A Shielded Synthesis System for Production of 2-Deoxy-2-[^{18}F]Fluoro-D-Glucose

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A remotely operated, shielded synthesis system for the production of 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}FDG) for clinical studies has been developed. Using this system, 25 mCi of ^{18}FDG are produced at the end of a 60-min synthesis from ~300 mCi of F-18 (total F-18 recovered from the target at the end of bombardment). The fractional distribution of F-18 among various components of the synthesis system has been measured. This yield of ^{18}FDG (25 mCi) is ample for two consecutive human studies in house or for shipment to collaborating institutions within a 3-hr (door to door) radius.

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We have previously reported the development of a new synthesis of 2-deoxy-2-fluoro-D-glucose (FDG) and its application to the labeling of this molecule with fluorine-18 (*1*,*2*). The initial choice of ^{18}FDG as a radiotracer and analog of 2-deoxy-D-glucose was a fortunate one and initial studies in animals (*3*,*4*) and man (*5*-*9*) have shown considerable promise. This has created a demand for ^{18}FDG, not only within our own institution but from collaborating institutions. Accordingly, a modification and simplification of the synthetic procedure previously reported was necessary not only from the standpoint of increasing the yield, but also in order to reduce the handling and radiation exposure to personnel involved in the synthesis.

The reaction of 3,4,6-tri-*O*-acetylglucal (TAG,**2**), with ^{18}F-F_2 is shown in Fig. 1. The adaptation of the synthetic procedure previously reported (*1*,*2*) to one compatible with the frequent production of 20–25 mCi of ^{18}FDG required the simplification and remote execution of the following steps.

Step 1. ^{18}F-F_2 production and recovery from target.

Step 2. Reaction of ^{18}F-F_2 with TAG.

Step 3. Separation of isomeric difluoro adducts (**3** and **4**) produced by Step 2.

Step 4. Hydrolysis of adduct **3** and H^{18}F removal.

Step 5. Purification of ^{18}FDG, **1**.

Step 6. Millipore filtration.

We have recently described a hot cell for the synthesis of labeled organic compounds and briefly outlined the improved ^{18}FDG synthesis as an example of the use of this new hot cell (*10*). We report here a detailed description of the procedure developed for the routine production of ^{18}FDG for in-house use and for shipment to collaborating institutions. Although this procedure is continually evolving we feel it is appropriate to provide details at this time because of numerous inquiries about targets, equipment, and procedures in use at B.N.L.

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MATERIALS AND METHODS

^{18}FDG shielded synthesis system. This is housed in the shielded synthesis box (*10*) shown in Fig. 2, which has been designed to accommodate the equipment necessary for carrying out the synthesis by remote operations. Figure 3 shows a diagram of the ^{18}FDG synthesis system, showing components housed inside and outside the shielded synthesis box. The components of the system are lettered in the diagram and listed below. Reference is made to the letters throughout the procedure. Such steps as solvent transfer, liquid chromatography, filtration, and solvent addition are accomplished by the remote application of vacuum or pressure. Stopcocks (which are turned by extension tools or a master-slave manipulator) or solenoid valves are used as required. For simplicity, the components of the synthesis system (excluding the Ne/F_2 target and ^{18}F-F_2 delivery line) will be broken down into those that, for reasons of safety, must be housed within the hot cell or shielded hood, and those that present no radiation hazard and are placed outside of the shielded area for easy access.

Components housed within the shielded synthesis area (letters following components are referred to in the synthesis procedure) are: reaction vessel (A); soda-lime trap (B); charcoal trap (C); Column 1 (D); rotary evaporator (E) and flask (F); Column 2 (G) and flask (H) and forecut (I); Millipore filtration assembly (J);

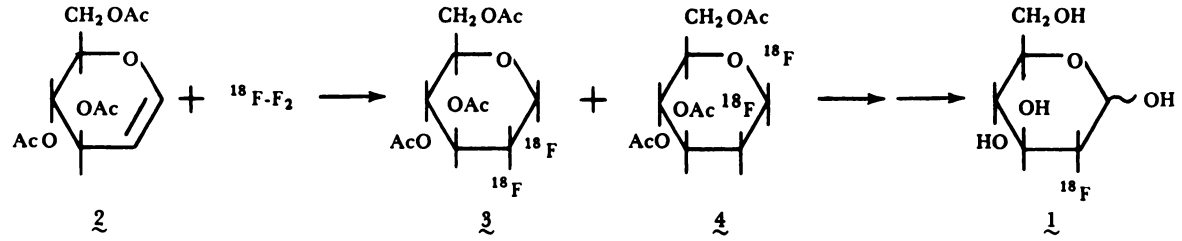

FIG. 1. Synthesis of ^{18}FDG.

three baths (0, 45, and 125°) on a sliding power jack assembly (K); ionization chamber (L); nitrogen inlet (M) and water bath (N); and flowmeter (X).

