GLUCOSE METABOLISM OF TWO STRAINS OF *MYCOPLASMA LAIDLAWII*

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ABSTRACT

CASTREJON-DIEZ, JAIME (Tulane University School of Medicine, New Orleans, La.), THELMA N. FISHER, AND EARL FISHER, JR. Glucose metabolism of two strains of *Mycoplasma laidlawii*. J. Bacteriol. **86**:627–636. 1963.—Two strains of *Mycoplasma laidlawii* were incubated in systems containing D-glucose-C^{14}; carbon dioxide, acetate, pyruvate, and lactate were isolated from appropriate fluids after resting-cell and growth experiments. In resting-cell experiments, radioactivity recoveries were shown to be 95% for *M. laidlawii* A and 89% for *M. laidlawii* (Adler). By growth studies, the radioactivity recovery for *M. laidlawii* A was 83% and for *M. laidlawii* (Adler) was 90.5%. Low specific activities of the products as compared with the specific activity of glucose suggested cellular pools, or that the dissimilation of other substances present in the complex growth medium yielded products which contributed to the dilution factors. Enzyme studies added support to the hypothesis that glycolysis is operative in these organisms. Experiments with D-glucose-*1*-C^{14} or D-glucose-*6*-C^{14} as substrate suggested that the hexose monophosphate shunt may be functional in *M. laidlawii* (Adler), particularly since glucose-6-phosphate dehydrogenase, ribose-5-phosphate isomerase, and transketolase were demonstrated. This pathway is absent in *M. laidlawii* A.

Because of difficulties experienced in growing and obtaining large cell yields of pleuropneumonia-like organisms (PPLO), studies regarding their metabolic activities have been somewhat restricted. Despite these limitations, pioneer investigators in the field of PPLO carbohydrate biochemistry (Dujardin-Beaumetz, 1900; Rodwell and Rodwell, 1954*a*, *b*, *c*) have reported numerous findings of significance. *Mycoplasma mycoides* was shown to accumulate acetate in growth fluids when glucose served as substrate (Dujardin-Beaumetz, 1900). In addition, resting cells tested aerobically were found to oxidize glucose, glycerol, pyruvate, and lactate to acetate and CO_2 (Rodwell and Rodwell, 1954*a*, *b*, *c*), while anaerobically the dismutation of pyruvate yielded lactate, acetate, and CO_2. In aerobic experiments, carbon recovery from glucose was 95%; anaerobically, 85% of the pyruvate could be accounted for in products. Finally, hexokinase and aldolase, two key enzymes of the glycolytic pathway, were found to be present in cell-free extracts of *M. mycoides* (Rodwell and Rodwell, 1954*c*). Recent data obtained from studies with *M. laidlawii* A (Neimark and Pickett, 1960) indicate that under growth conditions this organism dissimilates glucose to acetate and lactate although a carbon recovery of only 58% was reported, suggesting the possibility of additional products other than those listed or perhaps undetected assimilatory processes. Resting cells of *M. laidlawii* B (Tourtellotte and Jacobs, 1960) metabolized glucose to lactate, pyruvate, and acetate with an 89% recovery of carbon in the products.

When complex media are required for growth of microorganisms, theoretical carbon-recovery values are often realized with difficulty and with some question. It therefore seemed reasonable to calculate radioactivity recoveries following the dissimilation of C^{14}-labeled glucose for both resting-cell and growth-type studies, and to correlate these observations with the corresponding carbon data. The present report describes such experiments.

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MATERIALS AND METHODS

Organisms. Cultures of *M. laidlawii* A were obtained from D. G. Edward, Wellcome Research Laboratories, England. H. E. Adler, University of California at Davis, provided us with a nondocumented strain of *M. laidlawii.* The strains of *Mycoplasma* studied fulfilled criteria necessary for the characterization of these organisms (Freundt, 1958), namely, the production of acid from glucose, fructose, mannose, maltose, starch, dextrin, and glycogen.

Substrates and enzymes. C^{14}-labeled glucose was obtained from Nuclear-Chicago Corp., Des Plaines, Ill. Glucose-6-phosphate, fructose-1,6-diphosphate, and *Clostridium kluyveri* diaphorase were purchased from Mann Research Laboratories. Nicotinamide adenine dinucleotide (NAD^+), reduced NAD^+ (NADH), and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were supplied by Sigma Chemical Co., St. Louis, Mo.; glyceraldehyde phosphate dehydrogenase was obtained from Worthington Laboratories.

