consisted of vesicles, some of which stained positive for acid phosphatase. Most of the vesicles were shown to contain electron-dense particles consistent with the structure of ferritin.

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Appelmans, F., Wattiaux, R. & de Duve, C. (1955). *Biochem. J.* **59**, 438.

Bowers, W. E. & de Duve, C. (1967a). *J. Cell Biol.* **32**, 339.

Bowers, W. E. & de Duve, C. (1967b). *J. Cell Biol.* **32**, 349.

Bowers, W. E., Finkenstaedt, J. T. & de Duve, C. (1967). *J. Cell Biol.* **32**, 325.

The Role and Regulation of Poly- β -hydroxybutyrate Synthesis in *Azotobacter beijerinckii*

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When *Azotobacter beijerinckii* fixes nitrogen in glucose batch culture, towards the end of exponential growth the culture oxygen concentration falls to zero, coinciding with a rapid increase in the cellular content of poly- β -hydroxybutyrate (Ritchie, Senior & Dawes, 1969), which may attain values of 70% (w/w) of the cell mass (Stockdale, 1967). We have shown that poly- β -hydroxybutyrate biosynthesis in this organism involves the condensation of 2 acetyl-CoA molecules to give acetoacetyl-CoA, which is then reduced to D(-)- β -hydroxybutyryl-CoA, the substrate for poly- β -hydroxybutyrate synthetase, by the NAD(P)H-oxidizing acetoacetyl-CoA reductase (Ritchie, Senior & Dawes, 1971).

We suggest that poly- β -hydroxybutyrate biosynthesis is regulated by the intracellular NAD(P)H/NAD(P)⁺ concentration ratio, in view of our studies of the sensitivity of various enzymes of glucose catabolism and the tricarboxylic acid cycle to NADH or NADPH or both. Glucose 6-phosphate dehydrogenase, NAD(P)⁺-reducing, is an allosteric enzyme (modulator-independent co-operative) sensitive to ADP, ATP, NADPH and NADH inhibition, in which it resembles the enzyme from *Hydrogenomonas* (Schindler & Schlegel, 1969). 6-Phosphogluconate dehydrogenase, NADP⁺-specific, is inhibited by both NADH and NADPH, whereas glyceraldehyde 3-phosphate dehydrogenase, which shows a dual specificity for NAD⁺ and NADP⁺, is unaffected (Senior & Dawes, 1970). Citrate synthase is inhibited by NADH but can be reactivated by AMP. The NADP⁺-specific isocitrate dehydrogenase, co-purified with citrate

synthase, is inhibited by NADH, NADPH and ATP, and cumulative feedback inhibition by citrate and ATP is observed when the combined Entner-Doudoroff enzymes are assayed by pyruvate yield from 6-phosphogluconate. Isocitrate and *cis*-aconitate can replace citrate.

During exponential growth the tricarboxylic acid cycle fulfils two functions, the catabolic production of energy and the anabolic provision of α -oxoglutarate and oxaloacetate for amino acid and protein synthesis, in conjunction with nitrogen fixation. In oxygen limitation the NAD(P)H/NAD(P)⁺ concentration ratio might be expected to approach unity, with severe curtailment of both catabolic and anabolic functions of the tricarboxylic acid cycle by means of the regulatory functions we have described, and consequent decrease in nitrogen fixation for growth. The formation of poly- β -hydroxybutyrate may therefore serve, not only as a reserve deposition, but as a regulatory electron sink, reoxidizing sufficient NAD(P)H to permit both glucose conversion into acetyl-CoA and the operation of the tricarboxylic acid cycle to an extent sufficient to produce ATP, reducing equivalents and carbon skeletons for protein biosynthesis.

Ritchie, G. A. F., Senior, P. J. & Dawes, E. A. (1969). *J. gen. Microbiol.* **58**, iii.

Ritchie, G. A. F., Senior, P. J. & Dawes, E. A. (1971). *Biochem. J.* **121**, 309.

Schindler, J. & Schlegel, H. G. (1969). *Arch. Mikrobiol.* **66**, 69.

Senior, P. J. & Dawes, E. A. (1970). *Biochem. J.* **119**, 38P.

Stockdale, H. (1967). Ph.D. Thesis: University of Hull.

