JOURNAL OF BACTERIOLOGY, Mar., 1966
Vol. 91, No. 3

Formation of a Functional Electron Transport System During Growth of Penicillin-Induced Spheroplasts of *Haemophilus parainfluenzae*

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Received for publication 27 September 1965

ABSTRACT

WRIGHT, ELIZABETH A. (University of Kentucky College of Medicine, Lexington), AND DAVID C. WHITE. Formation of a functional electron transport system during growth of penicillin-induced spheroplasts of *Haemophilus parainfluenzae*. J. Bacteriol. **91**:1356–1362. 1966.—Penicillin in a lactose medium can be used to cause the formation of spheroplasts in *Haemophilus parainfluenzae*. The resulting spheroplasts grew under conditions which produced rapid formation of the electron transport system in the normal bacteria. The following elements that are incorporated into a functionally active electron transport system were formed in spheroplasts: formate and L-lactate dehydrogenases, 2-demethyl vitamin K_2, cytochromes b_1 and c_1, and the cytochrome oxidases. The catabolic enzymes aldolase, glyceraldehyde-3-phosphate dehydrogenase, and malic dehydrogenase showed slight increases in activity. These experiments indicated that spheroplasts can form a fully functional electron transport system essentially similar to that formed during normal growth. The various components of the electron transport system were formed at different rates in the growing spheroplasts.

Penicillin blocks the incorporation of precursors of the structural elements of the bacterial cell wall. Spheroplasts of *Haemophilus parainfluenzae* can be formed by cultivation of the organism in a medium containing penicillin and osmotically stabilized with lactose.

Weibull (17) indicated that the locus of the respiratory enzymes of *Bacillus megaterium* was the cell membrane by showing that difference spectra of the protoplast membranes before and after reduction with hydrosulfite exhibited the same maxima of 530, 558, and 600 mμ as in the spectra of the intact cells. Succinic, DL-lactic, and α-ketoglutaric dehydrogenases were also found in protoplast membrane fractions of *B. magaterium* by Storck and Wachsman (13). In *Staphylococcus aureus*, Mitchell and Moyle (7) found 90% of the cytochrome system, the acid phosphatase, and succinic and lactic dehydrogenase activities to be associated with a "small particle" fraction believed to be derived from the protoplast membrane. Similarly, the electron transport system of *H. parainfluenzae* is localized in the membrane-containing fraction of ruptured bacteria (25).

The present study is directed toward demonstration of the changes in the composition and function of membrane-bound elements under growth conditions which produce extensive formation of various parts of the electron transport system and in which cell wall polymerization and cell division are inhibited by penicillin.

MATERIALS AND METHODS

Growth of bacteria. The strain of *H. parainfluenzae*, the harvesting procedures, the conditions for the preservation of the bacteria, the methods of determining freedom from contamination with other bacteria, and the methods of determining viable-cell counts have been described (18). Two strains of *H. parainfluenzae*, one a mutant of the other, were used in this study. The parental type forms large amounts of cytochrome c_1 during growth, whereas the mutant forms detectable cytochrome c_1 only under special conditions of growth. The predominant cytochrome of the mutant is cytochrome b_1 (25).

The medium used in this study consisted of 4% (w/v) Proteose Peptone, 1.0% yeast extract (Difco), 204 mM NaCl, 18 mM KNO_3, 0.23 mM $Na_2S_2O_4$, and 40 mM tris(hydroxymethyl)aminomethane (Tris), *p*H 7.6. The medium was boiled and filtered, and 750 ml was added to 2.5-liter low-form Erlen-

meyer flasks. The medium was autoclaved for 1 hr at 188 C. After cooling, 750 ml of 0.56 M lactose (autoclaved separately) was added along with filter-sterilized nicotinamide adenine dinucleotide (NAD) to a final concentration of 1.5 μM. The flasks were then incubated at 37 C with rotary shaking as described (20) until the turbidity reached about 0.01. At this turbidity (10^8 viable cells per milliliter), penicillin was added to one flask, and both flasks were incubated without agitation for 16 to 18 hr. Periodically, 200-ml samples were withdrawn aseptically for analysis of turbidity, cytochromes, 2-demethyl vitamin K_2, dehydrogenases, and oxygen utilization. Spheroplasts after centrifugation were made to 10 ml with 50 mM phosphate buffer (*p*H 7.6) containing 0.28 M lactose. This resulted in rupture of the spheroplasts, and they were not washed further with buffer. The control samples were treated similarly.