Resting-cell preparations. For resting-cell experiments, the organisms were grown for 24 hr at 37 C in a medium composed of Beef Heart Infusion broth (Difco), 1% PPLO Serum Fraction (Difco), plus thallium acetate (1:200) or penicillin (500 units/ml). To prepare adequate inocula for resting-cell studies, one colony of the appropriate PPLO was removed from solid medium and cultured for 3 days at 37 C in 10 ml of liquid medium (Beef Heart Infusion broth containing PPLO Serum Fraction). The entire suspension was then transferred to 100 ml of fresh medium as used above, and these cultures were incubated for 24 hr at 37 C. After the growth interval, the total 100-ml culture was transferred to flasks containing 1 liter of the same type of medium, and such test cultures were incubated for 24 hr as before. The number of 1-liter cultures harvested depended on the amount of PPLO growth required for experimental purposes. Cells were centrifuged in the cold at 13,000 $\times$ *g*, washed once, and suspended in 0.02 M tris(hydroxymethyl)aminomethane (tris) buffer (pH 7.5). Suspensions were adjusted to contain 250 mg (wet weight)/ml.

Resting-cell experiments. Fermentation studies using 25 μmoles of unlabeled glucose and 2 μc of D-glucose-C^{14} were carried out in stoppered tubes (150 $\times$ 18 mm). Cell preparations [250 mg (wet weight)] were suspended in 10 ml of 0.01 M KCl. Reaction mixtures were incubated in a water bath at 37 C for 30 min, after which metabolic CO_2 was collected by flushing CO_2-free air through the system. Metabolic CO_2 was then trapped in 5 ml of 5 M NaOH. The CO_2 was plated as $BaCO_3$ and was counted in a gas-flow ultrathin-window nuclear counter to determine radioactivity. In all isotope experiments, the data are expressed as specific activity which is equivalent to counts per min per μmole. The mixture was centrifuged, and the supernatant fluid was used for chemical analysis. Organic acids were separated by column and paper chromatography.

Growth experiments. Fermentation studies were performed in two types of culture media. One medium, TC 199 (Microbiological Associates), containing 1% peptone (Difco) and 1% PPLO Serum Fraction, was selected for its low lactic acid content; the second medium, consisting of tryptic digest broth with PPLO Serum Fraction and containing approximately 12 μmoles of lactate per ml, was helpful in radioactivity-recovery experiments. Since the second medium contained a high concentration of lactate, the acid could serve as a pool in which radioactive metabolic lactate might be trapped, thereby permitting more isotope to appear in the products rather than in components arising from assimilatory processes.

When TC 199 medium was used, 15 ml of this basal solution containing 2 μc of D-glucose-C^{14}, D-glucose-*1*-C^{14}, or D-glucose-*6*-C^{14} were added per 200-ml milk-dilution bottle. After each fermentation mixture was inoculated with a small piece of agar supporting one PPLO colony, cultures were incubated for 48 hr at 37 C. The routine procedure for fermentation studies in tryptic digest broth (Beef Heart, Difco) was as follows. Portions (95 ml) of medium containing 50 μc of D-glucose-C^{14} and 5 ml of inocula taken from a 48-hr culture were added to 500-ml Erlenmeyer flasks equipped with stoppered outlets. Closed systems were incubated for 24 hr at 37 C. Metabolic CO_2 was collected as described above. Cultures were centrifuged, and cells and supernatant fluids were saved for further chemical studies. Cells were hydrolyzed with alcoholic KOH and extracted with ether to isolate the nonsaponifiable lipids. The ether layer was removed and concentrated under nitrogen. Samples of the concentrated ether phase and the remaining KOH layer were plated, and radioactivity was determined.

Paper chromatography. Acids were identified

as the hydroxamate derivative by the method of Seubert (1960). The chromatograms were developed in *n*-butanol–acetic acid–water (4:1:5) and in water-saturated butanol. Ketoacids were identified as the 2,4-dinitrophenylhydrazone derivative by the method of Seligson and Shapiro (1952). In these procedures, 1-ml samples of supernatant fluids from resting-cell experiments or from fermentation fluids were tested. Pyruvate was detected by paper chromatography as the 2,4-dinitrophenylhydrazone derivative and separated in butanol–ethanol–0.5 N NH_4OH (7:1:2) and in butanol saturated with 3% NH_4OH. The quinoxalinol derivative of pyruvate was identified in chromatograms developed in methanol.

Column chromatography. Acids were quantitatively separated by Celite column chromatography by the method of Swim and Krampitz (1954) using samples of 1 ml per column. Samples (0.2 ml) from each fraction were plated and counted to determine radioactivity.

Chemical analysis. Glucose was determined by the oxidase test using the reagents of Worthington Laboratories, as described by Fisher and Fisher (1959). Lactate was measured according to the procedure of Hullin and Noble (1953); pyruvate was measured by the method of Friedmann and Haugen (1943). Ketopentoses were determined by the carbazole procedure of Dische and Borenfreund (1951), heptoses by the cysteine-sulfuric acid method of Dische (1953), and fructose by the method of Roe (1934). Protein was measured using the Folin-Ciocalteau reagent, according to the method described by Kabat and Mayer (1948).

