# CYTOCHROME-LINKED FERMENTATION IN *BACTEROIDES RUMINICOLA*

D. C. WHITE,[1] M. P. BRYANT, AND D. R. CALDWELL

*Rockefeller Institute, New York, New York, and Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Maryland*

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ABSTRACT

WHITE, D. C. (Rockefeller Institute, New York, N.Y.), M. P. BRYANT, AND D. R. CALDWELL. Cytochrome-linked fermentation in *Bacteroides ruminicola*. J. Bacteriol. **84**:822–828. 1962—Previous studies showed that *Bacteroides ruminicola*, an anaerobic, saccharolytic, ruminal bacterium, ferments glucose with the production of succinic, acetic, and formic acids, requires a large amount of $CO_2$, and most strains require heme for growth. Difference spectra of cell suspensions of both heme-requiring strain 23, *B. ruminicola* subsp. *ruminicola*, and heme-independent strain GA33, *B. ruminicola* subsp. *brevis*, showed the presence of a cytochrome (absorption maxima at 560 mμ, near 530 mμ, and 428 mμ) similar to cytochrome *b*. This cytochrome and flavoprotein (trough at 450 mμ) in the cells, reduced by endogenous metabolism, were oxidized on addition of air, $CO_2$, oxalacetate, malate, or fumarate but no oxidation occurred in the presence of succinate, malonate, lactate, pyruvate, aspartate, citrate, $NO_3^-$, $SO_4^=$, 2-*n*-heptyl or hydroxyquinoline-N-oxide (HOQNO), amytal or azide. The oxidation of these cellular pigments by fumarate was not inhibited by $CN^-$, CO, malonate, succinate, amytal, or HOQNO. Glucose and reduced diphosphopyridine nucleotide (DPNH), but not succinate, reduced the pigments in frozen-thawed cells previously exposed to air for 4 hr at room temperature. The results suggest that this cytochrome and flavoprotein form an electron transport system for fumarate reduction to succinate by DPNH generated by glycolysis, and that succinate is produced via $CO_2$ condensation with pyruvate or phosphoenolpyruvate and with oxalacetate, malate, and fumarate as intermediates. A pigment similar to cytochrome *o* (absorption maxima at 570, 555, and 416 mμ) was observed when reduced cells were treated with CO and compared to reduced cells, but there was no detectable cytochrome oxidase activity. The function of this pigment is obscure. No peroxidase or catalase activity was detected in either strain. Pyridine hemochromogens of both strains indicate one major heme, a protoheme-like pigment, with absorption in the α region maximum at 556 mμ. As *B. ruminicola* is one of the most numerous of rumen bacteria and ferments a wide variety of carbohydrates of importance in ruminant rations, cytochrome must be of importance in electron transport in rumen contents, a highly anaerobic environment.

*Bacteroides ruminicola* appears to be one of the most important species of ruminal bacteria, as indicated by the wide range of carbohydrates fermented and by its presence among the predominant culturable bacteria in the rumen of animals fed a variety of rations (Bryant, 1959). It ferments glucose with the production of a large amount of succinate and requires a large amount of $CO_2$ for good growth (Bryant et al., 1958). The fact that a majority of strains require hemin for growth (Bryant and Robinson, *unpublished data*) led to the study of the hemoprotein in these bacteria.

## MATERIALS AND METHODS

The strains studied included type strain 23 of *B. ruminicola* subsp. *ruminicola*, which requires hemin for growth, and type strain GA33 of *B. ruminicola* subsp. *brevis*, which does not require hemin. The methods of isolation and culture and descriptions of characteristics of the strains have been described (Bryant et al., 1958).

Media from which cells were harvested were similar to that of Bryant and Robinson (1961) except that $3 \times 10^{-6}$ M hemin, 0.3% glucose, 1.0% Trypticase (BBL), and $3.6 \times 10^{-5}$ M $FeSO_4$ were added and the other casein hydrolysate, vitamins, cellobiose, $Na_2S$, and $(NH_4)_2SO_4$ were deleted. Hemin was deleted from the medium

[1] Present address: Department of Biochemistry, University of Kentucky, School of Medicine, Lexington.

used to culture strain GA33. In culture vessels similar to that of Allison et al. (1962), 1,500 or 3,000 ml of medium were inoculated with about 0.5% (v/v) of a culture of the strain incubated for about 24 hr at 37 C in the same medium.

