Plant Physiol. (1986) 82, 363–368
0032-0889/86/82/0363/06/$01.00/0

Carbon Assimilation in Carrot Cells in Liquid Culture[1]

Received for publication April 8, 1986 and in revised form May 28, 1986

JAN KANABUS, RAY A. BRESSAN, AND NICHOLAS C. CARPITA*
Department of Botany and Plant Pathology (J.K., N.C.C.) *and Department of Horticulture* (R.A.B.),
Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Assimilation of carbohydrates by carrot (*Daucus carota* L. cv Danvers) cells in liquid culture was studied to delineate the major metabolic pathways used in transformation of external carbohydrates to UDP-glucose. The cells grown on either sucrose or glucose for several years proved equally capable of utilizing each of these sugars. Sucrose was rapidly hydrolyzed extracellularly to glucose and fructose, and glucose was preferentially taken up. Uptake of fructose was slower and delayed until glucose was nearly depleted from the medium. Concentrations of cellular sugars, mainly glucose and sucrose, increased during late logarithmic phase of growth and decreased during the plateau phase. Continuous labeling of the cells with D-[^{14}C]glucose resulted in rapid accumulation of radioactivity in glucose-6-phosphate and UDP-glucose. Because there was virtually no uptake of sucrose, UDP-glucose was likely derived from glucose-1-phosphate in a reaction catalyzed by UDP-glucose pyrophosphorylase and not directly from sucrose. Concentrations of major nucleotides and nucleotide sugars were maximal during the early logarithmic phase of growth and decreased several-fold in the stationary phase. A modified 'energy charge' for adenylates calculated with the omission of AMP decreased steadily from 0.9 to 0.8 during the course of culture cycle. An analogous uracil nucleotide ratio was considerably lower (0.85) during early culture, decreased to about 0.7 for the entire logarithmic phase, and returned to initial values as cells entered stationary phase. The uracil nucleotide ratio may provide a useful index to assess the coupling between the energy available in phosphoanhydride bond in adenine nucleotides and the demand for sugar for polysaccharide synthesis through uridine diphosphate-sugar pools.

Plant cells in suspension culture offer an attractive model for a variety of biological studies. In connection with our long-term research goals, we became interested in some fundamental aspects of carbon utilization by cells. We have maintained in our laboratory two sublines of carrot cells, one grown on sucrose and the other on D-glucose as the major carbon source, and decided to investigate the fate of external sugar as it enters metabolic pool of the cells. Because growth, and its regulation, depends on availability of appropriate substrates it became important to determine the primary pathways leading from external supply of carbohydrates to UDP-glucose. UDP-Glc is at a pivotal point in the path of carbon from external or vacuolar stores to structural polymers such as cellulose and hemicelluloses, and possibly to storage polymers such as starch (11, 12), although ADP-Glc is most likely the immediate glucose donor for starch synthesis (7). There are two principal reactions generating UDP-Glc in 'sink' cells: (a) Glc-1-P + UTP ⇆ UDP-Glc + PPi, catalyzed by UDP-Glc pyrophosphorylase, and (b) Sucrose + UDP ⇆ UDP-Glc + fructose, catalyzed by sucrose synthase. It was not apparent which of these two routes would be used by cells derived from carrot root tissue and maintained in culture for several years. We therefore analyzed major sugars in culture media along with the cellular concentrations of simple sugars, Glc-1-P, Glc-6-P, and the major nucleotides and nucleotide sugars during the growth cycle of carrot cells. ATP is the main energy transducing compound of cells necessary for maintenance of adequate levels of other triphosphates, such as UTP and GTP. The adenylate 'energy charge' expressed as the ratio of: (0.5[ADP] + [ATP])/([AMP] + [ADP] + [ATP]) (1, 4, 26) is informative when describing the regulation of a number of metabolic processes by adenine nucleotides, although its general applicability has been questioned (20, 21). Energy transduction from the adenosine di- and triphosphates requires transfer of phosphate residues to other nucleotides. We calculated analogous ratios of uracil and guanine nucleotides to assess how tightly coupled the energy transfer between the nucleotide pools was with the formation of nucleotide sugars and, consequently, with the growth status of the cells at various stages of the growth cycle.

MATERIALS AND METHODS

Cell Culture. Cells of carrot (*Daucus carota* L. cv Danvers) were obtained from callus derived from root tissue (13) and were maintained in liquid culture in medium (pH 5) containing per liter: glycine, 2 mg; nicotinic acid, 0.5 mg; pyridoxine-HCl, 0.5 mg; thiamine-HCl, 0.5 mg; *myo*-inositol, 0.1 g; 2,4-D, 0.4 mg; sucrose, 30 g, or D-glucose, 31.6 g; and Murashige and Skoog salts (16; prepared commercially by GIBCO). Media containing sucrose were routinely autoclaved, while those containing glucose were filter-sterilized to avoid decomposition. Cells were subcultured weekly at about 0.8 g fresh weight per 100 ml of fresh medium in 500-ml Erlenmeyer flasks. Cultures were incubated at room temperature on a gyratory shaker at 110 rpm with a 2.5-cm displacement and under dim fluorescent light.

