Effects of Nutrition on Aspartic Acid Deaminase System of *Bacterium cadaveris.** (19898)

WILLIAM L. BOYD AND HERMAN C. LICHSTEIN.

From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis.

The enzymic constitution of bacterial cells is known to be influenced by several factors, perhaps the most important of which are the nature of the growth medium, the conditions of cultivation (*i.e.*, pH, oxygen tension, temperature, etc.), and the methods of harvesting and preparing the cell suspensions. Whereas some of these factors have been reasonably well investigated(1) others have been neglected by the microbial physiologist.

The present paper is concerned with the effects of the concentration of medium constituents on the activity of the aspartic acid deaminase system.

Materials and methods. The organism studied was *Bacterium cadaveris* (Gale) cultured at 30-32° C for 16-18 hours in a medium composed of yeast extract, pepticase, and dipotassium acid phosphate in varying concentrations. The pH of the media was adjusted to 7. The cells were harvested by centrifugation and when washed were suspended in distilled water at 30-32° C, centrifuged for 15 minutes, and resuspended in distilled water at the same temperature to give a cell concentration of 0.5-1 mg of bacterial nitrogen per ml. The deamination experiments were performed at pH 7 in M/20 phosphate buffer at 37°C, using adequate controls without added aspartic acid. The reaction was terminated by the addition of 0.2 ml of 25% trichloroacetic acid, the tubes centrifuged, and an aliquot of the supernatant fluid removed for ammonia determination by Nesslerization. Color was measured in a Klett-Summerson photoelectric colorimeter using a blue filter (400-465 mμ).

Results. The effect of the concentration of the 3 constituents of the growth medium on the aspartic acid deaminase system of *Bact. cadaveris* was determined in the following manner. The basal medium (1% each of yeast extract and pepticase, and 0.5% K_2HPO_4) was varied by holding the concentration of two ingredients constant and using 0, 1, 3 and 5% of the other constituent in parallel experiments. The deaminase activity of the harvested cells was determined before washing and after 1, 2, and 3 successive washes in distilled water, in order to ascertain the effect of nutrition on the stability of the cell suspensions.

TABLE I. Effect of Concentration of Medium Constituents on Activity of Aspartic Acid Deaminase System of *Bacterium cadaveris.*

Constituent varied	%	μg NH_3-N produced (60 min.) No. of washings 0	1	2	3
Yeast extract	0	54.2	60.3	51	11.3
	1	53.7	52.2	43.2	29.6
	3	54.8	51.3	45.2	42.1
	5	62.6	60.3	60.9	54.8
Pepticase	0	29.9	28.7	16.8	5.8
	1	29.3	26.4	28.1	17.1
	3	28.7	24.9	25.2	25.2
	5	23.2	24.9	25.1	24.5
Phosphate	0	56	22	9.9	3.2
	1	52.5	59.6	34.8	23.1
	3	58	58.6	11.9	10.2
	5	51.9	45.8	11.9	8.1

The data for 3 representative experiments are given in Table I. It may be seen that the aspartic acid deaminase activity of the cell suspensions before washing had not been altered significantly by varying the concentration of any one of the constituents of the growth medium from 0-5%. The initial activities varied from one experimental batch of cells to another, hence the lower activity of the pepticase cells in the experiment cited (Table I) has no significance. However, examination of the results obtained after 2 and 3 washings reveals marked differences in the activity of the cells harvested from the several media. It is apparent that the activity of the cells after 3 washings in distilled water increased in direct proportion to the concentration of either yeast extract or pepticase in the growth medium. For phosphate

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the greatest activity appeared at a concentration of 1% with a definite decrease in activity below and above this concentration.

Inasmuch as there were no significant differences in initial activity regardless of the concentration of medium constituents employed, and since marked differences were noted after successive washings, it may be concluded that the nutritional status of the cells, insofar investigated, affected either the stability of the enzyme system or the permeability of the cell membrane. The experiments were carried out with and without the addition of cofactors (1 mg yeast extract per tube) with no significant difference in results, indicating that coenzyme concentration was not limiting in these studies. It is important to note that both the amount of growth and the final pH varied only slightly among the various media studied.

Discussion. The results given indicate that the initial enzymic activity (aspartate deaminase system) of the bacterial cells is not influenced by the variations in nutrition studied. Upon the application of a criterion not previously employed, namely enzyme activity after successive washings, it became apparent that the nutrition during growth does affect significantly the activity of this enzyme system. Further work is necessary in order to elucidate the mechanism of these effects. Two possible explanations may, however, be offered. These are altered enzyme stability or differences in cell membrane permeability. In the latter case the effect may be due to either lowered permeability to the substrate or increased permeability resulting in loss of enzyme from the cell.

These results are significant not only because they suggest a more careful appraisal of the effects of nutrition on an enzyme system under investigation, but also because they reemphasize the fact that the enzyme constitution of a particular bacterial suspension is that portion of its potential which is selected by the nature of the growth medium, the conditions of cultivation, and the methods of harvesting and preparing the cell suspensions.

It appears to us not at all improbable that more rigid attention to these factors will lead to the discovery of enzyme systems predicted but hitherto not described, and to the stability of systems at present difficult to study.

Summary. Data are presented to show that certain variations in nutrition, while not affecting the initial activity of an enzyme system, may influence markedly the stability of this system to successive washings.

1. Gale, E. F., *Bact. Rev.*, 1943, v7, 139.

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Prevention of Growth Hormone-Induced Diabetes in Hypophysectomized Dogs by Adrenocortical Steroids.* (19899)

R. C. DE BODO, M. W. SINKOFF, S. P. KIANG, AND H. DEN.

From the Department of Pharmacology, New York University College of Medicine.

In an earlier investigation it was shown that growth hormone (Armour), in dosages of 1.0 mg/kg/day intramuscularly, completely abolished the insulin hypersensitivity of hypophysectomized dogs after several days administration, but always produced a concomitant diabetes and insulin resistance(1-3). In addition growth hormone usually produced toxic effects (anorexia, vomiting, lethargy, death). If growth hormone has a truly physiological role in carbohydrate metabolism, it was postulated that 1) administered growth hormone, in the proper dosages under proper conditions, should partially or totally abolish the insulin hypersensitivity of the hypophysectomized dog without producing diabetes and 2) could then be administered indefinitely without the

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