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Review Article

Glycoside Biosynthesis and the Biochemistry of Sugar Nucleotides

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AMONG the primary constituents of living organisms, carbohydrates probably rank first in abundance. There is no doubt that, among the carbohydrates, those that contain the glycosidic linkage constitute the great majority. The biological formation of this linkage is, therefore, of considerable importance. Studies on the biosynthesis of the glycosidic bond have been concerned largely with carbohydrates that are widely distributed in nature. Much of the knowledge gained in these studies, however, has been found to apply equally well to the biosynthesis of the heteroglycosides that are of particular interest to pharmaceutical scientists.

Despite the diversity of the glycosides studied, the mechanisms by which the glycosidic bond can be formed can be grouped into four categories. All of these involve the transfer of glycosyl groups and so are catalyzed by enzymes called glycosyl transferases.[1] Table I shows a representative glycosyl donor and acceptor for each type of reaction together with a common or trivial name for the enzyme catalyzing the reaction.

In reaction types I, II, and IV (Table I), the donor is a glycosyl ester in which the acid is either orthophosphoric (type I), pyrophosphoric (type II), or a nucleoside monophosphate (type IV). In type III, the donor is a glycoside in which the glycosyl group is attached to the hemiacetal carbon of a sugar residue through an oxygen atom. Widely held current belief is that the type IV reaction is the most important and most generally applicable for the formation of the glycosidic bond. The other reactions are either very restricted in their applicability to syntheses, or they function primarily in the breakdown of the glycosidic bond rather than in its synthesis. Because time and space do not permit an adequate consideration of reaction types I, II, and III, these will be dismissed with a few brief comments and reference to recent reviews on each type of reaction.

PHOSPHORYLASES

The reactions catalyzed by phosphorylases (2, 3) involve the transfer of some group, other than phosphate, from its linkage with an organic residue to inorganic phosphate. The group that is transferred may represent any one of a large number of different classes of substances, but for the purpose of this review, consideration will be

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[1] Nomenclature of enzymes, use of symbols, and abbreviations will follow the rules recommended by the International Union of Biochemistry (1). The following abbreviations will be used throughout the text: v = the initial steady-state velocity; V = the maximum value of v corresponding to saturation of the enzyme with substrate; K_m (Michaelis constant) = the substrate concentration at which $v = V/2$; NAD (DPN) = nicotinamide-adenine dinucleotide; $NADH_2$ = reduced form of NAD; NADP = nicotinamide-adenine dinucleotide phosphate; $NADPH_2$ = reduced form of NADP; P_i = inorganic phosphate; PP_i = inorganic pyrophosphate; A = adenosine; C = cytidine; G = guanosine; I = inosine; T = thymidine; U = uridine; dA = deoxyadenosine, etc.; AMP = adenosine monophosphate, etc.; ADP = adenosine diphosphate, etc.; ATP = adenosine triphosphate, etc. Unless otherwise indicated, the ionic form of each substance will be assumed to be the form prevailing at the pH of the experiment.

TABLE I.—ENZYMATIC REACTIONS LEADING TO THE FORMATION OF GLYCOSIDIC BONDS

Type	Glycosyl Donor	Glycosyl Acceptor	Reaction	Trivial Name
I	α-D-Glucose-1-phosphate	$(\alpha\text{-}1,4\text{-Glucosyl})_n$	α-D-Glucose-1-P + $(\alpha\text{-}1,4\text{-glucosyl})_n \rightleftharpoons (\alpha\text{-}1,4\text{-glucosyl})_{n+1} + P_i$	α-Glucan phosphorylase
II	5-Phospho-α-D-ribosyl pyrophosphate	Adenine	Adenine + 5-phosphoribosylpyrophosphate ⇌ AMP + PP_i	AMP pyrophosphorylase
III	Sucrose	$(\alpha\text{-}1,4\text{-Glucosyl})_n$	Sucrose + $(\alpha\text{-}1,4\text{-glucosyl})_n \rightleftharpoons (\alpha\text{-}1,4\text{-glucosyl})_{n+1}$ + D-fructose	Sucrose transglucosylase
IV	UDP-Glucose	$(\alpha\text{-}1,4\text{-Glucosyl})_n$	UDP-Glucose + $(\alpha\text{-}1,4\text{-glucosyl})_n \rightleftharpoons$ UDP + $(\alpha\text{-}1,4\text{-glucosyl})_{n+1}$	UDP-Glucose-α-glucan transglucosylase

limited to those reactions in which the glycosyl group is being transferred. Furthermore, because the major theme of this review is synthesis, rather than phosphorolysis, of glycosidic bonds, these reactions will be presented in their reverse direction. Hence, they may be defined as involving the transfer of the glycosyl group from its linkage with phosphate to an organic acceptor. The reaction may be formulated as follows:

$$X\text{-}P + Y \rightleftharpoons X\text{-}Y + P_i \quad \text{Reaction } 1$$

Here, X-P is the glycosyl donor; Y is the acceptor; X-Y is the new glycoside; and P_i is the inorganic phosphate.

In all of the known cases, the donor is a glycosyl-1-phosphate. Three classes of phosphorylases are known; these are presented below and will be seen to involve the formation of both *O*-glycosides and *N*-glycosides.

Polysaccharide Phosphorylases

The early studies on the phosphorolysis and then on the synthesis of glycogen and starch concerned the reaction catalyzed by this class of enzyme. Since their discovery, these enzymes have been found in many animal tissues, higher plants, and microorganisms (4). The recommended IUB nomenclature is "α-1,4-glucan: orthophosphate glucosyltransferase."[2] The synthetic reaction may be written as follows:

$$[\text{glucose}]_n + \text{glucose-1-P} \rightleftharpoons [\text{glucose}]_{n+1} + P_i \quad \text{Reaction } 2$$

The glucosyl residues are added to the nonreducing ends of the pre-existing polymer represented in Reaction 2 by $[\text{glucose}]_n$. The term "primer" is used to refer to this polysaccharide which is usually starch or glycogen. This is a convenient term but its use tends to obscure the fact that the primer is actually the second reactant in a bimolecular reaction. Linear α-1,4-linked chains of glucose down to 3 units in length have been shown to serve as primers. These are, however, much less effective than the larger branched-chain polymers.

[2] Enzymes catalyzing group-transfer reactions are named according to the pattern "donor:acceptor group-transferred-transferase."

Disaccharide Phosphorylases

This group of phosphorylases consists of three types, all of which have been found only in bacteria (5).

Sucrose Phosphorylase.—The nonspecific title of "disaccharide glucosyltransferase" is recommended for this enzyme (1). Studies on its mechanism of action indicate that it combines reversibly with the D-glucosyl moiety of various glucose donors and transfers this group to a large variety of acceptors. The general reaction, therefore, may be represented as follows:

$$\alpha\text{-D-glucosyl-1-R} + HOR' \rightleftharpoons \alpha\text{-D-glucosyl-1-R}' + HOR \quad \text{Reaction } 3$$

where R and R′ may be an appropriate ketose molecule, phosphate, arsenate, or hydrogen in the combination that would lead to an exchange reaction, phosphorolysis, arsenolysis, or hydrolysis.

Maltose Phosphorylase.—The reaction catalyzed by this enzyme involves the transfer of the glucosyl group with inversion of configuration as represented below:

$$\beta\text{-D-glucose-1-P} + \text{D-glucose} \rightleftharpoons \text{maltose} + P_i \quad \text{Reaction } 4$$

The enzyme is specific for β-D-glucose-1-P but can use either D-glucose or D-xylose as an acceptor. The recommended name for this enzyme is "maltose : orthophosphate glucosyltransferase" (1).

Cellobiose Phosphorylase.—This enzyme exhibits a specificity for glucose-1-phosphate as the glycosyl donor in the following reaction:

$$\alpha\text{-D-glucose-1-P} + \text{glucose} \rightleftharpoons \text{cellobiose} + P_i \quad \text{Reaction } 5$$

It lacks specificity for the glucosyl acceptor, however, and has not been given a systematic name. As in the case of the maltose phosphorylase reaction, the reaction catalyzed by this enzyme occurs with an inversion of configuration of the glycosidic bond.

Nucleoside Phosphorylases

These enzymes can be considered to be ribosyl or deoxyribosyl transferases (6). Enzymes that

react only with purine or with pyrimidine bases have been demonstrated. The recommended nomenclature (1) follows the pattern "nucleoside: orthophosphate ribosyl (or deoxyribosyl) transferase." These enzymes have been found in a wide variety of animal and microbial sources, and they catalyze the formation of *N*-glycosides according to the following reaction:

$$\alpha\text{-D-ribose-1-P} + \text{uracil} \rightleftharpoons \text{uridine} + P_i \quad \text{Reaction 6}$$

Since the glycosidic linkage is β in all of the nucleosides, this reaction represents another example of glycoside formation with inversion of configuration.

Energetics

In vitro measurements of equilibrium constants have shown that synthesis of glycosides by phosphorylase reactions is energetically feasible, except for the reaction leading to the formation of sucrose. The reason for this becomes readily apparent when the free energy of hydrolysis of sucrose is compared to that for the hydrolysis of other glycosidic bonds: at pH 7 in 0.15 *M* NaCl, the $\Delta F^{0\prime}$ for the hydrolysis of sucrose is -7750 cal./mole; for the hydrolysis of glucose-1-P, -5760 cal./mole; and for the hydrolysis of glycogen, -5030 cal./mole (7).

Table II presents some equilibrium constants for a reaction representing each type of phosphorylase reaction discussed above. These values are based on analytical concentrations rather than activities of the reactants and are, therefore, apparent equilibrium constants (K'). The values given for the polysaccharide phosphorylase reaction are the apparent equilibrium constants for the over-all reaction comprising a series of successive reactions each involving the addition (or removal) of a glucose residue.

The K' for each of the reactions in Table II is dependent on pH because of the difference in the acidic dissociation constants of inorganic phosphate and glucose-1-phosphate. Correction of these values for the conditions prevailing in a living cell would require much more information than is available at this time. Correction for such factors as pH, ionic strength, and metal complexing, however, can be accomplished by means of the mathematical expressions recently summarized by Johnson (7).

Despite the support given to a synthetic role for these reactions by free-energy data, other studies have provided much more convincing evidence in support of a phosphorolytic role or one primarily involved in the breakdown of glycosidic bonds. The metabolism of glycogen has been the subject of most intensive study in this regard, and the conclusions arising from such studies will be summarized in the discussion of glycogen synthesis by glucosyl transfer from a sugar nucleotide.

For the present, it can be said that the phosphorylase reactions are readily reversible and can be made to proceed in the phosphorolytic direction by appropriate adjustment of substrate concentrations. This behavior is in contrast to most of the reactions in which glycosidic bonds are formed by transfer of a glycosyl group from a sugar nucleotide. As will be seen later, most of the latter reactions are strongly exothermic and essentially irreversible. The organisms in which the disaccharide phosphorylases have been found do not accumulate the disaccharides formed by these enzymes; rather, they utilize these disaccharides very efficiently.

A mechanism for nucleotide biosynthesis that by-passes the nucleoside stage is discussed under *Pyrophosphorylases*. When coupled to the hydrolysis of the inorganic pyrophosphate produced in the reaction, this reaction becomes much more favored thermodynamically than the reaction catalyzed by the nucleoside phosphorylases. The pyrophosphorylase reaction, however, has not been demonstrated with deoxyribose derivatives.

TABLE II.—EQUILIBRIUM CONSTANTS OF PHOSPHOROLYTIC REACTIONS

Phosphorylase	Equilibrium Ratio	Conditions			Ref.
		K'	pH	Temp., °C.	
Polysaccharide	$\frac{[P_i]}{[\text{glucose-1-P}]}$ [a]	2.4	7.3	30	(8)
		10.8	5.0	30	
Sucrose	$\frac{[\text{sucrose}]\,[P_i]}{[\text{fructose}]\,[\text{glucose-1-P}]}$	0.05	6.6	30	(9)
Maltose	$\frac{[\text{maltose}]\,[P_i]}{[\text{glucose}]\,[\text{glucose-1-P}]}$	4.4	7.0	37	(10)
Cellobiose	$\frac{[\text{cellobiose}]\,[P_i]}{[\text{glucose}]\,[\text{glucose-1-P}]}$	4.4	7.0	37	(11)
Uridine	$\frac{[\text{uridine}]\,[P_i]}{[\text{uracil}]\,[\text{ribose-1-P}]}$	11	7.0	37	(12)

[a] Because the concentration of nonreducing end groups on the outer chains of the primer remains virtually unchanged, the concentration of the primer has no effect on the equilibrium constant over a wide range of concentrations.

Thus, it is possible that the phosphorylase reaction represents the major synthetic route to the deoxynucleosides.

PYROPHOSPHORYLASES

Of the numerous types of pyrophosphorylases (13), the only one to be discussed under this title is the group classified as pentosyltransferases (1). A typical reaction catalyzed by one of these enzymes is the following:

5-phosphoribosyl-1-PP_i + adenine $\rightleftharpoons$ AMP + PP_i Reaction 7

The recommended name for the enzyme that catalyzes Reaction 7 is "AMP:pyrophosphate phosphoribosyltransferase." Like the phosphorylases, these enzymes are named for the pyrophosphorolytic reaction.

Reactions similar to that shown in Reaction 7 have been demonstrated for other pyrimidines as well as purines, but they have not been demonstrated with the deoxyribose derivatives. These reactions lead to the formation of *N*-glycosidic bonds. Most of the measurements of equilibrium constants have been conducted with impure preparations and are, therefore, of doubtful validity. However, there is some evidence which indicates that some of these reactions favor synthesis, and others favor pyrophosphorolysis. Hydrolysis of the inorganic pyrophosphate by the action of pyrophosphatases is a highly exergonic reaction and will cause a drastic shift in the direction of nucleotide synthesis.

Another type of pyrophosphorylase instrumental in the formation of the glycosyl donors for glycoside biosynthesis *via* nucleoside diphosphate sugars will be discussed under *Biosynthesis of Sugar Nucleotides.*

TRANSGLYCOSYLASES

In this section, consideration will be limited to those reactions in which both the glycosyl donor and the product are glycosides having a sugar or alcohol residue attached to the hemiacetal carbon of a sugar molecule through an oxygen bridge (3). These reactions, therefore, do not lead to the net synthesis of glycosidic bonds, but they form new glycosidic bonds only at the expense of another such bond.

