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[2]; 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[3] 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 $FeCl_3$ test, is produced from *N*-acetylanthranilic acid when it is reacted with neutral hydroxylamine in the manner of LIPMANN AND TUTTLE[4] (C. RATLEDGE, unpublished result).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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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[1]. 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)[2]. 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[3]. Using larger doses of hydro-

cortisone, other investigators have demonstrated that acute or chronic treatment will significantly alter the uridine sugar nucleotide content in liver[4,5]. These observations are of interest since 5′-nucleotide sugars are currently viewed as essential precursors in biosynthesis of the extracellular constituents of connective tissue[6] and adrenocorticosteroids are known to modify or suppress formation of these macromolecular products[7,8].

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 experiments the 5′-nucleotides present in cold 10 % trichloroacetic acid extracts of polyvinyl sponge granulomas pooled from 15–20 control, hydrocortisone- or 6-mercaptopurine-treated animals were isolated and characterized by methods previously reported[2]. 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*-acetylgalactosamine). 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[2]. Quantitation of each amino sugar nucleotide present in 14-day control, hydrocortisone-, and 6-mercaptopurine-treated granulomas was made on the chromatographically isolated UDP-*N*-acetylhexosamines following acid hydrolysis (2 M HCl at 100° for 3 h)[3]. 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-^{14}C]glucosamine and [$^{14}C_6$]galactosamine to each hydrolysate prior to column fractionation[3].

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

TABLE I

5′-NUCLEOTIDE MONOSACCHARIDE CONTENT IN SPONGE GRANULOMAS AND NORMAL GUINEA-PIG LIVER

The 5′-nucleotide sugars were isolated from cold 10% trichloroacetic acid extracts, purified by column and paper chromatography and identified by chemical analyses. Drug-treated animals received parenteral hydrocortisone (4 mg/kg per day) or 6-mercaptopurine (25 mg/kg per day) for 14 days.

Compounds	*μmoles/100 g wet wt. tissue (mean ± S.D.)*			
	Sponge biopsy connective tissue			*Guinea-pig liver*
	14 day	*14 day + cortisol*	*14 day + 6-mercapto-purine*	
Total 5′-nucleotides	78 ± 6.0	89 ± 12.0	108.0 ± 10.5	404.0 ± 28.0
5′-Nucleotide sugars	8.30 ± 0.40	8.20 ± 1.20	10.10 ± 1.05	36.70 ± 2.17
UDP-hexoses	5.03 ± 0.35	2.34 ± 0.38	5.81 ± 0.63	16.88 ± 1.43
GDP-hexoses	1.66 ± 0.13	1.23 ± 0.40	2.42 ± 0.36	3.67 ± 0.70
UDP-*N*-acetylhexosamines	1.33 ± 0.09	4.10 ± 0.49	1.72 ± 0.18	15.41 ± 0.89
"UDP-glucuronic acid"	0.28	0.53	0.15	0.74

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*-acetylhexosamine levels[3]. Treatment with 6-mercaptopurine appeared from these analyses to have produced a modest increase in GDP-hexoses and UDP-*N*-acetylhexosamines 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 comparabe to that reported previously by other investigators[9].

Following treatment with parenteral hydrocortisone (Table II), the disproportionate increase in UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine indicates that utilization in final product formation, a shift in equilibrium of the UDP-*N*-acetylglucosamine 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*-acetylhexosamine content of 14-day granulomas. However, following chromatographic separation of the two hexosamines (Table II), UDP-*N*-acetylglucosamine accounted for 98% of total while the concentration of UDP-*N*-acetylgalactosamine had decreased to less than 0.03 μmole/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*-acetylglucosamine 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-mercapto-

TABLE II

AMINO SUGAR NUCLEOTIDE CONTENT IN SPONGE GRANULOMAS AND NORMAL GUINEA-PIG LIVER

The amount of each nucleotide present in the purified UDP-*N*-acetylhexosamine fraction was determined following acid hydrolysis of 2–3 μmoles of the mixture, and separation of the free hexosamines on a Dowex 50 (H^+) ion-exchange column. Figures in parentheses represent percent of each nucleotide found in the total amino sugar nucleotide pool.

Compounds	*μmoles/100 g wet wt. tissue (mean ± S.D.)*			
	Sponge biopsy connective tissue			*Guinea-pig liver*
	14 day	*14 day + cortisol*	*14 day + 6-mercaptopurine*	
UDP-*N*-acetylglucosamine	1.05 ± 0.11 (79)	3.61 ± 0.23 (88)	1.69 ± 0.18 (98)	12.30 ± 0.94 (79)
UDP-*N*-acetylgalactosamine	0.28 ± 0.03 (21)	0.49 ± 0.06 (12)	0.03 ± 0.01 (2)	3.20 ± 0.45 (21)

purine. A differential effect on the biosynthesis and/or utilization of UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine occurred under the experimental conditions 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[3,10]. 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 monosaccharides. 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 incorporation of individual monosaccharides into the carbohydrate-containing products formed by connective tissue cells.

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