On the Synthesis and Breakdown of a Glycogen-like Polysaccharide * in *Escherichia coli B*

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The following experiment was intended to elucidate the synthesis and breakdown of a glycogen-like polyglucose in bacterial cells.

Experimental. *E. coli* cells were cultivated in Friedleins medium with a restricted nitrogen source in the same way as previously described [1]. During the starvation phase, the cell concentration was 6×10^8 cells per ml, corresponding to 142 mg of cells per liter of culture (corrected dry weight). During this phase no net synthesis of protein occurred but glycogen was synthesized at an initially high rate. At the beginning of this phase, β-labeled ^{14}C-lactate was added. The cells were spun down 45 minutes after the addition of the labeled lactate. Then the cells were suspended in the Friedlein medium devoid of the carbon source. The nitrogen source contained ^{15}N to an excess of 15.6 %. After 90 min. in this medium the cells were harvested. The aeration, the harvesting procedures, and the chemical methods were the same as those previously described [2]. For measuring the radioactivity, the samples were spread on aluminium planchets and counted at infinite thinness in a windowless gas flow counter, flushed with a helium-*iso*butane mixture. Since the layer of material did not exceed 0.05 mg per cm^2, no correction was made for self-absorption.

Results. The specific activity of the glycogen carbon in the cells 45 min. after the addition of the labeled lactate was sixteen times higher than the specific activity of the protein carbon (Table 1, sample II). When these cells had been cultivated for 90 min. in the nitrogen-containing, carbonless medium (sample III), the specific activity of the glycogen carbon had decreased to about one sixth of the value in sample II. The glycogen per liter of culture decreased only to about two thirds of the value in sample II. During the same time there was a net synthesis of protein amounting to 12 % of the amount in sample II. The ^{15}N excess in the protein was 1.3 in sample III. The specific activity of the protein carbon in sample III was eight times the value in sample II.

The results may be interpreted in the same way as the results of Stetten and Stetten [3], working with rats injected once with labeled glucose. When the rats were killed a short time after the injection and their glycogen partially degraded by enzymes, it was found that the specific activity of the limit dextrins was less than the specific activity of the glucose split off. The interpretation may be that those glucose residues, which were the last ones to be formed, were preferentially attached to the ends of the glucose chains in the glycogen. The present results suggest that this "peripheral tier" of glucose residues was the first one to be utilized. Other explanations, taking an inhomogeneity of the glycogen into consideration, are also possible. Part of its carbon was possibly utilized in protein synthesis.

Table 1.

	I	II	III
C.p.m. per milliatom of glycogen carbon	0	110×10^3	17×10^3
C.p.m. per milliatom of protein carbon	0	7×10^3	57×10^3
^{15}N-excess of the protein	0	0	1.32
Protein-nitrogen, per cent of dry weight	6.7	6.4	7.3
Glycogen-glucose, per cent of dry weight	19.7	21.6	15.5

The roman numerals at the top of the table refer to the times at which the samples for analysis were taken. Sample I was taken at the moment of addition of the labeled lactate (205 min. after the inoculation of the nitrogen-deficient culture). Sample II was taken 45 min. after the addition of the labeled lactate, e. g. at the time the cells were spun down and resuspended in the nitrogen-containing, carbon-free medium. Sample III was taken 90 min. after the inoculation into this medium.

1. Holme, T. and Palmstierna, H. *Acta Chem. Scand.* **9** (1955) 1020.
2. Palmstierna, H. *Acta Chem. Scand.* **9** (1955) 195.
3. Stetten, M. R. and Stetten, D. Jr. *J. Biol. Chem.* **213** (1955) 723.

* For the sake of brevity called glycogen.