NATURALLY OCCURRING ACID-SOLUBLE NUCLEOTIDES[1]

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Received March 1, 1958

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I. INTRODUCTION

The acid-soluble nucleotides comprise one of the most active, varied, and versatile group of compounds in nature. They touch all phases of metabolism: carbohydrates, lipides, proteins, and nucleic acids, as well as the conservation and transfer of energy. Many processes—e.g., oxidation, reduction, dismutation, epimerization—take place only when the substrate has been "activated" by combination with a nucleotide. The balance between low-molecular-weight compounds and their polymers, and the diversion of intermediates to alternative pathways, may be controlled in part by the nucleotides. Their role as nucleic acid precursors and the genetic consequences of this are, of course, vital.

[1] The preparation of this review was supported in part by United States Public Health Service grant No. C2491 and in part by the Alexander and Margaret Stewart Trust Fund.

Basically derived from just two compounds, purine and pyrimidine, the various combinations and variations have increased the number of naturally occurring acid-soluble nucleotides known to more than 110. This can be compared with the 40 some monosaccharides (124) and approximately 80 amino acids known (205). The first such nucleotide was discovered over 100 years ago; currently they are being discovered at the rate of 20 or more per year. This progress is illustrated in figure 1.

This review will seek to classify, describe, and draw generalizations regarding the structures and functions of this class of compounds. Some have been treated often; many others have never been reviewed.

II. NOMENCLATURE

The term nucleotide is often used to denote several different things. In the strict sense, it is an *N*-glycoside phosphate, containing a purine or pyrimidine base, with phosphoric acid esterified to one of the hydroxyl groups of the sugar. In a broader usage, any cyclic nitrogen base, such as pyridine or isoalloxazine, may substitute for the purine or pyrimidine. A still wider usage would consider

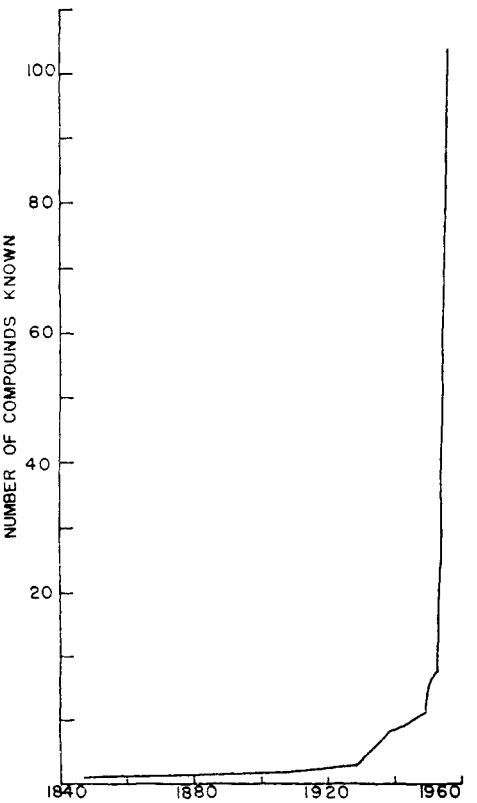


FIG. 1. Discovery of the natural acid-soluble nucleotides

almost any *N*-glycoside phosphate a nucleotide. It is in the strict sense that the term will be used in this paper.

The qualification "acid-soluble" is employed because the use of acid (2–4 per cent perchloric acid or 5–10 per cent trichloroacetic acid) is a common means of precipitating polymers, the insoluble fraction consisting of most of the protein, nucleic acid, and lipide of the tissue. The soluble portion then contains the tissue components of relatively low molecular weight, and it is to these that the discussion is restricted. Extraction with ethanol, dialysis, or any other suitable means of separation may, depending on the circumstances, achieve the same result. This distinction is, of course, only an operational fractionation for convenience in separating and analyzing this group of compounds and is not physiological.