Thunberg procedure. Dehydrogenase experiments with resting cells [25 mg of cells (wet weight)/tube] were carried out in Thunberg tubes, in a system containing 2 ml of 0.067 M phosphate buffer, 1 ml of 1:10,000 methylene blue, and 40 μmoles of substrate in 2 ml of water. Time of complete reduction from the moment the tube contents were mixed was recorded.

Preparation of cell-free extracts. Cell-free extracts were prepared by two methods. (i) To prepare sonic extracts, cells were washed once with 0.85% NaCl, resuspended in 0.02 M tris buffer (pH 7.5) at a concentration of 100 mg (wet weight) of cells/ml, and sonically treated for 15 min in a 10-kc Raytheon sonic oscillator. (ii) To prepare dodecyl sulfate-treated cells, cells were washed once with saline and resuspended in 0.02 M tris buffer; then 0.5% dodecyl sulfate was added. The preparation was kept in an ice bath for 15 min, and was dialyzed for 24 hr in tris buffer.

Enzymatic procedure. Hexokinase activity was measured colorimetrically by the method of Sols and Crane (1954). Since cell-free extracts had high adenosine triphosphatase activity, it was necessary to include 100 μmoles of fluoride in the reaction mixture to inhibit hydrolysis of adenosine triphosphate (ATP) by adenosine triphosphatase. It was then possible to measure hexokinase activity. Glucose, galactose, mannose, fructose, glucosamine, and acetylglucosamine, in 200-μmole amounts, were tested as substrates for this reaction. Triosephosphate dehydrogenase and aldolase were studied spectrophotometrically using 100 μmoles of tris buffer (pH 7), 1 μmole of arsenate, 100 μmoles of glyceraldehyde-3-phosphate or fructose-1,6-diphosphate, and 1.5 μmoles of NAD^+ plus 1 mg of protein as cell-free extract. The final volume was 3 ml, and the reaction was followed at 340 mμ in a Beckman DK lL recording spectrophotometer. The enzymatic activity of aldolase in crude extract was also measured colorimetrically (Bard and Gunsalus, 1950). Tests for lactic dehydrogenase were made spectrophotometrically at 340 mμ, measuring the conversion of pyruvate to lactate in the presence of 1 μmole of NADH. One unit is defined as a change of 0.01 in optical density per min at 340 mμ. The reverse reaction, whereby lactate is converted to pyruvate, was measured colorimetrically at 600 mμ when 2,6-dichlorophenol indophenol was added as hydrogen acceptor. One unit by this method was defined as a change of 0.01 in optical density per min at 600 mμ. Glucose-6-phosphate dehydrogenase was measured by using 20 μmoles of glucose-6-phosphate, 0.15 μmole of $NADP^+$, 5 units of *C. kluyveri* diaphorase, and 0.12 μmole of 2,6-dichlorophenol indophenol. Because of the presence of NADH and reduced $NADP^+$ (NADPH) oxidase in cell-free preparations, it was difficult to demonstrate the accumulation of reduced pyridine nucleotides. To resolve this problem, a diaphorase was included in the reaction mixture as well as 2,6-dichlorophenol indophenol, the latter serving as terminal hydrogen acceptor. Decrease in transmittance at 600 mμ was observed spectrophotometrically. Ribose-5-phosphate isomerase was measured by incubating 3 μmoles of ribose-5-phosphate, 50 μmoles of

TABLE 1. *Radioactivity recovery in experiments with resting cells of Mycoplasma laidlawii A using* D-*glucose-C*^{14}*

Compound	Total count		Product or substrate (*μmoles*)		Calculated CO_2	Specific activity	
	Disappeared	Produced	Determined	Calculated from C^{14} recovery		Theoretical†	Observed
Glucose	145,000		13.05				11,111
CO_2		1500	24.5	0.81		1852	61
Acetate		11,500	9.9	3.10	3.10	3704	1162
Pyruvate		10,750	4.4	1.93		5556	2443
Lactate		113,750	29.7	20.47		5556	3830
Total	145,000	137,500		74.21‡			
Recovery (%)		94.8		94.8	26.1		

* The system contained 250 mg of cells (wet weight), 100 μmoles of glucose, 2 μc of D-glucose-C^{14}, 100 μmoles of phosphate buffer (pH 7), 10 μmoles of $MgCl_2$, and 10 μmoles of KCl, in a total volume of 10 ml. The system was incubated for 30 min at 37 C.

† Theoretical values were calculated from the observed specific activity of the glucose carbon and the carbon content of the product.

