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Inhibition of Growth by Erythritol Catabolism in *Brucella abortus*

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The growth of *Brucella abortus* (US-19) in a complex tryptose-yeast extract medium containing D-glucose is inhibited by 10 mM erythritol. The enzymes of the erythritol pathway, except for D-erythrulose 1-phosphate dehydrogenase (D-*glycero*-2-tetrulose 1-phosphate:nicotinamide adenine dinucleotide (NAD^+) 4-oxidoreductase) were detected in the soluble and membrane fractions of cell extracts. Glucose catabolism by cell extracts was inhibited by erythritol, whereas, phosphorylated intermediates of the hexose monophosphate pathway were converted to pyruvic acid with oxygen consumption. Erythritol kinase (EC 2.7.1.27; adenosine 5'-triphosphate (ATP): erythritol 1-phosphotransferase) was found to be eightfold higher in activity than the hexokinase in cell extracts. In vivo, ATP is apparently consumed with the accumulation of D-erythrulose 1-phosphate (D-*glycero*-2-tetrulose 1-phosphate) and no substrate level phosphorylation. ATP levels dropped 10-fold in 30 min after addition of erythritol to log phase cells in tryptose-yeast extract medium with D-glucose as the carbon source. These data suggest bacteriostasis in the presence of erythritol results from the ATP drain caused by erythritol kinase.

Brucellosis is primarily a disease of domestic animals in which the bacteria localize in fetal tissues and fluids. The end result is abortion or premature birth due to endotoxin shock. Smith et al. (23) isolated a factor from fetal tissues, later identified as erythritol, which stimulated the in vitro growth of *Brucella abortus* and enhanced infections with *B. melitensis* (17). Erythritol apparently exerts a positive selective influence and partially explains the biochemical basis of tissue localization exhibited by *Brucella*.

Jones et al. (15) examined over 100 strains of *Brucella* and found that the growth of only one culture, the *B. abortus* strain (US-19) used for vaccine production in the United States, was inhibited by the presence of erythritol. Keppie et al. (18) showed that shake cultures of *B. abortus* (US-19) seeded with 2×10^7 organisms/ml were inhibited by 1 mM erythritol, whereas other strains of *B. abortus* were stimulated by 2 to 5 mM erythritol.

The intermediates in the pathway of erythritol catabolism in *B. abortus* (British 19) have been identified (25). Since erythritol utilization is widespread among the genus *Brucella*, we wanted to know why the polyol is not a carbon source for *B. abortus* (US-19) and, further, to determine the basis of the observed growth inhibition.

In this report we show that *B. abortus* (US-19) lacks D-erythrulose 1-phosphate dehydrogenase (D-*glycero*-2-tetrulose 1-phosphate:nicotinamide adenine dinucleotide [NAD^+] 4-oxidoreductase) and that this deficiency has profound effects on the physiology of the cell. A method for preparation of substrate amounts of D-erythrulose 1-phosphate (D-*glycero*-2-tetrulose 1-phosphate) is also described.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *B. abortus*, British strain 19 and US strain 19, were obtained from B. L. Deyoe, National Animal Research Laboratories, Ames, Iowa. Cells were grown on a rotary shaker in 250-ml Erlenmeyer flasks containing tryptose, yeast extract, vitamins, salts, and glucose or erythritol, as previously described (20). The cells were harvested in late log phase at an absorbancy measured at 620 nm (A_{620}, B & L spectrophotometer) of 6 to 7. One optical density unit corresponded to 1.4×10^{10} colony-forming units per ml, or 0.35 mg (dry weight) per ml. The cells were centrifuged at $6,000 \times g$ in a Sorvall RC-2B centrifuge for 20 min, washed once with 0.25 volume of 0.05 M *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES)-NH_4OH, pH 7.4, and resuspended to A_{620} of 40 in the same buffer by swirling with sterile glass beads. Bacterial suspensions to be used for cell extract preparation were stable at 3 to 5 C for up to 10 days.

Cell extract preparation. Cells were broken with a

Bronwill MSK cell homogenizer as described by Sperry and Robertson (25). After 4 min of homogenization, the beads were allowed to settle and the supernatant fluid was withdrawn and centrifuged at 7,700 × *g* for 20 min at 3 to 5 C to remove unbroken cells and debris. The supernatant (3.5 mg of protein/ml, pH 7.2) was decanted and will be hereafter referred to as the cell extract.

