Kinetics of Utilization of Organic Substrates by *Mycoplasma mycoides* subsp. *mycoides* in a Salts Solution: a Flow-microcalorimetric Study

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The metabolism of various organic substrates by suspensions of *Mycoplasma mycoides* subsp. *mycoides* in a salts solution was followed by microcalorimetry. Enthalpy changes associated with metabolism were in good agreement with theoretical values. Substrate utilization showed Michaelis kinetics, allowing saturation constants (K_m) and maximum specific rates of substrate utilization (V_{max}) to be determined. In cells grown on a complex medium containing glucose, K_m values were: glucose, fructose, *N*-acetylglucosamine, glycerol and pyruvate, $<5\ \mu\text{M}$; lactate, $20\ \mu\text{M}$; glucosamine, $130\ \mu\text{M}$, and mannose, $1\ \text{mM}$. Values of V_{max} for glycerol, pyruvate and lactate were similar and approximately twice those for glucose, mannose, glucosamine and *N*-acetylglucosamine; V_{max} for fructose was one-quarter of that for glucose. In cells grown on complex medium in which glucose was replaced by mannose, glucosamine or *N*-acetylglucosamine, V_{max} and K_m for the respective growth sugars and for glucose were not significantly affected. However, in cells grown in the presence of fructose, V_{max} for fructose increased to the value observed for glucose. It is suggested that *M. mycoides* is adapted to, and is constitutive for, the utilization of a single sugar (glucose), and a single amino sugar (*N*-acetylglucosamine), but that in the presence of fructose a fructose-utilizing pathway is induced.

INTRODUCTION

Mycoplasma mycoides subsp. *mycoides* is the causative agent of contagious bovine pleuropneumonia, a disease of economic significance in many African countries, India and elsewhere (Gourlay & Howard, 1979).

A fermentable sugar is essential for the growth of *M. mycoides* (Rodwell & Mitchell, 1979), which has been reported to use glucose, fructose, mannose, maltose, trehalose, glycogen or starch (Rodwell & Mitchell, 1979; Smith & Oliphant, 1982). However, in a medium without serum, we have shown that *M. mycoides* subsp. *mycoides* strain T₁ utilizes glucose, mannose and fructose, and that in pig or calf serum medium the apparent utilization of di- and polysaccharides of glucose is due to hydrolysis to glucose by serum enzymes (Miles & Lee, 1983). Glucosamine and *N*-acetylglucosamine may also replace glucose in media for *M. mycoides* strain T₁ (Miles & Lee, unpublished results). There is evidence that sugar transport in *Mycoplasma* spp. involves a phosphoenolpyruvate-dependent phosphotransferase system (PTS; Cirillo & Razin, 1973).

Resting cell suspensions of *M. mycoides* oxidize glucose, glycerol, pyruvate and lactate quantitatively to acetate and carbon dioxide (Rodwell & Rodwell, 1954); intermediate compounds of the tricarboxylic acid cycle, sugars other than glucose, fructose and mannose, and amino acids other than threonine and serine are not metabolized (Rodwell, 1967). Glycerol oxidation requires the presence of catalase and involves a flavoprotein-mediated oxidation to triose phosphate with concomitant formation of hydrogen peroxide (Rodwell, 1967).

Abbreviation: PTS, phosphotransferase system.

The measurement of heat production is a direct and sensitive method of observing the metabolic processes of micro-organisms, and microcalorimetry has been widely applied in studies of microbial growth, cell-drug interactions, etc. (Spink & Wadso, 1976; Beezer, 1980); it may be of potential value in growth studies of mycoplasmas (Ljungholm *et al.*, 1976).

In the work reported here we have investigated the effect on heat output of adding single substrates to *M. mycoides* strain T₁ grown in the presence of various sugars and suspended in a salts solution. The calorimetric data should allow determination of (1) the effect of substrate concentration on its rate of utilization, (2) maximum rates of substrate utilization, (3) associated enthalpy changes and (4) whether growth on a particular sugar leads to increased rates of utilization of that sugar, i.e. whether adaptation takes place. To ensure comparability between experiments the cells used in this study were stored in liquid nitrogen and for each growth-sugar were from a single batch of cells. The advantages of using liquid nitrogen stored cells in studies of this kind have been discussed previously (Beezer *et al.*, 1976).

METHODS

Organisms. *Mycoplasma mycoides* subsp. *mycoides* strain T₁ was kindly provided by Dr G. R. Smith, Nuffield Laboratories of Comparative Medicine, Regents Park, London, UK. This strain is a mild or attenuated strain and is widely used as a live vaccine in East Africa (Hudson, 1971).

