

C-11-Labeled Glucose and its Utilization in Positron-Emission Tomography

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Carbon-11-labeled glucose was prepared photosynthetically using the green alga *Scenedesmus obtusiusculus* Chod. The carbohydrates were extracted from the cells with dilute HCl and the glucose was isolated and purified using high-performance liquid chromatography. The manipulations in the hot cell are described. Analysis of the material (gas liquid chromatography and HPLC) showed that the glucose obtained was radiochemically pure. The total incorporation of the $^{11}\text{CO}_2$ added to the algae was 60–80%. The radiochemical yield of pure carrier-added glucose was approximately 25%, at 40 min after E.O.B. including the HPLC purification and sterile filtration. The C-11 glucose uptake in rat brain was compared with that of commercial D[U- ^{14}C]glucose, and preliminary PET studies with D-[^{11}C]glucose in a patient with a brain infarct are presented.

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D-Glucose is the sole energy source of the normal brain. There are advantages in using C-11 D-glucose, which is biologically indistinguishable from D-glucose, in studies of brain energy metabolism, even if the complexity of its metabolism makes the mathematical treatment of the obtained data cumbersome (1–3).

Regional cerebral glucose metabolism has been studied in vivo in animals using C-14 glucose (1) and in man with C-11 glucose (4–6). Other glucose analogs have also been used: C-14 2-deoxyglucose (7), C-11 2-deoxyglucose (8), 2-[^{18}F]fluoro-2-deoxyglucose (9–11), 3-[^{18}F]fluoro-3-deoxyglucose (12), and 3-(^{11}C)methylglucose (13). To investigate the possibility of using C-11 glucose in PET studies in humans, we have developed a photosynthetic method for the preparation of C-11 glucose in high chemical yields and high radiochemical purity. The uptake of the photosynthetically prepared C-11 glucose in rat brain has also been compared with that of commercial [U- ^{14}C]glucose. Preliminary positron tomograms using C-11 glucose in a patient with cerebral infarct are also presented.

Carbon-11 glucose has been obtained previously by photosynthesis (14–16) from $^{11}\text{CO}_2$ or by chemical synthesis using C-11 cyanide (17). In the photosynthetic procedures, leaves of Swiss chard (14,15) or broad bean (16) were found to give a mixture of C-11 glucose and C-11 fructose, and similar results were previously reported by us (18) when green algae were used.

The C-11 glucose obtained and purified as previously described (18) was used for positron-emission tomograms (PET) (6). Mathematical treatment of the resulting data gave a metabolic rate of glucose lower than those previously published (19). One factor contributing to this low value could be the assumed presence of approximately 30% fructose (18) or other carbohydrates (6). This necessitated specific purification methods for the glucose produced by the algae, and the aim of this work was therefore to design a routine method for preparation of pure C-11 glucose.

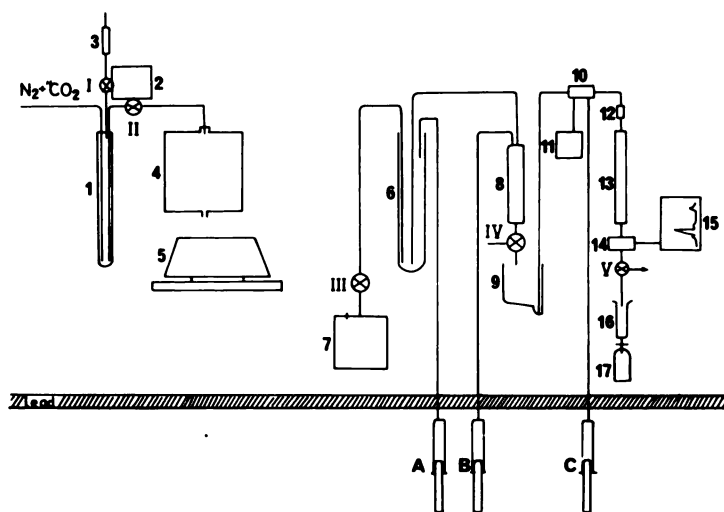
MATERIALS AND METHODS

Glucose preparation. Carbon-11 is obtained by the nuclear reaction $^{14}\text{N}(p,\alpha)^{11}\text{C}$. The photosynthesis was performed as previously described (18) using synchronized cultures of the unicellular green alga *Scenedesmus*