Turbidity. The turbidity of the bacteria in the growth medium was measured at 750 mμ in a Bausch & Lomb Spectronic-20 colorimeter with 13-mm test tubes. A linear relationship between turbidity measured in this manner and dry weight for these bacteria holds (23*a*) between absorbancy of 0.1 to 1.0 (0.5 to 5.0 mg/ml).

Dry weight. In these experiments the dry weight was determined directly by use of the technique described previously (23*a*).

Catabolic enzymes. The bacterial suspensions were subjected to sonic vibration for 2 min (21), and the cytoplasmic enzymes were separated by differential centrifugation (25). Glyceraldehyde-3-phosphate dehydrogenase was assayed as described by Velick (15); aldolase was assayed as described by Sibley and Lehninger (9); malic dehydrogenase was assayed as described by Ochoa (8).

Oxygen utilization. Oxygen utilization by the membranes or intact bacteria was measured by use of the Clark oxygen electrode (19).

Primary flavoprotein dehydrogenases. The primary respiratory dehydrogenases were measured spectrophotometrically (21).

Respiratory quinone. The quinone, 2-demethyl vitamin K_2 (DMK_2) has been isolated from *H. parainfluenzae* (6). It was extracted and assayed as described by White (22).

Cytochromes. Cytochromes were assayed by comparing the spectra of the reduced pigments with a similar suspension of bacteria in which the respiratory pigments were oxidized (18, 23). Cytochromes were reduced in the presence of 10 mM formate and 5 mM reduced nicotinamide adenine dinucleotide ($NADH_2$) and were measured after 3 to 5 min. After this interval, the cuvette containing the substrates has been made anaerobic by bacterial metabolism as measured with the oxygen electrode. The combination of these two substrates produces the maximal level of enzymatically reducible cytochrome with both the mutant and parental types (25). The complete reduction of cytochrome c_1 occurs on adding 2 mg of $Na_2S_2O_4$ to the cuvette. This measures the additional cytochrome c_1 that is not reducible enzymatically and exists in the cytoplasm apart from the membrane-bound electron transport system (11).

RESULTS

Action of penicillin on H. parainfluenzae. Growth of *H. parainfluenzae* in the proteose-peptone medium was completely inhibited by the addition of 0.1 unit (0.063 μg) of potassium penicillin G per ml. The turbidity of a log-phase culture was rapidly reduced after the addition of penicillin. At a concentration of 20 units per ml, the addition of penicillin to a rapidly growing population caused a constant 10-fold decrease in viable count per 55 min over six orders of magnitude at 37 C. This decrease in viable count in the presence of penicillin was completely reversible if penicillinase (100 μg/ml) was included in the culture medium. Various cell-free preparations of *H. parainfluenzae* were tested for the presence of β-galactosidase by the methods of Hu, Wolfe, and Reithel (3), and no enzymatic activity could be detected. Lactose does not stimulate respiration or growth of these bacteria. Consequently, lactose was tried as an osmotic stabilizer of penicillin-induced spheroplasts. When 0.28 M lactose was included in the medium and 200 units of penicillin per ml was added when the turbidity reached 0.1, spheroplasts were formed. A comparison of spheroplasts formed with penicillin in lactose-stabilized media and bacteria grown in the same media without penicillin is shown in Fig. 1. The narrowest diameter of the bacteria was increased fivefold by the formation of spheroplasts. These spheroplasts are easily ruptured by dilution in phosphate buffer that does not contain lactose. On rupture, deoxyribonucleic acid (DNA), detected by its deoxyribonuclease-sensitive transforming capacity for streptomycin resistance, was liberated (24). The spheroplasts were centrifuged at 0 C in the growth medium without rupture. However, we were unable to detect the accumulation of nucleotide-linked *N*-acetyl amino sugar compounds in penicillin spheroplasts by the method of Strominger (14).

