



Figure 1. The effect of added increments of serine and phosphoserine on the growth of *Streptococcus lactis* (RS) in the presence of homocysteine and in the absence of methionine and glycine.

methionine, glycine, and serine. Homocysteine was added at the rate of 10 mg per 100 ml of medium. The data show (figure 1) that, upon the addition of increasing levels of serine and phosphoserine (corrected for 1 moiety of phosphorus/serine molecule), phosphoserine is from 2 to 3 times more effective than serine. It is recognized that the stimulation of cell growth herein observed may be attributed to factors other than those involved in  $\beta$ -oxidation of serine and subsequent transmethylation to form methionine. The increase of cell numbers in the presence of phosphoserine may be a reflection of a response

to organically bound phosphorus in areas of metabolism other than the one under observation. The observations of Chargaff and Sprinson (*J. Biol. Chem.*, **151**, 273, 1943) and Kristoffersen and Nelson (*J. Dairy Sci.*, **37**, 635, 1954) concerning the inability of *Escherichia coli* and *Lactobacillus casei* to deaminate phosphoserine tend to eliminate the possible conversion of phosphoserine to a glycolytic intermediate, i.e., 3-phosphoglyceric acid or 3-phosphopyruvic acid. The possibility exists that phosphorylation of serine facilitates the incorporation of serine into cellular proteins.

## DEMONSTRATION OF AN IRON-ACTIVATED ALDOLASE IN SONIC EXTRACTS OF BRUCELLA SUIIS

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The requirement of iron for optimum growth of *Brucella suis* has been demonstrated (McCullough *et al.*, *J. Bacteriol.*, **53**, 5, 1947; Waring *et al.*, *J. Bacteriol.*, **66**, 82, 1953). Roessler *et al.* (*J. Biol. Chem.*, **194**, 207, 1952) reported the anaerobic formation of hexose phosphate inter-

mediates by *B. suis* in sonic extracts containing  $Fe^{++}$ ,  $Mg^{++}$ , and  $Mn^{++}$ . Since these data suggested an Embden-Meyerhof route of glucose dissimilation and a possible catalytic role of divalent metals, an investigation of the glucose metabolism of this organism was initiated.

The organism was grown in a medium consisting of casein partial hydrolyzate, yeast autolysate, and glucose with aeration by shaking at 37 C. Cells were harvested after 45–48 hours' incubation, washed twice with 0.85 per cent saline, and the metabolic activity of resting cells and sonic extracts examined. Sonic extracts were prepared by oscillating cells in 0.04 M bicarbonate buffer in a Raytheon 9 kc sonic oscillator for 30 minutes. Cell debris was removed by centrifugation at 10,000 rpm for 30 minutes and the supernatant liquid stored at –20 C. Aldolase activity was determined by the procedure of Sibley and Lehninger (J. Biol. Chem., **177**, 859, 1949), the absorbance of the chromogen produced being converted to alkali-labile phosphorus according to Bard and Gunsalus (J. Bacteriol., **59**, 387, 1950).

The addition of divalent metal trapping agents,  $\alpha, \alpha'$ -dipyridyl (0.004 M) or *o*-phenanthroline (0.001 M), to resting cells at pH 6.5 resulted in a 64 per cent inhibition of glucose oxidation. The finding of a metalloaldolase in yeast (Warburg and Christian, Biochem. Z., **314**, 149, 1943) and in bacteria (Bard and Gunsalus, J. Bacteriol., **59**, 387, 1950) implicated aldolase as the specific enzymatic site.

The effect of divalent metals on the aldolase in sonic extracts, presented in table 1, shows  $Fe^{++}$  and  $Mn^{++}$  to be the most effective adjuvants.  $Fe^{+++}$  (0.001 M) was slightly less effective than  $Fe^{++}$  (11.0  $\mu$ g alkali-labile phosphorus) under identical conditions. Cysteine was required for maximum activity whereas cysteine alone was inactive. Dipyridyl and phenanthroline, in the presence of  $Fe^{++}$ , inhibited aldolase. The maximum activities of the dialyzed and undialyzed preparations were not equal, suggesting that some loss of activity occurred during dialysis. The Michaelis constants for the undialyzed and dialyzed extracts were,  $K_m =$

TABLE 1  
Effect of divalent metallic ions on aldolase activity of sonic extracts

Additions*	Alkali-Labile Phosphorus†	
	Undialyzed	Dialyzed‡
Sonic extract . . . . .	3.5	1.0
+ 0.001 M $Fe^{++}$ . . . . .	17.3	13.6
+ 0.001 M $Co^{++}$ . . . . .	2.9	1.1
+ 0.001 M $Ni^{++}$ . . . . .	1.4	0.4
+ 0.001 M $Mn^{++}$ . . . . .	5.3	3.6
+ 0.001 M $Mg^{++}$ . . . . .	2.3	1.4
+ 0.001 M $Zn^{++}$ . . . . .	—	0.3

\* Protocol: per reaction tube: Veronal buffer, pH 7.1, 1.5 ml; 0.56 M hydrazine, pH 7.0, 0.25 ml; 0.14 M cysteine, 0.1 ml; 0.03 M fructose-1,6-diphosphate, 0.25 ml; extract, 0.1 ml undialyzed (3 mg protein), 0.25 ml dialyzed (5 mg protein); water and metallic ion solution to 2.7 ml; temperature, 37 C.

†  $\mu$ g alkali-labile phosphorus produced per mg biuret protein per hour.

‡ Aliquot of undialyzed extract dialyzed against deionized water, 18 hr at 5 C.

$3.3 \times 10^{-5}$  and  $3.7 \times 10^{-5}$  M  $Fe^{++}$ , respectively. The optimum pH, 7.0–7.5 for the dialyzed extract in Veronal buffer and 7.0–7.5 for the undialyzed extract in phosphate buffer, agrees with that reported for the aldolase of *Clostridium perfringens* (Bard and Gunsalus, J. Bacteriol., **59**, 387, 1950).

The "low" aldolase activity (compared to extracts from other microorganisms) of the extracts under investigation coincides with the low growth and respiration rate exhibited by this organism. Moreover, the presence of a triphosphopyridine nucleotide linked glucose-6-phosphate dehydrogenase in these extracts suggests that an alternate metabolic route also may be operative in *B. suis*.

## STUDIES OF THE MAHONEY (TYPE II BRUNHILDE) STRAIN OF POLIOMYELITIS VIRUS IN THE SYRIAN HAMSTER

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