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Interrelationships between Carbohydrate Metabolism and Nitrogen Assimilation in Cultured Plant Cells

II. The Effect of the Nitrogen Source and Concentration on Nutrient Uptake and Respiratory Activity in Cultured Sycamore Cells

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Summary. The effect of the nature and concentration of the nitrogen source on respiratory activity and removal of carbohydrate from the medium in suspension cultured sycamore (*Acer pseudoplatanus* L.) cells was determined. Comparison was also made of the rates of uptake of the two alternative nitrogen sources, nitrate and glutamate, at differing initial nitrogen concentrations within the range 7–14 mM. The initial pH of the culture medium before inoculation was 5.2; after inoculation the pH of both nitrate and glutamate cultures rose to reach an eventual level in the range 7.0–7.1. Glutamate was removed from the medium more slowly than nitrate. Under the particular conditions of culture used the growth of the cells was nitrogen limited. Sugar uptake from the medium continued for some time after the nitrogen in the medium was depleted. The data show that although cell division and protein content are nitrogen-limited, dry weight and fresh weight yields may also be determined in a complex interaction through carbohydrate availability. There were no obvious differences in respiratory activity between cultures grown on nitrate or glutamate.

Introduction

In the previous paper (Jessup and Fowler, 1976) we established that sycamore cells grown in suspension culture show similar changes in cell number, dry and fresh weight and soluble protein when supplied with nitrate or glutamate as sole nitrogen source. The data indicated that growth in these cultures was nitrogen limited since the maximum cell number attained was proportional to the amount of nitrogen supplied. The purposes of the present investigation were firstly, to determine the effect of the nitrogen source on respiratory activity and the removal of carbohydrate from the culture medium, and secondly to measure the rates of uptake of the nitrogen sources supplied.

Materials and Methods

Chemicals

All chemicals used were of 'AR' grade where possible and were purchased from B.D.H. Ltd. or Fisons Ltd. Enzyme and co-factor for the glutamate assay were purchased from Boehringer (Mannheim) Ltd.

Origin and Maintenance of Cell Suspension Cultures

The origin and maintenance of the sycamore (*Acer pseudoplatanus* L.) cell cultures were as described previously (Jessup and Fowler, 1976). The culture medium contained sodium nitrate or sodium glutamate as sole nitrogen source at an initial concentration of 7, 10 or 14 mM, and sucrose (0.058 M) as carbon source.

Analysis of Medium Constituents

Samples of culture were taken at intervals during the first 21 days after inoculation of cells into fresh medium. Cells were harvested by vacuum filtration through Miracloth (Calbiochem Ltd.) in a Hartley funnel supported in a Buchner flask. The pH of the filtrate was measured immediately, and samples of medium stored at −20° C for subsequent analysis of nitrate, glutamate and total carbohydrate as reducing sugar.

Nitrate was assayed according to Sarkissian and Fowler (1974).

Glutamate was estimated enzymatically (Bernt and Bergmeyer, 1974). The assay contained 1 ml Hydrazine-Tris buffer (0.28 M Tris, 1.4 M Hydrazine, pH 9.0), 1.1 μmoles ADP, 1.6 μmoles NAD, 24 μmoles EDTA and not more than 0.2 μmoles L-glutamate in a total volume of 3.25 ml. Reaction was started by the addition of 25 units of glutamate dehydrogenase (glycerol solution) and the change in optical density after 3 h at 25° C measured at 340 nm in a Gilford 250 recording spectrophotometer. A standard curve was prepared with known concentrations of L-glutamate.

Total reducing sugar was assayed after acid hydrolysis by the method of Somogyi (1952). Analysis of culture medium showed that 40–50% of the sucrose hydrolysed during autoclaving.

