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Comparative Biosynthesis of Mevalonic Acid by *Mycoplasma*

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ABSTRACT

SMITH, PAUL F. (University of South Dakota, Vermillion), AND C. V. HENRIKSON. Comparative biosynthesis of mevalonic acid by *Mycoplasma*. J. Bacteriol. **89**:146–153. 1965.—Three representative *Mycoplasma*, *M. laidlawii* strain B, *M. gallisepticum* strain J, and *M. hominis* strain 07, were examined for the presence or absence of enzymes associated with the biosynthetic pathway to mevalonic acid. *M. laidlawii* served as a control, because it synthesizes carotenoids from acetate. *M. laidlawii* was shown to contain a specific acetokinase and phosphotransacetylase for the synthesis of acetyl coenzyme A, and a β-ketothiolase and coenzyme A transferase for the synthesis of acetoacetyl coenzyme A. *M. gallisepticum* contained a specific acetokinase, phosphotransacetylase, and possibly an aceto coenzyme A kinase forming acetyl coenzyme A; it also contained a β-ketothiolase, a coenzyme A transferase, and a coenzyme A transphorase forming acetoacetyl coenzyme A directly or indirectly. The β-ketothiolase of *M. gallisepticum* was not affected by iodoacetamide, in contrast to the other two strains. *M. laidlawii* exhibited β-hydroxy-β-methylglutaryl coenzyme A condensing enzyme, and *M. hominis* did not. This activity of *M. gallisepticum* was masked by thiolase activity. *M. laidlawii* and *M. gallisepticum* contained a nicotinamide adenine dinucleotide phosphate-linked β-hydroxy-β-methylglutaryl coenzyme A reductase, and *M. hominis* did not. C^{14}-labeled acetate was incorporated into mevalonic acid only by *M. laidlawii* and *M. gallisepticum*. The lack of β-hydroxy-β-methylglutaryl coenzyme A condensing enzyme and reductase activities in *M. hominis* explains its growth requirement for sterol. The enzymatic block in *M. gallisepticum* must occur after mevalonic acid in the biosynthetic pathway to terpenoids.

The *Mycoplasma* behave as two nutritional types on the basis of lipid requirements. There are those which require a planar hydroxylated steroid, such as cholesterol, and those which synthesize their own terpenoid compounds, such as the carotenols (Smith, 1964). It is probable that both sterol and carotenol carry out analogous functions (Smith, 1963*a*) in the two types of organisms, because cholesterol spares the biosynthesis of carotenoids (Smith, 1963*b*). Although C^{14}-labeled acetate and mevalonate are incorporated into the unsaponifiable lipid of strains not requiring sterol, no such incorporation is evident in strains requiring sterol (Smith and Rothblat, 1962). Intermediates in the biosynthetic pathway to terpenoids, mevalonic acid, and squalene fail to satisfy the sterol requirement (Smith and Lynn, 1958). However, if tetraterpenoids (e.g., carotenoids) or polyterpenoids are the natural products of the *Mycoplasma*, squalene would not be a biosynthetic intermediate (Porter and Anderson, 1962). Rather, other colorless terpenes, such as geranyl geranyl pyrophosphate, phytoene, and phytofluene, are precursors to carotenoids. The presence of colorless oils with the chromatographic behavior of hydrocarbons has been detected in both sterol-requiring *Mycoplasma* (Tourtellotte et al., 1963) and *Mycoplasma* not requiring sterol (Rothblat and Smith, 1961). These colorless oils may represent the accumulation of intermediates by an incomplete biosynthetic pathway to tetra- or polyterpenoids, particularly in the sterol-requiring strains. Polyterpenols have recently been discovered in lactobacilli (Thorne, 1964), and many bacterial species are known to contain carotenoids.

