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EVIDENCE FOR A TRICARBOXYLIC ACID CYCLE IN *MYCOPLASMA HOMINIS*

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ABSTRACT

VANDEMARK, P. J. (Cornell University, Ithaca, N.Y.), AND P. F. SMITH. Evidence for a tricarboxylic acid cycle in *Mycoplasma hominis*. J. Bacteriol. **88**:1602–1607. 1964.—Resting cells of acetate-grown *Mycoplasma hominis* strain 07 oxidized the various intermediates of the tricarboxylic and glyoxylate cycles, with the exception of sodium citrate and glyoxylate. Extracts of these cells possessed isocitric dehydrogenase, isocitratase, α-ketoglutaric dehydrogenase, succinic dehydrogenase, fumarase, malic dehydrogenase, citratase, and acetyl coenzyme A kinase activities. With the assay conditions employed, condensing enzyme, malate synthetase, and phosphotransacetylase activities were negligible. Incubation of sodium acetate-*2-C*[14] with the various intermediates of the tricarboxylic acid cycle in the presence of cell-free extracts resulted in exchange of the isotope with these compounds as well as the formation of other labeled intermediates of the tricarboxylic acid cycle. Oxidation of sodium acetate-*2-C*[14] alone resulted in the formation of labeled succinate, fumarate, and malate.

The fermentative and nonfermentative strains of *Mycoplasma* appear to represent fundamentally different metabolic types. Fermentative strains appear to obtain energy by the dissimilation of carbohydrates via homolactic and heterolactic types of fermentation (Rodwell and Rodwell, 1954*a;* Tourtellotte and Jacobs, 1960; Neimark and Pickett, 1960; Gill, 1962; Castrejon-Diez, Fisher, and Fisher, 1963). However, the nature of energy-yielding pathways among the nonfermentative types is not well elucidated. Previous investigations of Smith (1960) indicated that the dissimilation of certain amino acids, although representing potential energy sources for nonfermentative strains, is primarily anabolic in nature. The studies of Lynn (1960) have shown that strains of this latter type are capable of oxidizing short-chain fatty acids. If the oxidation of fatty acids represents an energy source for these nonfermentative *Mycoplasma*, it would seem plausible that a terminal respiratory cycle, such as the tricarboxylic acid cycle, is functioning in these oxidations. Although previous investigators have failed to find evidence for a tricarboxylic acid pathway among *Mycoplasma* species (Lecce and Morton, 1954; Rodwell and Rodwell, 1954*b;* Tourtellotte and Jacobs, 1960), studies have been primarily limited to fermentative types of *Mycoplasma*, and it is feasible that the tricarboxylic acid cycle functions in fatty acid oxidation by the nonfermentative strains. The present report summarizes manometric, enzymatic, and isotopic evidence for a tricarboxylic acid cycle (or at least a portion of the tricarboxylic acid cycle) functioning in the nonfermentative *M. hominis* strain 07.

MATERIALS AND METHODS

Culture. *M. hominis* 07 was grown from a 10% inoculum in Difco PPLO broth medium containing 0.5% sodium acetate at 37 C both in stationary culture and with aeration by sparging with sterile air. The cells were harvested after 24 or 48 hr by centrifugation at 20,000 × *g* for 10 min. The cell crop was washed once in distilled water, and resting-cell suspensions were prepared by resuspension in approximately 1/500 of the original growth volume.

Manometric procedures. The oxidation of the tricarboxylic acid intermediates by resting-cell suspensions was followed by conventional manometric methods, with air as the gas phase at 37 C. The Warburg flasks in this study contained 67 μmoles of phosphate buffer (pH 7.4); approximately 5 mg of cells; cofactors including 1 mg of nicotine adenine dinucleotide (NAD), 1 mg of

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nicotine adenine dinucleotide phosphate (NADP), 0.5 mg of flavin mononucleotide (FMN), and 0.1 mg of coenzyme A (CoA); 50 μmoles of each of the tricarboxylic acid intermediates; and deionized water; in a total volume of 3.0 ml per flask. The center well of each vessel contained 0.15 ml of 40% potassium hydroxide to absorb carbon dioxide. The Warburg flasks were preincubated for 1 hr prior to tipping in substrates, to lower the endogenous activity of the cell suspensions, based on the findings of Lynn (1960).

