# Effect of Benzo(a)pyrene and Piperonyl Butoxide on Formation of Respiratory System, Phospholipids, and Carotenoids of *Staphylococcus aureus*

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*Staphylococcus aureus* formed an electron transport system when exponentially growing cells were aerated. Formation of the electron transport system occurred concomitantly with increases in the phospholipids and the carotenoids. The addition of piperonyl butoxide or benzo(a)pyrene at the onset of aeration (i) slowed the formation of the electron transport system, (ii) both inhibited cytochrome oxidase *o* synthesis and decreased its stability, (iii) simultaneously depressed the increase in total phospholipid (especially cardiolipin), and (iv) depressed the synthesis of the carotenoid rubixanthin. Benzo(a)pyrene was the more inhibitory of the two, both on the rate of synthesis of the electron transport system and on rubixanthin formation. Evidence obtained with the inhibitors suggested that inhibition of the lipid synthesis was related to the formation of the electron transport system.

From *Staphylococcus aureus*, membrane fragments containing the electron transport system and the lipids of the cell can be isolated (16). The electron transport system consists of primary dehydrogenases, cytochromes *b* and *a*, and cytochrome oxidase *o* (4, 16). The membrane contains phospholipids, glucolipids, vitamin $K_2$ isoprenologues, and carotenoids for which methods of quantitative analysis have been developed (5–10, 12, 15). *S. aureus* grows glycolytically on glucose in the absence of air (4, 17). Such cells contain no detectable electron transport system (4). If glycolytically growing *S. aureus* are aerated in the early exponential phase, there is a rapid synthesis of the respiratory pigments which occurs simultaneously with increases in the glucolipids, phospholipids, and carotenoids (4, 9). The purpose of the present study was to determine whether the modifications necessary for the synthesis of the electron transport system involve changes in the metabolism of the lipids or whether the lipid changes observed are a simultaneous but unrelated phenomenon. Inhibitors which affect lipid metabolism were examined for their effects on the synthesis of the electron transport system.

In this strain of *S. aureus*, piperonyl butoxide (PB) has been shown to inhibit the oxygen-requiring hydroxylation of δ-carotene to form the rubixanthins presumably by inhibiting the $P_{450}$ mixed-function oxidase system (8). Although PB and other mixed-function oxidase inhibitors greatly decrease the rate at which exponentially growing *S. aureus* forms membranes characteristic of cells grown at suboptimal temperatures (10), at 37 C PB does not affect the growth rate (8). A second lipophilic inhibitor, benzo(a)pyrene (BAP), the principal carcinogen from tobacco smoke, was shown to inhibit aerobic growth under conditions in which the less carcinogenic isomer benzo(e)pyrene (BEP) had no effect. Consequently, BAP was assayed for its effect on the metabolism of the lipids and the formation of the electron transport system.

## MATERIALS AND METHODS

**Materials.** PB was purchased from K & K Laboratories, Plainfield, N.J. BAP was purchased from Aldrich Chemical Co., Milwaukee, Wis. Other materials were supplied as previously described (4, 6, 7, 12, 16).

**Growth of S. aureus.** The strain, growth conditions, and harvesting procedures have been described (4, 7). The medium contained 0.3% (w/v) Trypticase (Baltimore Biological Labs.), 0.08% (w/v) yeast extract (Difco), 0.26 mM $K_2HPO_4$, 15 mM tris(hydroxymethyl)aminomethane, 4.1 mM glycerol, 3.8 mM sodium gluconate, 0.89 mM sodium acetate, 0.1 mM adenine, 0.1 mM xanthine, 0.1 mM uracil, 15 mM glucose, and the vitamins and iron preparations described previously (4). The *p*H

was 7.4. Foaming was controlled with antifoam A (4). The bacteria were grown in a 15-liter bottle fitted with a sparger and a tube (5-mm diameter) for withdrawing samples. By closing the venting tube and opening the tube for withdrawing medium, a 500-ml sample could be withdrawn aseptically in 30 sec. The culture vessel was filled with 6 to 10 liters and sparged with nitrogen on removal from the autoclave until aeration was begun. After the medium cooled to near 37 C, the vitamins, glucose, and iron were added, and the medium was inoculated with 200 ml of an exponentially growing anaerobic culture (4). The nitrogen used for sparging was deoxygenated by passage through hot copper filings (4). The medium was stirred vigorously with a Teflon-coated magnetic stirring bar throughout the experiment. The cells were grown at 37 C.

The bacterial density was estimated by measuring the turbidity at 750 nm in 13-mm test tubes (4). The turbidity was linearly related to the dry weight (4).

The PB and BAP were dissolved in dimethyl sulfoxide and added to the growth medium at the onset of aeration. The final concentration of dimethyl sulfoxide was less than 0.1% (v/v). Dimethyl sulfoxide present in the growth medium at 0.5% had no detectable effect on the growth rate or lipid metabolism.