A study was undertaken to examine representative strains of *Mycoplasma* for the presence or absence of the specific enzymes involved in terpenoid synthesis in an effort to elucidate the relationship of the various strains of *Mycoplasma* to one another and to bacteria. The present report presents evidence for the synthesis of mevalonic acid in two strains, and a block in the biosynthetic pathway in one strain.

MATERIALS AND METHODS

Three representative strains were employed in this study: *M. laidlawii* strain B, which does not require sterol and synthesizes carotenoids; and *M. gallisepticum* (Edward and Kanarek, 1960) strain J and *M. hominis* strain 07, which require sterol for growth. The organisms were grown and harvested as previously described (Henrikson and Smith, J. Gen. Microbiol., *in press*). Cell-free extracts were prepared as outlined by VanDemark and Smith (1964). The protein level of these extracts was determined by the trichloroacetic acid method of Stadtman, Novelli, and Lipmann (1951) with serum albumin as a standard.

The following methods were employed for the determination of various enzymatic activities: acetokinase (Rose, 1955); aceto-coenzyme A (CoA)-kinase (Jones and Lipmann, 1955); phosphotransacetylase (Stadtman, 1955); acetyl CoA deacylase (Gergely, 1955); coenzyme A transphorase (Barker, Stadtman, and Kornberg, 1955); CoA transferase by the measurement of increase in absorption at 310 mμ (Stern, 1955) and by coupling with thiolase and trapping of acetyl CoA with *p*-nitroaniline by use of freshly prepared aromatic amine acetylating enzyme from pigeon liver (Tabor, Mehler, and Stadtman, 1953; Kaplan and Lipmann, 1948); β-keto-thiolase (Stern, 1955). Cell-free extracts employed for aceto-CoA kinase were treated with Dowex-1 chloride to remove CoA. Phosphotransacetylase for use in the CoA transphorase system was prepared from a laboratory strain of *Aerobacter aerogenes* according to the method described by Stadtman (1955). The condensing enzyme forming β-hydroxy-β-methylglutaryl CoA (HMG CoA) from acetyl CoA and acetoacetyl CoA was measured both by determination of decrease in absorption of acetoacetyl CoA at 310 mμ according to the method of Carr (1962), except for the addition of a 5×10^{-4} M final concentration of iodoacetamide to inhibit β-keto thiolase activity (Rudney and Ferguson, 1951), and by determination of incorporation of 1-C^{14} acetyl CoA into the hydroxamate derived from the formed HMG CoA (Carr, 1962). The synthesis of HMG CoA from malonyl CoA and acetyl CoA was determined by measuring incorporation of 1 C^{14} acetyl CoA into HMG hydroxamate (Brodie, Wasson, and Porter, 1963). The synthesis of HMG CoA by incorporation of carbon dioxide into β-hydroxy isovaleryl CoA (Bachhawat, Robinson, and Coon, 1954, 1955; Coon, Robinson, and Bachhawat, 1955) was determined by measuring the radioactivity incorporated into HMG hydroxamate when cell extracts were incubated with sodium bicarbonate-C^{14} and isovaleryl CoA. The reductase transforming HMG CoA into mevalonic acid was determined with reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$) according to the method of Knappe, Ringelmann, and Lynen (1959), measuring the loss of absorption at 366 mμ. The system was controlled by blanking against a cuvette measuring reduced $NADPH_2$ oxidase activity. Incorporation of acetate-2-C^{14} into mevalonic acid by intact organisms was determined by incubating 20 mg of cellular protein with $NADPH_2$ (10 μmoles), adenosine triphosphate (ATP) (10 μmoles), coenzyme A (5 μmoles), magnesium chloride (10 μmoles), sodium fluoride (0.005 μmoles), sodium acetate (50 μmoles), and sodium acetate-2-C^{14} with specific activity of 2 mc/mmole (20 μc) for 1 to 3 hr at 37 C. A 0.1-g amount of unlabeled mevalonic acid was then added, and the mixture was heated at 70 C for 2 min to stop the reaction; this was followed by the addition of 15 ml of absolute ethanol to precipitate the protein which was removed by centrifugation. The supernatant fluid was concentrated to dryness *in vacuo* (Anderson and Porter, 1962). The dibenzylethylene diamine salt of mevalonic acid was formed by the method of Hoffman et al. (1957) and recrystallized to constant specific activity.