Components housed outside the shielded synthesis area are: needle valve for ^{18}F-F_2 delivery (O); trap assembly for forecut for Column 1 (P); solvent addition funnels for Column 1 (Q); addition funnel for rotary evaporator (R); vacuum controls (S); nitrogen control (T); saline addition line (U); extension tool for stopcock turning (V); and syringe assembly for Column 2 elution and solvent addition (W).

^{18}F-F_2 production and recovery from target. The targetry as well as the purity requirements for target gases have been described in detail (*11*,*12*). Briefly, the target is loaded with a commercially available mixture of approximately 1% F_2 in neon* and pure neon. The ^{18}F-F_2 delivered from the target is titrated as described previously (*11*) in order to determine the amount of F_2 carrier used.

Synthesis of ^{18}FDG. Before the run, all components of the system are assembled as shown in Fig. 3. Letters referring to a particular component of the system are used throughout this account and refer to Fig. 3. Connections are made with ⅛ in. o.d. Teflon tubing and swagelok fittings. The contents of the irradiated Ne/F_2 target containing 0.1% ^{18}F-F_2 in neon (~40-60 μmol F_2) were purged through a solution of recrystallized (from EtOH/hexane) 3,4,6-tri-*O*-acetylglucal (48-82 μmol) in 10 ml redistilled Freon-11, which is cooled with dry ice (A).† The gas leaving the reaction vessel is passed through a soda-lime trap (B) and a charcoal (6-8 mesh) trap at −78° (C).‡ Purging of the target (26.5 atm → 1 atm) requires 15-20 min. The Freon-11 solution is emptied onto Column 1 (D) (1 cm × 14 cm) using an extension tool (V). Column 1 is packed in hexane with deactivated silica gel,§ with ~10 ml of hexane in the reservoir over the silica gel. Vacuum is applied to the forecut trap (P) and the solvent eluted to the top of the silica gel into the forecut trap. To the column reservoir is then added 10 ml of ether:hexane (1:1) (addition funnel Q) and this is also eluted into the forecut trap. Thirty ml of the ether:hexane (1:1) is then added to the column reservoir, and the eluent is then directed into a 100-ml, round-bottom flask (F) attached to a rotary evaporator (E)‖ by evacuating the evaporator. Elution is continued until the solvent reaches the level of the top of the silica gel. The solvent is then evaporated (45° bath) from the difluoro adduct 3, and 3 ml of 2 *N* HCl followed by 1 ml of H_2O is added to the flask through addition funnel (R). The mixture is heated by an oil bath (K) at 125° for 12 min with the flask rotating. Charcoal (USP, 15 mg/0.5 ml H_2O), followed by 0.5 ml of H_2O is added to the flask, and heating is continued for 3 min. The flask is cooled (ice bath, K) to 25° initially, vacuum is applied, and the solution is evaporated to dryness at ~45°. To the residue is added (R) 2 ml of aqueous acetonitrile (0.3 ml H_2O/100 ml CH_3CN). This is evaporated to dryness and an additional 3 ml of aqueous acetonitrile is added. The solution is transferred from flask F to a dry column (G) (0.75 × 10 cm) of silica gel 60¶ and the flask rinsed with an additional 2 ml of aqueous acetonitrile. A syringe assembly (W) is used to transfer the product from (F) to (G), to add solvents onto the column and to elute the column by application of vacuum or pressure. The mixture is pushed through the column to the level of the silica gel, and 20 ml of aqueous acetonitrile are added. Elution is continued and ~7 ml forecut (I) is collected. The purified ^{18}FDG is eluted in the next 15 ml and is collected in a 100-ml, round-bottomed flask (H). The solvent is removed on the rotary

