2-Deoxy-2-[18F]Fluoro-L-Fucose, A Potential Agent for Regional Fucose Utilization Studies Associated with Glycoprotein Synthesis

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Introduction

Recent investigations in various fields have implicated the complex carbohydrate chains in glycoproteins in many important and interesting biological roles. Glycoproteins are widely distributed in animal tissues and exist in many plasma and membranes. The carbohydrate chains in the mammalia are composed of several sugars, glucose, mannose, glucosamin, mannosamin, N-acetyl glucosamin, sialic acid and fucose. In a number of such sugars, L-fucose plays a critical role where it is present in terminal positions of carbohydrate side chains. Administrated L-fucose is an excellent precussor of glycoprotein because L-fucose is not converted to other monosaccharides unlike such monosaccharides as glucose, galactose, mannose and glucosamine and is mainly incorporated to glycoprotein(Table 1).

The fluoroanalogue of L-fucose appears to be able to enter the cell to compete with the parent sugar in glycoprotein biosynthesis. The substitution of the hydroxyl group on C-2 of L-fucose with a fluorine atom will result in a molecule which isolates the fucose metabolism and thus will make it possible to study the same enzymic steps of fucose metabolism such as the fucose transport or the fucose kinase reaction. We felt that it would be useful to investigate a L-fucose analogue which makes differential measurements of changes in regional fucose metabolic activity associated with glycoprotein biosynthesis.

The present paper describes the synthesis of F-18 labeled 2-deoxy-2-fluoro-fucose for the radioactive species to suit the purpose as mentioned above. 2-deoxy-2-[^{18}F]fluoro-fucose was synthesized by reacting labeled acetyl hypofluorite with 3,4-di-O-acetyl fucal. Simple synthesis of 2-deoxy-2-[^{18}F]fluoro-fucose which used [^{18}F]-acetyl hypofluorite as a fluorinating agent can be provides a better radiochemical purity.

The another purpose of this paper is to evaluate the usefulness of the fluoro analogue for cancer diagnostic agent from a point of view of glycoprotein biosynthesis using positron emission computed tomography. The distribution of 2-deoxy-2-[^{18}F]fluoro-fucose in both tumor bearing and normal rats were investigated and metabolic pathway of the fluoro analogue was examined.

Materials and Methods

Synthesis of 2-deoxy-2-fluoro-fucose

α-L-(-)-fucose was obtained commercially from Aldrich Chemical Co.. 3,4-di-O-acetyl-L-fucal which is the precursor for 2-deoxy-2-[^{18}F]fluoro-fucose can be readily synthesized from L-fucose. The preparation of 3,4-di-O-acetyl-L-fucal was carried out of bromination of acetyl fucose, followed by reductive elimination with Zinc powder. This compound was identified by H-NMR measurements. The fluorination reaction was carried out on 3,4-di-O-acetyl-L-fucal by unlabeled reacting acetyl hypofluorite. Fluorine-argon gas (2/98 v/v) was bubbled through a glass reaction vessel containing a solution of aqueous ammonium acetate (7.1 mmol) in 50 ml of glacial acetic acid for 20 min to give a solution of acetyl hypofluorite. To the mixture of acetyl hypofluorite in glacial acetic acid thereby formed, a solution of 3,4-di-O-acetyl-fucal (1.6 mmol) 1 mol of acetic acid was added immediately and the reaction mixture was stirred by bubbling with Nitrogen gas for 10 min. After acetic acid was evaporated under reduced pressure in a water bath, the residue was dissolved in 10 ml of dichrolomethane and dried with anhydrous K_2CO_3. After passing through neutral almina (SEP PACK), the material was evaporated to dryness to yield 61.1% of 3,4-di-O-acetyl-2-deoxy-2-fluoro-fucose(Fig. 1). TLC (in ether:hexane 2:3 (v/v) on plates of Kieselgel 245) gave a single component Rf 0.26. Identification of the fluoro adduct was performed by 1H NMR measurement and mass spectrometry, 1H NMR d($CDCl_3$), 1.08(3H,J=7Hz,-CH3), 2.05(9H,d, J=7Hz,-OCOCH3), 4.21(1H,quartet,J=5Hz,CH-H), 5.08 - 5.52(3H,m,CH-H), 4.22(1H,d,J=Hz,CH-H); Mass(m/e); 292(M+). 273(M-F), 233(base peak). The solvent-free residue containing 50 mg of the fluoro adduct was suspended in 5 ml of 2.5 N-HCl and refluxed for 15 min (oil bath temperature:135°C). Activated charcoal (20 mg) was added, the acid was evaporated, and charcoal was filtrated. Thin-layer chromatography (CH_3CN:H_2O, 95:5) showed the product to have a purity of 95%, the impurities probably being partially hydrolized.