When *H. parainfluenzae* was incubated in the presence of penicillin in lactose-stabilized media, the spheroplasts formed in 1 to 2 hr and then grew. This is illustrated with the mutant type in Fig. 2. During the incubation period shown in Fig. 2, the dry weight of the control culture without penicillin increased from 1.38 to 16.7 mg/ml. In this same period, the dry weight of the penicillin spheroplasts increased from 1.03 to 4.97 mg/ml by actual measurement of the dry weight. After 4 hr of incubation in the presence of penicillin, there was less than one rod

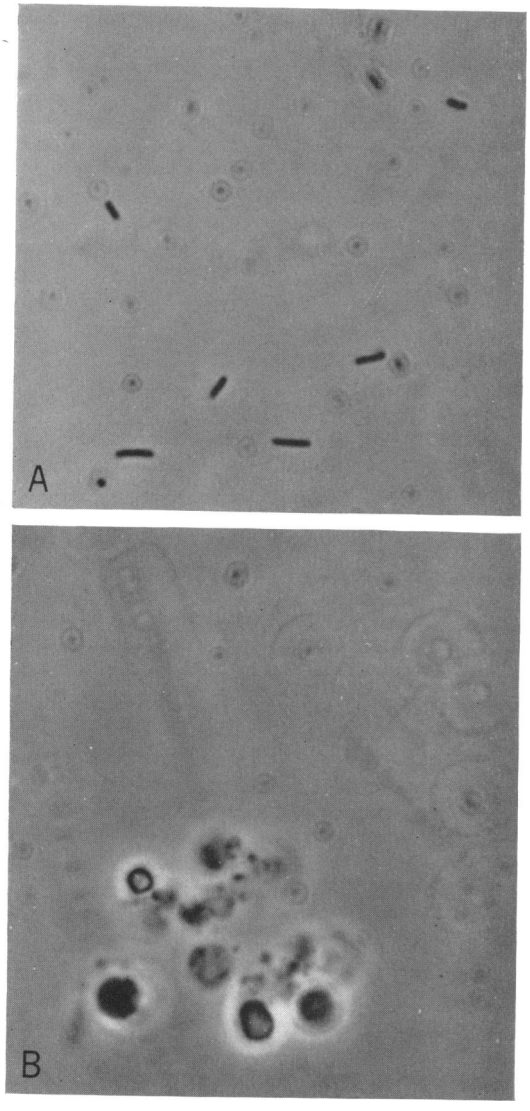

FIG. 1. *(A) Phase-contrast photomicrograph of Haemophilus parainfluenzae grown in Proteose Peptone-lactose medium for 14 hr. × 2,050. (B) Phase-contrast photomicrograph of H. parainfluenzae grown in Proteose Peptone-lactose medium containing 200 units of penicillin per ml for 14 hr. × 2,050.*

per 500 spheroplasts as counted in the phase-contrast microscope. The culture conditions chosen were those which cause the most remarkable changes in the membrane-bound electron transport system (18). The flasks were agitated to a turbidity near 0.1, the shaking was stopped, the penicillin was added to one flask, and then both flasks were incubated without agitation.

