STUDIES ON BACTERIAL ENZYMES.

III. Pneumococcus Maltase and Lactase.

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INTRODUCTION.

Intensive study of Pneumococcus at the Hospital of The Rockefeller Institute has resulted in a better understanding of the constituents of the bacterial cell. Among the active substances thus far studied may be mentioned, the endocellular hemotoxin (1), proteolytic enzymes (2), lipolytic enzymes (2), carbohydrate-splitting enzymes (2), bacteriolytic enzymes (2), and thermolabile cellular substances (3) concerned in oxidation and reduction processes; together with the carbohydrate soluble specific substance, and the antigenic protein fraction of the cell (4).

The carbohydrate-hydrolyzing enzymes previously recognized include the amylase, inulinase, and sucrase reported by Avery and Cullen (2) and the raffinase reported by Neill and Avery (5). The experiments reported here add maltase and lactase enzymes to the list of agents involved in the biochemical activities of living pneumococci.

While, as a general principle, it is true that hydrolysis to hexoses is a preliminary step in the acid fermentation of lactose and similar disaccharides by the more common bacteria, the assumption that this applies to all bacteria is based upon scant experimental evidence, and some authorities (5) (Kruse, Fischer, and others) have concluded that the rule is not universally applicable. A number of workers (6) have shown that the products of the fermentation of complex carbohydrates differ sometimes not only quantitatively, but qualitatively from those yielded in the fermentation of the component hexoses. Gayon and Dubourg (7) report differences in the products of the fermentation of

sucrose and of fructose which are especially difficult to explain if sucrose is hydrolyzed before being fermented by the types of bacteria they employed. Phenomena of this nature have been observed chiefly in "mixed acid" fermentations rather than in the type of acid fermentation induced by the "true" lactic acid bacteria most closely related to Pneumococcus. However, the existence of microorganisms inducing an unusual type of fermentation of the complex sugars makes it worth while to seek for experimental evidence of the hydrolyzing enzymes rather than to assume their presence from the fact that the disaccharide is fermented by the bacteria.

TABLE I.

Demonstration of Activity of Pneumococcus Maltase and Lactase.

Hydrolysis mixture		Hydrolysis mixture treated with		Change in pH due to fermentation of hexoses formed by pneumococcus enzymes
		Shiga dysentery bacilli	Typhoid bacilli	
		*p*H	*p*H	*p*H
Maltose	Active enzyme	7.0	No test	0.5
	Heat-inactivated enzyme	7.5	No test	
Lactose	Active enzyme	6.5	6.6	1.0
	Heat-inactivated enzyme	7.5	7.5	

EXPERIMENTAL.

Methods.

Enzyme Solutions.—Sterile extracts of pneumococcus cells were used to demonstrate the enzymes. The pneumococcus extracts were prepared by the method previously described (3). Broth cultures were centrifuged; the concentrated bacterial cells were collected and suspended in a small volume of the supernatant broth; the pneumococcus suspensions were frozen and thawed repeatedly to liberate the intracellular substances. The extracts were finally filtered through a Berkefeld candle. Hence, the pneumococcus extracts employed as enzyme solutions were not only sterile but were free from cell fragments.

Demonstration of Activity of Pneumococcus Maltase and Lactase.

The following experiment illustrates the fact that sterile extracts of pneumococci possess the property of hydrolyzing maltose and lactose.

0.2 cc. of a sterile solution of pneumococci was added to 3.0 cc. of sterile maltose and lactose solution. A control series was prepared with enzyme solution which had been inactivated by heat. Shiga dysentery bacilli, which attack neither maltose nor lactose, were used as fermenting agents in the detection of the hexose products of the enzyme action. Tests of the lactose hydrolysis mixtures were also made with typhoid bacilli.

The results given in Table I demonstrate that pneumococci possess an active maltase and an active lactase enzyme in addition to the previously described carbohydrate-splitting enzymes. It is interesting to note that the lactase enzyme is apparently more active than the maltase as indicated by the greater acid formation in the fermentation tests made upon the lactose hydrolysis mixture. Since the concentration of the pneumococcus solution was the same in both the maltose and lactose tests, this fact suggests that either the pneumococcus cell contains a more active lactase, or that the maltase enzyme if initially as active had deteriorated to a greater extent than the lactase at the time of the enzyme tests.

Comparison of the Activity of Different Carbohydrate-Splitting Enzymes of Pneumococcus.

The preceding experiment showed an apparent difference in the activity with which the sterile pneumococcus cell solution hydrolyzed maltose and lactose. It, therefore, seemed of interest to compare the apparent activity of other carbohydrate-splitting enzymes contained in the sterile cell solution with the activity of the maltase.

