JOURNAL OF BACTERIOLOGY, Dec. 1967, p. 1868-1874
Vol. 94, No. 6
Copyright © 1967 American Society for Microbiology
Printed in U.S.A.

# Membrane Lipid Changes During Formation of a Functional Electron Transport System in *Staphylococcus aureus*

FRANK E. FRERMAN AND DAVID C. WHITE
*Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40506*

Received for publication 15 September 1967

Addition of oxygen to a culture of anaerobically growing *Staphylococcus aureus* results in the formation of a membrane-bound, functional electron transport system. With the shift to aerobic growth, there is at least a 15-fold increase in cytochrome *a* and at least a 55-fold increase in cytochrome oxidase *o*. At the completion of the shift to aerobic growth, the cytochrome levels equal those found in bacteria grown with aeration throughout the entire growth cycle. Cytochromes $b_1$ and *o* are formed first. Their synthesis slows when cytochrome *a* becomes detectable. Concentrations of cytochromes $b_1$ and sometimes cytochrome *a* increase late in the adaptive period. Concomitant with this is a decrease in the oxygen tension at which the rate of oxygen utilization becomes dependent on the oxygen concentration. During the shift to aerobic growth, the protoheme content increases ninefold, and all the protoheme can be accounted for in enzymatically reducible cytochrome $b_1$ and cytochrome oxidase *o*. Protoheme, but not a functional cytochrome system, is synthesized by anaerobically growing *S. aureus*. Heme *a* appears only after a period of aerobic growth. During the shift to aerobic growth, there is a 1.6-fold increase in the vitamin $K_2$ content, with an alteration in the ratios of the 35 and 45 carbon side chain isoprenologues. A twofold increase in phosphatidyl glycerol and a 1.6-fold increase in cardiolipin occur with the shift to aerobic growth. Lysyl-phosphtidyl glycerol remains essentially constant in this period. Concentrations of mono- and diglucosyl diglycerides increase coordinately 1.3-fold during the shift to aerobic growth at a 2.5 to 1 M ratio.

Changes in oxygen tension in the growth environment of bacteria result in variations in the synthesis and composition of the membrane-bound respiratory chain (5, 11, 16, 19, 25, 26). *Staphylococcus aureus* derives its energy by glycolysis, when grown anaerobically, and contains no detectable respiratory system (4, 20). Oxygen induces the formation of a membrane-bound electron transport system (22) composed of flavoproteins, cytochrome $b_1$, cytochrome *a*, and cytochrome oxidase *o* (21, 31). The lipids of *S. aureus* have been characterized (2, 14, 15, 17, 18, 31), and are localized in the membrane containing the electron transport system (31), and the methods for their quantitative extraction and assay have been developed (31).

Coordinate lipid and membrane-bound pigment biosynthesis have been described in two systems. *Haemophilus parainfluenzae* synthesized demethyl vitamin $K_2$ and cytochrome $b_1$ coordinately (29). *Rhodopseudomonas spheroides* increases membrane phospholipid and photosynthetic pigments coordinately (12).

In this study, the shift of a culture of *S. aureus* from anaerobic to aerobic growth was used to examine the events in the formation of both the membrane-bound electron transport system and the lipids that are a part of this membrane. Oxygen induces the formation of the respiratory pigments. The synthesis of these pigments results in a progressively more effective electron transport system as measured by the ability to utilize oxygen at low oxygen concentrations. Concurrent with the formation of a progressively more effective electron transport system, changes in the membrane lipids occur: (i) doubling of the phosphatidyl glycerol (PG) and a 1.6-fold increase in the cardiolipin; (ii) a coordinate 1.3-fold increase in glucolipid with a molar ratio of diglucosyldiglyceride (DGDG) to monoglucosyldiglyceride (MGDG) of 2.5 to 1; and (iii) a 1.6-fold increase in vitamin $K_2$ with an increase in the proportions of the 45 carbon side chain isoprenologue.

The anaerobic synthesis of protoheme and of vitamin $K_2$ were unexpected findings. Jacobs (9–

11) reported that *S. epidermidis* cannot make protoheme anaerobically and Bishop et al. (1) reported that anaerobically grown *S. albus* contained no detectable vitamin $K_2$.

