

Changes in Membrane Lipid Composition in Exponentially Growing *Staphylococcus aureus* During the Shift from 37 to 25 C

G. H. JOYCE, R. K. HAMMOND, AND DAVID C. WHITE

Biochemistry Department, University of Kentucky Medical Center, Lexington, Kentucky 40506

Received for publication 10 July 1970

Lowering the temperature of growth of *Staphylococcus aureus* from 37 to 25 C decreased the growth rate and induced changes in the composition of the membrane lipids. Changes in lipid composition also occur in the transition between exponential and stationary growth phases at one temperature. To isolate the effects of lowering the temperature, exponentially growing *S. aureus* was abruptly switched from 37 to 25 C by transfer to cooler medium. Exponential growth continued at 25 C without a lag period but with a threefold increase in doubling time. In the period of exponential growth at suboptimal temperature, there was essentially no change in the fatty acid composition of the lipids, little change in the vitamin K₂ composition with perhaps a slight increase in the total level, and essentially no change in the phospholipid composition, but a marked stimulation of the synthesis of the rubixanthins. Growth of cells at 25 C was much more sensitive to the inhibition of rubixanthin formation by mixed-function oxidase inhibitors than cells growing at 37 C, suggesting some function for the rubixanthins at suboptimal temperatures. The striking increases in the proportions of monoenoic fatty acids observed at lowered growth temperatures in many biological systems are not detected in *S. aureus*.

The growth rate of many microorganisms decreases continuously with decreasing temperatures to a critical temperature below which the growth rate abruptly slows (22). After a shift to lower temperature, as long as the new temperature is above the critical temperature, the organism can maintain an exponential growth rate (6). In this period just after the shift in temperature, the organism undergoes numerous changes in its physiological processes (6, 25). Changes in the membrane lipids were among the earliest adaptations to lowered growth temperatures to be described (33). Many authors feel that these lipid changes are particularly significant to the cellular process which allows exponential growth to proceed at lowered temperatures (6, 14, 15, 25). The membrane vitamin K₂ isoprenologues (8, 9), the carotenoids (10), the fatty acids (38), and the complex lipids (37) of *Staphylococcus aureus* U-71 have been characterized and methods for their assay have been developed. This study will show that, after abruptly shifting the temperature of growth from 37 to 25 C, the most striking change in the membrane lipid composi-

tion during exponential growth was a remarkable stimulation of rubixanthin formation.

MATERIALS AND METHODS

Materials. Materials were acquired as in previous work with *S. aureus* U-71 (8-11, 37).

Growth of *S. aureus* U-71. The strain, culture conditions, medium, harvesting procedure and methods of preservation have been described (8-12). To rapidly shift the temperature of growth, a culture growing exponentially at 37 C was poured into a large volume of medium at 23 C so that the resulting temperature was 25 C. The temperature was then maintained at 25 C. The bacterial density was measured as the absorbance at 750 nm in 13-mm round tubes (37).

Lipid extraction and analysis. Lipids were extracted by the modified Bligh and Dyer procedure (4, 37). Fatty acids were isolated from the lipid extract by saponification and extraction (36).

The fatty acids were methylated (36) and separated on a 3.59-m column containing 15% (w/v) ethylene glycol adipate by gas chromatography under the conditions previously described (39). The lipid extract accounted for 99.5% of the fatty acids of *S. aureus* U-71, and the identification of 64 fatty acids has been described (38). Fatty acids are abbreviated as the

number of carbon atoms/the number of double bonds: A represents antiiso branching and I represents iso branching.

Phospholipids were analyzed by using a procedure developed by Short (27). Part of the lipid from cells grown with $H_3^{32}PO_4$ was chromatographed on silica gel-impregnated paper (Whatman SG-81) in a solvent of chloroform-methanol-diisobutyl ketone-acetic acid-water (23:10:45:4, v/v), and the lipids were located by autoradiography (27, 41). The lysyl-phosphatidyl glycerol (LPG; R_F value 0.22) was separated from the phosphatidyl glycerol (R_F value 0.55) and the cardiolipin (R_F value 0.74). The LPG was cut out and eluted, and the radioactivity was determined (27, 35, 41). The LPG isolated in this way contains no other lipids and yields 99% glycerol phosphoryl glycerol (GPG) after deacylation and analysis by chromatography on acid-washed aminocellulose paper (38). A second portion of the lipid was deacylated by mild alkaline methanolysis, and the glycerol phosphate esters were separated by chromatography in two dimensions on acid-washed amino cellulose paper (35, 38). The ^{32}P in each ester was determined. The GPG consists of GPG derived from LPG and phosphatidyl glycerol; the phosphatidyl glycerol was calculated from the proportion of LPG. Phosphatidic acid yields L- α -glycerol phosphate, and phosphatidyl glucose yields glycerol phosphoryl glucose after deacylation. Phosphatidyl glucose was recently characterized in the lipids of *S. aureus* by Short in this laboratory (27).

