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

The author wishes to thank Dr. S. Gardell for his advices and his help with the estimations.

1. Hedén, C. G. *Acta Pathol. Microbiol. Scand. Suppl.* **88** (1951) 19.
2. Gurin, S. and Hood, D. B. *J. Biol. Chem.* **131** (1939) 211.

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 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.

1. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 503.
2. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 1570.
3. Ågren, G. *Acta Physiol. Scand.* **17** (1949) 55.

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 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