After 15 to 20 hr of incubation at 37 C, the cells were harvested by centrifugation and were washed twice with ½ volume of 0.05 M phosphate buffer (pH 7.0). They were then resuspended in the buffer, made 30% (v/v) with glycerol, and kept under $N_2$ at 4 C or frozen at −70 C in the broken-cell preparations.

Spectra were measured using the difference spectra methods described by Chance (1954). In this technique, the absorption spectrum of the reduced respiratory pigments is measured against a similar cell suspension containing the oxidized pigments. A Cary model 14 spectrophotometer modified to utilize a more intense light source was used. Bacteria were measured at a cell density of between 10 and 30 mg of protein per ml. At these densities, the band width is less than 20 A at 560 mμ. The 0.1 to 0.2 optical density (OD) slide wire has an inherent noise level of $\pm 7 \times 10^{-4}$ OD and the spectra were traced by drawing a line through the middle of the recording error.

The contents of cuvettes were kept anaerobic by passing a stream of prepurified $N_2$ into the gaseous phase whenever the stoppers were removed, using the anaerobic techniques for culture transfer of the bacterium. The $N_2$ was passed through a column of hot reduced copper filings to remove traces of $O_2$. Solutions added to a cuvette were equilibrated with and kept under a gaseous phase of $N_2$. When substrate solutions were added to a cuvette, a similar volume of 0.05 M phosphate buffer (pH 7.0) was added to the balancing cuvette and both transfers were done under a stream of $N_2$.

Substrate solutions were adjusted to pH 7.0 and molar concentration. Reagent grade materials were used, except for sodium oxalacetate which was a gift from Chas. Pfizer & Co., Inc. (Brooklyn, N.Y.) to S. Granick and 2-*n*-heptyl-4-hydroxyquinoline-N-oxide (HOQNO), a gift from J. W. Lightbown.

Protein determinations were made by the biuret method (Gornall, Bardawill, and David, 1949) in the presence of 0.06% sodium deoxycholate.

Pyridine hemochromogens of the bacteria were measured in 50% pyridine plus 50% 0.09 M KOH containing 0.03% sodium deoxycholate, and a few mg of $Na_2S_2O_4$ were then added and the solution was stirred by bubbling with $N_2$.

Peroxidase assays were done utilizing the guaiacol reaction of Maehly and Chance (1954). Bacterial cells were shattered by pounding, after freezing with liquid nitrogen, and used on rewarming (Moses, 1955).

Catalase was assayed by the iodometric titrations of Herbert (1955) on whole cells or cells treated with toluene as described by Clayton (1959). The conditions for the assay were those of White (1962).

## RESULTS

*Difference spectra.* Figure 1 gives some difference spectra of strain GA33, the hemin-independent strain. It contains a cytochrome with a difference spectrum similar to cytochromes of the *b* type and henceforth referred to as the *b*-like cytochrome. The *b*-like cytochrome has absorption maxima at 560 mμ, near 530 mμ, and at 428 mμ, and a flavoprotein can be detected as the

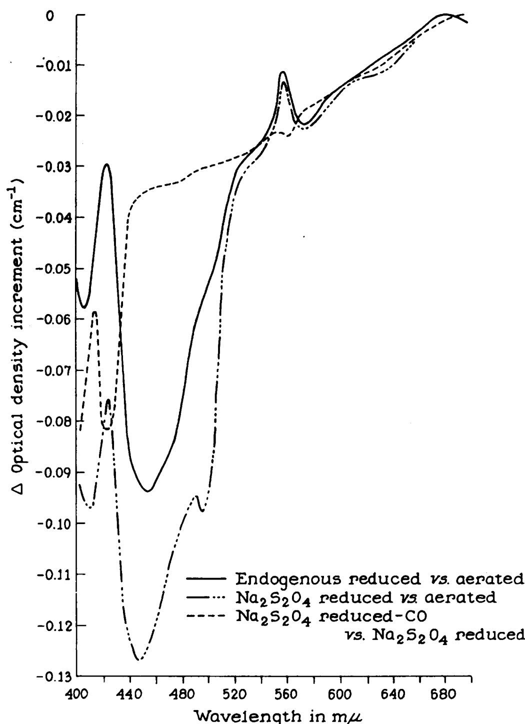


FIG. 1. *Difference spectra of a cell suspension of Bacteroides ruminicola, strain GA33, at room temperature and with cells representing 15 mg of protein per ml.*

