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

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The Occurrence of Phosphoproteins in Bacteria

Gunnar Ågren, Carl-Henric de Verdier and John Glomset

Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden

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 P³² 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 P³²-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

John Kahan

Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden

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 a 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

operation. Paper chromatography was fairly time-consuming and laborious to be applied for quantitative work.

The data now reported show that cardiac glycosides and their breakdown products may be separated quantitatively on a Dowex 50 column. Since other steroid derivatives may be eluted together with cardiac glycosides a reaction was elaborated for the specific determination of cardiac glycosides based on their specific sugar moiety. Common sugars are not eluted with the solvents.

The technique permits the estimation of the total amounts of primary glycosides, secondary glycosides and aglycones. The filter capacity of a 10 cm long column is 2–2 000 μ g, but the method can be scaled up for larger amounts. The mean recovery is 95–98 %, and the experimental error of a double determination is ± 2.59 %.

The separation may be regarded as a particular application of ion exchange resins based on the swelling properties of their network structure when the alteration of the size of pore spaces is carried out by suitable pretreatment and elution. This assumption may be supported by the fact that (1) the separation can be achieved with the resin in the free-acid form as well as in the sodium form, (2) the glycosides retarded could be eluted neither with acids nor alkalis, and (3) the eluting power of the same organic solvent seems to be altered by changing the prewashing agent.

The technique seems to provide a convenient standard method of assay for cardiac glycosides and their breakdown products in the presence of steroid derivatives. The method is simple to carry out; it is accurate, gives well reproducible results and may be used over a wide concentration range. A further advantage is that separation and analysis of the fractions can be completed within 30 minutes.

Methods for Studying Nucleotide Metabolism in Synchronized Cultures of Protozoa

Paul Plesner

Institute of Cytophysiology, University of Copenhagen, Denmark

In an attempt to study the nucleotide metabolism in different phases of the cell cyclus, and to correlate the pattern of the *in vivo* findings with the *in vitro* activity of some of the cellular enzymes, the technique of Zeuthen and

Scherbaum¹ has been employed to synchronize cultures of *Tetrahymena pyriformis*. An apparatus, that can accommodate 10 cultures of 150 ml each and expose them to the temperature shocks required, has been constructed. The cells are grown on a proteose-peptone-salt medium or on this medium after treating it with 5 grams of norite per liter and fortifying it with Kidder's vitamin B group² and 15 mg per liter of guanylic and uridylic acids each ("semisynthetic medium"). Norite absorbs all pyrimidines, purines and all their ribosides and ribotides.

The nucleotide metabolism has been studied *in vivo* by the incorporation of labelled adenine, guanine and uracil into the nucleotides of the living cells. In order to be able to incubate the cells with isotope within the limits of a well-defined phase of cell cyclus, the incubation time must not exceed 0.5 hour; to get a sufficiently high concentration of isotope in this period the semisynthetic medium is used, and simultaneously with the addition of the isotope, a dialysisbag, containing 1 g of Dowex-1 equilibrated to the semisynthetic medium without any nucleotide addition, is added. The Dowex is supposed to absorb the nucleotides, which up till this point has served as purine-pyrimidine source, while the labelled bases added should not be affected. At the conclusion of the incubation period, acetone powder is made of the cells, the powder extracted with 2 ml of buffer per 100 mg powder, and the extract dialyzed for 18 hours in the cold-room against 20 ml of the same buffer to which 100 mg of norite is added. The norite, which by now has extracted all the purine-pyrimidine bases and their derivatives, is eluted with 50 % ethanol at a slightly alkaline pH, and the eluate chromatographed on paper using a 96 % ethanol / *M* ammoniumacetate (750 : 300) solvent saturated with borate and the pH adjusted to 10 with NH_4OH . By this gentle method of differential dialysis, the activities of the acetone powder enzymes are retained and may be assayed *in vitro*. Chromatograms from different cell phases are presented as examples of the possibilities of this method. The variation in appearance of the chromatograms from phase to phase is discussed and values for the contents of nucleoside phosphorylase, phosphoglucomutase, aldolase and phosphoglycer-aldehyde dehydrogenase are presented.

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