Synthesis of 2-deoxy-2-[^{18}F]fluoro-fucose

Fluorine(^{18}F) gas was produced from the deutron bombardment of neon-fluorine gas mixture (99.95/0.05, v/v) by the 20Ne(d,α)18F nuclear reaction in a nichel target chamber at a pressure of 25 atom for 1 hour at a current of 10 μA using the Tohoku University Cyclotron. (^{18}F)fluorine gas was purged from the target through a grass reaction vessel containing a solution of ammonium acetate (0.07 mmol) in glacial acetic acid (15 ml) over a period of 25 min to give a solution of $CH_3COO^{18}F$ 40 mg (0.18 mmol) of 3,4-di-O-acetyl-fucal was added to this acetyl hypofluorite solution. Acetic acid was evaporated under reduced pressure in a 50 °C bath. 3 ml of aqueous acetonitrile (0.3%H_2O) was added to the labeled fluoro adduct and the mixture was then transferred to a column (0.75 × 10 cm) of silica gel. The column was eluted with the same solvent, a forecut taken and discarded, and the product eluted with 9 ml of

solvent. The solvent was evaporated. Saline was added and the solution passed through a Millipore filter (0.22 μm). The radioactive species had the same Rf and HPLC pattern on the two systems as that of the unlabeled sample which are synthesized by same method(Fig. 2).

Biological studies

Male Wistar rats bearing transplanted glioma cells (EA 285 s/c) and hepatoma cells(AH 109A) were used for tissue distribution studies. 2-deoxy-2-[^{18}F]fluoro-fucose were injected into rats through the tail vein and killed by neck dislocation at different time. The tumor and organs were removed, weighed and counted in an automated NaI well counter. Data were expressed as percentage injected dose per gram of tumor or tissue. Biochemical properties of 2-deoxy-2-fluoro-fucose were examined by means of radio-HPLC analysis. Male Wistar rats bearing transplanted glioma cells(EA285 s/c) subcutaneously and normal rats were used for these studies. After in vivo incubation of 2-deoxy-2-[^{18}F]fluoro-fucose for 10, 60, 90 and 120 min by intravenous administration, 1 - 2 g of the brain, liver, kidney or tumor was homogenized with a 2 volume of 0.2 N perchloric acid in a Teflon-glass homogenizer. The homogenate was centrifuged at 1500 rpm for 10 min. The precipitate was washed three times with 0.2 N perchloric acid and then unbound radioactivities and perchloric acid precipitable radioactivities were measured. $HClO_4$-soluble fractions(unbound radioactivities) neutralized with $NaHCO_3$ sol. were analized for the formation of sugar phosphates by radio-HPLC, TLC and paper electrophoresis. To make clear the metabolic pathway of the fluoro analogue of the fucose, 2-deoxy-2-[^{18}F]fluoro-fucose-1-phosphate and GDP-2-deoxy-2-[^{18}F]-fluoro fucose were synthesized chemically for the assay of the formation of the sugar phosphates in vivo of 2-deoxy-2-[^{18}F]fluoro-fucose. These chemical compounds, to some extent, make it possible to identify the unbound radioactivities. Autoradiographic studies the rats bearing transplanted intracranially glioma cells(EA 285 s/c) and the mice bearing transplanted subcutaneously lymphoma cells were performed. 2 - 10 mCi of 2-deoxy-2-[^{18}F]fluoro-fucose were injected into the rats and mice through the tail vein and they were decapitated 60, 120 min after injection, and frozen brains and subcutaneous tumor(mice lymphoma EL 4) were cut 20 μm thickness in a cryostat. Sections used for processing autoradiography were subsequently stained by hematoxylin.