**Assay of the cytochromes.** Cytochrome content was calculated from room temperature difference spectra of cell suspensions measured in 50 mM phosphate buffer (*p*H 7.6) at cell densities between 6 and 20 mg (dry weight) per ml (4). Cytochromes were estimated as follows. Cytochrome *b* plus *o* was estimated as the absorbancy increment between the maximum at 560 nm and a line connecting absorbancy at 580 and 540 nm, and cytochrome *a* was estimated as the absorbancy increment between the maximum at 603 nm and a line connecting 620 and 590 nm in the reduced versus oxidized difference spectra. Cytochrome oxidase *o* was estimated as the absorbancy increment between the maximum at 416 nm and the minimum near 430 nm, and cytochrome $P_{450}$ was estimated as the absorbancy increment between the maximum near 460 nm and a line connecting the minimum near 430 nm and 480 nm in the reduced and saturated with carbon monoxide versus reduced difference spectrum. The respiratory pigments were reduced enzymatically in the presence of 0.1 mM sodium L-lactate when the oxygen was exhausted. The respiratory pigments were oxidized by oxygen by mixing the suspension vigorously with a Vortex mixer (4). When the cells were washed with cold phosphate buffer and then resuspended in cold phosphate buffer with vigorous aeration by mixing, the endogenous respiration did not render the suspension anaerobic within the time necessary to complete a scan of the alpha region of the spectrum (4).

Protoheme was determined by forming the reduced pyridine hemochrome after extraction from the cytochromes (3).

**Extraction and analysis of the lipids.** Cells were harvested into an equal volume of ice containing sufficient HCl to bring the final *p*H to 2.0; they were then centrifuged, and the pellet was extracted by the modified Bligh and Dyer procedure (4). A portion of the lipids was separated by two-dimensional chromatography on silica gel-impregnated paper (12, 15). A second portion of the lipid was deacylated by mild alkaline methanolysis and separated by two-dimensional chromatography on amino cellulose paper (4, 12). Phosphatidylglycerol (PG) was determined as the difference between the lysyl-phosphatidylglycerol and the glycerol phosphorylglycerol derived from both lipids by mild alkaline methanolysis (10). Cardiolipin (CL), phosphatidic acid, and phosphatidylethanolamine were also assayed. Total lipid phosphate was assayed colorimetrically (4).

Carotenoids were extracted by the Bligh and Dyer method without making the medium acid. The lipid extract was then saponified, and the carotenoids were separated by chromatography on alumina-impregnated paper (7, 8). The pigments were eluted and assayed spectrophotometrically (7).

**Radioactivity.** The lipids were located by autoradiography and radioactivity was determined with a scintillation spectrophotometer as described previously (15, 19)

## RESULTS

**Effect of lipophilic agents on the growth of S. aureus.** In a series of studies designed to assess the effects of components of tobacco smoke on the formation of the electron transport system, the carcinogenic polycyclic hydrocarbon BAP (10 μM) was shown to have a marked effect on the growth rate of *S. aureus* when the function of the electron transport system was required, as during aerobic growth (Fig. 1). The less carcinogenic isomer BEP at 10 μM had no effect when grown with aeration in the absence of glucose. Neither BAP nor BEP was inhibitory when the cells were supplied with glucose in the nutrient broth medium. The presence of glucose in the growth medium inhibits both the synthesis and the function of the electron transport system in this strain of *S. aureus*. To assess the possible role of lipids in the formation of the membrane-bound electron transport system, BAP was examined for its effects on the events which accompany the shift from exponential anaerobic to exponential aerobic growth. The shift from anaerobic to aerobic growth involves the formation of the electron transport system (4). The addition of air under these growth conditions overcomes any inhibition of cytochrome formation by the residual glucose. As a control, PB, a lipophilic mixed-function oxidase inhibitor that has been shown to depress the aerobic hydroxylation of δ-carotene to rubixanthin in this strain of *S. aureus* (8), was examined. PB at 77 μM had no effect on the growth rate of *S. aureus*.

**Formation of the electron transport system.** The addition of air to an anaerobic, exponential-phase *S. aureus* culture stimulates the formation of a membrane-bound electron transport system (4). The electron transport system can be assayed either by the appearance of the ability to utilize oxygen in the presence of lactate or by the α absorbance of the respiratory pigments when

