for R-1-P and the inhibition constants for chloride, phosphate and sulphate have been determined. While GDP inhibits, when it is present at certain high concentrations, no such inhibitory effect has been observed for R-1-P. Since the constants obtained suggest that the binding is dependent primarily on the charge of the anion the experiments indicate that the group of the enzyme which "activates" R-1-P binds this and any other anion by a salt linkage. This linkage might be of the same type which is found in some synthetic anion exchangers. The results also indicate that when GDP serves as a substrate in the reaction it is attached to a group of the enzyme which is different and possibly of different nature from that to which R-1-P is attached.

## The Content of Polyglucose of Glycogenic Nature * during the First Hours of Growth in *Escherichia coli* B

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The following experiment is intended to elucidate the normal metabolism of *E. coli* B as measured by the "glycogen" content at different states of growth.

*Experimental.* Freeze-dried cells of *E. coli* B were inoculated into, and grown in a salt medium (pH 7) and cultivated for 18 hours with Na-lactate as the only source of carbon [1]. The volume of the culture was 5 liter. After centrifuging and washing the bacteria with fresh medium an amount of bacteria was inoculated into two 50-liter bottles, each containing 40 liter of the medium, to give a density of approximately $10^8$ bacteria/ml. The praeculture and the 40-liter cultures were constantly shaken at 37° C and aerated through sterile cottonwool filters at a rate of 1 liter per minute and liter of medium. The bacterial suspension was cooled to 6° C within three minutes after harvest of culture fluid. The specimens obtained at different times after inoculation were centrifuged at 0° C for two hours at 2 500 r.p.m. in an International refrigerated centrifuge with four 1-liter cups. The bottom was covered with a net of stainless steel and the cup was divided into four chambers by means of a baffle in order to prevent the bacteria from whirling up when the speed of the centrifuge slowed down. In this way it was possible to spin down the bacteria quantitatively with a loss not exceeding 1.7—2.4 %. The bacteria were washed with 0.9 % NaCl and freeze-dried.

The "glycogen" was isolated by the usual alkali treatment and precipitation with alcohol. The precipitate was hydrolyzed for three hours in 0.6 *N* HCl. The glucose thus obtained was purified by passing the hydrolysate through Dowex 50 in the H+-form and Dowex 2 in the acetate-form. No other sugar than glucose was found on the paper-chromatogram. The "glycogen" could be hydrolyzed by salivary amylase.

The estimations of glucose were performed according to Dische [2].

*Results.* A very rapid increase of "glycogen"/mg of dry cells was found, reaching its maximum at 30 minutes (Table 1). This indicates that the "glycogen" synthesis precedes the synthesis of the bulk of the cell-material.

*Table 1.*

| Time after inoculation minutes | Number of cells/ml $\times 10^{-6}$ | "Glycogen" % of dry weight | Mg "glycogen" per 1 000 ml culture | "Glycogen"/cell mg $\times 10^{-12}$ |
|---|---|---|---|---|
| 0 | 111 | 1.1 | 0.3 | 1.7 |
| 15 | 112 | 10.9 | 3.6 | 17.3 |
| 30 | 111 | 12.8 | 4.4 | 19.5 |
| 60 | 110 | 11.9 | 7.1 | 31.7 |
| 90 | 111 | 9.8 | 7.6 | 34.1 |
| 120 | 175 | 5.6 | 7.4 | 21.0 |
| 240 | 665 | 1.2 | 3.5 | 2.6 |
| 360 | 1 509 | 1.0 | 7.4 | 2.5 |

"Glycogen"/cell ratio reaches its maximum at 90 minutes after inoculation or immediately before the first cell divisions.

A very rapid decrease in "glycogen"/1 000 mls of culture was found between 90 and 240 minutes, indicating that "glycogen" synthesized during the "lag-phase" of growth was utilized during the first two hours of the logarithmic phase of growth. During this period the number of bacteria/ml increased six times.

Between 240 and 360 minutes after inoculation the amount of "glycogen"/cell remained approximately constant at a level slightly higher than that found in the bacteria at the

* For the sake of brevity called "glycogen".

time of inoculation. During this time the bacteria doubled their number only once.

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## The Occurrence of Phosphoproteins in Bacteria

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In connection with investigations of rat organ phosphoproteins [1,2], it was of interest to determine whether similar substances exist in bacteria and whether they are involved in bacterial growth. In these studies an aerobic (*Escherichia coli*) and a facultative anaerobic (*Lactobacillus casei* 7469) organism have been used.

*L. casei* was cultivated on a medium previously described [3] for *L. acidophilus* with the exclusion of the dried tomato juice. Moreover, in order to increase the utilization of isotopic phosphate added to the medium inorganic phosphate was also excluded, since preliminary experiments had shown that the phosphorus present in the Bactopeptone and liver furnished sufficient total phosphorus (0.03 %) for normal growth. To the medium was added 1 mC radioactive phosphate per liter, it having been shown that the presence of as much as 100 mC $P^{32}$ per liter had no demonstrable effect on growth or acid production over a period of 72 hours.

In order to determine whether phosphoserine was present in bacterial protein, the bacteria were centrifuged down and frozen 4 hours after inoculation, by which time the weight of the freeze-dried bacteria had increased two-fold. After grinding with Ballotini-beads in 30 % ethanol, the bacteria were once again dried and then extracted as described previously [1]. To the partial acid hydrolysate of the Schneider protein was added crystalline phosphoserine and the mixture was chromatographed successively on a Dowex 50 column, a Dowex 1 column, and in two solvent systems on paper [1]. In each procedure a $P^{32}$-containing fraction followed and could not be separated from crystalline phosphoserine. These experiments make it highly probable that phosphoserine is present in the Schneider protein fraction from *L. casei*.

Using phosphoserine, then, as a measure of the bacterial protein phosphorus, the specific activity of the latter has been determined at successive intervals after incubation and compared with the activity of other fractions.

Details of these experiments will be published later.

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## Estimation of Cardiac Glycosides in the Presence of Other Steroid Derivatives

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The chemical assay of cardiac glycosides has long presented the pharmacologist with a problem. The difficulties have chiefly been due to the presence of breakdown products and other steroid derivatives which had only little or no pharmacological activity but, in the lack of the specificity of the reactions, were estimated as cardiac glycosides.

Several attempts have been made to achieve separation of cardiac glycosides and their breakdown products by chromatographic means but the separation was associated with specific difficulties mainly due to some properties of the steroid skeleton. Firstly, cardiac glycosides are neutral compounds having an uniform stereochemistry with large relatively flat molecules. Secondly, they are unstable, being sensitive to oxidizing agents, strong acids and bases, heat and strong adsorbents. Thirdly, they have an unfavourable partition coefficient in the commoner solvent systems.

These properties make for non-ideal behaviour in chromatography systems. Fractional elution from aluminium columns resulted in large losses although aluminium has been applied with great success for the widest range of steroids. Partition chromatography methods gave good results and were reliable but they were capable of dealing with only rather limited groups of cardiac glycosides at each