Biosynthesis of Sialic Acids by Neisseria meningitidis*

ROBERT S. BLACKLOW AND LEONARD WARREN

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service, Bethesda 14, Maryland

(Received for publication, June 18, 1962)

Recent reports from this laboratory (2-4) and other laboratories (5, 6) have elucidated the pathway of biosynthesis of N-acetylneuraminic acid from N-acetyl-D-mannosamine and phosphoenolpyruvic acid in mammalian systems. The overall biosynthetic reaction for rat liver and bovine submaxillary gland is catalyzed by three enzymes: a kinase, a condensing enzyme, and a dephosphorylating enzyme. N-Acetyl-D-mannosamine is phosphorylated by a specific kinase and adenosine triphosphate to yield N-acetyl-D-mannosamine 6-phosphate. This condenses with phosphoenolpyruvic acid to give N-acetylneuraminic acid 9-phosphate according to Equation 1.

\[
\text{N-Acetyl-D-mannosamine 6-phosphate} + \text{phosphoenolpyruvate} + \text{H}_2\text{O} \xrightarrow{\text{Mg}^{2+}} \text{N-acetylneuraminic acid 9-phosphate} + \text{P}_i
\]

N-Acetylneuraminic acid 9-phosphate is then dephosphorylated to free N-acetylneuraminic acid by what appears to be specific phosphatase (1).

In 1958, Watson and Scherp (7) and Watson, Marinetti, and Scherp (8) demonstrated the presence of a polymer that contained sialic acid and a hexosamine in the polysaccharide capsule of Neisseria meningitidis, strain 1908 (Group C). In a previous preliminary communication (1) we have described a new enzyme system that is responsible for the biosynthesis of N-acetylneuraminic acid in extracts of this bacteria. Unlike the mammalian system, the extract of N. meningitidis, strain 1908, is able to synthesize N-acetylneuraminic acid from N-acetyl-D-mannosamine and phosphoenolpyruvic acid without having a phosphorylated N-acetylhexosamine or a phosphorylated N-acetylneuraminic acid as an intermediate. The reaction proceeds according to Diagram 1 and favors synthesis of N-acetylneuraminic acid.

This paper describes the isolation, purification, and properties of the enzyme involved in the synthesis of N-acetylneuraminic acid and the identification of the products of the reaction.

**EXPERIMENTAL PROCEDURE**

**Materials and Methods**

The tricyclohexylamine salt of phosphoenolpyruvic acid was purchased from Mann Research Laboratories, Inc. Reduced glutathione was purchased from the California Corporation for Biochemical Research. DEAE-cellulose was obtained from Distillation Products, Inc. and was prepared in the hydroxyl form according to the method of Peterson and Sober (10).

d-Mannosamine, N-acetyl-D-mannosamine, and C*-N-acetyl-D-mannosamine labeled in the acetyl group were prepared according to methods reported previously (2). N-Acetylneuraminic acid was isolated and crystallized from human plasma proteins by the procedure of Svennerholm (11). Alumina gel Cy was prepared according to the method of Wistatier (12) and was aged for 2 years.

N-Acetylneuraminic acid was measured by the thiobarbituric acid assay (13) with the crystalline NAN* isolated from human plasma protein as a standard. The thiobarbituric acid chromogen was extracted into 3 ml of cyclohexanone rather than 4.3 ml. N-Acetylneuraminic acid was also determined by the orcinol, Ehrlich, resorcinol, and diphenylamine tests (14-16). Optical densities were determined on a Beckman DU spectrophotometer with a microcell attachment (1-cm light path). Absorption spectra of chromophores were determined in a Cary recording spectrophotometer, Model 11 M.

Inorganic phosphate was measured by the method of Lowry and Lopez (17). Total phosphate was measured after digestion by the method of Fiske and SubbaRow (18). Protein was determined by the method of Lowry et al. (19) or by ultraviolet absorption (20) with crystalline bovine serum albumin used as a standard.

