nicotinic acid or quinolinic acid or both. At the concentrations used, these compounds had no appreciable effect on the growth rate of the organism, the final yield of cells, or the protein concentration of the cell-free extract. Cultures (1 liter each) were grown overnight with vigorous shaking at 37°. The bacteria were collected by centrifugation, washed, and disrupted by grinding with 2.5 g of alumina per g of cells. This mixture was then stirred with 5 volumes of 0.05 M potassium phosphate, pH 7.0, and centrifuged at 10,000 × g for 20 minutes. The supernatant solution was used as the enzyme source. The enzyme that forms nicotinic acid mononucleotide from quinolinic acid, “quinolinate decarboxylase,” was assayed by the PP-ribose-P-dependent decarboxylation of quinolinic acid-2,3,7,8-14C (9). Nicotinic acid mononucleotide pyrophosphorylase was measured as described by Imsande (6). Protein was determined by the method of Lowry et al. (10).

Table I presents the effects of the addition of various concentrations of nicotinic acid and quinolinic acid to the growth medium on the activity of the nicotinic acid mononucleotide synthesizing enzymes. The presence of nicotinic acid at 10⁻³ or 10⁻⁴ M in the medium almost abolishes the activity of quinolinate decarboxylase, while quinolinic acid at 10⁻⁴ M results in an approximately 1.5-fold increase in the specific activity of this enzyme. On the other hand, nicotinic acid mononucleotide pyrophosphorylase activity was not greatly affected by the presence of either compound in the medium, in confirmation of the previous report of Imsande (6). The data in Table II show that the effect of nicotinic acid supplementation on quinolinate decarboxylase activity is not due to an inhibition of the enzyme by nicotinic acid, NAD, or NADP, which could conceivably be present in higher concentrations in the nicotinic acid grown cells. The low activity of quinolinate decarboxylase in these extracts also cannot be attributed to formation of any other inhibitor, since the activity of mixed extracts (control + nicotinic acid) is additive. 

**REFERENCES**


Hexosamine Metabolism

II. ACID-SOLUBLE PRODUCTS IN RAT LIVER FOLLOWING PERFUSION WITH D-GLUCOSAMINE-1-¹C* 

Rudolph DeCillocco† and Frank Maley

From the Division of Laboratories and Research, New York State Department of Health, and the Department of Biochemistry, Albany Medical College, Albany, New York 12201

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The isotope distribution pattern of glucosamine-1-¹C, following intraportal injection into rat liver, revealed this compound to

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† Fellow of the American Diabetes Association.
be incorporated almost exclusively into N-acetyl-d-glucosamine 6-phosphate and uridine diphosphate N-acetylgalactosamine (1). However, a small amount of radioactivity was also detected in an early region (designated as A by McGarrah and Maley (1)) of the chromatographic elution pattern: although it was not possible to characterize this component because of the limited quantity available, autoradiographic studies suggested it to be sialic acid. Through the use of liver perfusion it has now been possible to establish the presence of sialic acid in rat liver, and to obtain a more definitive chromatographic pattern with glucosamine-1-\(^14\)C, than was previously described (1). Fig. 1 presents a typical Dowex 1-formate elution pattern of a neutralized, acid-soluble extract prepared from a rat liver perfused for 4 hours with glucosamine-1-\(^14\)C. The radioactive components were identified in the following manner.

Peak I—After concentration and lyophilization, this region was tentatively identified as sialic acid by chromatography in ethanol-ammonium acetate (pH 7.5) (5:2), n-butyl alcohol-propyl alcohol-HCl (1:2:1), and sec-butyl alcohol-acetic acid-water (4:1:5). The thioisobitaric acid method of Warren (3) gave a characteristic sialic acid color (\(\lambda_{max}, 549\) nm) and revealed about 0.5 \(\mu\)mol of this compound in 10 g of rat liver. Further characterization was obtained by treatment of the radioactive material in Peak I with purified NAN aldolase\(^1\) (4). A solution containing 112,800 c.p.m. of Peak I material, 5.5 \(\mu\)mol of carrier N-glycoyl-b-mannosaminic acid, 10 \(\mu\)mol of phosphate buffer (pH 7.4), and 6.4 units of NAN aldolase in a total volume of 1 ml was incubated at 37° for 18 hours. Lacit dehydrogenase assay revealed 3.98 \(\mu\)mol of pyruvate at the end of this time. Passage of the digestion mixture through a column of Dowex 1-formate layered on top of Dowex 50-H\(^+\) (each resin bed was 3 x 1 cm) to remove salt and unhydrolyzed sialic acid yielded 72,400 c.p.m. in the eluate. Initial identification of the eluted material was accomplished by subjecting it to electrophoresis in 1% borate (5). Autoradiography located the compound in the N-acetyl-d-mannosamine region. In order to distinguish between N-acetyl- and N-glycocoly-d-mannosamine, chromatography in n-butyl alcohol-acetic acid-water (6) was employed and revealed the radioactive material to migrate like N-acetyl-d-mannosamine. Thus, from the chromatographic and enzymic studies, Peak I appears to be composed mostly of N-acetyl-d-neuraminic acid. This compound is probably derived from UDP-GlcNac (7–9) and not from the hydrolysis of liver glycoproteins, since the specific activities of UDP-GlcNac, acid-soluble sialic acid, and sialic acid hydrolyzed from protein were estimated to be 1.50 \times 10^6, 1.72 \times 10^6, and 4.10 \times 10^5 c.p.m. per \(\mu\)mol, respectively. The sialic acid pool does not appear to arise during the course of the perfusion, since it was also detected in livers extracted within 15 seconds after rats were killed. A similar specific activity pattern was also obtained with a rat that received an intraperitoneal injection of glucosamine-1-\(^14\)C and was allowed to metabolize it over a 0.5-hour period.

