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 μg, 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 $\pm$ 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 Scherbaum [1] 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 group [2] 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 phosphoglyceraldehyde dehydrogenase are presented.

1. Zeuthen, E. and Scherbaum, O., *in* J. A. Kitching: *Recent Development in Cell Physiology*, Bristol 1954, p. 141.
2. Kidder, G. W. and Dewey, V. C. *in* A. Lwoff: *Biochemistry and physiology of protozoa*, New York 1951, p. 391.