**Assay:** Condensing Enzyme—The assay for the condensing enzyme of Neisseria meningitidis was based upon the conversion of N-acetyl-D-mannosamine and phosphoenolpyruvate to NAN. In this assay, 0.5 μ mole of N-acetyl-D-mannosamine and 0.5 μ mole of phosphoenolpyruvate, 1.0 μ mole of MnCl₂ and 5.0 μ moles of reduced glutathione, 30 μ moles of Tris-acetate buffer, pH 8.3, and enzyme in a volume of 0.20 ml were incubated for 1 hour at 37°C. The thiobarbituric acid assay for free NAN (13) was carried out on the entire vessel contents. Synthesis of product was linear with respect to time and enzyme concentration in the assay (Figs. 1 and 2). Synthesis of 0.01 μ mole of NAN led to an increase in optical density of 0.193 at 549 nm. A unit of enzyme is defined as that amount of enzyme that leads to the synthesis of 1 μ mole of free N-acetylneuraminic acid per hour under the conditions of the assay. For most assays, between 0.01 and 0.05 unit of enzyme was added.

**RESULTS**

**Purification of Condensing Enzyme**

**Preparation of Extract**—Eight 2-liter flasks containing 1 liter of Trypticase soy broth (Difco Laboratories) were inoculated

* The abbreviation used is: NAN, N-acetylneuraminic acid.
with 100 ml each of a 24-hour Trypticase soy culture of Neisseria meningitidis, strain 1908. Flasks were incubated on a rotary shaker at 37° for 18 hours. The cells were harvested by centrifugation at 4000 × g in an angle centrifuge, washed twice in 0.1 M Tris-chloride buffer, pH 7.6, and finally resuspended in 50 ml of this buffer and refrigerated at 4° overnight. The cell suspension was put through a French press and then centrifuged at 20,000 × g for 20 minutes. The supernatant solution was decanted, diluted with 2 volumes of 0.1 M Tris-chloride buffer, pH 7.6, and filtered at 4° in a Krueger-type filter. The extract could be stored at -15° for over 2 months without loss of activity. The protein concentration of the crude extract was critical for optimal purification; extracts with protein concentration between 10 mg per ml and 20 mg per ml gave the best purification, and all extracts were adjusted to this concentration with buffer.

**Acetone Fractionation**—All procedures were performed at -14° with precooled acetone. To 15 ml of the extract were added 150 μmoles of 1 M mercaptoethanol. Acetone (0 to 41%), 10.5 ml, was added slowly with constant stirring, the mixture was allowed to stand for 10 minutes at -14° and was then centrifuged for 10 minutes at 10,000 × g in a Servall SS-1 angle centrifuge. A heavy, reddish precipitate was discarded, and the clear supernatant solution was used for the next acetone step. Another 10.5 ml of cold acetone were added to the supernatant solution with constant stirring and after standing for 10 minutes, the mixture was centrifuged at 10,000 × g for 10 minutes. A fine, white precipitate was formed. The supernatant solution was discarded and the centrifuge tubes were allowed to drain for 60 seconds. Residual acetone was then wiped from the inside of the centrifuge tube. This precipitate (42 to 58% acetone) was dissolved in 5 ml of cold 0.05 M Tris-acetate buffer, pH 8.3, containing 0.005 M mercaptoethanol.

**Gel Adsorption and Elution**—All procedures were performed at 2°. Uniformly suspended alumina gel Cy, 3.5 ml (960 mg per ml dry weight), was diluted to 10 ml with distilled water and spun for 5 minutes at 8000 × g in a Servall RC-2 refrigerated centrifuge and the precipitate was used for adsorption of the acetone fraction. Acetone fraction, 4.8 ml, was added to the gel precipitate and the mixture was stirred for 10 minutes and then spun in an angle centrifuge for 5 minutes at 8000 × g. The condensing enzyme was adsorbed to the alumina gel Cy. Protein was eluted from the gel with 4.8 ml of 0.025 M potassium phosphate. The thiobarbituric acid assay was done on the contents of each vessel.

**FIG. 1.** Biosynthesis of N-acetylneuraminic acid with time by extracts of Neisseria meningitidis, strain 1908. The incubation vessels were as described under "Assay" with 20 μg of the 42 to 58% acetone fraction in each vessel. The incubations were stopped at times indicated by quick freezing in a dry ice-alcohol mixture. The thiobarbituric acid assay was done on the contents of each vessel.