Media. The growth medium consisted of (g l⁻¹ except where stated): tryptose (Oxoid), 25; yeast extract (Oxoid), 5; glycerol, 5; Na₂HPO₄, 8.1; KH₂PO₄, 1.9; NaCl, 2.5; benzylpenicillin, 0.125; glucose, 5; pig serum (Gibco, UK) inactivated at 56 °C for 30 min, 100 ml l⁻¹. Benzylpenicillin and glucose were sterilized by membrane filtration (pore size 0.22 µm); other components, except serum, were autoclaved after adjustment of the pH value to 7.6. The final pH value was 7.6. Where appropriate, glucose was replaced by filter-sterilized mannose, fructose, glucosamine or *N*-acetylglucosamine. The characteristics of growth of *M. mycoides* in this medium have been described elsewhere (Miles, 1983).

Viable counts. A surface drop-plate method (Postgate, 1969) was used for viable counts, with a solid medium of blood agar base (Oxoid), 30 g l⁻¹; glycerol, 10 g l⁻¹; pig serum, 240 ml l⁻¹ and benzylpenicillin, 0.125 g l⁻¹. Dilutions were prepared in one-quarter strength Ringer's solution (NaCl, 39 mM; KCl, 1.4 mM; CaCl₂, 1.1 mM; NaHCO₃, 0.60 mM; pH 7.0). Plates were incubated at 37 °C for 7 d.

Harvesting and storage of inocula. Samples (1 ml) of a liquid nitrogen stored stationary phase (72 h) glucose-grown culture were inoculated into 220 ml volumes of medium in 250 ml disposable tissue culture bottles (Sterilin). After 48 h static incubation at 37 °C, the cells were centrifuged (14 000 *g*, 30 min) at 4 °C, resuspended in half their original culture volume of one-quarter strength Ringer's solution containing 1.5 M-glycerol, and dispensed in 2 ml samples into 12 × 35 mm polypropylene screw-in cap ampoules (Sterilin). Ampoules were cooled to -90 °C at a rate of 15 °C min⁻¹, and then cooled rapidly to -196 °C in liquid nitrogen according to the procedure of Beezer *et al.* (1976). At the time of harvesting, cultures had an OD₅₄₀ (EEL colorimeter) of 0.06 to 0.10 and viable counts of 1 to 2 × 10⁹ c.f.u. ml⁻¹ depending on the growth-sugar. An OD₅₄₀ of 0.10 was equivalent to 0.13 g dry wt of cells l⁻¹.

Calorimetric incubations. The calorimeter used was a multichannel conduction calorimeter (prototype of the 2277 Bioactivity Monitor, LKB Produkter, Suurkuusk & Wadso, 1982) operated in the flow-through mode at a flow rate of 20 ml h⁻¹. The volume of the flow cell was 0.6 ml and calibration was by internal heaters. The long-term base-line stability of this instrument is ±0.1 µW (Suurkuusk & Wadso, 1982).

Flow lines were disinfected with ethanol (15 min) and washed with sterile distilled water (4 × 15 min). Incubations were done in sterile acid-washed 50 ml glass vessels with air condensers. Vessels were maintained at 37 °C in a water bath and contained 25 ml of magnetically-stirred medium of composition (g l⁻¹): Na₂HPO₄, 8.1; KH₂PO₄, 1.9; NaCl, 2.5; MgCl₂, 0.04; CaCl₂, 0.03; MnCl₂, 0.00015; ZnSO₄, 0.001; CuSO₄·5H₂O, 0.0005; FeSO₄·7H₂O, 0.001; benzylpenicillin, 0.125; and 160 U catalase (Sigma C-10) ml⁻¹. After a stable base line had been achieved in this medium, a liquid nitrogen stored ampoule was thawed in a water bath (4 min, 37 °C), and 1.5 ml of the contents was centrifuged (MSE Microcentaur; 11 000 *g*, 4 min), washed and resuspended in 1 ml of the salts solution plus catalase described above, and inoculated into the incubation vessel. In all cases viable counts of thawed cell suspensions were >85% of those before freezing.

Freshly prepared solutions of substrate were added 1 h after inoculation unless stated otherwise. No heat effects were observed on adding substrates (at the concentrations used here) to uninoculated salts solution. All experiments were done at least twice.