Preparation of Brucella membranes. Membranes of *Brucella* were prepared as described previously (25). Briefly, the cells were disrupted using a Bronwill MSK cell homogenizer. After the beads had settled, the supernatant was decanted and 1 mg each of ribonuclease (EC 3.1.4.22) and deoxyribonuclease (EC 3.1.4.5) were added with stirring for 20 min. The suspension was centrifuged at 3,020 × *g* for 10 min to remove unbroken whole cells and large cell fragments. The opaque supernatant fluid was centrifuged at 25,000 rpm in an SW41 rotor for 1 h at 4 C (Beckman model L2-65B). The high speed supernatant fluid was withdrawn and the membranes were suspended in 5 to 10 ml of 0.1 M HEPES-NH_4OH, pH 7.4, containing 10^{-4} dithiothreitol with the aid of a variable speed homogenizer (Tri-R Instrument Co.).

Measurement of respiration. The activity of the overall pathway was determined by oxygen consumption using standard manometric techniques. The main compartment of each 15-ml flask contained (in micromoles): HEPES-NH_4OH, pH 7.4, 50; reduced glutathione, 9.0; niacinamide, 0.82; dithiothreitol, 0.2; $MgSO_4$, 12.0; $MnSO_4$, 6.0; $(NH_4)_2HPO_4$, 25.0; adenosine-5'-triphosphate (ATP), 5.0; nicotinamide adenine dinucleotide (NAD), 10.0; as well as 1 ml of CE (3.5 mg protein/ml) and distilled water to 2.75 ml. The side arm contained 0.25 ml of substrate (10 to 25 μmol) and the reactions were incubated for 3 h after tipping in substrate. The reactions were terminated by addition of an equal volume of cold 0.6 M $HClO_4$. The suspensions containing denatured protein were centrifuged at 10,000 × *g* and 4 C for 10 min, and neutralized with 1 N KOH. The activity of the entire pathway could also be followed by the accumulation of pyruvate or release of [^{14}C]CO_2. With ^{14}C-labeled substrates the [^{14}C]CO_2 was trapped in the center well with 0.2 ml of a solution of ethanolamine in ethylene glycol monomethyl ether (1:2, vol/vol) which was added to 10 ml of scintillation fluid (7) and counted in a Packard Tri-Carb liquid scintillation spectrometer (model 3375B) with an efficiency of 75% for ^{14}C.

Enzyme assays. Glucokinase (EC 2.7.1.2.) was assayed by coupling to glucose 6-phosphate dehydrogenase (EC 1.1.1.49) in a 0.3-ml volume which contained 35 μg of cell extract protein, 1.0 IU of glucose 6-phosphate dehydrogenase and (in micromoles): HEPES-NH_4OH, pH 7.4, 10.0; ATP, 2.0; $MgCl_2$, 2.0; nicotinamide adenine dinucleotide phosphate (NADP), 0.2; and D-glucose, 2.0.

Erythritol kinase (EC 2.7.1.27) was measured by a coupled enzymatic assay (2) in a 0.3 volume which contained 35 μg of cell extract protein, 4 IU of pyruvate kinase (EC 2.7.1.40, type II, rabbit muscle), 20 IU of lactic dehydrogenase (EC 1.1.1.28 type III, beef heart), and (in micromoles): HEPES-NH_4OH, pH 7.4, 10.0; ATP, 1.0; $MgCl_2$, 2.0; phosphoenolpyruvate, 0.5; reduced NAD, 0.1; and erythritol, 2.0.

D-Erythritol 1-phosphate dehydrogenase (D-*erythro*-tetritol 1-phosphate:NAD^+ 2-oxidoreductase) and D-erythrulose 1-phosphate dehydrogenase (D-*glycero*-2-tetrulose 1-phosphate:NAD^+ 4-oxidoreductase) were assayed by coupling to dichlorophenol indophenol with diaphorase (EC 1.6.4.3 type III, pig heart). The reaction in a 0.3-ml volume contained 35 μg of cell extract protein, 2 IU of diaphorase and (in micromoles): HEPES-NH_4OH, pH 7.4, 10.0; dichlorophenol indophenol, 0.03; and NAD, 0.2. Addition of 2 μmol of $MgCl_2$ was necessary for activity of D-erythrulose 1-phosphate dehydrogenase. The reactions were monitored at an A_{600} until the blank rate was negligible. The reaction was started by addition of 0.5 μmol of substrate.