Oxygen Uptake

Oxygen uptake was measured at 25°C with a Clark-type oxygen electrode (Rank bros., Bottisham, Cambridge) (Fowler and Clifton, 1974). During early stages of growth, when the rate of uptake was low, samples of culture were concentrated by gentle suction through glass-fibre paper (Whatman GF/C) followed by immediate re-suspension of the cells in a smaller volume of the filtered medium.

Results

Effect of the Nitrogen Source on the pH of the Medium

Figure 1 shows the changes in the pH of the medium during the first 14 days of growth in cultures supplied with an initial concentration of 14 mM nitrate or glutamate as sole nitrogen source. Before inoculation the medium pH was 5.2. Immediately after inoculation there was a rapid increase of approximately 1 pH unit in the nitrate medium, but only a small rise in the glutamate medium. Subsequently the pH continued to rise slowly in both media until a constant value of 7.0 to 7.1 was reached. This was by 96 h in nitrate-grown and 240 h in glutamate grown cultures.

Uptake of the Nitrogen Source from the Culture Medium

The uptake of the nitrate and glutamate (assuming that disappearance from the medium represents uptake into the cells) from their respective media is shown in Figures 2 and 3. Nitrate was depleted rapidly and its removal from the medium was complete by 192 h for all initial concentrations used. Glutamate uptake was slower and complete by 288–336 h depending upon the intial concentrations used.

Uptake of Carbohydrate

Sucrose was supplied as the major carbon source at an initial concentration of 0.058 M. At equivalent initial nitrogen concentrations the rate of uptake of carbohydrate was consistently higher in nitrate grown cultures (Fig. 4A) compared with those grown on glutamate (Fig. 4B). Sucrose exhaustion was complete

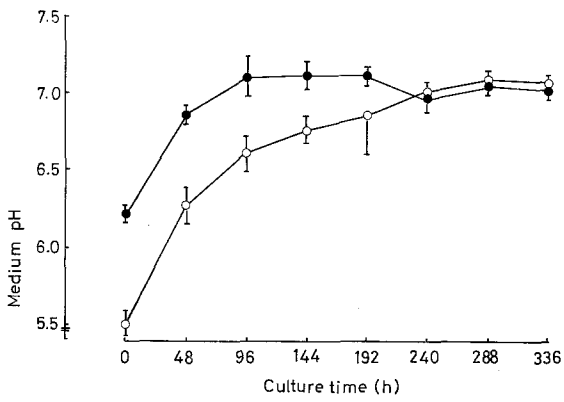

Fig. 1. Changes in the pH of the medium during the growth of sycamore cells in suspension culture with nitrate (●) or glutamate (○) as the nitrogen source. The initial concentration of the nitrogen source was 14 mM. The initial medium pH prior to inoculation was 5.2. The data presented are the means of three experiments ± the SE where these exceed the span of the points

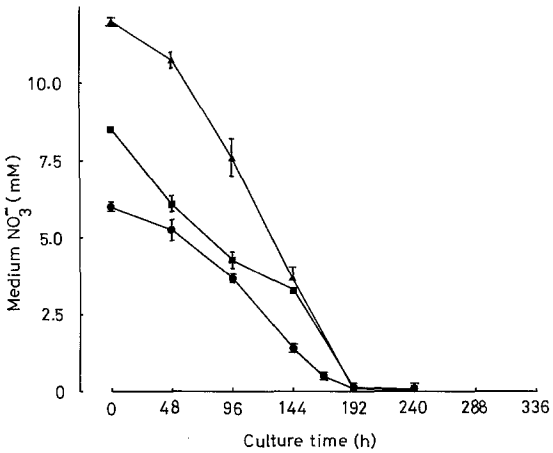

Fig. 2. The uptake of nitrate from the medium during the growth of sycamore cells in suspension culture. The initial nitrogen concentrations were 7 mM (●), 10 mm (■); and 14 mM (▲). The data presented are the means of three experiments ± SE where these exceed the span of the points