## MATERIALS AND METHODS

*Growth of bacteria. S. aureus* U-71 (31) was used in these experiments. The medium was as described (31). Purity of the culture was determined after each experiment by phase contrast microscopy and colonial morphology on nutrient agar plates.

The bacteria were grown in 22-liter bottles at 37 C. The culture was stirred with a Teflon-covered magnetic bar. Gases were added to the culture through a glass sparger, which was inserted through a rubber cork and extended 18 inches below the surface of the medium. Nitrogen was deoxygenated as described (28) and bubbled through the medium as it cooled after autoclaving. Copper tubing was used for conveying the nitrogen between the deoxygenating column and the culture bottle to minimize diffusion of oxygen. The medium was inoculated with 500 ml of *S. aureus* that was grown anaerobically as described (28). During the inoculation, the medium was bubbled vigorously with deoxygenated nitrogen. Over a 15-hr period of anaerobic growth, the culture reached a density of about $10^7$ cells/ml. Nitrogen was bubbled through the medium during the period of anaerobic growth at a rate of 50 ml/min per 18 liters. No oxygen could be detected in the effluent nitrogen by passing it through a reduced solution of 0.01% (w/v) resazurin at *p*H 7.6 for 17 hr. After the anaerobic samples had been withdrawn, aeration was begun at a rate of 10 liters/min per 15 liters of growth medium. Foaming was controlled with Antifoam B (Dow-Corning Corp., Midland, Mich.). When the bacterial density reached an absorbancy of 1.5, the aeration was increased to 20 liters/min. Samples (500 to 1,000 ml) were removed under pressure into a beaker containing one-half the volume of ice. The bacteria were harvested by centrifugation, washed twice, and resuspended in 0.05 M phosphate buffer, *p*H 7.6 (31).

*Assay of the protoheme.* Protoheme content of the bacteria was determined as the pyridine hemochrome, as described by Falk (5).

*Assay of the cytochromes.* Cytochromes were assayed by difference spectroscopy (25, 29). Cytochrome *a* was estimated from the absorbance increment between the maximum, at 605 mμ, and a line connecting 630 mμ and 595 mμ, in the oxidized minus reduced difference spectra. Cytochrome $b_1$ was estimated as the absorbance increment between the maximum, at 560 mμ, and a line connecting 540 and 580 mμ, in the reduced minus oxidized difference spectra. This estimation is complicated by the absorbance of cytochrome oxidase *o*. Cytochrome oxidase *o* has a maximum at 557 mμ (21) in this type of difference spectrum. The absorbance increment is consequently given as cytochrome $b_1$ plus cytochrome oxidase *o* in this paper. Cytochrome oxidase *o* was measured as the absorbancy increment between the maximum, at 416 mμ, and the minimum, at 430 mμ, in the carbon monoxide (saturated) reduced minus reduced difference spectrum. Since neither cytochrome $b_1$ nor cytochrome *a* reacts with carbon monoxide (21), the measurement of cytochrome oxidase *o* from the carbon monoxide reduced minus reduced difference spectrum is unequivocal. The suspension of bacteria with the respiratory pigments oxidized was kept at 0 C and was vigorously aerated by shaking in the air before each measurement. The reduced minus oxidized difference spectrum was also compared with an absolute spectrum of the reduced pigments compared to frosted glass (29). The cytochromes were reduced in the presence of 20 mM L-lactate. Reduction of the respiratory system in the presence of this substrate is complete (Table 1).

*Assay of oxygen utilization.* Oxygen utilization was measured with an oxygen electrode (26) using 20 mM L-lactate as substrate. L-Lactate was used because it gave the highest rate of oxygen uptake of the substrates tested (Table 2). These experiments indicated that the endogenous respiration of the washed bacterial suspensions was not sufficient to influence the difference spectroscopy. To determine the inhibition of respiration by 2-*n*-nonyl-4-hydroxyquinoline *N*-oxide (NOQNO), 0.03 ml of a 3.4 μM NOQNO solution in ethyl alcohol was added to 3.0 ml of a bacterial suspension, after the rate of oxygen utilization in the presence of L-lactate could be estimated accurately, but before the oxygen tension had dropped below 150 μM. The critical oxygen concentration was measured as described previously (27).

