# THE INDUCED SYNTHESIS OF β-GALACTOSIDASE IN *E.COLI*

## II. ANALYSIS OF THE ACCOMPANYING SYNTHETIC ACTIVITY BY MEANS OF ISOTOPES

by

SØREN LØVTRUP*

*Biophysics Laboratory, Department of Terrestrial Magnetism*
*Carnegie Institution of Washington, D.C. (U.S.A.)*

### INTRODUCTION

In the preceding paper[1] it was shown that when the synthesis of β-galactosidase is induced by lactose in *E.coli*, the increase in enzyme activity exceeds that to be expected from the increase of cells, but corresponds rather well to the assumption that both new and old cells acquire the enzyme. In nitrogen-free medium the addition of lactose stimulates a synthesis of the enzyme at the cost of the nitrogen reserves in the cells.

Among the many questions raised by these findings several would appear answerable by determining the incorporation of radioactive isotopes associated with the induced enzyme synthesis. An attempt has been made in the present paper to answer a few of the problems which might be solved in this way.

### MATERIAL AND METHODS

*Biological material.* The experiments were performed on *E. coli* B. Regarding the details of culture technique the reader may refer to ROBERTS *et al.*[2].

*Enzyme determinations.* The methods used for extraction and determination of the enzyme were the same as described in the preceding paper[1].

*Isotope technique.* Samples for determination of uptake of isotopes were centrifuged, washed in saline, and centrifuged again. In the experiments with labelled sulfate the cells were first extracted with 5 ml 5 % cold TCA to remove acid-soluble sulfur compounds (*cf.*[2]). The residue, assumed to contain only protein-bound S, was dissolved in 5 ml 0.1 *N* NaOH. Samples of 0.5–1 ml were taken directly for counting. In the experiments with $^{14}CO_2$ the sample for each time interval had to be in a separate, closed bottle. Normal aeration was thus not possible and the bottles were therefore shaken in an air thermostat at 37° C. The samples taken for counting were extracted with cold TCA, alcohol, alcohol-ether (1:1), and ether in succession, 5 ml of each liquid. The nucleic acids were extracted by hot 5 % TCA (100° C for 20 minutes). The residue, considered to be protein, was dissolved in 5 ml 0.1 *N* NaOH. Samples generally varying between 0.5–1 ml were taken from the two latter fractions for counting (*cf.*[2]).

* Present address: Department of Histology, University of Göteborg, Sweden.

*References p. 439.*

## RESULTS

### *Experiments with* $^{35}S$

#### *Enzyme and protein synthesis in complete medium*

In the first experiment to be reported a comparison was made between increase in dry weight (DW) and uptake of sulfate in the protein fraction in cells growing in glucose and in lactose, respectively. The bacteria were grown in a medium containing 14 $\mu$g S $\times$ ml$^{-1}$, *i.e.* about half the normal concentration, radioactive sulfate (447 counts $\times$ sec$^{-1}$ $\times$ ml$^{-1}$ or 32 counts $\times$ sec$^{-1}$ per mg S) and either glucose or lactose (1 mg $\times$ ml$^{-1}$). Samples for counting and density determinations were taken at regular intervals, and in the lactose experiments extra samples were taken for enzyme determinations.

The two growth curves are shown in Fig. 1, where also the increase in DW has been plotted against the uptake of protein S. It is seen that there is a linear relationship between these two quantities, identical within the limits of error for glucose and lactose-grown bacteria, and corresponding to 5 $\mu$g protein-S per mg DW. When the lag phase is over, the sole difference between the bacteria in the two cultures presumably is the synthesis of $\beta$-galactosidase. The result obtained thus leads to the hardly surprising conclusion that the amount of this enzyme is negligible compared to the total protein content of the bacteria.

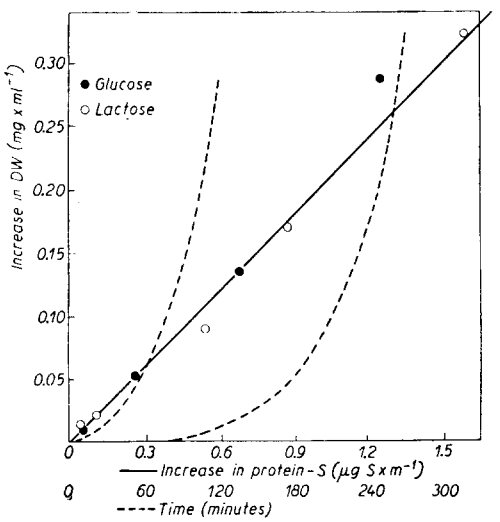


Fig. 1. Growth and increase in protein-S under normal conditions and during adaptation.