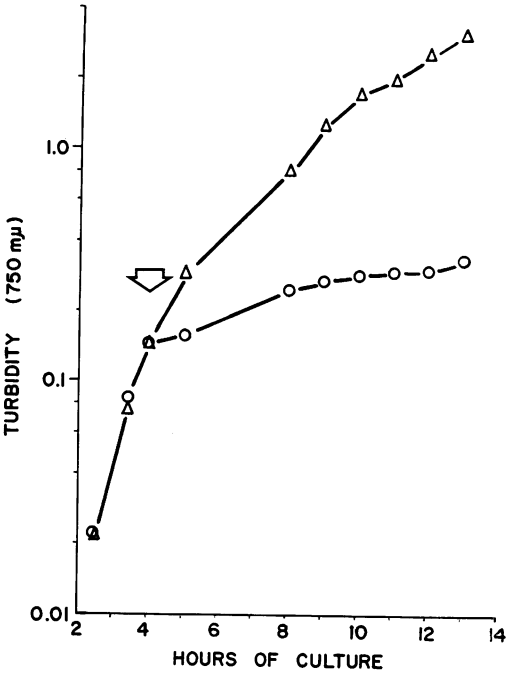

FIG. 2. *Change in turbidity during the growth of penicillin-induced spheroplasts of Haemophilus parainfluenzae (mutant type). Turbidity was measured at 750 mμ in the presence of 200 units of penicillin per ml (○) and compared with the control without penicillin (△). A similar growth pattern was obtained for the parental type of H. parainfluenzae.*

Both mutant and parental types behaved similarly.

Cytoplasmic catabolic enzyme systems. Enzymes involved in the degradation of carbohydrates in *H. parainfluenzae* were probably localized in the bacterial cytoplasm or may have been dissociated from the membrane (23*a*). Both the bacteria and spheroplasts were subjected to sonic vibration, centrifuged, and the supernatant portion was assayed. Control and spheroplast preparations of the mutant and parental type showed similar increases in activity. Aldolase activity measured as fructose 1,6 diphosphate hydrolysis, glyceraldehyde-3-phosphate dehydrogenase activity measured as $NADH_2$ formation, and malic dehydrogenase activity measured as $NADH_2$ disappearance all increased about twofold during the 6 to 8 hr of incubation.

Primary membrane-bound dehydrogenase. These dehydrogenases are the rate-limiting reactions in the membrane-bound electron transport system and can be synthesized differentially (21). During the growth conditions illustrated in Fig. 2, the

intact bacteria increased the L-lactate dehydrogenase activity 7-fold and the formate dehydrogenase activity 10-fold. The penicillin spheroplasts produced a twofold increase in the activity of the L-lactate dehydrogenase and a threefold increase in formate dehydrogenase activity during this same time span.

DMK_2 formation. The quinone found in *Haemophilus*, i.e., DMK_2, functioned as a member of the electron transport system (22). Under growth conditions where both the mutant and parental types increased the DMK_2 content 8- to 12-fold in intact cells, the mutant forming predominantly cytochrome b_1 increased the DMK_2 content 3-fold and the parental type 8-fold in the presence of penicillin (Fig. 3A).

Cytochrome formation. The growth conditions of the experiments illustrated in Fig. 2 were those which provoked a great synthesis of cytochrome (18). This synthesis resulted in the formation of an electron transport system which could support oxidation at the maximal rate at very low oxygen concentrations (20). The mutant of *H. parainfluenzae* which did not synthesize the large amounts of cytochrome c_1 allowed the examination of the effect of penicillin on cytochrome b_1 formation. The intact mutant cells increased the cytochrome b_1 concentration eightfold. Penicillin spheroplasts of this mutant increased the cytochrome b_1 content threefold. In this mutant strain, the formation of DMK_2 and cytochrome b_1 was coordinate as shown previously (23). The parental type under similar conditions formed an 18-fold increase in cytochrome c_1, of which all but about 5% was enzymatically reducible. In the same time period, a sevenfold increase in cytochrome c_1, of which 95% was enzymatically reducible, occurred in the penicillin spheroplasts of the parental type. These data are illustrated in Fig. 3B. The nonenzymatically reducible cytochrome c_1 was not membrane-bound (11). It accumulated during the stationary-growth phase and had no apparent function (18). As far as can be determined, the nonenzymatically reducible cytochrome c_1 and the c_1 that could be removed from the membrane were identical (25).