Results and Discussion

A method for the addition of fluorine to the double bond in acetylated glycals using acetyl hypofluorite has been shown to provide a convenient route for the synthesis of acetylated 2-deoxy-2-fluoro sugars. The reagent attacks the double bond predominately from the least hindered side to give a cis

adduct. We synthesized the fluoro sugar in two methods using acetyl hypofluorite, CH_3COOF in AcOH and CH_3COOF(gas) in Freon-11, and each yields were 11 % and 24 % respectively and the purities were same (approximate 87 %).

As mentioned above, L-fucose has a remarkable properties and simple metabolic pathway, and then a positron labeled fucose analogue can be to trace fucose transport or appropriate enzymatic steps in the alternative pathway.

Figure 3 shows the time courses in five representative tissues in normal rats and the radioactivity in blood showed rapid clearance of 2-deoxy-2-[^{18}F]fluoro-fucose. In the kidney, the highest uptake and slow clearance were observed. The liver uptake pattern was similar to the kidney. In the brain, extremely low radioactivities were shown because the fluoro analogue dose not cross the blood-brain-barrier readily. Generally, in many organs, uptake levels have a tendency to reach equilibrium after 60 min.

The isolated tumor tissues of rats injected with 2-deoxy-2-[^{18}F]fluoro-fucose were homogenized and the proteins precipitated with 0.2 N perchloric acid and the acid-soluble fraction was analized on radio-HPLC. The uptake of 2-deoxy-2-[^{18}F]fluoro-fucose was 0.38 % dose/g in rat glioma cells(EA285 s/c) sacrificed at 60 min after the administration and tumor-brain ratio was 6.13(Fig. 4).

The biochemical study of the fucose analogue in tumor tissue(EA285 s/c), the labeled rat glioma cells were extracted with perchloric acid and the most part of the accumulation of total radioactivity in the tumor tissue was found in the acid soluble fraction(>97 %). The analysis of the acid soluble fraction on radio-HPLC revealed that two radioactive peaks were observed. The facts gave suggestions that incorporation of the L-fucose analogue into the macromolecular fraction of EA285 s/c cells does not occurred. In the two radioactive peaks of the acid-soluble fraction on radio-HPLC analysis, peaks 1 consisted primarily of 2-deoxy-2-[^{18}F]fluoro-fucose, peak 2 consisted the metabolite of the fluoro analogue, which exhibited lability to acid hydrolysis, 2N HCl in a water bath at 80 °C for 15 min(Fig. 5).

Examinations by TLC of labeled EA285 s/c rat glioma cells extracted with the same manner confirmed the presence of the fluoro analogue (^{18}FDF) and the other radioactive spot as the sole detectable metabolite of it. In normal rat liver cells, the radioactive species had the same HPLC pattern and Rf values on the two systems as that of the tumor cell studies. Further in normal rat liver study, paper electrophoresis was performed, the result was also same as above(Fig. 6). These results suggest that 2-deoxy-2-[^{18}F]fluoro-fucose can be trapped in the cells which has rapid turnover of the fucosyl glycoprotein synthesis such as tumor cells or liver cells as a result of anabolizing the fluoro analogue (^{18}FDF) incompletely.

