Cultivation of Parasitic Leptospires: Effect of Pyruvate

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Sodium pyruvate (100 µg/ml) is a useful addition to the Tween 80-albumin medium for the cultivation of parasitic serotypes. It is most effective in promoting growth from small inocula and growth of the nutritionally fastidious serotypes.

Most parasitic leptospires grow well in the Tween 80-albumin medium (1, 2). However, variations in the growth-promoting quality of different lots of bovine serum albumin can result in media which are unsatisfactory for general use. This deficiency in the culture media is most apparent when cultivating the nutritionally fastidious leptospiral serotypes or when initiating growth from small inocula.

In an attempt to improve the albumin medium, the following compounds were tested for growth-promoting activity: glucose, acetate, glycerol, pyruvate, lactate, alanine, aspartate, glutamate, and glycine (Sigma Chemical Co., St. Louis, Mo.). Solutions of these compounds, at concentrations of 1 to 1,000 µg/ml, were sterilized by filtration. Inocula ranging from 1 to 10³ cells of ballum, hardjo, icterohaemorrhagiae, and canicola per ml were used in the screening of these compounds. Incubation was at 30°C and growth was measured nephelometrically.

Sodium pyruvate at concentrations of 50 to 500 µg/ml was the only compound tested that demonstrated significant growth-promoting activity in the Tween 80-albumin medium. This growth-enhancing property of pyruvate on parasitic leptospires has also been observed in protein-free media (3; C. D. Cox, personal communication). The lowest concentration of pyruvate tested in the Tween 80-albumin medium which gave a maximal response was 100 µg/ml, and this was the level of pyruvate used for subsequent testing. Since pyruvate was heat stable it was added to the basal medium (2) and sterilized by autoclaving.

A total of 14 parasitic serotypes were tested in the Tween 80-albumin medium containing 100 µg of pyruvate per ml at initial cell densities of 10⁴, 10³, and 10² leptospires per ml. The pyruvate effect was most evident with the lowest cell densities and frequently was not detectable with the larger inocula. It had little effect on either the growth rate or final cell yield. The serotypes tested are listed in order of response to pyruvate (greatest response to least response): hardjo Nebraska, ballum S102, tarassovi Pe-repelcin, gatuni LT839, arboreae Arborea, atchafalaya LSU 1013, atlantaes LT81, mankan Mankarso, bakeri LT79, fort-bragg Fort Bragg, pomona Pomona, icterohaemorrhagiae LT1144, copenhageni M20, and canicola Hond Utrecht IV. The nutritionally fastidious serotype hardjo did not initiate growth during the 30-day test period with an inoculum of 10³ cells per ml unless pyruvate was present. Serotype canicola, one of the less fastidious leptospires, initiated growth 2 days earlier in the pyruvate-containing medium with the 10³ cell inoculum.

The effect of pyruvate on the initiation of growth from single cells on solid medium was investigated. The solid medium was prepared by incorporating 1% agar into the Tween 80-albumin medium. A culture of canicola was diluted such that 0.1 ml contained 10 to 30 leptospires. The diluted culture (0.1 ml) was spread onto plates prepared with and without pyruvate. After 18 days, colonies of canicola were visible when pyruvate was present. No growth was discernable in the absence of this compound (Fig. 1). In another experiment plates with and without pyruvate (100 µg/ml) were streaked with hardjo and incubated at 30°C. As seen in Fig. 2 after 30 days of incubation, growth was not visible on the plates without pyruvate, whereas definite colonial growth was present on the pyruvate-containing medium.

The effect of pyruvate on the virulence of canicola was investigated. Serotype canicola NADL, a virulent strain, was isolated and
FIG. 1. Plates spread with serotype canicola Hond Utrecht IV and incubated 18 days at 30 C (A) with 100 μg of pyruvate per ml and (B) without pyruvate.

subcultured three times (1% inoculum vol/vol) in the presence and absence of pyruvate (100 μg/ml) and assayed for virulence in hamsters. The mean lethal dose (LD₅₀) of canicola was determined by using 21- to 30-day-old male golden hamsters (Lakeview outbred LUG-LAK, Lakeview Hamster Colony, Newfield, N.J.). Hamsters in groups of 10 were injected intraperitoneally with various numbers of cells, and the number of deaths were recorded during the 30-day observation period. The LD₅₀ of the pyruvate-grown cells was 14, whereas cells cultivated in the absence of this compound had an LD₅₀ of 60. Thus, pyruvate does not appear to adversely affect virulence. The possibility of this compound enhancing virulence merits further studies.

Sodium pyruvate (100 μg/ml) is a useful addition to the Tween 80-albumin medium for the cultivation of parasitic leptospires. It is particularly helpful for colonial studies of these organisms. Pyruvate is relatively inexpensive, and since it is heat stable it can be conveniently sterilized with the basal medium by autoclaving.

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