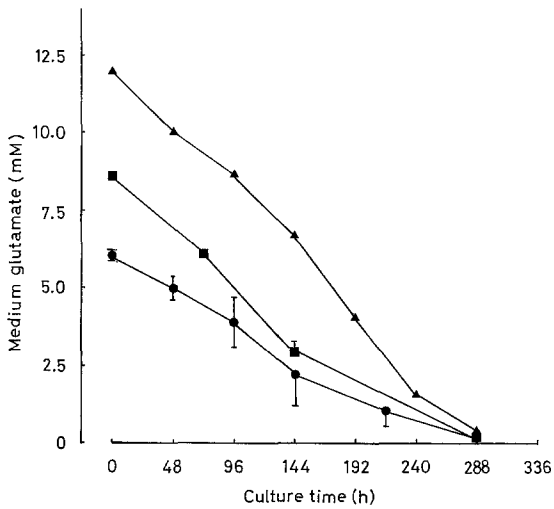

Fig. 3. The uptake of glutamate from the medium during the growth of sycamore cells in suspension culture. The initial nitrogen concentrations were 7 mM (●), 10 mM (■), and 14 mm (▲). The data presented are the means of three experiments ± SE where these exceed the span of the points

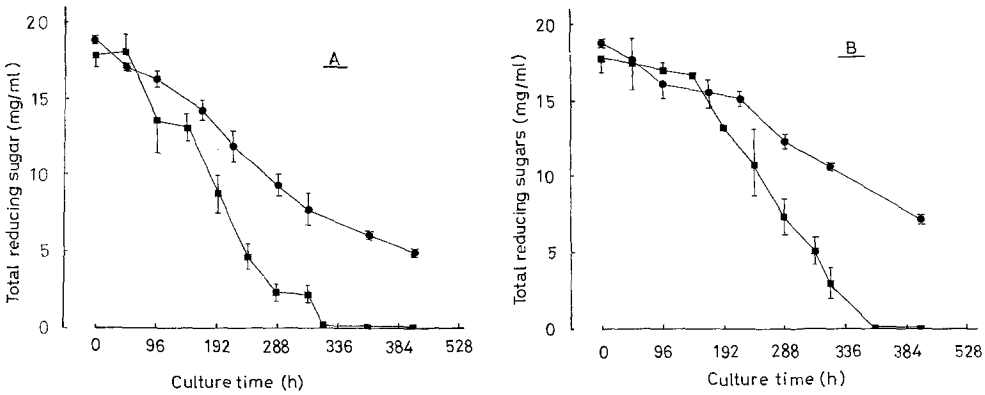

Fig. 4A and B. The uptake of sucrose from the medium by sycamore cells grown in suspension culture with nitrate **A** or glutamate **B** as the nitrogen source. The initial nitrogen concentration was 7 mM (■) or 14 mM (●). The data presented are the means of five experiments ± SE where these exceed the span of the points