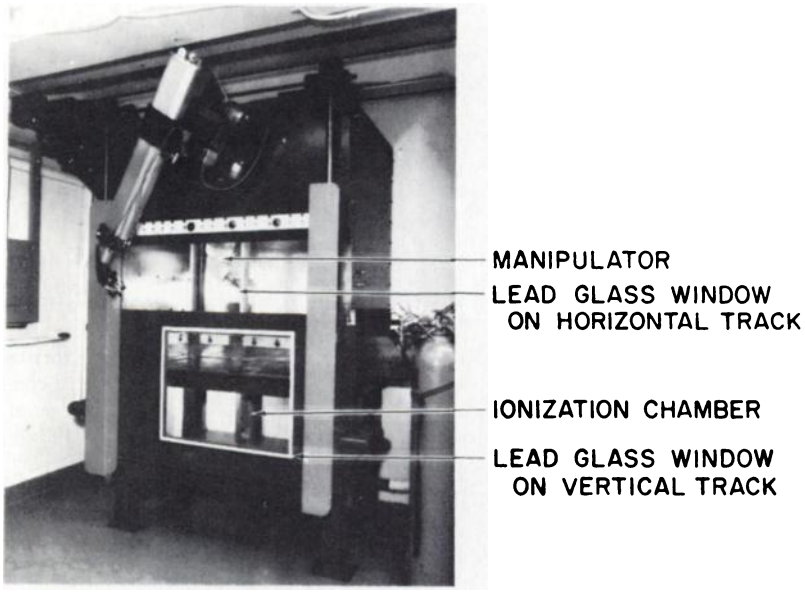

FIG. 2. Shielded box for housing ^{18}FDG synthesis system.

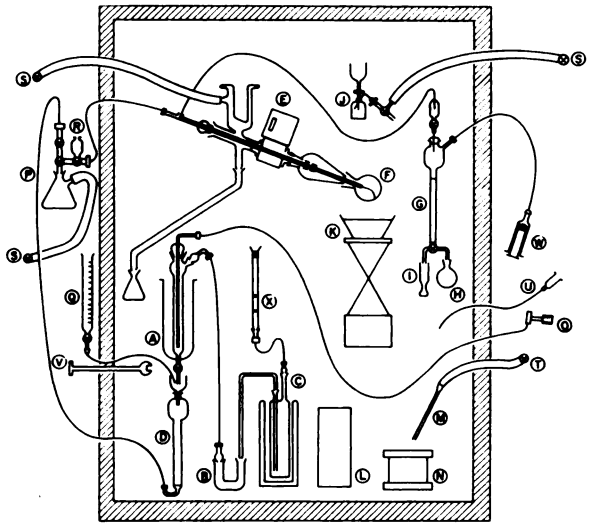

Fig. 3. Diagram of components of ^{18}FDG synthesis system.

evaporator, 1 ml of USP H_2O is added (R), and the solution evaporated to dryness. The flask is removed from the evaporator, put in a 45° water bath (N) and a stream of N_2 (M) is passed into the flask to remove all traces of solvent. Saline (USP) is added (U) and the solution is passed through a Millipore filter into an evacuated multiinjection vial (J).

The procedure typically produces ~1 mg of ^{18}FDG with specific activity of 20-25 mCi/mg at end of synthesis and a radiochemical purity of 96-98% (see Analysis of ^{18}FDG).

Remote manipulations. During the synthesis, the hot cell (*10*) is used in the completely closed configuration. The flow of ^{18}F-F_2 is controlled by the needle valve outside the hood. As described in the synthesis procedure, transfers of solutions are accomplished by application of pressure or vacuum. The master-slave manipulator (10) is used to remove the flask from the rotary evaporator (E) and to attach flask H to the rotary evaporator, in addition to turning the stopcocks on Column 2 (G) for the final chromatography.

F-18 recovery distribution and product analysis. Syntheses were carried out using 1-325 mCi of F-18 (recovered yield at end of bombardment), the low-level runs being used in analytical and development work. The components of the system that were assayed for F-18 activity after a run were the Freon-11 solution, the reaction vessel (A), a soda-lime trap (B) following the reaction vessel, and a charcoal trap at −78° (C) on the outlet of the soda-lime trap. The sum of the activities in these components, corrected to EOB, gives the recovery of F-18 from the target. Each of the components used in the synthesis was designed to fit into a Capintec CRC-4 ionization chamber (L), which was previously calibrated for fluorine-18. In this way, during a low level run the radioactivity could be monitored continually, with losses determined at all stages. Other components of the synthesis system (columns, forecuts, Millipore filters, etc.) were assayed either during low-level run or after a standard run when the activity had decayed to safe levels.

Analysis of 3 and 4. In order to determine the efficiency of recovery of the desired difluoro adduct 3 from Column 1 (D), gas-liquid chromatographic (GLC) analysis of the crude reaction mixture and column eluate was made.