Vitamin K_2 isoprenologues were isolated from the lipid extracts by initial chromatography on silica gel-thin-layer plates with a solvent of chloroform-isooctane (2:1, v/v), eluted and assayed spectrophotometrically (8). The isoprenologues of vitamin K_2 were separated by reversed phase chromatography on hexadecane-impregnated Kieselguhr plates (8). Cells were grown with 2-methyl-1,4- ^{14}C -naphthoquinone and the isoprenologues were located by autoradiography (8).

Carotenoids were isolated after saponification of a portion of the lipid extract, followed by extraction and separation on alumina-impregnated paper SS-288 (Schleicher & Schuell Co., Keene, N.H.). The pigments were then eluted and assayed spectrophotometrically (10). In some experiments, the major carotenoids were estimated from the absorption spectrum of the lipid in hexane before separation as described (12). The carotenoids of *S. aureus* U-71 have been identified (10).

Oxygen utilization. Oxygen utilization was measured polarographically with sodium lactate as the substrate (7).

RESULTS

Effect of temperature on the growth rate. The doubling time increased and the yield of cells decreased when *S. aureus* U-71 was incubated at suboptimal temperatures. The doubling times in the early exponential growth phase and the densities measured as absorbance at 750 nm in 13-mm round test tubes in the early stationary

growth phase at the following temperatures were: 37 C, 30 min and A 2.0; 35 C, 48 min and A 1.82; 30 C, 68 min and A 1.41; 25 C, 120 min and A 1.08; 20 C, 13.5 hr and A 0.55. The very long doubling time at 20 C (seven times longer than at 25 C) suggested that these cells were not comparable to cells grown between 35 and 25 C.

Lipid composition of the membrane at different temperatures. Cultures were grown at 20, 25, 30, and 35 C to the early stationary phase, and the lipid components of the membrane were assayed. There are changes in the lipid composition in the membrane between cells harvested in the exponential and stationary growth phases at the same temperature which must be differentiated from the changes in lipid composition induced by growth at different temperatures.

The total vitamin K_2 content per cell remains relatively constant during the growth cycle at 37 C, but the proportions of the K_2 -40 isoprenologue increases and the K_2 -0, K_2 -5, K_2 -25, and K_2 -30 decrease as the cells enter the stationary phase (9). Comparison of the vitamin K_2 content of cells grown at different temperatures and harvested in the early stationary growth phase is illustrated in Fig. 1. Lowering the temperature of growth from 35 to 25 C resulted in a 16% increase in total vitamin K_2 per cell. Cells grown at 20 C contained 28% less vitamin K_2 than cells grown at 25 C. The proportion of vitamin K_2 isoprenologues K_2 -0 through K_2 -30 increased from 4.3 to 15%, and the K_2 -45 increased from 7.5 to 10.6% of the total as the growth temperature was decreased from 35 to 25 C. The proportion of K_2 -40 decreased from 69.4 at 35 C to 55.3% of the total at 25 C.

The fatty acid composition of *Escherichia coli* shows remarkable changes as the temperature of growth is lowered. Marr and Ingraham (18) showed that lowering the temperature of growth from 37 to 10 C results in a 15% increase in the proportion of unsaturated fatty acids without significant change in the amount of total fatty acid. In *S. aureus*, surprisingly, there seems to be very little change in the fatty acid composition throughout the growth cycle at 25 C or between cells harvested in the stationary growth phase at 37 or 25 C. The difference between *S. aureus* and *E. coli* is striking. *S. aureus* contains about 3 μ moles of monoenoic fatty acid per g (dry weight) at both 25 and 37 C. *E. coli* contains 56 μ moles of monoenoic fatty acids per g (dry weight) at 37 C and 74 μ moles of monoenoic fatty acid per g (dry weight) at 25 C (18).

During the growth cycle there is a 15 to 20% increase in the total phospholipid in cells grown at 37 C. Cells harvested in the early stationary growth phase at 37 C contained about 56 μ moles

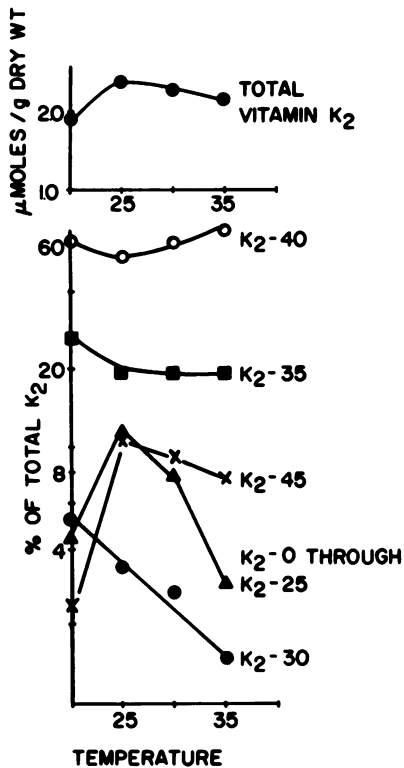


FIG. 1. Vitamin K_2 isoprenologues in *S. aureus* grown at 20, 25, 30, and 35 C. Cells were harvested in the early stationary phase of growth and the lipids were extracted. The vitamin K_2 was purified from the other lipids by thin-layer chromatography, and the isoprenologues were separated on hexadecane-impregnated Kieselghur (8). The upper ordinate scale is exponential.

of phospholipid per g (dry weight). Cells harvested at a comparable point in the growth cycle after incubation at 25 C contained 62 μ moles of phospholipid per g (dry weight).