RESULTS

The power-time (*p-t*) curve of glucose grown cells inoculated into salts plus catalase medium is shown in Fig. 1. There was an immediate peak of power (5.0 to 6.0 µW), followed by a decline to a low value (approximately 0.5 µW) which was maintained for several hours. There was no

Fig. 1. Representative p - t curves to show the effect of glucose and mannose on the power output of glucose-grown cells suspended in salts solution at a concentration of $6.7 \text{ mg dry wt l}^{-1}$. Cells were inoculated at 0 h. Curve (a) was the control (no sugar added). Glucose additions were (b) 20 mM at 1 h, (c) 2 mM at 1 h, (d) 20 μM at 1 h and 2 mM at 2.5 h and (e) 10 μM at 1 h and 20 μM at 2 h. Mannose additions were (f) 0.4 mM at 1 h, 0.8 mM at 1.5 h and 2 mM at 2 h.

change in viable count during the first 6 h of incubation. We interpret this peak as being due to metabolism of residual substrates, either intracellular, or extracellular and not removed during washing of cells after thawing. Glycerol may be the most important residual substrate as (1) it was used as a cryoprotectant during freezing at a high concentration (1.5 M), (2) maximum power output was similar to that after addition of glycerol and (3) the decline in power from the peak was similar to that after exhaustion of added glycerol.

After the decline of power to $0.5 \mu\text{W}$, addition of glucose to a concentration of 2 mM resulted in a rise in power output to a plateau value ($3.0 \mu\text{W}$) which was maintained for approximately 2 h before a gradual decline. At a higher concentration of glucose (20 mM) the decline was more rapid and occurred earlier (Fig. 1, Table 1). Plateau values were observed from approximately 8 min after addition of glucose; this time will reflect the time required for glucose to reach the calorimeter (4 min) and for the concentration in the calorimeter vessel to rise, as well as any time required for the cells to adapt to a maximum rate of metabolism.

When the initial concentration of added glucose was reduced to 20 or 10 μM , the plateau value reached was not reduced. However, 1.5 and 1 h respectively, after the addition of glucose, power output had declined to the value observed before addition (Fig. 1). Further addition of glucose caused power output to return to the plateau value (Fig. 1). It is clear, therefore, that after addition of small quantities of glucose the return of power output to that observed before addition was due to exhaustion of glucose.

For the period of glucose utilization (initial glucose concentration 10 or 20 μM), total heat production (Q_T), and hence the enthalpy change for glucose metabolism (ΔH_{met}), was estimated by graphical integration of the area under the p - t curve, assuming that power output at the plateau value started at the time of addition of glucose. If it is further assumed that the quantity of heat evolved (Q_i) at any time (t) is proportional to the glucose consumed, then glucose concentration at that time will be $(Q_T - Q_i) S_i / Q_T$, where S_i is the initial glucose concentration. Also, the rate of substrate utilization (V) at any time will be $dQ \cdot S_i / Q_T \cdot dt$, where dQ/dt is power output.

Using these relationships, double reciprocal plots of glucose concentration against rate of glucose utilization were made (e.g. Fig. 2). These plots were linear in the range 0 to 10 μM , suggesting that glucose metabolism follows Michaelis kinetics, and allowing determination (Table 2) of a maximum rate of glucose utilization (V_{max}) from the intercept on the ordinate axis ($1/V_{\text{max}}$), and a saturation constant (K_m) from the intercept on the abscissa ($-1/K_m$).

Table 1. *Increase in power output and specific rate of substrate utilization after addition of various substrates to M. mycoides*

Glucose-grown cells stored in liquid nitrogen were inoculated into a salts solution (final cell concentration $6.7 \text{ mg dry wt l}^{-1}$), and after 1 h substrate was added. In calculating the rate of substrate utilization, experimentally determined values of ΔH_{met} (Table 2) were used; it was assumed that the values of ΔH_{met} for mannose and glucosamine were the same as for glucose and *N*-acetylglucosamine respectively.

Substrate	Concn (mM)	Increase in power output (μW) after substrate addition			Rate of substrate utilization at 0.2 h [$\mu\text{mol (g dry wt cells)}^{-1} \text{ min}^{-1}$]
		0.2 h	1.0 h	2.0 h	
Glucose	20	3.1	1.2	0.5	42
	2	3.0	3.0	2.4	41
Fructose	20	3.0*	1.0	1.0	40*
	2	1.4*	1.1	1.1	18*
Mannose	20	4.2	4.1	4.0	57
	2	3.5	3.2	3.0	48
Glucosamine	2	3.5	3.5	2.5	49
<i>N</i> -Acetylglucosamine	2	3.2	3.0	3.0	45
Glycerol	40	6.3	6.3	6.3	124
	4	6.3	6.2	6.2	124
Pyruvate	4	2.2	2.2	2.1	116
Lactate	4	10.5	5.6	2.4	357
	0.4	3.5	3.5	3.4	119

* Measured at the transient peak of power (0.15 h), and probably due to glucose contamination of fructose (see text).

