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Respiratory Systems and Cytochromes in *Campylobacter fetus* subsp. *intestinalis*

SYDNEY HARVEY† AND JUNE LASCELLES*

Department of Microbiology, College of Letters and Science, University of California, Los Angeles, California 90024

Cell suspensions of *Campylobacter fetus* subsp. *intestinalis* grown microaerophilically in complex media consumed oxygen in the presence of formate, succinate, and DL-lactate, and membranes had the corresponding dehydrogenase activities. The cells and membranes also had ascorbate-*N,N,N',N'*-tetramethyl-*p*-phenylenediamine oxidase activity which was cyanide sensitive. The fumarate reductase activity in the membranes was inhibited by *p*-chloromercuriphenylsulfonate, and this enzyme was probably responsible for the succinate dehydrogenase activity. Cytochrome *c* was predominant in the membranes, and a major proportion of this pigment exhibited a carbon monoxide-binding spectrum. Approximately 60% of the total membrane cytochrome *c*, measured with dithionite as the reductant, was also reduced by ascorbate-*N,N,N',N'*-tetramethyl-*p*-phenylenediamine. A similar proportion of the membrane cytochrome *c* was reduced by succinate under anaerobic conditions, whereas formate reduced more than 90% of the total cytochrome under these conditions. 2-Heptyl-4-hydroxyquinoline-*N*-oxide inhibited reduction of cytochrome *c* with succinate, and the reduced spectrum of cytochrome *b* became evident. The inhibitor delayed reduction of cytochrome *c* with formate, but the final level of reduction was unaffected. We conclude that the respiratory chain includes low- and high-potential forms of cytochromes *c* and *b*; the carbon monoxide-binding form of cytochrome *c* might function as a terminal oxidase.

Various subspecies of *Campylobacter fetus* are pathogenic to humans and domestic animals; they have a respiratory type of metabolism, but as microaerophiles they only grow with oxygen at low partial pressure (15). *C. fetus* cannot grow anaerobically with fumarate or nitrate, whereas other species, for example, *Campylobacter sputorum*, acquire energy for anaerobic growth by coupling formate oxidation to the fumarate reductase system (14). The formate-fumarate reductase system has been investigated in detail in the strict anaerobe *Vibrio succinogenes* and involves low- and high-potential forms of cytochrome *b* (11-13).

Intact cells of *C. fetus* subsp. *jejuni* respire various substrates, and enzymes of the Krebs cycle have been demonstrated in extracts (6, 15). Also, cytochromes *b* and *c* have been identified spectroscopically, but the terminal oxidase system is unknown (6). Our work concerns respiratory systems in *C. fetus* subsp. *intestinalis* and was prompted by the exceptionally high concentration of cytochrome *c* found in cells grown microaerophilically. This paper describes some of the characteristics of the membrane-associated cytochromes of the organism with attention to their reduction by substrates under anaerobic conditions. The ultimate object of the work was to determine the function of the cytochromes in electron transfer from substrates to oxygen and to fumarate.

† Present address: Food and Drug Administration, Los Angeles, CA 90015.

MATERIALS AND METHODS

Organism and culture conditions. *C. fetus* subsp. *intestinalis* (Barr) was obtained from the culture collection of Virginia Polytechnic Institute and State University (Anaerobe Laboratory), Blacksburg. Stock cultures were maintained in tubes of fluid thioglycolate medium (Difco Laboratories, Detroit, Mich.) at 35°C and subcultured every 3 to 4 weeks. Inocula for large-scale cultures were grown in brucella broth (Difco) supplemented with 0.025% $FeSO_4 \cdot 7H_2O$ and dispensed in screw-cap tubes (16 by 125 mm) containing 10 ml of medium. After inoculation with 1 ml of stock culture, the tubes were incubated for 2 days at 32°C with gentle shaking; 4 ml of this culture was used to inoculate 400 ml of medium for mass cultures.

Growth and preparation of cell suspensions. The medium was brucella broth with 10 μM $FeSO_4$ (final concentration) added immediately before inoculation; stock solutions of $FeSO_4$ (0.1 M in 0.1 N H_2SO_4) were sterilized by autoclaving. The cultures were in 500-ml flasks containing 400 ml of medium; they were incubated aerobically at 32°C for 24 h with gentle shaking and then with more vigorous aeration for 18 h on a gyratory shaker at 150 rpm. This regimen

gave reproducible cultures of actively motile cells; the optical density at 540 nm was approximately 0.45, equivalent to 170 μg of cell protein per ml.

