*Carbohydrate Research*, 40 (1975) 119–127


# INHIBITION OF THE METABOLISM OF AMINO SUGARS WITH 2-DEOXY-2-(2-FLUOROACETAMIDO)-α-D-GLUCOPYRANOSE*

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(Received August 1st, 1974; accepted in revised form, September 19th, 1974)

## ABSTRACT

The metabolism of amino sugars in extracts of mouse cells was studied by the use of 2-acetamido-2-deoxy-α-D-glucopyranose, 2-acetamido-2-deoxy-α-D-mannopyranose, and *N*-acetylneuraminic acid as tracers with or without added 0.5mM 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose. The accumulation of uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) and cytidine 5′-(*N*-acetylneuraminyl monophosphate) was inhibited by the fluoro sugar.

## INTRODUCTION

The surfaces of mammalian cells have long been known to contain complex carbohydrate materials that often lack clearly recognizable roles. More recently, it was observed that cultured cells exposed to a variety of viral or environmental agents "transform" and acquire altered *in vitro* and *in vivo* growth properties[1]. Glycoprotein and glycolipid changes in cell-surface membranes appear to accompany certain of such transformations[2,3]. Whether the structural modifications of these macromolecules are the causes of the new cellular behavior, or are merely secondary consequences of the "transformation", has not yet been established.

Direct modification of cell-surface carbohydrates would be a first step in an attempt to establish any correlation between surface carbohydrates and their biological function in mediating cell behavior. Furthermore, it may be of use to resolve the question of causal relationship between changes in the biological properties and the carbohydrate components in the cell surface. External modification of carbohydrate material with glycosidases is hampered by limited access to substrate, owing to the apparently complex structure of the cell periphery[4]; moreover, glycosidases are often contaminated with extraneous enzymic activities. Furthermore, the enzymic modifications may be only transitory, as cells repair their surfaces.

Previous studies[5] have shown that, up to a final concentration of 0.5mM, 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose is nontoxic to mouse cells in

*Dedicated to Professor Michael Heidelberger in honor of his 87th birthday.

culture and may affect synthesis of cell-surface carbohydrate components. By use of the methods of Hultsch *et al.*[6], we have conducted studies with a cell-free system to clarify the detailed effects on amino sugar metabolism. We now report studies on altering amino sugar metabolism with a 2-acetamido-2-deoxy-α-D-glucopyranose analog: 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose. These studies were undertaken with the intent of using a nontoxic, metabolic modifier to establish a new steady-state pool of precursors for glycolipid and glycoprotein biosynthesis. Replacement of hydrogen by fluorine results in little steric change[7], but the vastly different electronegativity would be expected to have a definite effect on the interaction of a fluorine-containing molecule with an enzyme.

## EXPERIMENTAL AND RESULTS

*Materials.* — A continuous, fibroblastic cell-line, T AL/N, was established from AL/N mouse embryos[8], and the cells were grown in Dulbecco medium containing twice the normal concentration of vitamins and amino acids, with added 10% fetal-calf serum, on plastic, Falcon petri-dishes, in a humidified atmosphere (5% of carbon dioxide) in an incubator at 37° as described[9].

Adenosine 5′-triphosphate, uridine 5′-triphosphate, cytidine 5′-triphosphate, 2-amino-2-deoxy-α-D-glucopyranose, and 2-acetamido-2-deoxy-α-D-mannopyranose were all purchased from Calbiochem (La Jolla, Calif. 92626), and nicotinamide from Eastman Organic Chemicals (Rochester, N.Y. 14650). Labelled compounds were obtained from the following sources: 2-amino-2-deoxy-α-D-[$^{14}$C]glucopyranose (270 Ci·mol$^{-1}$) from Amersham/Searle Corp. (Arlington Heights, Ill. 60005), 2-[$^{14}$C]acetamido-2-deoxy-α-D-glucopyranose (41.7 Ci·mol$^{-1}$) from New England Nuclear (Boston, Mass. 02218), 2-acetamido-2deoxy-α-D-[$^{3}$H]mannopyranose (460 Ci·mol$^{-1}$) from ICN Life Science Group (Cleveland, Ohio 44128), *N*-acetyl-[$^{14}$C-4]neuraminic acid (50 Ci·mol$^{-1}$) from New England Nuclear, uridine 5′-(2-acetamido-2-deoxy-α-D-[$^{14}$C-1]glucopyranosyl pyrophosphate) (45 Ci·mol$^{-1}$) from New England Nuclear, and cytidine 5′-(*N*-acetyl-[$^{14}$C-4,5,6,7,8,9]neuraminosyl monophosphate) (180 Ci·mol$^{-1}$) from New England Nuclear. The 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose was synthesized by Mr. C. G. Butchard as described[10], and was kindly given to us by Dr. Paul W. Kent; analysis, as the per(trimethylsilyl) ether, on a column of SE-30 in argon (40 ml·min$^{-1}$) at a constant temperature of 200° showed only one component; hence, the fluoro derivative contained no detectable 2-acetamido-2-deoxy-α-D-glucopyranose (personal communication from Dr. Kent). 2,5-Diphenyloxazole (PPO) and 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP) were purchased from Packard Instrument Company (Downers Grove, Ill. 60515). Solvents and all other chemical compounds were of reagent grade.

