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The Incorporation of $^{14}CO_2$-carbon into the Glycogen of *Escherichia coli* B

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Under resting cell conditions *Escherichia coli* B ferments Na-lactate and accumulates a polysaccharide of glycogen-like nature. If aerated with a mixture of air and $^{14}CO_2$ in a 9:1 ratio (v/v) respectively, isotope is incorporated into the glucose units. A detailed description of the experimental conditions has been published previously [1]. A preliminary analysis by means of a bacterial degradation with *Lactobacillus casei* indicated that 95 % of the isotope was located in the 3 and 4 positions of the glucose molecule. However, the method of degradation did not permit differentiation of the individual carbon atoms in the glucose molecule. It is the purpose of this report to show the exact isotopic distribution pattern, since the value of this method for the preparation of glucose-3,4—^{14}C, rests upon an equal labeling of the 3 and 4 carbon atoms. In the standardized method the cells were allowed to ferment Na-lactate in the presence of $^{14}CO_2$ for 60 min. The glycogen was isolated by the usual alkaline hydrolysis method. Depolymerisation was accomplished by hydrolysis in 0.6 N HCl and the glucose thus obtained was purified by passing through Dowex 50 in the H^+-form and Dowex 2 in the acetate form. The ion exchangers must be used in the order mentioned. The glucose was further purified by chromatography according to Gardell [2]. The sugar was finally subjected to a bacterial degradation by means of *Leuconostoc mesenteroides*, which allows a complete separation of all C-atoms in the glucose molecule [3]. Carbon 1 is split off as carbon dioxide, carbon atoms 2 and 3 appear as ethanol, and carbons 4, 5 and 6 as lactic acid. Ethanol and lactic acid were chemically degraded by conventional methods. All samples were counted as CO_2 in a proportional gas counter [4].

The following specific activities for the different carbon atoms were found:

C-atom	c/min/mmole C
1	600
2	200
3	15 600
4	15 000
5	140
6	80

Total oxidation of glucose by the van Slyke combustion method gave 33 700 c/min/mole glucose. The counting accuracy was ± 2 % for the carbons 3 and 4, and ± 15 % for the other carbons. The activities were thus fairly evenly distributed in the 3 and 4 positions. Furthermore very little of the activity was found in the other carbon atoms.

The method described previously [1], thus provides a simple method of preparation of glucose-3,4-^{14}C.

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