For the biosynthesis of GDP-L-fucose, which is the precursor of the fucosyl glycoconjugates, two distinct routes are known(Fig. 7). The biosynthesis de novo of L-fucose, as its nucleotide derivative, occurs in mammals primarily via GDP-D-mannose. On the other hand, only in the presence of exogenous L-fucose, the alternative pathway is activated. The quantitative significance of the participation of L-fucose kinase and GDP-L-fucose pyrophosphorylase in the biosynthesis of fucose containing glycoproteins is controlled by the availability of exogenously supplied L-fucose in the diet or by turn over of fucose containing substances. Thus it is the main problem which step of the alternative pathway do the fluoro analogue metabolized.

Substitution of the hydroxyl group on C-6 position with fluorine atom should not greatly affect the substrate activity for appropriate enzymes such as L-fucose kinase but substitution of the 2-position, because of its proximity to the anomeric center, could lead to development of inhibitors of L-fucose anabolizing enzymes.

The autoradiographs obtained from sections of the brain of the rat bearing transplanted glioma showed preferential high uptake legion correspond to the location of the tumor 60 min and 120 min after i.v. injection of the 2-deoxy-2-[^{18}F]fluoro-fucose(Fig. 8). The ^{18}F-labeled 2-deoxy-2-fluoro-fucose will be a potencial brain tumor imaging radiopharmacentical connected with the behavior of cell membrane.

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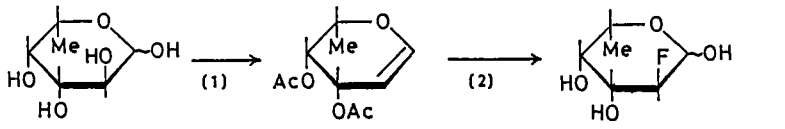
Table 1. BIOLOGICAL PROPERTIES OF L-FUCOSE

(1) L-Fucose is not converted to other monosaccharides
(2) Mainly incorporated to glycoprotein
(3) Plays a critical role where it is present in terminal positions of carbohydrate side-chains



L-Fucose | 3,4-di-O-acetyl-fucal | 2-deoxy-2-fluoro-fucose

(1) $(CH_3CO)_2O$/Pyridine , 25% HBr-AcOH sol. , Zn/AcOH

(2) AcOF/AcOH or AcOF/$CFCl_3$, 3N HCl

Solution of NH_4OAc (7 mmol) in acetic acid

↓ F_2/Ar (v/v 2%) gas was bubbled (room temperature)

↓ Di-O-acetyl-fucal (1.6 mmol) was added

reaction mixture

↓ solvent was evaporated in vacuo

residue

↓ CH_2Cl_2 was added

↓ K_2CO_3, SEP PACK

effluent

↓ solvent was evaporated in vacuo

↓ acid hydrolysis(3N HCl)

2-Deoxy-2-fluoro-fucose

Fig. 1. SYNTHESIS OF 2-DEOXY-2-FLUORO-FUCOSE

(1) $(CH_3CO_2)_2O$/Pyridine, 25 % HBr-AcOH sol., Zn/AcOH
(2) AcOF/AcOH or AcOF/$CFCl_3$, 3N HCl

A)

solution of NH_4OAc in acetic acid

↓ ^{18}F-F_2 gas was bubbled (room temperature)

↓ 3,4-di-O-acetyl fucal in acetic acid was added

reaction mixture

↓ solvent was evaporated in vacuo

residue

↓ 3N HCl was added for hydrolysis

aqueous solution

↓ active charcoal

↓ solvent was evaporated in vacuo

↓ $CH_3CN(0.3\%H_2O)$ was added

↓ Silica gel column

effluent

↓ saline was added

2-deoxy-2-^{18}F-fucose

B)