*Preparation of cell-free extract.* — To prepare a cell-free extract from T AL/N cells, monolayer cultures in the logarithmic phase of growth were rinsed twice with phosphate-buffered saline, and scraped from the petri plate with a rubber policeman in a small volume of 0.2M Tris hydrochloride (pH 7.4) buffer containing 2mM

magnesium chloride. Ten strokes of a Dounce homogenizer having a tight-fitting pestle were followed by centrifugation at 10,000*g* for 30 min. The clear, supernatant liquor, which gave more reproducible results than the total homogenate, was used as the cell-free extract.

*Enzymic activity of cell-free extract.* — Interference with early steps of the sequence of the metabolism of amino sugars in mammalian cells might be expected to affect the pool sizes of the "amino sugar nucleotides" that are the precursors of glycolipids and glycoproteins: uridine 5′-(2-acetamido-2-deoxy-α-D-galactopyranosyl pyrophosphate) and cytidine 5′-(*N*-acetylneuraminosyl monophosphate). To examine various portions of the metabolic sequence, radioactively labelled 2-acetamido-2-deoxy-α-D-glucopyranose, 2-acetamido-2-deoxy-α-D-mannopyranose, and *N*-acetylneuraminic acid were used in separate experiments as tracers. The effect of added 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose was then studied.

Volumes of 0.5 or 1.0 ml of the cell-free extract, together with added tracer, were incubated in a shaking water-bath at 37°. Radioactive 2-acetamido-2-deoxy-α-D-glucopyranose was added at 1 $\mu$Ci·ml$^{-1}$ to study its metabolism. Radioactive 2-acetamido-2-deoxy-α-D-mannopyranose was first diluted with unlabelled compound to a final specific activity of 230 Ci·mol$^{-1}$, and used at 4.6 $\mu$Ci·ml$^{-1}$ in order to study its utilization. *N*-Acetylneuraminic acid was used at 1 $\mu$Ci·ml$^{-1}$. The 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose was included at a final concentration of 0.5mM.

*Analytical methods.* — Aliquots (200 $\mu$l each) of the incubation mixture were withdrawn after the elapse of various times, added to an equal volume of ice-cold, absolute ethanol, and centrifuged to remove protein. The precipitated protein contained negligible radioactivity. (Ethylenedinitrilo)tetraacetic acid (sodium salt, EDTA) was added to the deproteinized, supernatant liquor to a final concentration of 1mM.

2-Acetamido-2-deoxy-α-D-glucopyranose and 2-acetamido-2-deoxy-α-D-mannopyranose were separated by high-voltage, paper electrophoresis in 1% sodium tetraborate (pH 9.1). A 150-$\mu$l aliquot was applied to Whatman 3MM paper and electrophoresed at 60–70 V·cm$^{-1}$ for 45 min. Under these conditions, 2-acetamido-2-deoxy-α-D-glucopyranose remains at the origin, 2-acetamido-2-deoxy-α-D-mannopyranose migrates 7 to 8 cm towards the cathode, and amino sugar phosphates, nucleotide derivatives, and *N*-acetylneuraminic acid migrate as an ill-resolved spot 15 cm from the origin.