*Bacterial dry weight and protein.* Dry weight of the bacteria was determined as described (31). Bacterial density was measured as absorbancy at 750 mμ as described previously (31).

*Lipid extraction.* Lipids were extracted by the methods described previously (31).

*Assay of vitamin $K_2$.* Vitamin $K_2$ isoprenologues were purified by silicic acid chromatography followed by preparative thin-layer chromatography (31). They were identified by co-chromatography with authentic standards as described previously (31). The proportions of each isoprenologue were determined after descending chromatography in the system utilizing Dow-Corning silicone 200 impregnated paper as described by Lester, White, and Smith (13). The quinones were located by observing quenching areas with a long wavelength ultraviolet lamp; they were

TABLE 1. *Reduction of cytochromes of Staphylococcus aureus with L-lactate and $Na_2S_2O_4$*

| Substrate | Absorbancy increment[a] | | |
|---|---|---|---|
| | Cytochrome *a* | Cytochrome $b_1 + o$ | Cytochrome oxidase *o* |
| L-Lactate | 0.019 | 0.013 | 0.160 |
| $Na_2S_2O_4$ | 0.019 | 0.013 | 0.160 |

[a] Cytochromes measured by difference spectra as described in Materials and Methods with bacterial suspensions containing 46.4 mg (dry weight) per ml.

TABLE 2. *Rate of oxygen utilization by Staphylococcus aureus with various substrates*

| Substrate | Rate of oxygen utilization (mμmoles per sec per mg of protein) |
|---|---|
| L-Lactate | 0.200[a] |
| Glucose | 0.133 |
| Succinate | 0.014 |

[a] Concentration of substrates was 0.02 M in the suspensions tested. Further substrate additions caused no increase in the rate of oxygen utilization.

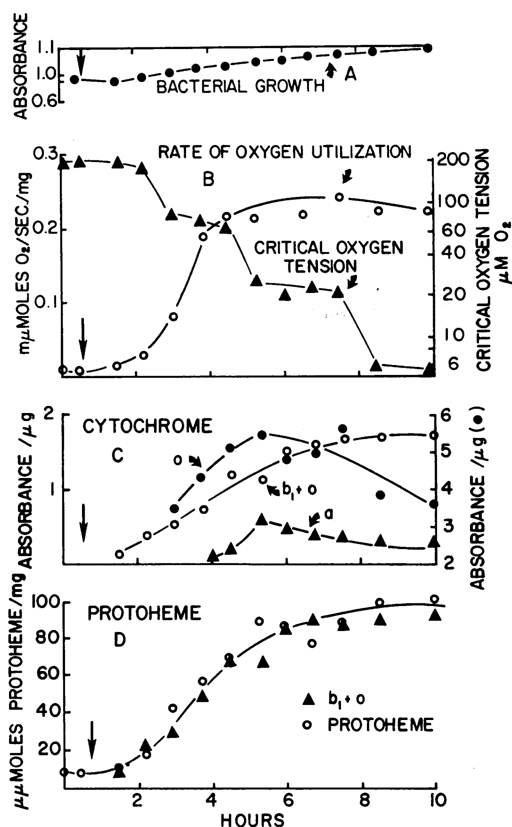


FIG. 1. *Comparison of the patterns of growth (A), respiratory oxygen utilization (B), respiratory pigment formation (C), and protoheme concentration (D) during induction of the electron transport system in Staphylococcus aureus by the shift from anaerobic to aerobic growth. Estimation of each parameter was performed as outlined in Materials and Methods and the time at which the oxygen was added is indicated by the large arrow. In part C, symbols are: Absorbancy increment (○) per μg of bacterial dry weight, with the maximum at 560 mμ in the difference spectra; contains the absorbance of both cytochrome $b_1$ and cytochrome o. Right hand ordinate (●) refers to cytochrome oxidase o. In part D, symbols are: (○) protoheme concentration in the bacteria estimated by pyridine hemochrome; and (▲) protoheme content of cytochromes $b_1$ plus o calculated from the α maximum in the difference spectrum with $\epsilon_{560}$ = 19.1 × 10³ (7).*

quickly circled with a pencil, cut out, and eluted with chloroform-isooctane (2:1, v/v). The extracts were then evaporated to dryness in a stream of nitrogen, redissolved in isooctane, and the absorption spectra were measured with the Cary 15 spectrophotometer.