When glucose was replaced by 2 mM-*N*-acetylglucosamine, glucosamine or mannose, or 4 mM-glycerol or sodium pyruvate, power output reached a plateau value which was comparable with that obtained for 2 mM-glucose and which was maintained for 1 h or more (Table 1).

Fructose (2 and 20 mM) gave a transient peak of power followed by a low plateau (Table 1). When, during this plateau, further equal additions of fructose were made, similar transient peaks followed before power output returned to the same plateau value. These transient peaks were attributed to glucose contamination of fructose which, although below 0.05% (w/w), would be significant in this system. The low plateau of power output was attributed to fructose metabolism and was obtained at low concentrations of fructose (200 and 20 μM) where the transient peak of power was absent.

Sodium L-lactate (4 mM) gave a peak of power output (10.5 μW), which was greater than that for other substrates, followed by a gradual decline (Table 1); however, lower levels of sodium lactate (0.4 mM) gave plateaus of power comparable with that for glucose.

As with glucose, low concentrations of *N*-acetylglucosamine (20 μM) or glycerol, sodium lactate or sodium pyruvate (40 μM) gave *p-t* curves which showed a plateau of power followed by a decline to the power output obtained before substrate addition. This decline was due to substrate exhaustion, as further addition of substrate caused power output to return to the plateau value; thus Q_T and hence ΔH_{met} could be determined in the same way as for glucose (Table 2). Also, double reciprocal plots of substrate concentration against rate of substrate utilization were linear (Fig. 2), allowing determination of K_m and V_{max} (Table 2).

The rate of utilization of fructose was too low to allow determination of Q_T , K_m or V_{max} by the procedure used for glucose. However, by doubling the organism concentration, plateau values of power output of 0.9, 1.8 and 2.4 μW were obtained at fructose concentrations of 2, 10 and 200 μM respectively, thus allowing estimation of K_m and V_{max} values for fructose (Table 2). In calculating V , it was assumed that the ratio of S_i/Q_T was the same as that observed in fructose-grown cells (see below).

In contrast to the results obtained with other sugars, the effect of low concentrations (20 μM) of mannose or glucosamine on power output was barely detectable. With these sugars, plateau

Fig. 2. Double reciprocal plots of substrate concentration versus rate of substrate utilization by cells suspended in a salts solution. The data were derived, as described in the text, from representative p - t curves of glucose-grown cells with glucose ($\circ$), N -acetylglucosamine ($\square$), glycerol ($\blacktriangledown$), mannose ($\bullet$) and glucosamine ($\blacksquare$) as substrates, and of fructose-grown cells with fructose ($\blacktriangle$) as substrate. Correlation coefficients of the linear regression lines were 0.993 with glucose as substrate, and ≥ 0.998 with all other substrates. Similarly derived data for glucose-grown cells with lactate and pyruvate gave correlation coefficients of 0.995 and 0.999 respectively.

Table 2. *Kinetics of the utilization of various substrates by M. mycoides in salts solution*

Experimental data were derived from p - t curves according to the methods given in the text. In calculating $V_{\max}$ it was assumed that ΔH_{met} for fructose was the same in glucose-grown cells as in fructose-grown cells and that ΔH_{met} values for mannose and glucosamine were the same as for glucose and N -acetylglucosamine respectively. Theoretical values of ΔH_{met} are for the conversion of substrate to acetate, CO_2 and water (see text), and are derived from published lists of heats of combustion (Weast, 1981).