Cultures were harvested by centrifuging for 20 min at 4,000 × *g* and washed in 0.1 volume of 40 mM Tris-hydrochloride buffer, pH 7.5, saturated with argon. The cells from 400 ml of culture were finally suspended in 4 to 5 ml of the same buffer and used immediately for measurements of respiration or stored at −20°C for preparation of cell extracts.

Preparation of extracts and cell fractions. Extracts were prepared by two passages through a French pressure cell (12,000 lb/in^2) with the addition of DNase and RNase (10 μg/ml). The homogenates were centrifuged for 10 min at 12,000 × *g* to remove large debris, and the supernatant was then centrifuged for 4 h at 70,000 × *g*. The supernatant from the high-speed centrifugation was removed and designated as cytoplasmic fraction. The pellet was rinsed with 40 mM Tris-hydrochloride buffer, pH 7.5, and suspended in this buffer containing 20% (vol/vol) glycerol (2.5 ml for material derived from a 400-ml culture). This fraction, designated as membrane, was kept at 0°C under argon, and enzyme activities were measured within 24 h.

Cytochrome spectra. Cytochromes were examined spectroscopically with an Aminco DW-2a spectrophotometer in the split-beam mode with a band pass of 1 nm. The concentration of total cytochrome *c* was calculated from the dithionite-reduced minus ferricyanide-oxidized spectra (548 to 537 nm), using Σ 17.3 mM^{-1} cm^{-1} (16). Difference spectrum measurements were also made with membranes reduced with 8 mM sodium ascorbate–0.8 mM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (ascorbate-TMPD). Carbon monoxide spectra were measured with dithionite-reduced preparations in anaerobic cuvettes; the (dithionite-reduced plus CO) minus (dithionite-reduced) spectra were recorded after the attainment of maximum absorbance, usually within 3 min.

Measurement of oxygen uptake. Oxygen uptake was measured with a Clark oxygen electrode (Rank Brothers, Bottisham, Cambridge, England). The reaction chamber contained cells, equivalent to 1 to 3 mg of protein, in 1 ml of air-saturated 40 mM potassium phosphate buffer, pH 7.5, and 20 mM substrate (sodium salt). Ascorbate-TMPD oxidase activity was measured in the presence of 20 mM sodium ascorbate and 2 mM TMPD. The cells showed no oxygen uptake without substrate, or with ascorbate or TMPD alone. Activities are expressed as nanomoles of oxygen per minute per milligram of protein.

Enzyme activities. Fumarate reductase was assayed with reduced methyl viologen as the electron donor in anaerobic cuvettes in an atmosphere of argon. Each cuvette contained in a final volume of 2.5 ml: membranes (approximately 0.3 mg of protein) in 40 mM Tris-hydrochloride buffer (pH 7.5), 0.16 mM methyl viologen, and 0.8 mM sodium dithionite (freshly prepared and added after the cuvettes had been flushed with argon). The reaction was started by the addition of 4 μmol of sodium fumarate from the stopper, and the decline in absorbance at 578 nm was measured (Σ 9.7 mM^{-1} cm^{-1}). Succinate and lactate dehydrogenase activities were measured with phenazine methosulfate and 2,6-dichlorophenolindophenol as the acceptor and 20 mM sodium succinate or DL-sodium lactate as the substrate (9). The same assay system with the omission of cyanide was used to assay formate dehydrogenase. Succinate and formate dehydrogenase activities were also assayed with ferricyanide as the acceptor, as described by Kröger and Innerhofer (12). Ferric iron reductase activity was measured with ferrozine as the trap for ferrous iron and with formate or succinate as the reductant (3). Isocitrate dehydrogenase activity was measured with NADP as acceptor in 40 mM Tris-hydrochloride buffer (pH 7.5) containing 3 mM $MnCl_2$. Malate dehydrogenase was assayed with oxaloacetate and NADH. Ascorbate-TMPD oxidase activity was assayed in the membranes by measuring oxygen consumption as described above for whole cells. Enzyme activities are expressed as nanomoles of product formed or oxygen consumed per minute per milligram of protein.