Acyl CoA preparations (C^{14} acetyl CoA, acetoacetyl CoA, succinyl CoA, butyryl CoA, isovaleryl CoA, malonyl CoA, and HMG CoA) were synthesized from CoA and the respective anhydrides, or, in the case of malonyl CoA, from thiophenyl malonate, by the method of Simon and Shemin (1953). The thiophenyl ester of malonic acid was synthesized from malonic acid and thiophenol according to the method of Trams and Brady (1960). β-Hydroxy-β-methylglutaryl anhydride was prepared by refluxing the acid with acetic anhydride according to Hilz et al. (1958). Hydroxamates were formed by incubating the acyl-CoA preparations or the anhydrides with neutral 2 M hydroxylamine. The hydroxamates formed in reaction mixtures were separated by ascending paper chromatography in aqueous phenol (Bachhawat et al., 1955) and detected both by spraying with 5% ferric chloride in 0.1 M hydrochloric acid, and by radioisotope counting. Adequate separation of mixed hydroxamates was achieved with the following R_F values: acetate, 0.75; isovalerate, 0.10; malonate, 0.09; HMG, 0.45.

Carbon-14 counting was performed in a Tracerlab proportional counter as previously described (Smith, 1963*b*), or in a Packard TriCarb liquid scintillation counter (model 3214). All chemicals, except those synthesized, were obtained from commercial sources and were of the highest purity obtainable. Diketene, the anhydride used for synthesis of acetoacetyl CoA, was obtained from K & K Laboratories, Inc., Jamaica, N.Y., and required redistillation immediately prior to use.

RESULTS AND DISCUSSION

The biosynthesis of mevalonic acid usually proceeds by the condensation of 1 mole of acetyl CoA with 1 mole of acetoacetyl CoA to form β-hydroxy-β-methylglutaryl CoA (HMG CoA) with subsequent reduction by a pyridine nucleotide-linked reductase to form mevalonic acid. The mechanism(s) of acetate activation and

TABLE 1. *Acetokinase activity of Mycoplasma*

Fatty acid	Hydroxamate formed by	
	M. laidlawii strain B	*M. gallisepticum* strain J
Acetic	2.83*	1.36
Propionic	<0.7	0.52
Butyric	<0.7	0.20
Valeric	<0.7	0
Caproic	0	0

* Numbers denote micromoles of hydroxamic acid formed per milligram of protein per minute. Each tube contained 750 μmoles of fatty acid as potassium salt, 50 μmoles of Tris buffer (*p*H 7.4), 10 μmoles of $MgCl_2$, 700 μmoles of neutral hydroxylamine, 10 μmoles of ATP, and 1 mg of enzyme protein, in a total volume of 1.0 ml. Reaction time was 1 or 2 min at 22 C.

TABLE 2. *Aceto CoA kinase activity of Mycoplasma*

Condition	Hydroxamic acid formed by	
	M. laidlawii strain B	*M. gallisepticum* strain J
Untreated cell extract	3.6*	4.4
Untreated cell extract + CoA	3.0	5.5
Dowex-treated cell extract	2.0	5.9
Dowex-treated cell extract + CoA	2.1	7.0

* Numbers denote micromoles of hydroxamic acid formed per milligram of protein in 20 min. Each tube contained 10 μmoles of ATP, 20 μmoles of potassium acetate, 100 μmoles of Tris buffer (*p*H 7.4), 200 μmoles of neutral hydroxylamine, 50 μmoles of KF, 10 μmoles of $MgCl_2$, 10 μmoles of glutathione, 0.1 μmole of CoA where indicated, and 1 or 2 mg of enzyme protein, in a total volume of 1 ml. Reaction was carried out at 37 C for 20 min.

acetoacetyl CoA formation were not known for the strains employed with the exception of *M. hominis* strain 07 (VanDemark and Smith, 1964); therefore this study includes data pertaining to acetate activation and acetoacetyl CoA formation in addition to HMG CoA synthesis and HMG CoA reduction.