Because of the relatively high bond energy in sucrose, transfer of either the glucosyl or the fructosyl residue of this disaccharide to a suitable acceptor with the formation of a new glycosidic bond would be expected to be favored energetically. Enzymes catalyzing such reactions are found in several microorganisms, and their presence is frequently manifested by the development of considerable viscosity in liquid culture media containing sucrose. A representative reaction of this type is that effected by an enzyme produced by *Leuconostoc mesenteroides:*

n sucrose $\rightleftharpoons$ (α-1,6-D-glucose)$_n$ + n D-fructose Reaction 8

Glycogenlike polymers as well as levans are formed by similar mechanisms by other organisms. In higher plants, the evidence supporting this mechanism for the biosynthesis of fructans is not entirely convincing. A serious challenge to this pathway in higher plants is the recent discovery of UDP-fructose in dahlia tubers (14). This is the first report of the occurrence of this substance in higher plants.

Also of considerable importance in this group are the so-called "branching enzymes." Of chief interest among these are the branching enzymes that transfer segments from linear portions of α-1,4-glucose chains to the 6-position of a nonterminal D-glucose unit. These enzymes are responsible for converting the linear polysaccharide to a branched structure such as amylopectin or glycogen.

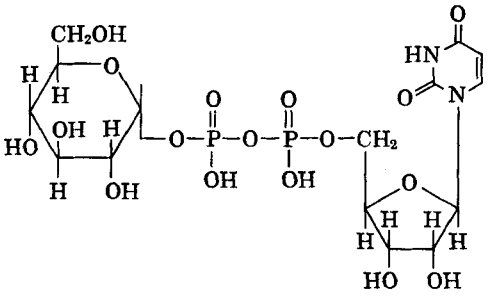
Certain transglycosylases, which are better known as hydrolases, have been shown to be capable of transferring a glycosyl group to a sugar or other alcohol with the formation of a new glycoside. Invertase, for example, has been shown to be a fructosyl transferase that can catalyze the formation of oligosaccharides as well as the hydrolysis of sucrose. Hydrolysis, then, may be considered as a special case of a transferase reaction in which the acceptor is water. In many instances, the preference shown for water appears to be due to the high concentration of water (55 *M*) as compared to other acceptors. Incubation of the enzyme with high concentrations of other acceptors in these cases promotes the synthesis of a new glycoside (15).

NUCLEOSIDE DIPHOSPHATE SUGAR REACTIONS

The events leading to the discovery of the involvement of nucleoside diphosphate sugars in carbohydrate metabolism were recently related by Leloir in the fourth Hopkins Memorial Lecture (16). After failing to obtain lactose synthesis by mammary gland extracts, he and his co-workers turned to the study of lactose utilization by *Saccharomyces fragilis* in the hope of obtaining some information that might aid their study of the synthesis of this carbohydrate. The organism was found to ferment lactose at a higher rate than a mixture of glucose and galactose, the components of lactose. An extract of this yeast

was found to convert galactose to galactose-1-P and then further to glucose-6-P. This latter transformation was complicated by the fact that it required two cofactors, one thermolabile and the other thermostable. The thermolabile cofactor was shown to be phosphoglucomutase, the enzyme that catalyzes the conversion of glucose-1-P to glucose-6-P. Glucose-1,6-diphosphate was known to be required for this conversion, but it did not fulfill the requirement for the thermostable cofactor from yeast.

While these studies were in progress, a report by Park and Johnson (17) appeared which described the accumulation of an acid-soluble labile phosphorus compound in *Staphylococcus aureus* that had been treated with penicillin. Preliminary characterization studies on the impure compound provided evidence for the presence of uracil, labile phosphate, stable phosphate, and possibly a pentose. Shortly after, Caputto *et al.* (18) reported the identification of the thermostable factor required for the conversion of galactose-1-P to glucose-6-P. This was shown to be uridine diphosphate glucose (UDP-glucose) (I).



UDP-Glucose
I

Further studies with stoichiometric rather than catalytic amounts of UDP-glucose showed that the enzyme preparation from *S. fragilis* converted UDP-glucose to UDP-galactose (19). Continuing studies on the compounds that accumulated in penicillin-treated *S. aureus* resulted in their identification as UDP-hexosamine linked to amino acids (20–22).

The first evidence for the transfer of the glycosyl groups from the sugar nucleotides was provided by Dutton and Storey (23) when they identified the thermostable factor in liver necessary for the formation of phenol glucuronides as a UDP-glucuronic acid compound. Shortly thereafter, Leloir and his co-workers demonstrated the transfer of the glucosyl group from UDP-glucose to glucose-6-P (24) and to fructose (25) to produce trehalose phosphate and sucrose, respectively.

Since the time of these early studies, nucleoside diphosphate sugar compounds have been implicated in a large number of carbohydrate transformations. The extent of this involvement will become apparent in the text that follows.

The major categories used in this discussion of sugar nucleotides include the following: occurrence, biosynthesis, transformations, and glycosyl transfer. The last three are reactions by which sugar phosphates are converted to glycosides as exemplified by the biosynthesis of lactose:

Biosynthesis glucose-1-P + UTP $\rightleftharpoons$ UDP-glucose + PP_i Reaction *9*

Transformation UDP-glucose $\rightleftharpoons$ UDP-galactose Reaction *10*

Glycosyl transfer UDP-galactose + glucose $\rightleftharpoons$ lactose + UDP Reaction *11*

The transformation reactions are obviously not an essential step in the biosynthesis of all glycosides.

Occurrence

In the past decade, there has been a great increase in the number of sugar nucleotides isolated from natural sources. The list of these compounds in Table III shows that this increase reflects a variation both in the nature of the sugar and in the identity of the purine or pyrimidine component. The wide variety of sugar derivatives emphasizes the need to include such derivatives within the definition of the term "sugar nucleotide."

Frequently, the occurrence of a sugar nucleotide in an organism is a good indication of the presence of a compound that contains that sugar in glycosidic linkage. Similarly, the presence of a sugar residue in a cellular component increases the possibility of the existence of a nucleoside diphosphate compound of that sugar.

Deliberately excluded from Table III are those nucleoside diphosphate compounds that contain a hexosamine, or derivative thereof, linked to various peptides. These do not appear to be involved in the biosynthesis of compounds normally classed as glycosides. Instead, they are involved in the synthesis of cell wall components in a manner not yet entirely understood. The citations of examples of enzymatic synthesis of the sugar nucleotides are restricted to syntheses from nucleoside triphosphates and sugar phosphates *via* the nucleotidyl transferases discussed under *Biosynthesis of Sugar Nucleotides*. Examples of formation of sugar nucleotides *via* modifications of the glycosyl residue of a previously existing sugar nucleotide are not included in Table III but are discussed in a separate

TABLE III.—OCCURRENCE AND ENZYMATIC SYNTHESIS OF SUGAR NUCLEOTIDES

Sugar Nucleotide	Occurrence		Enzymatic Synthesis	
	Source	Ref.	Source	Ref.
UDP-D-Glucose	*Saccharomyces fragilis*	(18)	*Saccharomyces fragilis*	(37)
	Guinea pig liver	(26)	Guinea pig liver	(26, 38)
	Higher plants	(27)	Brewer's yeast	(39)
	Banana	(28)	Rat mammary gland	(40)
	Vicia faba	(29)	Sugar beet leaves	(31, 41)
	Higher plant	(30)	Higher plants	(42)
	Sugar beet root	(31)	Mung bean	(43)
	Chlorella pyrenoidosa	(32)	*Impatiens holstii*	(44)
	Hansenula capsulata	(33)	Rat muscle	(45)
	Dahlia tuber	(14)	Rat liver	(46)
	Acetobacter xylinum	(34)	Pneumococcus	(47)
	Rice	(35)	Connective tissue	(48)
	Cryptococcus laurentii	(36)	Human leucocyte	(49)
			Hansenula holstii	(50)
			Trichomonas foetus	(51)
			Rice	(35, 52)
			Sheep thyroid	(53)
			Pea	(54)
			Arthrobacter sp.	(55)
			Eremothecium ashbyii	(56)
UDP-D-Galactose	Mung bean	(27)	Yeast	(61)
	Chicken liver	(57)	Higher plants	(42)
	Baker's yeast	(58)	Beef liver	(62)
	Dahlia tuber	(14)	Pneumococcus	(47)
	Escherichia coli	(59, 60)	*Streptococcus faecalis*	(63)
			Trichomonas foetus	(51)
			Escherichia coli	(64)
UDP-D-Fructose	Dahlia tuber	(14)		
UDP-D-Xylose	Higher plants	(27)	Mung bean	(65)
	Cryptococcus laurentii	(36)	Higher plants	(42)
UDP-L-Arabinose	Higher plants	(27)	Higher plants	(42)
UDP-L-Rhamnose	Pneumococcus	(66)		
UDP-Dihydroxyacetone	Pneumococcus	(67)		
UDP-D-Glucuronic acid	Liver	(23, 26, 68)	Mung bean	(70)
	Mung bean	(69)		
UDP-D-Galacturonic acid	Pneumococcus	(71)	Mung bean	(70)
	Mung bean	(72)		
UDP-D-Glucosamine			Rat liver	(46)
UDP-*N*-Acetylglucosamine	Yeast	(75)	Guinea pig liver	(26, 38)
	Guinea pig liver	(26)	Rat liver	(46)
	Bovine liver	(73)	Calf liver	(77)
	Mung bean	(69, 74)	*Staphylococcus aureus*	(77)
	Crustacean	(76)	Pneumococcus	(47)
	Dahlia tuber	(14)	*Streptococcus faecalis*	(63)
			Sheep thyroid	(53)
			Gastric mucosa	(78)
UDP-*N*-Acetylgalactosamine	Bovine liver	(73)		
	Animal tissues	(79)		
	Bacteria	(80)		
	Mung bean	(74)		
	Dahlia tuber	(14)		
UDP-*N*-Acetylglucosamine phosphate	Hen oviduct	(79)		
UDP-*N*-Acetylglucosamine sulfate	Hen oviduct	(79)		
UDP-*N*-Acetylglucosamine (4 ← 1)-D-galactose	Human milk and colostrum	(81)		
UDP-*N*-Acetylglucosamine (4 ← 1)-D-galactose-(4 or 2 ← 1)-L-fucose	Human milk and colostrum	(81)		
Pseudo-UDP-glucose			Brewer's yeast	(82)
Pseudo-UDP-galactose			Brewer's yeast	(82)
dUDP-Glucose			Pea	(54)
GDP-D-Mannose	Yeast	(83)	Yeast	(90)
	Hen oviduct	(84)	Mammary gland	(91)
	Molds	(85, 88)	*Aerobacter aerogenes*	(92)
	Mammary gland	(86)	*Hansenula holstii*	(50)
	Milk	(87)	*Arthrobacter* sp.	(55, 93)
	Red algae	(89)		
	Mung bean	(74)		
	Cryptococcus laurentii	(36)		

Sugar Nucleotide	Occurrence: Source	Ref.	Enzymatic Synthesis: Source	Ref.
GDP-D-Glucose	*Eremothecium ashbyii*	(88)	Mammary gland	(91)
	Mammary gland	(91)	*Hansenula holstii*	(50)
			Higher plants	(54)
			Eremothecium ashbyii	(56)
GDP-D-Fructose	*Eremothecium ashbyii*	(88)		
GDP-L-Galactose	Red algae	(89)		
GDP-D-Mannuronic acid	Brown algae	(94)		
GDP-L-Fucose	*Aerobacter aerogenes*	(95)		
	Mammary gland	(86)		
	Milk	(87)		
GDP-Colitose	*Escherichia coli*	(96)		
GDP-D-Glycero-D-mannoheptose	Yeast	(97)	Yeast	(92)
dGDP-D-Mannose			*Arthrobacter* sp.	(93)
CDP-D-Glucose			*Salmonella paratyphi*	(98)
			Pea	(54)
CDP-Ribitol	*Lactobacillus arabinosus*	(99)	Bacteria, yeast, *Chlorella*	(100)
CDP-Glycerol	*Lactobacillus arabinosus*	(101)	Bacteria, yeast, *Chlorella*	(100)
	Staphylococcus aureus	(102)		
CDP-Abequose	*Salmonella* sp.	(103)		
CDP-Tyvelose	*Salmonella* sp.	(103)		
CDP-Paratose	*Salmonella paratyphi*	(104)		
CMP-*N*-Acetylneuraminic acid	*Escherichia coli*	(105)	*Neisseria meningitidis*	(106)
			Submaxillary gland	(107)
			Animal tissues	(108)
			Sheep brain	(109)
CMP-*N*-Glycolylneuraminic acid			Sheep brain	(109)
CMP-3-Deoxyoctulosonic acid			*Escherichia coli*	(110)
TDP-D-Glucose	*Hansenula capsulata*	(33)	*Pseudomonas aeruginosa*	(111, 113)
			Streptococcus faecalis	(63, 112)
			Hansenula capsulata	(33)
			Pasteurella pseudotuberculosis	(114)
			Pea	(54)
			Arthrobacter sp.	(55)
TDP-D-Galactose			*Streptococcus faecalis*	(63)
			Mung bean	(115)
TDP-D-Mannose	*Streptomyces griseus*	(116)		
TDP-D-Ribose	*Streptomyces griseus*	(116)		
TDP-L-Rhamnose	*Escherichia coli*	(117)		
	Streptomyces griseus	(116)		
	Lactobacillus acidophilus	(118)		
	Bacteria	(119)		
TDP-6-Deoxy-D-glucose	*Escherichia coli*	(120, 121)		
TDP-D-Fucose	*Escherichia coli*	(120, 121)		
TDP-D-Glucosamine			*Pseudomonas aeruginosa*	(122)
TDP-*N*-Acetyl-D-glucosamine			Gastric mucosa	(78)
TDP-4-Keto-6-deoxy-D-glucose	*Escherichia coli*	(120, 121)		
TDP-4-Acetamido-4,6-dideoxy-D-glucose	*Escherichia coli*	(117)		
TDP-4-Acetamido-4,6-dideoxy-D-galactose	*Escherichia coli*	(119, 120)		
IDP-D-Glucose			Pea	(54)
			Animal tissues	(123)
IDP-D-Mannose			*Arthrobacter* sp.	(93)
			Animal tissues	(123)
ADP-D-Glucose	*Chlorella pyrenoidosa*	(32)	Wheat	(126)
	Corn	(124)	Rice	(35, 127)
	Rice	(35, 125)	Pea	(54)
			Arthrobacter sp.	(55)
ADP-D-Galactose	Corn	(128)	Corn	(129)
ADP-D-Mannose	Corn	(128)	Corn	(129)
			Mammary gland	(130)
ADP-*N*-Acetyl-D-glucosamine	Corn	(128)	Corn	(129)

section. Attention should be called also to the derivatives of cytidine which possess a single phosphate rather than a pyrophosphate linkage to the glycosyl group. Another distinguishing feature of these compounds is that they are formed from CTP and the unphosphorylated sugar derivative rather than a glycosyl phosphate.