To separate the other metabolites, descending chromatography of a duplicate, 150-$\mu$l aliquot was performed on Whatman No. 1 paper with 7:2:1 1-propanol–water–M sodium acetate (pH 5). By use of the radiolabelled compounds already described (see Materials), the following $R_{GlcNAc}$ values were found: 2-acetamido-2-deoxy-α-D-mannopyranose 1.00, 2-amino-2-deoxy-α-D-glucopyranose 0.71, *N*-acetylneuraminic acid 0.43, uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) 0.11, and cytidine 5′-(*N*-acetylneuraminosyl monophosphate) 0.11. A peak of the radioactivity appearing at an $R_{GlcNAc}$ value of 0.25 was assigned to amino sugar

6-phosphates[6]. These chromatographic and electrophoretic systems are complementary: 2-acetamido-2-deoxy-α-D-mannopyranose and 2-acetamido-2-deoxy-α-D-glucopyranose are separated by electrophoresis, and amino sugar 6-phosphates, *N*-acetylneuraminic acid, and nucleotide esters of amino sugars are resolved by chromatography. There was good agreement in analysis of duplicate samples by the two methods.

Protein was estimated by the method of Lowry *et al.*[11] by the use of crystalline, bovine serum-albumin as the standard.

*Measurement of radioactivity.* — Paper chromatograms and electrophoretograms were scanned in a Vanguard Model 880 strip scanner. Paper strips were cut, and counted in a Tri-Carb Model 4322 scintillation spectrometer in a toluene fluid containing PPO (5 $g \cdot l^{-1}$) and POPOP (0.3 $g \cdot l^{-1}$).

*Supplements to the cell-free extract.* — Unlike the behavior in the rat-liver extract of Hultsch *et al.*[6], in the extract of the cultivated mouse-cells, the synthesis of uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) is unaffected by a final concentration of 0–100mM nicotinamide: the portion of total radioactivity ranged, for uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) from 80.6% to 85.0%, for 2-acetamido-2-deoxy-α-D-glucopyranose 6-phosphate from 8.6% to 13.9%, for *N*-acetylneuraminic acid from 0.07% to 0.10%, and for 2-acetamido-2-deoxy-α-D-glucopyranose from 5.4% to 6.3%. The incubation was for 2 h at 37° in a reaction volume of 1 ml containing mM ATP, mM UTP, and 1 $\mu Ci \cdot ml^{-1}$ 2-acetamido-2-deoxy-α-D-glucopyranose, when the protein content was 16 $mg \cdot ml^{-1}$ (see Table I). Therefore, in all further experiments on mouse cell-extracts, nicotinamide was not included.

Adenosine 5′-triphosphate is required for the metabolism of 2-acetamido-2-deoxy-α-D-glucopyranose. Table I shows that the optimal concentration of adenosine 5′-triphosphate is 1mM for the synthesis of radioactive metabolites comigrating in electrophoresis which, under the condition of the experiment, include 2-acetamido-2-deoxy-α-D-glucopyranose 6-phosphate and uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) (see Analytical methods). Similarly, uridine 5′-triphosphate added at a final concentration of 1mM is optimal for the synthesis of the comigrating, radioactive metabolites (see Table II). Adenosine 5′-triphosphate at a concentration of 1mM stimulates the metabolism of 2-acetamido-2-deoxy-α-D-mannopyranose (experiments and results similar to those reported in Table I; data not shown). The optimal concentration of cytidine 5′-triphosphate for promoting rapid synthesis of cytidine 5′-(*N*-acetylneuraminosyl monophosphate) by the cell-free system was also found to be 1mM (see Table III).

*Kinetics of amino sugar metabolism.* — Fig. 1 shows an 8-h time-course of metabolism of radioactive 2-acetamido-2-deoxy-α-D-glucopyranose. The starting material was depleted within 30 min, as it was rapidly phosphorylated. The subsequent synthesis of uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) is consistent with the known metabolism of amino sugars. Because no adenosine 5′-triphosphate was added in this experiment, 2-acetamido-2-deoxy-α-D-manno-

TABLE I

DETERMINATION[a] OF THE OPTIMAL CONCENTRATION OF ADDED ADENOSINE 5′-TRIPHOSPHATE IN ITS EFFECT ON THE METABOLISM OF 2-ACETAMIDO-2-DEOXY-D-GLUCOSE

| *ATP* (*added μmoles*) | *Time* (*min*) | | | | |
|---|---|---|---|---|---|
| | *0* | *10* | *20* | *30* | *45* |
| 0 | n.d.[b] | 0.96 | 0.87 | 1.3 | 2.3 |
| 0.1 | n.d. | 2.8 | 2.9 | 3.7 | 4.7 |
| 0.3 | n.d. | 1.5 | 4.2 | 9.6 | 10.0 |
| 1.0 | 0 | 3.8 | 7.9 | 7.3 | 13.0 |
| 3.0 | n.d. | 2.0 | 5.6 | 4.4 | 12.5 |

[a]As % of total radioactivity as GlcNAc-6-P and UDP-GlcNAc. In a 1-ml reaction volume containing mM UTP and 1 $\mu Ci \cdot ml^{-1}$ of 2-acetamido-2-deoxy-D-glucose. The protein content was 7.3 $mg \cdot ml^{-1}$. For abbreviations, see legend to Fig. 1. [b]Not determined.