*Assay of lipids.* Phospholipids and glucolipids were separated by thin-layer chromatography, recovered, and assayed as described previously (31).

*Reagents.* All chemicals were of the best grade commercially available and were essentially as described previously (28, 31).

## RESULTS

*Change in growth rate.* Introduction of oxygen to a culture of *S. aureus*, grown anaerobically to a bacterial density of about 0.18 mg (dry weight) per ml, resulted in a shift to a more rapid growth rate (Fig. 1A). The bacteria continued to grow at the anaerobic growth rate until 1 to 2 hr after the introduction of air. Doubling time during the adaptation was dependent on the stage of anaerobic growth at the time the bacteria were shifted to aerobic growth. The newly established growth rate was maintained for at least another 10 hr. During the period of adaptation to aerobic growth, the bacteria can reach a density of 0.35 mg (dry weight) per ml. Doubling time for *S. aureus* in this medium, when grown with high aeration for the whole growth cycle, was about 50 min. During this growth period, the *p*H of medium decreased from 7.6 to 7.2.

*Formation of the electron transport system.* About the time that there was a noticeable increase in the growth rate after the shift to aerobic growth, enzymatically reducible cytochrome $b_1$ and cytochrome oxidase *o* were detectable (Fig. 1C). Under these growth conditions, the cytochrome pigments are completely reduced in the presence of L-lactate whenever they are detectable by reduction with $Na_2S_2O_4$. The rapid formation of cytochrome $b_1$ and cytochrome oxidase *o* continued for about 4 hr. At this time, the synthesis of these cytochromes slowed and cytochrome *a* became detectable. After cytochrome *a* had increased somewhat, the concentration of cytochrome oxidase *o* decreased. Concentration of cytochrome oxidase *o* decreased as the rate of synthesis of cytochromes $b_1$ and *a* increased. This indicated that cytochrome $b_1$ was being formed. Concentration of cytochrome *a* remained fairly constant after the initial period of synthesis (Fig. 1C). In experiments where the bacteria were

shifted to aerobic growth at lower bacterial densities, after the initial lag, the concentration of cytochrome *a* increased throughout the period of aerobic growth (Fig. 2D).

Total content of cytochromes in the bacteria, after the shift to aerobic growth had been completed, approximated the cytochrome content of bacteria grown with aeration throughout the entire growth cycle. Bacteria grown with vigorous aeration for the entire growth cycle (Table 1) contained 85% of the cytochrome *a*, 54% of the cytochromes $b_1$ and *o*, and 70% of the cytochrome oxidase *o* formed during the shift to aerobic growth (Fig. 1).

*Formation of the respiratory system.* Formation of the membrane-bound electron transport system was followed by the measurement of oxygen utilization in the presence of L-lactate. A rapid rate of oxygen utilization, together with reduction of the electron transport system, was found with L-lactate (Table 1 and 2). The capacity to utilize oxygen in the presence of L-lactate increased about 30-fold during the shift to aerobic growth (Fig. 1B).

*Change in critical oxygen concentration.* Maximal rate of oxygen utilization is a relatively poor measure of the changes in the composition of the electron transport system. In many experiments, the maximal rate of oxygen utilization increased much more rapidly than the formation of the cytochrome pigments. Changes in the composition of the electron transport system, after the appearance of cytochrome *a*, were not reflected in changes in the maximal rate of oxygen utilization. Maximal rate of oxygen utilization was assayed at high oxygen tensions (above 100 $\mu$M oxygen); bacteria actually grew at much lower oxygen tensions (1 to 10 $\mu$M oxygen). See reference 30. Composition of the electron transport system was in closer agreement with changes in the critical oxygen concentration. The critical oxygen concentration is that concentration at which the rate of oxygen utilization becomes first order with respect to the oxygen tension. The critical oxygen concentration provides a measure of the affinity of the intact electron transport system for oxygen (27). Affinity of the electron transport system for oxygen is a measure of the proportion of cytochrome oxidase that can be maintained reduced at low oxygen tension (3). The proportion of oxidase that is reduced is, in turn, dependent on the electron flux to the oxidases (3, 27). The critical oxygen concentration decreased 100-fold during the formation of the electron transport system (Fig. 2B). Kinetics of the fall of the critical oxygen concentration closely followed changes in the composition of the electron transport system. Decrease in critical oxygen