Thus far, none of the naturally occurring D-sugar nucleotides have been found to possess a β-glycosidic linkage. The β linkage occurs in the enzymatically synthesized UDP-β-L-arabinose, for example, but it should be noted that the configuration about the anomeric carbon of β-L-arabinose is the same as that in α-D-xylose. L-Sugars that have previously been found in nature are the only ones that occur in the naturally occurring L-sugar nucleotides.

Biosynthesis of Sugar Nucleotides

In this section, consideration will be given principally to reactions of the type represented by Reaction *12*.

$$\text{UTP} + \text{glucose-1-P} \rightleftharpoons \text{UDP-glucose} + \text{PP}_i \quad \text{Reaction } 12$$

Reactions of this type may be said to effect the *de novo* synthesis of sugar nucleotides in contrast to those reactions that involve modification (epimerization, oxidation, reduction, etc.) of the glycosyl moiety of a previously formed sugar nucleotide.

Enzymes that catalyze reactions of the type shown in Reaction *13* are commonly called pyrophosphorylases. Mechanistically, these reactions share certain properties with other reactions involving very different substrates but which are also classified as pyrophosphorolytic. The general equation for these reactions is shown below (written to show pyrophosphorolysis):

$$\text{A-B} + \text{PP}_i \rightleftharpoons \text{A-PP}_i + \text{B} \quad \text{Reaction } 13$$

For a comprehensive discussion of all types of pyrophosphorylases, the reader is referred to a recent review (13).

The term "pyrophosphorylase" is somewhat of a misnomer when applied to reactions of the type represented by Reaction *12* because the equilibrium of such reactions does not show any tendency to favor pyrophosphorolysis. Evidence presented later supports the widely held view that the primary function of these enzymes is in the synthesis of sugar nucleotides rather than in their pyrophosphorolysis. The nomenclature recommended for these enzymes gives cognizance to this view. Thus, the enzyme catalyzing the reaction shown by Reaction *12* would be named, "UTP: α-D-glucose-1-phosphate uridylyltransferase."

Table III presents a list of the nucleotidyl transferases that have been demonstrated to date. The sources are as diverse as the types of sugar nucleotides themselves. There are many examples of sugar nucleotides for which a nucleotidyl transferase has not yet been demonstrated. Similarly, there are a number of transferase reactions leading to sugar nucleotides whose natural occurrence has not yet been shown.

Munch-Peterson and his co-workers (37) were the first to demonstrate the enzymatic synthesis of a sugar nucleotide. These workers studied the pyrophosphorolytic reaction of UDP-glucose with a cell-free preparation from yeast. At the same time, this group also accomplished the enzymatic synthesis of UDP-galactose with an extract of *S. fragilis* (61). Munch-Peterson (39) performed much of the early work on the kinetic, thermodynamic, and mechanistic characterization of this reaction. Most of the other papers on sugar nucleotide biosynthesis simply present evidence for the forward or reverse reaction with a crude enzyme from the various natural sources. Strominger and Smith (77) urged caution in reporting new nucleotidyl transferases and stressed the need for a reasonable purification of the enzyme in order to avoid misinterpretations like the following:

$$\text{UTP} + \text{glucose-1-P} \rightleftharpoons \text{UDP-glucose} + \text{PP}_i \quad \text{Reaction } 14$$

$$\text{UDP-glucose} + \text{galactose-1-P} \rightleftharpoons \text{UDP-galactose} + \text{glucose-1-P} \quad \text{Reaction } 15$$

$$\text{Sum: } \text{UTP} + \text{galactose-1-P} \rightleftharpoons \text{UDP-galactose} + \text{PP}_i \quad \text{Reaction } 16$$

The occurrence of Reactions *14* and *15* in the same system would provide data that were consistent with the existence of a nucleotidyl transferase for the direct formation of UDP-galactose (Reaction *16*). Only catalytic amounts of UDP-glucose and glucose-1-phosphate would be present. With the multitude of reactions now known for the sugar nucleotides and their precursors, other similar errors in interpretation can arise from limited analytical data obtained with crude enzyme preparations.

The requirement for a divalent cation in the nucleotidyl transferase reactions is well established. Mg^{2+} has been shown to be generally most effective in stimulating enzyme activity. Mn^{2+} has been found to be a good substitute and Cu^{2+}, Co^{2+}, and Ni^{2+} have been found to serve as poorer substitutes for Mg^{2+} (39, 42, 43, 62, 65, 109, 115, 129).

It is difficult to make any generalizations concerning the specificity of nucleotidyl transferases for either the glycosyl phosphate or the nucleo-

side triphosphate because most of the studies have been conducted with enzymes purified to a minimum degree. An apparent lack of specificity could, therefore, have its origin in a number of complicating factors.

Ginsburg (43), working with an 800-fold purified enzyme from mung bean seedlings, demonstrated a specificity for the synthesis of UDP-glucose. Isselbacher (62) showed his preparation from beef liver to be active in the synthesis of UDP-galactose and not UDP-glucose. More recently, Frydman *et al.* (115) obtained evidence to show that the enzyme in mung bean extracts that catalyzes the synthesis of TDP-galactose is probably different from that which catalyzes the synthesis of TDP-glucose and UDP-galactose. Barber and Hassid (54) obtained evidence for the existence, in fresh green peas, of separate enzymes acting on α-D-glucose-1-P and GTP, ATP, CTP, UTP, TTP, ITP, and dUTP. Shoyab and co-workers (109) showed that their 30-fold purified enzyme from sheep brain catalyzed the synthesis of CMP-*N*-acetylneuraminic acid from *N*-acetylneuraminic acid and CTP, but that it was inactive with dCTP, CDP, CMP, ATP, dATP, ADP, AMP, UTP, UDP, TTP, ITP, and GTP.

Varying degrees of nonspecificity have been shown in a number of other studies. Strominger and Smith (77) purified an enzyme from liver that catalyzed the formation of UDP-*N*-acetylglucosamine but not UDP-*N*-acetylgalactosamine; a purified bacterial enzyme, however, was active in the formation of both of these sugar nucleotides. Preiss and Wood (93) demonstrated the synthesis of GDP-mannose with a 60- to 88-fold purified enzyme from an *Arthrobacter* species; synthesis of IDP-mannose and dGDP-mannose by this same preparation was believed to be mediated by the same enzyme. Passeron and his co-workers (129) obtained synthesis of sugar nucleotides from ATP and mannose-1-P, galactose-1-P, and *N*-acetylglucosamine-1-P with a partially purified preparation from corn. This preparation, however, was not active with mannose-1-P and GTP or UTP. Synthesis of both IDP-glucose and IDP-mannose was obtained by Verachtert *et al.* (123) with an enzyme that had been purified 300-fold.

Rabinowitz and Goldberg (82) achieved the synthesis of pseudo-UDP-glucose and pseudo-UDP-galactose with the nucleotidyl transferase from brewer's yeast. Their results with this and other enzymes suggested that the pseudouridine derivatives are acted upon by the same enzymes that act on the corresponding uridine derivatives. Pseudouridine is a C—C linked ribonucleoside (5-ribosyluracil) that has been found in ribonucleic acids (131).

Determination of the apparent equilibrium constants of the nucleotidyl transferase reactions has met with some difficulty because of the impurity of the enzyme preparations. Munch-Peterson (39) reported that K' = about 1 for the formation of UDP-glucose by a purified enzyme from brewer's yeast. Others have reported a similar value (42, 65, 77, 90). Such a value would be expected because the newly created bond is very similar to the one being broken, as shown in the two hypothetical reactions (Reactions *17* and *18*) whose sum equals the equation for the synthesis of UDP-glucose (Reaction *12*)

uridine-P-P-P + H_2O → uridine-P + PP_i Reaction *17*

uridine-P + glucose-1-P → uridine-P-P-glucose + H_2O Reaction *18*

Studies on the determination of the $\Delta F^{0\prime}$ of hydrolysis of the glycosyl ester bond in the sugar nucleotides have utilized the reaction in which the glucosyl group is transferred to fructose (Reaction *19*). When the $\Delta F^{0\prime}$ of this reaction is added to that of the hydrolysis of sucrose (Reaction *20*), the sum is equal to the $\Delta F^{0\prime}$ of hydrolysis of UDP-glucose as shown by Reaction *21:*

UDP-glucose + fructose → sucrose + UDP Reaction *19*

sucrose + H_2O → glucose + fructose Reaction *20*

Sum: UDP-glucose + H_2O → UDP + glucose Reaction *21*

It was discovered very early that the $\Delta F^{0\prime}$ of hydrolysis of the glycosyl ester linkage in the sugar nucleotides was very high because of the failure to observe reversibility in many reactions in which these compounds served as glycosyl donors. Owing to the relatively high $\Delta F^{0\prime}$ of hydrolysis of sucrose, Reaction *19* is one of the few reactions of its type with demonstrable and measurable reversibility.

Cardini *et al.* (132) reported a K' (pH 7.4, 37°) for Reaction *19* of about 5 using a preparation from wheat germ. This corresponds to a $\Delta F^{0\prime}$ of about −1000 cal./mole. Approximate values were reported because of contamination of their preparation with enzymes that catalyzed interfering reactions. Adding this value for the $\Delta F^{0\prime}$ of Reaction *19* to the value reported by Johnson (7) for the $\Delta F^{0\prime}$ of Reaction *20*, *viz.*, −7750 cal./mole, gives a value of −8750 cal./mole for the $\Delta F^{0\prime}$ of Reaction *21*.

Glaser (133) calculated a $\Delta F^{0\prime}$ of about

−8000 cal./mole for the hydrolysis of UDP-glucose at pH 7.4 using previously reported values of free energy changes for Reactions *22-24*.

glucose-1-P + H_2O → glucose + P_i Reaction *22*

sucrose + P_i → glucose-1-P + fructose Reaction *23*

UDP-glucose + fructose → UDP + sucrose Reaction *24*

Sum: UDP-glucose + H_2O → UDP + glucose Reaction *21*

A more recent value for the K' of Reaction *19* was reported by Avigad (134). Working with an enzyme preparation from Jerusalem artichoke tuber with no demonstrable sucrase activity, he found $K' = 1.4$ and 1.8 at pH 7.6 and 30° in two separate experiments. The average of these two values, 1.6, corresponds to a $\Delta F^{0'}$ of about −300 cal./mole. Adding this value to that given by Johnson (7) for the hydrolysis of sucrose, gives a value of −8050 cal./mole.

The widespread occurrence of pyrophosphatases lends further support to assigning a synthetic, rather than a pyrophosphorolytic role to the nucleotidyl transferases. The action of the former enzymes in catalyzing the highly exothermic hydrolysis of inorganic pyrophosphate adds considerable driving force to the synthetic reaction. It also reduces the concentration of inorganic pyrophosphate, thus acting kinetically to reduce the rate of pyrophosphorolysis. In nuclei, pyrophosphatase activity is very low or absent. Pyrophosphorolytic activity in this locus, therefore, might be significant.

The ready reversibility of Reaction *19* suggests this as an alternate route for the formation of UDP-glucose. DeFekete and Cardini (135) demonstrated the formation of both UDP-glucose and ADP-glucose by this reaction and showed the subsequent transfer of the glucosyl residue to starch with a preparation from corn endosperm. Murata *et al.* (127) obtained similar results with ADP with a preparation from rice.

Transformations of Sugar Nucleotides

The first reaction observed for UDP-glucose after its identification in 1950, was its conversion to UDP-galactose (19). Since that time, numerous other transformations of the sugar moieties of the sugar nucleotides have been discovered. These have been classified into seven categories, and each will be discussed separately.

Uridylyl Transfer.—The only example of this is Reaction *15*.

UDP-glucose + galactose-1-P ⇌ UDP-galactose + glucose-1-P Reaction *15*

The recommended name (1) for the enzyme catalyzing this reaction is "UDP-glucose:α-D-galactose-1-P uridylyltransferase."

The enzyme was first demonstrated by Kalckar and his co-workers in an extract of *S. fragilis* (61). Maxwell *et al.* found this transferase activity in extracts of rat and calf liver, but were unable to show this activity in extracts of rat brain or mammary gland (136). Smith and Mills, however, were able to demonstrate this enzyme in the mammary gland of lactating rats (40). Smith and his co-workers also demonstrated this enzyme in a noncapsulated strain of pneumococcus (47).

Much of the early interest in this enzyme resided in its employment for the preparation of UDP-galactose (136) and the determination of galactose-1-phosphate (137).

One very significant area of research was that of Kalckar and his co-workers, who studied galactosemia, a childhood genetic defect marked by an impaired ability to utilize galactose and a resultant accumulation of galactose-1-phosphate in the blood. The impaired metabolism was traced to a deficiency of this uridylyl transferase. An excellent review of the early work of this group has been written by Kalckar (138). Some very recent work with erythrocytes from the blood of normal and galactosemic humans showed a very small, but variable, utilization of galactose-1-^{14}C by the galactosemics. No radioactivity could be found in the UDP-hexose fraction of homozygotes. A small amount of radioactivity was found in this same fraction from heterozygotes; this was attributed to transfer of the uridylyl group from UDP-glucose to galactose-1-P (139).

In a galactose-negative mutant of *Escherichia coli*, K-12, gal 23, failure to utilize galactose was found to result from a genetic block in the synthesis of UDP-glucose from UTP and glucose-1-P. The UDP-glucose:α-D-galactose-1-P uridylyltransferase as well as galactokinase and a 4-epimerase were present in these cells (64).

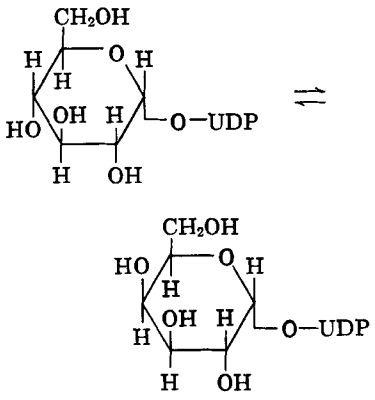
The occurrence of this uridylyl transferase was extended to higher plants when Pazur and Shadaksharaswamy demonstrated it in an extract of soybean sprouts (140).

Kurahashi and Sugimura (141) purified the enzyme sixty- to seventyfold from a galactokinase-less mutant of *E. coli*, K-12 and determined Michaelis' constants for all four compounds taking part in the reaction. They found $K' = 1.1$ at pH 8.75 and 30° for the reaction written in the direction of UDP-galactose formation (Reaction *15*). In testing the effect of cysteine, Mg^{2+}, and NaF on the transferase activity, they found that cysteine was required

and that the latter two supplements were inhibitory.