The standard numbering system for the purine nucleotides is illustrated in figure 2a for purine riboside 5′-phosphate. Two systems are in common use for the pyrimidine nucleus. Figure 2b shows pyrimidine riboside 5′-phosphate in the system used by *Chemical Abstracts*; figure 2c shows an older and widely used form which is patterned by analogy to purine nomenclature, not after standard rules. The *Chemical Abstracts* form will be used throughout this paper.

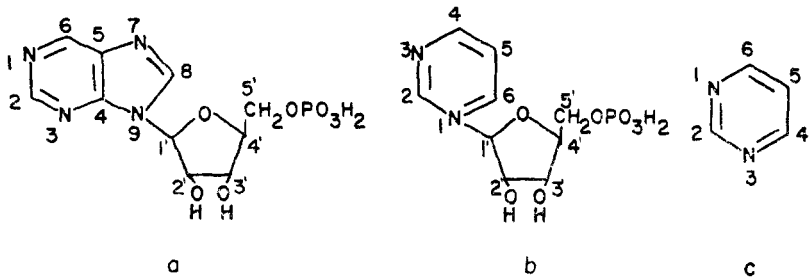


FIG. 2. Numbering systems for (a) purine riboside 5′-phosphate, (b) pyrimidine riboside 5′-phosphate, and (c) pyrimidine (old style).

TABLE 1

Structures of the pyrimidines

All are 2-OH

	5-H	5-CH_3
6-OH	Uracil	Thymine
6-NH_2	Cytosine	5-Methylcytosine

TABLE 2

Structures of the purines

	2-H	2-OH	2-NH_2
6-H	Purine		
6-NH_2	Adenine		
6-OH	Hypoxanthine	Xanthine	Guanine

The substitutions on the basic nucleus presented by the naturally occurring purines and pyrimidines are listed in tables 1 and 2, respectively. It is to be noted that, with only rare exceptions, the compounds under discussion contain either D-ribose or 2-deoxy-D-ribose.

III. METHODS

Classically, purines and pyrimidines and their derivatives were precipitated by heavy metals and as alkaloidal salts. These methods have been reviewed by Levene and Bass (166). For instance, silver ion brings down the purines under neutral or slightly acid conditions and the pyrimidines in basic solution. Precipitation by barium ion was a later development (see reference 160), specific for the nucleotides and other phosphate compounds. The barium salts of the di- and triphosphates are relatively insoluble in aqueous solution; the monophosphate salt is relatively insoluble in alcoholic solution. Zinc salts have also been used in this manner (99). These methods were the principal ones used throughout the period when the adenine nucleotides were the only ones known.

In 1950 Cohn introduced the use of ion-exchange resins (57), such as Dowex 1, for the separation of hydrolysis products of nucleic acids, and several years later Potter and his associates modified this procedure for the separation of the acid-soluble nucleotides (127). The latter workers eluted the column with an "extended gradient" of solvent, formic acid with or without added ammonium formate, gradually increasing the formate concentration over a wide range. This technique, the most commonly used at present, provides good separation of most of these compounds, although some overlapping peaks must be rechromatographed for complete separation. It is through the use of this method that systematic examination of a wide spectrum of the acid-soluble nucleotides has been made.

Another development, without which this technique would have been impractical, is the availability of ultraviolet spectrophotometers. Since the purines and pyrimidines absorb in the 260 mμ region, the progress of elution from ion-exchange columns can easily be followed. The characteristic ultraviolet spectrum of each nucleotide also helps in identifying the compound.

Other recently developed, but now standard, techniques such as paper chromatography and the use of radioactive isotopes have also been of great help in this field.

The great differences which may exist in the profile of the acid-soluble chromatogram when the tissue is prepared in different ways are not always appreciated. Figure 3 (top) shows a chromatogram of the Flexner–Jobling rat carcinoma when the tissue was removed in a cold room and homogenized in acid (260). Nucleotides at all three levels of phosphorylation are present. When this same tissue is frozen *in situ* and the acid-soluble fraction is prepared from the frozen material, it is seen (figure 3, bottom (163)) that the nucleotides are present mostly in the form of mono- and triphosphates, little diphosphate being detected. Differences also exist if muscle taken after decapitation, after *in situ* freezing, or after *in situ* freezing following nembutal anesthesia, or after decapi-

