specific radioactivity of glucose 6-phosphate, 6-phosphogluconate and gluconate in tissues. It has been used in this laboratory as an alternative to chromatography in checking the purity of radioactive preparations of glucose.

Glucoamylase of Dog Serum

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Dog serum contains at least two starch-degrading enzyme activities (Franzini & Bonini, 1967). In the conventional saccharogenic determination of dog serum α -amylase the presence of a glucoamylase gives falsely elevated values. The glucoamylase activity was totally separated from α -amylase activity by gel filtration. The late elution of α -amylase from both Bio-Gel P-300 and Sephadex G-200 aided this separation. The glucoamylase was eluted from both gel-filtration media with an apparent molecular weight 180 000.

The enzyme appears to have both glucoamylase and α -glucosidase activities and to attack α -(1 $\rightarrow$ 6)- as well as α -(1 $\rightarrow$ 4)-linkages. The profiles of the pH-activity curves for glucoamylase and α -glucosidase activity were similar. With starch and maltose as substrates, the optimum for both enzyme activities was at pH 6.1.

A calibrated amylose fraction, prepared by repeated gel filtration of alkali-hydrolysed starch, was used as substrate in kinetic measurements. A K_m value of 0.027 mM was obtained for this amylose fraction. With maltose and isomaltose as substrates K_m values of 0.35 and 36.6 mM respectively were obtained. Like other glucoamylases (Seetharam, Swaminathan & Radhakrishnan, 1970), the glucoamylase and maltase activities were inhibited by tris, turanose and erythritol.

We are grateful to the Medical Research Council of Ireland for their financial support.

Franzini, C. & Bonini, P. A. (1967). *Experientia*, **23**, 373.
Seetharam, B., Swaminathan, N. & Radhakrishnan, A. N. (1970). *Biochem. J.* **117**, 939.

Further Observations on Transcobalamin III in Normal Human Serum

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Vitamin B₁₂ is carried in human serum bound to macromolecules. These carriers are not fully saturated, so that when radioactive vitamin B₁₂ is

added to serum *in vitro* a certain amount becomes bound. Fractionation of such serum by DEAE-cellulose chromatography, after removal of the excess of exogenous vitamin B₁₂, results in two peaks, which have been designated transcobalamin I and transcobalamin II (Hall & Finkler, 1965). Fractionation of a similarly labelled serum on Sephadex G-200 also results in two peaks, one corresponding to 120 000 molecular weight and transcobalamin I and the other to 36 000 molecular weight and transcobalamin II (Hom, 1967). It has, however, been demonstrated in this laboratory that a hitherto unrecognized binder designated by us 'transcobalamin III' exists in normal human serum, and accounts for approx. 18% of the radioactivity bound (Bloomfield & Scott, 1971).

Careful comparison of the same serum fractionated by DEAE-cellulose and Sephadex G-200 showed that there is approx. 18% more radioactivity in the transcobalamin II peak by the former method of fractionation. This could be due to contamination of the transcobalamin II peak on DEAE-cellulose with transcobalamin I. However, when this fraction was reapplied to the same type of column after being dialysed and concentrated, it was shown to be contaminated with no more than 2% of transcobalamin I.

It can be shown by Sephadex G-200 chromatography that all of the low-molecular-weight binding proteins can be removed from serum by treatment with activated charcoal. If serum so treated (and thus containing only the high-molecular-weight binders) is run on DEAE-cellulose, contrary to what would be expected if there were only two types of binders, a peak of about 18% of the radioactivity was found in the transcobalamin II area. In addition, reapplication of this peak material on Sephadex G-200 shows that it is still of high molecular weight, and not simply due to dissociation of transcobalamin I during chromatography.

The possibility that the new transcobalamin III binder might be an artifact brought about by the polymerization of transcobalamin II seems unlikely. It is possible to cause the first DEAE-cellulose peak to split by applying the serum in different salt concentrations. Fractionation of this split peak by gel filtration consistently shows that, however it is divided by changing the ionic strength of the applied sample, the same proportions of high-molecular-weight and low-molecular-weight binders are obtained.

Bloomfield, F. J. & Scott, J. M. (1971). *Br. J. Haemat.* (in the Press).
Hall, C. A. & Finkler, A. E. (1965). *J. Lab. clin. Med.* **65**, 459.
Hom, B. L. (1967). *Clinica chim. Acta*, **18**, 315.