By calculating the ratio between enzyme activity and incorporation of sulfur (enzyme and protein synthesis) it is possible to estimate whether the synthetic activity during the lag phase is quantitatively different from that occurring after adaptation has occurred, *i.e.* during growth proper. The results of such calculations from two different experiments are shown in Fig. 2, from which it is seen that during the lag and early growth phase the ratio between these two quantities is higher than later on; the highest value measured being about seven times the final value. If as concluded above

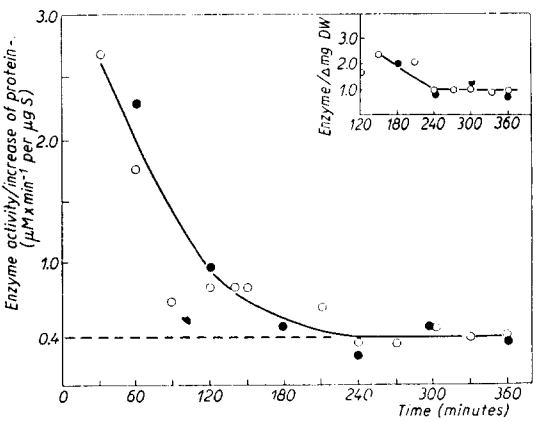


Fig. 2. Changes in the relative enzyme synthesis in complete medium. The two different symbols indicate two different experiments.

*References p. 439.*

the amount of enzyme is negligible compared to total protein, we may thus conclude that other proteins than the enzyme proper are synthesized during the lag phase, although the production of β-galactosidase enjoys a certain preference. This result is in conformance with the observation reported in the preceding paper, *viz.* that the amount of enzyme synthesized under such experimental conditions exceeds the amount to be expected from the increase in cell number (DW), *cf.* the graph inserted in Fig. 2, which shows the relative ratio between total enzyme and increase in DW. The two curves are very similar, but the latter cannot be traced back as far as the former, because no measurable growth occurred during the first 120 minutes.

*Enzyme and protein synthesis in N-free medium*

The bacteria used in the present experiments were starved to get as great effects as possible. The starvation was achieved by harvesting and washing the bacteria from a culture, and keeping the resulting pellet in the refrigerator for about two weeks. As will be seen from the experiments, the degree of starvation obtained under these conditions (minimum of liquid, no nutrient solution) during this long period is comparable with that reached in the preceding paper by keeping a glucose-exhausted culture in the refrigerator overnight.

The bacteria were suspended in 35 ml N-free medium. One portion of 3 ml was transferred to complete medium, containing 2.5 mg glucose per ml, and incubated for two hours. A sample of 10 ml was treated similarly, with the exception that N-free medium was used. A second portion of 10 ml was centrifuged and suspended in distilled water for one hour, while a third 10 ml sample was left as control. After the treatment the cells were centrifuged, washed with saline, and each portion added to N-free medium, containing 1 mg lactose and 7 μg S per ml. Radioactive sulfate was also added, the initial content being 637 counts × $sec^{-1}$ × $ml^{-1}$; *i.e.* 91 counts × $sec^{-1}$ per μg S. Samples for determination of enzyme activity and uptake of sulfate were taken during the following 3 hours. The results are shown in Fig. 3.

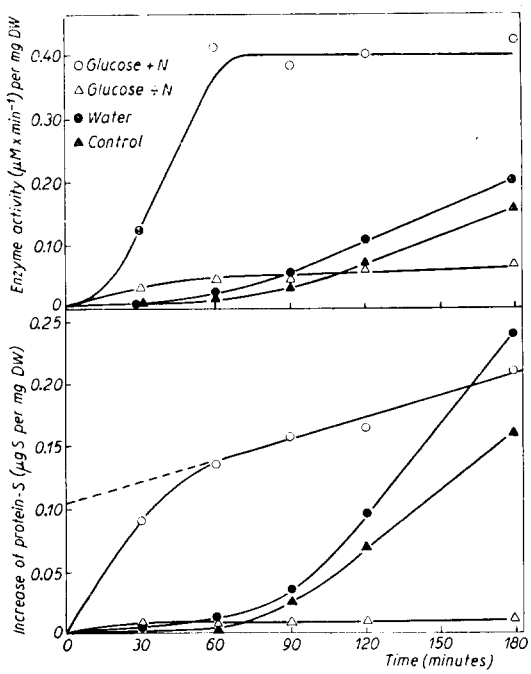


Fig. 3. Effect of preceding incubation with glucose in normal and in nitrogen-free medium and of extraction with water on the induced enzyme synthesis and on the incorporation of protein-S in nitrogen-free medium.

