PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 487th Meeting of the Society was held at the Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, on Saturday, 19 October 1968, when the following papers were presented:

COMMUNICATIONS

Ferrochelatase of Lettuce Chloroplasts

By R. F. G. J. KING and E. R. PAGE. (*Department of Biochemistry, University of Bath, and National Vegetable Research Station, Wellesbourne, Warwicks.*)

Protohaem ferro-lyase (EC 4.99.1.1), also known as ferrochelatase, has been reported in mammalian mitochondria (Nishida & Labbe, 1959; Porra & Jones, 1963*a,b*), in bacteria (Mazanowska, Neuberger & Tait, 1966) and in plant cells (Page, 1966; Jones, 1968). Most of the activity in mature green lettuce leaves (var. PI 177418) was found to be associated with the chloroplasts, which were obtained by extraction in an Ato-Mix blender at 0° with 0.3M-NaCl-100mM-tris, pH 7.8, and subsequent centrifugation. The method of assay used was that of Krantz, Gallien-Lartigue & Goldwasser (1963). The pH response of the lettuce enzyme was similar to that of the enzyme in French-bean seeds (Page, 1966) and to that reported for the mammalian mitochondrial enzyme, with an optimum activity at pH 7.8.

Utilization of either mesoporphyrin or protoporphyrin as substrates by the enzyme was achieved under reducing conditions with mercaptoethanol. The K_m for mesoporphyrin utilization was 100 μM , and that for Fe^{2+} was 6.5 μM .

Between the temperatures of 18° and 37° the rate of enzymic reaction increased with a Q_{10} of 2.9 and an E_{act} of 18.7 kcal./mole.

Solutions of protoporphyrin were prepared both with and without 2% (v/v) Tween 80. The presence of Tween 80 with the substrate resulted in considerable inhibition of enzymic activity.

By the addition of insoluble polyvinylpyrrolidone (Polyclar AT) during separation of the chloroplasts from the lettuce the activity of the enzyme towards mesoporphyrin and protoporphyrin was increased. This increase was not achieved by the use of soluble polyvinylpyrrolidone (Polyclar L).

Ultrasonic treatment of intact chloroplasts followed by centrifugation at 1500g resulted in an increase of 50% in the total combined activity of pellet and supernatant.

When soluble polyvinylpyrrolidone was added before ultrasonic treatment, the ratio of activity in the pellet to that in the supernatant after centrifugation at 1500g was altered, but the total was still 50% above the value for intact chloroplasts at the same concentration.

Jones, O. T. G. (1968). *Biochem. J.* **107**, 113.

Krantz, S. B., Gallien-Lartigue, O. G. & Goldwasser, E. (1963). *J. biol. Chem.* **238**, 4085.

Mazanowska, A. M., Neuberger, A. & Tait, G. H. (1966). *Biochem. J.* **98**, 117.

Nishida, G. & Labbe, R. F. (1959). *Biochim. biophys. Acta*, **31**, 519.

Page, E. R. (1966). *Rep. nat. Veg. Res. Sta. for 1965*, p. 30.

Porra, R. J. & Jones, O. T. G. (1963*a*). *Biochem. J.* **87**, 181.

Porra, R. J. & Jones, O. T. G. (1963*b*). *Biochem. J.* **87**, 186.

The Hexokinase of Boar Spermatozoa

By E. LINFORD. (*National Institute for Research in Dairying, Shinfield, Reading, Berks.*)

Mammalian sperm contain the enzymes of the glycolytic pathway and the tricarboxylic acid cycle (Mann, 1964). Present studies show that the two dehydrogenases of the pentose phosphate pathway are also present in boar sperm extracts. Two characteristics of sperm glycolysis are that fructose is the only exogenous sugar available and that after ejaculation the fructose in the seminal plasma cannot be replenished as it is used. Spectrophotometric assay of the hexokinase activity indicates that the rate of fructose phosphorylation in extracts from ultrasonically treated boar sperm exceeds the rate of fructose utilization by intact spermatozoa. Thus, the hexokinase activity *in vivo* seems to be limited, possibly by a permeability barrier to fructose entry or by some property of the enzyme. The sperm hexokinase has a high K_m for fructose (3mM), and hence the low concentration of fructose in the boar seminal plasma (0.7mM) limits the rate of fructose utilization.

Gel filtration of 105000g supernatant of boar