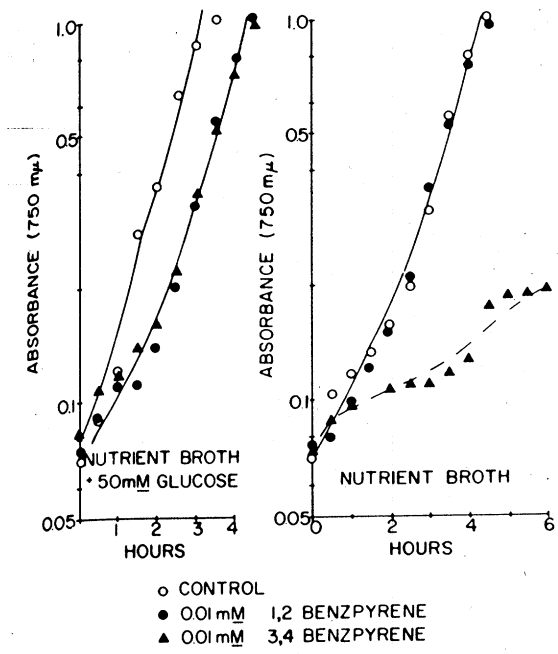


FIG. 1. *Growth rate of S. aureus measured as the absorbance at 750 nm in 13-mm round test tubes in nutrient broth (right) and nutrient broth plus 50 mM glucose (left). Benzo(a)pyrene and benzo(e)pyrene were added in ethanol such that the final ethanol concentration was less than 1%. A similar amount of ethanol was added to the control. Cells were grown in 30 ml of medium in 250-ml Erlenmeyer flasks equipped with side arms that were agitated at 37 C. An absorbance of 0.65 corresponds to 0.19 mg (dry weight) per ml (4).*

reduced in the presence of lactate (4). The difference spectra of suspensions of *S. aureus* 30 min after the introduction of air into an exponentially growing culture is illustrated in Fig. 2. The formation of the respiratory system that was induced in anaerobically growing *S. aureus* by the addition of air was essentially complete in one bacterial doubling (less than 60 min), as illustrated in Fig. 3. The $\alpha$ absorbance maxima of cytochrome *a* (603 nm) and cytochrome *b* plus cytochrome oxidase *o* (558 nm) can be detected in the reduced versus oxidized difference spectrum. The carbon monoxide combining pigments can be demonstrated in cells reduced in the presence of lactate and saturated with carbon monoxide as compared with cells with the respiratory pigments reduced. Cytochrome oxidase *o* (maxima at 416, 538, and 572 nm) and the mixed-function oxidase pigment "$P_{450}$," believed to function in carotenoid hydroxylation, can be detected.

Cytochromes *b*, *o*, and presumably $P_{450}$ contain protoheme as the prosthetic group. As expected, protoheme biosynthesis was stimulated by the addition of oxygen. Cells with a fully formed respiratory system contained about 40 nmoles of protoheme per g (dry weight). From the $\alpha$ maxima of cytochromes *b* plus *o*, assuming a molar extinction coefficient of 20,000 and one heme per mole, the protoheme content of the membrane was calculated to be 40 nmoles per g (dry weight).

When 10 $\mu$M BAP was added to the medium at beginning of aeration, the formation of the electron transport system was slowed (5 hr versus 1 hr in the control), although the growth rate was not seriously affected (Fig. 4). The respiratory system contained about as much enzymatically reducible cytochromes *b* and *a* as the control when fully formed. Less than half the cytochrome oxidase *o* formed in the control was formed in the presence of BAP, and after 3 hr the amount of cytochrome oxidase *o* per cell de-

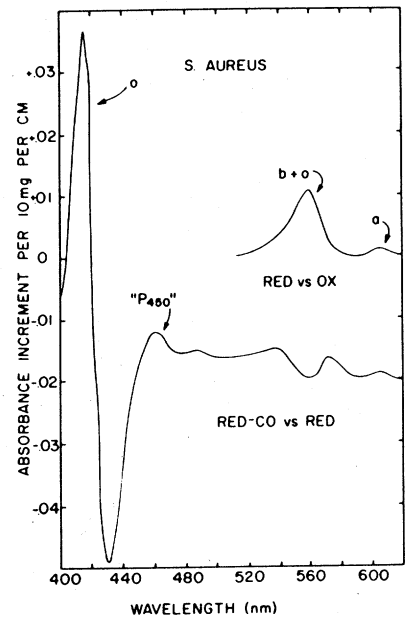


FIG. 2. *Difference spectra of S. aureus. Cells were harvested by centrifugation 1 hr after the initiation of aeration, washed, and suspended in 50 mM phosphate buffer (pH 7.6) at 4 C at a density of 12 mg (dry weight) per ml. L-Lactate (0.1 mM final concentration) was added to a portion of the cells to reduce the respiratory pigments (4). After anaerobiosis, these cells were compared to a suspension in which the pigments had been oxidized by vigorous agitation with a Vortex mixer in a Cary 14 recording spectrometer (Red vs Ox spectrum). Another portion of the cell suspension with the pigments reduced in the presence of L-lactate was saturated with carbon monoxide, and these cells were compared to cells with the pigments reduced in the presence of L-lactate (Red-CO vs Red spectrum). These methods have been described in detail (4).*