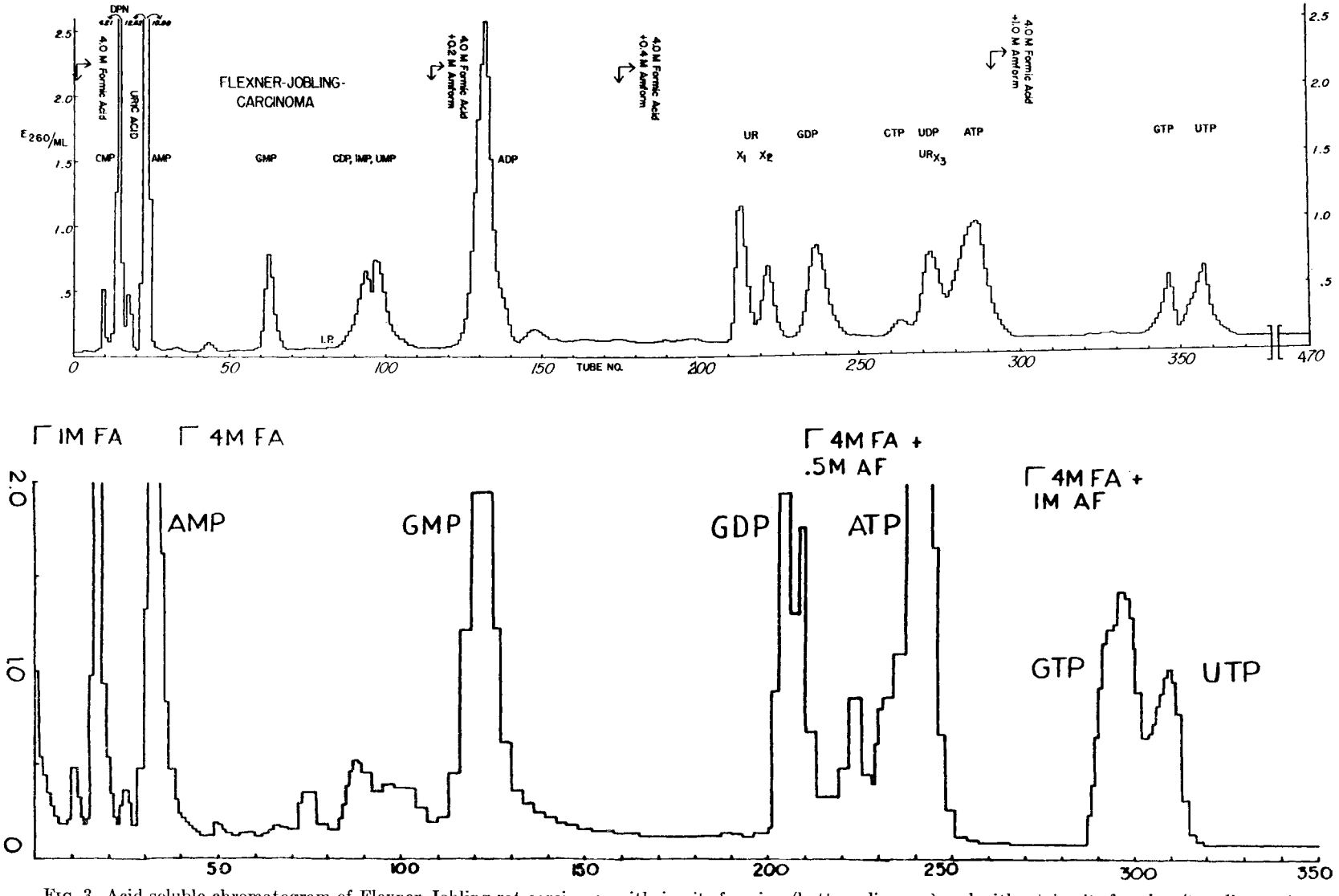


FIG. 3. Acid-soluble chromatogram of Flexner–Jobling rat carcinoma with *in situ* freezing (bottom diagram) and without *in situ* freezing (top diagram)

tation following magnesium anesthesia is analyzed. Which method results in the most physiological condition on analysis is sometimes a debatable point, since a multitude of factors are involved, including the facts that some tissue reactions are very rapid and that only the balance of synthetic and degradative processes is observed. The point is that the results obtained depend considerably on the method used. The consequences of this fact in attempting to discuss *physiological* relationships and quantities are obvious.