All enzyme assays were started by addition of substrate and were performed at 23 C with a Gilford 240 spectrophotometer.

Preparation of D-erythrulose 1-phosphate (D-glycero-2-tetrulose 1-phosphate). The reaction mixture (45 ml) contained (in millimoles): HEPES-NH_4OH, pH 7.4, 28.0; ATP, 1; [U-^{14}C]erythritol (5 μCi), 1.0; $MgCl_2$, 2.0; reduced glutathione, 0.2; $(NH_4)_2HPO_4$, 0.5; KOH, 1.9; and 96 mg of *B. abortus* (US-19) cell extract protein. The mixture was incubated with shaking at 37 C for 8 to 9 h, and terminated by addition of 5 ml of 3.3 M $HClO_4$, followed by centrifugation at 10,000 × *g* for 10 min to remove denatured protein. The supernatant fluid was adjusted to pH 6.8 with 2 N KOH and stored overnight at 4 C for precipitation of $KClO_4$. The pH was adjusted to 7.0 with 1 N NaOH after addition of 5 ml of 1 M barium acetate and the suspension was centrifuged at 10,000 × *g* for 10 min. The supernatant was adjusted to pH 2.0 with 6 N HBr before addition of 2 g of Norit. The Norit was removed by filtration using Whatman 3MM filter paper and the filtrate was adjusted to pH 6.7 with NaOH. The barium salt of the sugar phosphate was precipitated by addition of 4 volumes of ethanol. The precipitate was collected after standing overnight by centrifugation at 10,000 × *g* for 10 min, washed with 85% (vol/vol) ethanol, and dried in a vacuum dessicator. The yield was 55% based on ^{14}C recovery and an enzymatic assay using glycerol 3-phosphate dehydrogenase (10).

Preparation of 3-keto-L-erythrose 4-phosphate (L-glycero-3-tetrosulose 4-phosphate). The reaction mixture contained cell extract from *B. abortus* (British 19) prepared by centrifugation at 100,000 × *g* and other components as described previously (25).

Paper chromatography. Descending paper chromatography was performed on Whatman 3MM filter paper using the solvent system of Bandurski and Axelrod (4), containing methanol, ammonia and water (vol/vol/vol, 6:1:3). The chromatograms were air-dried and sprayed for polyols with the reagent of Bragg and Hough (6), and for phosphates with the spray of Bandurski and Axelrod (4).

Analytical methods. Periodate oxidations ere performed as described by Jackson (14), formaldehyde was determined with chromatropic acid (24),

and formate was determined using the thiobarbituric acid assay (5). Beta-keto acids were assayed with the *p*-nitroanilane diazo reagent (16). Protein was measured with the micro-biuret procedure (3). Pyruvate and ATP were measured enzymatically (8, 19). Erythritol was measured by a coupled enzyme assay which contained (in micromoles): triethanolamine hydrochloride (TEA-HCl-NH_4OH) buffer pH 7.2, 10.0; $MgCl_2$, 2; ATP, 1; phosphoenolopyruvate, 0.5, reduced NAD, 0.10; pyruvate kinase (3 IU); type III muscle lactate hydrogenase (16 IU); 0.5 IU of erythritol kinase (EC 2.7.1.27) partially purified from *Propionibacterium pentosaceum* (12); and up to 75 μl of solution containing 10 to 40 nmol of erythritol.

Luciferin-luciferase ATP assay. The procedure used was that of Chapman et al. (9), and the assays were performed on perchlorate extracts of the bacteria.

Effects of erythritol on growth and ATP level of B. abortus (US-19). Bacteria were grown with 1% (wt/vol) glucose to an A_{620} of 0.4 (A_{620} of 0.14 = 10^9 colony-forming units/ml) and duplicate flasks adjusted to 10 mM and 20 mM erythritol. Two-tenths milliliter of 35% $HClO_4$ was added to 1-ml samples of each of the cultures taken just before, 0.5 and 3 h after addition of erythritol. The solution was kept at 0 C for 15 min, neutralized with 0.5 ml of 2.6 N KOH, and centrifuged at 10,000 × *g* for 10 min with the supernatant fluid decanted and frozen until assay. Growth was measured by the increase in A_{620}.