Sugar used for cell growth	Substrate	$V_{\max}$ [μmol (g dry wt cells) $^{-1}$ min $^{-1}$]	K_m (μM)	ΔH_{met} (kJ mol $^{-1}$)	
				Experimental	Theoretical
Glucose	L-Lactate	138	20	440	487
	Pyruvate	133	4.3	270	283
	Glycerol	127	4.4	760	786
	Glucose	62	2.0	1100	1050
	Fructose	16	3.7	ND	
	N -Acetylglucosamine	43	1.2	1120	NA
	Glucosamine	49	130	ND	
	Mannose	65	1030	ND	
	Threonine	ND	<40	ND	
	Serine	ND	<40	ND	
Fructose	Glucose	57	2.1	1070	1050
	Fructose	51	2.3	1140	1060
Mannose	Glucose	58	1.9	1000	1050
	Mannose	73	980	ND	
N -Acetylglucosamine	Glucose	54	1.9	1070	1050
	N -Acetylglucosamine	35	1.2	1070	NA
Glucosamine	Glucose	54	1.8	1120	1050
	Glucosamine	43	140	ND	

ND, Not determinable from the experimental data; NA, not available as products of metabolism unknown.

values of power output were obtained at a number of substrate concentrations by making several additions of substrate during a single calorimetric experiment (e.g. Fig. 1). At the substrate concentrations used (0.02 to 10 mM), the effect of substrate utilization on substrate concentration was negligible. Double reciprocal plots of substrate concentration against rate of substrate utilization (Fig. 2) allowed calculation of $V_{\max}$ and K_m , but because of the low rate of substrate

utilization at low substrate concentrations Q_T , and hence ΔH_{met} , could not be determined. Therefore, in calculating values of V we have assumed that the ratio of S_i/Q_T is the same (on a molar basis) for mannose and glucosamine as for glucose and *N*-acetylglucosamine respectively. If this assumption is invalid, the values of V_{max} (but not K_m) for mannose and glucosamine (Table 2) are also invalid.

Threonine and serine, which were reported by Rodwell (1967) to be metabolized by resting cell suspensions of *M. mycoides*, both gave plateaus of power output of approximately 0.8 μW at a concentration of 40 μM . These power outputs represented a rate of substrate utilization too low to enable determination of ΔH_{met} ; however, as power output was not increased by increasing threonine or serine concentration to 4 mM, K_m for these amino acids was presumably < 40 μM .

Cells grown on media in which glucose was replaced by fructose, mannose, glucosamine or *N*-acetylglucosamine gave p - t curves for glucose (20 μM) which were similar to those for glucose-grown cells, and from which values of K_m , V_{max} and ΔH_{met} were determined (Table 2). Also, p - t curves representing the utilization of mannose, glucosamine and *N*-acetylglucosamine by cells grown on these substrates were similar to those of glucose-grown cells and kinetic parameters (Table 2) were determined in the same way. However, the rate of utilization of fructose by fructose-grown cells was significantly greater than by glucose-grown cells. Analysis of p - t curves by the procedure described for glucose allowed determination of ΔH_{met} , K_m and V_{max} for fructose (Table 2, Fig. 2).

In this study, with the exception of the initial peak of power observed before the addition of substrate (Fig. 1), power output in replicate p - t curves varied by no more than 10%. The results shown in Table 1 are from representative p - t curves, and in Table 2 the values given for the kinetic parameters are the means of those determined in three or four replicate experiments; average values were within 5% of individual values.

DISCUSSION

Values of ΔH_{met} from p - t curves (Table 2) are close to theoretical values for the conversion of glucose, fructose (in fructose-grown cells), pyruvate, lactate and, in the presence of catalase, glycerol to acetate and carbon dioxide. Thus the results support the original observations of Rodwell & Rodwell (1954) concerning carbohydrate metabolism in *M. mycoides*. The value of ΔH_{met} for *N*-acetylglucosamine is close to that for glucose, and presumably reflects the relatively small enthalpy change associated with its hydrolysis, and metabolism of the glucose so produced.

The analysis used here in the determination of K_m and V_{max} for glucose is a modification of that previously applied to the growth of bacteria (Belaich & Belaich, 1976; Belaich *et al.*, 1968) and yeast (Schaarschmidt & Lamprecht, 1978; Murgier & Belaich, 1971) on various substrates. In these analyses substrate utilization followed Michaelis kinetics, though in some cases there was deviation at high substrate concentration. In the experiments reported here, Michaelis kinetics were also found, though there were deviations at high concentrations of glucose and lactate. The rate of glucose utilization in the presence of saturating concentrations (2 and 20 mM, Table 1) was only 70% of V_{max} derived from Michaelis plots with low concentrations (Table 2), and may possibly be due to inhibitory effects of high glucose concentrations in salts solution; there was a more rapid decline in power output with 20 mM than with 2 mM-glucose (Fig. 1).

A high concentration of lactate (4 mM) added to cells gave peaks of power followed by a decline (Table 1). Rates of substrate utilization at these peaks were up to threefold higher than V_{max} derived from Michaelis plots with lactate concentrations below 40 μM (Table 2). We can offer no explanation for these results, except that high concentrations of lactate may affect cell permeability.

