Secretion of Photosynthetic Products by Carrot Tissue Cultures*

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Summary. Green carrot callus cultures when exposed to $^{14}\text{C}$O$_2$ in a liquid medium showed light-dependent $^{14}$C-incorporation into sucrose, glutamine and malic acid. About 5% of total $^{14}$C fixed in a 3 h period appeared in these products in the bathing medium; this was not due to tissue damage. Kinetic studies showed that the release occurred from a metabolic and not a storage compartment. The effects of DCMU, temperature and fluorooacetate demonstrated that release from this compartment was under respiratory and not photosynthetic control.

Introduction

Green plant tissue cultures are known to show photosynthetic CO$_2$-fixation (Roux and Tendille, 1954; Naef, 1968; McLaren and Thomas, 1967) and several studies have suggested the feasibility of autotrophic growth of callus and suspension cultures (Fukami and Hildebrandt, 1967; Sunderland, 1967; Bergmann, 1967; Corduan, 1970). Photosynthesizing algae and sugar cane leaf discs are known to release metabolic products into their bathing media (Watt, 1969; Hellebust, 1965; Schoolar and Edelman, 1970) and direct and indirect evidence has been obtained that a similar process occurs in colourless plant suspension cultures (Stuart and Street, 1969; Lescure, 1966) and callus cultures (Devillers, 1969).

We here report the results obtained using photosynthetic $^{14}$CO$_2$-fixation to investigate the release of metabolites from carrot tissue cultures; these studies form part of a program investigating photosynthesis and product secretion in cultured plant tissues (Schoolar and Edelman, 1970) in relation to autotrophic growth.

Materials and Methods

Callus Cultures. Two strains of carrot callus cultures (CRT 1 and CRT 2) grown on 15 ml agar medium in tubes and subcultured at monthly intervals were used in this investigation. CRT 1, the strain isolated by Gautheret in 1937 was grown on a defined medium based on that of Gautheret (1938) with 0.1 mg/l thiamine. CRT 2, isolated in December 1968 in this laboratory was grown on the coconut milk medium described previously (Hanson and Edelman, 1970). Cultures

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A. D. Hanson and J. Edelman: were grown at 26±2°C in 1300 lux of continuous fluorescent light. Cultured in these conditions, CRT 1 contained about 30 µg chlorophyll/g fresh weight and CRT 2 50 µg/g fresh weight; chlorophyll a/b ratios were 2.0 and 1.8 respectively.

Conditions of 14CO2 Incubation. Experiments on 14CO2 fixation and metabolite release were carried out routinely using calluses in the third and fourth weeks of a passage. About 300 mg of callus tissue cut into 50 mg pieces was immersed in 5.0 ml of Murashige and Skoog mineral element solution (1962) adjusted to pH 4.5 in 10 ml tubular vessels. Vessels were closed with a trimmed serum cap and pre-illuminated for 30 min in a water bath at 25°C, in 5000 or 15000 lux of water-screened tungsten light; continuous reciprocal shaking at 80 cycles/min agitated the medium. 20 µCi of Na214CO3 (50 mCi/m Mole) were injected giving a total CO2 concentration within the vessel of about 0.1%. Illumination was continued for 3 h.

Extraction and 14C Assay. Tissues were killed and extracted by grinding in sand in 24 volumes of ethanol containing a trace of CaCO3; after concentration of this extract chlorophyll was determined spectrophotometrically, according to the equations of Wintermans and De Mots (1965). The residue from ethanol extraction was re-extracted in 10 volumes of boiling 30% ethanol for 15 min and the two ethanolic extracts were pooled and aliquots assayed for 14C in a liquid scintillation spectrometer. The final insoluble residues were dried overnight at 70°C and counted on planchets at infinite thickness using a thin end-window GM tube under conditions previously determined as giving a counting efficiency of 1.47%. 14C products released into the bathing medium were assayed by scintillation counting after evaporating aliquots of acidified medium to dryness under an infra-red lamp in a stream of cool air to remove excess 14CO2.

Carbohydrate Analysis. Soluble carbohydrates were determined by the method of Dubois et al. (1956) after washing tissues for 90 min in running tap water to remove free space sugars (Glasziou, 1960). For starch estimation the ethanol-insoluble residues were treated with 2N H2SO4 at 100°C for 4 h and the resulting glucose was assayed by the same method.

Chromatography and Electrophoresis. Released products and ethanolic tissue extracts were fractionated into cationic, anionic and neutral fractions using the Dowex column procedure of Canvin and Beevers (1961) and the composition of the fractions was further investigated by paper and thin-layer chromatography and high-voltage paper electrophoresis combined with autoradiography. The identity of sucrose was checked by inversion using melibiase-free invertase (Koch Light Ltd.) and glutamine was identified by hydrolysis to glutamic acid with 6N HCl at 120°C for 4 h.

Respiratory Rates. Warburg manometry was carried out in darkness at 25°C using the techniques described by Umbreit, Burris and Stauffer (1964).

C6/C1 Ratios. These were determined in sealed 20 ml Warburg flasks using an incubation period of 3 h at 25°C in darkness. About 1 g of tissue was incubated in 2.0 ml Murashige and Skoog mineral element solution pH 4.5 containing 3 µCi of C-1 or C-6 labelled glucose of the same specific activity (45 mCi/m Mole). Evolved 14CO2 was trapped in the 0.1 ml of 10% KOH in the centre well and assayed in the scintillation spectrometer using a scintillant mixture based on that of Yardley (1964).

Results

1. Tissue Carbohydrate Analysis

The results of a quantitative carbohydrate analysis of GRT 1 and CRT 2 are shown in Table 1. Sucrose was the major stored carbohydrate in both strains; virtually no starch was detected in either strain.