The carotenoid content of cells depends on the growth phase. During late exponential-stationary phase, *S. aureus* increases the phytoene and carotene content per cell 10 to 40 times and the content of the rubixanthins 80 to 500 times at 25 C (10). Comparison of cells harvested in the late exponential growth phase showed a 3% increase in total carotenoid in cells grown at 25 C compared to cells grown at 35 C. Cells grown at 20 C contained 32% less carotenoid than the cells grown at 35 C. Between 35 and 25 C, the nonpolar carotenoids (phytoene, ζ -carotene, δ -carotene) decreased and the rubixanthins increased by 33%.

Function of the electron transport system. *S. aureus* forms a membrane-bound electron transport system consisting of cytochromes, quinones,

cytochrome oxidase, and primary dehydrogenases when grown with oxygen at 37 C, although the organism can grow glycolytically in the presence of glucose (7). The formation of the respiratory system can be induced in anaerobic cultures by aeration. Concomitant with the formation of the electron transport system there are changes in the membrane lipid components (7). Possibly growth at suboptimal temperatures could disrupt the integration between lipid synthesis and the formation of the electron transport system, and the cells might be forced to grow glycolytically. A functional electron transport system as evidenced by the ability to respire in the presence of lactate was formed at all temperatures of growth (Table 1). The electron transport system formed at each growth temperature could function at all the temperatures compatible with growth.

Temperature switch. To minimize the complexities of the lipid changes resulting from the transition between exponential and stationary growth phase and the changes induced by growth at suboptimal temperatures, experiments in which cells growing exponentially at 37 C were abruptly switched to 25 C were performed. When a culture of *S. aureus* growing exponentially at 37 C (doubling time 40 min) was poured into sufficient medium at 23 C to lower the temperature to 25 C, there was no lag in exponential growth, although the doubling time increased to 100 min (Fig. 2). The effects of abruptly lowering the growth temperature were examined to see what lipid changes were involved in the initial compensatory mechanisms.

Fatty acids. In the initial period after the temperature switch, there was exponential growth without a significant change in the proportions

TABLE 1. Effect of growth temperature on oxygen utilization

Growth temp	O ₂ utilization ^a					
	15 C	20 C	25 C	30 C	35 C	40 C
C						
20	0.75	1.15	1.50	1.95	2.70	
25	0.75	1.95	2.25	3.25	4.40	5.75
30	0.40	0.75	1.35	1.75	3.20	3.15
35	0.80	1.15	1.65	1.95	3.80	3.85

^a Potassium phosphate buffer suspensions of cells grown at 20, 25, 30, and 35 C were assayed for oxygen utilization at 15, 20, 25, 30, 35, and 40 C with an oxygen electrode (7) in the presence of 20 mM sodium L-lactate. Results are expressed in micromoles per second per gram (dry weight) at indicated temperature.

of the fatty acids (Fig. 2). The monoenoic fatty acids remained about 3% of the total during the period of exponential growth at 25 C.

Phospholipid composition. Exponentially growing cells when switched from 37 to 25 C did not alter the proportions of the phospholipids, although the total phospholipid increased slowly (Fig. 3).

Vitamin K₂ isoprenologue composition. The increase in the proportions of the shorter side-

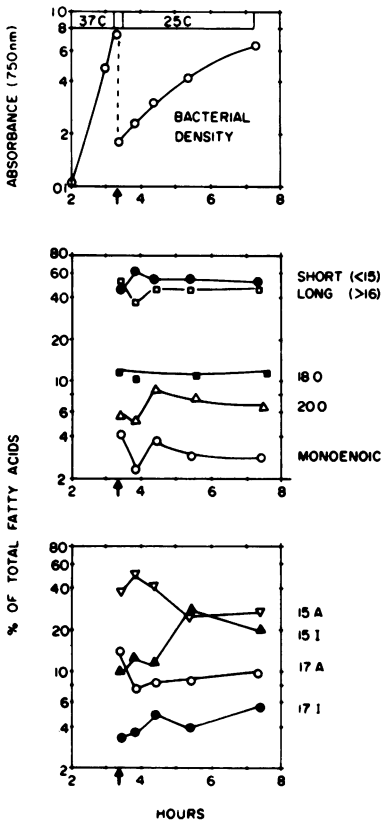


FIG. 2. Fatty acid changes during the switch from exponential growth at 37 C to exponential growth at 25 C. The culture was grown to a density of 0.21 mg (dry weight) per ml at 37 C in 1 liter of medium and then added to 4 liters of medium at 23 C (final temperature 25 C); 500-ml samples were removed at intervals. The samples were removed into an equal volume of ice and centrifuged; the cells were extracted, the lipid was saponified, and the fatty acids were recovered. The fatty acids were methylated and analyzed by gas-liquid chromatography on a 12-ft column of ethylene glycol adipate (36, 38). The upper figure illustrates the changes in bacterial density during growth at 37 C and after the switch to growth at 25 C (indicated by the arrow). Bacterial density was measured as the absorbance at 750 nm in 13-mm round tubes (37). The absorbance at 750 nm between 0.05 and 0.65 corresponds linearly to dry weights between 0.017 and 0.19 mg (dry weight) per ml.