Enzymatic methods. Cell-free extracts were prepared by sonic oscillation of resting-cell suspensions in a 10-kc Raytheon oscillator for 10 min as previously described (Smith, Van Demark, and Fabricant, 1963). The cell debris was removed by centrifugation at 25,000 × *g* for 30 min, and the resulting supernatant fluid was used for enzymatic analysis. The protein level of cell extracts was determined by the trichloroacetic acid method of Stadtman, Novelli, and Lipmann (1951), with crystalline bovine serum albumin as a standard.

Isocitric dehydrogenase activity was measured spectrophotometrically by following the reduction of NADP at 340 mμ according to the method of Ochoa (1948), and the specific enzyme activity was expressed as the micromoles of NADP reduced per hour per milligram of enzyme protein. Isocitratase and citratase were measured spectrophotometrically by the formation of the phenylhydrazones of glyoxylic and oxaloacetic acids, respectively, according to the method of Dixon and Kornberg (1959). The specific enzyme activity was expressed as the micromoles of phenylhydrazone formed per hour per milligram of enzyme protein. α-Ketoglutaric and succinic dehydrogenase were measured as the rate of dichlorophenol-indophenol reduction at 660 mμ according to the method of Slater and Bonner (1952) and Basford and Huennekens (1955), with the specific enzyme activity expressed as the micromoles of dye reduced per hour per milligram of enzyme protein. Fumarase activity was measured as the increase of absorption at 240 mμ when sodium malate was incubated with the sonic extract according to the method of Racker (1950), with the specific activity expressed as the micromoles of fumarate formed per hour per milligram of enzyme protein. Malic dehydrogenase was measured, according to a modification of the method of Mehler et al. (1948), as the reduction of NAD at 340 mμ when the enzyme preparation was incubated with sodium malate in tris(hydroxymethyl)aminomethane (tris) buffer at pH 9, and the specific activity was expressed as the micromoles of NAD reduced per hour per milligram of enzyme protein. Malate synthetase was measured according to the method of Dixon and Kornberg (1959) by following the rate of cleavage of acetyl CoA at 232 mμ, and specific activity was expressed as the micromoles of acetyl CoA cleaved per hour per milligram of enzyme protein.

Condensing enzyme was measured by chemical assay, based on the conversion of acetyl phosphate and oxaloacetate to citrate (Ochoa et al., 1951), and spectrophotometrically, based on the conversion of acetyl CoA and malate to citrate when incubated with the sonic-treated preparation and malic dehydrogenase (Stern et al., 1951). The latter activity was measured as the rate of NAD reduction resulting from the conversion of malate to oxaloacetate. The specific activity was expressed as the micromoles of citrate formed in the first method, or as the micromoles of NAD reduced in the latter method, per hour per milligram of protein.

Acetate activation and acetyl CoA kinase activities were measured as the rate of hydroxamic acid formation by the method of Jones et al. (1953), and the activity was expressed as the micromoles of hydroxamic acid formed per hour per milligram of enzyme protein. Acetyl CoA deacylase activity was measured by following the disappearance of acetyl CoA from an incubation mixture containing 0.1 μmole of acetyl CoA, 50 μmoles of cysteine, 500 μmoles of tris buffer (pH 8.0), and 8.0 mg of enzyme protein in a total volume of 5.0 ml of water. Samples (1 ml) were removed at 1-min intervals and added to 0.5 ml of 2 M NH_2OH, and the level of hydroxamic acid was determined by the method of Jones et al. (1953). Phosphotransacetylase was measured according to the method of Stadtman et al. (1951).

Isotopic methods. Solutions of sodium acetate-*2-*C^{14} (New England Nuclear Corp., Boston, Mass.) containing 18 μc and 100 μmoles of acetate per ml were used. In studies of the incorporation of C^{14} from sodium acetate-*2-*C^{14} into the various tricarboxylic acid intermediates, the incubation mixture contained 80 μmoles of tris buffer (pH 7.4), 10 μmoles of potassium phosphate (pH 7.4), 25 μmoles of magnesium chloride, 70 μmoles of

glutathione, 90 μmoles of adenosine triphosphate (ATP), 0.5 ml of CoA, 100 μmoles of potassium acetate, and sonic extracts containing approximately 10 mg of protein. The mixtures were incubated at 37 C for 45 min. The reactions were stopped by the addition of 0.2 volume of 5% trichloroacetic acid. The solutions were centrifuged, and the clear supernatant fluid was evaporated to dryness in vacuo. The samples were reconstituted to a volume of 1 ml with deionized water and chromatographed on a silicic acid column (Unisil; Clarkson Chemical Co., Williamsport, Pa.) by use of the solvent system of Swim and Krampitz (1954). The resulting fractions were analyzed for radioactivity as previously described (Smith, 1963), and labeled fractions were identified by paper chromatography with a solvent system of butanol-acetic acid-water (4:5:1). Malic, citric, and isocitric acids were also identified enzymatically, by use of purified malic dehydrogenase, citratase, and isocitratase preparations.