‡ Expressed as μmoles of C.

cysteine, and 100 μmoles of $MgCl_2$ with 0.1 ml of cell-free extract containing 0.4 mg of protein. After 10 min of incubation, ketopentoses were determined. Accumulation of sedoheptulose and fructose was also observed after incubation of cell-free extracts with a mixture of ribose-5-phosphate and ribulose-5-phosphate. Transketolase was demonstrated by detecting the production of glyceraldehyde phosphate when cell-free extracts were incubated with ribose-5-phosphate; the substrate had been incubated for 24 hr at room temperature to allow isomerization. Glyceraldehyde phosphate was demonstrated by use of glyceraldehyde phosphate dehydrogenase, 1.5 μmoles of NAD^+, 5 units of *C. kluyveri* diaphorase, and 0.12 μmole of 2,6-dichlorophenol indophenol as hydrogen acceptor.

RESULTS

Resting-cell experiments. Resting-cell preparations of *M. laidlawii* A dissimilated D-glucose-C^{14} (Table 1). The C^{14}-labeled tracer was found in several compounds, including metabolic CO_2, lactate, pyruvate, and acetate. Related experiments with *M. laidlawii* Adler (Table 2) indicate that C^{14}-labeled tracer could be demonstrated in products similar to those listed above. In Fig. 1, the large acid peak of acetate was only slightly labeled, having a specific activity of 260 counts per min per μmole. Because of the presence of large acid peaks with small amounts of radioactivity, a carbon balance was thought to be unreliable; instead, a radioactivity-recovery balance was calculated. The recoveries of these experiments are reported in Tables 1 and 2. The data indicate that specific activities of the products are low when compared with theoretical specific activities. Probably the resting cells had endogenous reserve material which the organisms metabolized concomitantly with glucose dissimilation. The radioactivity balances show a good recovery, 89% for the Adler strain and 95% for *M. laidlawii* A. Very low CO_2 recoveries (26% for both strains) were obtained, suggesting the possibility of CO_2 fixation.

Growth experiments. In preliminary growth experiments, stationary cultures of *M. laidlawii* were found to use 4 μmoles of glucose per ml, while cultures incubated on the shaker removed 3.5 μmoles of glucose per ml from the medium. Cultures incubated on the shaker used 67.5% of the glucose present in the medium as compared with 77% glucose dissimilated by stationary cultures. Initially, the reaction of the medium was pH 7.55, but culture fluids of both strains dropped to pH 7.05 after 72 hr of incubation. When the two strains were grown at 37 C in a medium without glucose, the pH of the growth fluid changed from 7.55 to 7.80.

Growth experiments using 2 μc of D-glucose-C^{14} per culture were set up for the two strains of *M. laidlawii* in TC 199–peptone–PPLO Serum

TABLE 2. *Radioactivity recovery in resting-cell experiments with Mycoplasma laidlawii (Adler)**

Compound	Total count		Product or substrate (μmoles)		Calculated CO_2	Specific activity	
	Disappeared	Produced	Determined	Calculated from C^{14} recovery		Theoretical†	Observed
Glucose	266,400		24.0				11,100
CO_2		1725	32.0	0.93		1850	54
Acetate		13,500	53.9	3.65	3.65	3700	250
Pyruvate		13,250	17.6	2.39		5550	753
Lactate		207,000	55.0	37.30		5550	3764
Total	266,400	235,475			3.65		
Recovery (%)		88.4		88.4	25.5		

* The system contained 250 mg of cells (wet weight), 100 μmoles of glucose, 2 μc of D-glucose-C^{14}, 100 μmoles of phosphate buffer (pH 7), 10 μmoles of $MgCl_2$, and 10 μmoles of KCl, in a total volume of 10 ml. The system was incubated for 30 min at 37 C.

† Theoretical values were calculated from the observed specific activity of the glucose carbon and the carbon content of the product.

Fraction medium. After 48-hr growth intervals, it was found that both organisms produced three radioactive acids, acetate, pyruvate, and lactate (Fig. 2), in addition to labeled CO_2. Radioactive material was not isolated as neutral products. The radioactivity recovery in these experiments was 60% for both PPLO strains. These data suggested that some of the products of glucose dissimilation could be used by the cells in other pathways. To test this hypothesis, glucose, pyruvate, lactate, or acetate was included in the medium in 1% concentrations. Cells obtained from these cultures had a yellow pigment which had not been observed when cells were grown in the absence of

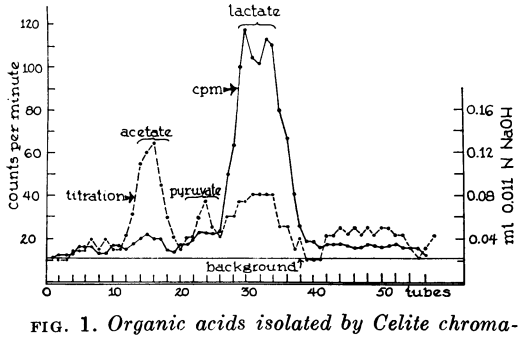

FIG. 1. *Organic acids isolated by Celite chromatography from 1 ml of supernatant fluid per resting-cell experiment. As substrate, 2 μc of D-glucose-C^{14} and 25 μmoles of unlabeled glucose were used. Cells [250 mg (wet weight) of Mycoplasma laidlawii (Adler)] were suspended in 10 ml of 0.01 M KCl in stoppered tubes and incubated for 30 min at 37 C before assay.*