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FIG. 1. Scheme of equipment in hot cell for C-11 glucose synthesis: 1. Trapping tube (containing dilute KOH solution); 2. Whitey Dual Valve Actuation (one valve open while the other is closed); 3. Ascarite trap; 4. Temperature-controlled reaction vessel; 5. Centrifuge; 6. Centrifuge tube (containing algae after photosynthesis with supernatant); 7. Container, under vacuum, for supernatant; 8. Ion-exchange column, Bio Rad AG11A8; 9. Evaporation tube; 10. Rheodyne injector Type 7126 with a loop of 1 ml; 11. Pump, LDC Constametric I; 12. Pre-column, Aminex HPX-85H 40 X 4.6 mm in a Micro Guardholder; 13. Chromatography column, Aminex HPX-87P 300 X 7.8 mm; 14. Radioactivity detector; 15. Chromatogram recorder; 16. Syringe equipped with a Millipore filter, 0.22 μ m; 17. Sterile vial, 10 ml; I, II, III: Whitey Ball Valves; IV, V: Altex electrovalves; A: Syringe for HCl (3.0 ml) injection; B: Syringe for water (5.0 ml) injection; C: Syringe for drawing neutralized algal extract into Rheodyne injector.



obtusiusculus Chod. The algae were grown as described by Kylin et al (20). For the photosynthesis, the algae were suspended in a buffer of morpholinoethanesulphonic acid (100 mM), CaCl₂ (0.5 mM), and MgCl₂ (5 mM) at pH 5.0.

After the photosynthesis and centrifugation (position 6 in Fig. 1) the supernatant was sucked to the waste container (7) and the cells boiled with dilute HCl as described earlier (18). The suspension was cooled in an ice bath, then transferred to and eluted through an ion-exchange column* (7 ml ion-exchange resin in a 10-ml syringe) (8). The resin was washed with water (3 ml) added by syringe (B). The eluate was collected in vessel 9, evaporated to 0.5–0.8 ml, then sucked into the injector loop (10) with syringe C for HPLC separation (see below).

Gas chromatographic analysis. Gas chromatography (GLC) of the derivatized sugars (see below) was performed using an instrument equipped with an OV 225 column (100 cm X 4 mm i.d.) and flame ionization detector (carrier gas: N₂, 25 ml/min). The C-14 gas radiochromatograms were obtained under similar conditions using a nuclear ratemeter† combined with a gas chromatograph.

To determine which sugars were present after the ion-exchange column, the neutral solution was evaporated to dryness and the residue dissolved in pyridine. This solution was treated with hydroxylamine followed by acetic anhydride, as described by Seymor et al. (21), thereby converting aldoses to peracetylated aldononitriles, and ketoses to peracetylated oxime derivatives. This mixture was then analyzed by GLC, as described above, and the chromatograms compared with those obtained from samples of authentic sugars.

For C-14 gas radiochromatography, a method for the derivatization more convenient than the above was used. Sodium borohydride (10 mg) was added to an aliquot of the neutral aqueous solution from the ion-exchange column (4–5 ml) and the solution was kept at room temperature for 2 hr. It was then acidified to pH 3.5 using a Dowex 50 H⁺ ion-exchange resin, filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in methanol (5 ml) and again evaporated. This was repeated twice to remove the boric acid, formed from NaBH₄, as the volatile methyl ester.

The resulting alditols were dissolved in 1 ml of a 1:1 mixture of acetic anhydride and pyridine, and the solution was heated at 100° for 30 min. Excess reagent was removed under reduced pressure and the residue was dissolved in a small amount of methylene chloride and transferred to a small sample tube, where it was concentrated to ~100 μ l for GLC analysis. Due to the time-consuming derivatization of the sugars, the GC analysis was performed using only C-14 labeled material.