The lability of some of the acid-soluble nucleotides also causes some difficulties. Pyrophosphate and other anhydride bonds are much more acid-labile than are phosphoester bonds. Thus, standing in acid any length of time will certainly cause some hydrolysis to more simple forms. Some compounds are so labile that they may decompose during chromatography, as Tsuyuki and Idler have reported (292). They were unable to isolate a certain complex nucleotide unless it was chromatographed in the cold. How many other compounds of similar lability may exist is unknown. Another compound, Coenzyme III (286), is known to decompose while standing on a charcoal column, apparently losing some labile substituent.

While methods must be accommodated to the purpose of the experiment, such departures from the physiological as described above must always be kept in mind.

IV. ADENINE NUCLEOTIDES AND DERIVATIVES[2] (TABLES 3, 4, AND 5)

A. Adenosine mono-, di-, tri-, tetra-, and pentaphosphates

The adenine nucleotides are more abundant than those of the other bases, and it is therefore not surprising that they were known long before the discovery of the others. Actually the first nucleotide isolated was inosinic acid (figure 4a), found in meat extracts by Liebig in 1847 (175). Since later workers have found this compound to be a rather minor constituent of intact tissue, Embden expressed the opinion that it really was an artifact arising through the deamination of adenylic acid (figure 4b) (84). Liebig's analytical values were correct except for phosphorus, which he failed to find; indeed, it was not until 1871 that phosphorus was recognized to be a normal constituent of living organisms. Much

[2] AMP = adenosine monophosphate.
ADP = adenosine diphosphate.
ATP = adenosine triphosphate.
IMP = inosine monophosphate.
IDP = inosine diphosphate.
DNA = deoxyribonucleic acid.
RNA = ribonucleic acid.
DPN = oxidized diphosphopyridine nucleotide.
DPNH = reduced diphosphopyridine nucleotide.
TPN = oxidized triphosphopyridine nucleotide.
TPNH = reduced triphosphopyridine nucleotide.
NMN = nicotinamide mononucleotide (nicotinamide riboside 5′-phosphate).
FAD = flavine adenine dinucleotide.
CoA = Coenzyme A.
PRPP = phosphoribosylpyrophosphate.
P = orthophosphate.
PP = pyrophosphate.
LMN = lyxoflavine mononucleotide.
LAD = lyxoflavine adenine dinucleotide.

TABLE 3

Adenine nucleotides and derivatives (see also tables 4 and 5)

Adenosine monophosphate	Triphosphopyridine nucleotide	ADP glutamic acid
Adenosine diphosphate	ADP-ribose	ADP aspartic acid
Adenosine tetraphosphate	Alpha DPN	*S*-Adenosylmethionine
Adenosine pentaphosphate	Monohydroxytrihydro DPN	*S*-Adenosylhomocysteine
Diadenylic acid diphosphate	Desamino DPN	*S*-Adenosylmethylpropylamine
Cyclodianhydrodiadenylic acid	Coenzyme III	Adenosine 3′-phosphosulfate
Adenosine 3′-phosphate	Flavine adenine dinucleotide	Adenosine 3′-phospho-5′-phospho-sulfate
Adenosine 3′-triphosphate	Lyxoflavine adenine dinucleotide	
Deoxyriboadenosine triphosphate	Coenzyme A (see also table 4)	1-(Ribosyl-5-phosphate)adenylic acid
Magnesium ATP*	Phosphoryl-CoA	
Sodium ATP	Desamino-CoA	2-Methoxyadenine
Potassium ATP	2-Methyladenosine 3′-phosphate	Inosine monophosphate
Calcium ATP	Adenylosuccinic acid	Inosine 3′-phosphate
Diphosphopyridine nucleotide	Adenylosuccinate-5′-phosphosul-fate-glutamic-serine	2-Methylinosine-3′-phosphate
		Magnesium IDP

* ATP = adenosine triphosphate. DPN = diphosphopyridine nucleotide.
ADP = adenosine diphosphate. IDP = inosine diphosphate.