Acetate activation. Two possible methods of acetate activation were examined, i.e., acetokinase and aceto CoA kinase. Both *M. laidlawii* and *M. gallisepticum* exhibited acetokinase activity which was rather specific for acetate (Table 1). These results are in general agreement with those of Castrejon-Diez, Fisher, and Fisher (1962). As reported by VanDemark and Smith (1964), it is unlikely that the other strain employed in this study, *M. hominis*, possesses an acetokinase due to its lack of phosphotransacetylase, which would be necessary for the organism to utilize the acetyl phosphate formed. Examination of *M. laidlawii* and *M. gallisepticum* for aceto CoA kinase, the acetate-activating enzyme of *M. hominis*, led to inconclusive results. The data (Table 2) indicate the absence of any stimulation of acetate activation by coenzyme A when added to Dowex-treated cell extracts of *M. laidlawii*. However, stimulation by CoA did occur with *M. gallisepticum* not only in Dowex-treated cell extracts, but also in the untreated enzyme preparation. It is possible that *M. gallisepticum* contains both an acetokinase and an aceto CoA kinase. Whether these two enzymes carry out the same function in the intact organism is worthy of study. Both organisms exhibiting acetokinase activity possess phosphotransacetylase (Table 3). This activity of *M. gallisepticum* is particularly high. Thus, all three organisms employed in this study possess adequate mechanisms for the formation of acetyl CoA. The acetyl CoA deacylase activity of *M. laidlawii* and *M. gallisepticum* is essentially nil (<0.02 μmole of acetyl CoA loss per milligram of protein per min) as determined by the method of Gergely (1955), and that of *M. hominis* is relatively great (VanDemark and Smith, 1964).

Acetoacetyl CoA formation. Three possible mechanisms of acetoacetyl CoA formation were examined in *M. laidlawii* and *M. gallisepticum:* (i) the transfer of CoA from acetyl CoA to butyrate by coenzyme A transphorase with the presumed subsequent oxidation of the butyryl CoA via the fatty acid oxidative pathway; (ii) the transfer of coenzyme A from succinyl CoA to acetoacetate by coenzyme A transferase; (iii) synthesis from 2 moles of acetyl CoA by β-ketothiolase. *M. hominis* has previously been shown to be able to form acetoacetyl CoA by reactions i and iii (VanDemark and Smith, *unpublished data*). Table 3 presents the evidence for the existence of coenzyme A transphorase and transferase in *M. gallisepticum*, and for the existence of only coenzyme A transferase in *M. laidlawii*. Coenzyme A transferase activity in both organisms is equivalent by two different methods. The β-ketothiolase activities of *M. laidlawii* and *M. gallisepticum* are shown in Fig. 1. These data represent cleavage of acetoacetyl CoA with the assumption that the enzyme in *Mycoplasma* carries out the reverse reaction as in other systems. The enzyme of *M. laidlawii* was inhibited about 50% by 5×10^{-4} M iodoacetamide, indicating its probable sulfhydryl nature. However, the enzyme of *M. gallisepticum* was unaffected

by this level of iodoacetamide, a result which gave rise to difficulty in demonstrating HMG CoA condensing enzyme in this strain. All three strains used in this study exhibited at least two mechanisms for the formation of acetoacetyl CoA.