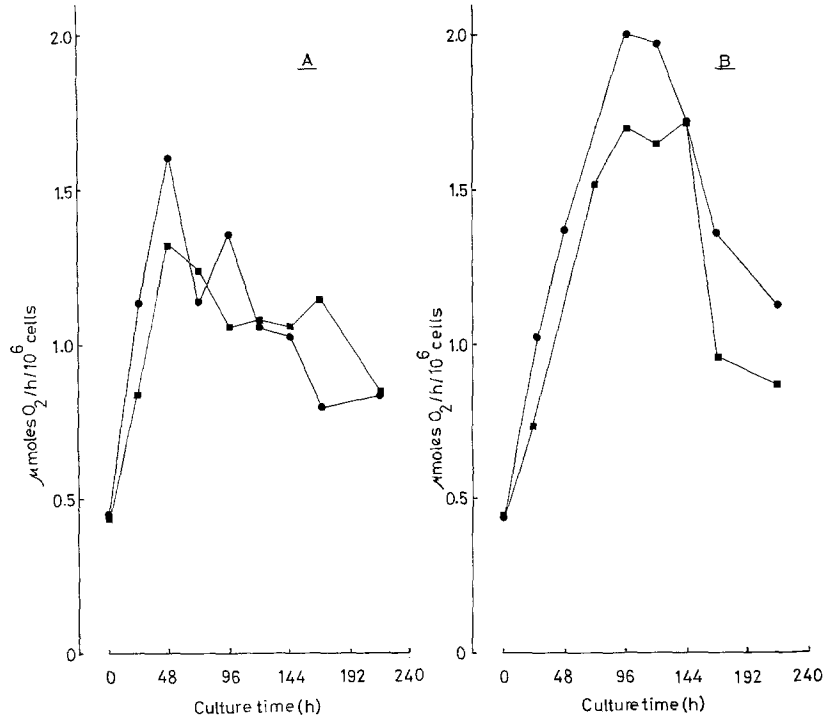

Fig. 5A and B. The rate of oxygen uptake per cell by sycamore cells grown in suspension culture with nitrate **A** or glutamate **B** as the nitrogen source. The initial nitrogen concentration was either 7 mM (■) or 14 mM (●). The data presented are for one experiment and the pattern is typical of that obtained in a number of experiments

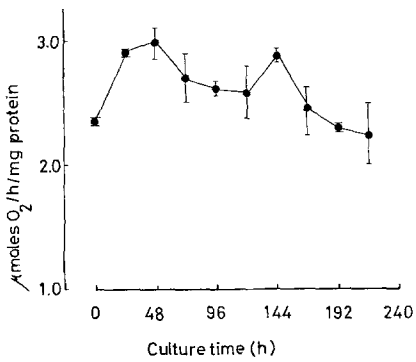

Fig. 6. The rate of oxygen uptake per mg soluble protein by sycamore cells grown in suspension culture with nitrate as the nitrogen source. The initial nitrogen concentration was 14 mM. The data presented are the mean of three experiments ± SE where these exceed the span of the points

on 14 mM nitrate and glutamate cultures by 336 h and 360 h respectively. In cultures with 7 mM nitrogen, carbohydrate was still present by 384 h, although at a higher concentration in the glutamate-grown culture.

Respiration Rates

The rate of respiration (Fig. 5) expressed on a per cell basis increased rapidly in all cultures during lag-phase, reaching a maximum at, or just prior to, the initiation of cell division. This was by 72 h in nitrate cultures and by 120 h in glutamate cultures. The maximum value represented roughly a three-fold increase

over 0 h values in nitrate cultures and an approximately four-fold increase in glutamate cultures. Changes in cellular protein content (Jessup and Fowler, 1976) closely paralleled these changes in respiration rate so that when oxygen uptake is expressed in terms of cellular protein, closely similar values are obtained for both nitrogen regimes. Figure 6 shows a typical pattern in 14 mM nitrate cultures. There is still a small rise during the first 48–72 h of growth, but this is much smaller than that observed when uptake is expressed on a cellular basis.

Discussion

pH Changes in the Medium

Suspension cultures of sycamore cells supplied with nitrate and glutamate showed similar changes in the pH of the medium during early stages of growth, rising from an initial value of 5.2 to 7.0–7.1 by 96 and 240 h respectively. Early rises in pH have been observed in sycamore cultures grown on nitrate or urea (Wilson, 1971) and in soybean (Hahlbrock and Kuhlen, 1972) and Paul's scarlet rose (Nash and Davies, 1972) cultures. In contrast with ammonium as nitrogen source there is a substantial decline in pH (Gamborg and Shyluk, 1970) which may lead to marked differences in the uptake of nutrients with a consequent effect on cell growth and the pattern of metabolism.

It is difficult to attribute these changes in pH to a specific cause, presumably they occur as the result of the complex interactions not only in the uptake of ions from the medium, but also possibly through the release by the cells of cations (Simpkins and Street, 1970) back into the culture medium. From the point of cur after this time, but these may be due to the view of the present studies it is reassuring that nitrate and glutamate caused similar changes in the pH of the medium, since the effects of pH on the uptake of inorganic nutrients from these media should then be comparable.