Growth Experiments. Cells in the early logarithmic phase of growth (4 d after being subcultured) were either pipetted directly, or filtered, resuspended and pipetted at 0.2 to 0.4 g fresh weight per 3 ml into sterile 125-ml Erlenmeyer flasks with 22 ml fresh medium. Two or three flasks were used at each experimental point, and sometimes several flasks were combined to obtain sufficient material. At desired times, cells were harvested by filtration on Whatman No. 4 or Fisher No. P8 paper disks, washed briefly with water and weighed. Dry weights were determined after drying of filtered cells at 105°C for 20 to 28 h.

Radioactive Labeling. Cells were filtered, washed, and then resuspended to desired density in medium containing 50 mM D-glucose. One to two g of cells in 25 ml was measured into 125-ml flasks. Before addition of the tracer compound, cells were preincubated for 1 to 2 h to allow recovery from transfer shock. During preincubation period there was a 25% drop in cellular

[1] Supported by United States-Israel Binational Agricultural Research and Development Project No. US-535-82; Journal Paper No. 10,691 of the Purdue University Agricultural Station.

sucrose while levels of cellular reducing sugars decreased by about 10% of the initial value. D-[U-^{14}C]glucose (200 mCi/mmol; 100 μCi per flask) was added, and cells were incubated on a shaker as described above for up to 4 h. At desired times, cells were filtered and washed with fresh medium containing 50 mM mannitol in place of glucose. Cells were then frozen in liquid N_2 and stored at −20°C for future analysis.

Extraction and Quantitation of Sugars, Sugar Phosphates, and Nucleotides. Frozen cells were extracted in ice cold 10% (w/v) TCA using 2 ml/g fresh weight in a Duall glass-glass homogenizer. Following 15 min of intermittent agitation, homogenates were centrifuged in 1.5-ml microcentrifuge tubes in an Eppendorf Centrifuge 5414 for 4 min. Six hundred μl portions of supernatants were neutralized by shaking for 1 min with 800 μl of a mixture of 1,1,2-trichlorotrifluoroethane (Freon) and *n*-trioctylamine (3:1; v/v) (18). Suspensions were centrifuged as above, and the upper aqueous phases were collected for analyses. In some experiments, D-[^{14}C]glucose or a mixture of [^{14}C]ATP and UDP-[^{14}C]Glc were added to the TCA solutions used for cell extraction to assess recovery of sugars or the extent of nucleotide decomposition, respectively. Upon HPLC of such extracts, a small amount of [^{14}C]ADP observed indicated that not more than 5% of ATP was degraded to ADP. No radioactivity was found in AMP or UMP. The ratio of recovery of [^{14}C] in UDP-Glc to that in ATP and ADP was constant to within a few percent of that seen in the original mixture. These tests verified that a 15 min exposure to about 7% ice-cold TCA during cell extraction did not result in gross alteration of the nucleotide composition of the soluble fraction of the cells. Radioactive glucose helped also in relating volume of the extract to weight of cells used for extraction. Nucleotides in neutralized cell extracts were separated by HPLC using a 250 × 4.8 mm column of Partisil-10 SAX (Whatman) at a flow rate of 1 ml/min with the following buffers: buffer A, 8 mM K-phosphate (pH 3.5); buffer B, 800 mM K-phosphate (pH 4.2). Elution was isocratic for 10 min with buffer A followed by a 70 min linear gradient from 100% A to 30% A and 70% B, and then 10 min under the final conditions. Nucleotides and nucleotide-sugars were quantified at 254 nm by peak integration with a Spectra Physics 4100 computing integrator. Fractions of 1 or 2 ml were collected, and radioactivity was measured by liquid scintillation spectroscopy.

Sugar Analyses. Reducing sugars were determined in diluted filtered media and in neutralized extracts by the method of Nelson (17) using modified copper reagent (23) with D-glucose as standard. Sucrose content was calculated in matched samples incubated for 2 h at 37°C with invertase (10 units/ml). Sucrose, glucose, and fructose were also quantified by GLC of TMSi derivatives. Samples containing up to 1 μmol of each sugar were dried and incubated at 75° C for 30 min with 100 μl of 'STOX' reagent (Pierce) containing 25 mg/ml hydroxylamine hydrochloride and 6 mg/ml phenyl-β-glucopyranoside (internal standard) in pyridine. After cooling the samples to ambient temperature, 100 μl of *N*-trimethylsilylimidazole (Pierce) were added, and the mixture was incubated at ambient temperature for 30 min. Two μl of the resulting solution were injected into a 0.2 cm × 2 m column of 3% OV-17 (Supelco) temperature programmed with a 3 min hold at 140°C then from 140°C to 250°C at 10°C/min, followed by a 10 min hold at 250°C. Injector and FID were at 260°C. Nitrogen flow was 30 ml/min.

