- 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