HPLC separation. The pump was equipped with stainless steel columns (150 X 4 mm i.d. for analytical purposes, and 300 X 8 mm i.d. for preparative purposes) with Swagelok connectors and Altex stainless steel frits (2 μ m). The detector had a 10.0 mm pathlength with a volume of 8 μ l and operated at 190 nm. An injector valve was used having a sample loop of 100- μ l (analytical experiments) or 1-ml volume (preparative experiments).

Analytical columns were packed with LiChrosorb-NH₂ (particle size 5 μ m); mobile phase acetonitrile/water 9:1; flow rate 2 ml/min. Preparative columns were either as above or used Aminex HPX-87P (particle size

9 μm) with a pre-column (micro guard system Aminex HPX-85). The mobile phase for the Aminex column was water at 50° with a flow rate of 0.85 ml/min.

For HPLC analysis of the C-14 algal extract, the Li-Chrosorb column was injected with 100- μl samples and the eluate was collected in 0.5-ml fractions. The radioactivity of each fraction was determined by liquid scintillation counting.

For preparative experiments the neutral solution from the ion-exchange column was evaporated to a volume of 0.5–0.8 ml, which was then injected onto the column. Detection involved both the absorbance at 190 nm and the radioactivity determination of each collected fraction. When the HPLC separation conditions had been determined using C-14 algal extract, C-11-labeled preparations were chromatographed under the same conditions and gave identical results. The C-11 glucose was collected 9–10.5 min after the injection (see Fig. 5).

Preparation of the injection solution. The glucose fraction of the eluate from the preparative Aminex column (1–1.5 ml) was diluted with sterile saline to a volume of 5 ml and filtered under aseptic conditions through a Millipore filter (0.22 μm) into a sterile injection vial.

Animal experiments. Male rats (Sprague Dawley, ~400 g) were injected i.v. with a solution containing ~0.5 mCi C-11 glucose from the algal photosynthesis and ~10 μCi [^{14}C]glucose. Ten minutes later, blood samples were taken, the animals were decapitated, and the brain was rapidly removed. Representative samples were taken from left and right frontal and temporal lobes for analysis of the radioactivity. The C-11 activity was first determined in the blood and in each brain fraction using a well counter; then, after allowing the C-11 activity to decay, the C-14 activity was determined in the

same samples after combustion, using liquid scintillation counting.

Positron camera. A number of patients were scanned with a ring-detector positron camera system, after the administration of C-11 glucose. The positron camera, specially designed for head examinations, consists of four rings with 96 BGO detectors in each ring (22). The circuitry accepts coincidences in plane and between adjacent planes, thus enabling seven planes covering about 10 cm to be viewed simultaneously. The small detector size, 12 \times 20 \times 30 mm, and a continuous wobbling motion allows a resolution of 8 mm in the reconstructed image. PET images from a patient with brain infarct are shown in Fig. 6. The scans represent an average of a whole dynamic study from 10 to 40 min after the injection of 9.5 mCi of C-11 glucose. Each image is reconstructed from about 4.2 million counts.

RESULTS AND DISCUSSION

The manipulations required for the preparation of C-11 glucose in a hot cell are shown schematically in Fig. 1. The synthesis is performed in a completely closed hood, which avoids radiation risks for personnel even when large quantities of $^{11}\text{CO}_2$ are used at the start of the synthesis. The trapping of the $^{11}\text{CO}_2$ from the target gas in dilute alkali solution is very efficient due to the high pH. The alternative procedure, to bubble the target gas directly into the algal suspension, gave a much lower yield of C-11 glucose, since only part of the $^{11}\text{CO}_2$ is trapped in a buffer at pH = 5.0.

The algae were grown in synchronous cultures essentially as previously described (18). In our experience, the light intensity and the gas flow recommended by Kylin et al. (20) are crucial for good cell growth. The buffer used in the photosynthesis is five times the concentration reported in our first publication (18). We found that the present buffer gives more reproducible yields. Also the cell density has been increased to twice that originally used, resulting in a 10–20% increase in the incorporation of the radioactivity into the cell fraction.