TABLE 4

S-Acyl-Coenzyme A compounds

Acetyl	Succinyl
Propionyl	Acetoacetyl
Butyryl (and similarly for C_6 to C_{22})	Isovaleryl
β-Hydroxybutyryl (and similarly for C_6 to C_{22})	β-Hydroxyisovaleryl
β-Ketobutyryl (and similarly for C_6 to C_{22})	β-Hydroxy-β-methylglutaryl
β-Unsaturated butyryl (and similarly for C_6 to C_{22})	α-Methylbutyryl
Cholyl	Tiglyl
β-Alanyl	α-Methylacetoacetyl
Aspartyl	β-Methylcrotonyl
Methylmalonyl	Phenylacetyl

TABLE 5

Compounds "activated" by formation of adenylates

Acetic acid	Benzoic acid	Cholic acid	Phenylacetic acid
Pantoic acid	Luciferin	Lipoic acid	Citrulline

Amino acids (carboxyl activation)

DeMoss	Berg	Hoagland	McCorquodale	Others, separately
Tryptophan	Glycine	Tryptophan	Tryptophan	Methionine
Histidine	Glutamic acid	Leucine	Methionine	Tyrosine
Phenylalanine	Serine	Alanine	Tyrosine	Tryptophan
Methionine	Threonine	Lysine	Leucine	Alanine
Tyrosine	Leucine	Valine	Isoleucine	
Leucine	Isoleucine		Valine	
Isoleucine	Arginine		Glycine	
Valine	Methionine		Cystine	
	Valine			
	Aspartic acid			
	Histidine			
	Phenylalanine			
	Tryptophan			
	Tyrosine			
	Proline			
	Lysine			
	Alanine			
	Cystine			

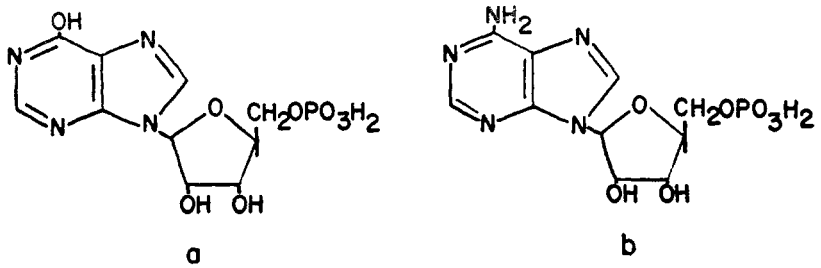


FIG. 4. (a) Inosine monophosphate; (b) adenosine monophosphate

later Liebig's compound was found by Haiser (108) and by Levene (167) to be the phosphate ester of hypoxanthine riboside. In the work prior to 1934, the ribose is portrayed as being bound to N-7, rather than to N-9. This was a convention begun by Emil Fischer (86) and did not indicate the actual position of bonding. Fischer recognized the tautomeric structure of the imidazole portion of purine, and was unable to distinguish between N-7 and N-9. Rather than writing both forms I and II, he adopted the convention of putting the double bonds be-

$$\overset{7}{\text{N}}\!=\!\overset{8}{\text{C}}\!-\!\overset{9}{\text{NH}}- \qquad \text{I}$$

$$-\text{HN}-\overset{8}{\text{C}}\!=\!\overset{9}{\text{N}}- \qquad \text{II}$$

tween positions 8 and 9, although his paper clearly states that this was merely a matter of convenience. In 1936–38 Gulland and coworkers (104, 105) provided the first unambiguous synthesis of N-7 methyl and N-9 methyl derivatives of the purines, and by comparing the ultraviolet spectra of these compounds with those of the natural products, clearly showed that the glycosidic bond was at N-9. The characterization of the basic structure of the nucleic acid derivatives has been reviewed by Levene and Bass (166).