Materials. Tryptose, yeast extract, and potato infusion agar were obtained from Difco Laboratories. All standard biochemicals and carbohydrates, unless otherwise indicated, were obtained from Sigma Chemical Co. [U-^{14}C]erythritol (3.6 mCi/mmol) was purchased from Amersham/Searle. All other chemicals were reagent grade and purchased from commercial sources.

D-Erythritol 1-phosphate and D-erythrulose 1-phosphate were generous gifts from C. E. Ballou, University of California, Berkeley.

RESULTS

Since the ability to catabolize erythritol is almost universal in *Brucella*, it was possible that a key enzyme was missing in the vaccine strain of *B. abortus* known to be inhibited in a complex growth medium by low concentrations of the polyol (15, 18). Alternatively, an intermediate of erythritol catabolism was regulating a glucose catabolic enzyme. Erythritol kinase (ATP:erythritol 1-phospho-transferase) and D-erythritol 1-phosphate dehydrogenase (D-*erythro*-tetritol 1-phosphate:NAD^+ 2-oxidoreductase) activities were present in cell extracts of *B. abortus* (US-19) as was found in *B. abortus* (British 19) (25); however, D-erythrulose 1-phosphate dehydrogenase (D-*glycero*-2-tetrulose 1-phosphate:NAD^+ 4-oxidoreductase) was not detected (Table 1). Erythritol kinase could be easily assayed by coupling the activity to the following dehydrogenase, but the rate was about 10-fold lower than that obtained with the kinase assay of Anderson and Wood (2). Diaphorase was included at 100-fold excess of reduced NAD oxidase in the dehydrogenase assays to increase the specificity using crude extracts. The validity of the assay was established by measuring glucose 6-phosphate dehydrogenase in cell extracts of *B. abortus*; the rate was similar after the reduction of NADP at 340 nm or reduction of dichlorophenol indophenol at 600 nm in the presence of diaphorase (unpublished observations).

TABLE 1. *Comparison of dehydrogenases of the erythritol catabolic pathway in two strains of B. abortus*

Enzyme	Activity (μmol/min per mg of protein)	
	US-19 cell extract	British 19 cell extract
D-Erythritol-1-PO_4 dehydrogenase	0.032	0.040
D-Erythrulose-1-PO_4 dehydrogenase[a]	0.000	0.023

[a] Two micromoles of $MgCl_2$ included in assay.

D-Erythrulose 1-phosphate dehydrogenase was only partially solubilized during disruption of *B. abortus* (British 19) and the next enzyme in the pathway, 3-keto-L-erythrose 4-phosphate dehydrogenase (L-*glycero*-3-tetrosulose 4-phosphate: ferricytochrome 1-oxidoreductase) was membrane bound, apparently coupled to the electron transport system (25). Cell extracts of *B. abortus* (British 19) were prepared to supply the missing enzyme(s) to cell extract of *B. abortus* (US-19). Addition of the membrane fraction from British 19 to the soluble fraction of US-19 (Table 2) supplied all the enzymes for the conversion of D-erythrulose 1-phosphate to CO_2 and dihydroxyacetone phosphate which is converted to pyruvic acid by the latter steps of the Embden-Meyerhof-Parnas pathway (21).

It was possible that 3-keto-L-erythrose 4-phosphate dehydrogenase was also missing in the membrane fraction of *B. abortus* (US-19). Membrane and soluble fractions of both British 19 and US-19 were prepared and incubated in combination with 3-keto-L-erythrose 4-phosphate (L-*glycero*-3-tetrosulose 4-phosphate) (Table 2). Since the enzyme is entirely in the membrane fraction of *B. abortus* (British 19) (25), the [^{14}C]CO_2 released when the soluble fraction of British 19 was incubated with the membrane fraction of US-19 indicated that only

TABLE 2. *Reconstitution and assay of erythritol catabolic pathway using soluble and membrane fractions of B. abortus (US-19) and B. abortus (British 19)*

