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Destruction of Reducing Sugars by Resting Bacterium coli.*

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The study of carbohydrates by the destructive action of yeast and bacteria has been carried out along different lines.[1] One of us[2] has caused differential sugar destruction by exposing test solutions to suspensions of resting yeast cells and then to bacterial growth. The present investigation was undertaken to study quantitatively the destruction of various sugars by resting bacteria grown upon sugar-free medium and medium enriched with various sugars.

A typical strain of *B. coli* (No. 4348 from the American Type Culture Collection) was grown upon a medium prepared as follows: Beef juice broth rendered sugar-free by the usual incubation with *B. coli,* filtered and adjusted to pH 7.6, 1.5% agar added. This mixture, alone or enriched with sugars, (0.5%) was slanted in culture tubes 1 inch in diameter. The slants were heavily seeded so that the entire surface was covered with organisms, and allowed to incubate at 37.5° C. for 18 hours. The growth was then removed with a small volume of physiological saline and the "resting" organisms washed at least twice with saline by centrifuging. About 0.45 to 0.55 cc. of packed organisms were suspended in 4 cc. of a solution containing 2.85% Na_2HPO_4 and 1.005% NaH_2PO_4. The suspension was brought to a temperature of 37.5° C. in a water bath, 2 cc. of an aqueous solution of carbohydrate† at the same temperature added, the contents of the tubes thoroughly mixed and incubation carried out for 10 minutes in the water bath. The tubes were immersed in melting ice until they were thoroughly chilled

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[1] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, 1932, **2**, 478. Baltimore.

[2] Hubbard, R. S., and Allison, C. B., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 408; *Clifton Med. Bull.*, 1928, **14**, 35; Hubbard, R. S., and Deegan, J. K., *J. Biol. Chem.*, 1928, **77**, lvii; 1930, **86**, 575; Hubbard, R. S., and Wilson, D. C., *Clifton Med. Bull.*, 1930, **17**, 57; Hubbard, R. S., and Kingsbury, M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 93.

† The amount of sugar in this solution was usually a little greater than was destroyed by the organisms as shown by preliminary experiments. It varied from about 0.003% to 0.6% for the different carbohydrates studied.

and the organisms thrown down by high speed centrifuging. The clear supernatant liquid was then decanted and its reducing power determined by the method of Folin and Wu, using as standards, solutions of the appropriate sugars in the phosphate solution. There was a slight blank value which varied between 0.5 and 1.5 mg. of glucose per 100 cc. This was determined in each test upon suspensions of organisms containing no sugar.

Among the carbohydrates studied (glucose, mannose, levulose, galactose, maltose, xylose, and arabinose) there were no significant differences in destructive ability of the organisms whether grown on sugar-free medium or medium enriched with glucose or mannose. In each case glucose and mannose were destroyed in large and approximately equal amounts (9 mg.). Levulose, galactose, and maltose were destroyed in much smaller quantities, 0.6, 0.2, and 0.2 mg., respectively. No demonstrable destruction of lactose, xylose, or arabinose was obtained.

When the organisms were grown upon medium enriched with levulose, maltose, xylose, or arabinose, their ability to destroy the corresponding sugar became very marked. No change was noted in their ability to attack the other sugars. When, however, either lactose or galactose were used to enrich the medium, the resulting organisms developed a reciprocal ability to destroy both sugars. Again no change was noted in their ability to destroy other sugars.

A number of rather interesting inferences may be drawn from the results. In spite of the fact that all common sugars are readily attacked by *B. coli* during growth, the ability to destroy some of them is usually latent or is present only to a limited extent in the organisms themselves. When exposed to these sugars for only a comparatively short period of time, the ability to destroy them is markedly increased. In some instances a new ability to attack certain sugars seemed to develop under these conditions, but it is possible that the sugars in question—arabinose, xylose, and lactose—may have been destroyed in the control experiments in amounts too small to be demonstrated by the technique used. It is practically impossible to believe that the results are due to the development of a variant of the organism in the presence of an unusual sugar in the culture medium, for the effect of the resting organisms upon sugars other than the specific one was in general not influenced by these variations in the methods of cultivation. We believe that the results can be most readily interpreted by assuming that *B. coli* is an organism with great ability to adapt itself to changes in the type of carbohydrate furnished it and that the ability to destroy such

carbohydrates becomes a definite property of the particular organism which has once developed it.

The actions of *B. coli* independent of the method of cultivation are also of interest. The organism behaved toward glucose and mannose in exactly the same manner, an observation which, as far as the authors know, has not been described in biological studies. Others have described a similar relationship between glucose and levulose based on experiments with yeast. A consideration of the structural formulas of these 3 sugars justifies interesting speculation regarding the relationships between the structure of carbohydrates and the mode of enzyme action.

In one instance the results seem to indicate the general method in which the sugar was destroyed. Organisms grown upon a medium containing either galactose or lactose developed an equal ability for destroying both sugars. It seems probable, therefore, that the attack was upon the whole lactose molecule rather than by means of hydrolysis of the sugar.

These results of quantitative studies of the destruction of sugars by resting *B. coli* seem to indicate that this method of study may be of considerable value in attacking problems in the physiology of bacteria.

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Quantitative Variations in Destruction of Glucose by Resting Bacterium coli under Different Experimental Conditions.*

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In spite of the extent to which masses of yeast cells have been used in determining the true glucose content of blood, quantitative data upon the various factors which may affect the reaction are meagre. There seems to be even less available information upon the destruction of glucose by bacterial cells. The present report concerns quantitative studies of some of the factors involved in the destruction of glucose by "resting" *B. coli*. The general methods were those described in the preceding paper.

* This material formed part of a paper read before the Syracuse meeting of the Western New York Branch of the Society for Experimental Biology and Medicine in May, 1932.