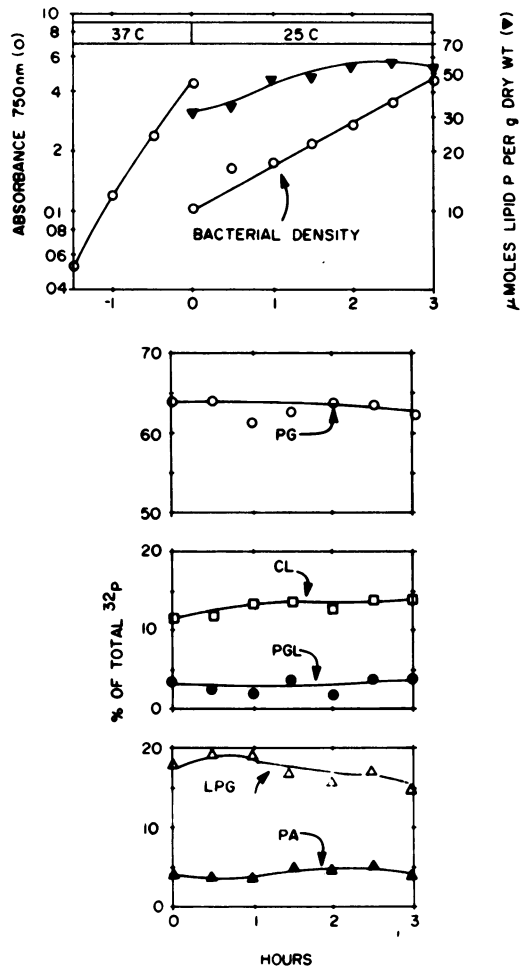


FIG. 3. Changes in the phospholipid composition during exponential growth during the switch from growth at 37 C to 25 C. Cells were grown at 37 C for 10 doublings in 1.6 liters of medium containing 900 μCi of $\text{H}_3^{32}\text{PO}_4$. At a density of 0.3 mg (dry weight) per ml, the culture was poured into 6.4 liters of medium at 23 C (resulting temperature 25 C) and 1-liter samples were withdrawn. The samples were harvested on ice and centrifuged; the lipids were extracted and separated by chromatography. Lysyl-phosphatidyl glycerol (LPG) was separated from the other lipids by ascending chromatography on silica gel-impregnated paper. Another portion of the phospholipids was deacylated and the glycerol phosphate esters were separated by two-dimensional chromatography on aminocellulose paper. The phosphatidyl glycerol (PG) was calculated as the difference between the glycerol phosphoryl glycerol and the LPG. The lipids were located by autoradiography, and the proportions of the ^{32}P in each lipid were determined. Each sample was run in duplicate, and the recovery of ^{32}P from the paper was $100 \pm 8\%$. The specific activity of the total phospholipid remained about 12,000 counts/min of ^{32}P per μmole of phosphate throughout the experiment.

chain isoprenologues and the decrease in the K_2 -40 seen in stationary-phase cells grown at 35 and 25 C (Fig. 1) were not detected in the initial period of growth after the temperature switch. Total vitamin K_2 increased about 20%. The proportion of ^{14}C in K_2 -45, K_2 -35, K_2 -25, K_2 -20, and K_2 -15 did not change significantly during the initial period of growth after a temperature switch (Fig. 4). There was a decrease in the ^{14}C in K_2 -0, K_2 -5, K_2 -10, and K_2 -30, and an increase in K_2 -40.