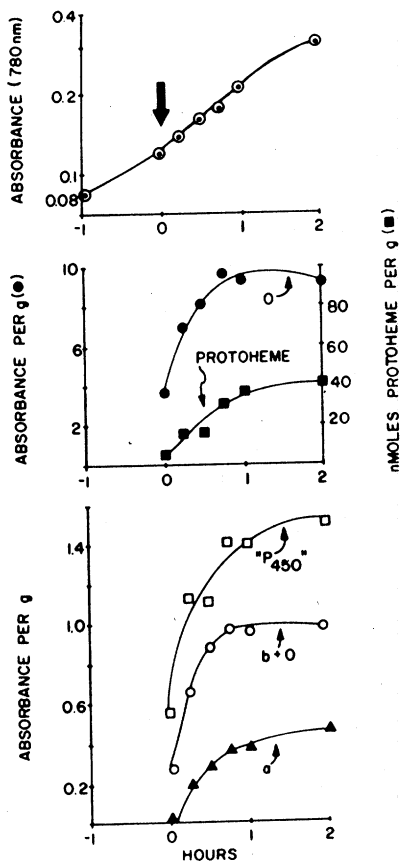


FIG. 3. *Formation of the electron transport system in exponentially growing S. aureus after the introduction of air. S. aureus was grown in 9 liters of medium that had been sparged with deoxygenated nitrogen (30 ml per min) from the time of removal from the autoclave until the aeration was started (indicated as ↓). The cells were grown for 14 hr to a density of 0.05 mg (dry weight) per ml and air was introduced at a rate of 20 liters per min. Samples of 500 ml were removed in 1 min onto an equal volume of ice and harvested by centrifugation. The cytochromes in washed cell suspensions were then examined by difference spectroscopy. Protoheme was measured as the reduced pyridine hemochrome.*

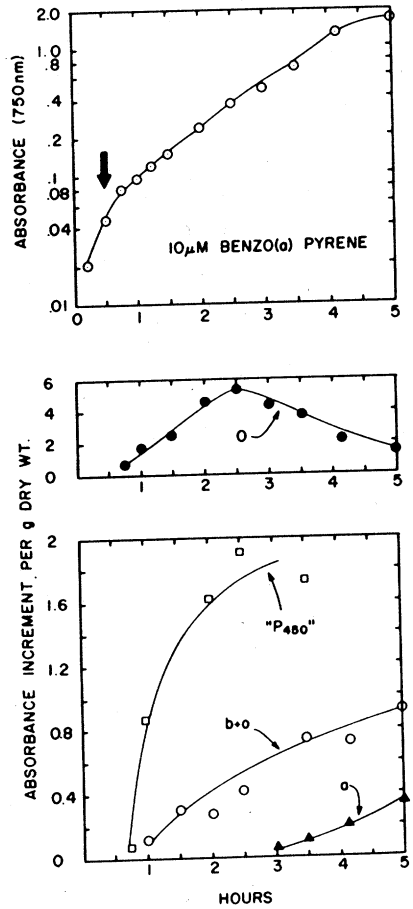


FIG. 4. *Formation of the electron transport system in S. aureus after the introduction of air (↓). Benzo(a)-pyrene dissolved in dimethyl sulfoxide was added when aeration was initiated. Final dimethyl sulfoxide concentration was 0.1% (v/v). The experiment was performed as in Fig. 3.*

creased. Only half the total level of protoheme was formed (about 20 nmoles per g, dry weight). Initially the $P_{450}$ appeared to be formed at the same level as in the control, although the rate of synthesis was slower. Three hours after the start of the adaptation to aerobic growth, the $P_{450}$ became impossible to estimate accurately, because a large amount of a carbon monoxide combining pigment with a minimum at 430 nm appeared (Fig. 5).

The effect of BAP was compared to another lipophilic inhibitor, PB (Fig. 6). The addition of 77 μM PB simultaneously with aeration had no detectable effect on the exponential growth rate of *S. aureus*. There was a delay in the synthesis of cytochromes *b* and *a* when compared with the control, although the levels of these cytochromes eventually reached the control levels. Protoheme seemed to be formed at the same level as in the control cultures. The synthesis of $P_{450}$ appeared to be markedly inhibited by PB. Cytochrome oxidase *o* formation appeared to be inhibited by PB as with BAP. These effects can be seen in the difference spectrum of cells to which PB was added with the onset of aeration (Fig. 7). Both BAP and PB markedly stimulated the formation of a lactate reducible pigment that had a minimum near 430 nm. Although not illustrated in Fig. 4 and 6, this was detected in the reduced versus oxidized difference spectrum.

