It was concluded, therefore, that the isolated material was N-acetylanthranilic acid. How it arises during the metabolism of shikimic or quinic acid is under current investigation. It is unlikely, however, to represent a main pathway for the metabolism of anthranilic acid, as in the ethereal extracts of this present culture large amounts of catechol were detected. Catechol presumably was being produced by an anthranilate hydroxylase as observed already in many instances; the subsequent degradation of catechol would then provide the carbon fragments necessary for cell growth.

N-Acetylanthranilic acid may be a precursor, or be related to a precursor, of anthranilic acid. As such it might have a connexion with the recent finding by Somerville and Elford that during the conversion of chorismic acid to anthranilic acid by the anthranilate synthetase of Escherichia coli a hydroxamate was formed. A hydroxamate, giving a positive FeCl₃ test, is produced from N-acetylanthranilic acid when it is reacted with neutral hydroxylamine in the manner of Lipmann and Tuttle (C. Ratledge, unpublished result). Hydroxamates obviously could be produced from other N-acylanthranilates and it will be of interest to learn if an N-acylanthranilate participates at all in the final stages of anthranilic acid biosynthesis.

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Department of Biochemistry, The University, Hull (Great Britain)

C. Ratledge

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Metabolism of 5'-nucleotide monosaccharides in inflammatory connective tissue

Subcutaneous implantation of polyvinyl alcohol sponge in guinea pigs stimulates rapid proliferation and maturation of inflammatory connective tissue. Maximum rates of formation of extracellular products occur at 14 days, and the sponge implant is completely organized by connective tissue in 42 days. During this period a series of age-dependent alterations in the tissue content of 5'-nucleotide monosaccharides have been demonstrated, the most prominent being a 2-fold increase in tissue levels of the UDP-N-acetylhexosamines (UDP-N-acetylglucosamine plus UDP-N-acetylgalactosamine). Parenteral administration of hydrocortisone (4 mg/kg per day) for 14 days further accentuated this change and also reduced the tissue content of UDP-hexoses (UDP-glucose plus UDP-galactose). These steroid-induced changes in the tissue content of 5'-nucleotide monosaccharides occurred in the absence of any detectable alteration in hepatic levels of these compounds. Using larger doses of hydro-

Biochim. Biophys. Acta, 156 (1968) 217-220
cortisone, other investigators have demonstrated that acute or chronic treatment will
significantly alter the uridine sugar nucleotide content in liver. These observations
are of interest since 5'-nucleotide sugars are currently viewed as essential precursors
in biosynthesis of the extracellular constituents of connective tissue and adreno-
corticosteroids are known to modify or suppress formation of these macromolecular
products.

To test the specificity of the effects of hydrocortisone on sugar nucleotide
metabolism, guinea pigs bearing polyvinyl sponge implants were treated with the
antimetabolite 6-mercaptopurine (25 mg/kg per day for 14 days). In individual ex-
periments the 5'-nucleotides present in cold 10% trichloroacetic acid extracts of poly-
vinyl sponge granulomas pooled from 15–20 control, hydrocortisone- or 6-mercaptopu-
rine-treated animals were isolated and characterized by methods previously re-
ported. The chromatographic systems employed separated the uridine hexose (UDP-
glucose plus UDP-galactose), guanosine hexose (GDP-mannose plus GDP-fucose), and
amino sugar nucleotides (UDP-N-acetylglucosamine plus UDP-N-acetylgalacto-
samine). They did not separate the individual monosaccharide sugar nucleotides. These
compounds were characterized by ultraviolet spectral analysis, phosphorus content,
and lability to specific phosphatases. The acyl sugar present in a nucleotide was
identified, following pH 2 hydrolysis, by paper chromatography and electrophoresis. Quantitation of each amino sugar nucleotide present in 14-day control, hydro-
cortisone-, and 6-mercaptopurine-treated granulomas was made on the chromato-
graphically isolated UDP-N-acetylhexosamines following acid hydrolysis (2 M HCl
at 100° for 3 h). The free hexosamines were separated on a Dowex 50 (H+) ion-
exchange column as described by GARDELL, and the amount of glucosamine and
galactosamine present in the column fractions determined. Complete separation and
recovery was checked by adding known quantities of carrier [1-14C]glucosamine and
[14C6]galactosamine to each hydrolysate prior to column fractionation.