The pseudouridine analog of UDP-glucose was effective as a substrate in this reaction with enzymes from calf liver and human red blood cell hemolyzates (82).

Epimerization.—The epimerization of the glucose residue of UDP-glucose was the first transformation of a sugar nucleotide to be demonstrated (19). (Scheme I.)



Scheme I

In many of the early studies, the conversion of galactose-1-P to glucose-1-P was followed. The enzyme preparations catalyzing this conversion were called "galactowaldenases" or, more simply, "waldenases." These names were chosen because of the catalysis of what appeared to be a Walden inversion about carbon atom 4 of glucose. Present knowledge reveals that the epimerization takes place while the sugar residue is linked to UDP and that the waldenase preparations were actually a combination of the galactose-1-P uridylyltransferase discussed above (Reaction *15*) and UDP-glucose 4-epimerase (Reaction *10*). In addition, results of studies on the mechanism of the epimerase reactions have shown that a Walden-type inversion does not occur.

The recommended name (1) for this epimerase is the one given above, "UDP-glucose 4-epimerase." Reports of the occurrence of this epimerase together with several others are assembled in Table IV. It will be noted that the substrates thus far involved in this type of reaction include uridine diphosphate hexoses, hexosamines, *N*-acetylhexosamines, uronic acids, and pentoses as well as thymidine diphosphate hexoses and *N*-acetylhexosamines. The list in Table IV also includes enzymes that vary in position specificity and therefore would be called 2-, 4-, and 5-epimerases. The widespread distribution of these enzymes is evident from their occurrence in plants, animals, and microorganisms. Excluded from presentation and discussion at this time are the reactions leading to the formation of deoxysugar nucleotides; these usually involve one or more epimerizations in additon to reduction.

TABLE IV.—OCCURRENCE OF NUCLEOSIDE DIPHOSPHATE SUGAR EPIMERASES

Reaction	Source	Ref.
UDP-glucose ⇌ UDP-galactose	Yeast	(19, 58, 142–145)
	Rat tissues	(136)
	Calf liver	(146, 147)
	Lactobacillus bulgaricus	(148–150)
	Mung bean	(42)
	Pneumococcus	(47)
	Escherichia coli	(64, 151, 152)
	Trichomonas foetus	(51)
	Tumor cells	(153)
UDP-xylose ⇌ UDP-arabinose	Mung bean	(42, 154)
UDP-glucosamine ⇌ UDP-galactosamine	Calf liver	(155)
UDP-*N*-acetylglucosamine ⇌ UDP-*N*-acetylgalactosamine	Rat liver	(156,[a] 157)
	Bacillus subtilis	(80, 157)
	Calf liver	(155)
	Rabbit skin	(158)
	Trichomonas foetus	(51)
	Chick embryo	(159)
UDP-*N*-acetylglucosamine ⇌ UDP-*N*-acetylmannosamine	Rat liver	(160)
UDP-glucuronic acid ⇌ UDP-galacturonic acid	Mung bean	(154, 162)
	Pneumococcus	(161)
UDP-glucuronic acid ⇌ UDP-iduronic acid	Rabbit skin	(158, 163)
TDP-glucose ⇌ TDP-galactose	*Streptococcus faecalis*	(164)
	Mung bean	(165)
	Pasteurella pseudotuberculosis	(114)
TDP-glucose ⇌ TDP-mannose	*Streptomyces griseus*	(166)
TDP-*N*-acetylglucosamine ⇌ TDP-*N*-acetylgalactosamine	*Pseudomonas aeruginosa*	(122)

[a] Comb and Roseman (160) were not able to confirm the results of Cardini and Leloir (156). They suggest that the conclusion of the latter authors was based on an erroneous identification of the reaction product.

A very common method for determining UDP-glucose 4-epimerase activity was to add UDP-glucose dehydrogenase and NAD to the incubation mixture. In this way, the conversion of UDP-galactose to UDP-glucose could be followed by measuring the absorbance due to $NADH_2$ formed as a result of the action of the dehydrogenase on UDP-glucose. This assay had the advantage of convenience, but it also had the disadvantage of indirectness.

Maxwell departed from this routine and omitted NAD from incubation mixtures containing the 4-epimerase from calf liver (146). When this was done, no reaction took place. This finding quickly led to the demonstration of a requirement for NAD by the liver enzyme. The waldenase preparation from yeast (19) and from *Lactobacillus bulgaricus* (148–150) had been found to be active without added NAD. In a study of the enzyme from yeast, Maxwell *et al.* found a correlation between activity and fluorescence during the various stages of purification (144). They noted that the fluorescence spectrum of the purified enzyme resembled that of $NADH_2$ and then proceeded to demonstrate the presence of bound NAD (145). Since that time, numerous examples of both types of enzymes have been found in a variety of organisms.

Those epimerases which require added NAD for activity have, with one exception, been found only in mammalian tissues: calf liver (146, 147, 155), rat liver (157), rabbit skin (158, 163), and tumor cells (153). The exception is the report of the occurrence in a fully encapsulated strain of pneumococcus (161).

The epimerases which are active without added NAD have, thus far, been found only in microorganisms: *S. fragilis* (19, 144, 145), *L. bulgaricus* (149, 150), *Bacillus subtilis* (80, 157), *Trichomonas foetus* (51), and *E. coli* (80, 152). These listings include epimerases that act on substrates other than UDP-glucose.

The requirement for NAD could not be satisfied by $NADH_2$, NADP, or $NADPH_2$ with the enzymes from calf liver (146) and rabbit skin (158). With the enzyme from rabbit skin, $NADH_2$ was found to act as an inhibitor. The NAD analogs, thionicotinamide dinucleotide, and acetylpyridine adenine dinucleotide, could substitute for NAD but were less effective. Unlike the liver enzyme, the yeast epimerase was not inhibited by $NADH_2$ in studies by Maxwell *et al.* (144).

Epimerases containing bound NAD could be made to release this cofactor only after drastic treatment. Kowalsky and Koshland (150) found that prolonged dialysis of the extract from *L. bulgaricus* and treatment with charcoal did not cause a loss of activity. Treatment with neurospora NAD-ase (DPN-ase), however, did cause a loss of activity that could be restored by adding NAD. Maxwell *et al.* (144) and Glaser (157) failed to deactivate the epimerases from yeast and *B. subtilis*, respectively, by treatment with NAD-ase. In both cases, however, treatment with *p*-chloromercuribenzoate was effective in removing activity. Restoration of activity required both cysteine and NAD for the yeast enzyme, while cysteine alone was adequate for the *B. subtilis* enzyme. The failure to demonstrate a requirement for NAD for restoration in the latter case leaves considerable doubt as to its removal by the *p*-chloromercuribenzoate treatment.

The first studies on the mechanism of the epimerization were conducted with the waldenase system from *L. bulgaricus*. These were conducted independently and reported simultaneously. One group studied the conversion of galactose-1-P to glucose-1-P in the presence of ^{18}O-labeled water (149); the other studied the same conversion in the presence of water doubly labeled with tritium and ^{18}O (150). In both cases, no label appeared in the glucose-1-P, thus eliminating a dehydration-hydration mechanism or a displacement involving hydroxyl ion.

After the discovery of the requirement for NAD by the calf liver enzyme, Maxwell attempted to trap an expected carbonyl intermediate by conducting the reaction in the presence of carbonyl trapping agents. She also conducted the reaction in the presence of NAD labeled in the *para* position with tritium and in the presence of $NADH_2$ labeled in both *para* positions with tritium (147). No carbonyl compound could be trapped, and no tritium was transferred to the glycosyl moiety. The same author, working with the yeast epimerase, failed to obtain any incorporation of tritium from labeled water or $NADH_2$ (145).

Using a somewhat different approach, Bevill and his co-workers (143) conducted the reaction with the yeast enzyme and with UDP-glucose labeled with tritium on the 4 and 6 carbon atoms of the glucose residue. (The number 6 carbon atom was unavoidably labeled in their labeling procedure.) These investigators observed no loss of tritium from the C-4 position. Wilson and Hogness (151) purified an enzyme from *E. coli*, showed it to contain bound NAD, but failed to find a peak for $NADH_2$ in its absorption spectrum. They measured the difference spectrum between the enzyme alone and the en-

zyme with substrate and found that a peak appeared at a wavelength very close to that at which $NADH_2$ exhibits a peak. Calculations based on the height of this peak indicated that 19% of the bound NAD was in the reduced form at equilibrium. It thus appears that the mechanism involves an NAD-dependent oxidation-reduction with the participants firmly bound so as to prevent equilibration with the medium components.

Support for the occurrence of a similar mechanism *in vivo* was obtained when Kohn *et al.* (167) administered galactose, uniformly labeled with ^{14}C and labeled with tritium at position 4, to rats and found the ratio of ^{14}C to tritium unchanged in the glucose obtained by hydrolysis of the liver glycogen.

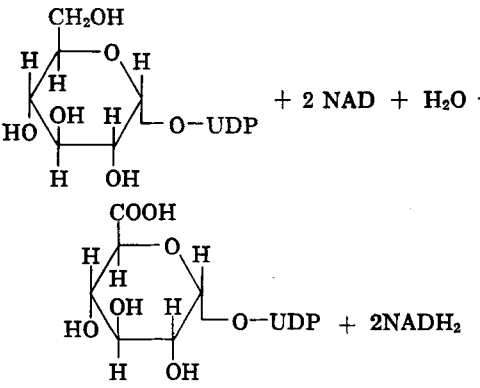
There are only two reports citing other requirements for the activity of the epimerases. Glaser (157) obtained a slight stimulation of the UDP-*N*-acetylglucosamine 4-epimerase of *B. subtilis* by Mg^{2+}. Darrow and Creveling (142) found that an organic or inorganic cation was required for maximum activity of the UDP-glucose 4-epimerase of yeast. Wilson and Hogness, on the other hand, observed no effect upon the addition of Mg^{2+}, Mn^{2+}, or EDTA (151). Most other investigators have found no necessity for adding such supplements.

The first estimate for the equilibrium constant for the UDP-glucose 4-epimerase reaction was made by Leloir with the yeast enzyme (19). He reported that the incubation mixture contained, at equilibrium, approximately 25% UDP-galactose and 75% UDP-glucose. For the reaction written in the direction of UDP-glucose formation, the K' would be about 3. Two more recent and more precise determinations, both employing a purified enzyme from *E. coli*, have given values of 3.5 for the same reaction. One of these determinations was conducted at 25° and pH 8.7 (152) and the other at 27° and pH's varying from 7.1 to 8.5 (151). The latter study showed essentially no effect of pH on the K' over the range studied.

In studies with enzymes from mung beans, Feingold *et al.* (154) found a $K' = 1.0$ for the formation of UDP-L-arabinose from UDP-D-xylose, and a $K' = 1.1$ for the formation of UDP-galacturonic acid from UDP-glucuronic acid. Tinelli *et al.* (114) reported a K' of about 1 for the formation of TDP-glucose from TDP-galactose by an extract of *Pasteurella pseudotuberculosis* type IV. Two reports on the K' for the formation of UDP-*N*-acetylglucosamine from UDP-*N*-acetylgalactosamine are not in agreement: one study with an extract of *B. subtilis* reported a $K' =$ about 2 and the other, with an extract of rabbit skin, reported $K' =$ about 5.

All of these equilibrium constants show that the free energy changes in the epimerase reactions are very small. It would be expected, therefore, that these reactions could function in either direction in a physiological system.

Oxidation (Dehydrogenation).—The enzymatic oxidation of nucleoside diphosphate sugars to the corresponding uronic acid derivatives has been demonstrated with extracts of the liver of various animals (168–170), pea seedlings (171), and *Streptococcus* (47, 172, 173) and *Arthrobacter* (174) species. Despite this widespread distribution, the only substrates for these enzymes have been found to be NAD and either UDP-glucose (47, 168–173) or GDP-mannose (174). The reaction consists of a two-stage oxidation at C-6 of the glucosyl (or mannosyl) residue to give the glucuronyl (or mannuronyl) derivative as shown in Reaction *25*.



Reaction *25*

The recommended nomenclature (1) follows the pattern "electron donor:electron acceptor, oxidoreductase." The enzyme catalyzing the reaction shown in Reaction *25* would, therefore, be called "UDP-glucose:NAD oxidoreductase." The name "UDP-glucose dehydrogenase" is also acceptable as a trivial name.

UDP-Glucuronic acid was first encountered as a thermostable factor in liver that was necessary for the formation of the glucuronide of *o*-aminophenol by liver homogenates (175). Results of preliminary studies (23) showed this factor to be a compound of uridine diphosphate and glucuronic acid; the structure shown in Reaction *25* was confirmed in further studies (68).

Early attempts to demonstrate the formation of this compound from glucuronic acid-1-P and UTP *via* a uridylyl transferase were unsuccessful. It is interesting to note that the reported forma-

tion of UDP-glucuronic acid and UDP-galacturonic acid with a uridylyl transferase from mung bean stands as the lone example of biosynthesis by this route (70). The alternate route, *viz.*, the oxidation of the glycosyl residue after attachment to the nucleoside diphosphate group—was first demonstrated by Strominger *et al.* with enzymes from the livers of various animals (168) and has since been demonstrated many times.

The stoichiometry of the dehydrogenase reaction was shown to be that given in Reaction *25* in studies with enzymes purified 180-fold from calf liver (168), 200- to 400-fold from calf liver (170), 1000-fold from pea seedlings (171), and five- to thirtyfold from the *Arthrobacter* species (174).

Kinetic studies with purified enzymes from a variety of sources gave Michaelis' constants for NAD and for UDP-glucose (169, 171, 176, 177) or UDP-mannose (174) that were generally in agreement with one another. Differences in K_m attributable to the nature of the buffer employed were pointed out in one study (169).

Inactivation of the liver enzyme by preincubation with inorganic pyrophosphate, *p*-chloromercuribenzoate, and certain carbonyl reagents was observed (169). Competitive inhibition by UDP-galactose and "uncompetitive" inhibition by UMP, UDP, and UTP was studied by Salitis and Oliver (176) and discussed with relation to evidence of disturbance of glucuronide synthesis in galactosemic infants. Maxwell and co-workers (169) observed no effect on addition of 10^{-3} *M* Zn^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, Co^{2+}, or sodium versenate.

Strominger and his co-workers found that the purified dehydrogenases from liver (170) and from pea seedlings (171) did not act on UDP-galactose, UDP-*N*-acetylglucosamine, UDP-*N*-acetylgalactosamine, GDP-mannose, glucose-1-P, glucose, or ethanol; NADP was also ineffective as a substrate for these enzymes. The GDP-mannose dehydrogenase purified by Preiss (174) was also inactive with NADP and with mannose-1-P, CDP-mannose, TDP-mannose, IDP-mannose, UDP-glucose, TDP-glucose, CDP-glucose, GDP-glucose, ADP-glucose, UDP-galactose, and TDP-galactose. This enzyme, however, did effectively utilize deoxy-GDP-mannose.