Oxygen utilization capacity. Figure 3C displays data showing the increase in respiratory activity stimulated on addition of formate to the intact bacteria or spheroplast membranes. There was essentially no endogenous respiratory activity with either the parental or mutant types when prepared as described in Materials and Methods. During the incubation period, the oxygen utilization capacity of the bacteria tested with the addition of formate increased about 11- to 25-fold

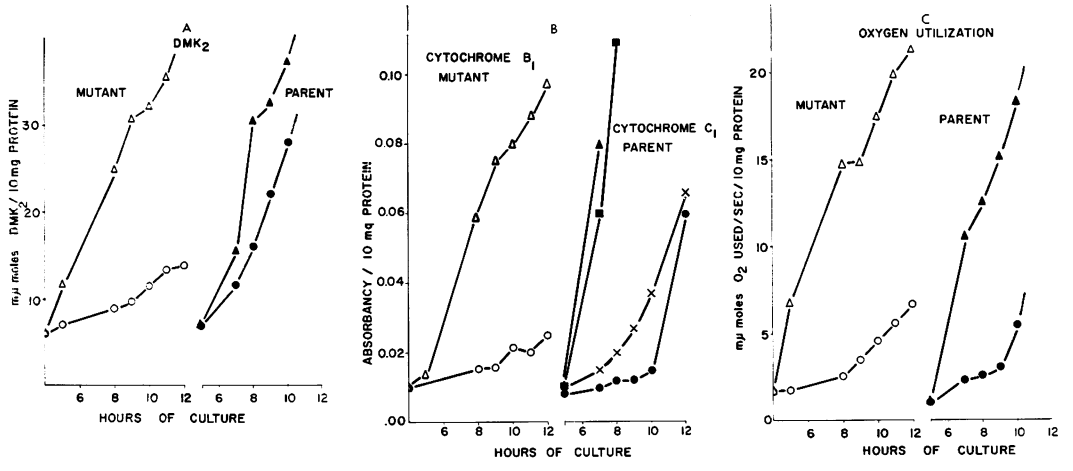

FIG. 3. *Changes in the DMK_2, cytochrome, and oxygen utilization capacity during the growth of penicillin-induced spheroplasts of Haemophilus parainfluenzae. (A) Left: formation of DMK_2 per 10 mg of protein in the mutant type incubated with (○) or without (△) penicillin. Right: formation of DMK_2 in the parental type incubated in the presence (●) or absence (▲) of penicillin. (B) Left: formation of cytochrome b_1 per 10 mg of protein in the mutant type incubated with (○) or without (△) penicillin. Right: formation of total cytochrome c_1 in the parental type incubated with (×) or without (▲) penicillin, or formation of formate plus $NADH_2$-reducible cytochrome c_1 in the parental type incubated with (●) or without (■) penicillin. (C) Left: change in the oxygen utilization in the presence of 10 mM formate expressed as millimicromoles of oxygen per second per 10 mg of protein in the mutant type incubated in the presence (○) or absence (△) of penicillin. Right: change in oxygen utilization in the presence of formate for the parental type incubated in the presence (●) or absence (▲) of penicillin.*

in both the mutant and parental types. Similarly, during the same incubation period, the oxygen utilization capacity that was observed on the addition of formate increased about ninefold in the parental type and about threefold in the mutant.