Radioactivity in Neutral Sugars was determined following descending chromatography of cell extracts on Whatman 3 MM filter paper in ethyl acetate:acetic acid:water (7:3:1, v/v/v) for 30 to 40 h. Chromatograms were air-dried, cut into 1.5 cm sections, and assayed for radioactivity by liquid scintillation spectroscopy. Paper sections containing sucrose were retrieved after counting, washed in several changes of toluene, and dried. Sucrose was extracted with water, hydrolyzed with invertase as described above, and rechromatographed to determine the distribution of radioactivity in the glucosyl and fructosyl moieties.

Enzymic Determinations of Sugar-Phosphates and UDP-Glucose. Glucose-6-P was measured enzymically. Samples of neutralized extracts containing up to 200 nmol of Glc-6-P were added to a reaction mixture containing a final concentration of 50 mM Tris-HCl (pH 7.6), 1 mM EDTA, 6 mM $MgCl_2$, 14 mM β-mercaptoethanol, and 0.4 mM $NADP^+$ in a final volume of 2 ml. Absorbance of the mixture at 340 nm was measured, and 10 to 20 μl of solution containing 1 unit of Glc-6-P dehydrogenase in 5 mM sodium citrate (pH 7.6) and 1% (w/v) BSA were added. Change in absorbance was measured to completion and compared to that seen with Glc-6-P standards. Glucose-1-P was measured in the above samples after Glc-6-P had been fully oxidized. Twenty μl of solution containing 2 units of phosphoglucomutase in 5 mM sodium citrate (pH 7.6) containing 1% BSA was added and additional increases in A_{340} were measured. In those cases, reaction mixtures also contained 10 μM Glc-1,6-bisP, a cofactor in the isomerization reaction. Commercial Glc-1-P was used as a standard. UDP-Glucose was assayed enzymically with UDP-Glc dehydrogenase in a reaction mixture containing 0.35 M glycine (pH 8.7), 5.6 mM EDTA, 1.5 mg/ml NAD^+, 0.05 unit/ml of UDP-Glc dehydrogenase from bovine liver, type IV (Sigma), and a test sample containing 5 to 10 nmol of UDP-Glc per ml of final reaction mixture. The enzyme was added after the initial measurement of A_{340}. Quantitation was based on UDP-Glc standards.

Chemicals. Enzymes were purchased from Sigma Chemical Co. Phosphoglucomutase (EC 2.7.5.1) was from rabbit muscle; glucose-6-P dehydrogenase (EC 1.1.1.49) from bakers yeast, type IX; UDP-Glc dehydrogenase (EC 1.1.1.22) from bovine liver, type IV; and invertase (EC 3.2.1.26) from *Candida utilis*, grade X. Nucleotides, nucleotide sugars, and sugar phosphates were also from Sigma and, when used as quantitative standards, their salt form and water content were taken into account. Phosphate buffers for HPLC were prepared by titrating measured volumes of 85% phosphoric acid (Fisher Chemical Co.) with a solution of analytical grade potassium hydroxide. After being cooled and adjusted to volume, buffers were filtered through Millipore type GS filter, 0.22 μm pore size. Other reagents were of analytical grade and were purchased from various major U.S. suppliers. D-[U-^{14}C]glucose (specific radioactivity of 200 to 300 mCi/mmol) and other miscellaneous ^{14}C-labeled compounds were from ICN.

RESULTS AND DISCUSSION

Growth Curves. Growth rates of carrot cells in liquid culture varied depending on the initial inoculum, but the growth curves were nevertheless typical of most other plant cells that we and others maintain (3, 5, 13). Logarithmic rate of growth occurred between d 3 and 9 after subculture (Fig. 1). There was little difference in growth rates between cells grown on sucrose and glucose. Cells grown for several years on glucose as sole carbon source retained their ability to utilize sucrose. Cells are not autotrophic so that when mannitol was substituted for glucose or sucrose, the cellular sugars decreased rapidly and the cells died within 2 to 3 d (data not shown).

Sugar Uptake and Utilization by the Cells. Levels of sucrose, glucose, and fructose were measured daily in media from sucrose-grown cultures. Sucrose disappeared from the media within 3 d, before the cells had begun logarithmic growth, and in its place appeared nearly stoichiometric amounts of fructose and slightly lower amounts of glucose (Fig. 2). This observation suggests that the cells secrete, or possess on their surface, invertase in excess so that sucrose is hydrolyzed faster than hexoses can be absorbed. Extracellular invertase activity and rapid extracellular sucrose hydrolysis have been observed in other cell cultures (24, 27).

