Effects of Oxygen and Heme on the Development of a Microbial Respiratory System

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Abstract

The effect of adding heme to anaerobically grown cells of a strain of Staphylococcus epidermidis, which was heme-deficient due to anaerobic growth, has been examined. Cells grown anaerobically in media containing heme exhibited a marked increase in several oxidative activities as compared with cells grown anaerobically without heme. The respiratory activity of whole cells and a cyanide-sensitive reduced nicotinamide adenine dinucleotide oxidase activity of cell-free extracts were increased fourfold. The content of enzymatically reducible pigments which exhibit difference spectra similar to cytochromes $b_1$ and $b_2$ was also markedly increased. These pigments are mostly sedimented at 100,000 × $g$ (1 hr). Hemin also caused a marked increase in respiratory activity when added directly to the anaerobic culture after the period of growth, but did not cause a similar increase in respiration when added to washed, resting-cell suspensions. Under the latter conditions, heme pigments were formed which exhibited difference spectra similar to, but not identical with, the spectra of pigments found in anaerobic cells grown in the presence of heme. When resting suspensions of cells grown anaerobically without heme were exposed to air, a rapid fourfold increase in respiratory activity and a limited increase in cytochrome-like pigments occurred. The presence of the heme precursor $\Delta$-amino-levulinic acid during this aeration resulted in a rapid and marked increase in heme pigments, but only a slight stimulation of respiratory activity. The possible implications of these results for the roles which heme and oxygen play in the development of the respiratory system of this organism are discussed.

In some microorganisms which can grow both aerobically and anaerobically, such as yeast and Pasteurella pestis, the content of respiratory enzymes and cytochrome pigments is markedly diminished by growth under conditions where oxygen is not available (4, 6, 17, 18). However, the role which oxygen plays in the development of the respiratory system in these facultative anaerobes is not known. With the yeast Saccharomyces cerevisiae, it has been established that oxygen has a marked effect on the formation of the respiratory system; exposure of resting suspensions of anaerobically grown cells to oxygen induces the formation of respiratory enzymes, cytochrome pigments, and even mitochondrial structures (15, 18, 20).

Our previous investigation with a strain of Staphylococcus epidermidis established that this organism also exhibited a much decreased oxidative activity and cytochrome content as a result of growth under anaerobic conditions. However, if anaerobic growth took place in media which had been supplemented with heme, the resulting cells exhibited a much higher respiratory rate (with glucose as substrate) and also contained large quantities of pigments resembling cytochromes $b_1$ and $b_2$, as compared with cells grown anaerobically without heme. This addition of heme also resulted in a markedly increased ability of anaerobic cultures to reduce nitrate. Thus, some functional cytochromes seemed to be formed in the absence of oxygen if heme was supplied (10, 11). These observations suggested that one of the important functions of oxygen in the development of the respiratory system in this microorganism was as a requirement for the biosynthesis of heme. Goldfine and Bloch (7) have suggested that the lack of hemoprotein synthesis during anaerobic growth of some facultative anaerobes may perhaps be ascribed to a requirement for oxygen in the synthesis of the prosthetic group, heme. A probable role for oxygen in heme synthesis is...
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biosynthesis is suggested by the observation that, in rat liver mitochondria, one of the terminal reactions in the biosynthesis of heme (the conversion of coproporphyrinogen to protoporphyrin) requires molecular oxygen (16). S. epidermidis forms large quantities of coproporphyrin when grown anaerobically (9). The observation that other bacteria, such as Escherichia coli, form cytochromes during anaerobic growth suggests that not all bacteria exhibit an oxygen requirement for heme synthesis (8).

In the present investigation, the electron transport system in extracts of S. epidermidis which had been grown anaerobically in the presence of heme was characterized more completely and was compared with the electron transport system found in aerobically grown cells. We also investigated the effect of adding heme to resting suspensions of cells which had been grown anaerobically without heme, and the ability of oxygen to induce the development of the respiratory system in resting suspensions of these cells.

