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Studies on Vitamin Nutrition of *Listeria monocytogenes*

by A. Cury, P.C.L. Portellada, and S.H. Huttor

Studies relating to the present work will permit establishment of a medium of determined composition in which culture of Listeria monocytogenes is possible. The basic medium used is composed of a mixture of 19 amino acids (the majority synthetic), dextrose and mineral salts. This medium supplemented with riboflavin, biotin, thiamine and thioctic acid (protogen), lipoic acid, pyruvate oxidation factor, acetate substitution factor) permits growth in series of the microorganism. Omitting any one of these factors of the medium, growth does not occur, which evidence indicates essential factors for the microorganism under experimental conditions.

The intensity of growth is not in practice modified by the addition to the medium of other vitamins and nutritives - pantothenic acid, pyridoxine, pyridoxal, pyridoxamine, paraaminobenzoic acid, vitamin B₁₂, folic acid, leucovorine (citrovorum factor) cocarboxylase, DPN, ATP, xanthopterine, hydrolyzed ribonucleic and desoxyribonucleic acids, purine bases (adenine, guanine, xanthine) and pyrimidines (uracil, thymine).

"Vitamin-free" casein hydrolysate added to the medium supplemented with the 4 essential vitamins already mentioned, increases intensity

of growth, which gives evidence of the presence in the casein hydrolysate of an "agent" which, not being essential to growth, exercises a stimulating action.

The influence of inoculum, agitation, pH and period of incubation were then studied. Maximum growth is obtained at the end of 48-72 hours of incubation. No growth was observed in pH of 5.0 and 10.0 since optimum pH is between 7.0 and 8.0. With large inoculum the growth begins sooner than with small inoculum, but the intensity of growth in both cases is identical after 48-72 hours of incubation. No effect was observed of the influence of agitation on growth.

The necessity of thioctic acid, which is a growth factor for Listeria monocytogenes, merits more detailed study. It was verified that the growth of the organism is proportional, within certain limits, to the concentration of thioctic acid (0.005 ug--0.09 ug/100 ml of medium), which fact can serve as basis for establishment of a method for quantitative determination of this factor. As thioctic acid is related to oxidation of pyruvate and consequently formation of acetate, the influence which these substances would have on the growth of Listeria monocytogenes was investigated. In the presence of high quantities of acetate (100 - 500 mg/100 ml of medium) the organism replaces the factor, that is, growth proceeds normally in the absence of thioctic acid; lesser doses of acetate (3 - 30 mg/100 ml of medium), in the absence of thioctic acid, have no action on growth but, associated with sub-optimum doses of thioctic acid, show a marked increase of growth. In analogous manner, in the presence of high quantities of pyruvate (300 - 1,000 mg/100 ml of medium) growth of the organism also proceeds normally in the absence of thioctic acid, and, an interesting fact, growth

observed in these cases is particularly intense, attaining levels much higher than were observed in any other condition.

The necessity of thioctic acid for Listeria monocytogenes will permit use of this bacterium in the study of mechanism of action of that factor not only in the metabolism of microorganisms in general, but also in higher beings.