Behaviour of Polyethylene Glycol on Dialysis and Gel-filtration

POLYETHYLENE glycol is now widely used for concentrating protein solutions by dialysis. We have used the material of molecular weight 15,000 (L. Light and Co., Ltd., Colnbrook, England) and have found that it penetrates dialysis sacs to an appreciable extent.

100 ml. distilled water was dialysed against polyethylene glycol in a sac of 24/32" 'Visking' tubing until the sac was apparently empty. The contents of the sac were rinsed out with distilled water and a residue of 27 mg was obtained on freeze-drying.

Although it seemed likely that this material must be of low molecular weight and would therefore separate from proteins on gel-filtration on 'Sephadex G-100' (Pharmacia, Uppsala, Sweden), this turns out not to be so. Fig. 1 shows the elution curves obtained with 50 mg polyethylene glycol (mol. wt. 15,000) and of 27 mg of the material which passed into the dialysis sac above. The column was prepared and eluted with 0.02 M-phosphate buffer pH 6.9, and the volume in which the sample was applied was 5 ml. in each case. The polyethylene glycol in the effluent was estimated by heating 1-ml. samples, diluted if necessary, for 30 min at 100° with 1.0 ml. 0.2 N KMnO_4 and 1.0 ml. 2 N H_2SO_4 and titrating with thiosulphate the iodine liberated on addition of excess KI to the cooled reaction mixtures. A standard curve was prepared relating decrease of the thiosulphate titre to weight of polyethylene glycol. The recovery of polyethylene glycol from both columns appeared to be 110 per cent.

Fig. 1 also shows the elution curves obtained in separate experiments on the same column for about 18 mg human haemoglobin (mol. wt. 68,000) run in 0.02 M phosphate buffer, pH 6.9, and applied in 2 ml. of the same buffer, and for about 30 mg pepsin C (= para-pepsin II, mol. wt. 40,700 (ref. 1)), run in 0.1 M acetate,

pH 5.6, and applied in 20 ml. of the same buffer. The haemoglobin in the effluent was estimated by its extinction at 540 m μ and the pepsin C by its proteolytic activity against haemoglobin.

It is seen that the fraction of the polyethylene glycol which passed the dialysis membrane has emerged in a slightly larger elution volume than the whole polyethylene glycol, indicating that it is a fraction of slightly smaller molecular size.

Both samples of the polyethylene glycol, however, have emerged in a volume considerably smaller than that at which the protein of molecular weight 40,700 is found and at about the same volume as the protein of molecular weight 68,000.

It is possible that polyethylene glycol exists in aqueous solution as a random coil and thus exhibits a larger effective molecular size than a protein of greater molecular weight in gel filtration, but this leaves unexplained the passage of the material through the dialysis membrane. Whatever the explanation of the phenomenon, it is apparent that gel-filtration is not necessarily a suitable means of separating polyethylene glycol from proteins of moderate molecular weight. Ion-exchange chromatography may be used for this purpose—we have found that, as expected, polyethylene glycol is not retarded on columns of diethylaminoethyl- or carboxymethyl-cellulose at pH 6.9.

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¹ Ryle, A. P., and Porter, R. R., *Biochem. J.*, **73**, 75 (1959).

Onuphic Acid—A Sugar Phosphate Polymer from the Tube of *Hyalinoecia tubicola*

ALTHOUGH sugar phosphate polymers are present in bacterial cell walls¹ and phosphorylated polysaccharides are produced by certain yeasts², reports of macromolecules composed of carbohydrate and phosphate in higher animals have been confined to the ribose phosphate backbone of the nucleic acids. During an investigation of the nature of skeletal structures in the invertebrates, however, it has been observed³ that in the tubes of the marine worm *Hyalinoecia tubicola* the phosphate is not present as inorganic salt but is closely associated with carbohydrates.

H. tubicola lives inside a tough, portable, quill-like tube made of a light, translucent substance which contains appreciable quantities of magnesium, calcium and phosphorus. In 1882, Schmiedeberg⁴ analysed the tubes of *Onuphis* (now *Hyalinoecia*) *tubicola* and found ash 37.5, Mg 5.9, Ca 2.4 and P 9.5 per cent. He suggested that the tube consisted of a carbohydrate substance 'onuphin', albuminoid and the acid phosphates of calcium and magnesium, and he commented that it was difficult to prepare onuphin free from phosphate without degrading it. Later, in a survey of inorganic deposits in the invertebrates, Clarke and Wheeler⁵ reported quantities of magnesium, calcium and phosphorus in *H. artifex* similar to those found from *H. tubicola*, and they explained the discrepancies in the proportions of bases to acid by assuming that the phosphate might be present as metaphosphate or polyphosphate.

Our analyses for ash (37.2 per cent) and for phosphorus (9.3 per cent) in the washed and dried tubes of *H. tubicola* agree closely with those of Schmiedeberg, but we have been unable to establish the presence of any orthophosphate, metaphosphate or polyphosphate in the supernatant after acid demineralization. The X-ray diffraction pattern of whole tubes contains two broad diffuse rings only; no well-defined, oriented or continuous reflexions suggesting crystalline salts have been observed.

After demineralization in 1 N HCl, the tube swells to a gelatinous mass in which, after washing, only a small

Fig. 1. Gel filtration on 'Sephadex G-100'. Column 2.3 cm x 40 cm. Upper, unfractionated polyethylene glycol; middle, polyethylene glycol from dialysis sac; lower, haemoglobin ○ and pepsin C ●.