The cells incubated in complete medium show a very high initial rate of enzyme synthesis (although a slight lag is seen, *cf.*[1]), and the final activity (0.40 μ*M* × $min^{-1}$ per mg DW) is twice the value for "semi-starved" cells. The enzyme synthesis ceases after 60 minutes, but the uptake of sulfur in the protein

*References p. 439.*

fraction, which is rapid while enzyme is produced, continues slowly throughout the experiment at a rate of $5.5 \cdot 10^{-4}$ $\mu$g S $\times$ $min^{-1}$ per mg DW.

The initial rate of both enzyme and protein synthesis are relatively high in the bacteria preincubated with glucose in N-free medium. This indicates, in agreement with the results in the preceding paper, that the supply of energy, improved by the incubation in glucose, determines the rate at which synthesis begins. Both the increase in enzyme activity and the uptake of sulfate cease almost completely after 30 minutes, the final enzyme level corresponding to 0.05 $\mu M$ $\times$ $min^{-1}$ per mg DW, *i.e.* about 25% cent of normal. This low enzyme content partly reflects the degree of starvation, but may also be due to losses in the nitrogen reserves as a result of the preincubation with glucose (*cf.* the experiments with $^{14}CO_2$-uptake).

The considerable decrease in initial rate of enzyme synthesis resulting from starvation (*cf.*[1]) is exhibited both by the controls and the water extracted bacteria. The same phenomenon is also shown by the curves for uptake of protein-S. These two groups run almost parallel, although the water extracted cells seem to do rather better than the controls. The enzyme activity in these cells is after 180 minutes close to the normal maximum content in "semi-starved" cells, when incubated in N-free medium, and it exceeds by far the amount of enzyme in the cells preincubated with glucose in N-free medium. When the uptake of protein-S is compared, its is seen that it approaches, and for the water-extracted cells even exceeds, that occurring in the bacteria in the first group, which were taken in the logarithmic growth phase. The source of nitrogen sustaining this extensive synthesis seems rather mysterious, but the most reasonable assumption is that it comes from autolyzing cells. In that case the enhancing effect of the water treatment might be easier understandable. To bring agreement between the results it seems necessary to assume that incubation of the exhausted cells with glucose reduces the degree of autolysis.

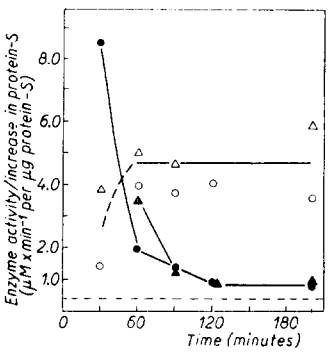


Fig. 4. Changes in the relative enzyme synthesis in nitrogen-free medium. The signification of the different symbols is the same as in Fig. 3.

Calculating the ratio between enzyme activity and incorporation of protein-S may also in this case give a measure of the "efficiency" of enzyme synthesis. In doing these calculations, it seems reasonable to correct the values in the first group for the uptake of S occurring after the enzyme synthesis has ceased. The results of the calculations are shown in Fig. 4. The variations are seen to be great, but even so it seems clear that there is a quantitative difference between the glucose-incubated cells on one hand, and the water extracted and the controls on the other. The enzyme synthesis which is of very short duration in the two first groups, is highly "efficient", corresponding to about 4–6 $\mu M$ $\times$ $min^{-1}$ per $\mu$g protein-S, as compared to 0.4 for adapted growing cells (*cf.* Fig. 2). In the two other groups, in which synthesis goes on for a longer period, the "efficiency" seems rather high in the beginning, but after 120 minutes a level around 1 $\mu M$ $\times$ $min^{-1}$ per mg protein-S has been reached. This decrease in "efficiency" is thus completely analogues to that occurring in complete medium (*cf.* Fig. 2), but the "efficiency" is somewhat higher after 180 minutes in N-free medium.

*References p. 439.*

### *Experiments with* $^{14}C$

#### *Uptake of $CO_2$ in growing, adapting and resting cells*

Normal "semi-starved" bacteria were harvested, washed in saline and resuspended in 350 ml nitrogen-free medium, boiled to remove $CO_2$. The experiment comprised three groups of four bottles. In the first group was added 40 ml bacterial suspension and 1 ml 10% lactose to each bottle, in the second group lactose was replaced by glucose, and in the third group was added 5 ml bacterial suspension, 35 ml complete medium and 1 ml glucose. To each bottle was further added $^{14}CO_2$ (30 μl of a solution containing about 25 millicurie per ml). After varying time intervals bottles were taken out for determination of DW (optical density) and uptake of $CO_2$. The DW in the first groups was 0.70 ± 0.03 mg DW per ml, in the third group DW increased from 0.11 to 0.54 mg per ml. The results of the countings are shown in Table I.