Cytochrome oxidases. The presence of cytochrome oxidases was established with certainty by examination of sufficiently large preparations of spheroplast membranes to detect the cytochrome oxidases spectrophotometrically. Figure 4 illustrates difference spectra of spheroplasts of the parental-type *H. parainfluenzae* grown in penicillin-containing media. In the difference spectra illustrated in Fig. 4, the maxima of reduced and reduced-carbon monoxide complexes of cytochrome oxidases a_1, a_2, and *o* are indicated. In experiments where the cytochrome oxidases were assayed by difference spectra at the beginning and end of a 6-hr incubation period similar to that illustrated by Fig. 2, the proportions of cytochrome oxidases a_1, a_2, and *o* were increased

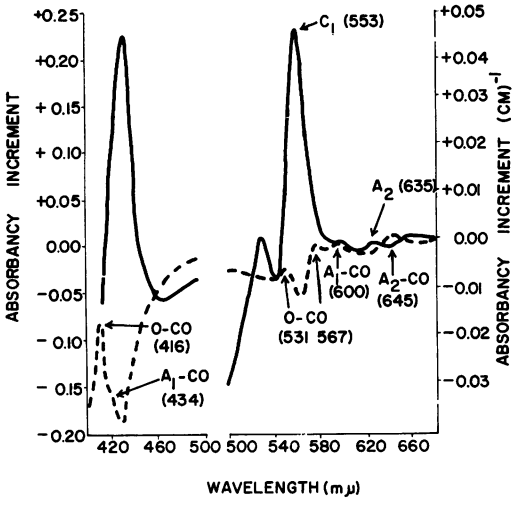

FIG. 4. *Difference spectra of Haemophilus parainfluenzae (parental type) spheroplasts. Solid line: difference in absorbancy between identical suspensions of membranes made anaerobic in the presence of 10 mM formate and 5 mM $NADH_2$, and oxidized with air by shaking vigorously. Dotted line: difference in absorbancy between suspensions reduced with formate and $NADH_2$ and bubbled with deoxygenated carbon monoxide for 5 min. Measurements were made in 50 mM phosphate buffer (pH 7.6) at 25 C at a bacterial density of 10 mg of protein per ml. Difference spectra of the mutant type of H. parainfluenzae are identical, except that the predominant cytochrome is cytochrome b_1 instead of c_1. Maxima characteristics of the cytochromes and cytochrome oxidases are indicated in the figure.*

at least 70-fold, at least 10-fold, and 4-fold, respectively, without penicillin and at least 30-fold, at least 3-fold, and 3-fold, respectively, in the parental type incubated with penicillin. In the mutant-type, cytochrome oxidases a_1, a_2, and *o* were increased at least 5-fold, at least 10-fold, and 10-fold, respectively, during an 8-hr growth period without penicillin and at least 6-fold, at least 6-fold, and 10-fold respectively, when incubated with penicillin for 8 hr.

DISCUSSION

There is evidence from a number of investigations that the bacterial cell wall is not required for many of the functions of the bacterial cell. The synthesis of inducible enzymes and the presence of a functional respiratory system in protoplasts was demonstrated by Wiame, Storck, and Vanderwinkel (26) and Landman and Spiegelman (4). Weibull (16) compared the intact cells and protoplasts of *B. megaterium* and showed that they had the same endogenous respiration and identical rates of glucose oxidation. A progressive uptake of P^{32} into the phospholipids of *Escherichia coli* spheroplast membranes and *B. megaterium* isolated membrane fractions was noted by Hill (2). Smith (10) gave evidence indicating that stretching the cell membrane containing the respiratory chain in *B. subtilis* does not inhibit electron transport, although swelling of the spheroplast decreases the reactivity of the dehydrogenases associated with the membrane. This work indicates that the attachment of the dehydrogenases to the membrane is easily broken by changes in membrane shape. Spiegelman (12), investigating the effect of osmotic changes in the medium of *B. megaterium* protoplasts, found that their enzyme-forming capacity remains unimpaired. This evidence indicates that much of the enzyme-forming capacity of the intact cell is retained in the spheroplast.