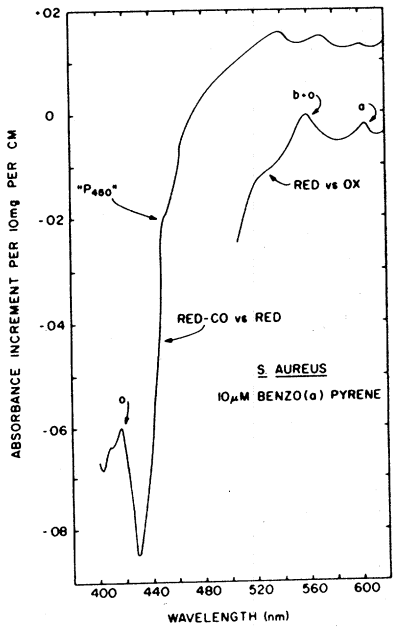


FIG. 5. *Difference spectra of S. aureus grown for 1 hr after aeration in the presence of 10 μM benzo(a)-pyrene as in Fig. 4. Data were analyzed as in Fig. 2.*

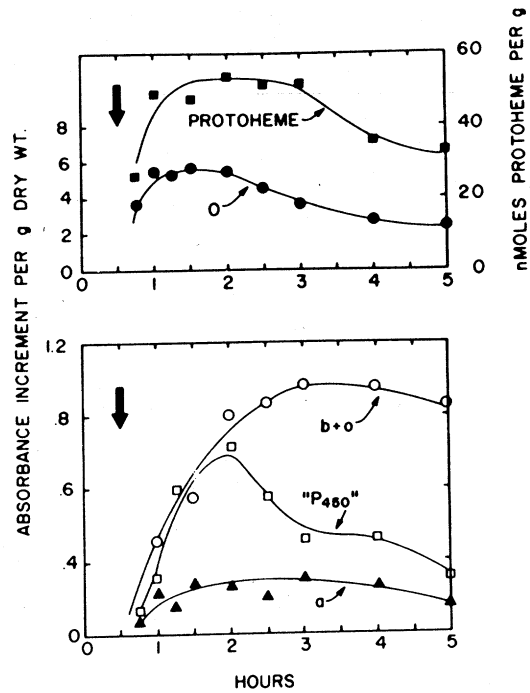


FIG. 6. *Formation of the electron transport system in S. aureus after the introduction of air (|). Piperonyl butoxide dissolved in dimethyl sulfoxide was added at the start of aeration. The final piperonyl butoxide concentration was 77 μM and the final dimethyl sulfoxide concentration was 0.1% (v/v). The experiment was performed as in Fig. 3.*

Despite the changes in the formation of the electron transport system induced in the presence of PB and BAP, the doubling time after the start of aeration was similar to the 45-min doubling time in the control cultures.

**Synthesis of the phospholipids.** With the onset of aeration, exponentially growing *S. aureus* increased the total phospholipids from about 50 to 90 μmoles per g dry weight (Fig. 8). This increase was complete in about 6 hr (about 4 times longer than was required for the formation of the electron transport system, Fig. 3). In the control culture, the total amount of PG and CL in the membrane increased 2.5 times, accounting for the increase in total phospholipid. When the lipophilic reagents PB and BAP were added to the cells at the onset of aeration, the following changes in the formation of the phospholipids occurred (Fig. 8). (i) The increase in total phospholipids per gram (dry weight) was not detected. (ii) There was a decrease in the proportion of CL and a greater increase in the proportion of PG. The actual amount of Cl in the membrane decreased 47% and the PG increased by 52%. (iii) There was no increase in the proportion of phosphatidic acid. (iv) The proportion of phosphatidylethanolamine fell and the proportion of lysyl-phosphatidylglycerol remained constant in all experiments.

**Synthesis of the carotenoids.** Rubixanthin synthesis is markedly stimulated by aeration (7),

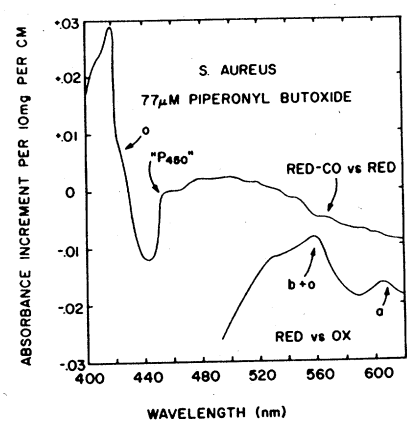


FIG. 7. *Difference spectra of S. aureus grown for 1 hr after aeration in the presence of 77 μM piperonyl butoxide. Data were analyzed as in Fig. 2.*

probably because of the involvement of an oxygen-requiring, mixed-function oxidase in the hydroxylation of δ-carotene to the rubixanthins (8). With the onset of aeration in exponentially growing cells, there was a rapid synthesis of rubixanthin (Fig. 9). Initially there was a twofold