TABLE II

DETERMINATION[a] OF THE OPTIMAL CONCENTRATION OF ADDED URIDINE 5′-TRIPHOSPHATE IN ITS EFFECT ON THE METABOLISM OF 2-ACETAMIDO-2-DEOXY-D-GLUCOSE

| *UTP* (*added μmoles*) | *Time* (*min*) | | | |
|---|---|---|---|---|
| | *0* | *10* | *20* | *30* |
| 0 | n.d.[b] | 34.0 | 49.1 | 54.2 |
| 0.1 | n.d. | 35.0 | 54.3 | 60.3 |
| 0.3 | n.d. | 37.6 | 63.5 | 72.4 |
| 1.0 | n.d. | 46.2 | 75.1 | 83.9 |
| 3.0 | 1.3 | 43.8 | 66.0 | 76.7 |

[a]As % of total radioactivity as GlcNAc-6-P and UDP-GlcNac. In a 1-ml reaction volume containing mM ATP and 1 $\mu Ci \cdot ml^{-1}$ of 2-acetamido-2-deoxy-D-glucose. The protein content was 10.3 $mg \cdot ml^{-1}$. Incubation at 37°. For abbreviations, see legend to Fig. 1. [b]Not determined.

TABLE III

DETERMINATION[a] OF THE OPTIMAL CONCENTRATION OF ADDED CYTIDINE 5′-TRIPHOSPHATE IN ITS EFFECT ON THE METABOLISM OF *N*-ACETYLNEURAMINIC ACID

| *CTP* (*added μmoles*) | *Time* (*min*) | | |
|---|---|---|---|
| | *15* | *30* | *45* |
| 0 | 0.25 | 0.6 | 0.4 |
| 0.1 | 1.2 | 1.4 | 1.4 |
| 0.3 | 1.0 | 4.9 | 3.6 |
| 1.0 | 4.0 | 9.3 | 14.5 |
| 3.0 | 1.9 | 7.0 | 12.3 |

[a]As % of total radioactivity as CMP-NANA. In a 1-ml reaction volume containing 1 $\mu Ci \cdot ml^{-1}$ of NANA. The protein content was 10.3 $mg \cdot ml^{-1}$. For abbreviations, see legend to Fig. 2.

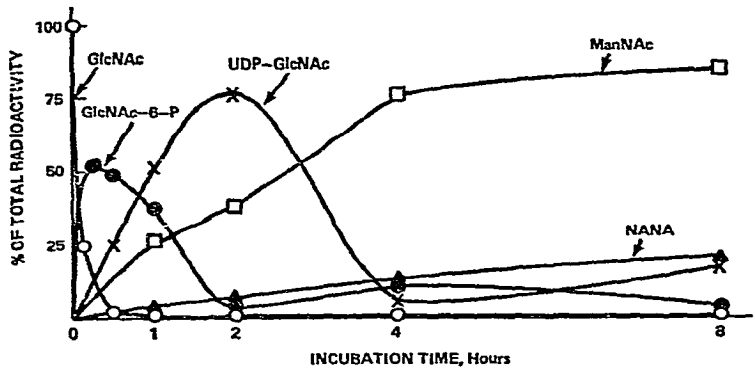


Fig. 1. Appearance of intermediates in the metabolism of amino sugar after adding radioactive 2-acetamido-2-deoxy-α-D-glucopyranose (GlcNAc) to the cell-free extract. Other abbreviations are: GlcNAc-6-P = 2-acetamido-2-deoxy-α-D-glucopyranose 6-phosphate, UDP-GlcNAc = uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate), NANA = *N*-acetylneuraminic acid, ManNAc = 2-acetamido-2-deoxy-α-D-mannopyranose. The incubation mixture contained mM UTP, 1 $\mu$Ci·ml$^{-1}$ of 2-acetamido-2-deoxy-D-glucose, and 8.8 mg·ml$^{-1}$ of protein.

pyranose accumulated. A steady synthesis of *N*-acetylneuraminic acid was observed. These results are in general agreement with the more rapid metabolism for rat liver-cell extracts observed by Hultsch *et al.*[6].