trough at 450 mμ. Addition of dithionite to cells already reduced by their endogenous metabolism reduces more of the flavoproteins, yet does not significantly increase the *b*-like cytochrome absorption. If reduced cells are treated with CO and compared with reduced cells, the spectrum of an "oxidase" with absorption similar to that of cytochrome *o* (maxima at about 570 mμ, about 555 mμ, 416 mμ, and minima at 425 mμ) becomes evident. While the maxima at about 570 and 555 mμ were small, they were always found in cells of both strains and the soret peak at 416 mμ was more definite. The absorption maximum at 500 mμ may represent an unknown pigment first described by Lindenmeyer and Smith (1957) and since reported in other species. The conventions for naming these respiratory pigments are those of Smith (1961).

That these are indeed respiratory pigments can be demonstrated by the ability to oxidize the *b*-like cytochrome and flavoprotein with air, then reversibly reduce them by treating the same sample with $N_2$. The 500-mμ pigment likewise can be oxidized with air and then reduced by displacing the air with $N_2$. The oxidase-like pigment can be demonstrated by bubbling with CO (treated to remove $O_2$ and $CO_2$) and then by removing the CO by bubbling with $N_2$. It will lose its characteristic absorption maxima. The hemin-independent strain GA33 and hemin-requiring strain 23 have identical respiratory pigments.

If the frozen-thawed bacteria are allowed to partially equilibrate with air, glucose and reduced diphosphopyridine nucleotide (DPNH), but not succinate, can increase the level of reduction of the *b*-like cytochrome and flavoprotein. To demonstrate this, the cells were held at room temperature and exposed to air for 4 hr. Presumably, by this time they exhaust their endogenous metabolic stores. Some lysis appears to have occurred by this time, as evidenced by an increased viscosity. Similar cells kept at 4 C under $N_2$ for 4 hr maintained the pigments in the reduced state and do not increase in viscosity.

Pyridine hemochromogens of both strains of bacteria indicate one major heme, a protoheme-like pigment with maximal absorption in the $\alpha$ region at 556 mμ. The hemin-independent strain contained about $5 \times 10^{-7}$ moles of protoheme per 10 mg of bacterial protein. The hemin-requiring strain, grown in medium containing excess hemin, contains about twice this amount.

*Cytochrome oxidase activity.* The presence of cytochrome *o* (a cytochrome oxidase)-like pigment in these obligate anaerobes is puzzling and no cytochrome oxidase activity could be detected. By use of a vibrating platinum electrode similar to that described by Chance (1954) in a chamber maintained at 30 C by circulating water and stirred with a 5-mm magnetic flea, measurements of the effect of the bacteria on the $O_2$ level of the suspending buffer were made. If 0.5 ml of cell suspension (150 mg of bacterial protein) were added to 4.5 ml of 0.05 M phosphate buffer (pH 7.0), no change in the $O_2$ level (220 μmole of $O_2$ per ml) could be detected after 20 min. Addition of glucose, malate, fumarate, succinate, citrate (0.1 M final concentration), or DPNH ($2 \times 10^{-4}$ M final concentration) had no effect on the $O_2$ tension. If the buffer was saturated with $O_2$ rather than air, there was a very slow $O_2$ concentration-dependent decrease in $O_2$ tension produced by the bacteria. This is typical of the behavior of flavoprotein and not of cytochrome oxidase (Chance, 1957). Again, both strains behaved similarly.

Neither $NO_3^-$ nor $SO_4^=$ oxidized the cytochromes reduced by endogenous metabolism, and incorporation of $1 \times 10^{-3}$ M KCN, azide, or amytal into growth medium resulted in little, if any, inhibition of growth of strain 23. These findings add further weight to the probability that cytochrome oxidase-like activity is not present in *B. ruminicola*.

*Peroxidase activity.* There was no detectable peroxidase activity as measured by the optical density change at 470 mμ with guaiacol (final concentration: 0.02 M) incubated for 5 min at 25 C in 0.05 M phosphate buffer (pH 7.0) with 24 mg of bacterial protein per ml and $H_2O_2$ concentrations of between 60 and 600 μmoles. Cells of both strains were shattered at liquid nitrogen temperature before addition to the guaiacol solution.