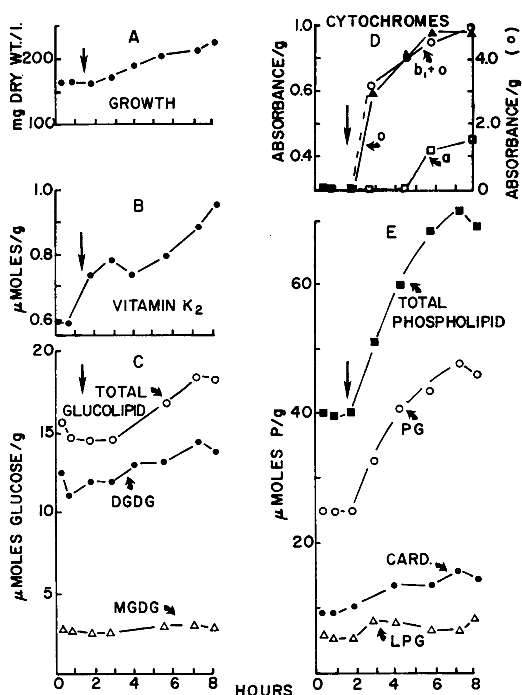


FIG. 2. *Changes in concentration of membrane components and bacterial growth of Staphylococcus aureus during the shift from anaerobic to aerobic growth. Beginning of aeration is indicated by arrows in (A) bacterial growth; (B) vitamin $K_2$ content; (C) total glucolipid, diglucocyldiglyceride (DGDG) and monoglucosyl-lsiglyceride (MGDG) content; (D) cytochrome content; and (E) total phospholipid, phosphatidyl glycerol (PG), cardiolipin, and lysyl-phosphatidyl glycerol (LPG) content. Components were isolated and separated as described in Materials and Methods and are plotted per gram (dry weight).*

concentration was triphasic. The first period of rapid fall paralleled the rapid appearance of cytochrome $b_1$ and cytochrome oxidase *o*. The second period of fall occurred concomitantly with the synthesis of cytochrome *a*. The final reduction in the critical oxygen content occurred with the second increase of cytochrome $b_1$.

*Change in sensitivity to NOQNO during adaptation to aerobic growth.* During the period of induction of the electron transport system, there was a change in the type of respiratory system. This was detected by the response in the rate of oxygen utilization to NOQNO. This inhibitor blocks electron transport in *S. aureus* between cytochrome $b_1$ and cytochrome oxidase (21). In anaerobically grown *S. aureus*, the rate of oxygen utilization in the presence of L-lactate was not inhibited by 34 $\mu$M NOQNO. This suggested a

transition from a flavoprotein-mediated respiratory system to a cytochrome-linked respiratory system with a terminal cytochrome oxidase (21). After the shift to aerobic growth, the ability of the bacteria to utilize oxygen became increasingly sensitive to inhibition by NOQNO. At 45, 90, and 135 min after the shift to aerobic conditions, the respiratory system was inhibited 47, 81, and 100%, respectively, in the presence of 34 $\mu$M NOQNO.

*Synthesis of protoheme*. Protoheme was detected (Fig. 1D) in anaerobically grown *S. aureus* at a level of 7 $\mu\mu$moles/mg (dry weight). The lack of inhibition of respiration by NOQNO indicated that no functional cytochrome system exists in anaerobically grown *S. aureus*. With the shift to aerobic growth, the protoheme content of each cell was increased ninefold. Protoheme content of cytochrome $b_1$ and cytochrome oxidase *o* approximated the total protoheme in the bacteria (Fig. 1D). Apparently, the protoheme associated with catalase activity that is induced by aeration (22) did not significantly affect these calculations.

*Vitamin $K_2$*. Vitamin $K_2$ increased 1.6-fold during the shift to aerobic growth (Fig. 2B). Rapid cytochrome oxidase *o* and cytochrome *a* synthesis continued until the molar ratios of vitamin $K_2$ to cytochrome oxidase *o* approached 16 to 1 and the vitmain $K_2$ to cytochrome *a* approached 50 to 1 (Fig. 3).