Substrate[a]	US-19		British 19		
	Soluble[b]	Membrane[c]	Soluble[d]	Membrane[e]	$^{14}CO_2$ (μmol)
D-Erythrulose-1-PO_4	+	−	−	−	0.54
D-Erythrulose-1-PO_4	+	+	−	−	0.72
D-Erythrulose-1-PO_4	+	−	−	+	4.49
D-Erythrulose-1-PO_4	−	−	+	+	5.40
3-Keto-L-erythrose-4-PO_4	+	−	−	−	1.91
3-Keto-L-erythrose-4-PO_4	−	−	+	−	0.66
3-Keto-L-erythrose-4-PO_4	+	−	−	+	5.15
3-Keto-L-erythrose-4-PO_4	−	+	+	−	3.92
3-Keto-L-erythrose-4-PO_4	−	−	+	+	6.28

[a] The reactions were run in 15-ml Warburg flasks as described in Materials and Methods. The incubations contained 10 μmol of [^{14}C]-D-erythrulose 1-phosphate (10,950 dpm/μmol) or 10 μmol of [^{14}C]-3-keto-L-erythrose 4-phosphate (9,840 dpm/μmol).

[b] Protein (7.1 mg).

[c] Protein (3.6 mg).

[d] Protein (7.2 mg).

[e] Protein (2.6 mg).

D-erythrulose 1-phosphate dehydrogenase was absent in the cell extract of US-19. The reason(s) for the threefold higher level of [^{14}C]CO_2 observed with the soluble fraction of *B. abortus* US-19 incubated with 3-keto-L-erythrose 4-phosphate than was observed with the British 19 soluble fraction is unknown. The enzyme may exist in multiple forms in US-19 or be partially solubilized by the disruption procedure. The accumulation of products and release of [^{14}C]CO_2 is the most convenient method of assaying the last two enzymes unique to the erythritol pathway. The dehydrogenase is the rate-limiting reaction in the pathway and the equilibrium appears to be pulled by the decarboxylase (L-*glycero*-3-tetrulosonic acid 4-phosphate 1-carboxy-lyase) (25).

Preparation of D-erythrulose 1-phosphate (D-glycero 2-tetrulose 1-phosphate). Extracts of *B. abortus* (US-19) were used to prepare D-erythrulose 1-phosphate which is otherwise synthesized by a 14-step procedure (10). The sugar phosphate was isolated as the barium salt in 55% yield and co-chromatographed with synthetic D-erythrulose 1-phosphate using methanol-ammonia-water (6:1:3, vol/vol/vol). The *B. abortus* (US-19) product served as a substrate for α-glycerol phosphate dehydrogenase but was not cleaved by fructose diphosphate aldolase indicating the lack of a *trans*-hydroxyl group (10). The sugar phosphate consumed 1.9 mol of periodate/mol of substrate and yielded 0.95 mol of formaldehyde and 0.94 mol of formic acid, consistent with the product being D-erythrulose 1-phosphate.

Inhibition of glucose metabolism in cell extract in B. abortus (US-19) by erythritol. To gain some insight into the growth inhibition caused by erythritol, the polyol was added to cell extracts metabolizing D-glucose or intermediates of the hexose monophosphate pathway, the primary pathway of glucose catabolism in *Brucella* (21). Erythritol inhibited O_2 uptake and the accumulation of pyruvic acid (Table 3) when added to reaction mixtures containing D-glucose; however, there was no inhibition observed with phosphorylated intermediates. These data could be interpreted to mean that erythritol, or an intermediate of erythritol catabolism, inhibited hexokinase in cell extracts or the incomplete erythritol pathway acted as an ATP drain. It appeared that hexokinase was not inhibited by the first two intermediates of the erythritol pathway since doubling the ATP level increased the pyruvate yield twofold (Table 4). Addition of yeast hexokinase such that the combined hexokinase activities were one-half that of erythritol kinase raised O_2 consumption and pyruvate accumulation to those levels observed with D-glucose alone or with phosphorylated intermediates. Further

TABLE 3. *Inhibition of B. abortus US-19 glucose metabolism in cell extracts by erythritol*

Substrate[a]	− Erythritol[a]		+ Erythritol[b]	
	O_2 Uptake (μmol)	Pyruvate (μmol)	O_2 Uptake (μmol)	Pyruvate (μmol)
Glucose	27.7	8.2	6.2	1.1
Glucose-6-PO_4	28.5	6.5	36.7	8.1
Fructose-6-PO_4	27.8	5.5	34.1	6.9
6-Phosphogluconate	23.1	6.2	30.1	7.4
Ribose-5-PO_4	11.3	3.6	16.7	4.0

[a] The reaction mixture and conditions were as described in Table 2. Each reaction contained 25 μmol of substrate.