Results and Discussion

Manometric studies. Initial investigations involved manometric studies of the oxidation of the various intermediates of the tricarboxylic acid and glyoxylate cycles by resting cells of acetate-grown strain 07. These cells oxidized the intermediates tested, with the exception of sodium citrate and glyoxylate (Table 1). These various oxidations of the tricarboxylic acid intermediates were inhibited by 10^{-2} M malonate and 10^{-6} M fluoroacetate. The failure to oxidize citrate could be explained either by its failure to permeate the *Mycoplasma* cell or by the possibility that it is chelating cations essential to the cell's respiration. It is interesting to note that the addition of 0.1% citrate to the growth medium was found to inhibit growth. At relatively high levels, glyoxylate is known to inhibit oxygen uptake by respiring microbial cells and tissue suspensions (D'Abramo, Romano, and Ruffo, 1958), thus perhaps providing an explanation for the failure of these *Mycoplasma* cells to oxidize this intermediate. In view of the possibility that the apparent depression of endogenous activity by citrate and glyoxylate may be due to other, perhaps more significant, mechanisms, the nature of this inhibition is being further investigated.

TABLE 1. *Oxidation of tricarboxylic acid intermediates by resting cells of acetate-grown Mycoplasma hominis 07*

Substrate	Q_{O_2}(N)*
Endogenous	84
Isocitrate	142
α-Ketoglutarate	122
Succinate	152
Fumarate	129
Malate	128
Glyoxylate	47
Acetate	117
Citrate	63
Pyruvate	143

* Each Warburg flask contained 67 μmoles of phosphate buffer (pH 7.4); approximately 5 mg of cell nitrogen; cofactors, including 1 mg of NAD, 1 mg of NADP, 0.5 mg of FMN, and 0.1 mg of CoA; and 50 μmoles of the specified tricarboxylic acid intermediate. The Q_{O_2} (N) value represents the microliters of oxygen uptake per milligram of cell nitrogen per hour.

TABLE 2. *Enzymatic activity of acetate-grown Mycoplasma hominis 07*

Enzyme	Activity*
Isocitric dehydrogenase	0.42
Isocitratase	5.3
α-Ketoglutaric dehydrogenase	1.6
Succinic dehydrogenase	3.8
Fumarase	2.0
Malic dehydrogenase	1.2
Malate synthetase	<0.001
Acetyl CoA kinase	0.22
Condensing enzyme	<0.001
Citratase	7.9

* Enzymatic activity, expressed as micromoles per hour per milligram of enzyme protein, was determined by product formed, as described in Materials and Methods.

Enzymatic studies. The results of investigations to demonstrate the various enzymes of the tricarboxylic acid and glyoxylic acid cycles in the cell-free extracts are summarized in Table 2. The data presented in Table 2 represent the composite data determined with a number of sonic preparations of strain 07. The relative enzymatic activity of these preparations did not vary greatly with different preparations. Variations in protein concentrations of these sonic extracts were corrected for by expressing the data in terms of specific activities. The isocitric dehydrogenase was NADP-specific and required the addition of

magnesium ions for maximal activity. The α-ketoglutaric and malic dehydrogenases were NAD-specific.

These enzymatic data indicate that, although the majority of the enzymes of the tricarboxylic acid and glyoxylate cycles are present in acetate-grown strain 07, two key enzymes of these cycles, the condensing enzyme and malate synthetase, are not readily demonstrated. It is possible that these enzymes were not readily demonstrated because the crude enzyme preparations used contain a high acetyl CoA deacylase activity. The minimal deacylase activity of the various sonic preparations was 25 μmoles of acetyl CoA deacylated per milligram of cell nitrogen per hour. In the chemical assay for condensing enzyme in which acetyl phosphate was incubated with oxaloacetate in the presence of transacetylase, CoA, and the sonic extract of strain 07, acetyl phosphate disappeared, but chemical analysis showed negligible citrate formation. Such a pattern of results could be caused by the high acetyl CoA deacylase activity of the crude *Mycoplasma* enzyme preparation used. Similarly, the failure to demonstrate condensing enzyme by the optical assay in which acetyl CoA was incubated with malate, NAD, malic dehydrogenase, and the 07 enzyme could be due to the breakdown of the acetyl CoA by the deacylase activity of the latter. Since the latter assay method was also complicated by the presence of a NADH oxidase in the crude enzyme preparation used, the values expressed in Table 2 for condensing enzyme activity were those obtained by the determination of citrate formation by the former assay method. Likewise, the deacylase activity made impossible the assay of malate synthetase activity by following the cleavage of acetyl CoA. A partial purification of the enzyme preparation to remove this contaminating deacylase would make possible the assay for condensing enzyme and malate synthetase by the conventional enzymatic methods. Unfortunately, enzyme purification of extracts of this organism is not feasible currently, owing to the low cell yields obtained on culturing this organism.