These studies do not reveal the extent to which the sialic acid arose from CMP-NAN during the isolation procedure, for, as reported (10, 11), the latter compound is extremely acid-labile and would have probably been hydrolyzed to N-acetyln neuraminic acid during the column chromatography, if not sooner.

Peaks II and III—These radioactive areas were identified initially as N-acetylglucosamine-1-P and N-acetylglucosamine-6-P, respectively, since they moved at the same rate as the corresponding compounds added to the column as markers. The phosphate esters in Peaks II and III could be distinguished by the acid lability of the phosphate in II (12) (0.1 N HCl for 10 minutes at 100°) and its resistance to a nonspecific phosphatase (Ploidase, Schwarz BioResearch, Inc.). In contrast, the phosphate ester in III was acid-stable and sensitive to Ploidase. The above properties are characteristic of N-acetylglucosamine 1- and 6-phosphate, respectively, and were employed to separate these compounds, especially when overlapping occurred. Passage of the dephosphorylated acylhexosamines through separate Dowex 1-formate-Dowex 50-H\(^+\) columns yielded sufficient material upon concentration to give a faint positive test for acylhexosamines (13). Borate electrophoresis, followed by autoradiography, revealed the dephosphorylated Peak II material to consist of equivalent amounts of reducing compounds that migrated like N-acetylglucosamine and N-acetylglactosamine. The latter possibly resulted from the pyrophosphorolysis of UDP-GalNac, but other routes of synthesis cannot be excluded at the present time. Additional evidence favoring the existence of a galactosamine derivative in Peak II has come from Dowex 50-Na\(^+\) chromatography (14) of this region after acid hydrolysis (2 \times 100° for 12 hours), and from autoradiograms of the acid-hydrolyzed products following paper chromatography in ethyl acetate-pyridine-water (6:2.5:2).

Dephosphorylated Peak III consisted mainly of components that migrated (upon electrophoresis in 1% borate) like N-acetylgalactosamine and N-acetylmannosamine, with the former compound predominating. The latter compound could be derived from UDP-GlcNac (15) or from N-acetylglucosamine (16). Paper chromatography in isopropyl alcohol-water (4:1) was utilized to separate the acylhexosamines from the hexoses and revealed that dephosphorylated Peaks II and III chromatographed identically with marker N-acetylglucosamine. These procedures would not, however, distinguish between the N-acetyl- and N-glycocolyhexosamines. Radioactive hexoses could not be detected.

Peak IV—This area coincides with UDP-GlcNac, which was

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\(^1\)The abbreviation used is: NAN, N-acetyln neuraminic acid.

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characterized previously (1). In contrast to the previously reported short term intraportal injection studies (1), where at least half of the radioactivity was found in N-acetylglucosamine 6-phosphate, the present studies revealed most of the glucosamine-1-\textsuperscript{14}C to be incorporated into UDP-GlcNAc. Of $4 \times 10^6$ c.p.m. incorporated into the acid-soluble fraction of the liver, $3 \times 10^6$ c.p.m. were found in IV. Little if any radioactivity was found in UDP-glucose.

The results obtained with 2- and 6-hour perfusions were similar, but with variations in the distribution of radioactivity in the indicated regions (Peaks I through IV). Diabetic livers, obtained after perfusion or intraportal injection, yielded much more radioactivity in Peaks II, III, and IV, with the specific activity of IV being about twice that in the normal. These results will be reported in more detail in a future publication.

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