[b] Each reaction contained 12.5 μmol of erythritol and 12.5 μmol of cosubstrate.

TABLE 4. *Reversal of glucose inhibition by erythritol in cell extracts of B. abortus (US-19)*

System[a]	O_2 uptake (μmol)	Pyruvate (μmol)
Glucose	22.8	6.2
Glucose + erythritol	6.1	0.9
Glucose + erythritol + 2X ATP	12.0	2.0
Glucose + erythritol + yeast hexokinase	28.6	7.2

[a] Each mixture contained 12.5 μmol of erythritol and 12.5 μmol of D-glucose.
[b] Yeast hexokinase (0.625 IU) was added.

support for lack of control at the hexokinase level is the observation that erythritol and D-glucose are co-utilized in vivo by strains of *B. abortus* which contain D-erythrulose 1-phosphate dehydrogenase and can use the polyol as a carbon source (D. C. Robertson, unpublished observations).

Kinetic parameters of both erythritol kinase and hexokinase were examined to determine relative rates of phosphorylation in binary mixtures of erythritol and D-glucose. The V_{max} of erythritol kinase was eightfold that of the hexokinase (Table 5) which roughly corresponded to the sevenfold decrease in pyruvate noted in mixtures of erythritol and D-glucose (Table 4).

In vivo effects of erythritol on B. abortus (US-19). If the inhibition of glucose catabolism in cell extracts was due to ATP depletion by erythritol kinase, a similar effect should be observed in whole cells. When erythritol was added to early log phase cells in tryptose-yeast extract medium, ATP levels dropped 10-fold in 30 min and growth ceased (Fig. 1). The reason for the twofold drop in ATP level in the control is unknown; however, it has been consistently observed. Apparently the phosphorylated intermediate(s) that accumulated were not toxic to the cells since growth resumed after the cells were removed by centrifugation and resuspended in fresh media without erythritol or plated on Trypticase soy agar (data not shown). Alternatively, the phosphorylated intermediate may be hydrolyzed and the inorganic orthophosphate used in other biosynthetic reactions. The difference in ATP levels in the controls and flasks to which erythritol was added simply reflects experimental variation.

DISCUSSION

The enzymes of erythritol catabolism detected in cell extracts of *B. abortus* (US-19) are shown in Fig. 2. D-erythrulose 1-phosphate dehydrogenase (D-*glycero*-2-tetrulose 1-phosphate:NAD^+ 4-oxidoreductase) was the only enzyme not detected; thus, cell extracts could be used to prepare D-erythrulose 1-phosphate (D-*glycero*-2-tetrulose 1-phosphate) without extensive enzyme purification.

3-Keto-L-erythrulose 4-phosphate dehydrogenase (L-*glycero*-3-tetrosulose 4-phosphate:ferricytochrome 1-oxidoreductase) was partially solubilized during disruption of *B. abortus* (US-19) as indicated by the higher yield of CO_2 when cell extract supernatant fluids were incubated with 3-keto-L-erythrose 4-phosphate (Table 2). These results are of interest since the enzyme is membrane-bound in *B. abortus* (British 19) and requires a functional electron transport system and a terminal electron acceptor for enzymatic activity (25). It is possible that the enzyme exists in multiple forms in US-19 or is more easily solubilized owing to the absence of the D-erythrulose 1-phosphate dehydrogenase which is also membrane bound in *B. abortus* (British 19) (25).

The growth inhibition of *B. abortus* (US-19) caused by ervthritol, apparently was not due to

TABLE 5. *Comparison of hexokinase and erythritol kinase in cell extracts of B. abortus (US-19)*

Substrate[a]	K_m (M)[b]	V_{max}[c]
Erythritol	4.0×10^{-4}	381
Glucose	4.3×10^{-5}	47

[a] CE was centrifuged at 40,000 × *g* for 20 min.
[b] K_m was determined from a Lineweaver-Burk plot.
[c] V_{max} was the maximal velocity recorded for the spectral assay, converted to nanomoles per minute per milligram of protein.