*Catalase activity.* Using the iodometric titration of Herbert (1955) on whole cells suspended in 0.01 M phosphate buffer (pH 6.8), *Katalase-fahigkeit* (protein) values of 0.01 and 0.20 were measured for strains 23 and GA33, respectively. Identical values were obtained with cells treated with toluene as described by Clayton (1959). Toluene would destroy any permeability barrier to $H_2O_2$. This low level of catalase activity probably represents nonspecific catalase activity of the hemoproteins of the cell.

*Cytochrome-linked fermentation.* Figure 2 shows

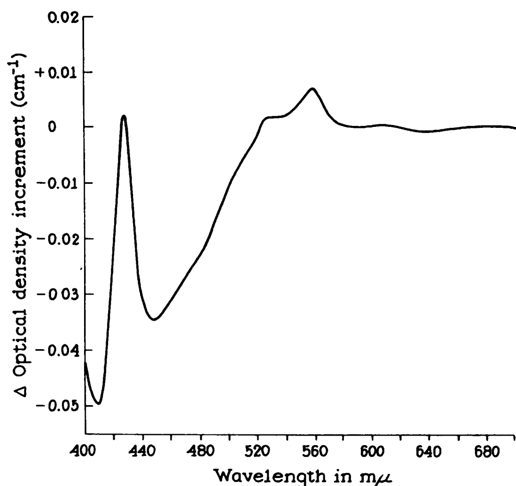


FIG. 2. *Difference spectrum of a cell suspension of Bacteroides ruminicola, strain 23 (15 mg protein per ml), oxidized by malate (0.05 M final concentration) compared with cells reduced by endogenous metabolism.*

the difference spectrum produced by adding malate anaerobically to cells reduced by their endogenous metabolism. The *b*-like cytochrome maxima and flavoprotein trough can be seen. The changes in OD measured at the $\alpha$ maximum (the difference between OD at 560 m$\mu$ and at 580 m$\mu$) and at the $\gamma$ maximum (the difference between OD at 428 m$\mu$ and 455 m$\mu$) were almost as large as those obtained by reducing cells in one cuvette with $Na_2S_2O_4$ and shaking the cells in the other cuvette vigorously in air. In this experiment, the cells were added as anaerobically as possible to stoppered cuvettes, and a base line was run to be sure that no oxidation had occurred. Substrate (0.1 ml of a 1 M solution) was then added anaerobically to one cuvette and 0.1 ml of buffer was added anaerobically to the other. Adding buffer to each cuvette as described produced no detectable change in OD (less than 0.0008 OD units). If a cuvette with 2 ml of cell suspension and with 1 ml of air as the gas phase was tipped as was done to mix the substrates, no detectable oxidation occurred in the 560-m$\mu$ region and a change of less than 0.004 OD was detected at 428 m$\mu$.

Data in Table 1 show the effect of certain substrates and inhibitors of oxidation of the reduced *b*-like cytochrome; $CO_2$, oxalacetate, malate, and fumarate oxidized the reduced flavoprotein and

TABLE 1. *Oxidation of endogenously reduced pigments in cell suspensions of Bacteroides ruminicola by addition of substrates**

| Substrate | Strain | $\alpha$ Δ OD (560 to 580 m$\mu$) | $\gamma$ Δ OD (428 to 455 m$\mu$) |
|---|---|---|---|
| Fumarate | 23 | 0.007 | 0.037 |
| | GA33 | 0.009 | 0.049 |
| Malate | 23 | 0.007 | 0.036 |
| | GA33 | 0.006 | 0.033 |
| Oxalacetate | 23 | 0.009 | 0.045 |
| $HCO_3^-$, $CO_2$ | 23 | 0.004 | 0.034 |
| | GA33 | 0.005 | 0.039 |
| CO + fumarate | GA33 | 0.004 | 0.025 |
| Fumarate + KCN | 23 | 0.004 | 0.035 |
| Succinate | 23 | <0.0008 | 0.0008 |
| Succinate + fumarate | 23 | 0.007 | 0.032 |
| Malonate | 23 | <0.0008 | 0.0008 |
| Malonate + fumarate | 23 | 0.007 | 0.057 |
| HOQNO | GA33 | <0.0008 | 0.0008 |
| HOQNO + fumarate | GA33 | 0.006 | 0.039 |
| Amytal | GA33 | <0.0008 | 0.0008 |
| Amytal + fumarate | GA33 | 0.004 | 0.037 |