Carotenoid composition. The most striking

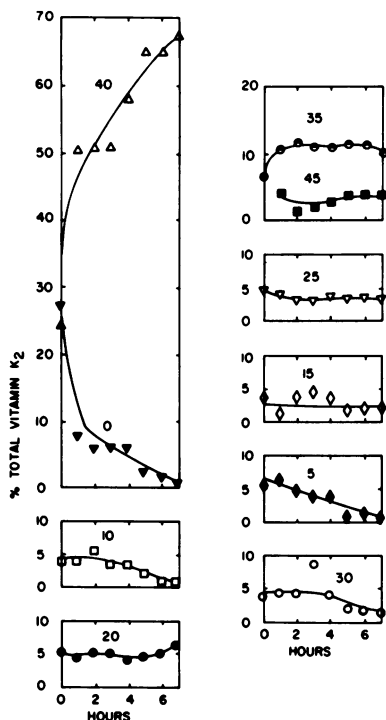


FIG. 4. Effect of temperature switch on the formation of vitamin K_2 isoprenologues in *S. aureus*. 0, Vitamin K_2 with no isoprenoid side chain; 5, vitamin K_2 with 5 carbon atoms in its isoprenoid side chain; 10 through 45, vitamin K_2 with 10, 15, 20, 25, 30, 35, 40, or 45 carbon atoms in the isoprenoid side chain. Cells were grown with 100 μCi of 2-methyl- ^{14}C -1, 4-naphthoquinone per 1.6 liter of medium for 3 hr at 37 C, and the flask was then poured into 6.4 liters of medium at 23 C (final temperature 25 C). Samples were withdrawn and the vitamin K_2 isoprenologues were assayed as in Fig. 1. The proportion of the total ^{14}C in the vitamin K_2 is plotted on the ordinate. The specific activity of the ^{14}C in the 2-methyl of vitamin K_2 is constant in all isoprenologues during exponential growth at 37 C (9). The cells contained 1.55 $\mu moles$ of vitamin K_2 per g (dry weight) at the beginning of growth at 25 C and 1.91 $\mu moles$ after 3.5 hr of exponential growth at 25 C (doubling time 182 min).

change in the membrane lipid composition during the initial period after an abrupt lowering to the growth temperature was the rapid synthesis of the rubixanthins (Fig. 5). Concomitant with this rapid synthesis was a rapid decrease in the proportions of the phytofluens without much change in the carotenes or the phytoene. In this experiment there was essentially no change in the total phospholipid content and a 30% increase in the total vitamin K_2 .

The striking increase in rubixanthins during exponential growth after the abrupt lowering of temperature suggested that these polar xanthophylls are important in the adaptation of the membrane to lowered temperature. It has been shown that the inhibitors of the P_{450} mixed-function oxidases in this strain of *S. aureus*

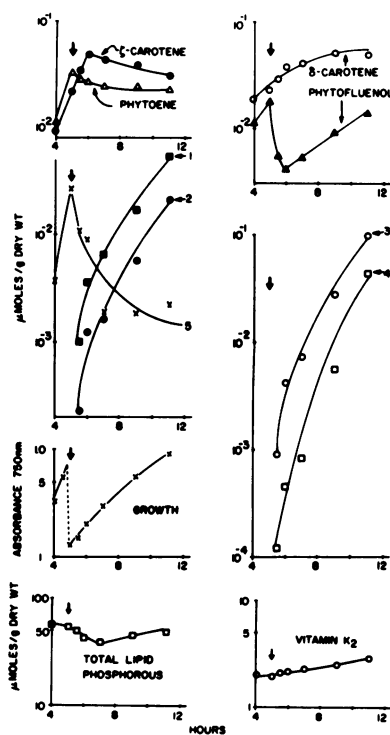


FIG. 5. Carotenoid changes during the switch from exponential growth at 37 C to growth at 25 C. Cultures were grown with aeration by vigorous shaking at 37 C to a density of 0.24 mg (dry weight) per ml and then poured into medium at 23 C (indicated by the arrow) and sampled as in Fig. 2. A portion of the lipid was analyzed for total phospholipid and vitamin K_2 (lower graphs). The remainder was saponified. The carotenoids were separated chromatographically, recovered from the alumina-impregnated paper, and assayed spectrophotometrically (10). Carotenoid 5 is a phytofluens-like carotenoid; 1, 3, and 4 are rubixanthin-like carotenoids and 2 is rubixanthin.

inhibited the aerobic hydroxylation of δ -carotene to rubixanthin without affecting the doubling time or the final cell yield in cells grown at 37 C (12). These inhibitors and the concentrations used were: 2-diethylaminoethyl-2,2-diphenyl valerate (SKF 525A), 50 $\mu\text{g/ml}$; 2,4-dichloro-6-phenylphenoxyethylamine (DPEA), 25 $\mu\text{g/ml}$; and piperonyl butoxide, 200 $\mu\text{g/ml}$.

Cells grown into the stationary phase at 25 C at the same concentration of DPEA, or at lowered concentrations of SKF 525A or piperonyl butoxide had reduced growth rates and lower final cell yields (Table 2).

The cells were harvested in the late stationary growth phase when the carotenoid content is highest (10). The total xanthophyll produced in the presence of the inhibitors was depressed at least 10 times, although the phytoene content was higher and the total carotenoid was not lowered. A portion of the piperonyl butoxide was bound to the membrane. Diphenylamine inhibits both vitamin K₂ and carotenoid formation at 37 C without affecting the growth rate (11). High concentrations are bound to the membrane (11). Diphenylamine at 25 C inhibited rubixanthin formation 10-fold and actually stimulated the growth rate and cell yield (Table 2). Rubixanthin can be formed in *S. aureus* by the hydration of carotene followed by cyclization of phytofluene in the absence of air (10). This process is 25 times less effective than the aerobic mixed-function oxidase system in cells grown at

37 C (10). At 37 C the aerobic doubling time is 45 min and the anaerobic doubling time is 120 min (10). In cells incubated at 25 C, the aerobic doubling time is 150 min but the anaerobic doubling time is 1,050 min, again suggesting a greater dependence on rubixanthin for growth at suboptimal temperatures. Adding β -carotene in dimethyl sulfoxide (about 1 μmole per g of dry weight at the end of the experiment) did not increase the doubling times of cells in the presence of DPEA, SKF 525A, or piperonyl butoxide incubated at 25 C.

