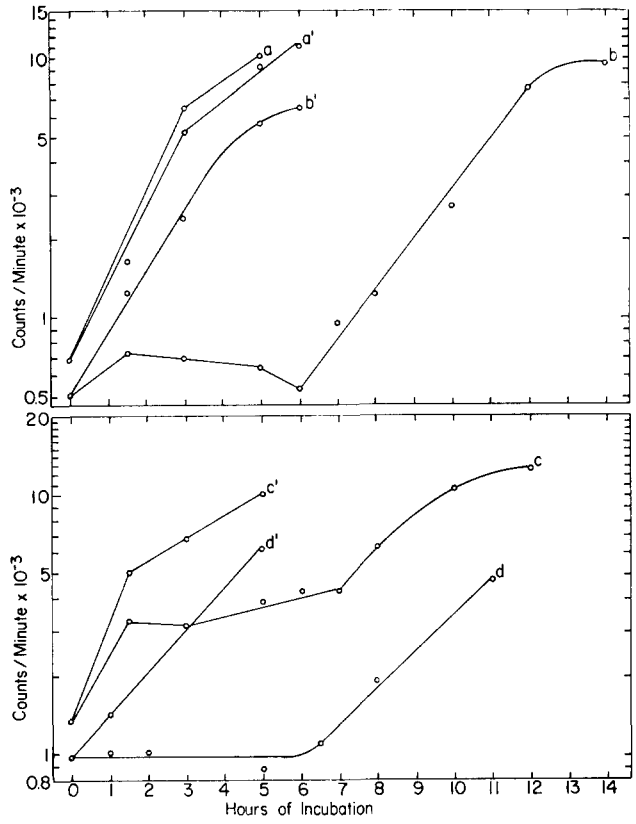

FIG. 3. Incorporation of acetate-^{14}C into fatty acids by membrane cultures of *E. coli* exposed to various gaseous environments at 37°C. Membrane cultures were incubated in air at 37°C on minimal agar medium containing acetate-^{14}C (10^6 dpm/culture) for approximately 9 hr preceding time zero on the figure. The radioactivity in fatty acids was determined by the method of Cronan (6). Cultures were incubated in a mixture of 1 atm of air and 4 atm of nitrogen (*a*); 1 atm of 100% oxygen (*b*); a mixture of 1 atm of 95% oxygen and 5% CO_2 plus 1 atm of air (*c*); a mixture of 1 atm of air and 4 atm of oxygen (*d*); and in air as paired controls (*a'*–*d'*). After 5 hr, cultures *b*, *c*, and *d* were transferred to air incubation at 37°C.

grown cultures (Table 4). The free sulfhydryl concentration in the extract was decreased by about 42% during the oxygen exposure. Synthetase specific activity was measured by an optical assay (11) at 30°C for partially purified fatty acid synthetase from air-grown cells and from cells after exposure to oxygen at 15.2 atm, as described in Materials and Methods. The specific activities for air-grown and oxygen-exposed cells were, respectively, 0.84 and 0.85 nmole of malonyl CoA incorporated into fatty acids/min/mg of protein (averages of three determinations each). The activity was proportional to the amount of protein over the protein concentration range tested, 0.76 mg to 1.74 mg per reaction (total reaction volume, 0.4 ml). The compositions of the fatty acids synthesized in air, in 4.2 atm of oxygen, and upon reincubation of the oxygen-exposed reaction mixture in air were similar (Table 5).

DISCUSSION

Oxygen at hyperbaric pressures was bacteriostatic, not lethal, for *E. coli* strain E-26. Rates of growth and fatty acid synthesis, but not composition of fatty acids synthesized, were altered by oxygen at 1 atm. Growth and synthesis of fatty acids continued in oxygen at 2.2 atm, but at a reduced rate. At 4.2 atm of oxygen, growth and synthesis of fatty acids stopped almost immediately.

The fact that more time was required for recovery to the normal growth rate when the period in hyperoxia was extended suggests that cumulative damage occurred in hyperoxia. The initial increases and subsequent decreases in optical density and in total radioactive fatty acids present in the intact cell mass after incubation at 2.2 or 4.2 atm of oxygen suggest that incomplete or deficient cells were produced and that these cells subsequently lysed.

Under hyperoxic conditions one might expect changes in the relative amounts of individual fatty acids if there were damage either to enzymes or to control mechanisms, or if *in situ* oxidation of fatty acids occurred. The kinds and relative proportions of fatty acids produced by growth of *E. coli* in air and in oxygen at 1 atm were very similar when cultures of comparable ages were compared. Com-