Changes in the tissue content of 5'-nucleotide monosaccharides in 14-day

<table>
<thead>
<tr>
<th>Compounds</th>
<th>µmoles/100 g wet wt. tissue (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sponge biopsy connective tissue</td>
<td>Guinea-pig liver</td>
</tr>
<tr>
<td>14 day</td>
<td>14 day + cortisol</td>
</tr>
<tr>
<td>Total 5'-nucleotides</td>
<td>78 ± 6.0</td>
</tr>
<tr>
<td>5'-Nucleotide sugars</td>
<td>8.30 ± 0.40</td>
</tr>
<tr>
<td>UDP-hexoses</td>
<td>5.03 ± 0.35</td>
</tr>
<tr>
<td>GDP-hexoses</td>
<td>1.66 ± 0.13</td>
</tr>
<tr>
<td>UDP-N-acetylhexosamines</td>
<td>1.33 ± 0.09</td>
</tr>
<tr>
<td>'UDP-glucuronic acid'</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Biochim. Biophys. Acta, 156 (1968) 217–220
control, hydrocortisone-, 6-mercaptopurine-treated animals, and normal guinea-pig liver are compared in Table I. In control and drug-treated animals, sugar nucleotides accounted for approx. 10% of total 5'-nucleotides isolated from 14-day sponge granulomas. As noted previously administration of hydrocortisone in two groups of animals reduced UDP-hexoses, and elevated UDP-N-acetyhexosamine levels\(^2\). Treatment with 6-mercaptopurine appeared from these analyses to have produced a modest increase in GDP-hexoses and UDP-N-acetyhexosamines in the sponge granuloma. Because of the known acid lability of UDP-glucuronic acid to the methods of extraction employed in this study, changes in the content of this nucleotide observed in the individual experiments should be considered only semi-quantitative estimates. The tissue content of total amino sugar nucleotides in guinea-pig liver is comparable to that reported previously by other investigators\(^9\).

Following treatment with parenteral hydrocortisone (Table II), the disproportionate increase in UDP-N-acetylgalactosamine and UDP-N-acetylgalactosamine indicates that utilization in final product formation, a shift in equilibrium of the UDP-N-acetylgalactosamine 4'-epimerase (EC 5.1.3.7) reaction, or both had occurred during administration of this agent. As noted (Table I) 6-mercaptopurine produced only a modest increase above control values in the total UDP-N-acetyhexosamine content of 14-day granulomas. However, following chromatographic separation of the two hexosamines (Table II), UDP-N-acetylgalactosamine accounted for 98% of total while the concentration of UDP-N-acetylgalactosamine had decreased to less than 0.03 \(\mu\)mole/100 g. A differential effect on amino sugar nucleotide metabolism in the sponge granuloma occurred as a result of treatment with 6-mercaptopurine and hydrocortisone. Inhibition of the UDP-N-acetylgalactosamine 4'-epimerase (EC 5.1.3.7) reaction by 6-mercaptopurine would explain the chemical values observed after administration of this drug. In control granulomas and in normal guinea-pig liver, the proportion of the two amino sugar nucleotides is comparable to equilibrium ratios reported for chemical and enzymatic studies on other mammalian tissues\(^9\).

The age-dependent change in nucleotide monosaccharides noted during maturation of inflammatory connective tissue\(^2\) can be modified in 14-day granulomas by the pharmacological action of hydrocortisone and the antimetabolite 6-mercaptopyrurine.

### TABLE II

<table>
<thead>
<tr>
<th>Compounds</th>
<th>(\mu)mole/100 g wet wt. tissue (mean (\pm) S.D.)</th>
<th>Sponge biopsy connective tissue</th>
<th>Guinea-pig liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14 day + cortisol</td>
<td>14 day + 6-mercapto-</td>
</tr>
<tr>
<td>UDP-N-acetylgalactosamine</td>
<td>1.05 (\pm) 0.11 (79)</td>
<td>3.61 (\pm) 0.23 (88)</td>
<td>1.69 (\pm) 0.18 (98)</td>
</tr>
<tr>
<td>UDP-N-acetylgalactosamine</td>
<td>0.28 (\pm) 0.03 (21)</td>
<td>0.49 (\pm) 0.06 (12)</td>
<td>0.03 (\pm) 0.01 (2)</td>
</tr>
</tbody>
</table>

Biochim. Biophys. Acta, 156 (1968) 217–220
purine. A differential effect on the biosynthesis and/or utilization of UDP-N-acetyl-
glucosamine and UDP-N-acetylgalactosamine occurred under the experimental con-
ditions employed in this study. In the sponge granuloma the same doses of these
two drugs have been shown to reduce total protein, collagen, mucopolysaccharide
and lipid content of the tissue. During administration of hydrocortisone and
6-mercaptopurine quantitative suppression of final product formation by connective
tissue cells produces specific alterations in the tissue levels of 5'-nucleotide mono-
saccharides. The results also indicate that important primary sites of action of each
of these drugs may be present at specific steps in the pathway leading to final in-
corporation of individual monosaccharides into the carbohydrate-containing products
formed by connective tissue cells.

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ROSEMAN and Associates for several of the 5'-nucleotide monosaccharide standards
and 14C-labeled hexosamines used in this study.

Rackham Arthritis Research Unit,
Department of Internal Medicine,
The University of Michigan Medical School,
Ann Arbor, Mich. (U.S.A.)

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