* The change in OD measured at the $\alpha$ maximum of cytochrome *b* (560 m$\mu$) and between the $\gamma$ maximum (428 m$\mu$) and trough of flavoprotein (455 m$\mu$). This represents the change produced in 15 mg of bacterial protein after anaerobic addition of substrate to one cuvette and a similar amount of phosphate buffer to the other, as described in Materials and Methods. In each case, a base line of reduced cells vs. reduced cells was run to establish that the pigments were not oxidized during addition of the cell suspension to the cuvettes. The reactions were complete within 5 min. All substrates were at a final concentration of 0.05 M except for HOQNO (2-*n*-heptyl-4-hydroxyquinoline-N-oxide) which was at $3 \times 10^{-5}$ M and amytal at $3 \times 10^{-3}$ M, and $HCO_3^-$ and $CO_2$ indicates the addition of 0.05 M final concentration of $NaHCO_3$ and saturation of the suspension with $CO_2$.

cytochrome in the bacterial suspensions. Compounds that failed to produce oxidation of these pigments included succinate, malonate, HOQNO, amytal, lactate, pyruvate, aspartate, citrate,

$NO_3^-$, $SO_4^=$, and azide. The fumarate oxidation of the pigments was not inhibited by $CN^-$, CO, malonate, succinate, amytal, or HOQNO. In some bacteria, HOQNO inhibits oxidation of cytochrome *b* (Lightbown and Jackson, 1956).

## DISCUSSION

Anaerobic bacteria have been known for some time to contain cytochrome pigments (reviewed by Newton and Kamen, 1961); however, strictly fermentative, saccharolytic, obligate anaerobes were not known to contain these pigments. This might very well be due to the fact that very little effort has been made to look for cytochromes in fermentative anaerobes other than in sporeformers of the genus *Clostridium*. Chaix and Fromageot (1942) showed that *Propionibacterium pentosaceum* contains cytochromes, including a cytochrome *b*, but this organism produces catalase, shows considerable oxygen uptake during catabolism of various substrates, and shows only a tendency toward anaerobiosis in growth.

The fact that *B. ruminicola* subsp. *ruminicola*, a saccharolytic, nonsporeforming anaerobe functioning in the rumen fermentation, requires hemin for growth led us to look for functional hemoproteins in the cells. In this bacterium and in *B. ruminicola* subsp. *brevis*, the subspecies not requiring exogenous hemin for growth, two cytochromes were detected having the characteristic absorption maxima of cytochrome *b* and one similar to cytochrome *o*.

Cytochrome *o* behaves as an oxidase in a number of bacterial species (Castor and Chance, 1959). In *Hemophilus* species, it reacts with $O_2$ and $NO_3^-$ (White and Smith, 1962). The function of the similar pigment in this obligate anaerobe is obscure, since no cytochrome oxidase activity could be detected and growth occurs readily in fairly high concentrations of $CN^-$, azide, and amytal. Growth of *B. ruminicola* requires an $E_h$ at which resorufin, the pink reduction product of resazurin added to media, is reduced to colorless. At pH 6.9, the $E_h$ of resorufin is $-0.042$ v (Twigg, 1945). The cytochrome *o*-like pigment has not been detected by means other than its CO complex, and CO does not interfere with activity of the *b*-like cytochrome. At present we can assign no functional activity to this pigment.

On the other hand, the *b*-like cytochrome and flavoprotein are reversibly oxidized and reduced in such a manner as to suggest that they are involved in an electron transport system which couples DPNH oxidation with fumarate reduction. Addition of DPNH to cell suspensions resulted in reduction of these pigments, while addition of fumarate resulted in their oxidation.

