ACTA CHEMICA SCANDINAVICA 10 (1956) 1557—1562

On the Glycogen in *Escherichia coli B*; its Synthesis and Breakdown and its Specific Labeling with ^{14}C

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A technique has been devised by which it is possible to label the glycogen in *E. coli B* cells with ^{14}C without labeling the protein to any appreciable extent.

It has been shown that those glucose residues that were the last to be incorporated during a net synthesis of glycogen in *E. coli B*, were the first to be split off by the cells when the conditions were changed in order to cause a breakdown of the glycogen.

If there is a pool of substances on the pathway from medium glucose towards glycogen, this pool will not be large enough to cause any measurable dilution of the medium glucose, when it is incorporated in the glycogen.

It appears that cells in the starvation phase in a nitrogen-starved culture do not break down glycogen simultaneously as they synthesize it.

After the injection of ^{14}C-labeled glucose into rats, Stetten and Stetten [1] found that after a short interval the rat liver glycogen was predominantly labeled in those glucose residues that were the first to be split off by enzymic degradation of the glycogen.

The methods employed by us [2,3] have made possible investigations of the synthesis and breakdown of glycogen in metabolizing bacterial cells. *Escherichia coli B* cells accumulate glycogen during nitrogen starvation. During the last part of this accumulation phase the carbon source was labeled with ^{14}C. The conditions were thereupon changed, so as to make the cells break down part of the glycogen that had accumulated. The results indicate that also in this case those glucose residues that were last incorporated in the glycogen were the first to be split off when the glycogen was degraded, in our case, by the cells.

METHODS AND RESULTS

Nitrogen-starved cultures of *E. coli B* were prepared as previously described [2]. Instead of Friedlein's sodium lactate-salt medium, the glucose-salt medium devised by Hook and coworkers was used [4]. The glucose concentration was 1 g/l instead of 4 g as in the original medium. This reduction had no influence on the length of the lag phase nor on the mean generation time in the log phase. It was possible to starve the cells of the nitrogen source by reducing the concentration of ammonium chloride to 50 mg/l of medium. As in nitrogen-starved cultures grown in lactate medium, the dry weight of cells per litre of culture remained constant during the starvation phase.

During the starvation phase, totally labeled ^{14}C-glucose was added to the culture, and the phase was allowed to proceed for another 45 min. in the labeled medium. The cultivation was then interrupted by cooling the culture rapidly to 4°C. The cell suspension was divided into two equal parts which were centrifuged separately. The wet weight of the cells obtained from these centrifugations [5] was the same for both portions. One portion was taken for analysis, and the cells of the other were incubated for a further 90 min. in a medium from which the glucose had been totally omitted. It contained the same amount of ammonium chloride as the complete medium. The sample taken at the end of this incubation period is called +90 (Table 1). Prior to inoculation the cells were washed once with the medium in which they were to be inoculated. During the nitrogen starvation an increase in ultra violet absorption of the cell-free culture medium was observed, just as in lactate-grown, nitrogen-starved cultures [3]. No such absorption was observed in the glucose-free medium. The results are summarized in Table 1 (expt. A).

Cells that had not been subjected to nitrogen starvation were also used (expt. B, Table 2). Cells from an 18 hours' praeculture in the complete medium were harvested and washed, and then inoculated into an ammonium chloride-free medium (volume: 6 l). The incubation was interrupted by cooling after 10 min. The cells were centrifuged and reincubated in a fresh medium (volume: 4 l) of the same composition, but in this case the glucose was totally labeled with ^{14}C. After an incubation period of 30 min. the culture was cooled and the cells centrifuged and washed. Then they were inoculated into a medium (volume: 2 l) containing ammonium chloride in excess (1 g/l) but no glucose, the incubation lasting in this case 60 min. The results are shown in Table 2.

Prior to inoculation into a new medium, the cells were washed once in the medium in which they were to be incubated. In both experiments the initial culture volume was 6 l. Each analysis is based on cells taken from about one third of the culture. When reinoculating cells into a new medium, the volume of this new medium was always the same as that from which they were harvested. In all particulars other than those described in the present paper, the methods employed were the same as those previously reported [2,3,5].