Acetate activation, as measured by the rate of hydroxamic acid formation, appears different from that previously reported by Castrejon-Diez et al. (1962). These workers found acetokinase activity in a number of *Mycoplasma* species but not in the strain of *M. hominis* tested. The activation of acetate by *M. hominis* 07 would appear to be an aceto-CoA-kinase, since our preparations lacked phosphotransacetylase, which would be essential for acetate utilization by cells possessing acetokinase but not aceto-CoA-kinase. Furthermore, this kinase activity, unlike that reported in *M. gallinarium* (Castrejon-Diez et al., 1962), is relatively specific for acetate with negligible activation of the other fatty acids tested with the exception of that with sodium propionate (Table 3).

Isotopic studies. Because of the difficulties of demonstrating condensing enzyme and malate synthetase by use of conventional enzymatic methods, the incorporation of C^{14} from sodium acetate-*2-*C^{14} by the enzyme preparation from strain 07 into the various tricarboxylic acid intermediates was undertaken. Table 4 shows the incorporation of sodium acetate-*2-*C^{14} by the sonic preparation into the key tricarboxylic acid intermediates when incubated alone or with glyoxylate, oxaloacetate, or citrate. The high level of radioactivity in malate when sodium acetate-*2-*C^{14} is incubated with glyoxylate is evidence for the presence of malate synthetase in *M. hominis* 07. The incorporation of C^{14} into citrate, especially when sodium acetate-*2-*C^{14} is incubated with citrate, could also be considered as presumptive evidence for a condensing enzyme in this organism. However, the high incorporation of radioactivity into the dicarboxylic acids,

TABLE 3. *Fatty acid activation by sonic extracts of Mycoplasma hominis 07*

Fatty acid*	Hydroxamic acid†
Formate	0
Acetate	2.80
Propionate	0.74
Butyrate	0.12
Isobutyrate	0
Valeric	0.07
Isovaleric	0

* The complete system contained 50 μmoles of tris buffer (pH 7.4), 10 μmoles of $MgCl_2$, 20 μmoles of ATP, 0.1 μmole of CoA, 20 μmoles of reduced glutathione, 50 μmoles of hydroxylamine, 20 μmoles of the listed fatty acid, and approximately 5 mg of enzyme protein, in a total volume of 2.0 ml.

† Expressed as micromoles per hour per milligram of protein.

especially succinate, might indicate that acetate is being incorporated by the reactions similar to those described by Seaman and Naschke (1955) and Davies (1958).

The oxidation of sodium acetate-*2-C^{14}* by whole cells was studied manometrically, and an analysis was made of the labeling in the various products formed, as described in Materials and Methods. Although radioactivity was found in essentially all of the intermediates of the tricarboxylic acid cycle during this oxidation, the major labeling occurred in succinate, malate, and fumarate.

One of the unusual characteristics of the oxidation of fatty acids by *Mycoplasma*, as reported by Lynn (1960), was the relatively long lag period which occurred before the rate of oxygen uptake exceeded endogenous activity. This characteristic lag period is illustrated in the curve of the rate of acetate oxidation (Fig. 1). However, the oxidation of isocitrate took place without this lag. This would appear to indicate that the lag period in fatty acid oxidation by *Mycoplasma* is due to a rate-limiting step prior to the tricarboxylic acid cycle. Since there is no lag in the activation of acetate by this organism (Fig. 1), the rate-limiting reaction in this fatty acid oxidation might be interpreted to be the incorporation of acetyl CoA to form citric acid.