White and Frerman (31) have reported three major isoprenologues of vitamin $K_2$ in *S. aureus*. The isoprenologues can be separated as described in Materials and Methods. Recoveries of the three major isoprenologues from the paper chromatograms were 92%. Four additional components were observed, which reacted immediately with neotetrazolium after reduction with $NaBH_4$. These spots were observed in vitamin $K_2$ preparations from both aerobically and anaerobically grown bacteria. From their chromatographic properties, these minor quinone components have shorter side chains than the three major isoprenologues. These components represent less than 8% of the total vitamin $K_2$. The proportions of the $K_2$ [45] increased and the $K_2$ [35] decreased during the 1.6-fold increase in total quinone content associated with the shift to aerobic metabolism (Table 3).

*Glucolipids*. Concentration of total glucolipid increased 1.3-fold during the shift to aerobic growth (Fig. 2C). During this increase, the DGDG to MGDG ratio remained near 2.5:1 (Fig. 3).

*Phospholipids*. When *S. aureus* was shifted to aerobic growth conditions, the amount of total phospholipid increased twofold (Fig. 2E). The PG increased twofold, and cardiolipin increased 1.6-fold. The LPG content remained relatively constant during the shift to aerobic growth. The molar ratio between PG and LPG varied between 4.1:1 and 8.9:1 (Fig. 3).

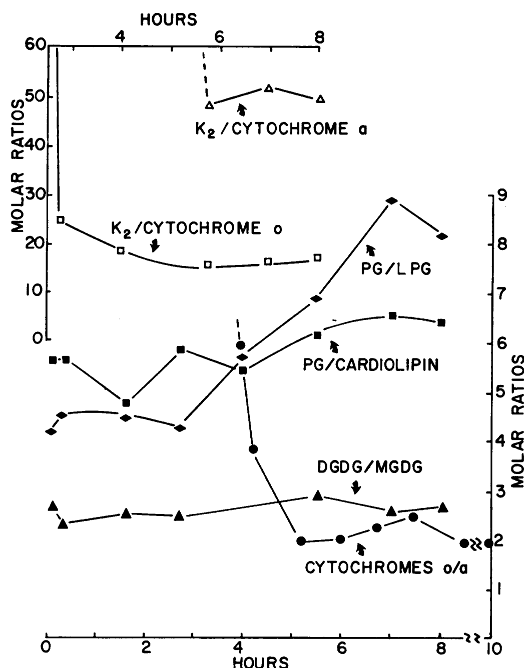


FIG. 3. *Variation in molar ratios of membrane components in the membrane-bound electron transport osytem of Staphylococcus aureus during the shift from anaerobic to aerobic growth. Molar ratios were calculated with extinction coefficients of 13 × 10³ (23), cytochrome a; 90 × 10³ (24), cytochrome oxidase o; and 19.28 × 10³ (8), vitamin K₂. Solid symbols refer to the right-hand axis. Ratio of cytochrome o to a taken from data in Fig. 1; remaining data from Fig. 2. Abbreviations are as in Fig. 2.*

During the period of the shift from anaerobic to aerobic growth, the highest levels of cytochromes, phospholipids, glucolipids, and vitamin $K_2$ achieved were approximately equal to levels of these components found in *S. aureus* grown with vigorous aeration throughout the entire growth cycle.

## DISCUSSION

Addition of oxygen to anaerobically growing *S. aureus* results in the formation of the membrane-bound electron transport system. Formation of the electron transport system involves synthesis of protoheme, cytochromes, cytochrome oxidase, phospholipids, glucolipids, and vitamin $K_2$ isoprenologues. These components are incorporated into a membrane-bound electron

TABLE 3. *Proportions of the major isoprenologues of Vitamin $K_2$ in anaerobically and aerobically grown Staphylococcus aureus*[a]

| Condition of growth | Vitamin | | |
|---|---|---|---|
| | $K_2$[35] | $K_2$[40] | $K_2$[45] |
| Anaerobic | 26.0[b] | 66.0 | 8.0 |
| Aerobic | 18.9 | 62.5 | 18.6 |

[a] Isoprenologues ($K_2$[35]: vitamin $K_2$ with a 35 carbon side chain) were separated by reversed phase paper chromatography as described in Materials and Methods, then eluted and assayed spectrophotometrically. The extinction coefficients, from synthetic vitamin $K_2$ isoprenologues (6), were $\epsilon_{245m\mu} = 18.96 \times 10^3$, $K_2$[35]; $\epsilon_{245m\mu} = 19.28 \times 10^3$, $K_2$[40]; $\epsilon_{245m\mu} = 19.37 \times 10^3$, $K_2$[45].

