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Effect of Erythritol on the In Vitro Growth and Respiration of *Listeria monocytogenes*

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In ruminants, such as cattle, sheep, and goats, common forms of listeriosis are abortion, stillbirth, or postnatal death of the young infected animal (M. L. Gray et al., Am. J. Vet. Res. **17**: 510, 1956; J. W. Osebold et al., J. Am. Vet. Med. Assoc. **137**:221, 1960). By experimental oral exposure of pregnant sheep and goats, M. L. Gray et al. have shown the reproductive system of these animals, especially the gravid uterus and its contents, to be highly susceptible to invasion by *Listeria monocytogenes*. In terms of tissue affinity, *L. monocytogenes* thus resembles those *Brucella* species which cause contagious abortion in cattle, sheep, and goats. Erythritol is localized in those ruminant tissues (placenta, chorion, fetal fluids) which are most susceptible to the *Brucella*, and the course of brucellosis in these animals may be explained by the presence of erythritol in the susceptible tissues (H. Smith et al., Nature **193**:47, 1962; J. Keppie et al., Brit. J. Exptl. Pathol. **46**:104, 1965). N. B. McCullough and G. A. Beal (J. Infect. Diseases **89**:266, 1951) found that erythritol was the most effective of nine carbohydrates tested as sole carbon sources for *B. abortus*, *B. suis*, and *B. melitensis* in a basal medium. Recently, it has been demonstrated that *B. abortus* preferentially metabolizes erythritol as a carbon and energy source even in the presence of high glucose concentrations (J. D. Anderson and H. Smith, J. Gen. Microbiol. **38**:109, 1965). The tendency for both *Brucella* and *Listeria* to localize in the placenta and associated tissues of pregnant ruminants, and the correlation between the presence of erythritol and the localization of the *Brucella* in these tissues, led us to determine whether *L. monocytogenes* attacks erythritol in vitro.

Seven strains of *L. monocytogenes* were used in all experiments. The designations of these strains, with their serotypes in parentheses, and their sources are as follows: strains 7973 (1), 5348 (2), 5105 (3), 5214 (4a), and 1071 (4b) from J. H. Schubert, Communicable Disease Center, Atlanta, Ga.; strains A4413 (4b) and 9037-7 (serotype unknown) from M. E. Friedman, Fort Detrick, Frederick, Md. Stock cultures were maintained on tryptose agar slants at 4 C and were transferred every 4 months.

Three types of experiments were carried out. These tested the ability of *L. monocytogenes* to (i) grow with erythritol as the sole carbohydrate, (ii) grow with erythritol and glucose as carbohydrates, and (iii) consume oxygen in the presence of erythritol.

All seven strains of *L. monocytogenes* tested grew well in Trypticase broth only if it was supplemented with glucose or other utilizable carbohydrate. An observable turbidity increase in this broth in response to added erythritol would, therefore, suggest the utilization of this substance. Experiments were performed with autoclaved phosphate-buffered Trypticase (2%) broth at *p*H 7.2 as the basic medium. Solutions of glucose and erythritol were rendered bacteria-free by passage through a membrane filter (0.45 μ; Millipore Filter Corp., Bedford, Mass.). Bacteria were grown in, and the turbidity was measured from, 13-mm cuvettes which contained a total volume of 3.0 ml after inoculation. Eight cuvettes were used for each strain: a control (Trypticase only), a glucose control (Trypticase plus 28 mM glucose), and six cuvettes containing Trypticase plus erythritol in concentrations of 0.2, 0.8, 3.1, 12.5, 50, and 200 mM. Each of the eight cuvettes was inoculated with approximately 10^7 cells (0.1 ml), grown in 2% Trypticase plus 28 mM glucose, and washed once by centrifugation in 2% Trypticase. Incubation at 37 C was stationary, except for mixing on a Vortex mixer just before each turbidity reading at 620 mμ. The growth response of strain A4413 is shown in Fig. 1; the other six strains gave similar responses. None of the seven strains showed a detectable increase in turbidity in Trypticase plus erythritol. All of these seven strains, however, demonstrated a marked turbidity increase in Trypticase plus glucose, reaching a maximum in 8 to 12 hr, at which time the *p*H was 6.0 to 6.1. A marked decrease in turbidity occurred during the next 12 hr, which might have been the result of death and lysis of the *Listeria* in the acid medium; H. Seeliger and G. Linzenmeier (Z. Hyg. Infektionskrankh. **136**:335, 1953) noted that these