DISCUSSION

The changes in composition of the membrane lipids in response to growth at lowered temperatures have been known for years (33). Lowering the temperature of the environment results in an increase in the proportions of monoenoic fatty acids in the cells (3, 6, 13-15, 23, 25). Psychrophilic strains show a greater proportion of unsaturated fatty acids than mesophilic strains of the same species (14, 15). However, in *E. coli* it is possible to observe exponential growth after lowering the temperature before there is a marked shift in the fatty acid composition, indicating that the changes in fatty acids in all species are not essential to growth at lowered temperatures (26). In *S. aureus* there was very little change in the proportion of monoenoic fatty acids with a decrease in the temperature of growth (Fig. 2).

Significant changes in the phospholipid com-

TABLE 2. Carotenoid biosynthesis and growth of *S. aureus* at 25 C in the presence of mixed-function oxidase inhibitors

Inhibitor concn ^a ($\mu\text{g/ml}$)	Yield ^b (mg of dry wt/ml)	Doubling time ^c (min)	Carotenoids ^d (nmoles/g of dry wt)				
			Rubixanthins + δ -carotene	ζ -Carotene	Phytoflueneols	Phytoene	Total
Control	0.58	150	440	240	200	980	1,860
SKF 525A (25)	0.36	240	50	60	70	920	1,100
DPEA (25)	0.19	330	50	110	150	2,400	2,700
PB ^e (13)	0.62	360	10	80	660	3,380	4,060
DPA (13)	0.74	90	90	80	80	1,400	1,650

^a The inhibitors were dissolved in dimethyl sulfoxide (final dimethyl sulfoxide concentration 0.1%, v/v) and added to 50 ml of medium in 250-ml Erlenmeyer flasks with 13-mm side arms. The flasks were shaken at 25 C for 24 hr. SKF 525A, 2-diethylaminoethyl-2,2-diphenyl valerate; DPEA, 2,4-dichloro-6-phenylphenoxyethylamine; PB, piperonyl butoxide; DPA, diphenylamine.

^b Yield determined from the maximum absorbance at 750 nm (37).

^c Doubling time was determined as the time to increase the absorbance between 0.05 and 0.1 at 750 nm in the early exponential growth phase.

^d Carotenoids measured in hexane from the lipid extract; rubixanthins and δ -carotene at 460 nm, $f = 125 \times 10^3$; ζ -carotene at 400 nm, $f = 120 \times 10^3$; the phytoflueneols at 375 nm, $f = 120 \times 10^3$; and phytoene at 286 nm, $f = 67 \times 10^3$ (12).

^e Phytoene determination includes bound PB (maxima at 287, 291, and 298 nm, $f = 6.75 \times 10^3$. SKF-525A, DPEA, and DPA adsorb below 250 nm.

position do not uniformly accompany shifts from higher to growth at lower temperatures (13, 14). In *S. aureus* there was a 10 to 20% increase in total phospholipid in the transition between exponential and stationary growth phase at 37 C. There was a 10% increase in total phospholipid between cells harvested in the stationary phase of growth at 37 and 25 C. When exponentially growing *S. aureus* was abruptly shifted from 37 to 25 C, there was a 15% increase in total phospholipid but essentially no change in the proportions of the lipids (Fig. 3). In other experiments there was essentially no change in total phospholipid per cell (as in Fig. 5). An increase in total phospholipid when *E. coli* is shifted to lower growth temperature has been reported (5, 23). In *E. coli* there is an increased rate of loss of ^{32}P in phosphatidyl glycerol, cardiolipin, and in phosphatidylethanolamine, a lipid from which ^{32}P is not lost during growth at 40 C when exponentially growing cells incubated with $\text{H}_3^{32}\text{PO}_4$ at 40 C are shifted to 10 C in nonradioactive medium (23).

In *S. aureus* the total vitamin K_2 and the proportions of the shorter side-chain isoprenologues increased in stationary-phase cells as the temperature of growth was lowered (Fig. 1). During the exponential growth period, after a shift from 37 to 25 C, there was a slight increase in total vitamin K_2 , and the proportions of the very short isoprenologues fell as the major isoprenologue $\text{K}_2\text{-40}$ increased (Fig. 3). The tendency to shift the proportions between the shorter and major isoprenologues is seen during exponential growth at 37 C (9). During exponential growth the total vitamin K_2 remains about 2.0 μmoles per g of dry weight (9). In strain U-71 of *S. aureus* there is little shift in the total vitamin K_2 level between aerobic and anaerobic growth and between exponential and stationary growth phases (9, 11).

The striking membrane lipid change after an abrupt decrease in temperature was the rapid synthesis of the rubixanthins (Fig. 5). The prolongation of the doubling time and the decreased growth yields of cells of *S. aureus* grown with the relatively specific mixed-function oxidase inhibitors at 25 C but not at 37 C [although rubixanthin formation is inhibited at both growth temperatures (12; Table 2)] suggested strongly that rubixanthin in the membrane allowed rapid growth at 25 C. Diphenylamine inhibited rubixanthin formation 10 times but actually stimulated growth at 25 C, suggesting that increased rubixanthin formation was not an obligatory feature of growth at suboptimal temperatures (Table 2). This concentration of diphenylamine

inhibits vitamin K_2 formation by 50% and rubixanthin formation 30 times at 37 C without affecting the growth rate. However, about 3 μmoles of diphenylamine per g (dry weight) accumulates in the membrane during growth at 37 C (11). This is about 1,000 times the total carotenoid concentration and may substitute for the rubixanthin in some way.