**FIG. 2.** Biosynthesis of N-acetylneuraminic acid dependence on enzyme concentration. The incubation vessels were as described under "Assay" with the amount of enzyme added (DEAE-cellulose eluate) as indicated in the figure. Incubation for 1 hour at 37°. The thiobarbituric acid assay was performed on the entire contents of the vessels.
eluate was placed on a column at 4°C. A 9.5 ml washing was done with a solution containing 0.05 M potassium phosphate, pH 7.2, in two 4.8-m1 batches. Calcium phosphate gel, magnesium hydroxide gel, and hydroxylapatite were also tested for adsorption and purification of the condensing enzyme. The acetone fraction, 8 units. The mixture was incubated for 6 hours at 37°C. At the conclusion of the incubation, the protein was precipitated with 2 volumes of alcohol and the mixture was centrifuged. The supernatant solution was applied to a column of Dowex 1-acetate (8% crosslinked; 100 to 200 mesh; 2.5 x 26 cm). The column was washed with 100 ml of water and 100 ml of 0.05 M formic acid and then eluted with a linear gradient of 500 ml of 1 M formic acid into 500 ml of 0.05 M formic acid. The yield of N-acetylneuraminic acid was 45.8 μmoles. Specific activity was determined on a sample of the lyophilized pooled eluate.

Specific activity

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4N-acetyl-b-mannosamine</td>
<td>C4N-acetylneuraminic acid</td>
</tr>
<tr>
<td>c.p.m. per μmole</td>
<td>6800</td>
</tr>
</tbody>
</table>

Formation of N-acetylneuraminic acid from C4N-acetyl-b-mannosamine

The incubation mixture contained in a total volume of 20 ml: C4N-acetyl-b-mannosamine, 100 μmoles, 6600 c.p.m. per μmole; phosphoenolpyruvate, 100 μmoles; MnCl2, 100 μmoles; reduced glutathione, 500 μmoles; Tris-acetate buffer, pH 8.3; 200 μmoles; and enzyme (acetone fraction), 8 units. The mixture was incubated at 37°C for 6 hours. At the conclusion of the incubation, the protein was precipitated with 2 volumes of alcohol and the mixture was centrifuged. The supernatant solution was applied to a column of Dowex 1-acetate (8% crosslinked; 100 to 200 mesh; 2.5 x 26 cm). The column was washed with 100 ml of water and 100 ml of 0.05 M formic acid and then eluted with a linear gradient of 500 ml of 1 M formic acid into 500 ml of 0.05 M formic acid. The yield of N-acetylneuraminic acid was 45.8 μmoles. Specific activity was determined on a sample of the lyophilized pooled eluate.
November 1962

R. S. Blacklow and L. Warren

3523

Table IV

Comparison of the amount of N-acetylneuraminic acid synthesized on large scale by comparison of chromophores formed in five colorimetric reactions

See text for experimental details.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Amount of NAcN biosynthesized</th>
<th>Amount of NAcN synthesized</th>
<th>Amount of NAcN authentic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orcinol</td>
<td>46.4</td>
<td>570</td>
<td>570</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>45.5</td>
<td>588</td>
<td>588</td>
</tr>
<tr>
<td>Ehrlich</td>
<td>45.8</td>
<td>530, 565</td>
<td>530, 565</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>46.4</td>
<td>520</td>
<td>520</td>
</tr>
<tr>
<td>Thiobarbituric acid</td>
<td>45.8</td>
<td>549</td>
<td>549</td>
</tr>
</tbody>
</table>

by the five colorimetric assays was in close agreement (Table IV). The biosynthetic N-acetylneuraminic acid, as well as authentic NAcN, was incubated with a crude preparation of NAcN-aldolase from Clostridium perfringens ATCC 10873 (22) as previously described (2). A material which co-chromatographed with authentic N-acetyl-D-mannosamine was the only N-acetylated compound formed upon enzymatic degradation of N-acetylneuraminic acid. The biosynthetic product contained no phosphate (18). The product was inactive in the thiobarbituric acid assay after pretreatment with sodium borohydride, indicating a free reducible 2-keto group (2).

Specificity of Condensing Enzyme—With purified enzyme preparations, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, N-acetyl-D-mannosamine 6-phosphate and their deacylated forms as well as D-mannosamine, D-mannose, D-glucose, D-galactose, D-glucose 6-phosphate, D-ribose, D-ribose 5-phosphate, D-erythrose, D-xylose, D-arabinose, UDP-D-mannose, UDP-glucose, and GDP-mannose could not substitute for N-acetyl-D-mannosamine. Pyruvate, lactate, 2-phosphoglycerate, oxaloacetate, and 3-phosphoglyceric acid could not substitute for phosphoenolpyruvate. In crude preparations, 3-phosphoglyceric acid could substitute for phosphoenolpyruvate presumably by its conversion to the latter. Unlike the crude rat liver extracts, UDP-N-acetylglucosamine could not substitute for N-acetyl-D-mannosamine in the crude extracts of N. meningitidis.