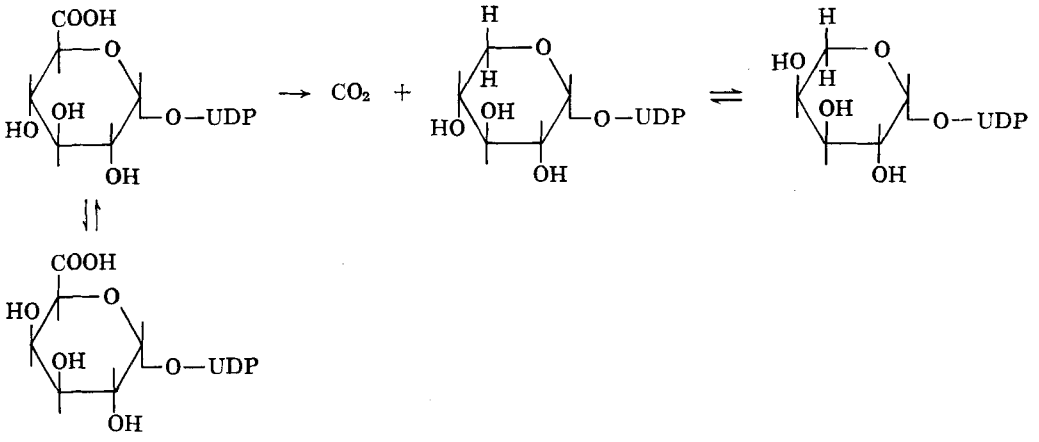
Certain analogs of both NAD and the sugar nucleotides have been found to be acted upon by these enzymes. Rabinowitz and Goldberg (82) demonstrated the reaction with pseudo-UDP-glucose. Strominger and Mapson found that the 2′,3′-*O*,*O*-diacetyl derivative of UDP-glucose was oxidized at about one-third the rate of UDP-glucose with the enzyme from pea seedlings (171). Goldberg *et al.* (177) studied the reaction of the 5-fluorouracil and 6-azauracil analogs of UDP-glucose with the liver dehydrogenase and found a considerable variation in the Michaelis' constants for these substrates at pH's greater than 7.0. These variations appeared to be related to the acidic dissociation constants of the substrates. Expression of each K_m in terms of concentration of the *undissociated* form of the pyrimidine base gave values that were very similar for the analogs and the normal substrate.

The deamino- and the acetylpyridine derivatives of NAD were found to support the two-step oxidation of UDP-glucose by the liver dehydrogenase (170).

Reversibility of the oxidation of UDP-glucose and GDP-mannose could not be demonstrated in studies with the enzyme from calf liver (170), pea seedlings (171), and the *Arthrobacter* species (174). Incubation of UDP-glucuronic acid and $NADH_2$ with the liver enzyme and with neurospora NADase (DPN-ase) to remove traces of NAD that might be produced as a result of the reverse reaction failed to give any evidence of reversal (170). In the study with the *Arthrobacter* enzyme, there was no incorporation of label from tritiated GDP-mannuronic acid into GDP-mannose in the presence of $NADH_2$ (174).

In all of the studies with these dehydrogenases, even the purest preparations catalyzed the two-step oxidation with no evidence of the formation of an intermediate oxidation state. Maxwell *et al.* (169) were unsuccessful in their attempts to trap an aldehydic intermediate by conducting the reaction in the presence of cyanide, hydroxylamine, or thiosemicarbazide. The failure to isolate a hydroxamate also tends to exclude an oxidative pathway through an acetal or mercaptal and on to a lactone, amide, or acyl mercaptal. These results leave the possibility of the intermediate existing only tightly bound to the enzyme during the oxidative process.

Decarboxylation.—Results of studies on the biosynthesis of xylans in higher plants provided some of the earliest evidence for the decarboxylation of a hexuronic acid. These studies consisted of feeding specifically labeled hexoses, pentoses, and glucuronic acid to plants and determining the activity of the carbon atoms of xylose obtained by hydrolysis of the plant xylans. The early studies have been adequately reviewed by Gibbs (178); since they are not primarily concerned with sugar nucleotides, no further detail will be presented here.



Scheme II

The first demonstration of the decarboxylation of UDP-glucuronic acid was accomplished by Neufeld *et al.* (162) with enzyme preparations from mung bean and a few other plants. The products of the reaction employing UDP-glucuronic acid as the substrate were UDP-galacturonic acid, UDP-xylose, and UDP-arabinose. Because of the presence of epimerases acting on both the uronic acid and the pentose nucleotides, these results left unsolved the question of whether one or both of the uronic acid derivatives were decarboxylated.

This same group of investigators was later able to separate the UDP-glucuronic acid 4-epimerase activity from particulate fractions of mung bean seedlings and radish root by preparing digitonin extracts (154). These extracts catalyzed the decarboxylation of UDP-glucuronic acid but not UDP-galacturonic acid. Because of the presence of UDP-pentose 4-epimerase, however, both the D-xylose and L-arabinose derivatives were formed. Kinetic studies revealed that the product of the decarboxylation in the early stages of the reaction was 85–90% UDP-D-xylose; this later epimerized to the L-arabinose derivative. Extrapolation of the results to zero time gave a value of 100% UDP-D-xylose. The sequence now believed to occur is shown in Scheme II.

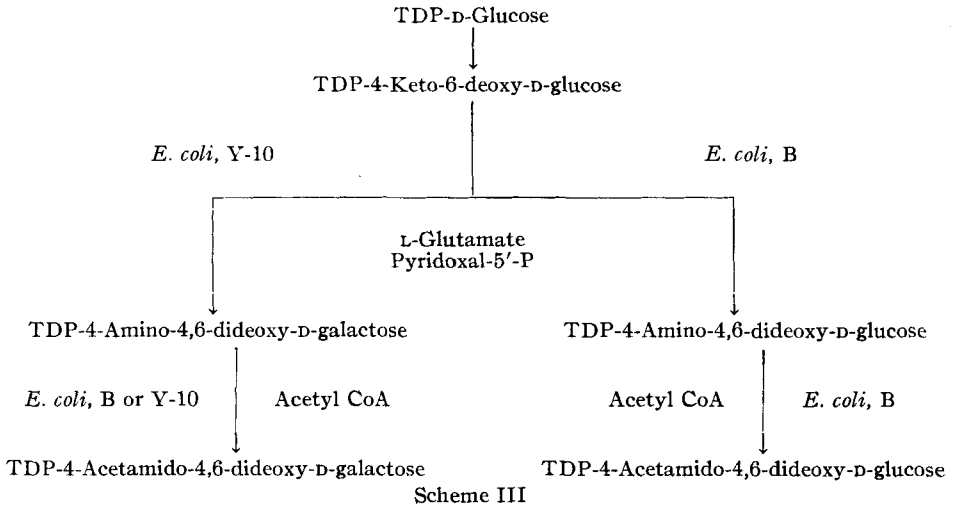
The equilibrium constant for the UDP-pentose 4-epimerase was found to be 1.0, but the K' for the decarboxylation reaction was not determined. Observations revealed, however, essentially a quantitative conversion to the pentose derivatives.

The decarboxylase is not included among the enzymes listed by the International Union of Biochemistry (1). Application of the rules of this body to its naming would provide the name "UDP-glucuronic acid carboxy-lyase."

Amination.—Table III presents numerous examples of both the occurrence and the biosynthesis of aminosugar nucleotides from the corresponding aminosugar phosphate and nucleoside triphosphate. These aminosugars are formed by reactions involving amino transfer from the amide group of glutamine to fructose-6-P (179, 180) or direct amination of fructose-6-P with ammonia (181).

Another route to the aminosugar nucleotides has been demonstrated by Matsuhashi and Strominger (182) in their study of the enzymatic synthesis of TDP-4-acetamido-4,6-dideoxy-D-glucose and TDP-4-acetamido-4,6-dideoxy-D-galactose, two nucleotides found in *E. coli*, strain B, and *E. coli*, strain Y-10, respectively. Extracts of each of these organisms were able to convert TDP-glucose to the respective 4-amino analog of the deoxysugar nucleotide associated with the organism. Glutamate was found to be the most active amino group donor; it could not be replaced by NH_4Cl plus ATP nor, to an appreciable extent, by glutamine (with a purified preparation) or several other amino acids. A requirement for pyridoxal-5′-phosphate was demonstrated. However, stoichiometric amounts of pyridoxamine-5′-phosphate did not serve as a replacement for the amino donor, glutamate. Acetyl coenzyme A was excluded from these reaction mixtures to prevent the reactions from continuing to the acetamidosugar nucleotides.

The actual acceptor for the amino group was shown to be TDP-4-keto-6-deoxy-D-glucose. This substance was utilized in preference to TDP-glucose by enzymes from both organisms. In reactions with this substrate, the requirement for L-glutamate and pyridoxal-5′-phosphate was again demonstrated. The specificity of the amination was retained by the enzymes from both



Scheme III

organisms. The reaction sequence is summarized in Scheme III.

Acetylation.—Kornfeld and Glaser (122) demonstrated the reaction shown in Reaction *26* with a partially purified enzyme from both *Pseudomonas aeruginosa* and *Streptomyces griseus*.

TDP-glucosamine + acetyl CoA $\rightleftharpoons$ TDP-*N*-acetylglucosamine + CoA Reaction *26*

The same preparation also catalyzed the acetylation of α-D-glucosamine-1-P but did not work with glucosamine-6-P or glucosamine.

The acetylation of both TDP-4-amino-4,6-dideoxy-glucose and -galactose was shown by Matsuhashi and Strominger (182). It was of interest to find that the acetylation of the galactose derivative was catalyzed by extracts of both *E. coli*, strain B and strain Y-10; this is in contrast to the reaction which immediately precedes this (see Scheme III) and which is specifically catalyzed by extracts of *E. coli*, Y-10.

The acetylation reaction constitutes one route to the acetamido-sugar nucleotides, but it is not the only route. The biosynthesis of several of these compounds has been shown to occur *via* the nucleotidyl transferase (pyrophosphorylase) reaction involving a nucleoside triphosphate and the acetamido-sugar phosphate (Table III).

Formation of Deoxysugar Derivatives.—Early studies on the biosynthesis of deoxyhexoses showed that these sugars retained the labeling pattern of radioactive hexoses administered to systems capable of synthesizing deoxyhexoses. These results indicated that the formation of deoxyhexoses from hexoses occurred without rearrangement of the carbon skeleton, but they did not provide any insight into the mechanism of the changes.

Evidence for the involvement of sugar nucleotides appeared in 1960 as a result of studies by several investigators. Ginsburg (183, 184) demonstrated a $NADPH_2$-dependent conversion of GDP-mannose to GDP-fucose with an extract of *Aerobacter aerogenes*. Pazur and Shuey (112, 185) and Kornfeld and Glaser (111, 186) independently demonstrated a similar conversion of TDP-glucose to TDP-rhamnose with bacterial extracts. In the short time since these reports, numerous other such transformations have been described. These are presented in Table V.

The diversity of these reactions with respect to the source of the enzymes, the nature of the nucleotide, as well as the nature of the sugar residue is apparent from the entries in Table V. The complexity of each of the reactions shown might not be so apparent unless careful consideration is given to the structures of the sugar moieties in both the substrate and the product. When this is done, it can be seen that each of the reactions may involve multiple epimerizations as well as reduction at one or more carbon atoms. Because of this complexity, it is very likely that more than one enzyme is required to effect the over-all reaction presented; it is equally likely that intermediate compounds exist in these conversions.

Most of the studies have shown a requirement for stoichiometric amounts of $NADPH_2$ (112, 121, 183, 184, 186, 190, 192–194, 196), $NADH_2$ (193), or a generating system for the latter (111, 166). An additional requirement for catalytic amounts of NAD (182, 186, 188, 196) or $NADPH_2$ (193) was also found in several investigations.

Studies in which these substances were omitted from the reaction mixtures provided the first substantial evidence for the existence of inter-

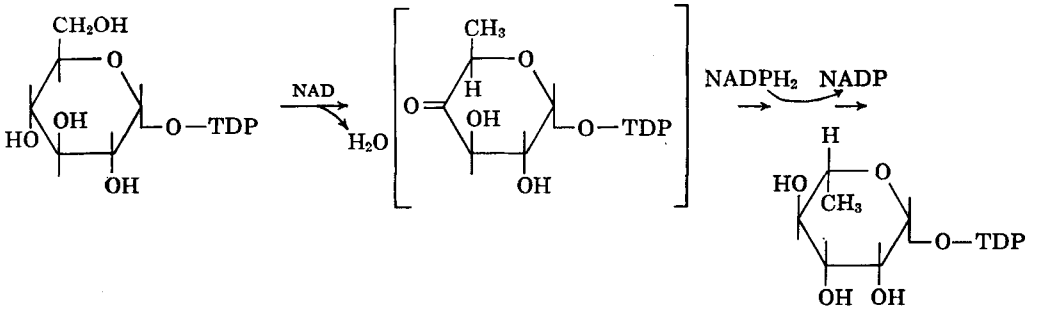
mediates. The results of earlier kinetic studies on the formation of GDP-fucose from GDP-mannose showed a lag in the disappearance of $NADPH_2$ consistent with the formation of an intermediate that did not require the net consumption of $NADPH_2$ (184). Further studies with this system demonstrated the dependence of the reaction on $NADPH_2$ and the accumulation, in the absence of $NADPH_2$, of an intermediate tentatively identified as GDP-4-keto-6-deoxy-D-mannose (183, 190). An independent study with an extract of *E. coli*, strain B, showed that TDP-glucose was converted to TDP-rhamnose when stoichiometric amounts of $NADPH_2$ were present, but that a sugar nucleotide tentatively identified as TDP-4-keto-6-deoxy-D-glucose was formed when $NADPH_2$ was absent (121, 187). Extracts of *E. coli*, strain Y-10, on the other hand, catalyzed only the formation of TDP-4-keto-6-deoxy-D-glucose in both the presence and absence of $NADPH_2$ (121, 187). This strain is not capable of producing TDP-rhamnose and is apparently blocked prior to, or just at, the step that requires $NADPH_2$.