In glucose-grown cells, K_m for the utilization of glucose, fructose, *N*-acetylglucosamine, pyruvate and glycerol was < 5 μM (Table 2), which is comparable with the values quoted by Pirt (1975) for energy substrates in growing bacteria. However, mannose and glucosamine gave high values. In glucose-grown cells, values of V_{max} for glycerol, lactate and pyruvate were similar, as were the values for sugars and amino sugars (Table 2), except the rate for fructose was one-

quarter of that for glucose. The value of K_m for glucose utilization obtained here ($2\ \mu\text{M}$) is considerably less than the estimate of $50\ \mu\text{M}$ reported by Cirillo & Razin (1973) for *M. mycoides* and *M. gallisepticum*; however, in their experiments the lowest initial glucose concentration was $500\ \mu\text{M}$.

In cells grown on substrates other than glucose, values of K_m and $V_{\max}$ for glucose were similar to those of glucose-grown cells (Table 2), indicating that the pathway of glucose utilization is constitutive. Values of K_m and $V_{\max}$ for mannose, glucosamine and *N*-acetylglucosamine, in cells grown on mannose, glucosamine and *N*-acetylglucosamine respectively, were also similar to those of glucose-grown cells (Table 2), suggesting that no inducible components exist in the pathways for utilization of these sugars. In cells grown on fructose, however, $V_{\max}$ for fructose utilization increased to a value close to that for glucose. As the growth of glucose-grown cells inoculated into fructose medium was essentially the same as in glucose medium, this adaptation does not reflect selection of a small proportion of mutant cells able to utilize fructose more rapidly, but rather that the pathway for fructose utilization has some inducible component(s).

The high value of K_m for mannose utilization ($1\ \text{mM}$), compared to that for glucose utilization ($2\ \mu\text{M}$) by both glucose and mannose-grown cells, suggests that mannose is not utilized as an energy substrate by this strain of *M. mycoides* *in vivo*. While little information is available on the concentration of specific sugars in the serum and cells of cattle, mannose has not been reported to be present in mammalian blood (Altman & Dittmer, 1974). It is also possible, as there is a high affinity for *N*-acetylglucosamine ($K_m\ 1\ \mu\text{M}$) and a relatively low affinity for glucosamine ($K_m\ 130\ \mu\text{M}$), that glucosamine might not be used to a significant extent *in vivo*.

The results suggest, therefore, that *M. mycoides* subsp. *mycoides* is adapted to, and is constitutive for, the utilization of a single sugar (glucose) and a single amino sugar (*N*-acetylglucosamine), but that in the presence of fructose, a fructose-utilizing pathway is induced. It is of interest that, at birth, calves have a high level of blood-fructose (approximately $3\ \text{mM}$), but that this concentration declines rapidly to $0.15\ \text{mM}$ within 24 h (Young *et al.*, 1970). In contrast, blood-glucose levels are relatively high (2 to $5\ \text{mM}$) in foetal and adult cattle (Coldman & Good, 1967; Young *et al.*, 1970).

The high affinity shown by *M. mycoides* for pyruvate and glycerol ($K_m\ 4.3$ and $4.4\ \mu\text{M}$ respectively), and perhaps also lactate ($K_m\ 20\ \mu\text{M}$), suggests that they are important *in vivo*. As $V_{\max}$ for these substrates is high, and comparable with that for glucose, they may act as energy sources. Kahane *et al.* (1978) have suggested that under aerobic conditions ATP might be generated from pyruvate oxidation in *M. hominis*.

The values of K_m and $V_{\max}$ derived here will be determined by the rate-limiting step in the metabolism of each substrate, and are dependent on the substrate used. This suggests that it is transport or some other early metabolic step, unique to the utilization of each substrate, which is rate-limiting. Belaich *et al.* (1968) argued that the values of K_m and $V_{\max}$, derived from microcalorimetric studies of growing cells, related to transport rather than to subsequent metabolism. If the values of K_m and $V_{\max}$ determined here relate to transport, the low affinity towards mannose and glucosamine might indicate that there are no specific PTS components for these sugars in *M. mycoides*, and that their transport involves glucose- and/or *N*-acetylglucosamine-specific components. The observed increase in fructose-specific PTS activity in fructose-grown cells as compared to glucose-grown cells of *M. capricolum* (Mugharbil & Cirillo, 1978) suggests that it is a PTS component(s) of the fructose pathway that is inducible in *M. mycoides*.

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