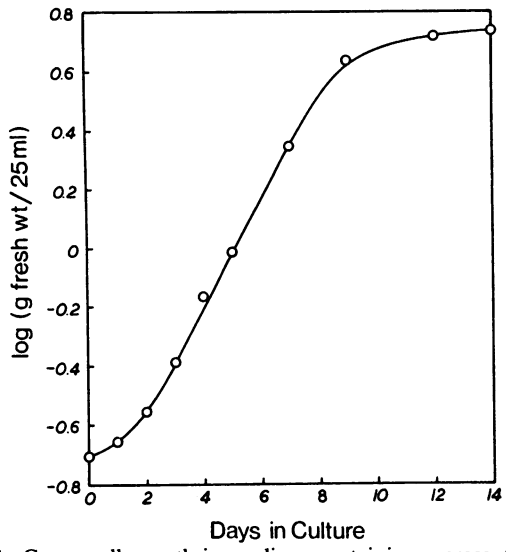

FIG. 1. Carrot cell growth in medium containing sucrose. Ordinate, logarithm of cell fresh weight in g per flask (25 ml culture); abscissa, days in culture after transfer to fresh medium.

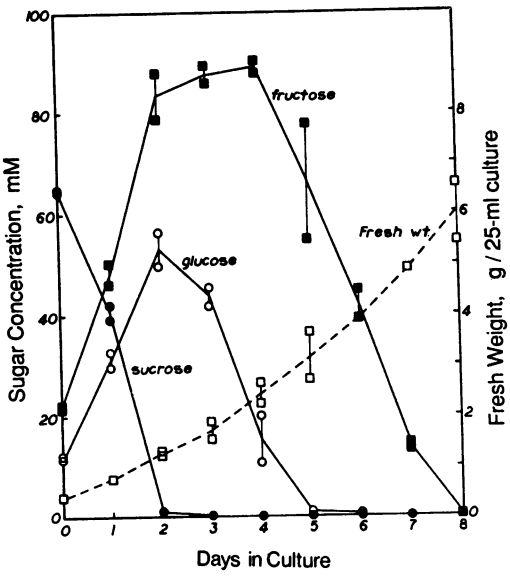

FIG. 2. Sugar utilization by carrot cells in suspension culture. Left ordinate, sugar concentration in media (mmol/L): (●——●), sucrose; (■——■), fructose; (○——○), glucose. Right ordinate, cell fresh weight per 25 ml culture medium (□- - -□). Abscissa, d in culture after transfer to fresh medium.

Fructose was not absorbed appreciably before virtually all of the glucose had been eliminated from the medium (Fig. 2). Even then, the maximum rate of fructose uptake (about 0.2 mmol/g fresh weight·24 h) was about half that of glucose (about 0.4 mmol/g fresh weight·24 h).

Cellular Sugars. Cellular sugars were measured in several growth-cycle experiments either as sucrose and reducing sugars or by GLC of TMSi derivatives of sucrose, glucose, and fructose. The initial sugar levels were variable depending on the stage of growth of the 'mother' culture. Concentrations of sucrose and glucose were about 18 and 10 μmol/g fresh weight, respectively, during early logarithmic growth, but increased to almost 60 μmol/g fresh weight at the end of logarithmic growth when some carbohydrate was still available in the medium. This was then followed by a rapid decline in concentrations of all sugars. These results would suggest that factors other than sugar availability were responsible for slowing of growth thus allowing for a transient accumulation of free sugars in the cells. Cellular concentrations of fructose (about 0.5 to 3 μmol/g fresh weight) showed no apparent relation to growth cycle. A final decrease in cellular sugar concentrations coincided with the plateau phase of growth when appreciable deterioration of the cultures occurred and when sugars were finally depleted from the medium. Under conditions of our experiments, media became depleted of Pi within 4 d of culture (data not shown), and other medium components such as hormones and vitamins may have become limiting (2, 24). Cells from midlogarithmic stage of growth transferred to a buffer containing only carbohydrate also deteriorated within 12 h (data not shown).

Nucleotides and Nucleotide-Sugars. The major nucleotides identified by HPLC in TCA-soluble extracts of the cells were di- and triphosphates of adenosine, guanosine, and uridine, UDP-Glc, and GDP-Glc. Nucleoside monophosphates were obscured by small amounts of other cochromatographing compounds, but were most likely in amounts much lower than the other nucleotides (8–10, 14, 19, 25). CDP and CTP were not identified in the elution profiles. Direct enzymic assays of UDP-Glc in the extracts indicated that only about one-half of the UV-absorbing material comprising the HPLC peak co-eluting with UDP-Glc standard was indeed UDP-Glc; UDP-Ara, UDP-Gal and UDP-Xyl most likely contributed to the size of UDP-sugar peak. HPLC data from one typical experiment are summarized in Figure 3. It is apparent that cells accumulated the above compounds to considerable concentrations during the early logarithmic phase. Later, during the midlogarithmic phase, these concentrations decreased and continued to do so during the plateau phase. From observation of the cells during growth cycle, it is apparent that, during early logarithmic phase, growth is largely due to cell division. The main part of the exponential phase consists, however, of cell expansion which would tend to increase the extent of vacuolization. Therefore, the cytoplasmic concentrations of nucleotides may be more nearly constant for most of the growth cycle than is suggested by the data in Figure 3.