TABLE I

Uptake of $^{14}CO_2$ (counts × $sec^{-1}$ × $ml^{-1}$)

| *Time (minutes)* | *Lactose* | | *Glucose* | | *Glucose + nitrogen* | |
|---|---|---|---|---|---|---|
| | *Nucl. acids* | *Protein* | *Nucl. acids* | *Protein* | *Nucl. acids* | *Protein* |
| 30 | 0.10 | 0.17 | 0.12 | 0.62 | 1.84 | 4.22 |
| 60 | 0.08 | 0.22 | 0.12 | 0.66 | 4.83 | 7.92 |
| 120 | 0.34 | 1.16 | 0.15 | 0.92 | 10.1 | 17.6 |
| 180 | 0.65 | 2.58 | 0.20 | 1.01 | 11.3 | 20.1 |
| $\frac{\text{Protein}}{\text{Nucl. acid}}$ (30 + 60 + 120) | 3.0 | | 5.6 | | 1.7 | |

In the first group a slight gradual increase occurs in the rate of uptake The final uptake in the nucleic acid and the protein fractions are 3 and 2.5 times higher, respectively, than in the second fraction. In this a very rapid uptake occurs during the first 30 minutes, after which the uptake continues at a very low rate. In the third group the rate of incorporation is very high, continuing linearly during the first two hours, after which a decrease occurs (isotope dilution?). The results clearly demonstrate that both nucleic acids and proteins are synthesized. One point of interest in this connection is to determine the ratio between protein and nucleic acid synthesis. The value of this ratio may be obtained from the results in Table I. Using only the three first values, because of the lack of linearity after 180 minutes, we get 3.0, 5.6 and 1.7 respectively for the three groups. The value for the third group may be considered quite accurate, because of the linearity and the high counting values, and the value for the first group has been confirmed by results to be reported below. The value for the second group is higher than for the first group in the present experiments, but this has not been confirmed. It would seem warranted to conclude from these results that the specific adaptive metabolism is characterized by a relatively high protein synthesis.

The second experiment to be reported was similar to the one first described, with the exception that nitrogen-free-medium was used in all three groups, and that no carbohydrate was added to the last group. Furthermore the amount of $^{14}CO_2$ was twice as high as in the preceding experiments.

*References p. 439.*

It is seen in Fig. 5 that the very rapid initial uptake in the presence of glucose has been confirmed, but after 60 minutes only a very slight further uptake occurs. In the medium continaing no carbohydrate there is a very slow uptake during the first three hours, but after this time no further uptake occurs. The final values are about half of those in the glucose medium. The initial rate of uptake is much lower in lactose than in glucose, but the uptake continues during the whole experimental period, and the uptake after 300 minutes is about 3 times higher in both fractions. The protein/nucleic acid ratio is the same in all three cases, taking the values at 180 minutes we get 3.3, 3.5, and 3.2 respectively.

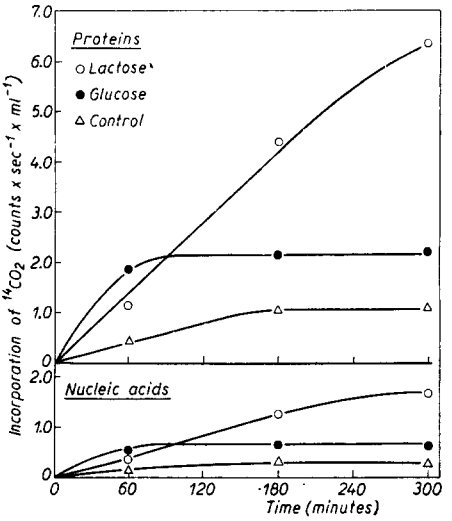


Fig. 5. Incorporation of $^{14}CO_2$ in the protein and nucleic acid fractions in nitrogen-free medium before and after addition of lactose and glucose, respectively.

These results thus show that the presence of glucose allows an uptake of $CO_2$ in the protein and nucleic acid fractions over and above that occurring in the absence of any carbohydrate. Moreover, in the presence of lactose this process goes even further. It seems permissible to conclude that the addition of lactose mobilizes reserves which are not normally available. Thus lactose apparently may both initiate the synthesis of β-galactosidase and the mobilization of nitrogen reserves. The uptake of $CO_2$ apparently continues longer than the normal duration of adaptive enzyme synthesis. Unfortunately no enzyme determinations were made to check this point.