MATERIALS AND METHODS

The organism used, the conditions of growth, the preparation of cell suspensions, and the spectrophotometric techniques have been described previously (10). Carbon monoxide difference spectra were obtained by adding hydrosulfitc to both sample and reference cuvettes and carbon monoxide to the sample cuvette. Cell suspensions were disrupted by means of a Branson Sonifier (Branson Instruments, Danbury, Conn.) or by the "x" press (5). Reduced nicotinamide adenine dinucleotide (NADH) oxidase activity was determined by following the rate of decrease in optical density at 340 mU in the presence of 0.025 M potassium phosphate buffer (pH 7.0), 400 µg of NADH per 3 ml, and various concentrations of extract. Protein was determined by the method of Lowry et al. (14), Δ-Aminolevulinic acid hydrochloride was purchased from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

Localization of pigments in cells grown anaerobically with heme. To characterize further the cytochrome-like pigments in cells grown anaerobically with heme, the distribution of these pigments was determined after fractionation of cell-free extracts by use of an ultracentrifuge. A suspension of cells grown anaerobically in media containing added heme (10) was broken in the "x" press and was sedimented at 10,000 g for 20 min to remove intact cells and large fragments. A portion of this supernatant fluid (13.5 ml) was then sedimented at 100,000 g for 1 hr. The resulting pellet was resuspended in the original volume (13.5 ml), and the difference spectra of these various fractions were recorded (Fig. 1). An examination of the heights of the peaks in these spectra revealed that more than half of the cytochrome b1-like component with a peak at 558 mU was sedimented by centrifugation at 100,000 g for 1 hr. This observation is consistent with the view that this pigment resembles a typical cytochrome as it is associated with a particulate portion of the cell.

Comparison of NADH oxidation in extracts of cells grown aerobically and anaerobically in the presence and absence of heme. We previously reported that whole cells grown anaerobically with heme exhibit a much higher respiratory rate (with glucose as substrate) than cells grown anaerobically without heme. This respiratory rate was about 60% of the rate exhibited by aerobically grown cells. To characterize further the respiratory system of these cells, we examined the NADH oxidase activity of cell-free extracts. This activity was about four times higher in cells grown anaerobically with heme than in cells grown anaerobically without heme (Table 1). This is in accord with our previous conclusion, that anaerobic growth in the presence of heme results in a marked increase in respiratory enzymes. It is interesting that cells grown anaerobically in the presence of heme, which contain pigments resembling cytochromes b and o, exhibit only about 40% of the NADH oxidase activity of the cells grown aerobically, which contain cytochromes of the b, o, and a type (10). Further work is needed before the difference in oxidative capacity of these two types of cells can be under-
Table 1. NADH₂ oxidase activity in extracts of cells grown under various conditions

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>Amt (µmoles) of NADH oxidized per min per mg of protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>0.0480</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0.0046</td>
</tr>
<tr>
<td>Anaerobic + hemin</td>
<td>0.0180</td>
</tr>
</tbody>
</table>

* Cell-free extracts were prepared by disrupting cell suspensions in the sonic oscillator and then removing the remaining whole cells and cell debris by centrifugation at 2,000 × g for 20 min.

stood. The NADH₂ oxidase activity of cells grown anaerobically in the presence of hemin was inhibited approximately 65% by 10⁻³ M cyanide. This is the same degree of inhibition noted by Taber and Morrison (19) for the NADH₂ oxidase activity in extracts of aerobically grown S. aureus.

Effect of addition of hemin to washed, resting suspensions of cells grown anaerobically without hemin on cytochrome content and respiratory capacity. Since our previous studies indicated that the presence of added hemin during the entire period of anaerobic growth caused a marked increase in respiratory catalysts, it was important to establish whether a similar effect of hemin could be observed when hemin was added after growth to resting suspensions of cells grown anaerobically without hemin. A washed suspension of these cells [8 mg (dry weight) per ml] prepared as previously described (10) was incubated in the presence of hemin (30 µg/ml) and 0.033 M phosphate buffer (pH 7.0) for 60 min under an atmosphere of nitrogen gas. The suspension was then washed five times by centrifugation in 0.85% saline (0°C) to remove loosely bound hemin. The difference spectra of the resulting cells are shown in Fig. 2 and 3, along with the spectra of cells which had not been treated with hemin. The spectra of anaerobic cells grown in the presence of hemin is included for comparison. The respiratory rate of these cell suspensions, and of a suspension of aerobically grown cells, was also examined (Table 2). As described previously, cells grown anaerobically in the presence of hemin exhibit about 55% of the respiratory activity of aerobically grown cells. They also contained pigments resembling cytochrome b₅ in the reduced minus oxidized spectrum (peaks at 558, 524, and 427 m,), and cytochrome o in the carbon monoxide spectrum (peak at 417 m, and trough at 435 m,). The cells which were grown anaerobically without hemin, but which were incubated with hemin under resting-cell conditions, also exhibited pigments. Although these

![Graph showing difference spectra](http://jb.asm.org/)

**FIG. 2.** Reduced minus oxidized difference spectra of cell suspensions exposed to hemin during the growth period or after the growth period under resting-cell conditions. All suspensions contained 2.35 mg of cells (dry weight) per ml, and were prepared as described in the text or previously (10). The sample cuvette was reduced with a few crystals of hydrosulfite, and the reference cuvette was aerated.