Xanthophylls are thought to function in non-photosynthetic bacteria in protecting the cells from photochemical damage. The photochemical activity is damaging only in the presence of oxygen (19, 24, 31). Mutants unable to form xanthophylls or cultures in which xanthophyll synthesis is inhibited by diphenylamine are much more sensitive to visible light than the pigmented cells (16, 20, 30). The action spectrum of the stimulation of aerobic carotenogenesis in one *Mycobacterium* resembles a flavoprotein and in another a cytochrome (2). Visible light does not stimulate carotenogenesis in some bacteria (21, 34) as in *S. aureus* U-71 (10). Visible light, however, has been shown to stimulate carotenogenesis in some other strains of *S. aureus* (31, 32).

Carotenoids may have other functions. In some mycoplasmas, the steroids can replace the xanthophylls in some structural role in the membrane (28) and there is a rapid turnover of the glucose moiety of the carotenoid glucosides that apparently is related to glucose transport (29).

In many bacteria carotenogenesis is maximal under growth conditions in which growth is maximal (21, 34), and these lipids tend to accumulate in the stationary phase of growth (10, 21, 32). Lowering the temperature of growth from 37 to 22 C results in an inhibition of carotenoid formation in some strains of *S. aureus* (32), although in other strains, including U-71, lowering the temperature of growth stimulated carotenogenesis (39; Fig. 5).

The one striking adjustment of *S. aureus* growing exponentially after an abrupt temperature decrease was the rapid synthesis of the polar carotenoids. This suggested that these lipids may have a function similar to that of the unsaturated fatty acids in other organisms in allowing membrane function at suboptimal temperatures.

ACKNOWLEDGMENTS

This investigation was supported by contract 12-14-100(9517) (73) with the Agricultural Research Service, U.S. Department of Agriculture, administered by the Eastern Utilization and Development Division, Philadelphia, Pa., and by Public Health Service grant GM-10285 from the National Institute of General Medical Sciences.

R. K. Hammond was supported by grant 5-TO1-GM-01026 from the National Institute of General Medical Sciences to the Department of Biochemistry, University of Kentucky Medical Center.