## DISCUSSION

It is unquestionable that the clue to a deeper understanding of the mechanism of induced synthesis of enzymes is the application of isotopes. A promising beginning has been made by HOGNESS, COHN AND MONOD[3] in a study of the precursors and turnover of β-galactosidase in *E.coli*. The results presented here deal with another aspect of the synthetic mechanism, but should be regarded merely as an orientation.

A few definite results have been obtained, however, illustrating the peculiarities of adaptive metabolism. It was reported in the preceding paper that when induction of β-galactosidase synthesis occurs in *E.coli* with lactose as the only energy source, a certain surplus synthesis of enzyme occurs during the early growth period, besides that occurring in the lag phase. This means that the ratio between enzyme synthesis and increase in DW decreases with time. Using radioactive sulfate it has been demonstrated that the ratio between enzyme and protein synthesis (enzyme activity/uptake of protein-S) behaves similarly. This ratio may be determined even before any measurable growth occurs. Values approaching seven times the normal for this ratio have been attained after 30 minutes incubation. Measuring the same ratio when adaptation occurs in the absence of nitrogen, still higher values (15–20 times

*References p. 439.*

normal) were found. These results thus indicate that metabolism during the initial phase of adaptation is specifically oriented towards synthesis of the particular enzyme.

With radioactive $CO_2$ it was possible to demonstrate that the ratio between protein and nucleic acid synthesis is twice as high during adaptation in the absence of nitrogen than during normal growth. This finding further stresses the special character of the processes during the initial phase of induced synthesis. Another result obtained by the work with $^{14}CO_2$ is that in the absence of nitrogen the uptake of $CO_2$ in (synthesis of) proteins and nucleic acids is considerably higher with lactose than with glucose. It thus seems that during the induced synthesis nitrogen reserves are mobilized which are not normally accessible.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the assistance, advice and encouragement given by all the members of the staff during his visit at the Biophysics Laboratory, Carnegie Institution of Washington.

SUMMARY

By following simultaneously the uptake of $^{35}S$ and enzyme synthesis in *E. coli* B during adaptation to lactose it was found that the ratio: Enzyme synthesis/protein synthesis is much higher during the earlier phases of adaptation than later on.

Using $^{14}CO_2$ it could be shown that the ratio: Protein synthesis/nucleic acid synthesis is higher in adapting than in growing cells. These results thus demonstrate that synthetic activity during adaptation is quantitatively different from that occurring during normal growth.

RÉSUMÉ

Chez *E. coli* B on a déterminé simultanément l'incorporation de $^{35}S$ et la synthèse induite de β-galactosidase pendant l'adaptation sur lactose. On a trouvé ainsi que pendant la phase de latence et au début de la croissance, le taux de la relation: Synthèse enzymatique/synthèse protéinique était considérablement augmenté.

En utilisant $^{14}CO_2$ on a trouvé que le taux de la relation: Synthèse protéinique/synthèse acide nucléique était plus haut pendant l'adaptation que pendant la croissance normale.

Ces résultats montrent que l'activité synthétique pendant l'adaptation (induction) se distingue quantitativement de celle pendant la croissance.

ZUSAMMENFASSUNG

Bei *E. coli* B wurde gleichzeitig die Aufnahme von $^{35}S$ und die induzierte Synthese von β-Galaktosidase während der Adaptation auf Laktose beobachtet. Es wurde dadurch gefunden, dass die Verhältniszahl: Enzymsynthese/Proteinsynthese während der Latenzphase und während der frühen Wachstumsphase beträchtlich erhöht ist.

Durch Anwendung von $^{14}CO_2$ konnte nachgewiesen werden, dass die Verhältniszahl: Proteinsynthese/Nukleinsäuresynthese während der Adaptation höher ist als während des Wachstums.

Diese Ergebnisse zeigen, dass die synthetische Aktivität während der Adaptation (Induktion) quantitativ verschieden ist von derjenigen, die während des normalen Wachstums besteht.

REFERENCES

1 S. LØVTRUP, *Biochim. Biophys. Acta*, 19 (1956) 247.
2 R. B. ROBERTS, P. H. ABELSON, D. B. COWIE, E. T. BOLTON AND R. BRITTEN, *Studies of Biosynthesis in E. coli*. Carnegie Institute of Washington D.C. (1955).
3 D. S. HOGNESS, M. COHN AND J. MONOD, *Biochim. Biophys. Acta*, 16 (1955) 99.

Received June 30th, 1955