

reactions currently used for the determination of these compounds. Application of the reaction to the determination of creatine and creatine phosphate in biological material is now being studied.

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¹ Sprince, H., and Rowley, G. R., *Science*, **125**, 25 (1957).

² Ruhemann, J., *J. Chem. Soc.*, **97**, 2025 (1910).

A Simple Method for the Concentration of Fluids containing Protein

THIS method is essentially based on the well-known principle of dialysis against substances of high molecular weight.

Fluid containing protein is placed in a glass tube or container, preferably with a small protuberance at the bottom end having approximately the smallest volume to which the fluid is to be concentrated. The size and shape of the glass container should be such as to provide the greatest possible dialysing surface. Using small glass tubes and narrow tubing, even a few millilitres of fluid containing protein can be successfully concentrated. The polyethylene glycol ('Carbowax' 20 M, made by Union Carbide, also available from G. T. Gurr), a waxy, flaky substance, with a molecular weight of 20,000, is broken up and an appropriate quantity of the dry granulated material is poured into the dialysis tubing. This can easily be achieved by inflating the tubing and using a funnel. 'Visking' dialysis tubing was used throughout, but any similar tubing would be suitable. The proportion of polyethylene glycol to fluid containing protein is not critical, but there should not be less than 1 part of polyethylene glycol to 10 parts of fluid. Polyvinylpyrrolidone (PVP) in dry powder form was also tried and gave satisfactory results; it is, however, much more expensive. The dialysis tubing containing

the polyethylene glycol is then bent double (or more) and is placed in the glass vessel containing the glycol (Fig. 1). The length of the tubing should be such as to allow for the rising column of water inside it. It is advisable to moisten the polyethylene glycol inside the tubing with a small quantity of water just before it is placed in the fluid containing protein. The level to which the tubing should be pushed down depends on the final concentration required. The concentrating process ceases, of course, when the fluid containing protein reaches the level of the bottom of the tubing. The whole procedure is thus automatic. With larger quantities of fluid the tubing can be bent several times, providing a larger dialysing area and accelerating the process. The electrolyte content of the concentrate will be the same as in the fluid containing protein at the start, as there is a free passage of electrolytes across the membrane.

The method is rapid, reliable, and no special equipment is required. It has also the great advantage that the concentrate can be collected from clean glass and need not be scraped out from the inside of a sticky tube. Under suitable conditions a concentration, for example, of 10 ml. of urine to about 0.25 ml. is easily achieved in 3-4 hr. The high efficiency of the method is also due to the fact that the hydrophilic agent, namely, polyethylene glycol, does not contain water and has, therefore, full absorptive capacity. No denaturation effects were observed.

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Unfermentable Sugars in Barley Mash fermented by the Amylo Process

IN an earlier communication¹ concerning the production of alcohol from starchy materials by the amylo process, it was demonstrated that sugar consumption with barley fell considerably below that of the other raw materials by failing to exceed 85 per cent even with barley grains from which the bran had been removed. This was attributed to the presence of a considerable amount of unfermentable sugars. We now wish to report on the composition of residual unfermentable sugars in the fermented mash of barley, including the isolation of laminaribiose and of a β -linked glucosyl trisaccharide from this mash.

Polished barley grains were cooked with 0.03 per cent hydrochloric acid at 3 kgm./sq. cm. for 60 min. Alcohol fermentation of the cooked mash (total sugar 10.9 per cent) was carried out by the amylo process with the amylo mould (*Rhizopus javanicus* Takeda) and alcohol yeast (*Saccharomyces cerevisiae* sp.). After completion of fermentation, the mash (total residual sugar 1.6 per cent) was deproteinized and concentrated *in vacuo*. The acidic hydrolysis of this concentrate produced pentose (xylose and arabinose) and glucose as the sugar components detectable by paper chromatography; the pentose-glucose ratio of 44:56 was estimated on the basis of the paper chromatographic analysis devised by A. E. Flood *et al.*² The concentrated sugar mixture (300 ml.) equivalent to 4,000 ml. of fermented mash was then separated into xylose, arabinose, galactose (?), three pentosyl oligosaccharides, five glucosyl oligosaccharides and higher pentosyl and glucosyl oligosaccharides by a combination of carbon column³ and paper chromatographic techniques. Three pentosyl oligosaccharides had R_F

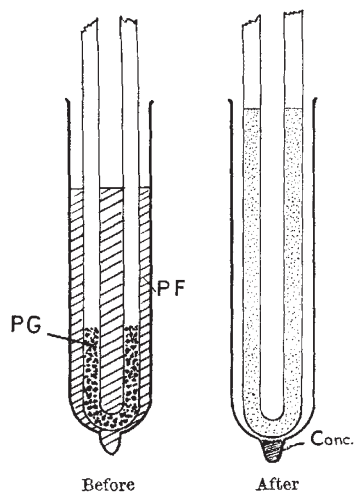


Fig. 1. Apparatus for concentrating fluids containing protein. PF, fluid containing protein; PG, polyethylene glycol; Conc., concentrate