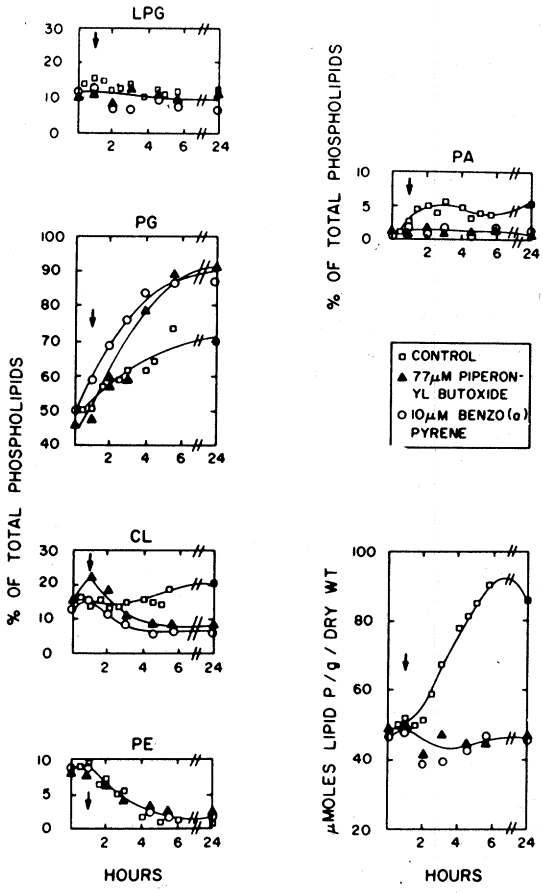


FIG. 8. *Phospholipid synthesis in S. aureus after aeration in medium (□), in medium plus 77 μM piperonyl butoxide (▲), and in medium plus 10 μM benzo(a)pyrene (○). Cultures were grown anaerobically as in Fig. 3 in the presence of 2 mCi of $H_3{}^{32}PO_4$ per 7 liters for at least seven doublings before aeration. At intervals, 1,000 ml of culture was withdrawn rapidly onto an equal volume of ice containing sufficient HCl to a final pH of 2.0; the culture was centrifuged and the lipids were extracted. A portion of the lipid was deacylated by mild alkaline methanolysis, and the glycerol phosphate esters derived from the lipids were separated on acid-washed aminocellulose paper by chromatography in two dimensions (12). The lipids were located by autoradiography, and the proportion of $^{32}P$ in each ester was determined. A second portion of the phospholipids was applied to silica gel-impregnated paper, and the lysyl-phosphatidylglycerol was separated from the rest of the lipids (10, 12). The phosphatidylglycerol content was determined as the difference between the glycerol phosphorylglycerol and the lysyl-phosphatidylglycerol. The total phosphate in the lipids was determined colorimetrically. The specific activity of the $^{32}P$ remained about 6,100 counts per min per μmole of phosphate throughout the experiment.*

drop in the phytoene concentration that rapidly recovered to a slightly elevated level as the adaptation to aerobic growth continued. Rubixanthin can be formed anaerobically in this strain of *S. aureus* by the hydration to phytofluenol and the subsequent cyclization to rubixanthin (7, 8). The phytofluenol level dropped as the adaptation to aeration continued, suggesting that the anaerobic hydration pathway of rubixanthin formation was also stimulated during the adaptation to aerobic growth.

The two lipophilic inhibitors had striking effects on the synthesis of carotenoids during the adaptation to aerobic growth. As expected, PB added at the onset of aeration depressed the synthesis of the rubixanthins (Fig. 10). In this experiment, the rubixanthins and the δ-carotene were not separated, so that the increase in δ-carotene obscured the decrease in rubixanthin formation. After 6 hr of aeration, the δ-carotene represented 90% of the absorbancy at 460 nm in the xanthophyll preparation. There was an early increase in the phytofluenol, after which the level dropped to the control level, suggesting that the anaerobic pathway of rubixanthin formation was functional in the presence of PB. Phytoene accumulated and the total carotenoid level was roughly twice the level in the control culture throughout the shift to aerobic growth.

BAP also had a striking effect on carotenoid formation (Fig. 10). The total carotenoid level was 40 times the control level after 4 to 6 hr of aerobic growth. Synthesis of δ-carotene and the rubixanthins was inhibited, and by 6 hr after the onset of aeration none of these xanthophylls was detectable in the cells. The great increase in carotenoids in cells grown with BAP was comprised of the hydrocarbons phytoene and δ-carotene, and the phytofluenols.