The facts that $CO_2$, oxalacetate, and malate also oxidize the *b*-like cytochrome and flavoprotein of *B. ruminicola* seem best explained on the basis of the probable mechanisms involved in its glucose fermentation. *B. ruminicola* requires a large amount of $CO_2$ and produces mainly succinic, acetic, and formic acids during growth in media containing glucose (Bryant et al., 1958). In studies on *Cytophaga succinicans*, which requires substrate amounts of $CO_2$ for fermentation of glucose and produces proportions of succinic, acetic, and formic acids similar to those of *B. ruminicola*, Anderson and Ordal (1961*a*, *b*) concluded that $CO_2$ was essential because it provided, through condensation with phosphoenolpyruvate, oxalacetic acid which is reduced to succinic acid using available hydrogen generated in glucose degradation. They suggested that *B. ruminicola* and other ruminal anaerobes producing similar fermentation products carry out a similar $CO_2$-dependent fermentation of glucose. The present results (that addition of $CO_2$, oxalacetate, malate, and fumarate to cells in which the *b*-like cytochrome and flavoprotein are reduced by endogenous metabolism results in oxidation of the pigments) further suggest that *B. ruminicola* and *C. succinicans* ferment glucose via similar mechanisms. It seems probable that $CO_2$, oxalacetate, and malate are precursors of fumarate, which accepts electrons from the flavoprotein *b*-like cytochrome system. It seems probable that the cytochrome and flavoprotein would not be involved in reduction of oxalacetate by DPNH.

Anderson and Ordal (1961*b*) suggested that 3 moles of adenosine triphosphate (ATP) could be generated per mole of glucose fermented by *C. succinicans* when only substrate-linked phosphorylations were considered. It is possible that electron transport between DPNH and fumarate, involving flavoprotein and the *b*-like cytochrome, is coupled with a high-energy phosphate generating system, and this could result in production of an additional mole of ATP per mole of glucose fermented. Elsden (*see* Gunsalus and Shuster, 1961) considered this as a possibility in *Propionibacterium* and *Veillonella* fermentations.

*B. ruminicola* appears to be one of the most im-

portant species of ruminal bacteria (Bryant et al., 1958); therefore, cytochrome-linked electron transport must be of importance in the highly anaerobic ruminal environment. It would be of interest to determine whether the many other species of predominant anaerobic ruminal bacteria, which produce fermentation products similar to *B. ruminicola,* also contain a cytochrome that is involved in fumarate reduction. These species include *Bacteroides succinogenes, B. amylophilus, Borrelia* sp., *Succinimonas amylolytica, Succinivibrio dextrinosolvens,* and *Ruminococcus flavefaciens* (*see* Bryant, 1959, for references). Wolin et al. (1960) isolated, via enrichment cultures of rumen contents, an anaerobic vibrio that contains a cytochrome *c* and a cytochrome *b* which are oxidized by malate, fumarate, or nitrate (Jacobs and Wolin, 1961). However, this organism does not ferment carbohydrates, and its functional significance in the rumen is not known.

The results suggest that *B. ruminicola* may have a fumaric reductase (succinic dehydrogenase) similar to that of the anaerobe *Veillonella alcalescens* (*Micrococcus lactilyticus*) and different from that of certain aerobically grown bacteria and mitochondria, because succinate and malonate did not inhibit oxidation of the pigments by fumarate and succinate did not reduce the pigments. Peck, Smith, and Gest (1957) showed that the fumaric reductase in cell-free extracts of *V. alcalescens* catalyzed the reduction of fumarate to succinate far faster than the reverse reaction, and fumarate reduction was not inhibited by succinate and was only slightly inhibited by malonate. It should be emphasized that the present study involved washed cells of *B. ruminicola,* and studies of its fumaric reductase using cell-free extracts might yield different results. It is of interest that studies of the purified fumaric reductase of *V. alcalescens* indicate that it is a flavoprotein and it does not contain heme (Warringa and Giuditta, 1958).

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## LITERATURE CITED

ALLISON, M. J., M. P. BRYANT, I. KATZ, AND M. KEENEY. 1962. Studies on the metabolic function of branched-chain volatile fatty acids, growth factors for ruminococci. II. Biosynthesis of higher branched-chain fatty acids and aldehydes. J. Bacteriol. **83**:1084–1093.

ANDERSON, R. L., AND E. J. ORDAL. 1961*a*. *Cytophaga succinicans* sp. n., a facultatively anaerobic, aquatic myxobacterium. J. Bacteriol. **81**:130–138.