Protein determination. Protein was determined by the modification of the Lowry method with bovine serum albumin as the standard (4).

Materials. Solutions of 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) were in ethanol. This compound, TMPD, and *p*-chloromercuriphenylsulfonate were obtained from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Observations with intact cells. Freshly harvested cells consumed oxygen with formate, D- and L-lactate, succinate, and ascorbate-TMPD (Table 1). Except with formate, respiration was abolished by 0.2 mM KCN. Oxygen uptake with formate was unaffected by 2 mM KCN and was only slightly inhibited by 5 mM KCN. Apparently, reductant derived from formate may not be obligatorily mediated by the terminal oxidase used with the other hydrogen donors.

The respiratory activities with succinate and lactate decayed within 24 h of storage at 0°C in air or argon, whereas oxygen consumption with

TABLE 1. *Respiratory activity of freshly harvested cells*[a]

Substrate	Respiratory activity	
	Without cyanide	With 0.2 mM cyanide
Formate	132	132
DL-Lactate	18	0
L-Lactate	10	0
D-Lactate	19	0
Succinate	11	0
Ascorbate-TMPD	78	0

[a] The reaction vessel contained cells equivalent to 1.5 to 3 mg of protein per ml; cyanide was added after a linear rate of oxygen consumption was attained (30 to 60 s). The activities are expressed as nanomoles of oxygen per minute per milligram of protein.

formate and with ascorbate-TMPD was stable for at least 48 h in cells stored under argon.

Spectroscopic examination of freshly harvested cells showed a predominance of cytochrome *c*, with an α-band maximum at 548 to 549 nm (dithionite reduced minus ferricyanide oxidized). The same maximum was shown in cells reduced with ascorbate-TMPD, but the extent of reduction was approximately 70% of that attained with dithionite.

Cytochromes in cell fractions. Crude extracts were divided by centrifugation into cytoplasmic and membrane fractions; approximately 50% of the total cytochrome *c* was found in the latter.

Examination of the membranes by difference spectroscopy showed a predominance of cytochrome *c* (α-band maximum, 548 nm), with a trailing shoulder centered at approximately 558 nm, indicating the presence of cytochrome *b* in a relatively minor amount. Figure 1 shows the dithionite-reduced spectra measured against membranes oxidized with ferricyanide (trace a) and against membranes reduced with ascorbate-TMPD (trace b). The latter spectrum is presumed to represent low-potential forms of cytochrome which are not reducible by ascorbate-TMPD. In various batches of membranes, the proportion of cytochrome *c* which was reducible by ascorbate-TMPD was approximately 60% of the total (Table 1). Difference spectrum measurements up to 650 nm gave no evidence of cytochrome *a* (data not shown).

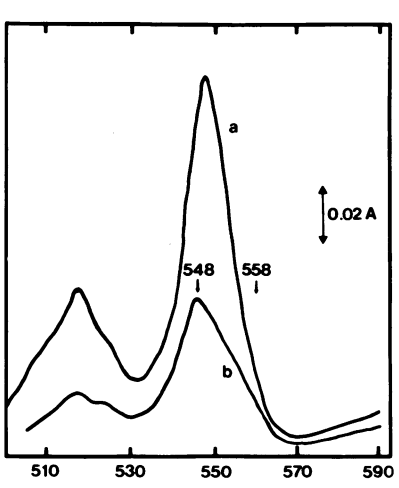

FIG. 1. *Difference spectra of membranes reduced with dithionite against ferricyanide-oxidized membranes (a) and against ascorbate-TMPD-reduced membranes (b). The protein concentration was 2 mg/ml.*

The presence of a carbon monoxide-binding form of cytochrome *c* was indicated by spectroscopy of the dithionite-reduced membranes (Fig. 2A and B). The carbon monoxide difference spectrum of the membranes had maxima at 410, 530, and 561 nm, with troughs at 432 and 548 nm. The carbon monoxide-binding form of cytochrome *c* was predominant, but the presence of minor amounts of other carbon monoxide-binding pigments could not be excluded.