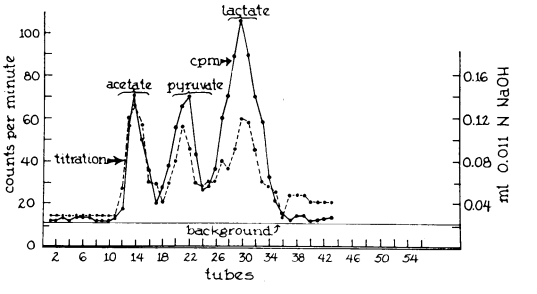

FIG. 2. *Organic acids isolated by Celite chromatography from 1 ml of supernatant growth fluid per experiment with Mycoplasma laidlawii (Adler). The culture medium consisted of TC 199, peptone, and PPLO Serum Fraction; 2 μc of D-glucose-C^{14} were added per 15 ml of culture medium.*

such substrates. The characteristics of this yellow compound agree with those described for the pigment isolated from *M. laidlawii* B by Rothblat and Smith (1961). In isooctane the pigment had absorption maxima at 412, 437, and 467 mμ, and absorption minima at 422 and 453 mμ.

Radioactivity recovery data (Tables 3 and 4) indicate that 83% of the C^{14} substrate for *M. laidlawii* A and 91% for *M. laidlawii* (Adler) could be accounted for in products. The small amount of radioactivity found in cells may be explained on the basis of internal pools or as assimilated cell constituents. The carotenoid pigment was also radioactive.

To study glucose metabolism in greater detail, fermentation experiments were performed using

TABLE 3. *Carbon recovery in growth studies with Mycoplasma laidlawii A**

Compound	Total count		Substrate	Product	Carbon (μmoles)	
	Disappeared	Produced			Substrate	Product
			μmoles	*μmoles*		
Glucose	7,561,619		177.6		1065.6	
CO_2		514,400		72.5		72.5
Acetate		1,741,825		123		246
Pyruvate		892,275		42		126
Lactate		2,862,250		135		405
Cell pools and assimilation		252,700		—†		35.8
Carotene		11,500		—†		1.6
Total --	7,561,619	6,273,950			1065.6	886.9
Recovery (%)		83				83

* The growth medium was composed of tryptic digest broth, 1% PPLO Serum Fraction, and 50 μc of D-glucose-C^{14}. The fermentation was started with a large inoculum and carried 24 hr at 37 C. The final volume was 100 ml. Values were calculated from data based on the radioactivity recovered in products as compared with the specific activity of glucose. The specific activity of the glucose carbon was 7095 counts per min per μmole of carbon.

† Values were not determined.

TABLE 4. *Carbon recovery in growth studies with Mycoplasma laidlawii (Adler)**

Compound	Total counts		Substrate	Product	Carbon (μmoles)	
	Disappeared	Produced			Substrate	Product
			μmoles	*μmoles*		
Glucose	5,782,794		134.1		804.6	
CO_2		545,500		76.0		76.0
Acetate		1,390,000		96.7		193.4
Pyruvate		0		0		0
Lactate		3,111,000		144.4		433.2
Cell pools and assimilation		155,500		—†		21.6
Carotene		20,800		—†		2.9
Total	5,782,794	5,222,800				727.1
Recovery (%)						90.5

* The growth medium was composed of tryptic digest broth, 1% PPLO Serum Fraction, and 50 μc of D-glucose-C^{14}. The fermentation was started with a large inoculum and carried 24 hr at 37 C. The final volume was 100 ml. Values were calculated from data based on radioactivity recovered in products as compared with specific activity of glucose. The specific activity of glucose carbon was 7187 counts per min per μmole of carbon.

† Values were not determined.

either D-glucose-*1*-C^{14} or D-glucose-*6*-C^{14}. Metabolic CO_2 collected from cultures of *M. laidlawii* A when D-glucose-*6*-C^{14} was substrate contained only 158 counts per min; CO_2 radioactivity values obtained in *M. laidlawii* (Adler) growth studies carried out under similar conditions were also low (90 counts per min). When D-glucose-*1*-C^{14} served as substrate, metabolic CO_2 of *M. laidlawii* A was labeled slightly; the CO_2 had a specific activity of 220, while *M. laidlawii* (Adler) showed a specific activity of 1340. When specific activities were compared, it was observed that CO_2 was labeled to a similar degree by *M. laidlawii* A with both glucose substrates. On the other hand, CO_2 produced by *M. laidlawii* (Adler) when D-glucose-*1*-C^{14} served as substrate had a considerably higher

specific activity than the CO_2 collected from the D-glucose-*6*-C^{14} fermentation (Tables 5 and 6).

