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

sperm extracts consistently gives two distinct peaks of enzyme activity. One peak is eluted at a position corresponding to a molecular weight of 100 000, whereas the other peak is eluted at the void volume from Sephadex G-150 (mol.wt. > 500 000). No significant change from one peak to the other was observed when either form was re-run separately on Sephadex, even when run in the presence of high salt concentrations (0.75M). No difference in the K_m of fructose or glucose could be detected between the two forms of hexokinase.

Horizontal starch-gel electrophoresis separates hexokinase into three bands. One of these migrates very slowly from the origin and is characteristic of sperm hexokinases. Katzen (1967) discovered this slowly migrating form of hexokinase in testis and epididymal extracts and deduced that it resulted from the sperm in these tissues. Separate electrophoresis of each of the two peaks from gel filtration strongly suggests that this 'sperm-type' hexokinase band on electrophoresis is identical with the higher-molecular-weight enzyme peak from gel filtration. The low mobility of the 'sperm-type' band may well be due to its being a relatively large molecule, retarded by the sieving action of starch gel, rather than to its charge or chemical properties.

This work is supported by a grant from the Pig Industry Development Authority.

Katzen, H. M. (1967). In *Advances in Enzyme Regulation*, vol. 5, p. 335. Ed. by Weber, G. Oxford: Pergamon Press Ltd.

Mann, T. (1964). *The Biochemistry of Semen and of the Male Reproductive Tract*. London: Methuen & Co. Ltd.

Characteristics of Growth of a *Pseudomonas* Species in Culture Media containing Chlorhexidine

By MURIEL BENTLEY, A. DAVIES, BARBARA S. FIELD and W. ROBERTS. (*Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire*)

Pseudomonads are able to grow in media containing many types of bactericide. We have used a strain isolated by Mr R. Hall that will grow aerobically in media containing up to 200 µg. of chlorhexidine/ml. In the absence of bactericide, cultures consisted mainly of motile bacteria that gave smooth colonies on agar; on transfer to media containing chlorhexidine the organisms became non-motile and had rough colonial morphology. When cultures grown in chlorhexidine were transferred to media containing less, or no, chlorhexidine, growth occurred either immediately or after a lag during which viability was roughly

constant, but on transfer to a medium containing more chlorhexidine viability first decreased, then remained constant, then increased. After this growth phase viability again decreased, usually fairly rapidly, and the greatest absorption of bactericide occurred. The ratio of viable cells to the total cell mass was always lower in the presence than in the absence of bactericide. Electron micrographs of sections revealed, amongst apparently normal bacteria, many greatly damaged bacteria; large numbers of empty vesicles of varying size were present; some had the complete bacterial wall structure, some only part of it. The vesicles probably came from deformed cells, some having no cytoplasm and some in which the cytoplasm was replaced by electron-dense granules.

Suspensions of washed bacteria grown in medium containing radioactively labelled nutrients without chlorhexidine release labelled components from their metabolic pool on boiling, on treatment with acids or on addition of chlorhexidine. Similar suspensions prepared from cultures grown in presence of chlorhexidine have a metabolic pool extractable by boiling or treatment with acids but only partly extractable or not extractable by chlorhexidine depending on the concentration during growth.

At pH 7 cultures in media containing chlorhexidine moved with an electrophoretic mobility of either 1.0×10^{-4} or 1.0×10^{-5} cm.²sec.⁻¹V⁻¹. It is suggested that in a normal population a small proportion of the pseudomonads can grow in chlorhexidine, and these are selected when chlorhexidine is added; only the most highly adapted of their progeny survive; on transfer to drug-free medium, the culture reverts to the original composition; resistance to chlorhexidine is due to non-absorption by the cells. This is in accordance with the hypothesis of Hamilton (1968).

Hamilton, W. A. (1968). *J. gen. Microbiol.* **50**, 441.

A Mutant Strain of *Escherichia coli* Requiring Lipic Acid for Normal Growth

By P. A. WHITTAKER* and G. S. PARKER. (*Department of Botany, University of Hull*)

A mutant of *Escherichia coli* K-12, W-945 (original strain from Professor W. Hayes) was obtained by u.v. irradiation. This mutant, named UV-3, has an exceptionally small colony form when grown on Oxoid nutrient agar and low cytochrome concentrations (particularly cytochrome a_2), and low concentrations of respiratory quinones, and low

* Present address: School of Biology, University of Sussex, Brighton.