Effect of carbon and nitrogen growth limitation upon nutrient uptake and metabolism in batch cultures of *Catharanthus roseus* (L) G. Don

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**Abstract.** Cell suspension cultures of the Madagascan Periwinkle, *Catharanthus roseus* (L). G. Don were grown as batch cultures in two different types of media; in one medium the limiting nutrient was inorganic nitrogen, and in the other it was carbon. The response of the cells to these growth-limiting conditions was monitored by measuring cellular fresh weight, dry weight and protein accumulation, cell viability, medium sugar and nitrate levels, and the activities of certain intracellular enzymes throughout growth in batch culture. The enzymes investigated were glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), hexokinase (EC 2.7.1.40), phosphofructokinase (EC 2.7.1.11), nitrate reductase (EC 1.6.6.1), glutamate dehydrogenase (EC 1.4.1.2) and glutamine synthetase (EC 6.3.1.2). The effect of culturing the cells under different nutritional regimes was apparent in all aspects of growth; only some enzyme activities were unaffected. Cell viability remained at a high level for several days after growth limitation in both types of culture. The possibility that protein degradation in nitrogen-limited batch cultures is under very stringent control is discussed.

**Introduction**

An extensive literature now exists regarding the accumulation of indole alkaloids in cultured cells of *Catharanthus roseus* (Zenk, 1978; Barz and Ellis, 1981), the ultimate objective of much of this work being the development of an industrial process for indole alkaloids of commercial significance (Fowler, 1982). Major progress towards this goal may be seen in the work on the pathways for, and hormonal regulation of the biosynthesis of serpentine and ajmalicine (Zenk, 1978; Pfitzner and Stockigt, 1982). In addition, cultural variation in the chemical nature and level of the indole alkaloids has been explored by Kurz and co-workers (1980). A notable omission in these studies has been work on the primary metabolism of *C. roseus* cells. This is important in two respects; firstly, there are indications that the nature and level of carbon and nitrogen supplied as substrate may influence secondary metabolism not only in cell cultures of *C. roseus* (Zenk, 1977) but also in cultures of other species (Schuler, 1981). Secondly, the importance of growth rate and biomass accumulation to the efficiency of an industrial process implies that the mode of conversion of primary substrates into essential cell material requires detailed attention. This paper reports
on initial studies directed towards furthering our understanding of primary metabolism in *C. roseus*.

The operation of the classical pathways of glycolysis, pentose phosphate pathway and citric acid cycle have been previously demonstrated in plant cell cultures (Fowler, 1978; Wilson, 1971), as well as the incorporation of nitrate and ammonia via nitrate reductase, glutamine synthetase and glutamate synthase (Dougall, 1977). In this work, we aimed to manipulate the concentration of substrates supplied to *C. roseus* in such a way that culture growth was either carbon- or nitrogen-limited. The effects of such limitations on parameters such as growth, nutrient uptake and key assimilatory enzyme activities were then monitored for the duration of one culture cycle. The effects of varying primary substrates upon secondary metabolism will be reported in a later paper.

**Materials and methods**

**Stock cultures and experimental material.** Batch suspension cultures were initiated and routinely maintained on Gamborg’s B5 medium (Gamborg et al 1968) as previously fully described (Morris and Fowler 1980). Samples from 13 day old suspension cultures were filtered under gentle suction through 100 μ nylon mesh, and inoculated into either (i) Gamborg’s B5 medium containing 2mM NH₄⁺ 30mM NO₃⁻ and 2% (w/v) sucrose (the standard B5 medium, designated N30) or (ii) B5 medium with 2mM NH₄⁺ and the concentrations of NO₃⁻ and sucrose adjusted to 5mM and 3% (w/v), respectively (designated N5).

**Chemicals and enzymes.** All chemicals used were of ‘AR’ grade where possible, and were purchased from Fisons or BDH. Enzyme cofactors and purified enzymes were purchased from Boehringer (Mannheim, W. Germany) Ltd., or Sigma Ltd., U.K.

**Measurement of growth and viability.** Fresh and dry weights were determined by filtering 3 ml of culture through a Whatman No. 1 filter paper supported on a stainless steel mesh, directly weighing for fresh weight and then drying overnight at 60°C prior to dry weight measurement. Protein estimations were made on extracts prepared for enzyme assays using the Folin method (Layne 1957), with bovine serum albumin as a standard. Viability was estimated using fluorescein diacetate according to the method of Widholm (1972).

**Analysis of medium sugars and nitrate.** Culture medium was separated from cells by filtering suspensions under gentle suction through Whatman No. 1 filter paper. Medium was stored at −20°C and subsequently analysed for sucrose, fructose, glucose and nitrate. Sucrose and fructose were determined