![Graph showing difference spectra](http://jb.asm.org/)

**FIG. 3.** Carbon monoxide difference spectra of cell suspensions prepared as in Fig. 2. All suspensions contained 2.35 mg of cells (dry weight) per ml, and were prepared as described in Fig. 2.

Table 2. Respiration of cells exposed to hemin under growth or resting-cell conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Amt of O₂ consumed per hr per mg of cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells grown anaerobically without hemin</td>
<td>10</td>
</tr>
<tr>
<td>Cells grown anaerobically without hemin; hemin added after growth</td>
<td>13</td>
</tr>
<tr>
<td>Cells grown anaerobically with added hemin</td>
<td>37</td>
</tr>
<tr>
<td>Cells grown aerobically</td>
<td>67</td>
</tr>
</tbody>
</table>

* Respiration rate was determined manometrically with glucose as substrate, as described previously (10).
pigments were grossly similar to those present in cells grown with hemin, there were some differences. For instance, in the reduced minus oxidized spectra, prominent peaks appeared at 560 and 427 m\(\mu\) in the cells incubated with hemin under resting conditions, and at 558 and 427 m\(\mu\) in the cells grown in hemin. In addition, the ratio of the height of the Soret peak to the \(\alpha\) peak was much smaller in cells grown in the presence of hemin. In the carbon monoxide spectra, the cells grown with hemin exhibited a Soret peak at 418 m\(\mu\), whereas those incubated with hemin under resting conditions exhibited a peak at 421 m\(\mu\). As described previously, the pigments in cells grown anaerobically with hemin could be reduced either with hydrosulftite or by endogenous substrates. With cells to which hemin had been added under resting-cell conditions, reduction of pigments by endogenous substrate was not readily obtained, and rapid reduction was only observed upon the addition of hydrosulftite. However, it is possible that the inability of these pigments to be reduced rapidly by physiological substrates is a consequence of the low respiratory capacity of these cells (see below). It seems likely that most of the pigments formed on addition of hemin to washed, resting suspensions of cells grown anaerobically without hemin are not cytochromes, but may rather be hemochromogens formed by combination of heme with some nitrogenous constituents of the cell other than the apoenzymes of the cytochromes.

The addition of hemin to these resting cells also caused no substantial increase in respiratory rate (with glucose as substrate) over anaerobically grown cells which had never been incubated in hemin (Table 2). This does not appear to be caused by the inability of hemin to penetrate the cell, since cell-free extracts (prepared by sonic oscillation) of washed suspensions which had been incubated in hemin exhibited hemochromogen pigments spectrally similar to those of the whole cells. These extracts were prepared from cells which had been extensively washed, and the pigments remained in the supernatant solution after removal of whole cells and cell debris by centrifugation at 10,000 \(\times g\) for 20 min. Therefore, we tentatively concluded that the pigments are not bound to the cell wall and that hemin can penetrate the cell under the incubation conditions described.

Thus, we were not able to demonstrate any significant effect of hemin on respiratory activity when it was added to washed, resting suspensions in phosphate buffer of cells grown anaerobically without hemin, nor did the heme pigments which were formed under these conditions have the properties of cytochromes. This is in sharp contrast to the effect which hemin exerts when it is present during the growth of this organism under anaerobic conditions. The cells which are thus obtained exhibit a much higher respiratory rate than those which have not been in contact with hemin, and their pigments do have the properties of cytochromes.

Effect of adding hemin directly to the culture after the period of anaerobic growth. Our previous experiments (10) reported a marked effect of hemin on the respiratory capacity of anaerobically grown cells when hemin was added to the growth medium at the time of inoculation and was, therefore, present during the entire period of anaerobic growth. The experiments reported above indicated that a similar effect of hemin could not be readily demonstrated when hemin was added to washed, resting suspensions of cells grown anaerobically without hemin. In an attempt to gain further information regarding this phenomenon, we determined the effect of hemin when it was added directly to cultures after anaerobic growth in the absence of hemin.