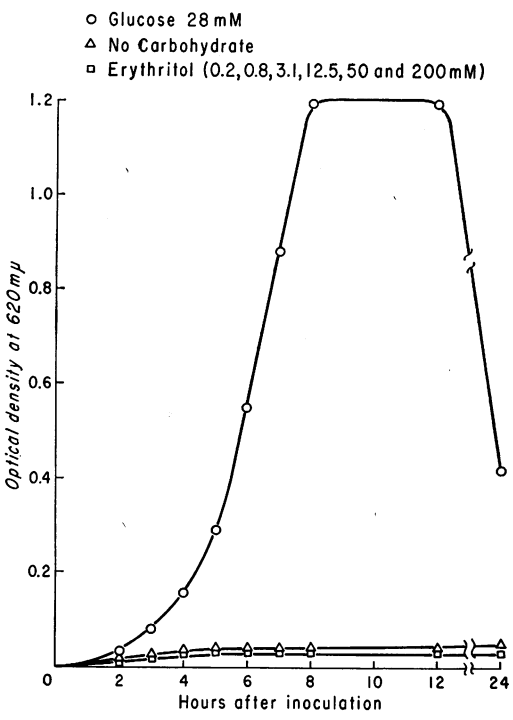

FIG. 1. *Growth response of Listeria monocytogenes A4413 in phosphate-buffered Trypticase broth. Erythritol or glucose was present as indicated.*

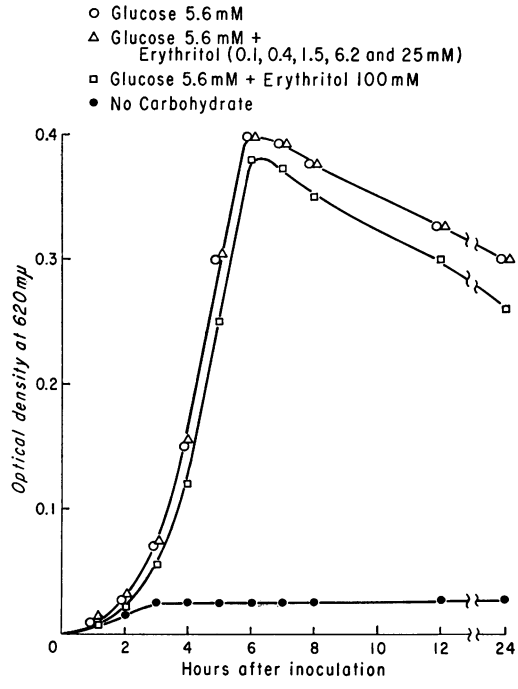

FIG. 2. *Growth response of Listeria monocytogenes A4413 in phoshate-buffered Trypticase broth. Glucose with or without erythritol was included as indicated.*

microorganisms often die at *p*H values below 5.6.

Similar experiments were done in which erythritol was added in final concentrations of 0.1, 0.4, 1.5, 6.2, 25, and 100 mM to Trypticase plus glucose (5.6 mM). Erythritol, if utilized by the *Listeria*, should support a greater turbidity in those cuvettes containing erythritol, glucose, and Trypticase than in a cuvette containing only glucose and Trypticase. The results of this experiment with strain A4413 are shown in Fig. 2; the other six strains behaved similarly. The same turbidity increases were observed in the presence or absence of erythritol (0.1 to 25 mM) in Trypticase broth plus 5.6 mM glucose. The turbidities observed from growth in Trypticase plus glucose plus erythritol never exceeded those obtained in Trypticase plus glucose. Slightly lower turbidities were apparent when 100 mM erythritol was present in this medium, suggesting that this concentration of erythritol was inhibitory.

Finally, manometric experiments were performed in the Warburg apparatus to determine whether resting *Listeria* consumes oxygen in the presence of erythritol. Cells were grown in Brain Heart Infusion (11.2 mM glucose) plus 8 mM erythritol with shaking for 5 hr at 37 C; they were washed once in 0.1 M phosphate buffer (*p*H 7.2) by centrifugation at 4 C, and resuspended in this buffer to an optical density at 620 mμ of 0.60 (0.045 mg of bacterial N per ml). The Warburg test flasks received 1.0 ml of bacteria in 0.1 M phosphate buffer, 1.8 ml of distilled water, and 0.2 ml of erythritol or glucose (10 μmoles). The center well contained 0.1 ml of 10% KOH; incubation was at 37 C. Control flasks without carbohydrate yielded no measurable oxygen uptake. Values for $Q_{O_2}(N)$ of 700 to 900 were obtained for the seven strains of *L. monocytogenes* in the presence of glucose. In contrast, none of these strains consumed a detectable quantity of oxygen in the presence of erythritol.

In summary, we have obtained no evidence that erythritol is metabolized in vitro by *L. monocytogenes*. Although virulence was not determined for these *Listeria* cells in this laboratory, in view of the consistent failure of these bacteria to metabolize erythritol, it seems likely that listeriosis, in contrast to brucellosis, is unrelated to the presence of erythritol in the susceptible host tissues.

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