Formation of β-hydroxy-β-methylglutaryl CoA. Demonstration of the condensation of acetyl CoA with acetoacetyl CoA was attempted by both

TABLE 3. *Phosphotransacetylase, coenzyme A transphorase, and coenzyme A transferase activities of Mycoplasma*

Enzyme tested*	Enzymatic activity	
	M. laidlawii strain B	*M. gallisepticum* strain J
Phosphotransacetylase (acetyl phosphate loss)	0.21†	11.14
Coenzyme A transphorase (butyryl CoA loss)	<0.001	0.022
Coenzyme A transferase (acetoacetyl CoA formation):		
Increase absorption at 310 mμ	0.017	0.015
Formation of *p*-nitroacetanilide	0.017	0.016

* Reaction conditions: Phosphotransacetylase: In each tube in a total volume of 1.0 ml were: 10 μmoles of Tris buffer (*p*H 8.0); 6 μmoles of acetyl phosphate; 0.1 μmole of CoA; 10 μmoles of cysteine; enzyme protein in amounts varying from 0.02 to 1.0 mg. After 5 min at 25 C, 50 μmoles of potassium arsenate were added, followed in 15 min by 500 μmoles of neutral hydroxylamine. Coenzyme A transphorase: per cuvette—75 μmoles of potassium arsenate; 30 μmoles of potassium acetate; 0.5 μmole of butyryl CoA; 2 units of *Aerobacter aerogenes* transacetylase, and 1.0 mg of enzyme protein. Blank cuvette lacked butyryl CoA, and control cuvette contained 30 μmoles of potassium butyrate in place of potassium acetate. Coenzyme A transferase: (i) Increase absorption at 310 mμ: per cuvette (in a total volume of 1.5 ml)—10 μmoles of Tris buffer (*p*H 8.0); 8 μmoles of $MgCl_2$; 100 μmoles of potassium acetoacetate; 0.003 μmole of CoA; 10 μmoles of glutathione; 1 μmole of succinyl CoA; 0.3 mg of enzyme protein. Blank cuvette lacked potassium acetoacetate. (ii) *p*-Nitroacetanilide formation: per cuvette (in a total volume of 1.5 ml)—10 μmoles of Tris buffer (*p*H 8.0); 8 μmoles of $MgCl_2$; 100 μmoles of potassium acetoacetate; 0.003 μmole of CoA; 10 μmoles of glutathione; 1 μmole of succinyl CoA; 0.1 μmole of *p*-nitroaniline; 1 mg of pigeon liver enzyme; 0.6 mg of enzyme protein. Control cuvette lacked *p*-nitroaniline.

† Numbers indicate μmoles of substrate used or product formed per milligram of protein per minute.

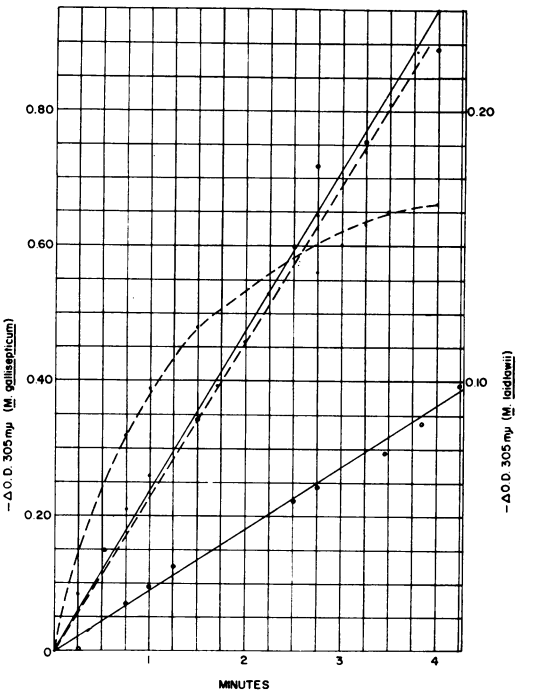

FIG. 1. *β-Ketothiolase activity of Mycoplasma and the effect of iodoacetamide. M. laidlawii without* (●- - -●) *and with* (○——○) *iodoacetamide; M. gallisepticum without* (●——●) *and with* (○- - -○) *iodoacetamide. Each cuvette contained (in a total volume of 1.5 ml): 200 μmoles of Tris buffer (pH 8.0); 8 μmoles of $MgCl_2$; 150 μmoles of coenzyme A; 10 μmoles of glutathione (pH 7.0); 0.5 μmole of acetoacetyl CoA; and 1 mg of enzyme protein. Blank cuvette contained 50 μmoles of potassium acetoacetate in place of acetoacetyl CoA.*