The results of an experiment using 2-acetamido-2-deoxy-α-D-mannopyranose as the tracer are shown in Fig. 2. Further metabolism of the starting material occurred, because of added adenosine 5′-triphosphate in the incubation. The tracer was phosphorylated in 1–2 h, and *N*-acetylneuraminic acid was synthesized. Cytidine 5′-(*N*-acetylneuraminosyl monophosphate) accumulated steadily. *N*-Acetylneuraminic acid as the tracer in similar experiments was converted, in >33% yield, into cytidine

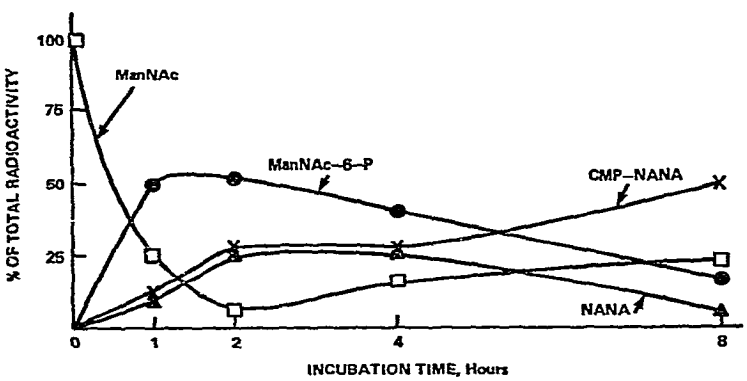


Fig. 2. Appearance of intermediates in the metabolism of amino sugar after addition of radioactive 2-acetamido-2-deoxy-D-mannose (ManNAc). Abbreviations are: ManNAc-6-P = 2-acetamido-2-deoxy-α-D-mannopyranose 6-phosphate, CMP-NANA = cytidine 5′-(*N*-acetylneuraminosyl monophosphate). For other abbreviations, see Fig. 1. The incubation mixture contained mM ATP, mM CTP, 4.6 $\mu$Ci·ml$^{-1}$ of 2-acetamido-2-deoxy-D-mannose, and 8.8 mg·ml$^{-1}$ of protein.

5′-(*N*-acetylneuraminosyl monophosphate) when the concentration of protein was >4 mg·ml$^{-1}$ and mM cytidine 5′-triphosphate was present (data not shown).

*Effect of 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose and 2-amino-2-deoxy-α-D-glucopyranose.* — When 0.5mM 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose was included in incubations with radioactive 2-acetamido-2-deoxy-α-D-glucopyranose as the tracer, as shown in Fig. 3A, there was 50% inhibition of synthesis of uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate). A substantial inhibition

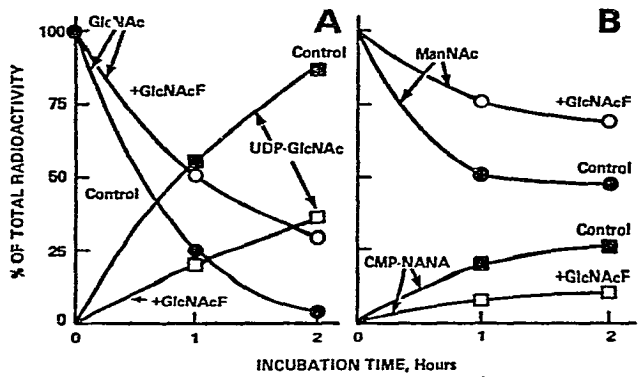


Fig. 3. Appearance of intermediates in the metabolism of amino sugar after addition (as in Fig. 1) of radioactive 2-acetamido-2-deoxy-D-glucose (3A), or, as in Fig. 2, 2-acetamido-2-deoxy-D-mannose (ManNAc) (3B); in the absence (control) or presence of 0.5mM 2-deoxy-2-(2-fluoroacetamido)-α-D-glucose (GlcNAcF). The incubation mixture contained mM UTP, 1 μCi·ml$^{-1}$ of 2-acetamido-2-deoxy-D-glucose, and 16 mg·ml$^{-1}$ of protein (Fig. 3A); and 1 mM ATP, 4.4 μCi·ml$^{-1}$ of 2-acetamido-2-deoxy-D-mannose, and 16 mg·ml$^{-1}$ of protein (Fig. 3B). For abbreviations, see Figs. 1 and 2.

of the "nucleotide sugar" was also observed when a final concentration of 0.25mM 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose was used. In Fig. 3B, it may be seen that, when radioactive 2-acetamido-2-deoxy-α-D-mannopyranose was used as the tracer, its metabolism also showed inhibition by 0.5mM 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose, although to a lesser extent than when 2-acetamido-2-deoxy-α-D-glucopyranose was used as the tracer. In a separate experiment (data not shown), there was no effect of the fluoro sugar on the conversion of *N*-acetylneuraminic acid into cytidine-5′-(*N*-acetylneuraminosyl monophosphate).