TABLE 2 SYNTHESIS OF FATTY ACIDS BY *E. coli* INCUBATED WITH ACETATE-^{14}C IN AIR AND IN OXYGEN AT 1 ATM

Fatty Acid*	In Air			In Oxygen		
	2 hr†	10 hr	24 hr	2 hr†	10 hr	24 hr
10:0	(trace)	trace (trace)	trace (trace)	(trace)	trace (trace)	trace (trace)
12:0	(3.1)‡	4.2§ (3.1)	4.3 (3.8)	(2.2)	4.3 (2.5)	4.7 (4.0)
14:0	(5.1)	6.6 (8.2)	6.5 (12.6)	(5.1)	11.1 (6.0)	12.6 (12.4)
14:0 (3-OH)	(11.3)	7.5 (4.4)	9.9 (6.8)	(5.4)	trace (4.1)	6.8 (3.1)
15:0	(trace)	1.8 (trace)	1.2 (trace)	(trace)	trace (trace)	trace (trace)
16:0	(27.8)	31.6 (32.9)	29.8 (29.8)	(28.2)	33.4 (35.2)	28.8 (31.2)
16:1	(12.6)	23.6 (19.6)	17.3 (15.6)	(12.7)	22.7 (15.0)	14.1 (12.0)
17:0 (cyc)	(10.8)	10.8 (11.3)	12.2 (12.1)	(9.8)	7.8 (8.9)	16.2 (13.5)
18:0	(7.2)	trace (2.5)	3.1 (4.9)	(8.3)	trace (2.7)	trace (4.7)
18:1	(16.9)	13.8 (15.5)	12.7 (11.5)	(22.8)	20.2 (21.4)	16.9 (15.5)
19:0 (cyc)	(5.8)	1.0 (2.5)	3.0 (3.8)	(5.4)	trace (3.9)	trace (3.7)

* Number of carbon atoms: number of bouble bonds. Cyc refers to presence of a cyclopropane ring.
† Radioactivity was too low for accurate analysis; therefore, only mass analyses are shown.
‡ Results in parentheses are percentages of the total mass of all fatty acids with chain length greater than 10.
§ Results are expressed as percentages of the total radioactivity which was incorporated into all fatty acids with chain length greater than 10.

TABLE 3 INCORPORATION OF ACETATE-^{14}C INTO FATTY ACIDS BY *E. coli* GROWN IN AIR, AFTER EXPOSURE TO HYPEROXIA, AND AFTER REINCUBATION IN AIR*

Fatty Acid†	In Air	Plus Oxygen at 4 atm for		Reincubated in Air for			Statistical Comparison of All Values	
	8 hr	1.5 hr	5 hr	1.5 hr	3 hr	6 hr	Average ± SD	Range
10:0	trace	trace	trace	trace	trace	trace		
12:0	3.9	3.4	4.2	4.2	3.4	3.7	3.8 ± 0.4	3.4–4.2
14:0	7.7	7.4	5.8	6.8	5.9	5.7	6.6 ± 0.9	5.7–7.7
14:0 (3-OH)	trace	3.5	4.1	3.9	4.1	5.6	3.7 ± 1.5	1.0–5.6‡
15:0	trace	1.1	trace	trace	trace	1.9		
16:0	32.5	35.6	34.5	37.6	34.2	34.0	34.7 ± 1.7	32.5–37.6
16:1	26.4	24.0	23.6	23.5	26.4	23.2	24.5 ± 1.5	23.2–26.4
17:0 (cyc)	7.2	5.8	5.6	4.4	7.1	9.3	6.6 ± 1.7	4.4–9.3‡
18:0	trace	trace	trace	trace	trace	trace		
18:1	22.4	19.2	22.0	19.7	18.6	14.3	19.4 ± 2.9	14.3–22.4‡
19:0 (cyc)	trace	trace	trace	trace	trace	2.3		

* Results are expressed as percentages of the total radioactivity incorporated into all fatty acids with chain length greater than 10.
† Number of carbon atoms: number of double bonds. Cyc refers to presence of a cyclopropane ring.
‡ Values having a percentage standard deviation greater than 10% and a range greater than 2% in absolute magnitude.

TABLE 4 EFFECTS OF EXPOSURE OF INTACT CELLS TO OXYGEN AT 3.2 ATM ON SYNTHETASE SPECIFIC ACTIVITY MEASURED IN CELL-FREE EXTRACTS OF *E. coli*

Growth Environment	10 min	20 min	10 min	20 min
	*cpm incorporated**		*specific activity*†	
1 atm air	1957	2645	0.22	0.18
Oxygen‡	2402	3402	0.21	0.18

* Incorporation of malonyl-1,3-^{14}C CoA into fatty acids. Reaction mixtures contained: imidazole–HCl, pH 7.4, 30 μmoles; 2-mercaptoethanol, 5 μmoles; TPNH, 1 μmole; acyl carrier protein, 0.2 ml of heated enzyme extract; acetyl CoA, 0.06 μmole; malonyl-1,3-^{14}C CoA (209,000 dpm), 0.205 μmole; and enzyme extract, 1.417 mg of protein (air control) or 1.836 mg of protein (oxygen) in a total volume of 0.5 ml. Control (zero reaction time) counts of 189 cpm for air and 307 cpm for oxygen have been subtracted. Data are averages of duplicate analyses.
† Nanomoles of malonyl CoA incorporated into fatty acids/mg protein/min, corrected for 65% counting efficiency.
‡ Membrane cultures exposed to a mixture of 1 atm of air and 3 atm overpressure of oxygen for 3 hr.

plete inhibition of fatty acid synthesis in hyperoxia and reinitiation of control rates of synthesis upon return to air were achieved without detectable changes in the types and relative amounts of fatty acids synthesized. Cellular fatty acids of *E. coli* were stable *in situ* to oxygen under conditions which completely prevented growth and synthesis. However, it should be noted that *E. coli* does not contain measurable amounts of highly unsaturated acids, which are especially susceptible to peroxidation.

