- Hochachka, P. W. (1965). *Archs Biochem. Biophys.* **111**, 96.
- Latner, A. L. & Skillen, A. W. (1968). *Isoenzymes in Biology and Medicine*, 1st ed. New York and London: Academic Press.
- Markert, C. L. & Faulhaber, I. (1965). *J. exp. Zool.* **159**, 319.

Immunoenzymology of Purified Urate Oxidase

By D. A. FITZPATRICK, O. FITZGERALD and K. F. MCGEENEY. (*Department of Medicine and Therapeutics, University College, Dublin 4, Irish Republic*)

Urate oxidase (EC 1.7.3.3) was first obtained in pure form by Mahler, Hübscher & Baum (1953) from the mitochondrial fractions of pig liver. Baudhuin, Beaufay & de Duve (1965) have since shown that the enzyme, when present, occurs in the core of the peroxisomes. It was considered that a method of preparation from the mitochondrial fraction could be developed that first removed the contaminating mitochondrial protein.

Pig liver mitochondrial fraction, which contains the peroxisomes, was prepared by differential centrifugation. The mitochondria were lysed and solubilized by homogenization and treatment with deoxycholate. Removal of the contaminating mitochondrial proteins was then effected by high-speed centrifugation, the peroxisome pellet containing 90% of the urate oxidase. Subsequent treatment, including gel filtration, yielded a pure preparation of the enzyme.

Purified preparations of the enzyme were used to immunize both guinea pigs and rabbits. The antisera obtained were tested for cross-reaction with urate oxidase prepared from other sources. Reactions of complete identity were obtained with the enzyme from a mammalian source (bovine kidney) and partial identity with enzyme extracted from non-mammalian vertebrate sources (*Rhombus maximus* and *Pleuronectes platessa*). The antisera, however, failed to react with various microbial urate oxidases.

The stability of purified urate oxidase from mammalian and microbial sources was studied by heating samples (a) at 50°C for 10min and (b) at 70°C for 30min, and comparing the antigenic integrity with that of unheated controls. Pig liver enzyme and yeast enzyme differed from the fungal enzyme by giving a precipitin line on immunodiffusion after being heated at the lower temperature. Bacterial enzyme differed from all three, giving a positive result after being heated at either temperature.

D. A. F. holds a fellowship from the Medical Research Council of Ireland.

- Baudhuin, P., Beaufay, H. & de Duve, C. (1965). *J. Cell Biol.* **26**, 219.
- Mahler, H. R., Hübscher, G. & Baum, H. (1955). *J. biol. Chem.* **216**, 625.

Measurement of Glucose Specific Radioactivity in Plasma

By J. COSTELLO, J. M. SCOTT and E. BOURKE. (*Departments of Clinical Medicine and Biochemistry, Trinity College, Dublin 2, Irish Republic*)

Accurate determination of glucose specific radioactivity is essential for investigations of the kinetics of glucose metabolism in man and experimental animals. Two main procedures have been used previously. First, radioactive glucose has been separated from other labelled compounds present by chromatography. The second type of procedure involves the chemical conversion of glucose into one of a variety of derivatives. These methods frequently have several undesirable features, including several recrystallizations and low recoveries; this is of crucial importance in man, where only tracer quantities of isotope can be administered.

In this communication we report an enzymic method of determining the specific radioactivity of both [1-¹⁴C]glucose and [U-¹⁴C]glucose in plasma. The method involves a single procedure, is reproducible and is specific for glucose. It is based on the use of three coupled enzyme reactions to convert glucose into ribulose 5-phosphate and carbon dioxide.

In initial studies it was found that the total reaction goes virtually to completion spectrophotometrically. The sequential reaction was also demonstrated by radioautography. Subsequently the reaction was carried out in counting vials and radioactive glucose was determined by the disappearance of ¹⁴CO₂ after acidification. It was found that, over a tenfold concentration range of plasma glucose, the reaction always went to over 98% completion. Comparable results were obtained with both [1-¹⁴C]glucose and [U-¹⁴C]glucose. If [1-¹⁴C]glucose is used this results in total loss of all radioactivity due to glucose as ¹⁴CO₂. [U-¹⁴C]Glucose can also be used, resulting in a loss of one-sixth of the radioactivity. Also, the plasma volume can be increased from 0.1 to 1.0ml without loss of sensitivity, and in practice gives excellent recoveries when other ¹⁴C-labelled compounds are present. The entire radioactivity assay is carried out in a scintillation vial and involves only one critical addition, namely the plasma or standard.

The method can be adapted for measuring the

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

By K. F. McGEENEY and D. O'DONNELL. (*Department of Medicine and Therapeutics, University College, Dublin 4, Irish Republic*)

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

By J. M. SCOTT and F. J. BLOOMFIELD. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*)

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.