The concentrations of TAG and 3 were determined by GLC, which had previously been calibrated using the pure substances [column, DC-710 (10%), 6 ft × ⅛ in., flow 13 ml/min at 195°C]. Retention times for TAG were 14.16 min (major) and 17.7 (minor), and for 3, 13.04 min. The mannose isomer (4) had a retention time of 18.94 min under the same conditions. Relative amounts of F-18-labeled 3, 4, and decomposition products in the crude reaction mixture were also determined by thin layer chromatography** using ether:hexane (1:1) eluent (R_f = 0.5 (3) and 0.16 (4)).

Analysis of ^{18}FDG. The chemical quantity of ^{18}FDG produced is conveniently determined by gas chromatographic analysis of the trimethylsilyl derivative as described previously (*4*) after calibration of the instrument with known quantities of silylated FDG. Thin layer chromatography (TLC) is used to determine the radiochemical purity of the ^{18}FDG. TLCs were run on Silica Gel 60 and Silica Gel No 6060 plates. Using $CHCl_3$:CH_3OH:H_2O (30:9:1), FDG gave an R_f of 0.09 with the former and 0.21 with the latter; in CH_3CN:H_2O (95:5) the R_f was 0.42 with the former and 0.25 with the latter. The 2-4% contaminant in the product is well separated on all of these TLC systems and runs ~0.3R_f units ahead of the FDG.

RESULTS AND DISCUSSION

Stoichiometry of TAG and F_2. It has been our experience that F_2/Ne mixtures have variable F_2 concentrations and also may contain gaseous contaminants that consume F_2 when the target gas is irradiated (*12*). Therefore, we titrate at least one loaded and irradiated target for each new gas cylinder to determine how much F_2 is delivered through the system. Since we have found that the extent of both chemical and radiochemical impurities depends on the ratio of TAG to F_2 used in the synthesis (see discussion below), this is an important preliminary step to establish the optimum quantity of carrier F_2 to add to the target. The use of 20% excess of TAG over F_2 minimizes yield losses due to over-fluorination.

Recovery of F-18 from the target. The keeping of records of the total F-18 activity recovered for each run and of its distribution within various components of the synthesis is an important aspect of maintaining reliable production. The recovery of F-18 from the system was taken as the sum of the activities from the Freon-11 solution,†† the reaction-vessel walls, the soda lime, and the cold

TABLE 1. DISTRIBUTION OF FLUORINE-18 RECOVERED FROM THE Ne/F_2 TARGET*

	Distribution of F-18 (%)
Percent recovery† of F-18 from Ne/F_2 target ..... 57.0 ± 2	Freon-11 solution ..... 71.1 ± 1.8
	Reaction-vessel walls ..... 10.3 ± 0.3
	Soda lime ..... 12.7 ± 1.3
	Charcoal trap ..... 5.0 ± 0.6

* The yield data represent an average ± s.d.m. of 17 runs in which the total dose on the target for each run was 24 μA-hr (12 μA × 2 hr).

† % Recovery = (Freon-11 + reaction vessel walls + soda lime + charcoal trap)/(calculated yield of F-18‡) × 100

‡ Based on ^{20}Ne(d,α)^{18}F excitation function (*11*).

TABLE 2. YIELD DATA FOR SYNTHESIS OF ^{18}FDG (STEPS 2-6)

Step no.	Description	Yield (%)*
2	^{18}F-F_2 + TAG	71.1 ± 1.8
3	Isolation of Adduct 3	29.7 ± 0.8
4	Hydrolysis of 3 + $H^{18}F$ removal	14.9 ± 0.4
5	Purification of ^{18}FDG	10.9 ± 1.1
6	Millipore filtration of ^{18}FDG	10.5 ± 1.2

* The yield for each step is reported as a percentage of the total F-18 activity recovered from the target, and each number is the average ± s.d.m. for 17 runs. Total dose on the target for each of these runs was 24 μA-hr (12 μA × 2 hr).

charcoal trap, and is shown in Table 1 for a number of production runs. The activity remaining on the vessel walls and trapped in the soda lime is probably $H^{18}F$, although this has not been unequivocally demonstrated. The activity trapped in the cold charcoal is usually $C^{18}F_4$ or a mixture of $C^{18}F_4$ and $N^{18}F_3$ (*12*). The distribution of F-18 within various components of the system is reasonably reproducible. For doses ranging from 24-30 μA-hr, the recovery varies within a range of 45-65% for different targets. A number of factors may be responsible for recoveries of less than 100%, including the loss of ~8% of the ^{18}F-F_2, which remains in the line after purging, losses due to exchange with target walls and delivery lines, and low production due to deuteron-beam penetration in the neon target (*13*). A high level of activity in the charcoal trap usually represents a serious problem, and some of the factors responsible for this have been determined (*12*).