Uptake of Nitrogen from the Medium

The rate of uptake of nitrogen by sycamore cultures varies with the form in which it is supplied. The present work shows that glutamate is more slowly removed from the medium that nitrate. In addition Wilson (1971) has shown that urea is absorbed more rapidly than nitrate under similar culture conditions. It is not clear whether these differences are due to the operation of distinct and separate uptake systems with characteristic kinetics for each nitrogen source or whether there is an effect by the nitrogen source on cell metabolism which then directly or indirectly determines subsequent demands for nitrogen from the medium.

Specific nitrogen uptake systems have been identified in tobacco (Heimer and Filner, 1971) and sycamore (King, 1974), and King and Hirji (1975) have reported several amino acid transport systems in cultured soybean root cells. Several workers (Wilson, 1971; King et al., 1973; Young, 1973) have suggested that in the medium commonly used for their culture (Stuart and Street, 1969) the growth of sycamore cells is nitrogen limited; our work with either nitrate or glutamate as nitrogen source supports this view. In both cases the time at which complete uptake of nitrogen is achieved (Figs. 2 and 3) corresponds closely with the point at which increases in cell number and soluble protein cease (Jessup and Fowler, 1976). Small increases in cell number do occur after this time, but these may be due to, the break up of small aggregates of cells in which the number of cells may previously have been underestimated (Henshaw et al., 1965), lack of synchrony in later cell divisions (Fowler, 1971) or to the fact that intracellular nitrate lags behind depletion of nitrate in the medium (unpublished results). There is a proportionality between the maximum cell number, protein content and amount of nitrogen supplied (Jessup and Fowler, 1976) which has also been reported by Wilson (1971), King et al. (1973) and Young (1973).

Sugar Uptake

The absorption of sugar continues after nitrogen uptake is complete, but total removal was observed during the normal growth period (504 h) only at the highest nitrogen concentrations. It is interesting to note that dry and fresh weights continue to rise after cell number and soluble protein have reached their maximum levels (Jessup and Fowler, 1976), and that the time at which sugar uptake is completed corresponds to the point at which dry weight begins to decline. This suggests that although cell division and protein content are nitrogen-limited, dry weight and fresh weight yields may also be determined by the carbohydrate availability with the present medium and growth conditions. Similar evidence has been presented by other workers (Wilson, 1971; Nash and Davies, 1972).

Respiration Rate

The rapid increase in oxygen uptake prior to the start of cell division, and its close relationship to changes

in cellular protein have been widely reported (Givan and Collin, 1967; Nash and Davies, 1972; Fowler, 1975). We have observed a similar relationship in sycamore cells supplied with nitrate or glutamate although there are interesting differences in the absolute values of these parameters.

Glutamate grown cells are generally larger and have a higher cellular protein content than the corresponding nitrate-grown cultures. Street et al. (1976) have also noted differences in cellular protein levels in continuous cultures of sycamore cells supplied with urea or nitrate and urea.

We were unable to demonstrate any significant difference between the rates of oxygen uptake expressed on a protein basis under the two nitrogen regimes, although such differences might be expected as a result of the varying demands on carbohydrate oxidation for NAD(P)H by the nitrogen assimilatory systems of the respective cultures. However it is possible that the resultant differences may be so small against the total uptake that the sensitivity of our analytical techniques is too low to detect them.

Suspension cultures of sycamore are able to utilise several sources of nitrogen including urea, nitrate, glutamate and combinations of these. We have established that the patterns of growth and associated nutrient uptake are essentially similar in nitrate and glutamate supplemented media at several initial concentrations and that growth is nitrogen limited. Such a homogeneous system in which growth can be precisely controlled and monitored constitutes a useful system for the study of metabolic control.

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