A Simple Method for Preparation of Glucose-3,4-^{14}C

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As shown before [1] *Escherichia coli B* accumulates a glycogen-like polysaccharide during the lag phase of growth. This finding could be used to label the glycogen-glucose preponderantly in the position C_3 and C_4. One way of obtaining such a labeling would be to grow the cells on lactate-1-^{14}C, expecting the pyruvate formed to enter the classical Embden-Meyerhof pathway towards glucose. Another possibility would be to circumvent the cumbersome synthesis of labeled lactate and cultivate the cells on non-labeled lactate in the presence of $^{14}CO_2$, expecting a CO_2-incorporation by way of the Wood-Werkman reaction and the tricarboxylic acid cycle to occur. Our experiments were carried out with this last assumption in mind.

Method: The cells were precultivated aerobically under shaking in a 6-litre Erlenmeyer flask containing 5 l of Friedleins lactate medium [2] (medium I) and harvested in the cold after 18 hours. About 4 g of cells (wet weight) were inoculated into a 6-litre Erlenmeyer flask containing 4 l of the medium mentioned, from which, however, the ammonium chloride had been omitted (medium II). It should be stressed that the cells must be kept in the cold until the moment of inoculation. A slight underpressure was effected in the vessel and a quantity of ^{14}C-labeled carbon dioxide was introduced corresponding to 2 g of barium carbonate. This will give an atmosphere containing about 10 % of carbon dioxide. After closing, the flask was vigorously shaken for 15 minutes before the inoculation. For practical reasons the cells were introduced by means of a syringe containing the cells suspended in 50 ml of cold medium II. The cells were allowed to grow for 60 minutes under very vigorous shaking.

Growth may either be interrupted by cooling the content of the flask to 4° or by adding formaldehyde to a final concentration of 0.5 %. The cells were harvested by centrifugation in the cold [1] and lyophilized. The glycogen was isolated and hydrolysed as previously described [1]. After purification by chromatography according to Gardell [3], the glucose was subjected to biological degradation in order to determine the ^{14}C-content in the separate carbon atoms of the glucose molecule * [4].

Results. The yield of glucose varied between 80 and 126 mg. In one experiment the distribution of the activity in the glucose obtained was as follows:

Carbon atoms 3,4	116 c.p.m. per μg
Carbon atoms 2,5	1.8 c.p.m. per μg
Carbon atoms 1,6	2.6 c.p.m. per μg

More than 95 % of the activity has thus entered carbon atoms 3 and 4. It must be stressed that the method of degradation used does not differentiate between the carbon atoms 3 and 4. Further work is in progress to find the ratio between the specific activities incorporated into C_3 and C_4. The isotope entering the positions 3 and 4 was diluted between 10 and 15 times. The specific activity of the protein carbon was in one experiment 21 c.p.m. per μg protein carbon. The specific activity of the glycogen carbon was in the same experiment 198 c.p.m. per μg of glucose carbon, showing that very little of the activity was lost to the protein. The remaining $^{14}CO_2$ in the atmosphere and the culture medium may be trapped by conventional methods and reutilized for further cultures of the same type as described, thus stepping up the actual yield of glucose-3,4-^{14}C considerably. The limiting factor is the amount of CO_2 expired by the cells. According to preliminary experiments it does not seem impossible to step up the yield to 20 %.

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2. Friedlein, F. *Biochem. Z.* **194** (1928) 273.
3. Gardell, S. *Acta Chem. Scand.* **7** (1953) 201.
4. Wood, H. G., Lifson, N. and Lorber, V. *J. Biol. Chem.* **159** (1945) 475.

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