This study establishes that penicillin in the presence of lactose can cause disruption of structural elements that are a part of the rigid wall of *H. parainfluenzae*, which leads to a change in shape. Since structures that are part of the remaining spheroplast membrane are vital to the growth of this bacterium (23*a*), the possible changes in membrane formation produced by such a change in the structural relationships were examined. In the presence of penicillin and lactose under conditions of growth that stimulate the rapid synthesis of the cytochromes and cytochrome oxidases, the bacteria can form sufficient material to increase their dry weight fivefold. There is an increase in the primary dehydrogenases for formate and L-lactate, DMK_2,

TABLE 1. *Relative increases in the proportions of membrane-bound components of the electron transport system of Haemophilus parainfluenzae incubated in the presence and absence of penicillin*

Type	Increase in dry wt	Dehydrogenases		DMK_2	Cytochromes		Cytochrome oxidases			Oxygen utilization with formate
		Lactic	Formic		b_1	c_1	a_1	a_2	o	
Mutant*										
Spheroplasts	5	2	3	3	3	—	>6	>6	10	3
Normal	12	7	10	8	8	—	>5	>10	10	11
Parental†										
Spheroplasts	5	2	3	8	—	7	>30	>3	3	9
Normal	11	8	10	12	—	18	>70	>10	4	25

* Incubated 8 hr as in Fig. 2.
† Incubated 6 hr as in Fig. 2.

enzymatically reducible cytochromes b_1 and c_1, and cytochrome oxidases a_1, a_2, and o. All these elements must be membrane-bound to function (11, 25). These elements of the membrane-bound electron transport system synthesized in the growing spheroplasts are capable of being reduced by formate with the subsequent utilization of oxygen. Substrate-stimulated oxygen utilization requires that each member of the electron transport system be functional (25). The formate-stimulated capacity for oxygen utilization also increases. Comparison of the increases in measured dry weight, oxygen utilization, dehydrogenase activity, DMK_2, cytochromes, and cytochrome oxidases during spheroplast and normal cell growth are summarized in Table 1.

These experiments indicate that, despite the changes in the spatial arrangements within the bacteria necessitated by change in shape, the bacteria can form a fully functional electron transport system essentially identical to that formed during normal growth. This identity involves the cytoplasmic catabolic enzymes, the primary flavoprotein dehydrogenases, the quinone, the cytochromes, and the cytochrome oxidases. It is also interesting that, just as in the intact bacteria, these elements can be formed differentially under the conditions of spheroplast growth.

ACKNOWLEDGMENTS

This project was supported by Public Health Service grant GM 10285 from the Institute of General Medical Sciences. One of the authors (E. A. W.) was supported by a fellowship from the Lederle Laboratories, Pearl River, N.Y.

We wish to thank J. Wilson for the photomicrographs in Fig. 1.

LITERATURE CITED

1. HERBST, E. J., AND E. E. SNELL. 1949. The nutritional requirements of Hemophilus parainfluenzae 7901. J. Bacteriol. **58**:379–386.
2. HILL, P. B. 1962. Incorporation of orthophosphate into a phospholipid by isolated membrane fractions of bacteria. Biochim. Biophys. Acta **57**:386–389.
3. HU, A. S. L., R. G. WOLFE, AND F. J. REITHEL. 1959. The preparation and purification of β-galactosidase from *Escherichia coli* ML 308. Arch. Biochem. Biophys. **81**:500–507.
4. LANDMAN, O. E., AND S. SPIEGELMAN. 1955. Enzyme formation in protoplasts of *Bacillus megaterium*. Proc. Natl. Acad. Sci. U.S. **41**: 698–704.
5. LEIDY, G., I. JAFFE, AND H. E. ALEXANDER. 1962. Emergence of competence (for transformation) of three *Haemophilus* species in a chemically defined environment. Proc. Soc. Exptl. Biol. Med. **111**:725–731.
6. 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 **3**:949–954.
7. MITCHELL, P. D., AND J. M. MOYLE. 1956. Permeation mechanisms in bacterial membranes. Discussions Faraday Soc. **21**:258–265.
8. OCHOA, S. 1955. Malic dehydrogenase from pig heart, p. 735–739. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 1. Academic Press, Inc., New York.
9. SIBLEY, J. A., AND A. L. LEHNINGER. 1949. Determination of aldolase in animal tissues. J. Biol. Chem. **177**:859–872.
10. SMITH, L. 1962. Structure of the bacterial respiratory-chain system. Respiration of *Bacillus subtilis* spheroplasts as a function of the osmotic pressure of the medium. Biochim. Biophys. Acta **62**:145–152.
11. SMITH, L., AND D. C. WHITE. 1962. Structure of the respiratory-chain system as indicated by