Another study on the formation of TDP-rhamnose from TDP-glucose with an extract of *P. aeruginosa* revealed an additional requirement (186). In this case, TDP-glucose remained unchanged when pyridine nucleotides were absent from the incubation mixture. The addition of catalytic amounts of NAD caused a disappearance of TDP-glucose but failed to support the production of TDP-rhamnose. Under these conditions, an intermediate having the properties of TDP-4-keto-6-deoxy-D-glucose accumulated. The reaction sequence shown in Scheme IV was proposed to represent the findings of this study.

Evidence for the existence of other keto-deoxyhexose nucleotide intermediates has been obtained in several other studies (182, 188, 192, 193, 195, 196). These intermediates have not been isolated because of their lability. However, substantial data on their properties and reactions provided reasonable support for their identity.

TABLE V.—OCCURRENCE OF ENZYMES CATALYZING THE FORMATION OF DEOXYHEXOSE NUCLEOTIDES

Reaction	Source	Ref.
TDP-D-glucose ⇌ TDP-L-rhamnose	*Streptococcus faecalis*	(112, 185, 187)
	Bacillus subtilis	(112)
	Alfalfa seeds	(112)
	Pseudomonas aeruginosa	(111, 186)
	Escherichia coli	(112, 114, 119, 121, 187, 188)
	Streptomyces griseus	(166)
	Salmonella weslaco	(114, 119)
	Salmonella berlin	(114)
	Alcaligenes faecalis	(114)
TDP-D-glucose ⇌ TDP-4-keto-6-deoxy-D-glucose	*Escherichia coli*, Y-10	(121)
TDP-D-glucose ⇌ TDP-3-acetamido-3,6-dideoxy-hexose	*Xanthomonas campestris*	(189)
TDP-D-glucose ⇌ TDP-4-acetamido-4,6-dideoxy-glucose	*Escherichia coli*, Y-10	(182)
TDP-D-glucose ⇌ TDP-4-acetamido-4,6-dideoxy-galactose	*Escherichia coli*, B	(182)
GDP-D-mannose ⇌ GDP-L-fucose	*Aerobacter aerogenes*	(190)
GDP-D-mannose ⇌ GDP-L-colitose	*Escherichia coli*	(191, 192)
GDP-D-mannose ⇌ GDP-D-rhamnose	Gram-negative bacteria	(193)
GDP-D-mannose ⇌ GDP-D-talomethylose	Gram-negative bacteria	(193)
UDP-D-glucose ⇌ UDP-L-rhamnose	Mung bean	(194)
	Tobacco	(195)
CDP-D-glucose ⇌ CDP-4-keto-6-deoxy-D-glucose	*Pasteurella pseudotuberculosis*	(188)
CDP-D-glucose ⇌ CDP-L-ascarylose	*Pasteurella pseudotuberculosis*	(196)



Scheme IV

These intermediates are the result of an intramolecular oxidation-reduction and a dehydration of the substrate. Therefore, the requirement for stoichiometric amounts of an external oxidant or reductant would not be expected. Catalytic amounts of such agents, however, might be required to mediate the reaction, and indeed, this appears to be the case with certain systems (186). In other systems, a stimulation by NAD, but not an absolute requirement for this compound, was shown in the formation of GDP-fucose (183, 184, 190). In studies with an extract of mung bean, all attempts to demonstrate a requirement for NAD or $NADH_2$ in the conversion of UDP-glucose to UDP-rhamnose were unsuccessful (194).

While there is no basis for expecting a universal requirement for NAD in the intramolecular oxidation-reduction, the results of a recent study indicate that some enzymes might contain tightly bound NAD that cannot be removed by the milder procedures. Such was the case for the enzyme from *E. coli*, strain B (188). This behavior is very similar to that observed with the UDP-glucose 4-epimerase from yeast. (See under *Epimerization.*)

In one study (193), the conversion of GDP-D-mannose to GDP-4-keto-D-rhamnose (an intramolecular oxidation-reduction) required catalytic amounts of $NADPH_2$. This could not be replaced by NADP, NAD, or $NADH_2$. The conversion of this intermediate to a mixture of GDP-D-rhamnose and GDP-D-talomethylose required stoichiometric amounts of $NADH_2$ or $NADPH_2$.

Investigations of the specificity of the enzymes involved in deoxyhexose nucleotide formation indicate that absolute specificity is the rule rather than the exception (188, 194, 196). Attempts to demonstrate reversibility have been unsuccessful (188, 193). In some cases, the keto-deoxyhexose intermediate appears to be a branch point from which deoxyhexose formation or amino-deoxyhexose formation can occur (182). (See under *Amination* and *Acetylation.*)

Present knowledge of the biosynthesis of the dideoxyhexoses (191, 192, 196) goes no further than that for the deoxyhexoses. The problem of the mechanism of the epimerizations that occur in deoxyhexose formation requires much further work. A comprehensive review of the biosynthesis of deoxy sugars appeared in 1963 (197).

Other Transformations.—The transformations of sugar nucleotides discussed in the preceding sections constitute the major types of reactions that lead to the formation of new sugar derivatives.

Evidence for the formation of phosphate and sulfate esters of sugar nucleotides is lacking despite the natural occurrence of such compounds (79).

Several hydrolytic and phosphorolytic reactions of sugar nucleotides have been reported (198–201). Reaction *27* represents one example (200).

$$\text{ADP-glucose} + P_i \rightleftharpoons \text{ADP} + \text{glucose-1-P} \quad \text{Reaction } 27$$

These reactions are believed to be involved in the breakdown of sugar nucleotides rather than in their synthesis because hydrolysis or phosphorolysis is strongly favored.

The introduction of a lactic acid residue to give a muramic acid derivative and the subsequent stepwise addition of one or more amino acid residues are transformations of sugar nucleotides that have not been considered because these substances ultimately find their way into glycopeptides rather than the simpler types of glycosides dealt with in this presentation. Much has been accomplished in this area, however, and many of its problems are currently receiving considerable attention (202–212).

Glycosyl Transfer from Sugar Nucleotides

The reactions that have taken place up to this point have served to provide the necessary thermodynamic potential for the glycosyl residues to participate in the formation of new glycosidic bonds. Some of the reactions have, in addition, brought about modifications of the glycosyl residues, thereby creating new structures capable of entering into glycosidic linkage with other substances.

The final step in the formation of a new glycosidic bond is the transfer of the glycosyl group of the sugar nucleotide to an acceptor, as shown by the general equation

$$\text{R-O-glycosyl} + \text{XOH} \rightleftharpoons \text{glycosyl-O-X} + \text{ROH} \quad \text{Reaction } 28$$

where R is a nucleoside di- (or mono)phosphate, and XOH is a suitable acceptor.

Such reactions are catalyzed by a class of enzymes called glycosyltransferases. The recommended nomenclature (1), therefore, follows the convention given in *Footnote 2.*

The relatively high energy of the glycosyl ester linkage of the sugar nucleotides (about 8000 cal./mole) as compared to the usual glycosidic linkages (4000–5000 cal./mole) causes these reactions to proceed strongly in the direction of glycoside synthesis.

Several of these reactions have been shown to require a divalent cation: Mg^{2+} is preferred in the greatest number of cases, while Mn^{2+}, Co^{2+},

Ni^{2+}, and Fe^{2+} have been found to support some activity in certain instances. In many cases, no requirement for a cofactor or activator has been shown.

The nature of the reactants and products in these reactions are quite diverse, and many of the reactions have important characteristics that are not shared by the others. For this reason, the glycosyl transfer reaction leading to the formation of each glycoside will be discussed separately according to the nature of the product.

Trehalose.—Trehalose, 1-(α-D-glucopyranosyl)-α-D-glucopyranoside, was the first disaccharide shown to be formed by a glycosyl transfer reaction involving a sugar nucleotide (24). Its synthesis (as the phosphate) was effected by an enzyme from yeast according to the following reaction:

UDP-glucose + glucose-6-P → trehalose-P + UDP Reaction *29*

The yeast preparation also contained a phosphatase that was specific for trehalose phosphate. The action of this enzyme in catalyzing the highly exergonic hydrolysis of the phosphate ester linkage makes the reaction between UDP-glucose and glucose-6-P quantitative.

Some difficulty was encountered in obtaining reliable values for the K' of the reaction shown in Reaction *29*. In a study with the yeast enzyme, purified fifteen- to twentyfold, Cabib and Leloir (213) reported a minimum value of 40 for K'. This corresponds to a $\Delta F^{0\prime}$ of about -2200 cal./mole and it gives a maximum value of -5800 cal./mole for the $\Delta F^{0\prime}$ of hydrolysis of the glycosidic bond of trehalose phosphate when -8000 cal./mole is used as the $\Delta F^{0\prime}$ of hydrolysis of UDP-glucose. In the same study, these authors failed to demonstrate reversibility of this reaction; they also failed to observe a reaction with glucose-1-P as acceptors.

Similar syntheses of trehalose have been reported with extracts of the fat body of certain insects (214–216).

Sucrose.—The enzymatic synthesis of sucrose by a reaction involving sugar nucleotides was first demonstrated with an enzyme from wheat germ (25). The report of this reaction appeared very shortly after the announcement of trehalose synthesis:

USD-glucose + fructose ⇌ sucrose + UDP Reaction *30*

Reaction *30* was later shown to be catalyzed by extracts of several plants (132).

Another reaction that was catalyzed by an extract of wheat germ, but which had earlier escaped detection because of the presence of phosphatases in the extract, is Reaction *31* (217):

UDP-glucose + fructose-6-P ⇌ sucrose-P + UDP Reaction *31*

Enzymes exhibiting specificity for fructose (31, 134), for fructose-6-P (41, 218), or capable of utilizing either of these compounds (219–221) have since been demonstrated in a variety of plants. In addition, an enzyme from green peas has been shown to utilize xylulose and rhamnulose as acceptors for the glucosyl group (220).

Specificity for the nucleoside diphosphate has also been studied. Avigad (134) found that the enzyme from Jerusalem artichoke would react with UDP and not with ADP, CDP, TDP, or GDP in Reaction *30* studied in reverse. Gabrielyan *et al.* (222) obtained sucrose synthesis with CDP-glucose as well as with the 3-*N*-methyl, the 4-thio, and the 6-aza analogs of UDP-glucose.

The ready reversibility of Reactions *30* and *31* (in the absence of phosphatases) stems from the relatively high energy of the glycosidic bond of sucrose. The $\Delta F^{0\prime}$ of hydrolysis of this bond has been estimated to be -6570 cal./mole (223) and -7750 cal./mole (7). Cardini *et al.* (132) reported a K' = about 5 for Reaction *30;* this corresponds to a $\Delta F^{0\prime}$ of about -1000 cal./mole. More recently, Avigad (134) found K' = 1.4 and 1.8 for Reaction *30* in two different experiments with an enzyme preparation having no demonstrable sucrase activity. The equilibrium for Reaction *31*, when coupled to the action of a phosphate, would be shifted far to the right.

Neufeld and Hassid (224) summarized the results of *in vivo* labeling experiments and suggested that Reaction *31* is the principal route for sucrose biosynthesis and that Reaction *30* represents a mechanism for the breakdown of sucrose with conservation of the energy of its glycosidic bond in the form of UDP-glucose. Subsequent transfer of the glucosyl residue of UDP-glucose to a suitable acceptor would cause a net transfer of the glucosyl group from sucrose to starch, for example. Their predictions were substantiated in recent experiments in which radioactivity from sucrose was transferred to starch and phytoglycogen through the intermediaries, UDP-glucose or ADP-glucose (52, 127, 135, 225).

Lactose.—The biosynthesis of lactose takes place according to the sequence shown by Reactions *9–11*. This route was clearly established by Watkins and Hassid (226) with particulate preparations from mammary glands of guinea pigs and cows. Reference to Tables III and IV will show that there are numerous examples of

the uridylyl transferases and 4-epimerases that catalyze Reactions *9* and *10*, respectively.

An earlier report described the transfer of the galactosyl group from UDP-galactose to glucose-1-P with the formation of lactosyl phosphate (227). This transfer was catalyzed by an enzyme from bovine mammary tissue. However, it has not been generally accepted because it has not been confirmed, and it would give rise to a labeling pattern inconsistent with that found by Wood *et al.* (228) in their study of labeling patterns given by different precursors.

A soluble preparation from bovine milk has recently been found to catalyze Reaction *11*. This galactosyl transferase requires Mg^{2+} or Mn^{2+} for activity (229).

Other Disaccharides.—Smith and Mills (230) obtained a particulate preparation from types II, III, and VIII pneumococcus that catalyzed the synthesis of a nonreducing disaccharide from UDP-glucose. The product was tentatively identified as 1-(β-D-glucopyranosyl)-β-D-glucopyranoside.

Frydman and Neufeld (231) found a galactosyl transferase in an extract of unripe pea seeds that catalyzed the following reaction:

UDP-galactose + myoinositol ⇌ 1-*O*-(α-D-galactopyranosyl)-myoinositol Reaction *32*

The product, galactinol, is known to occur in sugar beet. A study of this reaction showed that Mn^{2+} was required and that it could be replaced to some extent with Co^{2+} or Fe^{2+}, but not with Mg^{2+}, Ca^{2+}, Zn^{2+}, or Ni^{2+}. The enzyme was specific for UDP-galactose but was capable of utilizing the following inositols, in order of decreasing effectiveness: *myo-*, *dextro-*, *levo-*, and *scyllo*-inositol. Other sugars, hexitols, and alcohols were tested and found to be ineffective as acceptors.

Glycogen.—The biosynthesis of the α-1,4-glycosidic linkage of glycogen takes place according to

UDP-glucose + $(glucose)_n$ → glucosyl-α-(1 → 4)-$(glucose)_n$ + UDP Reaction *33*

The enzyme that catalyzes this reaction, UDP-glucose:α-1,4-glucan α-4-glucosyltransferase, is commonly known as "glycogen synthetase," and it has been found in various animal tissues (45, 49, 232–253), insects (254–256), crustaceans (253, 257), yeast (258, 259), bacteria (260), and a mold (253).

The biosynthesis of phytoglycogen from sugar nucleotides has been studied with enzymes from corn (225) and an *Arthrobacter* species (55). These studies are discussed under *Starch* since the enzymes and reactions resemble more strongly those functioning in starch synthesis.

In all cases, UDP-glucose has been found to be the preferred glucose donor in the glycogen synthetase reaction. With the synthetases from muscle, liver, and yeast, ADP-glucose was found to be about one-half as effective as UDP-glucose; and CDP-glucose, IDP-glucose, and ADP-maltose were found to be inactive as glucose donors (261). TDP-Glucose was found to be about one-twentieth as effective as UDP-glucose with the muscle enzyme (242), and it was estimated to be less effective in histochemical demonstrations of glycogen synthesis by mammalian skeletal muscle sections (245). Pseudo-UDP-glucose was shown to serve as a substrate for the enzyme from rat liver (82).