[b] Results are given in per cent of total vitamin $K_2$.

transport system that shows progressively increasing affinity for oxygen, as well as increasing sensitivity to the inhibitor NOQNO. Both of these features are characteristic of the shift to respiration involving cytochromes and cytochrome oxidases (21).

One of the most striking characteristics of the adaptation to aerobic growth is that the components of the electron transport system can be formed into a functional complex at widely varying proportions. In functional systems, molar ratios of cytochrome oxidase *o* to cytochrome *a* can vary between >200:1 and 2:1 (Fig. 3). Vitamin $K_2$ to cytochrome oxidase *o* can function within molar ratios between >800:1 and 16:1 (>120:1 and 45:1 for vitamin $K_2$ to cytochrome *a*). Phospholipid composition can change and the membrane be functional when the molar ratios of PG to LPG are between 4:1 and 9:1.

It has been proposed that the membrane-bound electron transport system must be formed by the stochiometric agglomeration of large subunits of constant composition. This hypothesis has been developed from degradation studies of beef heart mitochondria (7). During the shift to aerobic growth in *S. aureus*, widely different molar proportions of the various components of this membrane-bound system are functional. This observation, which has been made by monitoring the actual formation of the membrane-bound system in a living organism, clearly indicates that any such subunits added to the membrane cannot be of constant composition. The electron transport system in *S. aureus* could be formed either by the addition of subunits of differing composition or by the extensive modification of a basic membrane. The prediction from this study, that the membrane of *S. aureus* exists as a mosaic of domains with differing composition, is presently under investigation.

## ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM-10285 from the National Institute of General Medical Sciences. For part of the study, F. E. F. was supported by fellowship grant GM-24098 from the same source.

The authors wish to thank O. Isler of Hoffman-LaRoche, Nutley, N.J. for the synthetic homologs of vitamin $K_2$

## LITERATURE CITED

1. BISHOP, D. H., K. B. PANDYA, AND H. K. KING. 1962. Ubiquinone and vitamin $K_2$ in bacteria Biochem. J. **83**:606–614.
2. BRUNDISH, D. E., N. SHAW, AND J. BADDILEY. 1966. Glycosyl diglycerides in gram-positive bacteria. Biochem. J. **99**:546–549.
3. CHANCE, B. 1957. Cellular oxygen requirements. Federation Proc. **16**:671–680.
4. COLLINS, F. M., AND J. LASCELLES. 1962. The effect of growth conditions on oxidative and dehydrogenase activity in *Staphylococcus aureus*. J. Gen. Microbiol. **29**:531–535.
5. ENGLESBERG, E., A. GIBOR, AND J. B. LEVY. 1954. Adaptive control of terminal respiration in *Pasteurella pestis*. J. Bacteriol. **68**:146–151.
6. FALK, J. E. 1964. Porphyrins and metalloporphyrins, p. 182. Elsevier Publishing Co., New York.
7. GREEN, D. E., AND J. F. PERDUE. 1966. Membranes as expressions of repeating units. Proc. Natl. Acad. Sci. U.S. **55**:1295–1302.
8. ISLER, O., R. RÜEGG, AND A. LANGEMANN. 1960 Natural quinones with isoprenoid side chains. Chem. Weekblad. **56**:613–621.
9. JACOBS, N. J., J. JOHANTGES, AND R. H. DEIBEL. 1963. Effect of anaerobic growth on nitrate reduction by *Staphylococcus epidermidis*. J. Bacteriol. **85**:782–787.
10. JACOBS, N. J., AND S. F. CONTI. 1965. Effect of hemin on the formation of the cytochrome system of anaerobically grown *Staphlococcus epidermidis*. J. Bacteriol. **89**:675–679.
11. JACOBS, N. J., E. R. MACLOSKY, AND S. F. CONTI. 1967. Effects of oxygen and heme on the development of a microbial respiratory system. J. Bacteriol. **93**:278–285.
12. LASCELLES, J., AND J. F. SZILÁGYI. 1965. Phospholipid synthesis by *Rhodopseudomonas spheroides* in relation to the formation of photosynthetic pigments. J. Gen. Microbiol. **38**:55–64.
13. LESTER, R. L., D. C. WHITE, AND S. L. SMITH. 1964. The 2-demethyl-vitamin $K_2$'s. A new group of naphthoquinones isolated from *Hemophilus parainfluenzae*. Biochemistry **2**:949–954.
14. MACFARLANE, M. 1962. Characterization of the lipoamino acids as O-amino acid esters of phosphatidyl glycerol. Nature **196**:136–138.