Thunberg experiments with resting cells. The above experiments suggested the possibility that the hexose monophosphate shunt may be operative in *M. laidlawii* Adler, but not in *M. laidlawii* A. To investigate this possibility further, dehydrogenase activity was studied by the Thunberg technique, testing resting cells with various substrates. It was observed that methylene blue was rapidly reduced by resting cells of *M. laidlawii* (Adler) in the presence of glucose, but this could not be demonstrated by resting cells of *M. laidlawii* A under the same conditions (Table 7).

Enzymatic studies. Because resting-cell data strongly implicated glycolysis as an energy-yielding mechanism for these cells, studies were undertaken to determine the presence of key enzymes of that pathway. Hexokinase was measured colorimetrically as described above. With both organisms, hexokinase reacted strongly with glucose, moderately with galactose (Fig. 3), but not at all with fructose or mannose. The results for fructose and mannose are not shown, since the plot of this data would lie along the abscissa. It was observed that glucosamine served as substrate for a short time, after which no further activity could be detected. When glucose and glucosamine were tested together, there was a high initial rate which quickly leveled off, in contrast to the reaction with glucose alone in which the activity remained constant for some time. These data strongly suggest that the enzyme phosphorylates glucosamine, but in doing so it becomes irreversibly bound to the inhibitor (Fig. 4). Acetylglucosamine did not

TABLE 5. *Specific activities of products resulting from glucose metabolism of Mycoplasma laidlawii A in growth experiments with glucose labeled in various positions**

Compound	Specific activity					
	D-Glucose-C^{14}		D-Glucose-*1*-C^{14}		D-Glucose-*6*-C^{14}	
	Theoretical	Observed	Theoretical	Observed	Theoretical	Observed
Glucose		9200		12,900		9040
Carbon dioxide	1533	1050	0	220	0	158
Acetate	3066	2620	6450	2620	4520	1282
Pyruvate	4599	2030	6450	1990	4520	2899
Lactate	4599	3440	6450	2570	4520	4380

* Growth experiments were carried out in 15 ml of TC 199 medium containing 1% peptone and 1% PPLO Serum Fraction. The incubation time was 48 hr. Theoretical values were calculated from the determined radioactivity of the carbon in the glucose molecule. The position of labeling in the product is assumed on the basis of glycolysis of glucose.

TABLE 6. *Specific activities of products resulting from glucose metabolism of Mycoplasma laidlawii (Adler) in growth experiments with glucose labeled in various positions**

Compound	Specific activity					
	D-Glucose-C^{14}		D-Glucose-*1*-C^{14}		D-Glucose-*6*-C^{14}	
	Theoretical	Observed	Theoretical	Observed	Theoretical	Observed
Glucose		9200		12,900		9040
Carbon dioxide	1533	1250	0	1340	0	90
Acetate	3066	831	6450	4880	4520	1050
Pyruvate	4599	798	6450	1165	4520	1700
Lactate	4599	4093	6450	4150	4520	3420

* Growth experiments were carried out in 15 ml of TC 199 medium containing 1% peptone and 1% PPLO Serum Fraction. The incubation time was 48 hr.

TABLE 7. *Dehydrogenase activity of whole cells**

Substrate	*Mycoplasma laidlawii* A	*M. laidlawii* (Adler)
	min	*min*
Control	120+†	120+†
Glucose	120+†	22
Lactate	60	60
Pyruvate	95	80
Malate	109	120+†

* Results are given as the length of time required for complete reduction of methylene blue. The experiment was carried out by the conventional Thunberg technique with 25 mg of cells (wet weight)/tube. The system contained 130 μmoles of phosphate (pH 7), 40 μmoles of substrate, 1 ml of 1:10,000 methylene blue, and cells, in a total volume of 6 ml. Control tubes with water instead of cells were also included.

† No reduction after 120 min of incubation.