Previously, 3 min of illumination time was used in the photosynthesis, but it was found later that 5 min illumination gave as much as 20% higher incorporation in the algae. HPLC analysis of the neutralized algal extract showed that when the longer illumination time was used the amount of glucose also increased compared with the other radioactive components formed.

The isolation of C-11 glucose has also been simplified compared with our previous procedure (18). We found that the chloroform extraction could be avoided and that a clear colorless and neutral solution containing more than 90% of the radioactivity incorporated into the algae was obtained after elution through the ion-exchange column as described above. Incorporation was 60–80%

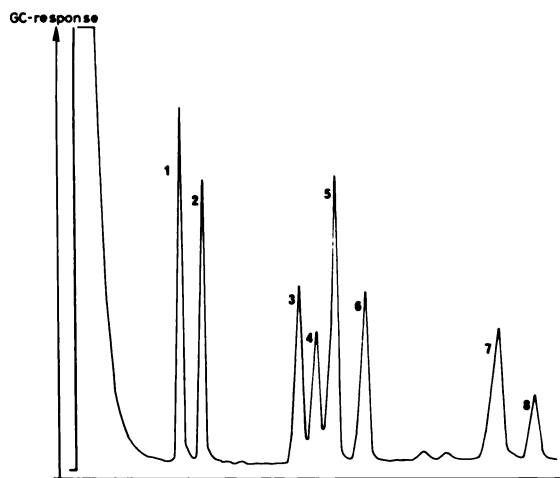


FIG. 2. Gas chromatogram of alditol acetates prepared from authentic samples of the sugars: (1) arabinose, (2) xylose, (3) mannose, (4) galactose, (5) glucose, (6) sorbose (iditol), (7 and 8) mannoheptulose.

based on the amount of $^{11}\text{CO}_2$ given to the algae, and this solution was ready for final purification 15 min after E.O.B.

For the analysis of the carbohydrates formed, the algae were incubated with $\text{Na}_2^{14}\text{CO}_3$ under conditions identical to those used for the C-11 procedures. After removal of the cell debris from the acidic solution, and neutralization as described above, the carbohydrate content was analyzed by GC and HPLC. The detailed analysis was performed using the C-14-labeled material. Control experiments starting from $^{11}\text{CO}_2$ showed that the C-11-labeled and the C-14-labeled material from the algae were identical.

To identify all possible monosaccharides present in the neutral solution after the ion-exchange column, the

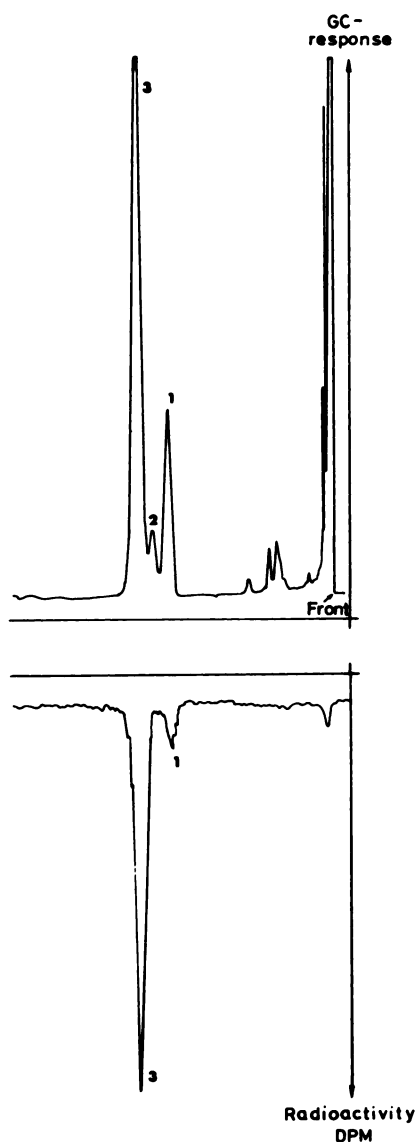


FIG. 3. Gas chromatogram (upper) and gas radiochromatogram (lower) of alditol acetates from algal extract: (1) mannose, (2) galactose, (3) glucose; other peaks have not been identified. Glucose peak in radiochromatogram represents about 90%, and mannose peak about 5%, of recorded radioactivity.