Cofactor Requirements and Inhibitors of Condensing Enzyme—The requirements for the biosynthesis of N-acetylneuraminic acid are shown in Table V. With crude extracts, metals and —SH groups are not necessary, but after the gel step (see "Purification"), metals were needed for optimal activity, as were sulfhydryl groups. Manganous ions are the most effective, and under conditions of the assay, the optimal concentration is $5 \times 10^{-4}$ M. Magnesium and cobaltous ions could substitute for manganous ions but were only 50% as effective at optimal concentrations. The optimal metal ion concentrations are shown in Fig. 3. Zinc, cupric, cuprous, ferric, and lead ions could not substitute for manganese ions in concentrations ranging from $2 \times 10^{-4}$ M to $2 \times 10^{-4}$ M. EDTA at a concentration of $1 \times 10^{-4}$ M could inhibit completely the activity of a system activated by $5 \times 10^{-4}$ M manganous ions. Addition of an excess of manganous ions to $2 \times 10^{-4}$ M could reactivated the system inactivated by EDTA.

Glutathione was necessary for maximal activity of the purified preparations, and sulfhydryl groups were generally included in the buffers in which the acetone fractionation was carried out and against which the lyophiilized DEAE-cellulose fraction was dialyzed. In preparations of the lyophiilized fraction dialyzed in the absence of sulfhydryl groups, optimal activity was achieved with a glutathione concentration of $5 \times 10^{-4}$ M (Fig. 4). In lyophiilized fractions prepared this way, $5 \times 10^{-4}$ M mercaptoethanol was only 82% as effective as glutathione at the same concentration, whereas cysteine was only 62% as effective at the same concentration, and homocysteine was only 15% as effective as glutathione at its optimal concentration. For most of the other studies with lyophiilized DEAE-cellulose eluate, the enzyme was dialyzed in the presence of $5 \times 10^{-4}$ M mercaptoethanol, and then only $2.5 \times 10^{-4}$ M glutathione was needed for optimal activity. With an acetone fraction which was prepared in the absence of sulfhydryl groups, the reaction was inhibited 82% by $1 \times 10^{-4}$ M p-chloromercuribenzoate. This inhibition was overcome by $2 \times 10^{-4}$ M glutathione.

Table V

Requirements for biosynthesis of N-acetylneuraminic acid by enzyme purified from extracts of Neisseria meningitidis, strain 1008

The complete vessel contained in a volume of 0.20 ml: phosphoenolpyruvate, 0.5 µmole; N-acetyl-D-mannosamine, 0.5 µmole; MnCl₂, 1 µmole; reduced glutathione, 5.0 µmoles; Tris-acetate buffer, pH 8.3, 30 µmoles; enzyme (lyophiilized DEAE-cellulose eluate), 20 µg. For this experiment, the lyophiilized enzyme was dialyzed for 3 hours against 0.05 M Tris-acetate buffer, pH 7.6 in the absence of —SH groups. The mixture was incubated at 37°C for 1 hour, and the thiobarbituric acid assay was carried out on the entire contents of the vessel.

<table>
<thead>
<tr>
<th>Omission</th>
<th>N-Acetylneuraminic acid formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>33 µmoles</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>1 µmole</td>
</tr>
<tr>
<td>Glutathione</td>
<td>6 µmoles</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>&lt;1 µmole</td>
</tr>
<tr>
<td>N-Acetyl-D-mannosamine</td>
<td>&lt;1 µmole</td>
</tr>
<tr>
<td>Enzyme</td>
<td>&lt;1 µmole</td>
</tr>
</tbody>
</table>
Stoichiometry—For every mole of N-acetyl-n-mannosamine and phosphoenolpyruvic acid converted to N-acetylneuraminic acid, 1 mole of inorganic phosphate was formed (Table VI).

Characteristics of Condensing Reaction—The pH optimum with Tris buffer lies between 8.0 and 8.4 with a maximum at 8.3 (Fig. 5). The $K_m$ value under the conditions of the assay for N-acetyl-d-mannosamine is $6.25 \times 10^{-2} \text{ M}$ and that for phosphoenolpyruvate is $4.2 \times 10^{-2} \text{ M}$, as calculated from Lineweaver-Burk plots (25) (Fig. 6).