The potential biological significance of the small differences in fatty acid composition (Tables 2 and 3) are unknown. The differences are probably insignificant in view of the variability reported with differences in nutrition and duration of incubation (16). As shown in Table 2, for example, there was a tendency in both oxygen- and air-grown cells for the percentage of 16:1 to increase and for 18:0 and 18:1 to decrease in older cultures. The variabil-

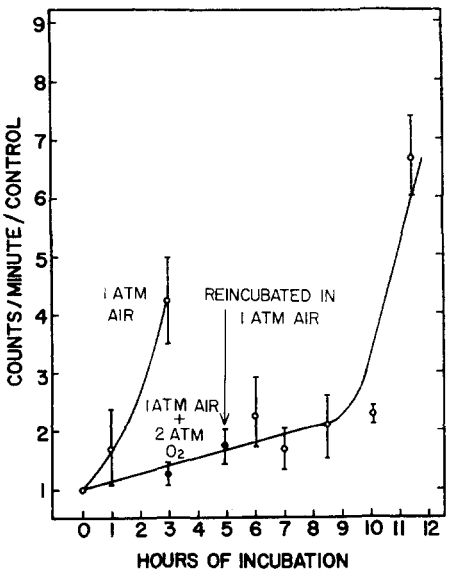

FIG. 4. Incorporation of acetate-^{14}C into fatty acids by membrane cultures prepared as described in Fig. 3 and exposed at 37°C to a mixture of 1 atm of air and 2 atm overpressure of oxygen, compared with air controls. Three experiments were normalized for plotting by dividing the cpm at given time intervals by the cpm in the control at zero time.

ity between air-grown and oxygen-exposed cells was of the same order of magnitude as the variations related to aging, and the differences would not appear to be growth-limiting.

The pattern of fatty acids reported here is similar to that found by Gavin and Umbreit (10) for another strain of *E. coli*, but the concentrations of individual acids are substantially different from those reported by Weinbaum and Panos (15) for strain E-26; however, the studies are not directly comparable since nutrition was different. A chain length of 18 carbons was preferentially synthesized by the extracts, while fatty acids with 16 carbons were predominately produced by intact cells. This is in agreement with previously published results (11).

Fatty acid synthesis may have ceased in hyperoxia simply because of feedback controls which became operative as a result of growth interruption. Reversible oxidation of sulfhydryl groups of synthetase or acyl carrier protein also may have prevented synthesis in hyperoxia. Reversible oxidation of sulfhydryl groups of synthetase could not be demonstrated in this study because sulfhydryl-reducing reagents were required for the assay. Effects on acetyl carboxylase or on acyl carrier protein would not be apparent in this study, nor would deficiencies in the cellular concentrations of NADPH or ATP, or in the cell's ability to transport metabolites into the cell. However, except for the possibility of reversible sulfhydryl oxidation, the data indicate that the apoenzymes of synthetase were stable to oxygen at pressures which completely inhibited synthesis.

TABLE 5 COMPOSITION OF FATTY ACIDS SYNTHESIZED BY *E. coli* SYNTHETASE* IN AIR AND IN OXYGEN FROM MALONYL-1,3-^{14}C CoA

Fatty Acid†	Retention Time, Relative to C_{16}‡	Air, 20 min	4.2 atm O_2, 20 min	4.2 atm O_2, 20 min, Plus Air, 20 min
		% of total fatty acids		
16:0	1.00	2.6	3.3	6.0
18:0	1.77 ± 0.05	10.6	9.4	6.2
18:1	2.10 ± 0.04	50.4	43.1	49.0
20:0	3.19 ± 0.15	7.9	6.5	7.4
Unknown§	3.73 ± 0.05	28.6	37.7	31.4

* Partially purified as indicated in Materials and Methods and analyzed by the radioactive assay of Lennarz et al. (11) with 2.16 mg of protein in a total reaction volume of 0.47 ml. Nonradioactive carrier (palmitate) was added and the mixture was saponified, purified, methylated, and analyzed by gas–liquid chromatography.

† Number of carbon atoms:number of double bonds. Cyc refers to presence of a cyclopropane ring.

‡ 183 cm × 3.5 mm glass column packed with 12% diethylene glycol succinate Anakrom ABS 60–80 mesh (Barber-Colman), column temperature 175°C, helium flow rate 80 ml/min.

§ This compound was not 22:0 or greater, since the retention times for 22:0 and 24:0 were 6.29 and 11.87, respectively, relative to 16:0. The absolute retention time of 16:0 was 3.66 min.

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