3,4-di-O-acetyl fucal in $CFCl_3$

↓ ^{18}F-FOAc gas was bubbled (room temperature)

reaction mixture

↓ solvent was evaporated in vacuo

residue

↓ 3N HCl was added for hydrolysis

aqueous solution

↓ AG11A8, active charcoal, SEP PACK

effluent

↓

2-deoxy-2-^{18}F-fucose

Fig. 2. SYNTHETIC METHOD OF 2-DEOXY-2-^{18}F-FLUORO FUCOSE

(1) ^{18}F-Fluorine gas production
$^{20}Ne(d,\alpha)$ ^{18}F reaction on ^{20}Ne containing 0.1 % F_2

(2) ^{18}F-acetylhypofluorite
A) a solution of NH_4OAc in acetic acid
B) a colum of KOAc/AcOH

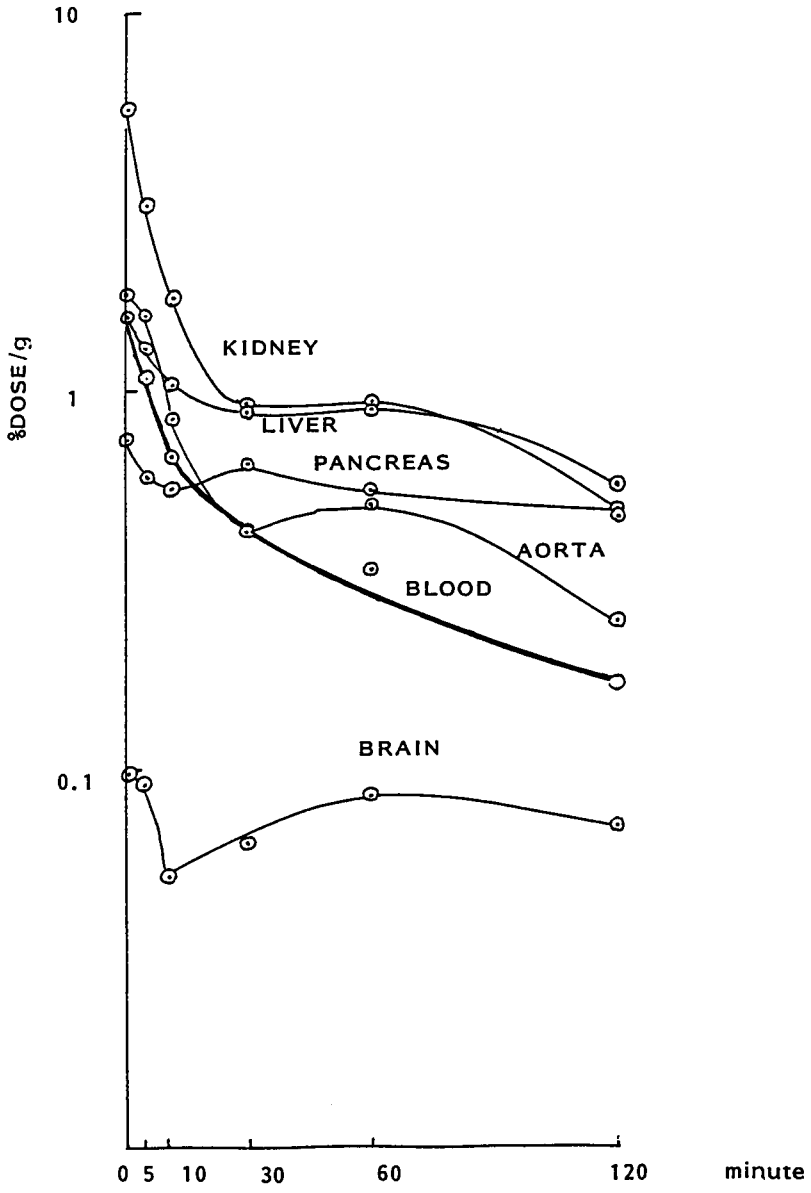


Fig. 3. NORMAL RAT BIODISTRIBUTION OF 2-DEOXY-2-^{18}F-FUCOSE

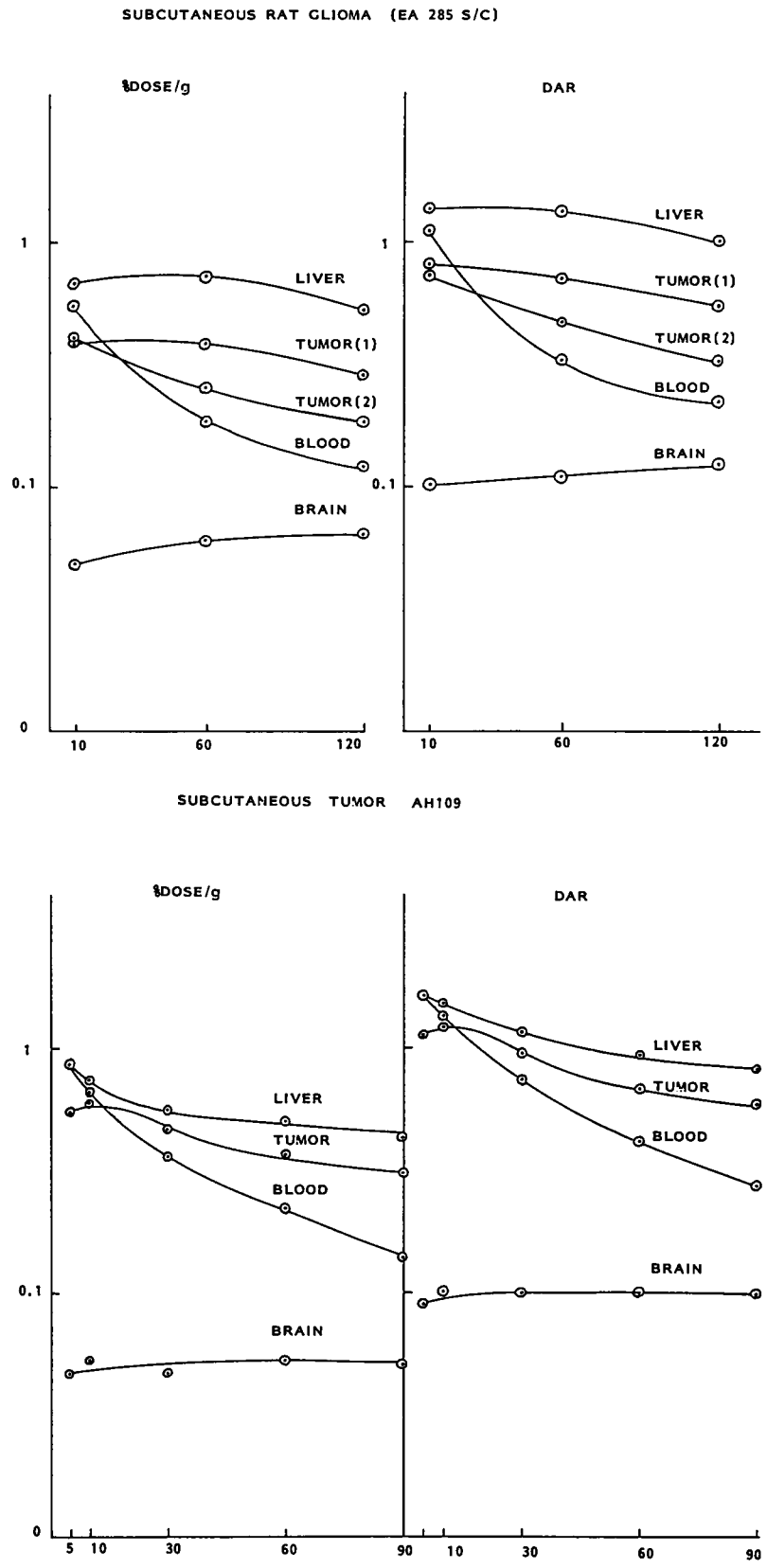
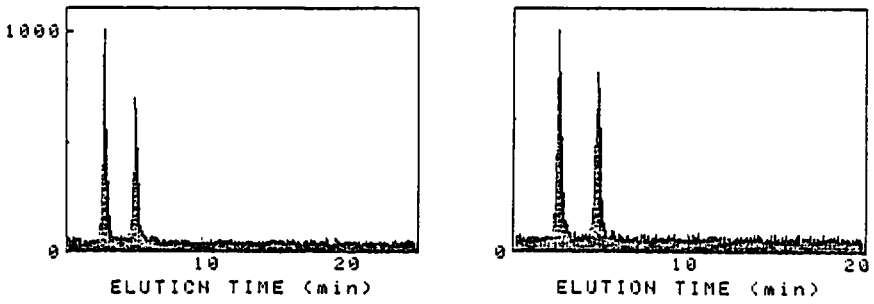