Energy Charge. The concept of energy charge was first developed by Atkinson (1) and Bomsel and Pradet (4) and has since proven of value in interpreting metabolic energy status of a variety of cells and tissues (19). Energy charge is defined as the ratio of the sum of [ATP] and its equivalents in the form of ADP to the total adenine nucleotides ([AMP] + [ADP] + [ATP]). We were unable to measure AMP. Therefore, we opted for a modified quotient: ([ATP] + 0.5 [ADP])/([ATP] + [ADP]) which should reflect closely the 'energy charge' ratios because concentrations of AMP are not expected to be high (8–10, 19, 25). Such modified energy charge ratios for adenine nucleotides decreased steadily from about 0.9 on d 1 to about 0.8 on d 14 after subculture (Fig. 3A).

Aside from metabolic control over the activity of several enzymic pathways, the adenylate energy charge is indicative of the stores of phosphoanhydride bonds that can be transferred to other nucleotides. Formation of UDP, UTP, and other nucleotide di- and triphosphates is catalyzed by nucleoside mono- and diphosphate kinases analogous to adenylate kinase (22). Further, formation of UDP-sugars and other nucleotide-sugars required for synthesis of most of the polysaccharides of a cell constitute the principal drain on UTP and, ultimately, ATP. Uracil and guanine nucleotide ratios, while not construed to constitute an energy charge, should reflect the extent to which high-energy bond formation is coupled to the transfer of that energy to other nucleotides participating in the formation of nucleotide-sugar substrates responsible for assimilation of carbohydrate. Changes observed in the guanine nucleotide ratio paralleled those of the adenine nucleotides (Fig. 3, A and B), but the uracil nucleotide ratios were nearly 0.9 initially, then decreased to about 0.7 during rapid growth, and rebounded to 0.85 as the cells reached the

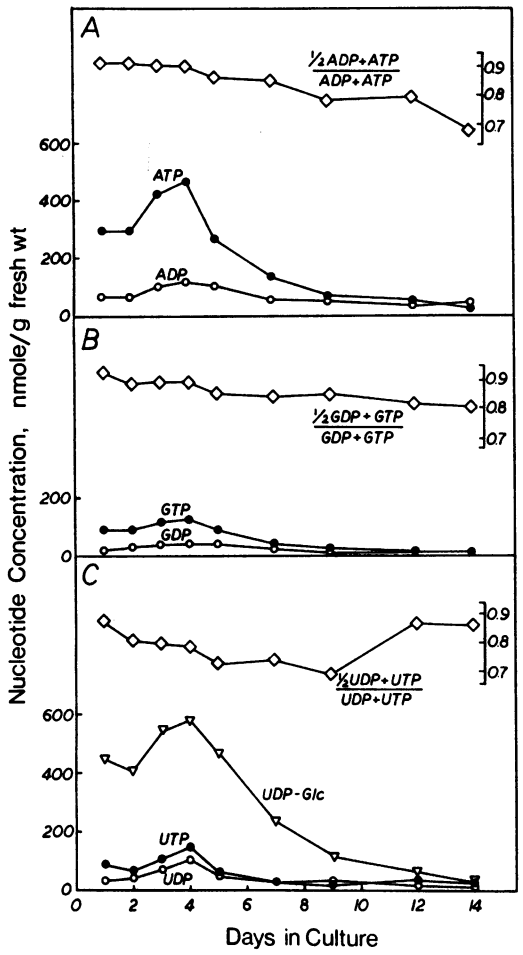

FIG. 3. Nucleoside triphosphate (●) and nucleoside diphosphate (○) levels in carrot cells during growth cycle in sucrose containing medium. Left ordinate, nucleotide concentration in nmol/g fresh weight (inset: nucleotide ratios [◇]). Right ordinate, modified nucleotide ratio (see text). Abscissa, d in culture after transfer to fresh medium. A, adenylates; B, guanylates; C, uridylates. Concentrations of UDP-Glc are shown with triangles in panel C.

plateau phase of growth (Fig. 3C). This depression during logarithmic phase of growth could be attributed, first, to a massive transfer of uridine from UTP to UDP-Glc and, second, to generation of UDP in polysaccharide synthetic reactions utilizing UDP-Glc. Both processes would lead to a reduced UTP/UDP ratio. Our results are largely in agreement with those of Meyer and Wagner (14) who reported that, in cell suspensions of tobacco, UDP-Glc and UDP-Gal (which do not resolve on their HPLC column) constituted the most abundant form of all nucleotides measured; nucleoside monophosphates comprised but a small portion of the total; and nucleotides and UDP-sugars attained maximum concentrations during the transition from the lag phase to the proliferative phase. The adenylate energy charge ratio reported by these authors (0.90–0.93 on d 4 of growth cycle) is in close agreement with our data, and the uracil nucleotide ratio calculated from their data shows a depression during early logarithmic phase similar to that observed in carrot cells (Fig. 3C). Similar results were recently reported by the same authors for cultures of *Datura innoxia* (15). These data indicate that the adenine and uracil nucleotide pools are not strictly coupled and, therefore, additional sites of regulation of UDP-glucose metabolism and carbon assimilation should be investigated.