The importance of phosphate and phosphate-containing compounds was noted early in the investigation of fermentation. Harden and Young in 1905 (113) found phosphate present in the glycolysis medium after fermentation and increased the rate of glycolysis by furnishing extra phosphate.

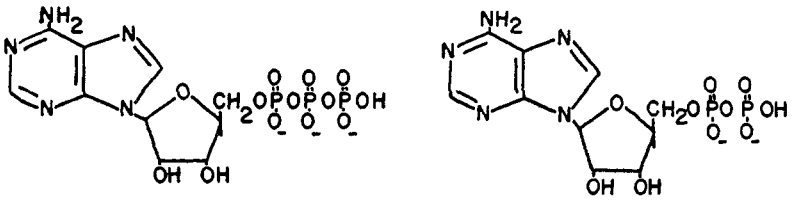
Bass (18), in 1914, found an acid-hydrolyzable adenine complex in human red blood cells, and in the same year Embden and Laquer (82) made the observation that one of the fractions of their glycolyzing muscle preparation consisted of an organic phosphate of adenine and ribose. The same group isolated the compound from muscle in 1927 and characterized it as adenosine monophosphate (figure 4b) (83). Although at first most workers identified it with the adenosine monophosphate derived from yeast nucleic acid by alkaline hydrolysis, work over the next few years clearly showed it to be different. Muscle AMP was more soluble in water, had a higher melting point, had a smaller levorotation, and exhibited a different rate of phosphate hydrolysis in acid. Finally, the work of Klimek and Parnas (141) and of Levene's group (166) clearly showed that the 5′-position was phosphorylated in muscle AMP and the 3′-position in yeast AMP.

While studying the rates of acid hydrolysis of the phosphorylated carbohy-

drates, Lohmann found an easily hydrolyzable phosphate compound (181) which he later isolated and identified as inorganic pyrophosphate (182). Its significance was not clear and was made more indefinite by the finding that exogenously added pyrophosphate failed to show behavior identical with the preformed muscle pyrophosphate (182).

After a more thorough study Lohmann found that in the tissues the pyrophosphate group is combined with AMP. The instability of this compound in alkali explained why Embden (83), who used calcium hydroxide in his isolation method, obtained only AMP from muscle. Almost simultaneously with Lohmann's discovery, the existence of adenosine triphosphate was also announced by Fiske and Subbarow (87).

Neutral or alkaline hydrolysis of ATP causes its decomposition into AMP and phosphate, while acid hydrolysis yields adenine, ribosephosphate, and phosphate. On the basis of both enzymatic and chemical studies, the structure was established as one in which the three phosphoric acid residues are esterified with each other at the 5′-position of the ribose (figure 5a) (185).



a b

FIG. 5. (a) Adenosine triphosphate; (b) adenosine diphosphate

In dialyzed extracts of lobster muscle, Lohmann found a two-step enzymatic hydrolysis of ATP. One phosphate was split off first, leaving adenosine diphosphate (figure 5b), subsequently found in all tissues under appropriate conditions (184).

Certain aspects of the older literature on this subject have been reviewed by Lutwak-Mann (188).

Recently two more compounds in the same series have been isolated. Adenosine tetraphosphate was first detected in commercial preparations of ATP (196, 197) and subsequently in horse muscle (168) and salmon liver (293). Liebermann found that it could not substitute for ATP in phosphorylation reactions (168). Finally, Sacks has found adenosine pentaphosphate in a commerical ATP preparation (254). Whether these compounds are physiological or are artifacts is not yet known. While they might have arisen from nonspecific enzymatic phosphorylation, or by a nonenzymatic, metal-ion-catalyzed reaction (187), their possible metabolic roles should not be overlooked.