Control experiments without or with 2-amino-2-deoxy-α-D-glucopyranose at mM concentration showed no effect on the metabolism of radioactive 2-acetamido-2-deoxy-α-D-glucopyranose: the portion of total radioactivity incorporated as 2-acetamido-2-deoxy-α-D-glucopyranose was 5.5% and 4.8%, respectively; as 2-acetamido-2-deoxy-α-glucopyranose 6-phosphate, 92.4% and 92.8%, respectively; and as uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate), 2.1% and 2.4%, respectively. The incubation was for 1 h at 37° in a 1-ml reaction volume containing mM ATP and 1 μCi·ml$^{-1}$ of 2-acetamido-2-deoxy-α-D-glucopyranose, when the protein content was 4.0 mg·ml$^{-1}$. When 2-amino-2-deoxy-α-D-glucopyranose was added to the incubation mixture as a radioactive tracer at a concen-

tration of 1 $\mu$Ci·ml$^{-1}$, in the presence of mM adenosine 5′-triphosphate, 99.9% of the counts remained in the tracer after 1 h at 37°, showing that there had been no metabolism at all, because of competition by D-glucose for 2-amino-2-deoxy-α-D-glucopyranose phosphorylation[12].

## DISCUSSION

Cell extracts from cultivated, mouse-embryo cells are suitable to the study of a sequence of enzymic processes in the metabolism of amino sugars. By judicious choice of tracer compounds, and by omission or addition of nucleotide triphosphates, portions of this sequence can be studied separately.

We observed that, in the presence of 0.5mM 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose, the accumulation of "nucleotide sugars" can be lessened: with radioactive 2-acetamido-2-deoxy-α-D-glucopyranose, its conversion into uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) was inhibited by 50% (see Fig. 3A); with radioactive 2-acetamido-2-deoxy-α-D-mannopyranose, its conversion into cytidine 5′-(*N*-acetylneuraminosyl monophosphate) was also inhibited (Fig. 3B). There was no effect from the presence of 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose on the conversion of *N*-acetylneuraminic acid into cytidine 5′-(*N*-acetylneuraminosyl monophosphate). Therefore, the effect seen when using tracer 2-acetamido-2-deoxy-α-D-mannopyranose must be on its phosphorylation or on the synthesis of *N*-acetylneuraminic acid.

With 2-acetamido-2-deoxy-α-D-mannopyranose as the tracer, it reappeared in 4 to 8 h (see Fig. 2), coincident with the disappearance of 2-acetamido-2-deoxy-α-D-mannopyranose 6-phosphate; the reappearance of the former is probably due to the hydrolysis of the latter metabolic intermediate.

The general effect of 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose in cell-free extracts is on the accumulation of the "nucleotide sugars" uridine-5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) and cytidine-5′-(*N*-acetylneuraminosyl monophosphate).

The 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose used did not contain any detectable amount of 2-acetamido-2-deoxy-α-D-glucopyranose or 2-amino-2-deoxy-α-D-glucopyranose. Even mM 2-amino-2-deoxy-α-D-glycopyranose had no effect on the metabolism of the amino sugar, so were it produced by breakdown from the fluoro derivative, this compound could not account for the results. The selective loss of the fluorine atom to afford 2-acetamido-2-deoxy-α-D-glucopyranose under the physiological conditions employed is highly unikely[13]. Therefore, the demonstrated effect of 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose on the amino sugar metabolism is not attributable to trivial causes.

Thus, it may be concluded that, in addition to a previously reported effect of 2-deoxy-α-D-*arabino*-hexopyranose[14], 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose can modify amino sugar metabolism, and can be usefully employed at nontoxic levels in mouse cells[5] in order to explore changes in glycolipid and glycoprotein biosynthesis.

ACKNOWLEDGMENTS

We thank Dr. Paul W. Kent for the generous gift of 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose, and for providing analytical data as to its purity. We also thank Dr. R. Stuart Tipson for help with the preparation of the manuscript. Alan M. Schultz was a recipient of a NCI Postdoctoral Fellowship.

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