This comparison was made by the fermentation method used in the preceding experiment. Equal amounts of the pneumococcus solution were added to sterile solutions of maltose, lactose, sucrose, and raffinose. The hexose products of the enzyme action were detected by use of Shiga dysentery bacilli as fermenting agents. There is an objection to this method in such comparisons since the tests may involve differences in the ease of fermentation of the different hexoses (glucose, fructose, and galactose) yielded in the hydrolysis of the different sugars. However, these differences are almost always simply differences in rate of acid formation, which vanish when a greater time is allowed for hexose fermentation by the dysentery bacilli. The results of the experiment are summarized in Table II.

Without presuming to attach too precise a quantitative value to the data in Table II, one can yet be certain that the differences in acid

formation in the test solutions indicate differences in the degree to which the various sugars have been hydrolyzed by the pneumococcus enzymes. The acid produced in the maltose mixture was unquestionably much less in quantity than that in any of the other test solutions. This indicates that the pneumococcus cell solution hydrolyzes maltose less actively than either of the other two disaccharides, and, more surprising, less actively than the trisaccharide, raffinose. One cannot be sure whether these experimental results are due to a relative inability of pneumococcus cells of this particular strain to hydrolyze maltose or

TABLE II.

Comparison of the Activity of Different Carbohydrate-Splitting Enzymes of Pneumococcus.

Hydrolysis mixture		Change in pH due to acid fermentation of hexose formed by pneumococcus enzymes
Test sugar	Amount of pneumococcus cell solution	
		pH
Maltose	0.4 cc. enzyme solution	0.5
	0.1 cc. enzyme solution	0.3
Lactose	0.4 cc. enzyme solution	1.7
	0.1 cc. enzyme solution	0.6
Sucrose	0.4 cc. enzyme solution	1.4
	0.1 cc. enzyme solution	0.6
Raffinose	0.4 cc. enzyme solution	1.0
	0.1 cc. enzyme solution	0.5

whether the maltase enzyme is especially labile and is destroyed more readily during the manipulation involved in the preparation of the enzyme solution. Maltose is apparently very readily hydrolyzed by the intact cells, for it seems to be as rapidly fermented as lactose in tests with the living bacteria.

Comparative tests of the heat lability and of the resistance to oxidation of the maltase, lactase, and sucrase were made on the enzyme solutions. In all such tests, the cell solution lost its maltase activity entirely after a heating and oxidation treatment which was insufficient to destroy all of the active lactase and sucrase. The

results, however, were not conclusive since they were complicated by the greater initial activity of the lactase and sucrase in the original cellular solution.

DISCUSSION.

Experimental proof of most of the microbial carbohydrases has been made with yeasts and fungi, although sucrase has been described from a number of different bacteria. An extended search of the literature, however, reveals very few actual demonstrations of active maltase and lactase enzymes in sterile solutions prepared from bacteria. The results of the experiments described prove the existence of active lactase and maltase in sterile, filtered solutions of the intracellular substances of pneumococci. The recognition of these two enzymes adds to the list of cellular substances known to be involved in the metabolic functions of living pneumococci.

The pneumococcus is closely related to the saprophytic streptococci involved in the common souring of milk. Microbiologists (Jensen (9), and others) who are interested primarily in the biochemical activities of bacteria point out the fact that pneumococci and the lactic streptococci (*Streptococcus lacticus*) possess sufficient characters in common to justify their inclusion in the same biological group of "lactic acid streptococci." The lactic acid fermentation of lactose and other disaccharides is generally believed to involve two reactions: (1) the hydrolysis of the disaccharide to its component hexoses; (2) the conversion of the hexoses to lactic acid. With living cells it is impossible to demonstrate the products of the first reaction as only the final acid product can be detected. Like the intermediate products of many chemical reactions, the hexoses never accumulate in detectable amount, since the living bacteria convert them to acid as rapidly as they are formed from the disaccharide. Indeed, the actual proof that two reactions are involved rests upon the fact that of the two processes (hydrolysis and fermentation) effected by the living cell, only the hydrolytic property retains its activity in sterile filtered solutions of the intracellular substances. Apparently, the power of the bacterial cell to attack hexoses is most intimately bound up with its morphological integrity, since solutions of the cellular substances, although

exhibiting many of the activities of the intact bacteria, seem to be devoid of action upon glucose (9).

Pneumococcus, in its present state of adaptation, is generally considered to have no extended independent existence outside of the animal body and its association with carbohydrates would seem to be limited to the foodstuffs encountered during its growth in the mouth. It is interesting, therefore, to find that the strictly parasitic Pneumococcus contains an endocellular equipment of carbohydrases that rivals the generous endowment of purely saprophytic fungi.

SUMMARY.

Lactase and maltase are constituents of the pneumococcus cell.

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