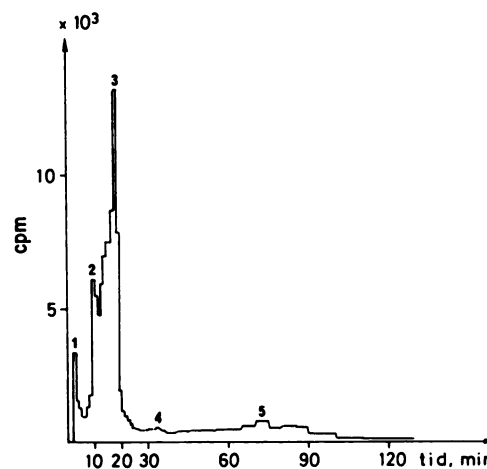


FIG. 4. Analytical HPLC of the C-14-labeled algal extract. Column: LiChrosorb-NH₂ 125 X 4 mm; mobile phase: acetonitrile/water 9:1; flow rate: 2 ml/min. (1) unknown, (2) mannose, (3) glucose, (4 and 5) unknown.

sugars were converted to peracetylated oxime derivatives as described by Seymor et al. (21). The GLC analysis of this mixture demonstrated the presence of mannose, galactose, and glucose in proportions 15:5:80, and the absence of fructose (<1%) (cf. Ref. 18).

For the C-14 gas radiochromatography, a more convenient method than the above was used in the derivatization. The sugars were reduced to the corresponding alditols and these were peracetylated before the chromatographic analysis. By this procedure pentoses, hexoses, and heptoses could be separated to give three dis-

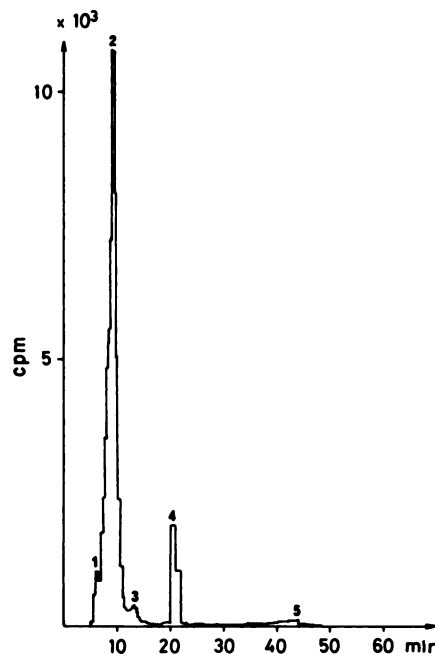


FIG. 5. Preparative HPLC of C-14-labeled algal extract. Column: Aminex HPX-87P 300 X 8 mm; mobile phase: water; flow rate: 0.85 ml/min. (1) unknown, (2) glucose, (3) mannose, (4 and 5) unknown. Glucose fraction was collected between 9 and 10.5 min after injection.

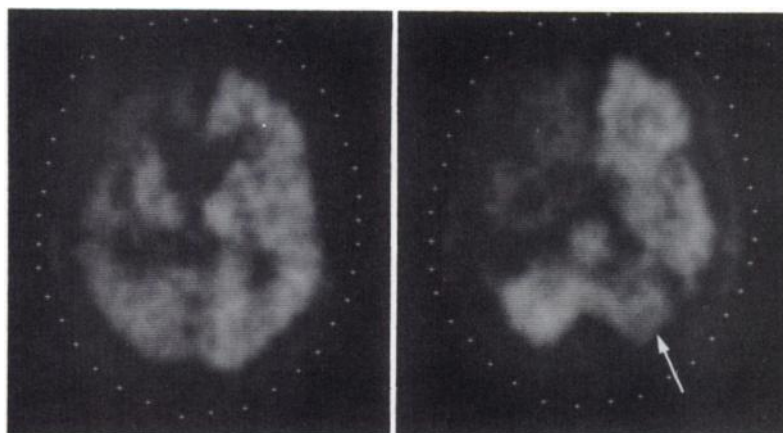


FIG. 6. Positron tomograms of brain (9.5 mCi C-11 glucose i.v.) in patient with cerebral infarct involving right frontal and temporal lobes. Darker areas (= low uptake, upper left in both images) indicate decreased metabolism. In left image dilated ventricles are also seen. In right image metabolism is also low in functionally related contralateral cerebellar hemisphere (arrow), although a CT scan indicated normal structure.