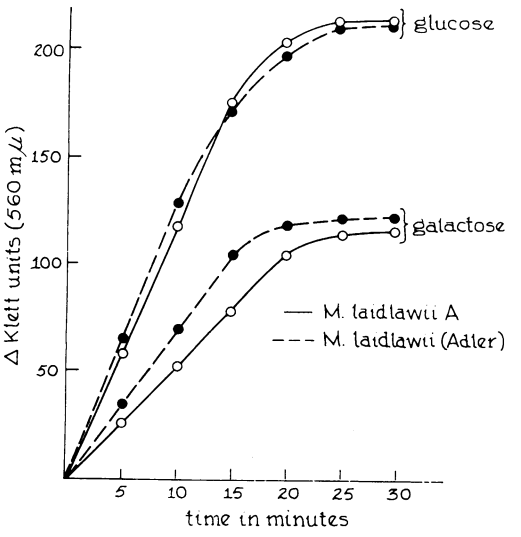

FIG. 3. *Hexokinase activity of two strains of Mycoplasma laidlawii. The reaction mixtures, adjusted to pH 7.2, contained 25 μmoles of adenosine triphosphate, 50 μmoles of $MgCl_2$, and 0.003% bromothymol blue. To start the reaction, 100 μmoles of NaF, 200 μmoles of substrate, and 0.1 ml of cell-free extract containing 0.2 mg of protein were added. The final volume was 10 ml.*

serve as substrate for the enzyme, and when mixed with glucose it did not inhibit the reaction.

Triosephosphate dehydrogenase and aldolase activities of the two PPLO strains were measured. Both extracts gave a rapid initial rate when glyceraldehyde-3-phosphate or fructose-1,6-diphosphate were used as substrates. The two organisms also gave positive reactions for aldolase when tested colorimetrically. In the latter reaction, *M. laidlawii* A produced 168 μg of alkali-labile phosphorus per hr per mg of protein, and *M. laidlawii* Adler produced 88 μg of phosphorus per hr per mg of protein. Lactic dehydrogenase activity was checked by two methods. By the reaction measuring conversion of pyruvate to lactate, the specific activity of this enzyme was found to be 11.3 units per mg of protein for *M. laidlawii* A and 11.0 units per mg of protein for the Adler strain. With the colorimetric procedure, the specific activity observed was 11.9 units for strain A and 4.5 units per mg of protein for the Adler strain.

That the hexose monophosphate shunt was operative in *M. laidlawii* (Adler) was strongly suggested by experimental data mentioned above. To explore this possibility further, assays of certain key enzymes of this pathway were made. Glucose-6-phosphate dehydrogenase was demonstrated in crude cell-free extracts and was found to be $NADP^+$-specific. Transketolase was shown to be present (Fig. 5) in these preparations by the procedure described in Materials and Methods.

Ribose-5-phosphate isomerase was studied, and it was observed that ketopentoses increased as the volume of the cell-free preparation increased (Fig. 6). A mixture of ribose-5-phosphate and ribulose-5-phosphate was incubated with varying volumes of cell-free extract. Heptose and fructose accumulated (Table 8). The cell-free extract of *M. laidlawii* (Adler) had appropriate enzymatic activities, supporting the contention that the hexose monophosphate shunt may be operative in this organism since important enzyme components of the shunt are present.

DISCUSSION

The data presented indicate that, during glucose dissimilation by resting-cell suspensions of two strains of *M. laidlawii*, metabolism of endogenous materials as well as glucose occurs simulta-

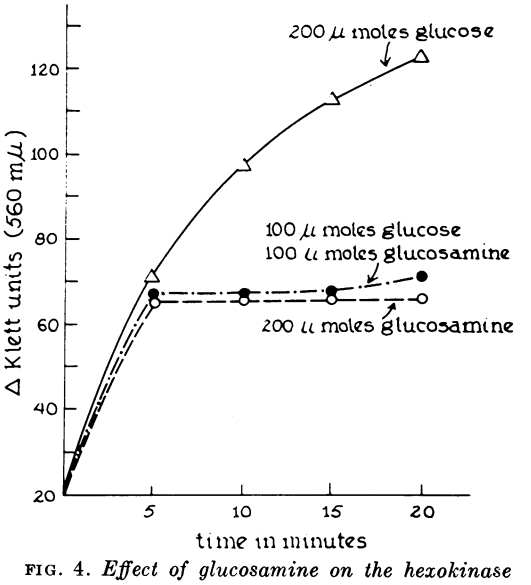

FIG. 4. *Effect of glucosamine on the hexokinase reaction of Mycoplasma laidlawii (Adler). The reaction mixtures, adjusted to pH 7.2, contained 25 μmoles of adenosine triphosphate, 50 μmoles of $MgCl_2$, and 0.003% bromothymol blue. To start the reaction, 100 μmoles of NaF, 100 or 200 μmoles of substrate or inhibitor, or both (as indicated), and 0.1 ml of cell-free extract containing 0.2 mg of protein were added. The final volume was 10 ml.*

neously. This fact is observed by the dilution of radioactivity of glucose-dissimilation products such as CO_2, lactate, acetate, and pyruvate. These products have lower specific activities than might be expected when one considers the original specific activity of glucose. In experiments with both organisms, very low CO_2 recoveries suggest CO_2 fixation by these PPLO.