Anaerobic cultures were grown under the same conditions as previously described (10), except that the glucose concentration in the growth medium was reduced from 0.5 to 0.25%. After a growth period of 16 hr, a small volume of hemin solution was added directly to the culture flask, to give a final concentration of 0.3 \(\mu g/ml\), and precautions were taken to introduce as little air as possible. (Preliminary growth experiments revealed that cultures were near the end of the logarithmic phase of growth after 16 hr.) A similar volume of 0.01 \(\mu g\) KOH solution (KOH is used to dissolve the hemin) was added to a separate anaerobic culture to serve as a control. Incubation of both flasks was continued for 1 hr at 37 C under anaerobic conditions. Both flasks were then chilled to 0 C, and the cells were removed by centrifugation, washed, and resuspended in phosphate buffer. The two suspensions were then tested manometrically for respiratory activity with glucose as substrate as described previously (10). The results of several experiments revealed that cultures to which hemin had been added yielded cells which exhibited an average \(Qo_2\) of 22, whereas those to which hemin had not been added exhibited a \(Qo_2\) of 9. Thus, hemin had a definite and marked effect in causing an increase in respiratory activity under these conditions. Studies are in progress to define further the conditions under which this effect of hemin can be observed.

Effect of aeration on the development of respiratory activity and cytochrome content of cells
grown anaerobically without hemin. When resting suspensions of cells grown anaerobically without hemin were aerated in the presence of buffer and glucose, they developed an increased respiratory rate when tested with glucose as substrate. Most of this increase occurred within 1 hr after exposure to air (Table 3). The maximal $Q_o_2$ achieved under these conditions varied between 24 and 33. This usually represented about a fourfold increase over cells which had not been exposed to air. During the aeration period, no significant increase in density of the suspensions could be detected. Additional aeration beyond a 2-hr period did not result in an increase in $Q_o_2$ even if more glucose was added to the aerated suspensions. The respiratory rates achieved under these conditions are considerably lower than those exhibited by cells grown aerobically ($Q_o_2$ of 66) and also somewhat lower than those exhibited by cells grown anaerobically in the presence of hemin ($Q_o_2$ of approximately 44) (10). The difference spectra of the anaerobic cells aerated for 2 hr are shown in Fig. 4 and 5, and are compared with the spectra of cells which were not aerated. A small but definite increase in pigments resembling cytochrome $b_1$ (peaks at 558 and 428 m$\mu$ in reduced minus oxidized spectra) and cytochrome $o$ (peak at 416 and trough at 435 m$\mu$ in the carbon monoxide spectra) was caused by aeration. It is significant that the amounts of these pigments (as indicated by the height of the peaks) in the anaerobic cells aerated for 2 hr were only a fraction of those obtained in aerobically grown cells. For instance, the same density of aerobically grown cells would have revealed peaks at 556 and 428 m$\mu$ in the reduced minus oxidized spectra four to five times higher than those shown in Fig. 5 (10). These results indicate that a rapid, but limited, increase in $Q_o_2$ occurred when resting suspensions of anaerobic cells were exposed to air at 37 C. This increase was also accompanied by a limited increase in pigments resembling cytochromes. Experiments are in progress to determine whether these changes in respiratory activity are dependent upon the synthesis of new protein.

**Effect of the heme precursor $\Delta$-aminolevulinic acid on the development of respiratory activity and pigment content during the aeration of anaerobically grown cells.** When $\Delta$-aminolevulinic acid (ALA) was added to resting suspensions when they were exposed to air for short periods of

![Fig. 4. Reduced minus oxidized difference spectra of cells grown anaerobically and exposed to air for 2 hr. Cell suspensions (prepared as described in Table 4) were adjusted to a concentration of 7 mg/ml in 0.05 M phosphate buffer, pH 7.0. A few crystals of sodium hydrosulfite were added to the sample cuvette, and the reference cuvette was aerated vigorously.](image)

![Fig. 5. Carbon monoxide difference spectra of cells grown anaerobically and exposed to air for 2 hr. Cell suspensions as in Fig. 4.](image)

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**Table 3. Respiratory rate of cells grown anaerobically after various periods of aeration**