15. MACFARLANE, M. 1962. Components of *Staphylococcus aureus* and *Salmonella typhimurium*. Biochem. J. **82**:40P.
16. MOSS, F. 1956. Adaptation of the cytochromes of *Aerobacter aerogenes* in response to environmental oxygen tension. Australian J. Exptl. Biol. Med. Sci. **34**:395–406.
17. POLONOVSKI, P., R. WALD, AND M. PAYSANT-DIAMENT. 1962. Les lipides de *Staphylococcus aureus*. Ann. Inst. Pasteur **103B**:32–42.
18. POLONOVSKI, M., R. WALD, AND F. PETEK. 1965. Isolement des glucolipides de *Staphylococcus aureus* et identification du diglucosyldiglyceride. Bull. Soc. Chim. Biol. **47**:409–416.
19. SCHAEFFER, P. 1952. Reschérches sur le metabolisme bactérien des cytochromes et des porphyrines. I. Dispartion particelle des cytochromes par culture anaérobie chez certaines bactéries aérobies facultative. Biochim. Biophys. Acta **9**:261–270.
20. STRASTERS, K. C., AND K. C. WINKLER. 1962. Carbohydrate metabolism of *Staphylococcus aureus*. J. Gen. Microbiol. **33**:213–229.
21. TABER, H. W., AND M. MORRISON. 1964. Electron transport in *Staphylococci*. Properties of a particle preparation from exponential phase *Staphylococcus aureus*. Arch. Biochem. Biophys. **105**:367–379.
22. THOFERN, E. 1961. Über synthese Struktur und Funktion Bekterieller Haminsysteme. Ergeb. Mikrobiol. Immunol. Exptl. Therap. **34**:213–274.
23. VANNESTE, W. H. 1966. Molecular proportions of the fixed cytochrome components of the respiratory chain of Keillin-Hartree particles and beef heart mitochondria. Biochim. Biophys. Acta **113**:175–178.
24. WEBSTER, D. H., AND D. P. HACKETT. 1966. The purification and properties of cytochrome *o* from *Vitreoscilla*. J. Biol. Chem. **241**:3308–3315.
25. WHITE, D. C. 1962. Cytochrome and catalase patterns during growth of *Haemophilus parainfluenzae*. J. Bacteriol. **83**:851–859.
26. WHITE, D. C. 1963. Respiratory system in the hemin-requiring *Haemophilus* species. J. Bacteriol. **85**:84–96.
27. WHITE, D. C. 1963. Factors affecting the affinity for oxygen of cytochrome oxidase in *Hemophilus parainfluenzae*. J. Biol. Chem. **238**:3757–3761.
28. WHITE, D. C. 1966. The obligatory involvement of the electron transport system in the catabolic metabolism of *Hemophilus parainfluenzae*. Antonie van Leeuwenhoek J. Microbiol. Serol. **32**:139–158.
29. WHITE, D. C. 1965. Synthesis of 2-demethyl vitamin $K_2$ and the cytochrome system in *Haemophilus*. J. Bacteriol. **89**:299–305.
30. WHITE, D. C. 1967. Effect of glucose on the formation of the membrane-bound electron transport system in *Haemophilus parainfluenzae*. J. Bacteriol. **93**:567–573.
31. WHITE, D. C., AND F. E. FRERMAN. 1967. Extraction, characterization, and cellular localization of the lipids of *Staphylococcus aureus*. J. Bacteriol. **94**:1854–1867.