The cytochrome *c* in the cytoplasmic fraction could not be distinguished spectroscopically from that in the membranes. At least 60% of the total was reduced by ascorbate-TMPD but not by ascorbate alone (Table 2). Also, the cytoplasmic cytochrome gave a carbon monoxide-binding spectrum similar to that found in the membranes.

Enzyme activities in cell fractions. Various enzyme activities were assayed in the membrane and cytoplasmic fractions (Table 2). Ascorbate-TMPD oxidase activity was found only in the membrane fraction and was completely inhibited by 0.01 mM KCN and by 0.1 mM sodium azide. Succinate, formate, and lactate dehydrogenase activities were also found only in the membrane fraction, as were fumarate reductase and ferric iron reductase with formate or succinate as the reductant. Malate dehydrogenase (NAD) and isocitrate dehydrogenase (NADP) activities were located in the cytoplasmic fraction.

Fumarate reductase and succinate dehydrogenase activities. The fumarate reductase of *V. succinogenes* is a membrane-associated flavoprotein which also has succinate dehydrogenase activity (11-13). The reductase has a high affinity for fumarate and is associated with an iron-sulfur protein which is inhibited by *p*-chloromercuriphenylsulfonate. Our observations indicate that membranes of *C. fetus* have a similar enzyme, responsible for the fumarate reductase and succinate dehydrogenase activities. The dehydrogenase activity measured with phenazine methosulfate and 2,6-dichlorophenolindophenol or with ferricyanide as the electron acceptor was strongly inhibited by fumarate. In the presence of 10 mM succinate, 60 and 86% inhibitions were observed with 1 and 2.5 mM fumarate, respectively. Succinate dehydrogenase activity with ferricyanide as the acceptor was completely inhibited by 0.5 mM *p*-chloromercuriphenylsulfonate, but no inhibition was observed with phenazine methosulfate and 2,6-dichlorophenolindophenol as the acceptor system. Fumarate reductase activity was also inhibited by the mercurial at the same concentrations. In these various respects, the enzyme ac-

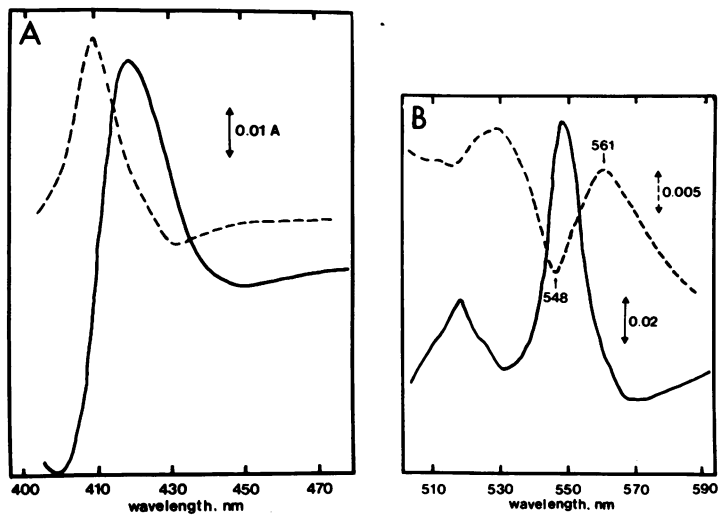

FIG. 2. *Carbon monoxide difference spectra of membranes reduced with dithionite (----) and dithionite-reduced minus air-oxidized membrane difference spectrum (——). The protein concentration was 2 mg/ml.*

TABLE 2. *Enzyme activities and cytochrome c in cell fractions*[a]

Component	Cell fraction	Protein (U/mg)[b]
Ascorbate-TMPD oxidase	Membrane	473
Fumarate reductase	Membrane	627
Formate dehydrogenase	Membrane	934
Succinate dehydrogenase	Membrane	307
Lactate dehydrogenase	Membrane	61
Formate-iron reductase	Membrane	6
Succinate-iron reductase	Membrane	7
Isocitrate dehydrogenase	Cytoplasm	215
Malate dehydrogenase	Cytoplasm	18
Cytochrome *c*, total	Membrane	3.2
	Cytoplasm	1.9
Cytochrome *c*, ascorbate-TMPD reducible	Membrane	1.9
	Cytoplasm	1.3

[a] Assays were done as described in the text, and the values are the average of at least three determinations on preparations from different batches of cells.