tinct groups of peaks (Fig. 2). To obtain this chromatogram, the authentic sugars were reduced, acetylated, and chromatographed, first separately and then as a mixture. Iditol was obtained from the reduction of sorbose, and the heptitols by the reduction of mannoheptulose.

When the carbohydrate solution from the algae photosynthesis was subjected to the same reduction, acetylation, and GLC analysis, three peaks appeared in the chromatogram, indicating the presence of mannose, galactose, and glucose (Fig. 3). No peaks due to pentoses or heptoses could be detected. Simultaneous recording of the radioactivity (Fig. 3) showed a small radioactive peak (2–3% of total radioactivity) near the solvent front, a peak corresponding to mannose (~5%), and a major peak corresponding to glucose (90%) of the total radioactivity. The galactose present was not radioactively labeled.

Analytical HPLC (C-14-labeled material) yielded the chromatogram shown in Fig. 4. This shows a small radioactive peak (4–5%) eluting at void volume and a small peak, corresponding to mannose, partially overlapping the major glucose peak. Other radioactive components with considerably higher capacity factors were eluted clearly separated from glucose.

Preparative chromatography using the larger Li-Chrosorb column gave good separation with acetonitrile/water 9:1 as mobile phase. However, this also resulted in unacceptably long retention times and therefore could not be used for C-11 experiments. With a mobile phase of acetonitrile/water 65:35, the retention time was acceptable but glucose and mannose could not be separated. Also the presence of acetonitrile in the mobile phase further complicates the final purification, since this solvent must be carefully removed by evaporation if the glucose is to be used for human studies.

An Aminex HPX-87P column, 8 mm i.d. × 300 mm with water as the mobile phase, was found to give a satisfactory separation of glucose, mannose, and other radioactive components (Fig. 5). The radioactive glucose was collected between 9 and 10.5 min after injection, which is acceptable for synthesis of C-11-labeled com-

pounds. The C-14-labeled glucose of this fraction was collected, reduced, and acetylated as described above and the derivative analyzed by radio-GLC as above. This gave only one radioactive peak in the chromatogram corresponding to glucose. We therefore conclude that the glucose obtained is radiochemically pure. The radiochemical yield of pure glucose was about 25%, based on the amount of $^{11}\text{CO}_2$ introduced to the alga. In a typical experiment, 250 mCi $^{11}\text{CO}_2$ was introduced into the synthesis, yielding 15–16 mCi C-11 glucose. The complete procedure—from the introduction of the radioactivity into the algal suspension to the final sterile injection solution—consumed about 40 min.

The resulting radioactive glucose solution was found to be sterile and free from pyrogenic material (*Limulus* and rabbit tests), and could therefore be used for positron-emission tomography studies in humans.

To show that the photochemically prepared C-11 glucose was biochemically identical to the commercially available [U- ^{14}C]glucose, a mixture of C-11 glucose prepared as described above (presumably randomly labeled with C-11) (23) and [U- ^{14}C]glucose was injected i.v. into the rats. Ten minutes later the animals were killed and the radioactivity was determined in the blood and in four different tissue samples from each brain. The animal experiments were performed with three subsequently prepared C-11 glucose samples using two rats per preparation. The cumulative results from these experiments show that the ratio of C-11 to C-14 in the blood was the same as the C-11/C-14 ratio measured in the brain tissue. It can therefore be concluded that the glucose prepared photosynthetically has an uptake in the brain identical to that of commercial [U- ^{14}C]glucose.

Figure 6 shows positron tomograms from a patient with a cerebral infarct. He received 9.5 mCi C-11 glucose i.v.

FOOTNOTES

* Bio-Rad AG 11A8.

† ESI.

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