Goldemberg (261) also studied acceptor preference and found that glycogen was highly preferred; other poly- and oligosaccharides can be used by rat muscle glycogen synthetase, but at a reduced rate. Revised values for relative reaction rates after correction for numerical errors were reported by Leloir (262).

It should be noted that glycogen synthetase catalyzes only the formation of α-1,4-glucosidic bonds (233). The formation of the branch points of glycogen (α-1,6-bonds) requires the participation of the so-called "branching enzyme" which is a glucosyl transferase of the type discussed under *Transglycosylases*.

Although glycogen synthesis *via* phosphorylase action is energetically feasible, much evidence has been accumulated to preclude this route as the major synthetic pathway to glycogen. Glycogen phosphorylase is currently believed to function almost exclusively in glycogen breakdown under normal conditions, and glycogen synthetase is given the synthetic role. The evidence for these conclusions has been summarized recently (224, 263, 264) and consists generally of the following observations: glycogen synthesis takes place when the ratio of inorganic phosphate to glucose-1-phosphate is much greater than that which exists at equilibrium for the phosphorylase reaction; agents, such as epinephrine and glucagon, that increase glycogen breakdown increase the activity of phosphorylase and reduce glycogen synthetase activity (265); agents that enhance glycogen synthesis, *e.g.*, K^+, decrease the activity of phosphorylase; insulin increases the activity of glycogen synthetase (266, 267) while it has no effect on phosphorylase activity (265); and the phosphorylase activity is greatly diminished in

liver and muscle afflicted with certain diseases characterized by an abnormally high tissue glycogen content.

The synthesis of glycogen by glycogen synthetase proceeds almost irreversibly (45, 235). Depending on the values used for the $\Delta F^{0\prime}$ of hydrolysis of UDP-glucose (−8050 or −8750 cal./mole) and the α-1,4-glycosidic bond of glycogen (−5030 cal./mole), a $\Delta F^{0\prime}$ of −3020 or −3720 cal./mole for the synthetase reaction is obtained; these values correspond to K' of approximately 150 and 470, respectively.

Most of the glycogen synthetase activity reported has been associated with particulate fractions, especially fractions that contain glycogen. The enzyme cannot be easily separated from glycogen, and it usually suffers a loss of activity when separation is attempted. Hauk and Brown (234) reported that the enzyme from rabbit muscle could be obtained in the soluble fraction if their preparations were frozen first. Steiner (240) found that dissociation of the rat liver enzyme from glycogen was accompanied by loss of activity; reactivation could be accomplished, however, by the addition of glucose-6-P or potassium fluoride to the supernate. The reactivated enzyme was thus obtained in a soluble fraction. In view of the more recent discovery of two forms of glycogen synthetase (243), many of the early interpretations and conclusions concerning the properties of glycogen synthetase might be reconsidered.

A very important segment of the work on glycogen metabolism has been the study of factors that might participate in a physiological control system for the synthesis of this primary carbohydrate. Evidence for the existence of two forms of glycogen phosphorylase, *A* (active) and *B* (inactive), and of factors controlling the interconversion of these two forms is well known and can be found in most of the recent biochemistry textbooks or review series (268). Similar control mechanisms have been demonstrated for glycogen synthetase.

The discovery of the stimulation of glycogen synthetase by glucose-6-P (233) provoked thoughts of the participation of this substance in a control system. The stimulatory power, however, was not found to be consistent in different investigations. Traut and Lipmann (253) contributed toward a clarification of some of the controversies that arose by demonstrating a wide variation among the enzymes obtained from several different species.

In addition to its stimulatory effect, glucose-6-P has also been found to protect the synthetase against inactivation (240) and to reactivate preparations that had lost activity as a result of long storage (240, 242).

Evidence was provided for the existence of two different forms of glycogen synthetase when it was found that extracts prepared from insulin-treated rat hemidiaphragms exhibited increased activity when measured in the absence of glucose-6-P. The synthetase activity of normal and insulin-treated hemidiaphragms was the same when measured in the presence of glucose-6-P (269, 270). Administration of insulin to alloxan-diabetic rats also caused an increased synthetase activity and a decreased dependency on glucose-6-P (239). This effect of insulin did not appear to be explained by increased concentrations of glucose-6-P in the tissues or the extracts (239, 270–272).

Larner and his associates, in a series of studies (266, 273–276), unequivocally established the existence of two forms of glycogen synthetase in preparations from rat, rabbit, and dog. One was dependent on glucose-6-P (D form), and the other was independent of glucose-6-P (I form) for its activity. The interconversion of these two forms was shown to take place according to

$$\text{I form} \underset{\text{dephosphorylation}}{\overset{\text{ATP} + \text{Mg}^{2+}\text{ phosphorylation}}{\rightleftharpoons}} \text{D form} \qquad \text{Reaction } 34$$

The conversion of the I to the D form was enhanced by the addition of adenosine-3,5-cyclophosphate to the system containing ATP + Mg^{2+} (276, 277). Appleman *et al.* (277) also obtained an increase in the D form by treatment with Ca^{2+} or with trypsin.

Kinetic studies (275) with the highly purified D form (600-fold) and I form (150-fold) from rabbit skeletal muscle gave the following results. For the I form, the addition of glucose-6-P or glucose-6-P plus Mg^{2+} caused a seven- to eightfold reduction in the K_m for UDP-glucose; the addition of Mg^{2+} alone caused a slight reduction in the K_m, and none of the additions affected V. For the D form, the addition of glucose-6-P or glucose-6-P plus Mg^{2+} caused a twentyfold decrease in the K_m and a forty- to fiftyfold increase in V, while the addition of Mg^{2+} alone caused a slight increase in K_m and did not affect V. Lesser stimulatory or inhibitory effects were given by Ca^{2+}, SO_4^{2-}, and PO_4^{3-}.

A study of more than 30 compounds showed that the structural features required of an activator were: hydroxyl groups at carbon atoms 2, 3, and 4, a phosphate attached to carbon 6, and a pyranose ring structure (278).

Epinephrine was found to reduce the activity of both the I and the D form of glycogen synthetase in intact rat diaphragms. Insulin, on the other hand, increased the activity of the I form and decreased the activity of the D form (265). Danforth and Harvey (279) found an inverse relationship between the amount of the I form of the enzyme and the level of glycogen in the isolated rat diaphragm. Other interrelationships between factors controlling glycogen phosphorylase and glycogen synthetase activity are thoroughly discussed in the proceedings of a recent symposium (264).

Starch.—The biosynthesis of the α-1,4-glucosidic linkages of starch was thought for sometime to be effected by a phosphorylase. The following observations, however, supported arguments against this pathway.

The inorganic phosphate levels in plant cells are such that phosphorolysis, rather than synthesis, of starch would occur (280); starch synthesis takes place in the plastids, whereas phosphorylase is found in the soluble portion of the cytoplasm (281).

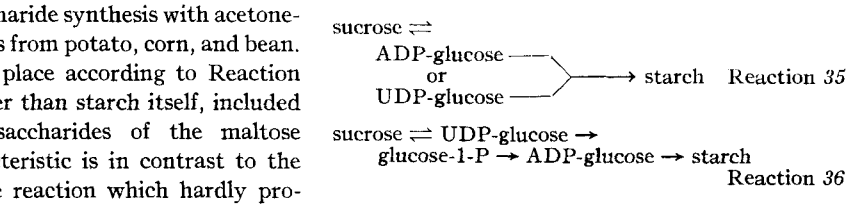
Following the demonstration of glycogen synthesis by a glycosyl transfer reaction with UDP-glucose as the donor, Leloir *et al.* (282) undertook studies to demonstrate a similar mechanism for starch synthesis. They succeeded in obtaining starch and oligosaccharide synthesis with acetone-dried starch granules from potato, corn, and bean. The reaction takes place according to Reaction *33*. Acceptors, other than starch itself, included maltose and oligosaccharides of the maltose series. This characteristic is in contrast to the glycogen synthetase reaction which hardly proceeds when maltose is used as the acceptor.

Only α-1,4-glycosidic linkages were formed in this reaction. The introduction of branch points (α-1,6-linkages) would require the participation of a transferring enzyme of the type discussed under *Transglycosylases*.

Shortly after the discovery of starch synthesis *via* glucosyl transfer from UDP-glucose, it was found that ADP-glucose was approximately ten times more active as a glucose donor in this reaction (283). IDP-glucose, CDP-glucose, and GDP-glucose were not active as glucose donors. The subsequent discovery of the natural occurrence and biosynthesis of ADP-glucose (see Table III) provided additional support for the assignment of a major synthetic role to this substrate. Other evidence in support of ADP-glucose came from the experiments of Kauss and Kandler (32). These investigators determined the ratio of ADP-glucose to UDP-glucose in *Chlorella* cells 15 sec. after the initiation of photosynthesis. The determinations were made with cultures at four different temperatures, and the results were compared to corresponding values for the ratio of sucrose to starch. The findings were consistent with the view that starch synthesis was dependent on ADP-glucose and sucrose synthesis on UDP-glucose. In all of the other studies on starch synthesis, ADP-glucose has always been found to be a more effective substrate than UDP-glucose.

All of the enzymes for the synthesis of starch from glucose-1-P and UTP were found in rice grains (52). However, Murata *et al.* (125) isolated ADP-glucose from rice grains and showed it to be three times as active as UDP-glucose in starch synthesis. The synthesis of starch from either UDP-glucose or ADP-glucose was also demonstrated with a preparation from corn endosperm (135). Enzyme preparations from waxy mutants of maize and from soybean leaves, in contrast, utilized only ADP-glucose in starch synthesis (284, 285).

The incorporation of the glucose residue of sucrose into starch in the presence of ADP or UDP was observed with preparations from both corn (135) and rice (52, 127). Two possible sequences were proposed to explain these observations (135):



The route shown in Reaction *35 via* ADP-glucose was demonstrated by Murata *et al.* (127) with a preparation from rice grains. These same workers reported the very interesting observation that glucose transferred from ADP-glucose went largely into the amylopectin fraction and glucose transferred from UDP-glucose went about equally into the amylose and amylopectin fractions. Interestingly, only ADP-glucose was found to serve as a glucose donor with a preparation from the maternal tissue of a waxy mutant of maize; this mutant contains in its endosperm starch that is almost entirely amylopectin (284).

The enzymes for starch synthesis are closely associated with starch granules, and there are no reports of successes in dissociating the enzymatic activity from the granules. Two recent reports described the enzymatic synthesis of glycogen with soluble preparations from corn (225) and an *Arthrobacter* species (55). In both cases, the

enzymes were specific for ADP-glucose and had an absolute requirement for a primer; this requirement could be satisfied by animal or phytoglycogen.

Cellulose.—Surprisingly little has been accomplished in the study of the biosynthesis of cellulose. None of the work reported in this area had utilized extracts of higher plants until very recently. For some time, the study by Glaser (286, 287) with particulate preparations from *Acetobacter xylinum* provided the only direct evidence for the involvement of UDP-glucose in cellulose synthesis. In this work, he was able to demonstrate the transfer of glucose residues from UDP-glucose to a soluble cellodextrin with the formation of β-1,4-linkages. The cellodextrin primer was essential, and reversibility could not be demonstrated. Only 1–2% of the radioactivity supplied as UDP-glucose was recovered in the product.

Elbein *et al.* (288) very recently described the enzymatic incorporation of glucose from GDP-glucose into cellulose with a particulate preparation from beans. Other sugar nucleotides were ineffective as glucose donors.

Brummond and Gibbons (289) were also recently able to demonstrate cellulose synthesis with a particulate preparation from *Lupinus albus*. They obtained 50–100% better incorporation of labeled glucose from UDP-glucose than from GDP-glucose. Experiments conducted in the presence of both of these sugar nucleotides, only one of which was labeled at one time, showed that there was essentially no interference with the rate of incorporation of the radioactivity by the unlabeled substrate. Such evidence was offered as support for the presence of two enzymes, each specific for a single donor.

The recent demonstration of the occurrence of UDP-glucose in the perchloric acid extract of the cellulose pellicles and in the liquid culture medium of *A. xylinum* is additional support for the involvement of this nucleotide in cellulose biosynthesis (34).

Chitin.—This β-D-1,4-linked polymer of *N*-acetylglucosamine is an important polysaccharide in the skeletal structure of crustaceans and in the structure of fungal cell walls. Its biosynthesis was studied by Glaser and Brown (290) with a particulate preparation from *Neurospora crassa*. These studies showed its formation by the following reaction:

$$\text{UDP-}N\text{-acetylglucosamine} + (\text{acetylglucosamine})_n \rightleftharpoons (\text{acetylglucosamine})_{n+1} + \text{UDP} \quad \text{Reaction } 37$$

A soluble chitodextrin was required as a primer; chitodextrins of high molecular weight were more effective than those of lower molecular weight. Reversibility could be demonstrated by employing a very high concentration of UDP. The enzyme could be solubilized by treatment with *n*-butanol, but the soluble enzyme catalyzed only the formation of the lower molecular weight chitodextrins.

The incorporation of acetylglucosamine into chitin by a similar reaction was also demonstrated with preparations from crustaceans (76).

Other Homopolysaccharides.—The formation of an insoluble β-D-1,3-glucan, similar to callose, from UDP-glucose was shown with a particulate preparation from mung bean seedlings (291). The enzyme could be solubilized by digitonin treatment. Studies with the purified enzyme established a requirement for glucose or for certain glucosides; these substances were shown not to function as primers or acceptors, but their exact role was not determined.

The synthesis of another β-1,3-glucan, paramylon, by a glucosyl transferase from *Euglena gracilis* was recently reported (292). The enzyme, called "paramylon synthetase," was associated with particulate matter, but it could be extracted with sodium deoxycholate. Activity was obtained without the addition of a primer, even after treating the preparation with a β-1,3-glucanase to hydrolyze any residual glucan. The enzyme appeared to be specific for UDP-α-D-glucose; no incorporation of glucose was obtained from ADP-glucose, TDP-glucose, or UDP-β-D-glucose.

Xylans are generally believed to be formed by xylosyl transfer from UDP-xylose. The natural occurrence of this sugar nucleotide as well as its biosynthesis have been discussed earlier (see Table III and *Transformations of Sugar Nucleotides*). However, there is only one report of the enzymatic synthesis of a β-1,4-xylose polymer (293). In this study, the product was actually a xylose oligosaccharide with three to six xylose residues. Each oligosaccharide was formed from an acceptor that contained one fewer xylose residues (two to five). The transfer of the xylosyl group from UDP-xylose was catalyzed by a particulate preparation from asparagus; the transfer of only one xylose unit was effected.

The transfer of mannose residues from GDP-mannose to yeast mannan has been accomplished with a particulate enzyme (294).