The results obtained from a nitrogen-starved culture with lactate as the carbon source are reproduced in Fig. 1. Isotopic lactate was added in the same way as described in expt. A. The cells treated in this way were incubated in a medium free of lactate, but containing the nitrogen source in excess. The methods and results have already been described in a preliminary paper [2] (expt. C).

Table 1. Experiment A.

Time after inoculation in min.	Glycogen-glucose per litre of culture (mg)	Counts per min. per μg	
		glycogen carbon	protein carbon
0	1.0	—	—
130	11.0	0	0
175	13.4	113	6
+90	10.2	23	24

The isotope was added at 130 min. after inoculation which in this case was the beginning of the starvation phase. At 175 min. after inoculation the cells were harvested and reincubated for 90 min. in the carbon-less, ammonium chloride-containing medium. During the starvation phase there was no increase in protein nitrogen per litre of culture (8.36 mg/l at 130 min. and 8.32 mg at 175 min.). The number of cells, however, increased during this time from 3.4 to 5.3×10^8 cells per ml. The specific activity of the glucose carbon in the medium was 593 c.p.m. per μg.

An experiment was performed in order to follow the breakdown of glycogen and the protein synthesis in a medium free of carbon but containing the nitrogen source in excess. The methods were the same as those described in expt. C, except that no isotope was added, *i.e.* the cells were praecultured for 270 min. in a nitrogen-deficient medium with lactate as the carbon source, and then incubated in a medium of the same basic composition, but containing 1 g/l of ammonium chloride and no lactate. The dry weight of cells per litre of culture and the cell concentration during the nitrogen starvation was the same as during the starvation phase in expt. C. The results are shown in Fig. 2 (expt. D).

In all cases the specific activity of the protein carbon has been estimated in the following way. Nitrogen determinations were performed on the protein (hydrolyzed for 2 h in 6 N HCl), and the specific activity was calculated per μg of protein nitrogen. These values were converted to specific activity per μg of protein carbon by using a N:C ratio of 0.23. The N:C ratio was determined in four samples and found to be 0.23 ± 0.02.

DISCUSSION

The results given in Table 1 and Fig. 1 (expts. A and C) show that if labeled carbon was added during the starvation phase in a nitrogen-starved culture, it was possible to label the glycogen in *E. coli B* without labeling the protein to any appreciable extent.

When cells labeled in this way were incubated in a medium containing ammonium chloride but no carbon source, glycogen was broken down and some protein synthesized, as shown in Fig. 2.

A lowering of the glycogen to about 75 % of the original value caused most of the label in the glycogen to disappear. This means that those glucose residues

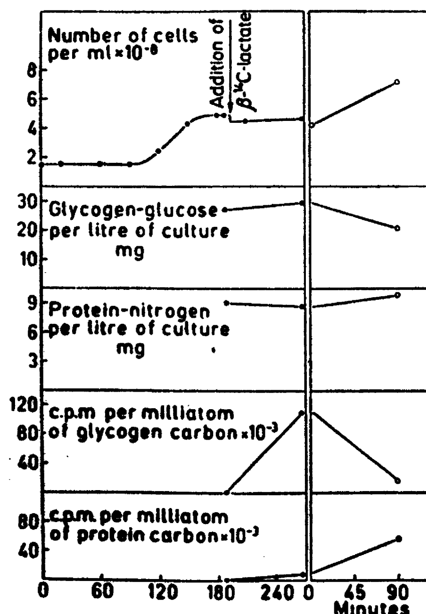

Fig. 1. Experiment C. The curves on the left-hand side of the double line show the course of events during nitrogen starvation. Part of the cells were reincubated in a medium containing the nitrogen source in excess but from which the carbon source was omitted (the curves on the right-hand side of the double line).

that were the last to be incorporated in the glycogen were the first to be split off by the cells, when incubated in a medium containing nitrogen. Under the circumstances the flow of carbon in the cell was directed away from glycogen towards the protein carbon, as shown by the increasing specific activity of the protein carbon.