The present study must be extended to determine whether terminal respiration via the tricarboxylic acid and glyoxylate cycles is characteristic of nonfermentative *Mycoplasma* strains and is perhaps inducible in certain fermentative strains of this microbial group. However, in the case of *M. hominis* 07 at least, the presence of this terminal respiratory cycle represents a portion of the pathway involved in the dissimilation of fatty acids. Such a pathway of fatty acid oxidation, when coupled with the oxidative phosphorylation made available by electron transport to oxygen, would appear to provide this organism far more energy than the substrate phosphorylation potentially available in the degradation of arginine and glutamine (Smith, 1960; Schimke and Barile, 1963). Supporting this latter premise is the increased growth of strain 07 in the presence of acetate and butyrate, as demonstrated by Smith and Lynn (1958).

As the studies of the *Mycoplasma* are extended, it becomes more apparent that their metabolic

TABLE 4. *Incorporation of radioactive carbon from sodium acetate-2-C^{14} into tricarboxylic acid cycle intermediates by extracts of Mycoplasma hominis 07*

Intermediate added*	Radioactivity (count/min) in				
	Malate	Citrate	Succinate	Fumarate	Isocitrate
None	610	80	1,420	970	10
Glyoxylate	3,710	740	5,480	3,430	1,220
Oxaloacetate	230	210	1,250	510	90
Citrate	6,400	1,720	5,410	260	320

* The complete system included 80 μmoles of tris buffer (pH 7.4), 10 μmoles of potassium phosphate (pH 7.4), 25 μmoles of $MgCl_2$, 70 μmoles of glutathione, 90 μmoles of ATP, 0.8 μmole of CoA, 100 μmoles of potassium acetate (containing 18 μc of sodium acetate-*2-C^{14}*), 80 μmoles of the tricarboxylic acid intermediates listed, and *Mycoplasma* extract (containing approximately 10 mg of protein), in a total volume of 5.0 ml.

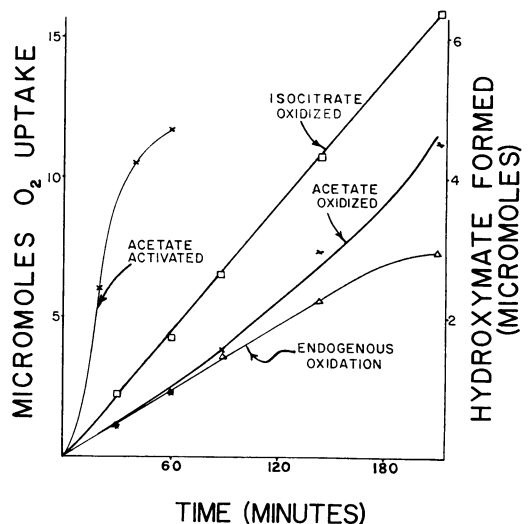

FIG. 1. *Comparison of the lag on acetate oxidation with acetate activation and isocitrate oxidation by Mycoplasma hominis strain 07. Acetate and isocitrate oxidation were followed manometrically with resting cells as described in Materials and Methods. Acetate activation was followed as the formation of hydroxamic acid by a sonic preparation of strain 07 (5 mg of enzyme protein) when incubated with 100 μmoles of tris buffer (pH 7.4), 50 μmoles of sodium acetate, 30 μmoles of glutathione, 10 μmoles of ATP, and 100 μmoles of hydroxylamine. The reaction was stopped, and hydroxamic acid was measured by the addition of $FeCl_3$ according to the method of Jones et al. (1953).*

complexity is not simple or primitive, but approaches that of the true bacteria.

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LITERATURE CITED

BASFORD, R. E., AND F. M. HUENNEKENS. 1955. Studies on thiols. I. Oxidation of thiol groups by 2,6-dichlorophenol indophenol. J. Am. Chem. Soc. **77**:3873–3877.

CASTREJON-DIEZ, J., T. N. FISHER, AND E. FISHER. 1962. Acetokinase reaction in several pleuropneumonia-like organisms. Biochem. Biophys Res. Commun. **9**:416–420.

CASTREJON-DIEZ, J., T. N. FISHER, AND E. FISHER. 1963. Glucose metabolism of two strains of *Mycoplasma laidlawii*. J. Bacteriol. **86**:627–636.

D'ABRAMO, F., M. ROMANO, AND A. RUFFO. 1958. Inhibition of citrate oxidation by glyoxylate in rat-liver homogenates. Biochem. J. **68**: 270–275.

DAVIES, D. D. 1958. Synthesis of succinate from acetate by an enzyme system of pig heart muscle. Nature **181**:339–341.