Uptake and Metabolism of D-Glucose. Incorporation of radioactivity from exogenous 50 mM [^{14}C]glucose into the TCA-insoluble fraction of the carrot cells was nearly linear for at least 60 min while accumulation of [^{14}C] in TCA-soluble pools slowed steadily, indicating a gradual saturation of the soluble pool (Fig. 4). Paper chromatography of neutralized cell extracts indicated that, during the first 10 to 20 s of labeling, the ^{14}C from external glucose was found mainly in glucose (40% of total) and in some unidentified anionic compounds remaining at the origin of the chromatograms (48%). Nevertheless, over 2% of the label was already in sucrose at that time. These values were obtained with cultures which were filtered and frozen as soon as radioactive glucose had been added (about 10 to 20 s). After 5 min, 65% of the label was at the origin and only 21% and 6% in glucose and sucrose, respectively. From then on, radioactivity accumulated in sucrose while the proportion of radioactivity found in glucose and in the immobile fraction steadily decreased. This occurred despite the fact that, during the 4 h of incubation, concentrations of cellular glucose and fructose remained nearly constant while that of sucrose decreased by 30% of the initial value. Cellular pool of fructose was about half that of glucose and it became labeled slowly. Only 3% of the total radioactivity was in fructose after 4 h of labeling as compared with 12% for glucose. Nevertheless, the ratio of radioactivity found in fructosyl and glucosyl moieties of sucrose (determined from chromatography of hydrolyzed cellular sucrose) increased from 0.6 at 5 min to close to 0.9 at 30 min, and to 0.98 by 4 h of labeling indicating that the fructosyl moiety in newly synthesized sucrose came directly from a rapidly labeled pool of glucose and that most of the free fructose was outside this metabolic pool.

The neutralized TCA-soluble fraction was also chromatographed on an anion-exchange HPLC column, and radioactivity was measured in major peaks. The cells incorporated radioactiv-

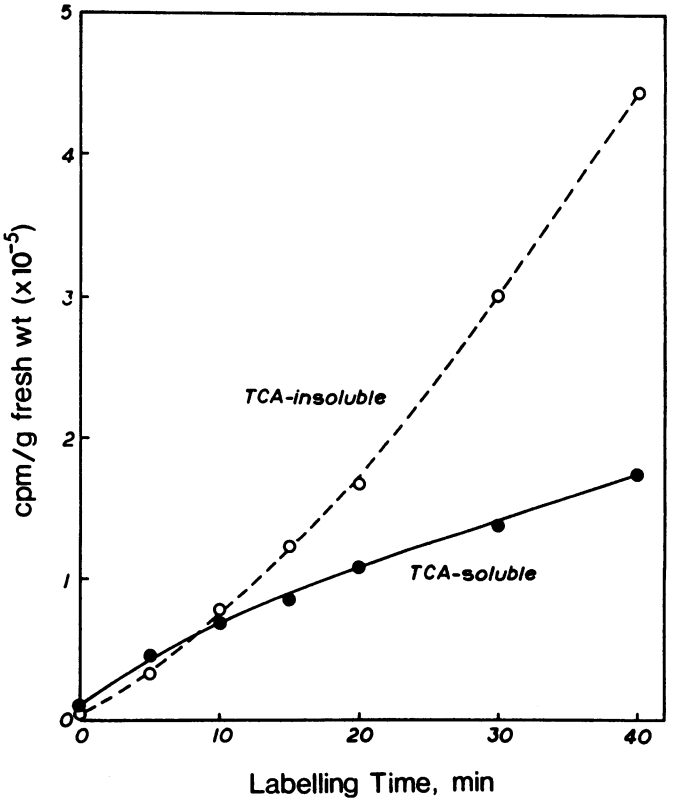

FIG. 4. Radioactivity from D-[^{14}C]glucose in TCA-soluble (——) and TCA-insoluble fraction (– – –) of carrot cells incubated in medium containing 50 mM D-glucose. Ordinate, cpm/g fresh weight; abscissa, time of labeling (min).