The results were less marked when cells grown for 18 h in Hook's medium were allowed to accumulate a certain amount of glycogen and were thereupon incubated in a labeled medium (Table 2, expt. B). In these cells the label did not disappear from the glycogen to the same extent when incubated in the carbon-free medium with the nitrogen source in excess. The results show, however, that the specific activity of the glycogen decreased to about 40 % of the original value when the glycogen per litre of culture decreased from 8.9 to 5.1 mg. This shows that the glycogen was not evenly labeled, and that, as in the other experiments, those glucose residues that were the last to be incorpo-

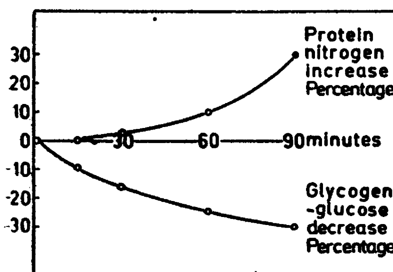

Fig. 2. Experiment D, illustrating the increase in protein nitrogen and decrease in glycogen during the second incubation period in an experiment performed in the same way as expt. C (Fig. 1, the curves on the right-hand side of the double line). The values are represented as increase or decrease in per cent of the values at the moment of inoculation.

Table 2. Experiment B.

Time after inoculation in minutes	Glycogen-glucose per litre of culture (mg)	Counts per min. per µg	
		glycogen carbon	protein carbon
0	1.1	—	—
10	5.8	0	0
40	8.9	348	117
110	5.1	140	320

Media: 0—10 min.: N-free Hook medium, glucose unlabeled.
10—40 min.: N-free Hook medium, glucose labeled.
40—110 min.: N-containing (NH_4Cl), glucose-free Hook medium.
The specific activity of the medium glucose carbon was 590 c.p.m. per µg.
From zero time to the end of the second incubation period (40 min.) the protein nitrogen per dry weight and litre of culture increased from 7.7 mg to 9.1 mg, showing that the cells contained reserves of nitrogen that were incorporated in the protein. At 110 min. the protein nitrogen per litre of culture was 11.5 mg.

rated in the glycogen were the first to be split off. In this case, too, there was an increase in the specific activity of the protein carbon during the last incubation period.

During the time the cells were cultivated in isotope medium in expt. A (130 to 175 min. after inoculation) the glycogen increased from 11.0 to 13.4 mg/l of culture. If this increase had been due solely to glucose of the same specific activity as that of the medium glucose, the specific activity of the glycogen carbon would have been 106 c.p.m. per µg at 175 min. The specific activity noted was 113 c.p.m. per µg of glycogen carbon. This means that during nitrogen starvation there was no large pool of substances on the pathway from glucose to glycogen, at any rate not during the starvation phase. Furthermore, the results may be taken as an indication that in nitrogen-starved cultures there was no appreciable breakdown of glycogen during the starvation phase.*

In expt. B the specific activity of the glycogen should have been 205 c.p.m. per µg when calculated in the same way. The specific activity observed was 348 c.p.m. This means that under the circumstances some of the non-labeled glycogen carbon present at the moment when the cells were inoculated into the labeled medium was broken down during the following incubation period. A net synthesis and a breakdown of glycogen seem to have occurred simul-

* Another possible, but rather unlikely explanation shou'd perhaps be pointed out. If some of the nonlabeled glycogen was broken down, *and* there was a pool of substances on the pathway from medium glucose towards glycogen, the non-labeled substances in this hypothetic pool may have compensated for a loss of non-labeled glycogen, present at the moment when the isotope was introduced. Since the specific activity of the hypothetic pool must have increased during the time in the labeled medium, the culture must have been harvested in this experiment exactly at the time when as much non-labeled carbon had disappeared from the glycogen as had been incorporated in the glycogen from the pool.

taneously in this experiment. This may possibly be attributed to the fact, that there was a considerable protein synthesis during this period in spite of the absence of any nitrogen source in the medium (*cf.* the text under Table 2). As shown by previously reported experiments [3,5] glycogen is utilized by the cells when protein is synthesized.

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Received August 27, 1956.