In heart, it was first observed by Embden (81) and later confirmed by Beattie (19) and by Ostern (222) that there is a nucleotide with the composition of a

diadenosine pentaphosphate, an extremely labile complex, which, except under special precautions, easily breaks down to a mixture of ADP and ATP. So easily does this happen that its existence was denied altogether by Lohmann and Schuster (186). Similar adenosine polyphosphate compounds were reported in yeast (295) and in red blood cells (301). Kiessling and Meyerhof isolated a dinucleotide containing two moles of adenine and two moles of difficultly hydrolyzable phosphate (139). With phosphopyruvic acid and a rabbit muscle extract the pyrophosphate derivative was formed (figure 6). In 1950, Ohlmeyer (220, 221) found that the decrease in the velocity of fermentation due to exhaustion of the phosphate supply could be lessened if a thermostable and protein-free fraction from fresh yeast was added to the glycolysis system. This factor enabled

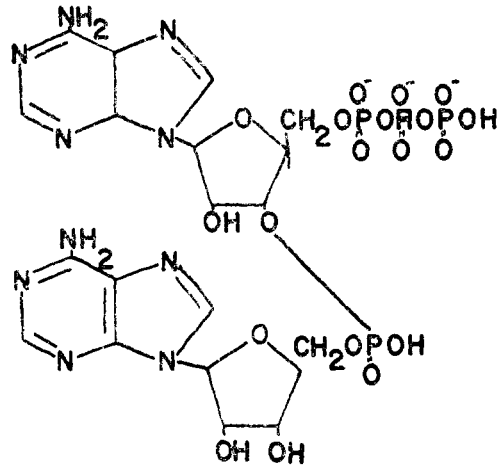


FIG. 6. Diadenylic acid diphosphate

the fermentation system with traces of phosphate to work about three times faster than addition of ATP would allow. The compound seemed to act by forming a phosphate compound which yields phosphate rapidly during the fermentation. It was homogeneous on an ion-exchange column. Ohlmeyer tentatively identified it with the Kiessling–Meyerhof dinucleotide on the basis of chemical behavior and analytical data. Recently Sutherland and Rall (287) isolated from liver homogenates a cyclodianhydrodiadenylic acid which is produced with ATP and epinephrine or glucagon in a particulate fraction of the tissue. It is a cofactor for phosphorylase activation (figure 7).

Until 1950 ATP, as known from animals and microörganisms, had not been isolated from plant tissue, although ATP from these sources functioned perfectly well in plant enzyme systems. In investigating this matter, Albaum, Ogur, and coworkers at first found an ATP in which the ratio of components was the same as in animal ATP, but which was deaminated more slowly. There were some indications that it was bound to protein (4). It was also known that most of the acid-labile phosphate was not free in solution, but was bound to protein in some way. Finally, however, these workers isolated from mung bean seedlings ADP

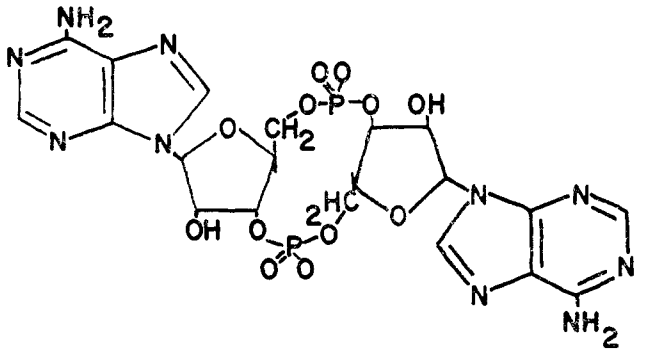


FIG. 7. Cyclodianhydrodiadenylic acid

and ATP which were identical in all respects with the corresponding animal compounds (5). Methods sufficient for this purpose in animal tissues were quite unsuccessful, and there are still indications that much, if not all, of the ADP and ATP present in these plants is bound to some tissue component.

Adenosine 3′-triphosphate was discovered in the autotrophic bacterium *Thiobacillus thiooxidans* by LePage and Umbreit (164), who were unable to find it in any of several other bacterial species studied. It was identified by its composition, by the rate of hydrolysis of the phosphates, and by the lack of copper complex formation. Barker and Kornberg were unable to confirm this (17), but used methods different from those of the original authors and reported lower yields.