[b] Units of enzyme activity are expressed as nanomoles per minute; units of cytochrome are expressed as nanomoles.

tivities in *C. fetus* resembled those in *V. succinogenes*.

Reduction of membrane cytochromes by substrates. The reduction of membrane cytochromes by formate and by succinate was determined under anaerobic conditions by difference spectroscopy with air-oxidized membranes as the reference. With freshly prepared membranes, the reduced cytochrome spectrum was evident within 1 min of the addition of substrate and had reached maximum levels within 5 min. More than 90% of the total cytochrome *c* was reduced with formate as the electron donor, whereas approximately 60% of the total was reduced with succinate (data not shown). The reduction level attained with succinate was similar to that attained with ascorbate-TMPD.

The observations with substrate-reduced cytochromes together with those described above with the chemical reductant (Fig. 1) suggested that the membranes contained two forms of cytochrome *c*. One form, designated as high potential, is accessible to the reductant derived from formate and from succinate, whereas the second form is reduced only by formate. Experiments with HQNO supported the notion of multiple cytochromes. With succinate as the reductant, the development of the cytochrome *c* maximum at 548 nm was greatly diminished by HQNO, and the absorbance maximum of reduced cytochrome *b* at 558 nm became evident (Fig. 3A). With formate as the reductant, the final level of reduced cytochrome *c* was not affected by HQNO (Fig. 3B). However, the attainment of the final level was delayed for several minutes, and during this period the absorbance maximum of cytochrome *b* at 558 nm was observed (data not shown).

DISCUSSION

In Fig. 4, a scheme of electron transfer in membranes of *C. fetus* subsp. *intestinalis* under anaerobic conditions is outlined. We suggest that the membranes have low- and high-potential forms of cytochrome *c*, and possibly two forms of cytochrome *b*, which are reducible with formate as the electron donor. Succinate is able to reduce the high-potential forms of cytochromes *b* and *c*, and ascorbate-TMPD can also reduce the latter. Reduction of cytochrome *c* by succinate was prevented by HQNO, and the spectro-

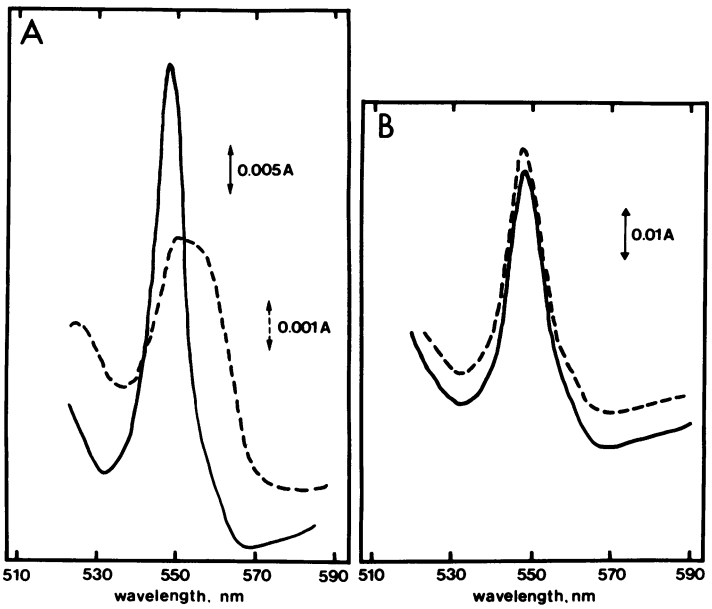

FIG. 3. *Effect of HQNO on reduction of membrane cytochromes by succinate (A) and by formate (B). Substrates were added in anaerobic cuvettes under argon, and measurements were made against air-oxidized membranes. HQNO (final concentration, 40 μM) was added 1 min before the substrate, and the spectra were recorded when no further increase in absorbance was observed, (——) Control without HQNO; (----) control with HQNO. The protein concentration was 2 mg/ml.*