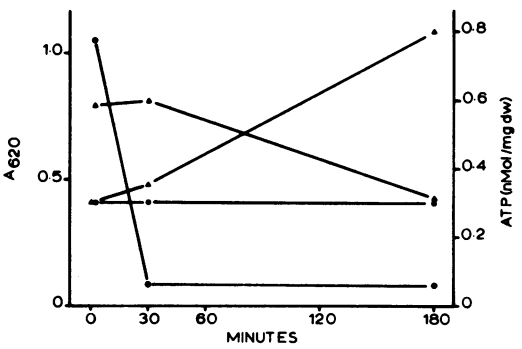

FIG. 1. *Drop in ATP and inhibition of growth by addition of 10 mM erythritol to tryptose-yeast extract medium containing 10 mM glucose. Symbols:* (○) *ATP levels in cells exposed to 10 mM erythritol-10 mM glucose;* (△) *ATP levels in cells and 20 mM glucose;* (●) *turbidity of the culture (A_{600}) in the presence of 10 mM erythritol-10 mM glucose;* (▲), *turbidity of culture in 20 mM glucose.*

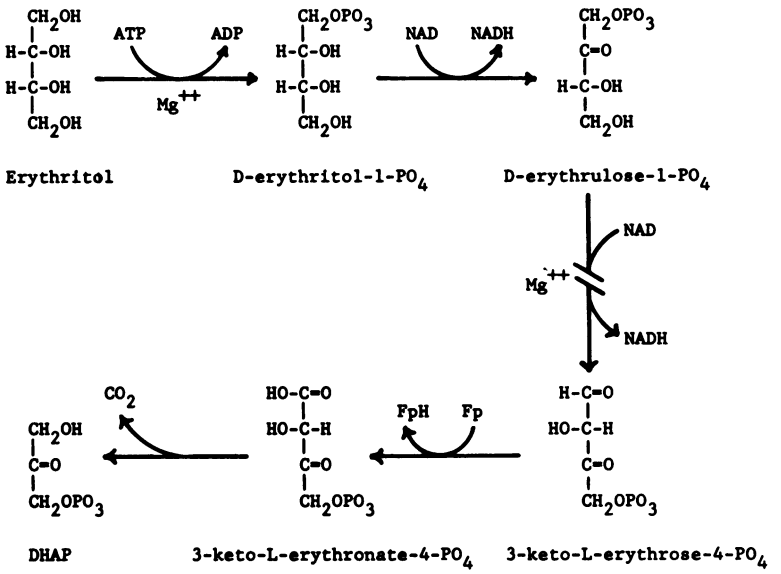

FIG. 2. *Enzymes of the erythritol pathway detected in B. abortus (US-19).*

accumulation of toxic phosphorlated intermediates. ATP levels in cell extracts were depleted due to high erythritol kinase activity and in vivo, intracellular ATP was shown to drop 10-fold after addition of erythritol to a complex growth media. It has not been ruled out that the energy charge (9) is a factor in the regulation of hexokinase. Even though the bacteria were grown with glucose as the carbon source, the erythritol catabolic enzymes were present at levels that were comparable to strains that utilize the polyol as a carbon source. The enzymes of the pathway have been detected in both *B. abortus* (British 19) and *B. abortus* (US-19) grown on a variety of sugars which might suggest they are constitutive, but mutants are needed to settle the matter. It is possible that some intermediate of hexose metabolism induces the enzymes of the erythritol pathway similar to induction of hexose-catabolizing enzymes in *P. aeruginosa* (13).

It is interesting that *B. abortus* (US-19) and *B. abortus* (British 19) are attenuated strains derived from the highly virulent strain 2308 (11); the former strain is inhibited by erythritol, and the latter can use the polyol as a carbon source. Limited genetic information on *Brucella* makes it difficult to explain the basis of the apparent mutation in *B. abortus* (US-19). The key to tissue localization by these bacteria seems to be the presence of erythritol, and Smith et al. (22) has proposed that the ability to catabolize erythritol is one of the major biochemical lesion(s) in *B. abortus* (US-19) but is probably not the only cause of decreased virulence. A unique enzyme system may have evolved in *Brucella* through centuries of association with domestic cattle, sheep, swine, and goats. Whether erythritol utilization is a virulence factor for these bacteria will perhaps be revealed by future studies on the regulation of the erythritol pathway and by further defining its role in the physiology of the cell.

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