spectrophotometric and isotope-labeling methods. Fig. 2 shows that the results of the condensation reaction, as evidenced by change in rate of disappearance of acetoacetyl CoA, measured as loss of absorption of thiol ester bond at 310 mμ (Fig. 2). The initial rate represents spontaneous breakdown of acetoacetyl CoA at the alkaline *p*H of the reaction: the second rate results from the cleavage of acetoacetyl CoA by β-keto thiolase activity incompletely inhibited by iodoacetamide; and the final rate results from the condensation reaction occurring upon the addition of acetyl CoA. Only with *M. laidlawii* was such a result obtained. The thiolase of *M. gallisepticum* was not inhibited by the iodoacetamide resulting in such rapid cleavage of acetoacetyl CoA that no rate change could be observed upon addition of acetyl CoA. Thus, one could not determine whether the HMG CoA condensing enzyme was present in this latter organism by this method.

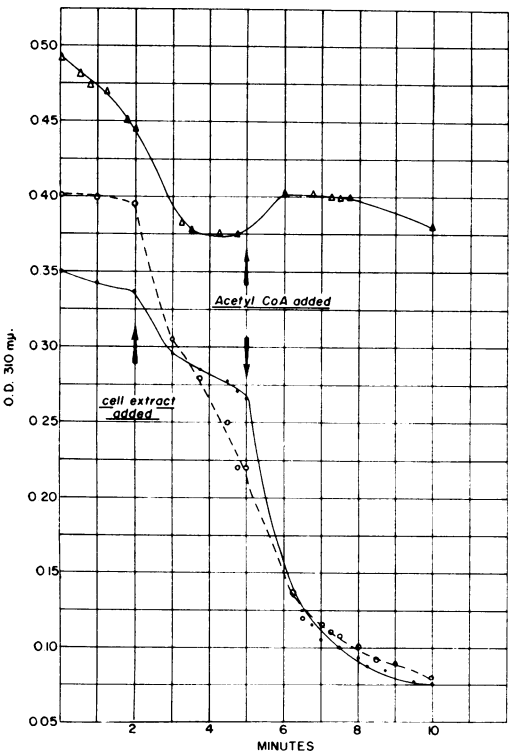

FIG. 2. *Evidence for β-hydroxy-β-methylglutaryl CoA condensing enzyme measuring disappearance of acetoacetyl coenzyme A upon addition of acetyl coenzyme A. Mycoplasma laidlawii* (●——●); *M. gallisepticum* (○- - -○); *M. hominis* (△——△). *Each cuvette contained (in a volume of 1.5 ml) 50 μmoles of Tris buffer (pH 8.0); 5 × 10⁻⁴ M iodoacetamide; 0.5 μmole of acetoacetyl CoA; and, at indicated times, 1 mg of enzyme protein and 0.25 μmole of acetyl CoA. Blank cuvette contained 50 μmoles of potassium acetoacetate in place of acetoacetyl CoA.*

No increase in rate of acetoacetyl CoA disappearance, indicative of a condensation reaction, was evident with *M. hominis* upon addition of acetyl CoA. The β-keto thiolase activity of this organism was effectively inhibited by iodoacetamide. The concentration of iodoacetamide used reportedly has no significant effect on the HMG CoA condensing enzyme (Rudney and Ferguson, 1951).