Equilibrium and Reversibility—When excess enzyme and N-acetyl-d-mannosamine were incubated with limiting amounts of phosphoenolpyruvate, there was essentially complete conversion of the phosphoenolpyruvate to N-acetylneuraminic acid (Table VII). The reaction appears to be irreversible. Incubation of N-acetylneuraminic acid and inorganic phosphate or arsenate with the enzyme, glutathione, and manganous ions did not lead to the disappearance of NAN. Incubation of phosphoenolpyruvate and N-acetyl-n-mannosamine to effect synthesis of N-acetylneuraminic acid aldolase-catalyzed reaction.

Fig. 4. Glutathione requirement for the synthesis of N-acetylneuraminic acid. Each vessel was as described under “Assay” with 10 $\mu$g of lyophilized DEAE-cellulose eluate dialyzed in the absence of sulfhydryl groups. Glutathione concentration of 6.25 $\mu$M was incubated at 37°C for 1 hour. The thiobarbituric acid assay was performed on the entire vessel contents.

Table VI

| N-Acetyl-d-mannosamine$^a$ | Phosphoenolpyruvate$^b$ | NAc$^c$ | P$^d$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$moles</td>
<td>$\mu$moles</td>
<td>$\mu$moles</td>
<td>$\mu$moles</td>
</tr>
<tr>
<td>Initial</td>
<td>1.87</td>
<td>1.90</td>
<td>0</td>
</tr>
<tr>
<td>Final</td>
<td>1.42</td>
<td>1.42</td>
<td>0.45</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.45</td>
<td>-0.48</td>
<td>+0.45</td>
</tr>
</tbody>
</table>

$^a$ Measured by the assay of Reissig et al. (23).

$^b$ Measured by the enzymatic method of Kornberg and Pricer (24).

$^c$ Measured by the thiobarbituric acid method (13).

$^d$ Measured by the assay of Lowry and Lopez (17).

Fig. 5. Optimal pH for the synthesis of N-acetylneuraminic acid. Each incubation vessel contained in a volume of 0.2 ml: N-acetyl-d-mannosamine, 0.5 $\mu$ mole; phosphoenolpyruvate, 0.5 $\mu$ mole; MnCl$_2$, 1 $\mu$ mole; glutathione, 5 $\mu$ moles; enzyme (acetone fraction), 20 $\mu$g; and Tris-acetate buffer, 50 $\mu$ moles, pH as indicated. Activity at 100% is equivalent to 0.041 $\mu$ mole of NAN synthesis in 1 hour at 37°C. The thiobarbituric acid assay was performed on the entire vessel contents.

The enzyme system described here which synthesizes N-acetylneuraminic acid from N-acetyl-d-mannosamine and phosphoenolpyruvate is different from that previously described for rat liver and bovine submaxillary gland (2, 6). Whereas in the mammalian systems, biosynthesis of N-acetylneuraminic acid proceeds through the phosphorylated intermediates, N-acetyl-d-mannosamine 6-phosphate and N-acetylneuraminic acid 9-phosphate, in the bacterial system there is a direct condensation of N-acetyl-d-mannosamine with phosphoenolpyruvic acid to give the final product, N-acetylneuraminic acid. A comparison of the condensing enzymes of bovine submaxillary gland and N. meningitidis, strain 1908, shows that both reactions are essentially irreversible and strongly favor the synthesis of N-acetylneuraminic acid. The cofactor requirements of both enzymes and the nature of the inhibitory substances described strongly suggest that both contain sulfhydryl groups that are essential for activity. Metal ions are required for activity of both enzymes. Although Mn$^{++}$ and to a lesser degree Co$^{++}$ and Mg$^{++}$, are all effective in the bacterial enzyme, Mg$^{++}$ is most effective in the mammalian condensing enzyme. The pH optima of the two enzymes are different.

The $K_m$ values for phosphoenolpyruvic acid in the two condensing enzymes are equal, but the affinity of N-acetyl-d-mannosamine 6-phosphate in the mammalian system is 25 times that of N-acetyl-d-mannosamine in the bacterial system of Neisseria meningitidis.

Davis (26) has suggested that enzymes acting on charged substrates may have advantages in terms of such properties as specificity and affinity. The higher affinity of N-acetyl-d-mannosamine 6-phosphate for its condensing enzyme is in agreement with this hypothesis.

