NOTES

EFFECT OF GLUCOSE CONCENTRATION IN THE GROWTH MEDIUM ON SOME METABOLIC ACTIVITIES OF *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes grown in the presence of 1.0% glucose was only one-fourth to one-ninth as virulent for mice via the respiratory route as when grown in the presence of 0.2 to 0.6% glucose. Also, increased glucose concentration in the growth medium caused a decrease in the dehydrogenase activity of the cells, suggesting a relationship between the virulence of the cell and its oxidative ability (Friedman and Kautter, J. Bacteriol. **83**:456, 1962). Further studies on glucose concentration and metabolic activities of *Listeria* are reported here.

Warburg experiments confirmed the relationship between glucose and oxidative activity. Figure 1 shows the oxygen consumption during the oxidation of 10 μmoles of glucose by resting cells from cultures grown for 16 hr on a shaker in brain heart infusion (BHI; 0.2% glucose) or in the following Peptone C media: modified NS-3 (0.2% glucose), NS-3 (0.6% glucose), and NS-4CA (1.0% glucose). There was no measurable endogenous respiration. Data on CO_2 production and the results of Warburg cup analyses are presented in Table 1. Pyruvate, lactate, acetoin, and CO_2 accounted for all the glucose utilized by NS-4CA cells; less of the glucose utilized was recovered in the form of these compounds when the other cell preparations were employed. Cells from the modified NS-3 medium oxidized pyruvate (pH adjusted to 6.5) and succinate at much slower rates [Q_{O_2} (N) of 17.7 and 8.1, respectively] than they oxidized glucose. Pyruvate was not utilized for growth. The results of the glucose-oxidation experiments again raise the question of whether the oxidative abilities of some pathogenic bacteria may be related to their virulence.

Increased glucose concentration also was associated with a decrease in catalase activity. By use of the iodometric method for residual H_2O_2 (Jolles, Münch. med. Wochschr. **51**:2083, 1904; quoted by Sumner and Somers, *Chemistry and Methods of Enzymes*, 2nd ed., Academic Press, Inc., New York, 1947), specific activities of catalases in 16-hr-old resting cells from various media were calculated and compared with the aerosol virulence of these cells for the mouse (Table 2). The catalase of *Listeria* is of the classical heme-iron type, sensitive to sodium azide or potassium cyanide. The data for strain A4413 supported the contentions that catalase activity is related to virulence (Huddleson,

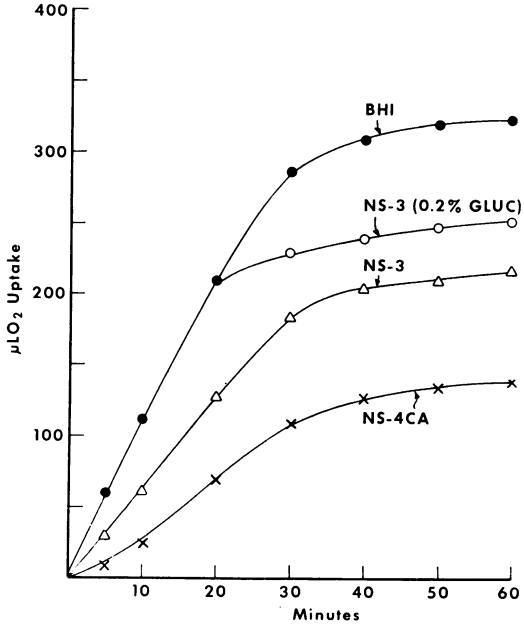

FIG. 1. *Oxidation of glucose by resting cells of Listeria monocytogenes A4413, grown in different media. The Warburg vessel contents/3 ml: 1 mg of cell nitrogen, 10 μmoles of glucose, pH adjusted to 7.0 with 1 ml of 0.067 M potassium phosphate buffer, 0.1 ml of 2 N KOH in the center well. The temperature was 36 C.*

TABLE 1. *Metabolism of glucose by resting cells of Listeria monocytogenes A4413**

Growth medium	$Q_{O_2}(N)$†	μmoles O_2/ μmole glucose	End products (μmole/μmole glucose)				Carbon recovery
			Pyruvate	Lactate	Acetoin	CO_2	
							%
BHI	444–773	1.4	0.15	0.17	0.03	0.96	34
NS-3 (0.2% glucose)	594	1.2	0.23	0.43	0.07	0.97	54
NS-3 (0.6% glucose)	375–475	0.9	0.61	0.33	0.21	0.75	74
NS-4CA	193–315	0.6	1.2	0.55	0.13	0.44	104

* Warburg vessel contents/3 ml: 1 mg of cell nitrogen, 10 μmoles of glucose, pH adjusted to 7.0 with 1 ml of 0.067 M potassium phosphate buffer, 0.1 ml of 2 N KOH in the center well, except in the case of CO_2. The vessels were shaken 60 to 80 min at 36 C.

† $Q_{O_2}(N)$ = μliters of O_2 taken up per mg of bacterial nitrogen per hr. Range of values represents three to five replicate experiments except for NS-3 (0.2% glucose), which was run once.

Brucellosis in Man and Animals, rev. ed., Commonwealth Fund, New York, 1943; Rockenmacher, Proc. Soc. Exptl. Biol. Med. **71**:99, 1949). On the other hand, strain Cornell grown in BHI was less than one-fifteenth as virulent for the mouse via the respiratory route as was strain A4413 (highest virulence), yet contained catalase that was one-and-one-half times more active. Strain AT-14 was one-eighteenth as virulent as strain A4413; strain 9037-7 was avirulent. Gutekunst, Delwiche, and Seeley (J. Bacteriol. **74**:693, 1957) found that high glucose in the medium for *Pediococcus* caused a decrease in pH that suppressed the activity but not the formation of the enzyme. With *Listeria*, however, significant pH changes were prevented by adequate buffering during growth and during catalase assay.

TABLE 2. *Catalase activity of resting cells of Listeria monocytogenes**

Strain	Medium	Specific activity†	Mouse LD_{50}‡ $\times 10^8$
A4413	NS-4CA	.149	22.8
A4413	NS-3	.355	2.6
A4413	BHI	.343	6.0
9037-7	BHI	.189	Avirulent
AT-14	BHI	.199	>108.8
Cornell	BHI	.476	>89.6

* Reaction mixture: 50 ml of 0.01 N H_2O_2 in 0.01 M phosphate buffer (pH 6.8), 1 ml of cell suspension containing 0.6 mg of bacterial nitrogen.

† Specific activity $= \frac{K_0/\text{ml enzyme}}{\text{mgN/ml enzyme}}$.

‡ The LD_{50} values were determined by respiratory exposure of mice, with the Henderson apparatus.

USE OF A TETRAZOLIUM SALT FOR AN EASILY DISCERNIBLE SEPARATION OF SEROLOGICAL PHASES

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On the basis of reaction to somatic antisera, cultures of *Salmonella* are assigned a group classification. Further speciation is accomplished by the identification of the flagellar antigens. In general, *Salmonella* contain two types of flagellar antigens, either of which may predominate in a given instance or exist in equal proportions. A complete identification requires that both phases be identified. Aside from time-consuming cultural means, a number of serological techniques exist to enhance suppressed or weak phases. All methods are based on the fact that flagellar antibody, in a semisolid matrix, will repress the development of homologous antigen, permitting heterologous phases to grow and spread.

In our laboratory, a modification of the Craigie tube is employed (Edwards and Ewing, *Identification of Enterobacteriaceae*, Burgess Publishing