Product analysis. GLC analyses of the crude reaction mixture after F_2 addition to TAG showed that from 60-75% of the initial TAG cannot be accounted for in terms of unreacted TAG, **3**, and **4**. The presence of a waxy solid in the reaction mixture after the fluorination is often detected, however, and may arise from polymerization of the TAG. Thin layer chromatography of the activity in the Freon-11 solution showed that ~33% of the radioactivity is not **3** or **4**, and remains unidentified. It is, however, removed by column chromatography (Column 1). This also removes all of the mannose adduct **4**, but only part of the unreacted TAG. Adjustment of the stoichiometry of F_2 and TAG so that all of the TAG is consumed results in an increase of radiochemical impurities at the expense of the desired adduct **3**. This was tested by deliberately adding excess ^{18}F-F_2 to part of a reaction mixture and observing an increase of radiochemical impurities in the hydrolysis product, ranging from 5% (typical for a 20% excess of TAG) to 22%. The excess TAG is decomposed on hydrolysis, turning brown and forming an insoluble tar. The hydrolysate is decolorized with activated carbon. The efficiency of the column in recovery of **3** from the crude reaction mixture is 62-72%, and under our conditions attempts to recover more **3** cause contamination by **4**. Thin layer chromatography shows that the ratio of **3** to **4** in the crude reaction mixture before the first column chromatography is 3:1.

A final purification of the ^{18}FDG after hydrolysis is required to remove the radiochemical impurity(s) presumably caused by over fluorination. This is accomplished conveniently and rapidly by a modification of the technique of "flash chromatography" described by Still and coworkers (*14*) and gives a product with consistently high purity (96-98%). The procedure (~7 min) obviates the need for space-consuming, high-pressure liquid-chromatographic equipment. The radioactivity losses during the synthesis itself are also presented in Table 2. Typical radiochemical yields of ^{18}FDG are 10-12% at EOB.

Monitoring of the radiation level at the external wall of the shielded synthesis box during the synthesis of ^{18}FDG using 300 mCi of ^{18}F-F_2 shows a background level of 0.0 mR/hr. The synthesis system as it is designed does not require entry into the shielded hood until the multiinjection vial is removed. This virtually eliminates exposure of chemists involved in the routine synthesis.

In summary, the essential requirement for a production setup for ^{18}FDG, such as the one we describe here, is that the design must consist of *simple* experimental setups that can be operated with a minimum of handling and transfer of radioactive materials. It is also desirable that only the steps involving high levels of radioactivity be housed within the shielded area and that all other components of the system, such as vacuum controls and solvent addition, be housed outside the shielded area. The ^{18}FDG synthesis previously described (*2*) has been modified to allow its incorporation into a shielded synthesis area where all steps are remotely executed. The present production of 25 mCi of ^{18}FDG at the end of a 60-min synthesis is ample for two consecutive patient studies at BNL or for shipment to collaborating institutions within a 3-hr (door to door) shipping time. The present synthesis system could be used with higher levels of F-18 without modification should the need arise.

FOOTNOTES

* Matheson Research Purity.

† It is important that the solution of TAG and Freon-11 not be allowed to condense water vapor after it is cooled with dry ice. To prevent such condensation, a slow flow of the target gas through the TAG and Freon-11 is begun before the dry ice is added to the cooling jacket of the vessel.

‡ The charcoal traps are reusable without activation.

§ Baker 3404, deactivated by adding 1.15 ml of H_2O and 65 g of silica gel and warming to ~50° under vacuum on a rotary evaporator for ~1 hr.

‖ Brinkman Model M.

¶ Merck No. 9385.

** Eastman Chromagram sheets (No. 6060).

†† While on low-level runs, the Freon-11 solution was counted directly, high-level production experiments precluded its direct assay. The value of the activity in the Freon-11 was obtained by summing other components of the system, which were assayed several hours after synthesis: Freon-11 = Column 1 + 2 (Column 2 + forecut + F + H + product + Millipore filter). The factor of 2 is used because half of the F-18 is lost as $H^{18}F$ due to hydrolysis of the difluoro adduct (**3**) and evaporation.

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