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.

The effect of acid and alkali upon the destruction of glucose was first investigated. No destruction was demonstrated if glucose was added to organisms suspended in 0.01 N hydrochloric acid, and very little if 0.001 N acid was used. In distilled water some destruction took place, but in 0.001 N sodium hydroxide this was more pronounced. If the strength of alkali was increased the amounts destroyed lessened, and in 0.02 N alkali no destruction of glucose could be demonstrated. It seemed clear, therefore, that a buffered solution of moderate alkalinity would furnish suitable conditions for carrying out studies of the decomposition of sugars and, accordingly, the alkaline buffered phosphate solution described in the preceding paper was adopted. It is the same one used in earlier work upon the destruction of glucose in blood filtrates by incubation with the *B. coli.*

The effect of varying the number of organisms and the concentration of sugar was next investigated. It could easily be shown that the amount of glucose destroyed paralleled quite closely the concentration of the bacterial suspension, but it was necessary to control conditions carefully to demonstrate the effect of varying

TABLE I.

Removal of glucose from 6 cc. of phosphate solution by exposure to a heavy suspension of *B. coli* for 10 minutes at 37.5°.

Exp.† No.	Glucose in solution incubated	Glucose found after incubation	Glucose removed by organisms‡	
			difference	Amt.
	%	%	%	mg.
1	0.100	0.001	0.100	6.0 (+)
	0.150	0.012	0.137	8.2
	0.200	0.062	0.137	8.2
	0.250	0.106	0.143	8.6
2	0.100	0.001	0.100	6.0 (+)
	0.150	0.021	0.138	8.3
	0.200	0.066	0.133	8.0
	0.300	0.150	0.149	8.9
	0.400	0.280	0.119	7.1

† In each experiment the same concentration of organisms (approximately 0.5 cc. packed cells) was present in every test.

‡ In all tables results have been corrected for the blank, (+) used when all the glucose present was destroyed, and under "difference" the difference between the % present at the beginning of the experiment and that found in the supernatant fluid after incubation and removal of the organisms indicated.

All standard and unknown solutions contained the same concentration of phosphate.

It is probable that there was a high % of error when the initial sugar content was large, because it was then necessary to dilute the solution greatly (4 to 10 times) before the colorimetric determination was carried out and the effect of any error was, therefore, greatly increased. The variations in the last 2 columns are all smaller than would be occasioned by an error of 5% in the determination of glucose.

concentrations of glucose in the presence of a constant concentration of bacteria. A large volume of packed washed organisms was suspended as evenly as possible in salt solution, and equal volumes pipetted into centrifuge tubes. These organisms were then centrifuged, the supernatant fluid decanted and the organisms resuspended in buffer solution containing various concentrations of glucose. The 2 protocols in Table I show how closely results can be duplicated when conditions are carefully controlled. The reaction is a definitely quantitative one, and when a large concentration of organisms is used the amount of glucose present does not measurably affect the amount of it which is destroyed.

Since the results obtained under controlled conditions were so concordant, other factors were varied and the effect of the variations determined. Table II shows the results when different incubation periods were used. Very short periods were not investigated because it was felt that the accuracy of the results would be very low, since some destruction of glucose undoubtedly takes place when the organisms are first immersed in the cooling bath. Long incubation was not carried out because we wished to avoid multiplication of the organisms. The results in the table show that the reaction appeared to proceed more rapidly during the first part than during the later stages of incubation. It seems probable that the apparent retardation of the reaction was not due to exhaustion of the organisms, for when bacteria which had been used in one experiment were washed with salt solution, placed on ice over night and used again, they showed no demonstrable change in their ability to destroy glucose. It seems more probable that the retardation shown was due to an accumulation of waste products either in the organisms themselves or in the medium in which they were suspended.

The effect of temperature was then investigated. The results are

TABLE II.

Effect of variations in the time of incubation at 37.5° upon removal of glucose.

Incubation	Packed Organisms	Glucose in solution incubated	Glucose removed by 0.5 cc. organisms	
			difference	Amt.
min.	cc.	%	%	mg.
5	0.45	0.050	0.050	3 (+)
10	0.47	0.050	0.050	3 (+)
5	0.49	0.100	0.083	5.0
10	0.43	0.100	0.100	6 (+)
20	0.47	0.100	0.100	6 (+)
5	0.45	0.200	0.085	5.1
10	0.45	0.200	0.141	8.5
20	0.43	0.200	0.206	12.4

shown in Table III. It is evident that the speed of the reaction increases as the temperature increases and, therefore, it is almost certain that the removal of glucose from phosphate solutions by *B. coli* is not an adsorption phenomenon.

TABLE III.
Effect of variations in temperature upon the destruction of glucose.

Temperature	Incubation	Packed organisms	Glucose in solution incubated	Glucose removed by 0.5 cc. organisms difference	Amt.	
	min.	cc.	%	%	mg.	
27.5° C.	5	0.45	0.050	0.050	3	(+)
	10	0.38	0.050	0.050	3	(+)
	5	0.40	0.100	0.075	4.5	
	10	0.40	0.100	0.107	6.4	
	20	0.40	0.100	0.100	6	(+)
	5	0.50	0.200	0.061	3.7	
	10	0.40	0.200	0.113	6.4	
	20	0.36	0.200	0.170	10.2	
47.5° C.	5	0.48	0.050	0.050	3	(+)
	10	0.40	0.050	0.050	3	(+)
	5	0.45	0.100	0.097	5.8	
	10	0.42	0.100	0.100	6	(+)
	20	0.42	0.100	0.100	6	(+)
	5	0.50	0.200	0.104	6.2	
	10	0.42	0.200	0.229	13.7	
	20	0.40	0.200	0.200	12	(+)

The experiments reported show that the destruction of glucose by masses of *B. coli* is a reaction or series of reactions which can be studied quantitatively and the physical chemical characteristics of which can, at least to some extent, be determined. When a large concentration of organisms was used and the reaction proceeded rapidly, the amount of sugar destroyed was a function of the number of cells, but not of the concentration of glucose present. The reaction velocity was not linear, for relatively more glucose was destroyed in 5 than in 20 minutes. The enzyme responsible for the destruction could not be washed out of the cells readily and apparently was not used up during the reaction, for the same organisms when used in successive tests showed no demonstrable change in their efficiency. The rate of the removal of glucose varied directly with the temperature and, therefore, at least one of the reactions involved could not be classed as an adsorption phenomenon.