The Preparation and Culture of Spontaneously Fused Tobacco Leaf Spongy-Mesophyll Protoplasts

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Fully expanded leaves of 50–60-day-old tobacco plants (*Nicotiana tabacum* var. *xanthi*) were surface-sterilized with 0.5% (w/v) Cetavlon solution (15 min), 70% (v/v) ethanol (30 s) and then 2.5% (w/v) sodium hypochlorite (30 min) followed by four washes in sterile distilled water. Leaf pieces, lower epidermis removed, were incubated aseptically in a Millipore-filtered cellulase (4%, w/v) (Onozuka P.1500) and Macerozyme (0.5%, w/v) enzyme mixture in 25% (w/v) sucrose solution for 3 h at 20°C (Power & Cocking, 1970). Incubation was terminated by twice washing the leaf pieces with the culture medium containing 23% (w/v)

sucrose and maintaining them in this medium overnight. Spontaneously fused tobacco protoplasts (Power & Cocking, 1971) were released by lowering a sterile cover slip on to a leaf piece floating in the medium and applying gentle pressure (Power, Cummins & Cocking, 1970). This gave a mixed culture of 30% fused protoplasts and 70% non-fused protoplasts, which were cultured aseptically in Petri dishes at 20°C and low light-intensity (4–60 ft-candles). Two culture media were employed: a medium similar to that used by Nagata & Takebe (1970) containing 2,4-D, 6-benzyladenine, glycine, *myo*-inositol and thiamin hydrochloride, but without mannitol; and a commercially prepared modified Whites medium (S-3; Biocult Laboratories Ltd.); both with added 23% (w/v) sucrose. For nuclear examination fused protoplasts were fixed in acetic acid–ethanol (1:3, v/v) for 3 h and stained with acetocarmine or acetoorcein.

After 24 h culture in the modified Nagata & Takebe (1970) medium systrophy occurred markedly within these spontaneously fused protoplasts, the nuclei coming together and remaining so during subsequent culture. Systrophy was less evident in Whites medium, but a general increase in size in both media preceded the first division of the fused protoplasts, which took place after about 4 days' culture. In fused protoplasts containing two or three nuclei, some nuclear division was synchronous and concomitant with the initiation of cell-wall regeneration. Fused protoplasts regenerated a wall and a few underwent repeated divisions, but there was no synchrony of regenerated cell division within the culture as a whole. Nuclear fusion could occur during any one of these divisions in these intraspecies fusion bodies. These results show that wall regeneration readily occurs; and that nuclear fusion probably, after common spindle formation, is possible in these spontaneously fused plant protoplasts, because in many instances the nuclei are closely associated with each other and enter mitosis synchronously.

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Nagata, T. & Takebe, I. (1970). *Planta*, **92**, 301.

Power, J. B. & Cocking, E. C. (1970). *J. exp. Bot.* **21**, 64.

Power, J. B. & Cocking, E. C. (1971) *Sci. Prog., Lond.*, **59** (in the Press).

Power, J. B., Cummins, S. E. & Cocking, E. C. (1970). *Nature, Lond.*, **225**, 1016.

Fluorescent Derivatives of 3-Hydroxy-L-kynurenine in the Lens of Man, the Baboon and the Grey Squirrel

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It has been known for a long time that the lenses of certain species of animal, including the primates and the Scuridae, are coloured yellow (Walls, 1942).

The human lens has been found to contain a number of fluorescent compounds, of which the most concentrated is the *O*- β -D-glucoside of 3-hydroxy-L-kynurenine (van Heyningen, 1971). This shows a strong blue–white fluorescence on paper. It is split by hydrolysis with β -glucosidase, or 1M-hydrochloric acid, to give 3-hydroxy-L-kynurenine and D-glucose. There is also present in the human lens a closely related glucoside with the α -amino group of 3-hydroxy-L-kynurenine blocked (but not by acetylation).

I have now examined the lens of the baboon (*Papio* species) and the grey squirrel (*Sciurus carolinensis leucotis*). Cooper & Robson (1969*a,b*) found evidence that the pigments in the lens of man, the baboon and the squirrel were very similar to each other, but probably not the same. I have confirmed that the fluorescing material from the lens of man, the baboon and the squirrel is yellow and has an absorption peak at about 368 nm.

Examination by paper electrophoresis and chromatography showed that the major fluorescent compound in baboon lens was the same as that in the human lens. The presence of glucosides in these lenses is surprising, as it is generally held that phenolic glucosides are not formed in animal tissues.

The lens of the squirrel, on the other hand, contained a main fluorescent compound different from that in the primate lens. It fluoresced green–yellow on paper and had all the properties expected of *N*-acetyl-3-hydroxy-L-kynurenine (Dalglish, 1952). The presence of the acetyl group was confirmed by hydrazinolysis followed by dansylation (Schmer & Kreil, 1969). The acetyl dansyl hydrazide formed was identified by two-dimensional chromatography on polyamide layer sheets (Hartley, 1970).

Thus 3-hydroxy-L-kynurenine occurs in the lens of the primate (man and baboon) as the *O*-glucoside, and in the squirrel lens as the *N*-acetyl derivative.

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