ANDERSON, R. L., AND E. J. ORDAL. 1961*b*. $CO_2$-dependent fermentation of glucose by *Cytophaga succinicans*. J. Bacteriol. **81**:139–146.

BRYANT, M. P. 1959. Bacteral species of the rumen. Bacteriol. Rev. **23**:125–153.

BRYANT, M. P., AND I. M. ROBINSON. 1961. Some nutritional requirements of the genus *Ruminococcus*. Appl. Microbiol. **9**:91–95.

BRYANT, M. P., N. SMALL, C. BOUMA, AND H. CHU. 1958. *Bacteroides ruminicola,* sp. nov. and *Succinimonas amylolytica,* gen. nov.—species of succinic acid-producing anaerobic bacteria of the bovine rumen. J. Bacteriol. **76**:15–23.

CASTOR, L. N., AND B. CHANCE. 1959. Photochemical determinations of the oxidases of bacteria. J. Biol. Chem. **234**:1587–1592.

CHAIX, P., AND C. FROMAGEOT. 1942. Les cytochromes de *Propionibacterium pentosaceum*. Bull. soc. chim. biol. **24**:1125–1127.

CHANCE, B. 1954. Spectrophotometry of intracellular respiratory pigments. Science **120**: 767–776.

CHANCE, B. 1957. Cellular oxygen requirements. Federation Proc. **16**:671–680.

CLAYTON, R. K. 1959. Permeability barriers and the assay of catalase in intact cells. Biochim. et Biophys. Acta **36**:35–39.

GORNALL, A. G., C. J. BARDAWILL, AND M. M. DAVID. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177**:751–766.

GUNSALUS, I. C., AND C. W. SHUSTER. 1961. Energy-yielding metabolism in bacteria, p. 1–58. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. II: Metabolism. Academic Press, Inc., New York.

HERBERT, D. 1955. Catalase from bacteria (*Micrococcus lysodeikticus*), p. 784–788. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. II. Academic Press, Inc., New York.

JACOBS, N. J., AND M. J. WOLIN. 1961. The cytochrome content of an anaerobic vibrio. Bacteriol. Proc., p. 166.

LIGHTBOWN, J. W., AND F. W. JACKSON. 1956. Inhibition of cytochrome systems of heart muscle and certain bacteria by the antagonists of dihydro-streptomycin 2-alkyl-4-

hydroxy quinoline N-oxide. Biochem. J. **63**: 130–137.

LINDENMEYER, A., AND L. SMITH. 1957. Some oxidative enzymes of anaerobically grown yeast. Federation Proc. **16**:212.

MAEHLY, A. C., AND B. CHANCE. 1954. The assay of catalases and peroxidases, p. 357–424. *In* D. Glick [ed.], Methods of biochemical analysis, vol. I. Interscience Press, New York.

MOSES, V. 1955. Tricarboxylic acid cycle reactions in the fungus *Zygorrhynchus moelleri*. J. Gen. Microbiol. **13**:235–251.

NEWTON, J. W., AND M. D. KAMEN. 1961. Cytochrome systems in anaerobic electron transport, p. 397–423. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. II: Metabolism. Academic Press, Inc., New York.

PECK, H. D., JR., H. O. SMITH, AND H. GEST. 1957. Comparative biochemistry of the biological reduction of fumaric acid. Biochim. et Biophys. Acta **25**:142–147.

SMITH, L. 1961. Cytochrome systems in aerobic electron transport, p. 365–396. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. II: Metabolism. Academic Press, Inc., New York.

TWIGG, R. S. 1945. Oxidation-reduction aspects of resazurin. Nature **155**:401–402.

WARRINGA, M. G., AND GIUDITTA, A. 1958. Studies on succinic dehydrogenase. IX. Characterization of the enzyme from *Micrococcus lactilyticus*. J. Biol. Chem. **230**:111–123.

WHITE, D. C. 1962. Cytochrome and catalase patterns during the growth of *Haemophilus parainfluenzae*. J. Bacteriol. **83**:851–859.

WHITE, D. C., AND L. SMITH. 1962. Hematin enzymes of *Haemophilus parainfluenzae*. J. Biol. Chem. **237**:1332–1336.

WOLIN, M. J., E. A. WOLIN, N. JACOBS, AND G. WEINBERG. 1960. A new cytochrome-producing anaerobic vibrio. Bacteriol. Proc., p. 77.