Fig. 4. TUMOR TISSUE DISTRIBUTIONS OF 2-DEOXY-2-(18F)FLUORO-FUCOSE



60min after i.v injection

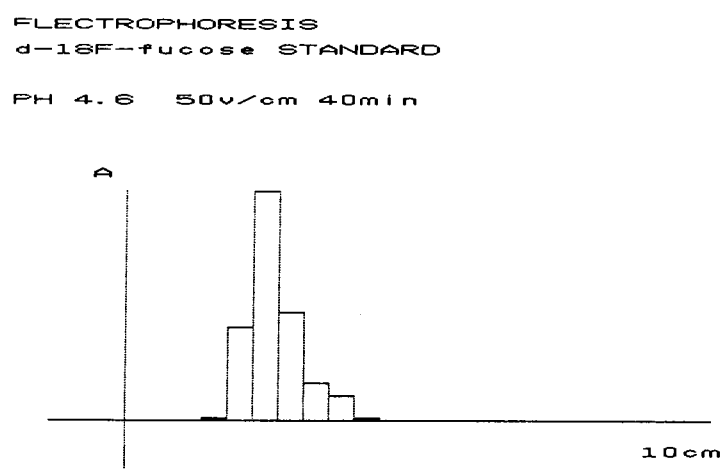
120min after i.v injection

RAT LIVER UNBOUND RADIOACTIVITY

HPLC RADIAL PACK SAX ,ELUENT 0.1M AcOH/AcONa buffer o.15 NaCl

FLOW RATE 2.0 ml/min

Fig. 5. RAT LIVER UNBOUND RADIOACTIVITY HPLC ANALYSIS



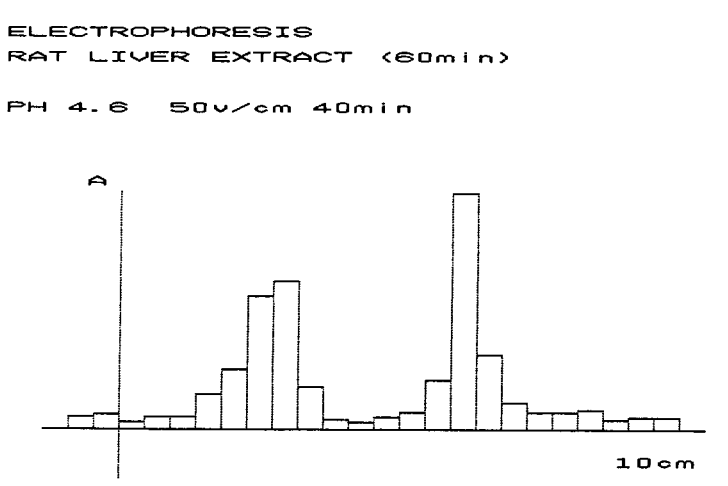
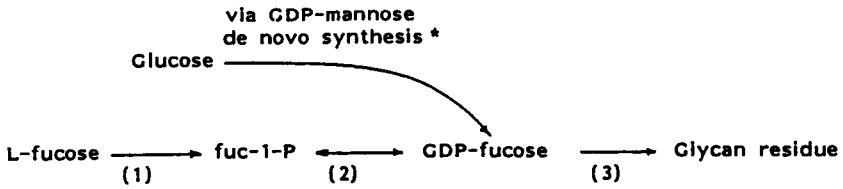