ity into the nonacidic fraction (void-volume peak), glucose phosphates and UDP-glucose within seconds. Glc-6-P and UDP-Glc were identified in HPLC eluates by enzymic methods to confirm the identity of the peaks. They were also quantified by the same methods in unfractionated neutralized extracts. As mentioned above, the direct assays of UDP-Glc in the extracts suggested that the HPLC peak containing UDP-Glc most likely contained also substantial amounts of other UDP-sugars. For calculation of specific activities, we used concentrations of all UDP-sugars based on the size of the UDP-Glc peak and the response factor determined for a UDP-Glc standard. The total radioactivity in the corresponding fractions was measured after subtraction of apparent baseline radioactivity from valley-to-valley. Similar calculations for glucose phosphates involved determination of Glc-6-P and Glc-1-P enzymically and the baseline-corrected total radioactivity in the corresponding HPLC peak. When plotted against time (Fig. 5), the two sets of data indicate the presence of fast, saturable component and a slower, linear one. It is likely that the slow components reflect a gradual increase in specific radioactivities due to a second, presumably vacuolar, glucose pool that slowly accumulated labeled glucose and reintroduced it into the metabolic compartment. A similar pattern of labeling has been observed in cotton fibers where calculations based on a computer simulation predicted the isotope dilution effect of a large vacuolar pool of glucose labeled slowly (6). The broken line in Figure 5 indicates the average specific radioactivity of glucose in the medium determined at each corresponding time point. This specific radioactivity was essentially constant throughout the experiment. The fact that the specific radioactivities for the glucose phosphates appear to exceed that for external glucose is most likely an artifact due to the presence of co-chromatographing compounds, such as Fru-6-P, whose contribution is not eliminated by our method of background subtraction. In general, these data suggest a rather complicated pattern of compartmentation of neutral sugars, sugar phosphates, and nucleotide-sugars in carrot cells that should be explored further.

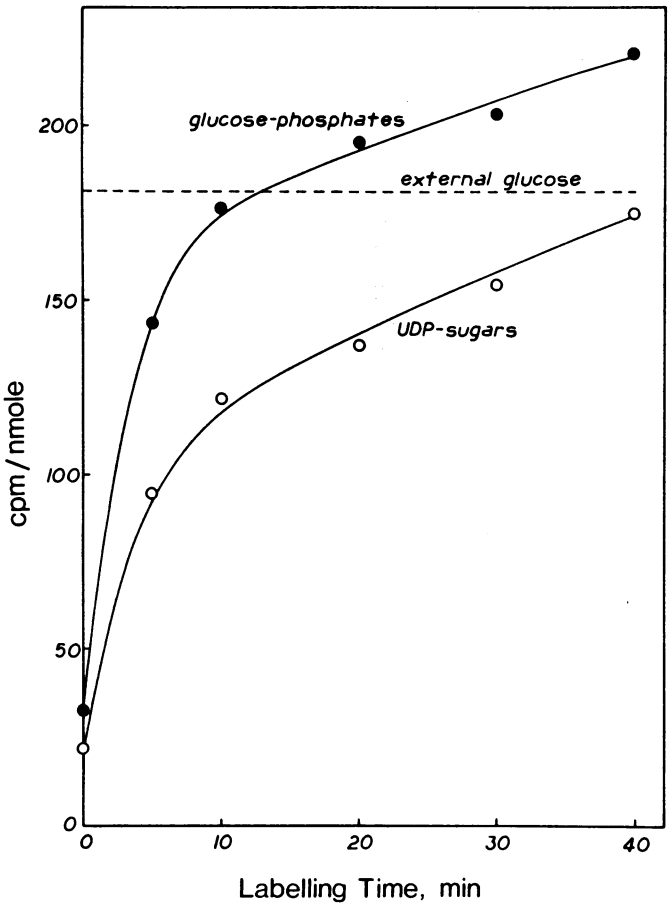

FIG. 5. Radioactivity from D-[^{14}C] glucose in glucose phosphates (●) and UDP-sugars (○). Same experiments as in Figure 4. Total net radioactivity per peak was obtained by valley-to-valley integration of radioactivity in 1-ml fractions from HPLC. This was divided by the estimated amounts of Glc-6-P plus Glc-1-P from enzymic assays and of UDP-sugars from HPLC peak integration at 254 nm, respectively. A horizontal dashed line represents average specific radioactivity of glucose in the medium during the experiment (mean ± SD = 181.6 ± 3.7) determined in filtered media.

CONCLUSIONS

These results presented above indicated that (a) sucrose is not an immediate source of carbon for carrot cells in liquid culture; (b) hexoses released by the action of extracellular invertase provide most, if not all, of the carbon; (c) cells exhibit a preference for glucose, but will utilize fructose when glucose is depleted from the medium; (d) UDP-Glc is synthesized primarily from assimilated glucose, ATP, and UTP in reactions catalyzed by hexokinase, phosphoglucomutase, and UDP-Glc pyrophosphorylase; and (e) sucrose, an intermediate form of carbon storage in the cell, is synthesized from UDP-Glc and fructose intracellularly and is not assimilated intact from the growth medium.

It should be emphasized that these conclusions apply only to these cells. However, in our recent studies of carbon utilization in other culture systems, we found that tobacco, proso millet, and maize cells also hydrolyze sucrose quickly, and that hexose provides the major source of reduced carbon. It seems to us that analyses of cellular levels of neutral sugars, sugar phosphates, uracil nucleotides, and UDP-sugars, and the external carbon sources are necessary for understanding the relationship between energy metabolism and carbon assimilation. To adequately describe the carbon flow, labeling experiments would have to be done during the first minutes if not seconds after addition of the label so that the rapidly labeled pools could be more precisely analyzed.