Both the bacterial and mammalian condensing enzymes are clearly different from N-acetylneuraminic acid aldolase (22). Pyruvate is inactive in the condensing enzyme reactions and biosynthesis of N-acetylneuraminic acid is favored, unlike in the N-acetylneuraminic acid aldolase-catalyzed reaction.
Figure 6. Dependence of N-acetylneuraminic acid synthesis on the concentration of N-acetyl-D-mannosamine and phosphoenolpyruvate. Each vessel contained in a volume of 0.2 ml: Tris-acetate buffer, pH 8.3, 30 μmoles; MnCl₂, 1 μmole; reduced glutathione, 5 μmoles; enzyme, 10 μg of lyophilized DEAE-cellulose eluate. Upper curves: 0.5 μmole of N-acetyl-D-mannosamine and concentrations of phosphoenolpyruvate as indicated. Lower curves: 0.5 μmole of phosphoenolpyruvate and concentrations of N-acetyl-D-mannosamine as indicated. Incubation, 1 hour at 37°. Thiobarbituric acid assay was performed on the entire contents of each vessel.

Table VII
Formation of N-acetylneuraminic acid from limiting amounts of phosphoenolpyruvate

<table>
<thead>
<tr>
<th>Phosphoenolpyruvate added</th>
<th>N-Acetylneuraminic acid formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmoles</td>
<td>μmoles</td>
</tr>
<tr>
<td>0.027</td>
<td>0.027</td>
</tr>
<tr>
<td>0.047</td>
<td>0.048</td>
</tr>
<tr>
<td>0.095</td>
<td>0.092</td>
</tr>
</tbody>
</table>

The biosynthetic pathways for formation of the hexosamine precursors of sialic acid also appear to be different in bacterial and mammalian systems. Roseman, Hayes, and Ghosh (27) have shown that in bacteria, N-acetyl-D-mannosamine 6-phosphate is formed from N-acetyl-D-glucosamine 6-phosphate, whereas in mammals, N-acetyl-D-mannosamine is formed by epimerization of N-acetyl-D-glucosamine (28) and UDP-N-acetyl-D-glucosamine (29, 30). It is remarkable that N-acetyl-D-mannosamine is formed as its 6-phosphate in bacteria and must be dephosphorylated before conversion to N-acetylneuraminic acid, whereas in the mammal, N-acetyl-D-mannosamine is formed as such and must be phosphorylated before it can be converted to N-acetylneuraminic acid 9-phosphate (2, 6) and N-acetylneuraminic acid (2). In both types of systems, N-acetylneuraminic acid and not N-acetylneuraminic acid 9-phosphate is activated to form cytidine 5'-monophosphate-N-acetylneuraminic acid (31, 1, 32).

The biological significance of the differences in the mammalian and bacterial systems is not known at the present time.

Summary

The biosynthesis of N-acetylneuraminic acid has been studied in extracts of Neisseria meningitidis, strain 1908 (Group C). An enzyme has been partially purified 125-fold which catalyzes the condensation of N-acetyl-D-mannosamine with phosphoenolpyruvate to give N-acetylneuraminic acid and inorganic phosphate according to Equation 2.

\[
N\text{-Acetyl-D-mannosamine} + \text{phosphoenolpyruvate} + \text{H}_2\text{O} \xrightarrow{\text{Mn}^{++}} \text{N-acetylneuraminic acid} + \text{P}\]

Mn⁺⁺ ions are required; Co⁺⁺ and Mg⁺⁺ are less effective. Sulhydryl compounds such as glutathione are required for maximal activity. The reaction strongly favors the formation of N-acetylneuraminic acid and appears to be essentially irreversible.

Biosynthesis of N-acetylneuraminic acid and its precursors in mammalian and bacterial systems is compared.

Acknowledgments—The authors would like to acknowledge the invaluable technical assistance of Miss C. W. Spearing, and wish to thank Dr. S. E. Mergenhagen of the National Institutes of Health for preparation of the extracts and growth of Neisseria meningitidis, strain 1908.

References
20. Warburg, O., and Christian, W., Biochem. Z., 310, 394 (1941).
Biosynthesis of Sialic Acids by *Neisseria meningitidis*

Robert S. Blacklow and Leonard Warren


Access the most updated version of this article at
http://www.jbc.org/content/237/11/3520.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/237/11/3520.citation.full.html#ref-list-1