## DISCUSSION

Formation of the electron transport system in exponentially growing *S. aureus* was essentially complete within 1 hr after the onset of aeration (Fig. 3). The electron transport system was assayed as the enzymatically reduced cytochromes *b*, *a*, and *o*. The $P_{450}$ as well as the total protoheme were also assayed. In the presence of 77 μM PB, synthesis of cytochrome *b* was 90% and cytochrome *a* was 80%; synthesis of cytochrome oxidase *o*, the $P_{450}$, and the protoheme was maximal for that growth condition within 2 hr after the onset of aeration (Fig. 6). However, the cells formed only 33% of the $P_{450}$ and 50% of the cytochrome oxidase *o* formed in the control. In cultures in which the induction of the electron

transport system was measured in the presence of 10 μM BAP, formation of the cytochromes was delayed: 62% of the total cytochrome *b*, none of the cytochrome *a*, 60% of the $P_{450}$, and all of the cytochrome oxidase *o* were formed within 2 hr after aeration (Fig. 4). After 5 hr of aerobic growth, the cells formed the same total amount of cytochrome *b* and *a* as the control. Cells grown with BAP formed only half the total cytochrome oxidase *o*, 25% more $P_{450}$, and a curious chromophore that distorted the difference spectrum in the 430-nm range (Fig. 4 and 5). The enzymatically active cytochrome oxidase *o* appeared to decrease per cell as incubation continued (Fig. 4). Cytochrome *b* plus *o* accounted for the total protoheme in the cells. No proto-

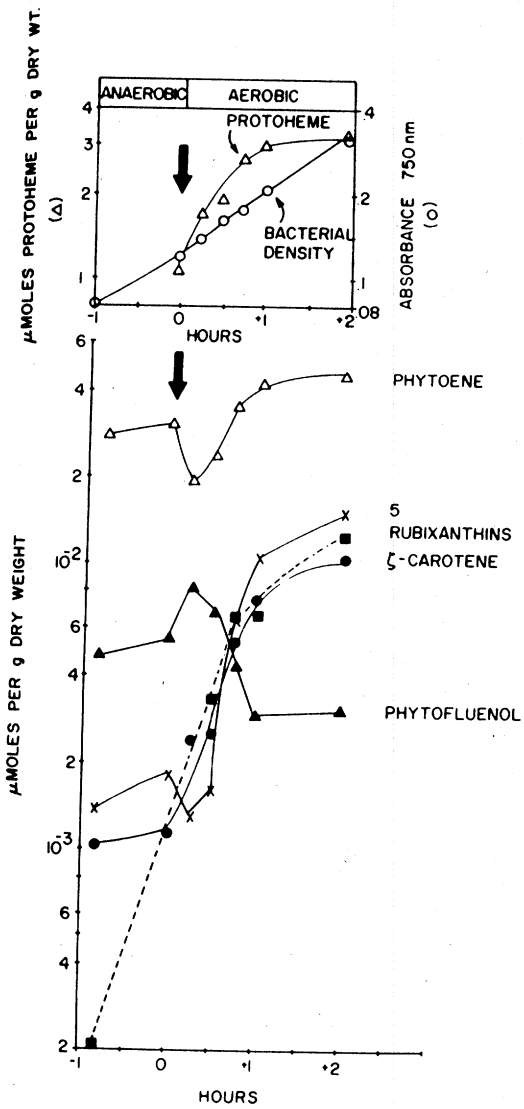


FIG. 9. *Carotenoid synthesis in exponentially growing S. aureus after aeration (|). Cells were grown and harvested, and the lipids were extracted as in Fig. 8, except that the suspension was not acidified before centrifugation. The lipids were then saponified, and the carotenoids were separated on alumina-impregnated paper as described (8). The figure 5 indicates a phytofluenol-like carotenoid (7). The carotenoids were examined in cells from which the data of Fig. 3 were taken.*

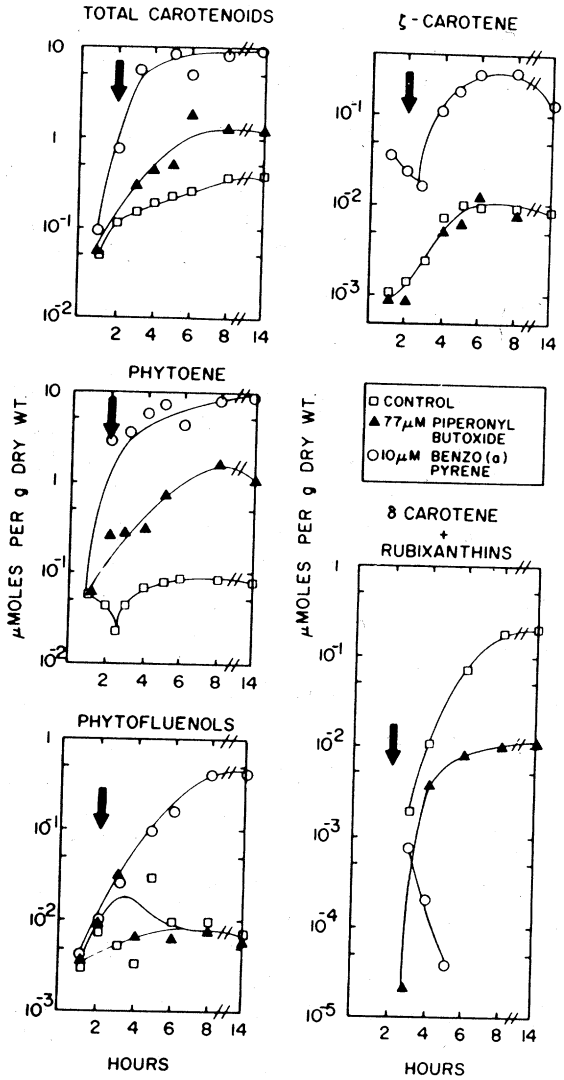


FIG. 10. *Carotenoid formation in exponentially growing S. aureus with the onset of aeration (|) in the control medium (□), in the control medium plus 77 μM piperonyl butoxide added with the beginning of aeration (▲), and in the control plus 10 μM benzo(a)pyrene added with the beginning of aeration (○). Carotenoids were analyzed as in Fig. 9.*