Enzymatic studies with crude cell-free extracts indicate that glucose metabolism of the saprophytic PPLO is very similar to that reported by Rodwell and Rodwell (1954*a*) for *M. mycoides*. Glucose is presumably dissimilated to pyruvate via glycolysis, pyruvate being decarboxylated to acetate and CO_2. *M. laidlawii* A and *M. laidlawii* (Adler) dissimilate glucose to lactate, while for the most part pyruvate, acetate, and CO_2 may arise as a result of dissimilation of endogenous pools in the cells. Although glycolysis appears to be important to the saprophytic *Mycoplasma* for energy-yielding purposes, this may not be their only method of glucose degradation.

The recovery of radioactive CO_2 from D-glucose-*1*-C^{14} and from D-glucose-*6*-C^{14} suggests that there may be a mechanism for oxidation of acetate; however, this may not be very active under the conditions employed. In the case of *M. laidlawii* (Adler), the high specific activity of CO_2

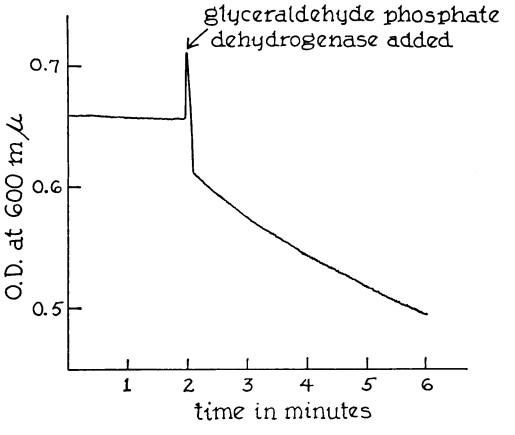

FIG. 5. *Transketolase activity of cell-free extracts of Mycoplasma laidlawii (Adler). The assay system contained 4 μmoles of ribose-5-phosphate, 10 μmoles of $MgCl_2$, 20 μg of cocarboxylase, 0.12 μmole of 2,6-dichlorophenol indophenol, 5 units of Clostridium kluyveri diaphorase, 0.3 μmole of NAD^+, and cell-free extract containing 0.4 mg of protein. After 10 min of incubation, 0.2 mg of glyceraldehyde phosphate dehydrogenase was added.*

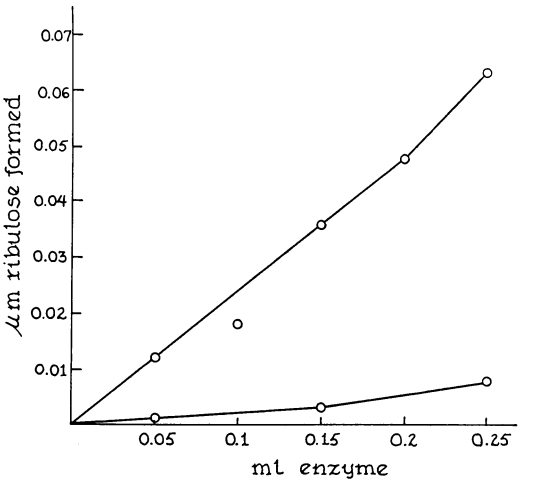

FIG. 6. *Ribose-5-phosphate isomerase activity of cell-free extracts of Mycoplasma laidlawii (Adler). The assay system contained 3 μmoles of ribose-5-phosphate, 100 μmoles of $MgCl_2$, 50 μmoles of cysteine, and varying volumes of cell-free extract containing 4 mg of protein/ml. The final volume was 1 ml, and the incubation time was 10 min. The controls were heated 30 min in a boiling-water bath. The bottom curve is a plot of the control results.*

TABLE 8. *Transketolase-transaldolase activities of extracts of Mycoplasma laidlawii (Adler)**

Volume of cell-free extract	Product	
	Heptose	Fructose
ml	*μmole*	*μmole*
0.1	0.01	0.22
0.15	0.02	0.31
0.2	0.035	0.50

* The system contained tris buffer (pH 7.5), 50 μmoles of cysteine, 100 μmoles of $MgCl_2$, 3 μmoles of a mixture of ribose-5-phosphate and ribulose-5-phosphate, and 0.1 to 0.2 ml of cell-free extract, in a total volume of 1 ml. The cell-free extract contained 4.1 mg of protein per 1-ml sample. The system was incubated for 10 min at 37 C.

when D-glucose-*1*-C^{14} was metabolized and the rapid reduction of methylene blue in the presence of glucose suggest that the hexose monophosphate shunt mechanism may be operative. This hypothesis is further substantiated by the demonstration of glucose-6-phosphate dehydrogenase, ribose-5-phosphate isomerase, and transketolase present in cell-free extracts of this organism. The fact that fructose accumulates when cell-free ex-

tracts are incubated with a mixture of ribose-5-phosphate and ribulose-5-phosphate also indicates that the shunt mechanism is present in this strain.

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