<table>
<thead>
<tr>
<th>Period for which cells were aerated</th>
<th>Amt of O$_2$ consumed per hr per mg of cells$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>$\mu$liters</td>
</tr>
<tr>
<td>None</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>90</td>
<td>21</td>
</tr>
<tr>
<td>120</td>
<td>24</td>
</tr>
<tr>
<td>240</td>
<td>21</td>
</tr>
</tbody>
</table>

$^*$ The cell suspensions used were prepared as follows. Suspensions of cells grown anaerobically without hemin were prepared as previously described (10). These cells (which had been washed once in cold saline) were adjusted to a concentration of approximately 5 mg/ml (dry weight) in 0.025 M potassium phosphate (pH 7.0) and 0.008 M glucose. A 50-ml amount of this suspension was placed in a 500-ml Erlenmeyer flask, on a rotary shaker at 37 C. Samples were removed at the time intervals indicated, cooled to 0 C, and tested manometrically for respiratory activity as indicated previously (10).
time, the resulting cells exhibited a marked increase in pigment content as indicated by the difference spectra (Fig. 6 and 7). The appearance of these spectra suggests that ALA causes at least a threefold increase in the content of these pigments, which are doubtlessly some kind of heme compound. Further experiments indicated that the formation of large quantities of heme compounds in the presence of ALA did not occur if the cells were exposed to air at 0 °C or if they were held under anaerobic conditions at 37 °C. Whether or not these heme pigments are true cytochromes or merely hemochromogens is not known. In Table 4, the respiratory rate of these cells is shown. As indicated, the addition of ALA caused only a slight stimulation in the QO2 of the resulting cells. (This stimulation, although small, was noted repeatedly in several different experiments.) Thus, although ALA caused a marked (at least threefold) increase in heme biosynthesis,

**Table 4.** Respiratory rate of cells grown anaerobically and exposed to air in the presence and absence of Δ-aminolevulinic acid (ALA)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Amt of QO2 consumed per hr per mg of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells not exposed to air</td>
<td>9.5</td>
</tr>
<tr>
<td>Cells exposed to air without addi-</td>
<td>17.0</td>
</tr>
<tr>
<td>tion of ALA</td>
<td></td>
</tr>
<tr>
<td>Cells exposed to air in the pres-</td>
<td>19.5</td>
</tr>
<tr>
<td>ence of ALA</td>
<td></td>
</tr>
</tbody>
</table>

* Cell suspensions were prepared as in Fig. 6. Respiratory activity was determined as in Table 3.

**FIG. 7.** Carbon monoxide difference spectra of anaerobically grown cells exposed to air in the presence and absence of Δ-aminolevulinic acid. Suspensions prepared as in Fig. 6.

**Fig. 6.** Reduced minus oxidized difference spectra of anaerobically grown cells exposed to air in the presence and absence of Δ-aminolevulinic acid (ALA). These suspensions were prepared as follows: 70 ml of a suspension of anaerobically grown cells (prepared in the presence of glucose and phosphate buffer as in Fig. 5) was placed in a 500-ml Erlenmeyer flask and was incubated at 37 °C for 45 min (without shaking). Where indicated, ALA was added to the incubation mixture at a level of 10 mg per 70 ml. After 45 min, the flasks were chilled to 0 °C, and the cells were removed by centrifugation, washed in cold saline, and resuspended in 0.05 m phosphate buffer, pH 7.0. The spectra were recorded at a cell density of 4.7 mg/ml (dry weight) after addition of hydrosulfite to the sample cuvette and aeration of the reference cuvette. Cells not exposed to air were held at 0 °C and did not receive ALA.

**DISCUSSION**

Growth of this strain of *S. epidermidis* under anaerobic rather than aerobic conditions causes a marked decrease in cytochrome content and respiratory activity. However, our results indicate that the addition of hemin to the anaerobic growth medium causes a marked increase in cytochrome-like pigments and oxidative activities which appear similar to those formed during aerobic growth. For instance, cells grown anaerobically in the presence of added hemin contain
pigments which exhibit difference spectra identical to the cytochromes of the b, and a type present in aerobically grown cells. (However, no cytochrome of the a type was detected in anaerobically grown cells.) These pigments are readily reducible by endogenous substrates and are apparently associated with the particulate portion of the cell (partly sedimented at 100,000 x g for 1 hr). In addition, cells grown anaerobically in the presence of added hemin exhibit a marked increase in respiratory activity as compared with those grown anaerobically without hemin. This is indicated by a sixfold increase in Q02 of whole cells (with glucose as substrate) and a fourfold increase in the cyanide-sensitive NADH2 oxidase activity of cell-free extracts. Thus, the addition of hemin to the medium in which anaerobic growth occurs causes a marked increase in a respiratory system which has at least some of the properties of the normal respiratory system found in aerobically grown cells. The addition of hemin to aerobically grown cultures causes no increase in the high level of respiratory activity and cytochrome content of the resulting cells (10, unpublished observations).

