14284

Serological Properties of Products Synthesized from Sucrose by Enzymes from Different Strains of Leuconostoc Bacteria

EDWARD J. HEHRE.

*From the Department of Bacteriology and Immunology, Cornell University Medical College, New York City.**

Previous reports[1,2] established the fact that sterile enzyme solutions obtained from *Leuconostoc mesenteroides* have the capacity to synthesize a serologically reactive polysaccharide from sucrose. These studies also showed, for one strain of leuconostoc, that the polysaccharide which was formed in the absence of cells agreed in major chemical respects and in serological properties with the dextran elaborated in cultures of the living bacteria. The purpose of the present paper is to present more complete evidence on the serological agreement between the products formed by the enzyme solutions and those formed in living culture. The new experiments were made with representatives of 2 serologically different types of leuconostoc bacteria.[3,4] Strains of one type are characterized by the fact that their sucrose broth culture fluids cross-react in high dilution with types 2, 12 and 20 antipneumococcus serums; whereas the culture fluids of strains of the second type react in high dilution with types 2 and 20 antipneumococcus serums, but react only in low dilution with type 12 serum.

Sterile enzyme solutions were prepared[2] from sucrose broth cultures of 6 strains of *Leuconostoc mesenteroides* representing the 2 described serological types. Material synthesized from sucrose by the enzymes was obtained for serological study as follows: 1 part of each enzyme solution was added aseptically to 3 parts of a sterile solution of 5% sucrose in 0.1 M acetate buffer (pH 5.6); the enzyme-sucrose mixtures were then incubated for 5 days at 23°C, together with appropriate controls. The sterility of all the mixtures was proved by rigorous microscopic and cultural tests.

The incubated enzyme-substrate mixtures, together with supernatant fluids obtained from sucrose broth cultures of the 6 different strains, were tested in a series of dilutions against types 2, 12 and 20 antipneumococcus rabbit serums. The serum-antigen mixtures were incubated for 1½ hours at 37°C and were then observed for occurrence of precipitation. The type-specificity of the reactions was controlled by tests against types 1 and 8 pneumococcus antiserums.

The results of the serological tests of the incubated enzyme-sucrose mixtures and of the sucrose broth culture fluids are summarized in Table I. The results of the tests of the incubated control mixtures (enzyme plus glucose, enzyme plus buffer, heated-enzyme plus sucrose) are omitted because none of them showed any increase of reactive material over the small amount originally contained in the enzyme solutions. The reactions obtained with the crude enzyme-sucrose mixtures and culture fluids can be accepted as the reactions of dextran polysaccharides, since dextran is the only leuconostoc product known to cross-react with types 2, 12 and 20 antipneumococcus serums.

It is evident (Table I) that the products formed from sucrose by the enzymes from the 6 different strains showed the same likenesses and differences in serological properties as occurred among the products formed in living cultures of the strains from which the enzymes had been derived. That is, the

* Aid was received by a grant from the Ruth B. Ettinger Fund.

1 Hehre, E. J., *Science*, 1941, **93**, 237.

2 Hehre, E. J., and Sugg, J. Y., *J. Exp. Med.*, 1942, **75**, 339.

3 Sugg, J. Y., and Hehre, E. J., *J. Immunol.*, 1942, **43**, 119.

4 Neill, J. M., Sugg, J. Y., Hehre, E. J., and Jaffe, E., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 339.

TABLE I.
Serological Comparison of Products Formed from Sucrose by Sterile Enzyme Solutions with Products Formed in Living Cultures.

Strain of leuconostoc	Reactivity *vs.* types 2, 20 and 12 antipneumococcus serums			
	Enzyme-sucrose mixtures incubated 5 days at 23°C		Supernatant fluids of sucrose broth cultures	
	2 and 20	12	2 and 20	12
B	++++	+	++++	+
M	+++	0	++++	+
O	++++	+	++++	+
A	+++	+++	+++	+++
C	++++	++++	++++	++++
K	+++	+++	++++	++++

++++, precipitation with 1:5000 or 1:10000 dilution of the antigens.
+++, " " 1:500 or 1:1000 " " " "
+, " " 1:10 or 1:20 " " " "
0, no precipitation with 1:10 " " " "

enzyme-sucrose mixtures of strains *B, M* and *O,* like the culture fluids of those strains, had only slight capacity to react with type 12 in comparison to their capacity to react with types 2 and 20 antipneumococcus serum; whereas the enzyme-sucrose mixtures of strains *A, C* and *K,* like the culture fluids of those particular strains, had as high capacity to react with the type 12 as with the types 2 and 20 antiserums.

It is important to note that this comparison, based on the *ratio* of reactivities with 3 different antiserums, is an unusually critical sort of test which is capable of detecting a minor serological difference even among products which appear alike if tested against a single antiserum. Consequently, the agreement shown in Table I in respect to the *ratio* of reactivities against the 3 types of antipneumococcus serum is an especially significant index of close serological likeness between the materials formed from sucrose by sterile enzymes and the materials formed in living cultures of the strain of leuconostoc bacteria from which the enzyme had been derived.

14285 P

Protection of Normal Rats Against Death from Water Intoxication with Adrenal Cortical Substances.*

ROBERT GAUNT.†‡

From the Departments of Biology, Princeton University and Washington Square College, New York University.

It is well known that, in general, resistance to stress is low in adrenalectomized animals and that protection can be afforded by appropriate replacement therapy. Similarly adrenalectomized animals are susceptible to the intoxicating effects of excess water, and studies have been made on the effects of various steroid substances in repairing this deficiency.[1] Attempts to increase the re-

* This investigation was aided by a grant from the Josiah Macy, Jr., Foundation.

† John Simon Guggenheim Memorial Fellow.

‡ The author wishes to express his indebtedness to Professor W. W. Swingle, in whose laboratory this work was conducted, for the laboratory facilities and cortical extracts used here; and to Dr. H. W. Hays, Ciba Pharmaceutical Products, Inc., for supplying desoxycorticosterone (Percorten).

1 Eversole, W. J., Gaunt, R., and Kendall, E. C., *Am. J. Physiol.,* 1942, **135,** 378.