studies with *Haemophilus parainfluenzae*. J. Biol. Chem. **237**:1337–1341.
12. SPIEGELMAN, S. 1957. Nucleic acids and the synthesis of proteins, p. 232–267. *In* W. D. McElroy and B. Glass [ed.], Chemical basis of heredity. Johns Hopkins Press, Baltimore.
13. STORCK, R., AND J. T. WACHSMAN. 1957. The association of enzymes with the protoplast membrane of *Bacillus megaterium*. Biochem. J. **66**:19–20.
14. STROMINGER, J. L. 1957. Microbial uridine-5′ pyrophosphate *N*-acetylamino sugar compounds. I. Biology of the penicillin-induced accumulation. J. Biol. Chem. **224**:509–523.
15. VELICK, S. 1955. Glyceraldehyde-3-phosphate dehydrogenase from muscle, p. 401–406. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 1. Academic Press, Inc., New York.
16. WEIBULL, C. 1953. The isolation of protoplasts from Bacillus megaterium by controlled treatment with lysozyme. J. Bacteriol. **66**:688–695.
17. WEIBULL, C. 1953. Characterization of the protoplasmic constituents of Bacillus megaterium. J. Bacteriol. **66**:696–702.
18. WHITE, D. C. 1962. Cytochrome and catalase patterns during the growth of *Haemophilus parainfluenzae*. J. Bacteriol. **83**:851–859.
19. WHITE, D. C. 1963. Respiratory systems in the hemin-requiring *Haemophilus* species. J. Bacteriol. **85**:84–96.
20. WHITE, D. C. 1963. Factors affecting the affinity for oxygen of cytochrome oxidases in *Haemophilus parainfluenzae*. J. Biol. Chem. **238**:3757–3761.
21. WHITE, D. C. 1964. Differential synthesis of five-electron transport primary dehydrogenases in *Hemophilus parainfluenzae*. J. Biol. Chem. **239**:2055–2060.
22. WHITE, D. C. 1965. The function of 2-demethyl vitamin K_2 in the electron transport system of *Hemophilus parainfluenzae*. J. Biol. Chem. **240**:1387–1394.
23. WHITE, D. C. 1965. Synthesis of 2-demethyl vitamin K_2 and the cytochrome system in *Haemophilus*. J. Bacteriol. **89**:299–305.
23*a*. WHITE, D. C. 1966. The obligatory involvement of the electron transport system in the catabolic metabolism of *Haemophilus parainfluenzae*. Antonie van Leeuwenhoek. J. Microbiol. Serol. **32**:139–159.
24. WHITE, D. C., AND S. GRANICK. 1963. Hemin biosynthesis in *Haemophilus*. J. Bacteriol. **85**:842–850.
25. WHITE, D. C., AND L. SMITH. 1964. Localization of the enzymes catalyzing hydrogen and electron transport in *Hemophilus parainfluenzae* and the nature of the respiratory chain system. J. Biol. Chem. **239**:3956–3963.
26. WIAME, J. M., R. STORCK, AND E. VANDERWINKEL. 1955. Biosynthesis induite l'arabokinase dans les protoplasts de *Bacillus subtilis*. Biochim. Biophys. Acta **18**:353–357.