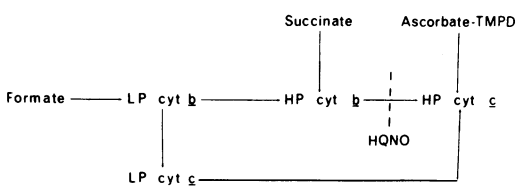

FIG. 4. *Scheme for electron transfer in membranes of C. fetus subsp. intestinalis. LP, Low-potential form of cytochrome c; HP, High potential form of cytochrome c. cyt b and cyt c, Cytochromes b and c, respectively.*

scopic observations indicated that electron transfer from reduced cytochrome *b* was inhibited. Reduction of cytochrome *c* with formate as the electron donor occurred in the presence of HQNO, indicating that the reductant from this substrate can bypass the form of cytochrome *b* involved with succinate as the reductant. Electron transfer from formate may involve low-potential forms of cytochromes *b* and *c* (Fig. 4), but the observations would also be consistent with a bypass involving cytochrome *c* only.

The membranes had high formate dehydrogenase and fumarate reductase activities, raising the question of why *C. fetus* fails to grow anaerobically in media supplemented with formate and fumarate. Growth of other species of *Campylobacter* occurs under such conditions, although at a slower rate and with a lower growth yield than with oxygen present (14, 15). Possibly, oxygen may be concerned in the biosynthesis of an essential metabolite which is unavailable to the organism even in a rich medium. Heme is one such possibility; in some bacteria and in mitochondria, the oxidation of coproporphyrinogen III to protoporphyrin occurs only in the presence of oxygen (7). Another possibility is that oxidation of cytochrome *c* by a terminal oxidase has an essential role in energy conservation in the organism.

The ascorbate-TMPD oxidase activity in the membranes and in whole cells of *C. fetus* was sensitive to low concentrations of cyanide and to azide and thus resembled that found in aerobes such as *Azotobacter* spp. (8). In addition, the microaerophile may also have a cyanide-insensitive terminal oxidase, since formate oxidation by intact cells was not inhibited by cyanide at concentrations which completely abolished respiration with other substrates (Table 1). However, the presence of an alternative oxidase needs to be established by more detailed kinetic studies of oxygen utilization. The CO-binding form of cytochrome *c* may serve as a terminal oxidase. This pigment was observed in the membranes and in the cytoplasm and apparently comprised a major proportion of the total. Soluble forms of CO-binding cytochrome *c* have

been found in many bacteria (19). In *Beneckea natriegens,* the cytochrome is located in the periplasm and is the major CO-binding pigment detectable; the high redox potential (E_0' plus 320 mV) is consistent with a terminal oxidase function (10, 17). Methylotrophic species of *Pseudomonas* and *Hyphomicrobium* also contain CO-binding forms of cytochrome *c* which mediate in electron transfer from methanol dehydrogenase to a cytochrome a-a_3 terminal oxidase (1, 5, 18). The apparent abundance of the pigment in *C. fetus* subsp. *intestinalis* warrants a detailed study of its function. In *C. sputorum,* CO-binding cytochromes have been reported, but whether the pigment was of the *b* or *c* type could not be determined from the spectroscopic data (14). (This pigment has now been identified as cytochrome *c* [H. G. D. Niekus, E. Van Doorn, W. De Vries, and A. H. Stouthamer, J. Gen. Microbiol. **118**:419–428, 1980.])

Our work does not clarify the reasons for oxygen toxicity in *Campylobacter* spp. Evidently, in an atmosphere of air at a low cell density, the terminal oxidase(s) is insufficient to maintain the concentration of dissolved oxygen below the inhibitory level. Possible loci of oxygen damage might be iron-sulfur proteins which participate in electron transfer reactions at low redox potentials and which are readily auto-oxidizable (20). One such protein might be that associated with fumarate reductase which is sensitive to *p*-chloromercuriphenylsulfonate. The auto-oxidation of iron-sulfur proteins could give rise to radicals such as peroxides or superoxide anions, which are likely to contribute to the complexities of oxygen toxicity (2, 15). The observation that iron salts and certain ferric iron chelates improve the tolerance of *C. fetus* to oxygen (2) might possibly be attributable to interactions with iron-sulfur centers, and in this context further investigation of the iron reductase system in the membranes could provide some insight.