Confirmation of the spectrophotometric results indicating the condensation reaction in *M. laidlawii* was obtained by incubating cell-free extracts with acetoacetyl CoA and 1-C^{14} acetyl CoA, and measuring the amount of C^{14} incorporation into the HMG hydroxamate derived from the HMG CoA formed. Significant incorporation of C^{14} occurred only in the case of *M. laidlawii* (Table 4). Although it could be concluded from these experiments that *M. hominis* probably lacks the condensing enzyme, the failure to inhibit β-keto thiolase activity in *M. gallisepticum* renders the data for this organism inconclusive.

Another mechanism which could result in the synthesis of HMG CoA is the condensation of 2 moles of acetyl CoA with 1 mole of malonyl CoA and the liberation of carbon dioxide (Brodie et al., 1963). Attempts were made to demonstrate the incorporation of C^{14} acetyl CoA into HMG CoA with all three organisms used by incubating 5 μmoles of acetyl CoA-C^{14} prepared from acetic anhydride 1-C^{14} (specific activity, 2.73 mc), 2.5 μmoles of malonyl CoA, and about 10 mg of enzyme protein in 0.067 M potassium phosphate buffer (*p*H 7.0), and isolating the HMG CoA or HMG-enzyme complex synthesized by forming the hydroxamate. These attempts failed; thus, under the conditions of the test, this pathway does not exist in *Mycoplasma*.

Bachhawat et al. (1954, 1955) gave evidence for the formation of HMG CoA by incorporation of carbon dioxide into β-hydroxy isovaleryl CoA. Attempts were made to demonstrate the existence of this mechanism in the three strains of *Mycoplasma*. Isovaleryl CoA was used in place of β-hydroxy isovaleryl CoA, because the anhydride was available and the former compound gives rise to the latter in the overall pathway (Coon et al., 1955). None of the organisms incorporated

TABLE 4. *Incorporation of 1-C^{14} acetyl coenzyme A into β-hydroxy-β-methyl-glutaryl coenzyme A by condensation with acetoacetyl coenzyme A*

Experimental group*	*Mycoplasma laidlawii* strain B	*M. gallisepticum* strain J	*M. hominis* strain 07
	count/min	*count/min*	*count/min*
Test			
Acetohydroxamic acid..	214	460	700
HMG hydroxamic acid.	494	11	22
Control			
Acetohydroxamic acid.	780	280	359
HMG hydroxamic acid.	39	11	12

* Reaction conditions: 100 μmoles of Tris buffer (*p*H 8.0); 10 μmoles of $MgCl_2$; 5 μmoles of acetoacetyl CoA; 5 μmoles of acetyl CoA-C^{14} prepared from acetic anhydride (specific activity, 2.73 mc/mmole); 50 μmoles of cysteine; 5 × 10^{-4} M iodoacetamide; 10 mg of enzyme protein. After 60 min at 37 C, 5 ml of 2 M neutral hydroxylamine were added, and after 40 min more carrier HMG hydroxylamine and 15 ml of absolute ethanol were added. After drying at *p*H 4.0, the hydroxamates were extracted with ethanol and chromatographed.

significant amounts of C^{14} into the HMG CoA when 10 to 20 mg of protein were incubated for 1 hr at 37 C with 500 μmoles of tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 8.1), 20 μmoles of magnesium chloride, 25 μmoles of cysteine, 10 μmoles of ATP, 200 μmoles of potassium bicarbonate, 5 μc of sodium bicarbonate (specific activity, 10 mc/mmole) and 25 μmoles of isovaleryl CoA. However, *M. hominis* did incorporate significant radioisotope into unidentified products which were not reactive with hydroxylamine.

The inability to demonstrate the formation of HMG CoA by *M. gallisepticum* by enzymatic methods necessitated the attempt to show incorporation of acetate-*2*-C^{14} into mevalonic acid. The reaction was carried out with intact cells of all three organisms as described in Materials and Methods. Successful demonstration of acetate incorporation into mevalonic acid was made with *M. laidlawii* and *M. gallisepticum*. No incorporation occurred with *M. hominis* (Table 5). It was concluded that the failure to inhibit β-keto thiolase in *M. gallisepticum* masked or prevented enzymatic demonstration of the HMG CoA formation. However, acetate incorporation into mevalonic acid would indicate that the condensing enzyme is present.

