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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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.

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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)