Colominic acid, a homopolymer of *N*-acetylneuraminic acid, is synthesized by an unusual pathway in that the glycosyl donor is a nucleoside *mono*phosphate derivative, CMP-*N*-acetylneura-

minic acid. Aminoff *et al.* (295) obtained a particulate preparation from *E. coli*, K-235, that catalyzed the incorporation of *N*-acetylneuraminic acid residues into colominic acid. The preparations contained small amounts of endogenous or bound colominic acid, so conclusions regarding primer requirements cannot be made.

Smith and co-workers (296) reported the synthesis of a polymer of D-galacturonic acid from UDP-galacturonic acid by an enzyme from type I pneumococcus. The product, however, did not appear to be the natural capsular polysaccharide of this organism.

Mucopolysaccharides.—Mucopolysaccharides are complex polysaccharides composed of uronic acids and amino- or acetamino-sugars sometimes esterified with sulfuric acid. This class of polysaccharides includes such substances as hyaluronic acid, heparin, and chondroitin sulfate.

Hyaluronic acid was the first polysaccharide shown to be synthesized from a sugar nucleotide (297). In this study, Glaser and Brown demonstrated the incorporation of radioactivity from UDP-*N*-acetylglucosamine-^{14}C into a low molecular weight mucopolysaccharide having the structure of hyaluronic acid. The reaction was catalyzed by an extract of Rous chicken sarcoma and was conducted in the presence of UDP-glucuronic acid to insure a supply of glucuronic acid residues.

The synthesis was studied in more detail by Markovitz *et al.* (173) with a particulate preparation from group *A* Streptococcus. This organism produces a capsule composed of hyaluronic acid. Radioactivity from tritium-labeled UDP-*N*-acetylglucosamine and UDP-glucuronic acid was incorporated into the polymer. The residue which became labeled in the product was the same as the one labeled as a nucleotide precursor. A requirement for Mg^{2+} was demonstrated, but the preparations were not sufficiently purified to permit any conclusions concerning a requirement for a primer.

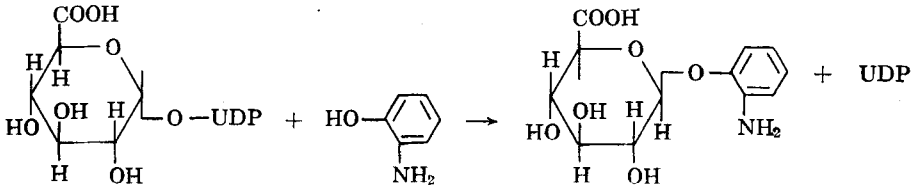
Silbert (298) obtained incorporation of radioactivity into a nonsulfated, acetylated polysaccharide that resembled heparin from the following precursors: UDP-glucuronic acid-^{14}C, UDP-*N*-acetyl-^{3}H-glucosamine, and UDP-*N*-acetylglucosamine-^{14}C. A microsomal preparation from mouse mast cell tumors provided the enzymes for this incorporation. The results showed that maximum incorporation requires the presence of both UDP-glucuronic acid and UDP-*N*-acetylglucosamine, that the glycosyl moieties of these two precursors are incorporated in equimolar amounts, and that UDP-glucosamine cannot substitute for UDP-*N*-acetylglucosamine.

Similar results were obtained by Silbert (299) in a study of the biosynthesis of a polysaccharide described as being a desulfated chondroitin sulfate. In this case, UDP-glucuronic acid and UDP-*N*-acetylgalactosamine were the substrates; the enzyme was obtained from chick embryo epiphyses. Perlman *et al.* (159) obtained a particulate preparation from this same source that contained, in addition to the transferase, a 4-epimerase that acted on UDP-*N*-acetylgalactosamine and a sulfotransferase that catalyzed the incorporation of sulfate into the polysaccharide. The latter two enzymes could be removed by washing the particles. The residue was capable of catalyzing the formation of a nonsulfated polysaccharide; this was offered in support of the belief that sulfation occurs after polymerization. The earlier isolation of UDP-*N*-acetylglucosamine sulfate from hen oviduct was support for arguments in favor of sulfation of the glycosyl residue prior to incorporation into a polymer (79).

Sussman and Osborn (300) studied the activity of a galactosyl transferase at various stages during the developmental process of the slime mold, *Dictyostelium discoideum*. This enzyme catalyzes the transfer of galactose residues from UDP-galactose to a mucopolysaccharide. The enzyme was found to be absent in vegetative cells; it appeared later in the developmental process, reached its peak activity just prior to fruiting, and then disappeared.

Lipopolysaccharides.—Considerable progress has been made in the study of the biosynthesis of the lipopolysaccharide of *Salmonella typhimurium*. The lipopolysaccharide in the cell wall of this organism contains D-glucose, a heptose, D-galactose, D-mannose, L-rhamnose, and abequose. A mutant, lacking UDP-galactose 4-epimerase, was unable to produce a galactose-containing lipopolysaccharide when grown in the absence of D-galactose. The galactose-deficient lipopolysaccharide was found to contain only D-glucose and the heptose; presumably, the other three sugars are incorporated, in sequence, after D-galactose (103). The failure to incorporate D-galactose because of a deficiency of the galactosyl transferase was precluded by the demonstration of an enzyme capable of transferring galactose from UDP-galactose to the galactose-deficient lipopolysaccharide (301, 302).

Another mutant, lacking phosphoglucose isomerase, was unable to produce UDP-glucose when grown on a glucose-deficient medium. Consequently, it produced a glucose-deficient lipopolysaccharide. This mutant was shown to contain an enzyme capable of transferring glucose residues from UDP-glucose to the glucose-deficient lipopolysaccharide (303). This enzyme



Scheme V

as well as the galactosyl transferase from the epimerase-less mutant have recently been obtained in the soluble form (303).

Teichoic Acids.—Two types of teichoic acids have been described. The teichoic acid in the cell walls of *Staphylococcus aureus* is a ribitol phosphate polymer with *N*-acetyl-D-glucosamine residues at position 4 of each D-ribitol-5-phosphate unit; D-alanine ester residues are attached to position 2 or 3 of the ribitol; and the *N*-acetylglucosaminyl residues are attached by both α- and β-glycosidic linkages.

The teichoic acid found in the cell walls of *Staphylococcus albus* is a glycerol phosphate polymer consisting of a chain of about 18 units. Approximately every third unit bears an *N*-acetylgalactosaminyl group bound to carbon atom 2 by an α- or β-glycosidic linkage; the remaining glycerol units bear D-alanyl groups attached by ester linkages to carbon atom 2; and most of the glycosidic linkages are of the α-configuration.

Nathenson and Strominger (304) obtained an enzyme from *S. aureus* that catalyzed the transfer of *N*-acetylglucosamine residues from UDP-*N*-acetylglucosamine to a modified polyribitol phosphate. The best acceptor was teichoic acid (ribitol-type) that had been treated with a β-aminidase. Both α- and β-glycosidic bonds were created in this transfer; the relative proportion of each of these types was about the same as the proportion that exists in the natural polymer. There were indications that the transfer to each type of linkage, α and β, was catalyzed by a different enzyme. The reaction required a divalent cation; Mg^{2+} was the most effective, but it could be replaced by Co^{2+}, Ca^{2+}, and, to a very slight degree, Mn^{2+}.

Glucuronides.—The transfer of the glucuronic acid residue from UDP-glucuronic acid to *o*-aminophenol was the first example of a glycosyl transfer from a sugar nucleotide (175). (Scheme V.)

Glucuronyl transferases have since been found in a wide variety of animal tissues and have been shown to utilize many different types of acceptors. Among the latter are phenols (48, 305–307), menthol (23, 308), tetrahydrocortisone (309) phenolphthalein (309), L-thyronine (309), anthranilic acid, and *o*-aminobenzoic acid (306–308). Most of the glucuronides have been shown to possess the β-glycosidic linkage as depicted in Scheme V. When organic acids serve as the acceptors, as in the last two examples above, the linkage is through an ester bond.

A stimulatory effect of Mg^{2+} was first reported with a crude enzyme preparation from rabbit liver (305). This was confirmed recently in studies with a purified soluble enzyme (310). A stimulation of the glucuronyl transferase by ATP and ADP-*N*-acetylglucosamine was also observed (311).

The apparent lack of specificity of many of the glucuronyl transferases might be attributed to some degree to the presence of more than one enzyme in the crude preparations that have been employed in most studies. Attempts to gain an insight into this question have been hampered by the difficulty in solubilizing these enzymes without loss of activity. Solubilization has been achieved recently and the results of studies with soluble purified enzymes support the existence of at least two types of transferases (310, 312). Other support for the existence of separate transferases was provided by experiments which showed that transferase activity with different acceptors was inhibited to different degrees by sulfhydryl group inhibitors and by other substances that acted as competitive substrates (313, 314). None of these results, however, revealed a highly specific enzyme.

Glucuronides are of rare occurrence in plants (315) and transferase enzymes that result in their formation from UDP-glucuronic acid have been demonstrated in only one case (316). The enzyme was obtained from the leaves of the French bean and, in contrast to animal transferases, it was found to be specific for quercitin. Plants appear to show a preference for glucoside formation with phenols and other aglycons. Rhamnose is also commonly found linked to these aglycons in higher plants.

An alternate pathway for the formation of glucuronides in animals is the transfer of the glucuronyl residue from a previously existing glucuronide to a new acceptor by the enzyme, β-glucuronidase. This reaction may be classed as a transglycosylase reaction and it has been shown to effect the formation of glucuronides of alcohols, but not of phenols (317, 318).

Glucuronidation affects the chemical and physi-

TABLE VI.—OCCURRENCE OF ENZYMES CATALYZING THE FORMATION OF ARYL GLYCOSIDES

Acceptor	Donor	Product	Source	Ref.
Anthranilic acid	UDP-glucose	Ester glucoside	Lentils	(321, 322)
			Bacillus megaterium	(323)
Hydroquinone	UDP-glucose	Arbutin	Wheat germ	(324–328)
			Schistocerca cancellata	(329)
Hydroxyhydroquinone	UDP-glucose	β-Glucoside	*Schistocerca cancellata*	(329)
Methoxyhydroquinone	UDP-glucose	β-Glucoside	*Schistocerca cancellata*	(329)
Catechol	UDP-glucose	β-Glucoside	Wheat germ	(324, 325)
			Schistocerca cancellata	(329)
Resorcinol	UDP-glucose	β-Glucoside	Wheat germ	(324, 325)
			Schistocerca cancellata	(329)
o-Nitrophenol	UDP-glucose	β-Glucoside	*Schistocerca cancellata*	(329)
p-Nitrophenol	UDP-glucose	β-Glucoside	*Musca domestica*	(330)
o-Aminophenol	UDP-glucose	β-Glucoside	*Schistocerca cancellata*	(329)
			Musca domestica	(330)
m-Aminophenol	UDP-glucose	β-Glucoside	*Schistocerca cancellata*	(329)
Salicyl alcohol	UDP-glucose	Salicin	Wheat germ	(324, 325)
Arbutin	UDP-glucose	β-Gentiobioside	Wheat germ	(324, 325, 327)
Quercitin	UDP-glucose	β-Glucoside	Mung bean	(331)
	TDP-glucose	β-Glucoside	Mung bean	(331)
Quercitin-β-glucoside	TDP-rhamnose	Rutin	Mung bean	(331)

cal properties of the acceptor and is believed to play an important physiological role in detoxication, elimination, and absorption (319, 320).

Simple Glycosides.—Phenols, alcohols, acids, and other substances frequently exist in plants as glycosides. Glucose is the most common sugar found linked to these aglycons; rhamnose is the next most frequently encountered sugar in these combinations. Enzymes that catalyze the transfer of glycosyl groups to these acceptors from sugar nucleotides have been obtained from higher plants, microorganisms, and insects.

Table VI presents a list of substances that have been shown to act as acceptors in enzymatic reactions leading to glycoside formation from nucleotide sugars.

Like many of the glucuronyl transferases from animals, these enzymes seem to exhibit a lack of specificity. Trivelloni (329) obtained two fractions from the fat body of the locust which were shown to exhibit specificity toward hydroquinone or *o*-nitrophenol; this specificity, however, was not absolute.

Barber found that the glucosylation of quercitin by an enzyme from mung bean could use UDP-glucose or TDP-glucose equally well as the glucosyl donor (331). The further transfer of the rhamnosyl group to quercitin-β-glucoside, however, was successful only with TDP-rhamnose. Goncalves (332) found UDP-glucose to be a more effective glucose donor than TDP-glucose in a study of glucosylation by a wheat germ extract.

The formation of the gentiobioside of hydroquinone has been studied with the wheat germ preparation (324, 325, 327). Separate enzymes for each successive glucosylation were demonstrated by Yamaha and Cardini (327). A study of rutin formation by Barber (331) likewise showed that separate enzymes were involved in the glucosylation of quercitin and in the subsequent rhamnosylation of the quercitin-β-glucoside.

Pridham and Saltmarsh (333) have recently reviewed the biosynthesis of phenolic glucosides in plants with much emphasis on *in vivo* feeding experiments.

Miscellaneous Glycosides.—Burton *et al.* (334) obtained incorporation of galactose from UDP-galactose into an endogenous lipid with a microsomal preparation from rat brain. With similar preparations, Cleland and Kennedy (335) demonstrated the transfer of galactose to sphingosine. Cell-free extracts of *P. aeruginosa* were shown to catalyze the transfer of rhamnose from TDP-rhamnose to (3-hydroxydecanoyloxy) decanoic acid with the formation of a rhamnolipid (336). Zeleznick *et al.* (337) incubated TDP-rhamnose-^{14}C with protoplast membranes of *Streptococcus pyogenes* and obtained incorporation of the rhamnose moiety into a polysaccharide material. Pazur and Anderson (338) obtained similar results with cell wall fragments of the same organism. Mannose-containing lipids were formed upon incubation of GDP-mannose-^{14}C with an extract of *Micrococcus lysodeikticus* (339). Three of these lipids were isolated. One was shown to be a mannosylmannosyl (1 → α) diglyceride; the other two were of unknown composition.

The glucosylation of the hydroxymethylcytosine residues of DNA has been accomplished with enzymes from phage-infected *E. coli.* (340). The phages, themselves, contain DNA in which the hydroxymethylcytosine residues contain one or more glucose units attached through α- or β-

linkages. The *E. coli* cells normally do not contain enzymes for such glucosylations. Upon infection with a specific phage (T2, T4, or T6), however, the synthesis of specific glucosyl transferases is triggered. In all cases, UDP-glucose serves as the glucose donor. The specificity of the various transferring enzymes has been studied (341).

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