Acknowledgments—We thank Drs. A. Poljakoff-Mayber and A. M. Mayer, Department of Botany, Hebrew University, Jerusalem, Israel, for valuable discussions, Ms. Barbara Mullen for dedicated technical assistance, and Mrs. Anna Olek for the artwork.

LITERATURE CITED

1. ATKINSON DE 1968 The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry 7: 4030–4034
2. BALAGUÉ C, A LATCHÉ, J FALLOT, J-C PECH 1982 Some physiological changes occurring during the senescence of auxin-deprived pear cells in culture. Plant Physiol 69: 1339–1343
3. BINZEL ML, PM HASEGAWA, AK HANDA, RA BRESSAN 1985 Adaptation of tobacco cells to NaCl. Plant Physiol 79: 118–125
4. BOMSEL JL, A PRADET 1967 Etude de adénosine 5′-mono-, di- et triphosphates dans les tissus végétaux. II. Evolution in vivo de l'ATP, l'ADP et l'AMP dans les feuilles de blé en fonction de différentes conditions de milieu. Physiol Vég 5: 223–236
5. BRESSAN RA, PM HASEGAWA, AK HANDA 1981 Resistance of cultured higher plant cells to polyethylene glycol-induced water stress. Plant Sci Lett 21: 23–30
6. CARPITA NC, DP DELMER 1981 Concentration and metabolic turnover of UDP-glucose in developing cotton fibers. J Biol Chem 256: 308–315
7. GHOSH HP, J PREISS 1966 Adenosine diphosphate glucose pyrophosphorylase. A regulatory enzyme in the biosynthesis of starch in spinach leaf chloroplasts. J Biol Chem 241: 4491–4504
8. GRONEWALD JW, JB HANSON 1982 Adenine nucleotide content of corn roots as affected by injury and subsequent washing. Plant Physiol 69: 1252–1256
9. HAMPP R, M GOLLER, H ZIEGLER 1982 Adenylate levels, energy charge and phosphorylation potential during dark-light and light-dark transition in chloroplasts, mitochondria and cytosol of mesophyll protoplasts from *Avena sativa L.* Plant Physiol 69: 448–455
10. HEMRIKA-WAGNER AM, LHW VAN DER PLAS 1965 Adenine nucleotide contents in callus-forming potato tuber discs with different respiratory charac-

teristics. Physiol Plant 64: 253–258
11. HOPPER JE, DB DICKINSON 1972 Partial purification and sugar nucleotide inhibition of UDP-glucose pyrophosphorylase from *Lilium longiflorum* pollen. Arch Biochem Biophys 148: 523–535
12. LELOIR LF, MAR DE FEKETE, CE CARDINI 1961 Starch and oligosaccharide synthesis from uridine diphosphate glucose. J Biol Chem 236: 636–641
13. MATTHEWS BF, JM WIDHOLM 1978 Regulation of lysine and threonine synthesis in carrot cell suspension cultures and whole carrot roots. Planta 141: 315–321
14. MEYER R, KG WAGNER 1985 Analysis of the nucleotide pool during growth of suspension cultured cells of *Nicotiana tabacum* by high performance liquid chromatography. Physiol Plant 65: 439–445
15. MEYER R, KG WAGNER 1985 Nucleotide pools in suspension-cultured cells of *Datura innoxia*. I. Changes during growth of the batch culture. Planta 166: 439–445
16. MURASHIGE T, F SKOOG 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497
17. NELSON N 1944 A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153: 375–380
18. POGOLOTTI AL JR, DV SANTI 1982 High-pressure liquid chromatography. Ultraviolet analysis of intracellular nucleotides. Anal Biochem 126: 335–345
19. PRADET A, P RAYMOND 1983 Adenine nucleotide ratios and adenylate energy charge in energy metabolism. Annu Rev Plant Physiol 34: 199–224
20. PURICH DL, HJ FROMM 1972 Studies on factors influencing enzyme responses to adenylate energy charge. J Biol Chem 247: 249–255
21. PURICH DL, HJ FROMM 1973 Additional factors influencing enzyme responses to the adenylate energy charge. J Biol Chem 248: 461–466
22. ROSS CW 1981 Biosynthesis of nucleotides. *In* A Marcus, ed, The Biochemistry of Plants, Vol 6. Academic Press, New York, pp 169–205
23. SOMOGYI M 1952 Notes on sugar determination. J Biol Chem 195: 19–23
24. THOM M, A MARETZKI, E KOMOR, WS SAKAI 1981 Nutrient uptake and accumulation by sugarcane cell cultures in relation to the growth cycle. Plant Cell Tissue Organ Culture 1: 3–14
25. THOMAS KC, PSS DAWSON 1977 Energy charge values of *Candida utilis* growing under energy-excess and energy-limiting conditions. FEMS Microbiol Lett 1: 347–349
26. TURNER JF, DH TURNER 1975 The regulation of carbohydrate metabolism. Annu Rev Plant Physiol 26: 159–186
27. ZAMSKI E, RE WYSE 1985 Stereospecificity of glucose carrier in sugar beet suspension cells. Plant Physiol 78: 291–295