Although this effect of hemin is obtained when hemin is present during the entire period of anaerobic growth, a similar effect was not observed when hemin was added to washed, resting suspensions of cells grown anaerobically in the absence of hemin. Under these conditions, hemin does not cause a marked increase in respiratory activity, and the hemochromogen pigments formed do not have the properties of typical cytochromes. However, a marked effect of hemin on the respiratory capacity of anaerobic cells was observed when it was added directly to the culture medium after anaerobic growth. We do not yet know why the effect of hemin can be observed under these latter conditions, but not when it is added to washed, resting suspensions. If we assume that the apoenzymes of the cytochromes, minus the heme prosthetic group, are present in cells grown anaerobically without hemin, our results would suggest that, when in the growth medium, cells are more capable of reactions required to couple hemin to preformed apoenzymes. On the other hand, this effect of hemin may require the synthesis of new apoenzyme, and this process would also be favored by keeping the cells in the growth medium. Another possibility is that components of the respiratory chain other than hemoproteins, such as vitamin K2, are missing from anaerobically grown cells (1). The addition of hemin to fully grown cultures while the cells remain in the growth medium may also cause an increased synthesis of these components. A further investigation of the effect of hemin under different conditions should yield significant information regarding the mechanism by which hemin exerts its effect.

Regarding the question of the presence or absence of the apoenzymes of the cytochromes in cells grown anaerobically without hemin, it is interesting to compare our results with resting cells with previous results obtained with a mutant strain of S. aureus, which is heme-deficient because of a genetic block in heme biosynthesis. With this organism, the addition of hemin after growth to resting suspensions grown without hemin does cause a restoration of respiratory and catalase activity (12). Chang and Lascelles (3) observed the formation of cytochrome b1 when hemin was added to cell-free preparations of particles of this mutant grown in the absence of hemin. These results would suggest that this mutant can form the apoenzymes of the cytochromes during growth in the absence of hemin. It is not yet possible to interpret our own results with anaerobically grown cells of S. epidermidis in terms of whether or not the apoenzymes of the cytochromes are formed during anaerobic growth in the absence of hemin. Perhaps the formation of the apoenzymes of the hemoproteins is dependent upon the presence of the prosthetic group, heme, during a period when protein synthesis is occurring. Recent studies with mammalian hemoglobin synthesizing systems indicate that heme does have an enhancing effect on globin formation (2, 13; C. L. Hammel and S. P. Bessman, Federation Proc. 23:317). Of course, in the case of S. epidermidis, heme may be playing a more indirect role in producing the observed increases in the respiratory system of anaerobically grown cells.

Our experiments in which resting suspensions of cells grown anaerobically without hemin were exposed to air indicate that this exposure results in a rapid, but limited, fourfold increase in respiratory capacity of whole cells, as determined with glucose as substrate. There is also a limited increase in pigments resembling cytochromes during this period of aeration. Although their exact significance must await further investigation, these results suggest that oxygen is capable of inducing some changes in the respiratory system under resting-cell conditions. One of the goals of future work will be to determine whether this effect of oxygen is primarily to allow heme synthesis, or whether oxygen plays another role independent of its proposed role in heme synthesis in inducing the formation of the components of the respiratory system.

The effect of the heme precursor Δ-aminolevulinic acid during this exposure to oxygen was of
interest, since it demonstrated that these anaerobically grown cells, although they are deficient in heme compounds, can rapidly synthesize large amounts of heme if they are exposed to oxygen and an appropriate heme precursor. The fact that this increased synthesis of heme has a slight stimulatory effect on the development of respiratory capacity may have some implications regarding the role of heme in the formation of the respiratory system. Certainly, it is clear that during the period when resting suspensions are exposed to air, the marked increase in heme formation brought about by the addition of \( \Delta \)-amino-levulinic acid is not accompanied by a marked increase in rate of development of the respiratory system. Further studies are in progress to answer some of the questions raised by these observations.

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**LITERATURE CITED**