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LITERATURE CITED

1. **Anthony, C.** 1975. The microbial metabolism of C_1 compounds. The cytochromes of *Pseudomonas* AM1. Biochem. J. **146**:289–298.
2. **Bowdre, J. H., N. R. Krieg, P. S. Hoffman, and R. M. Smibert.** 1976. Stimulatory effect of dihydroxyphenyl compounds on the aerotolerance of *Spirillum volutans* and *Campylobacter fetus* subspecies *jejuni.* Appl. Environ. Microbiol. **31**:127–133.
3. **Dailey, H. A., Jr., and J. Lascelles.** 1977. Reduction of iron and synthesis of protoheme by *Spirillum itersonii* and other organisms. J. Bacteriol. **129**:815–820.
4. **Dulley, J. R., and P. A. Greene.** 1975. A simple technique for eliminating interference by detergents in the Lowry method of protein determination. Anal. Biochem. **64**:136–141.
5. **Harder, W., and M. M. Attwood.** 1978. Biology, physiology and biochemistry of Hyphomicrobia. Adv. Microb. Physiol. **17**:303–359.
6. **Hoffman, P. S., N. R. Krieg, and R. M. Smibert.** 1979. Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni.* Physiological aspects of enhanced aerotolerance. Can. J. Microbiol. **25**:1–7.
7. **Jacobs, N. J.** 1974. Biosynthesis of heme, p. 125–148. *In* J. B. Neilands (ed.), Microbial iron metabolism. Academic Press, Inc., New York.
8. **Jurtshuk, P., Jr., T. J. Mueller, D. N. Mcquitty, and W. H. Riley.** 1978. The cytochrome oxidase reaction in *Azotobacter vinelandii* and other bacteria. FEBS Meeting (Copenhagen, 1977), vol. 49, p. 99–121. Pergamon Press, Oxford.
9. **King, T. E.** 1963. Reconstitution of respiratory chain enzyme systems. XI. Use of artificial electron acceptors in the assay of succinate dehydrogenase enzymes. J. Biol. Chem. **238**:4032–4036.
10. **Knowles, C. J., P. H. Calcott, and R. A. MacLeod.** 1974. Periplasmic CO-binding *c*-type cytochrome in a marine bacterium. FEBS Lett. **49**:78–83.
11. **Kröger, A.** 1978. Fumarate as terminal acceptor of phosphorylative electron transport. Biochim. Biophys. Acta **505**:129–145.
12. **Kröger, A., and A. Innerhofer.** 1976. The function of menaquinone, covalently bound FAD and iron-sulfur protein in the electron transport from formate to fumarate of *Vibrio succinogenes.* Eur. J. Biochem. **69**: 487–495.
13. **Kröger, A., and A. Innerhofer.** 1976. The function of the *b* cytochromes in the electron transport from formate to fumarate of *Vibrio succinogenes.* Eur. J. Biochem. **69**:497–506.
14. **Niekus, H. G. D., W. De Vries, and A. H. Stouthamer.** 1977. The effect of different dissolved oxygen tensions on growth and enzyme activities of *Campylobacter sputorum* subspecies *bubulus.* J. Gen. Microbiol. **103**:215–222.
15. **Smibert, R. M.** 1978. The genus *Campylobacter.* Annu. Rev. Microbiol. **32**:673–709.
16. **Smith, L.** 1978. Bacterial cytochromes and their spectral characterization. Methods Enzymol. **53**:202–212.
17. **Weston, J. A., and C. J. Knowles.** 1973. A soluble CO-binding *c*-type cytochrome from the marine bacterium *Beneckea natriegens.* Biochim. Biophys. Acta **305**:11–18.
18. **Widdowson, D., and C. Anthony.** 1975. The microbial metabolism of C_1 compounds. The electron transport chain of *Pseudomonas* AMI. Biochem. J. **152**:349–356.
19. **Yamanaka, T., and K. Okunuki.** 1974. Cytochromes, p. 349–400. *In* J. B. Neilands (ed.), Microbial iron metabolism. Academic Press, Inc., New York.
20. **Yoch, D. C., and R. P. Carithers.** 1979. Bacterial iron-sulfur proteins. Microbiol. Rev. **43**:384–421.