Fig. 6. ELECTROPHORESIS FOR THE METABOLITE OF 2-DEOXY-2-(18F)FLUORO FUCOSE IN VIVO NORMAL RAT LIVER



(1) Fucokinase

(2) GDP-fucose pyrophospholyrase

(3) Fucosyl transferase

* under the control of exsogeneous availability

Fig. 7. ENZYMES OF FUCOSYL GLYCOPROTEIN SYNTHESIS

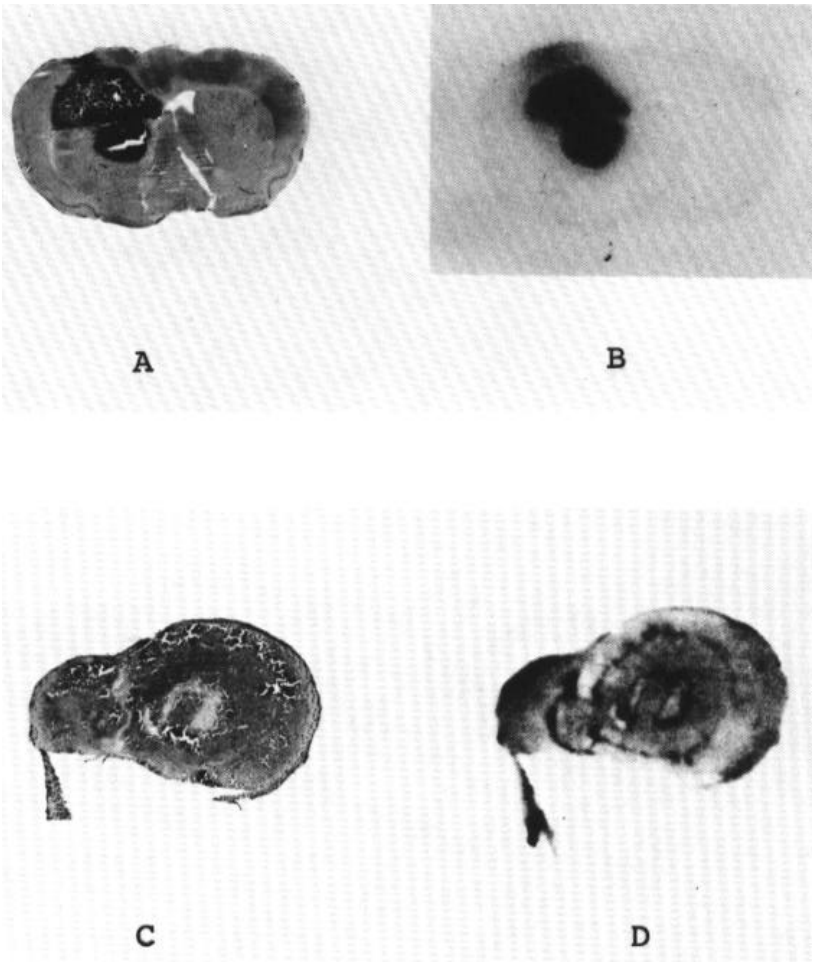


Fig. 8. Representatives of autoradiograms of tumors

A) Hematoxylin-Eosin stained section of rat glioma (EA285 s/c)
B) Autoradiograph of section of the rat glioma
C) Hematoxylin-Eosin stained section of mouse lymphoma(EL4)
D) Autoradiograph of section of the mouse lymphoma