β-Hydroxy-β-methylglutaryl coenzyme A reductase. The final step(s) in the synthesis of mevalonic acid is the reduction of HMG CoA with reduced pyridine nucleotide, and release of coenzyme A, the reaction(s) being mediated by an HMG CoA reductase (Knappe et al., 1959). Figure 3 presents evidence for the existence of this enzyme in *M. laidlawii* and *M. gallisepticum*, and its absence in *M. hominis*. The specificity of this reductase for pyridine nucleotide was not examined, because of the extremely active reduced nicotinamide adenine dinucleotide ($NADH_2$) oxidase present in all preparations. Loss of absorption by $NADPH_2$ above that caused by the relatively weak $NADPH_2$ oxidase in the preparation gave a measure of reductase activity. The reductase could be effectively inhibited by 5×10^{-4} M iodoacetamide.

The block in the enzymatic pathway for the biosynthesis of terpenoids occurs at the level of the HMG CoA condensing enzyme in *M. hominis* explaining its growth requirement for sterol. Whether the other enzymes in this pathway beyond the reductase are present must await further investigation. On the other hand, the other sterol-requiring strain, *M. gallisepticum*, possesses a full complement of enzymes for the synthesis of mevalonic acid. Undoubtedly some other enzyme(s) in the biosynthetic pathway to terpenoids is absent. This subject is currently under investigation. The control organism in this study, *M. laidlawii*, which synthesizes carotenoids contains all the necessary enzymes of this pathway. The fact that terpenoids are common components of many bacterial species may indicate that *Mycoplasma* are related to bacteria, and that, during the course of their development, some might have lost all or part of the genetic material coding for the enzymes of terpenoid biosynthesis.

TABLE 5. *Incorporation of sodium acetate-2-C^{14} into mevalonic acid*

No. of crystallization	*Mycoplasma laidlawii* strain B	*M. gallisepticum* strain J	*M. hominis* strain 07
2nd	3,505*	356	19
3rd	1,581	245	18
4th	915	226	12
5th	716	254	—
6th	652	—	—
7th	670	—	—

* Numbers denote counts per minute per milligram of dibenzylethylene diamine salt of mevalonic acid.

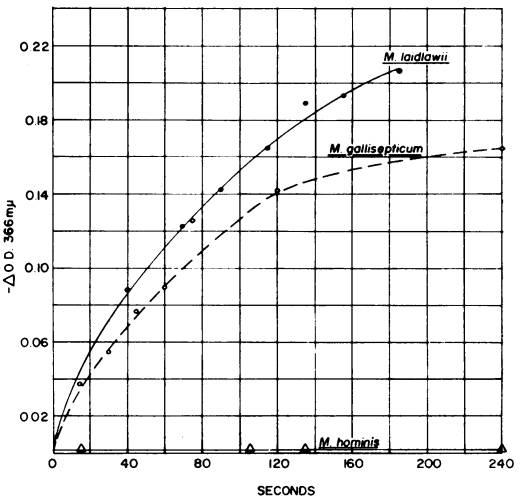

FIG. 3. *Loss of absorption by $NADPH_2$ as a result of reduction of β-hydroxy-β-methylglutaryl coenzyme A to mevalonic acid. Each cuvette contained (in a volume of 1.5 ml) 100 μmoles of potassium phosphate buffer (pH 7.0); 30 μmoles of sodium thioglycolate; 10 μmoles of potassium ethylenediaminetetraacetate; 20 μmoles of $NADPH_2$; 1 μmole of β-hydroxy-β-methylglutaryl CoA, and 1 to 4 mg of enzyme protein. Blank cuvette lacked HMG CoA.*

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