heme was in nonenzymatically reducible pigments.

The two inhibitors slowed the formation of the electron transport system (slightly in the case of PB, markedly in the case of BAP), decreased the total cytochrome oxidase formed by half and rendered it unstable, and increased (BAP) or decreased (PB) the amount of $P_{450}$.

Previous work suggested that changes in phospholipid metabolism occurred when the composition of the membrane-bound electron transport system of *Haemophilus parainfluenzae* was modified (18). The lipid changes involved a transient 5 to 10% increase in total phospholipid, changes in the proportions of the lipids, a decrease in the rate of turnover of the phospholipids, and change in the rate of incorporation of $H_3{}^{32}PO_4$ into the phospholipids (18). In *S. aureus*, in the 1 hr necessary for the formation of the electron transport system (Fig. 1), the total phospholipids increased 15 to 20% (Fig. 8). This confirms previous work (4). The increase involved primarily PG and CL without much change in the PG/CL ratio (Fig. 8). When the formation of the electron transport system was inhibited in the presence of PB or BAP, the increase in total lipid did not occur and the ratio of PG to CL was decreased (Fig. 8). The inhibitor diphenylamine, which depressed rubixanthin synthesis 90% but had no effect on phospholipid metabolism, did not affect the formation of the electron transport system (9). The formation of the respiratory system in *S. aureus* was complete in 1 hr, but the changes in the lipids continued for 5 hr, suggesting that only a small portion of the total lipids of the cell was changed during the formation of the electron transport system. Evidence for heterogeneity in phospholipid metabolism and distribution in the bacterial membrane has been found. There was a biphasic turnover of the phosphates in CL in this strain of *S. aureus* during exponential growth (12), and a portion of the membrane of *H. parainfluenzae* which contained a phospholipid composition different in composition and time of synthesis from the rest of the cell can be isolated (13, 14).

These initial studies suggest that the metabolism of a portion of the phospholipids and carotenoids (especially the rubixanthins) was involved in the formation of the membrane-bound electron transport system. It appears that these lipids were involved in the formation and stability of the cytochrome oxidase *o* portion of the electron transport system.

The inhibition of synthesis of the electron transport system was greater with BAP than with PB, although both had similar effects on phospholipid metabolism (Fig. 4, 6, and 8). A difference was detected in the effects of PB and BAP on carotenoid formation. With the formation of the electron transport system, there was a rapid synthesis of carotenoids, especially the rubixanthins (Fig. 9). BAP had a more pronounced inhibitory effect on δ-carotene and rubixanthin synthesis and caused a much greater synthesis of hydrocarbon carotenes than PB (Fig. 10). Again it appeared that only a small portion of the total rubixanthin was involved in some crucial role in the synthesis of the electron transport system, since decreasing the total rubixanthin formation 10-fold by growth with 74 μM diphenylamine had no effect on the formation of the electron transport system (9). A second portion of the rubixanthin seemed to be involved in the growth of *S. aureus* at suboptimal temperatures (10). When rubixanthin content was depressed 95% with PB, the growth rate at 25 C decreased 2.4 times (10).

The effect of these inhibitors on the induction of cytochrome $P_{450}$ was complicated by the method of assay employed. Absorbance of the carbon monoxide complex of the reduced pigment overlapped with absorbance of the Soret region of the carbon monoxide complex of reduced cytochrome oxidase *o* (Fig. 2). It appeared that PB not only inhibited the hydroxylating activity of $P_{450}$ (8), but it also inhibited the synthesis of the hydroxylating system as well (Fig. 6). BAP induces $P_{450}$ in the mammalian endoplasmic reticulum (1) and apparently stimulates $P_{450}$ synthesis in *S. aureus* (Fig. 4). The hyroxylation of BAP reduces its carcinogenic activity (11), and the simultaneous injection of PB and BAP greatly increases the toxicity of BAP (2).

The data of Fig. 1 indicated that BAP was inhibitory under growth conditions that involve a functional membrane-bound electron transport system. BAP is inhibitory, whereas the less carcinogenic isomer BEP is not. Perhaps this *S. aureus* membrane system could offer insight into the effect of some carcinogens on membrane formation and function.


## ACKNOWLEDGMENTS

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