DIXON, G. H., AND H. L. KORNBERG. 1959. Assay methods for key enzymes of the glyoxylate cycle. Biochem. J. **72**:3P.

GILL, J. W. 1962. Culture and metabolism of *Mycoplasma gallisepticum*. J. Bacteriol. **83**: 213–218.

JONES, M. E., S. BLOCK, R. M. FLYNN, AND F. LIPMANN. 1953. Acetyl coenzyme A synthesis through pyrophosphoryl split of adenosine triphosphate. Biochim. Biophys. Acta **12**:141–149.

LECCE, J. G., AND H. E. MORTON. 1954. Metabolic studies on three strains of pleuropneumonia-like organisms isolated from man. J. Bacteriol. **67**:62–68.

LYNN, R. J. 1960. Oxidative metabolism of pleuropneumonia-like organisms. Ann. N.Y. Acad. Sci. **79**:538–542.

MEHLER, A. H., A. KORNBERG, S. GRISOLIA, AND S. OCHOA. 1948. The enzymatic mechanism of oxidation reductions between malate or isocitrate and pyruvate. J. Biol. Chem. **174**:961–977.

NEIMARK, H. C., AND M. J. PICKETT. 1960. Products of glucose metabolism by pleuropneumonia-like organisms. Ann. N.Y. Acad. Sci. **79**:531–537.

OCHOA, S. 1948. Biosynthesis of tricarboxylic acids by carbon dioxide fixation. III. Enzymatic mechanisms. J. Biol. Chem. **174**:133–157.

OCHOA, S., J. R. STERN, AND M. SCHNEIDER. 1951. Enzymatic synthesis of citric acid. II. Crystalline condensing enzyme. J. Biol. Chem. **193**: 691–702.

RACKER, E. 1950. Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. Biochim. Biophys. Acta **4**:211–214.

RODWELL, A. W., AND E. S. RODWELL. 1954*a*. The pathway of glucose oxidation by *Asterococcus mycoides*, the organism of bovine pleuropneumonia. Australian J. Biol. Sci. **7**:37–46.

RODWELL, A. W., AND E. S. RODWELL. 1954*b*. The breakdown of pyruvate by *Asterococcus mycoides*, the organism of bovine pleuropneumonia. Australian J. Biol. Sci. **7**:31–36.

SCHIMKE, R. T., AND M. F. BARILE. 1963. Arginine metabolism in pleuropneumonia-like organisms isolated from mammalian cell cultures. J. Bacteriol. **86**:195–208.

SEAMAN, G. R., AND M. D. NASCHKE. 1955. Reversible cleavage of succinate by extracts of Tetrahymena. J. Biol. Chem. **217**:1–12.

SLATER, E. C., AND W. D. BONNER. 1952. The effect of fluoride on the succinic oxidase system. Biochem. J. **52**:185–196.

SMITH, P. F. 1960. Amino acid metabolism of PPLO. Ann. N.Y. Acad. Sci. **79**:543–550.

SMITH, P. F. 1963. The carotenoid pigments of *Mycoplasma*. J. Gen. Microbiol. **32**:307–319.

SMITH, P. F., AND R. J. LYNN. 1958. Lipid requirements for the growth of pleuropneumonia-like organisms. J. Bacteriol. **76**:264–269.

SMITH, S. L., P. J. VAN DEMARK, AND J. FABRICANT. 1963. Respiratory pathways in the *Mycoplasma*. I. Lactate oxidation by *Mycoplasma gallisepticum*. J. Bacteriol. **86**:893–897.

STADTMAN, E. R., G. D. NOVELLI, AND F. LIPMANN. 1951. Coenzyme A function in and acetyl transfer by the phosphotransacetylase system. J. Biol. Chem. **191**:365–376.

STERN, J. R., B. SHAPIRO, E. R. STADTMAN, AND S. OCHOA. 1951. Enzymatic synthesis of citric acid. II. Reversibility and mechanism. J. Biol. Chem. **193**:704–720.

SWIM, H. E., AND L. O. KRAMPITZ. 1954. Acetic acid oxidation by *Escherichia coli:* evidence for the occurrence of a tricarboxylic acid cycle. J. Bacteriol. **67**:419–425.

TOURTELLOTTE, M. S., AND R. E. JACOBS. 1960. Physiological and serologic comparison of PPLO from various sources. Ann. N.Y. Acad. Sci. **79**:521–530.