LITERATURE CITED

1. Batra, P. P. 1967. Mechanism of photoinduced carotenoid synthesis. Induction of carotenoid synthesis by antimycin A in the absence of light in *Mycobacterium marinum*. *J. Biol. Chem.* **242**:5630-5635.
2. Batra, P. P., and H. C. Rilling. 1964. On the mechanism of photoinduced carotenoid synthesis: aspects of the photo-inductive reaction. *Arch. Biochem. Biophys.* **107**:485-492.
3. Bishop, O. G., and J. L. Still. 1963. Fatty acid metabolism in *Serratia marcescens*. IV. The effect of temperature on fatty acid composition. *J. Lipid Res.* **4**:87-90.
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. DeSiervo, A. J. 1969. Alterations in the phospholipid composition of *Escherichia coli* B during growth at different temperatures. *J. Bacteriol.* **100**:1342-1349.
6. Farrell, J., and A. Rose. 1967. Temperature effects on microorganisms. *Annu. Rev. Microbiol.* **21**:101-120.
7. Frerman, F. E., and D. C. White. 1967. Membrane lipid changes during formation of a functional electron transport system in *Staphylococcus aureus*. *J. Bacteriol.* **94**:1868-1874.
8. Hammond, R. K., and D. C. White. 1969. Separation of vitamin K₂ isoprenologues by reversed phase thin layer chromatography. *J. Chromatogr.* **45**:446-452.
9. Hammond, R. K., and D. C. White. 1969. Formation of vitamin K₂ isoprenologues by *Staphylococcus aureus*. *J. Bacteriol.* **100**:573-578.
10. Hammond, R. K., and D. C. White. 1970. Carotenoid formation by *Staphylococcus aureus*. *J. Bacteriol.* **103**:191-198.
11. Hammond, R. K., and D. C. White. 1970. Inhibition of vitamin K₂ and carotenoid synthesis in *Staphylococcus aureus* by diphenylamine. *J. Bacteriol.* **103**:611-615.
12. Hammond, R. K., and D. C. White. 1970. Inhibition of carotenoid hydroxylation in *Staphylococcus aureus* by mixed-function oxidase inhibitors. *J. Bacteriol.* **103**:607-610.
13. Kates, M. 1964. Bacterial lipids. *Advan. Lipid Res.* **2**:17-90.
14. Kates, M., and R. M. Baxter. 1962. Lipid composition of mesophilic and psychrophilic yeasts (*Candida* species) as influenced by environmental temperature. *Can. J. Biochem. Physiol.* **40**:1213-1227.
15. Kates, M., and P. O. Hagen. 1964. Influence of temperature on fatty acid composition of psychrophilic and mesophilic *Serratia* species. *Can. J. Biochem.* **42**:481-488.
16. Kunisawa, R., and R. Y. Stanier. 1958. Studies on the role of carotenoid pigments in a chemoheterotrophic bacterium, *Corynebacterium poinsettiae*. *Arch. Mikrobiol.* **31**:146-156.
17. Liaaen-Jensen, S. 1965. Biosynthesis and function of carotenoid pigments in microorganisms. *Annu. Rev. Microbiol.* **19**:163-182.
18. Marr, A. G., and J. L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. *J. Bacteriol.* **84**:1260-1267.
19. Mathews, M. M. 1962. Studies on the localization, function and formation of the carotenoid pigments of a strain of *Mycobacterium marinum*. *Phytochem. Phytobiol.* **2**:1-8.
20. Mathews, M. M., and W. R. Siström. 1960. The function of the carotenoid pigments of *Sarcina lutea*. *Arch. Mikrobiol.* **35**:139-146.
21. Nandy, S. C., and S. N. Sen. 1963. Effect of environment on growth and carotenogenesis of *Sarcina litoralis*. *Can. J. Microbiol.* **9**:601-611.
22. Ng, H., J. L. Ingraham, and A. G. Marr. 1962. Damage and derepression in *Escherichia coli* resulting from growth at low temperatures. *J. Bacteriol.* **84**:331-339.
23. Okuyama, H. 1969. Phospholipid metabolism in *Escherichia coli* after a shift in temperature. *Biochim. Biophys. Acta* **176**:125-134.
24. Rilling, H. C. 1962. Photoinduction of carotenoid synthesis of a *Mycobacterium* sp. *Biochim. Biophys. Acta* **60**:548-556.
25. Rose, A. H. 1968. Physiology of micro-organisms at low temperatures. *J. Appl. Bacteriol.* **31**:1-11.
26. Shaw, M. K., and J. L. Ingraham. 1965. Fatty acid composition of *Escherichia coli* as a possible controlling factor of the minimal growth temperature. *J. Bacteriol.* **90**:141-146.
27. Short, S. A., and D. C. White. 1970. Metabolism of the glucose containing lipids of *Staphylococcus aureus*. *J. Bacteriol.* **103**:126-132.
28. Smith, P. F. 1963. The carotenoid pigments of mycoplasma. *J. Gen. Microbiol.* **32**:307-319.
29. Smith, P. F. 1969. The role of lipids in membrane transport in *Mycoplasma laidlawii*. *Lipids* **4**:331-336.
30. Stanier, R. Y., and Cohen-Bazire, G. 1957. Role of light in the microbial world: some facts and speculations. *Symp. Soc. Gen. Microbiol.* **7**:56-89.
31. Steuer, W. 1957. Die bedeutung verschiedener "Umwelteinflüsse" auf die Sussamensetzung des Gasamptpigmentes von *Micrococcus pyogenes*. *Zentralbl. Bakteriol. Parasitenk. Abt. I. Orig.* **168**:558-566.
32. Steuer, W. 1962. Untersuchungen über die Pigmentbildung von *Micrococcus pyogenes*. *Zentralbl. Bakteriol. Parasitenk. Abt. I. Orig.* **163**:210-216.
33. Terroine, E. F., R. Bonnet, G. Kopp, and J. Véhot. 1927. Sur la signification physiologique des raiasons ethyleniques des acides gras. *Bull. Soc. Chim. Biol.* **9**:605-620.
34. Thierry, O. C., and J. J. Cooney. 1966. Physiological factors influencing growth and pigment synthesis by *Micrococcus roseus*. *Can. J. Microbiol.* **12**:691-698.
35. White, D. C. 1968. Lipid composition of the electron transport membrane of *Haemophilus parainfluenzae*. *J. Bacteriol.* **96**:1159-1170.
36. White, D. C., and R. H. Cox. 1967. Identification and localization of the fatty acids in *Haemophilus parainfluenzae*. *J. Bacteriol.* **93**:1079-1088.
37. White, D. C., and F. E. Frerman. 1967. Extraction, characterization, and cellular localization of the lipids of *Staphylococcus aureus*. *J. Bacteriol.* **94**:1854-1867.
38. White, D. C., and F. E. Frerman. 1968. Fatty acid composition of the complex lipids of *Staphylococcus aureus* during formation of the membrane-bound electron transport system. *J. Bacteriol.* **95**:2198-2209.
39. White, D. C., and A. N. Tucker. 1969. Phospholipid metabolism during changes in the proportions of membrane-bound respiratory pigments in *Haemophilus parainfluenzae*. *J. Bacteriol.* **97**:199-209.
40. Wilson, T. E., and A. G. Nuenz. 1965. Factors influencing pigment production by *Staphylococcus aureus*. *Tex. Rep. Biol. Med.* **23**:147.
41. Wuthier, R. E. 1966. Two-dimensional chromatography on silica gel-loaded paper for the microanalysis of polar lipids. *J. Lipid Res.* **7**:544-550.