Recently, adenosine 3′-phosphate has been found in the venom of the tiger snake and related species (74, 75). While it might represent a degradation product of either host or prey nucleic acid, adenosine and inosine are also present, but neither pyrimidine compounds nor other phosphorylated nucleosides. This would seem to point to the physiological occurrence of this compound, whatever unknown purpose it may serve.

The chemical synthesis of AMP has been achieved by Baddiley and Todd (9) by the reaction of 2′,3′-isopropylideneadenosine and dibenzylphosphorochloridate, and ADP and ATP were prepared by Khorana from AMP by reaction with 85 per cent phosphoric acid and dicyclohexylcarbodiimide (138). (See Baddiley (13) for other references on the chemistry of the nucleotides.)

Several metal-ion complexes with the adenosine nucleotides are known. Magnesium ion (116, 152) and calcium ion (73) complexes of ATP are well known and are fairly stable. Magnesium ion is a necessary cofactor for most ATP reactions, and since the optimum ratio of magnesium ion to ATP is unity, it is believed that the complex may be the actual substrate of the reaction (116). Both sodium ion and potassium ion form comparable complexes, and an appreciable fraction of ATP is present in the form of such complexes in the range of pH and of ion concentrations found in physiological fluids. Both ion-complexes are equally stable, but form less stable complexes with ADP (206).

From molecular models of such complexes it appears that magnesium ion is almost completely surrounded by the phosphate atoms, that both sodium ion

and calcium ion are enclosed to a considerable extent, but that potassium ion seems to rest in a rather shallow depression. Melchoir (206) concludes that the shape of these complexes is determined by the size of the ion, and that if a particular shape is required or favored by an enzyme, then inhibition by other cations might occur if they formed complexes which had a different shape. Indeed, numerous examples are recorded of the inhibition of reactions by metal ions by unknown mechanisms.

Sable (253), Lieberman (174), and Klenow (140) and their respective coworkers have found enzymes from various sources which will catalyze the transfer of ATP phosphate to deoxy-AMP prepared by the hydrolysis of deoxyribonucleic acid. Compounds with the properties of deoxy-ADP and deoxy-ATP have been isolated from such reaction mixtures. Only one report of the isolation of a naturally occurring purine deoxyribotide has appeared. LePage (162), applying *in situ* freezing to rat tumors, isolated what is probably deoxy-ATP. The amount of this compound found was less than 1 per cent of the ATP of the tissue. No evidence was obtained of the presence of deoxy-AMP or deoxy-ADP.

While a complete and detailed discussion of the functions of the adenine nucleotides is beyond the scope of this review, a summary of their functions is pertinent. They may be considered to have two main types of function,—as coenzymes and as nucleic acid precursors.

The function first noticed was phosphate transfer. (For recent reviews, see 6 and 144.) Examples are known in which ATP transfers phosphate to alcohols (e.g., glucose), enols (e.g., enol-pyruvic acid), acids (e.g., acetic), and phosphate (e.g., nucleoside mono- and diphosphates). Pyrophosphate may be transferred to thiamin and to ribose 5-phosphate. ADP transfers phosphate in only one known case, the myokinase reaction: $2ADP \rightleftharpoons AMP + ATP$. These reactions are part of that thermodynamic economy whereby energy is produced, transported, and converted to work in the organism. These thermodynamic considerations were recognized by Lipmann (176), who introduced the terms high- and low-energy phosphate bonds (or, better, high- and low-energy phosphate compounds, since the energy does not reside in the bond alone). In his "phosphate cycle," inorganic phosphate is first introduced into a low-energy ester linkage, and then high-energy compounds are generated by oxidation. If these compounds are spontaneously hydrolyzed, the free energy is dissipated, but in the presence of suitable coupling mechanisms, such as the adenine nucleotides, this energy can be taken from the place where it is generated to where it is needed for endergonic reactions. Here the same hydrolysis products may be formed indirectly by a series of reactions with small free-energy changes, the free energy being made available to the organism for the performance of metabolic work.

In certain cases (to be discussed in detail below) ATP forms anhydrides with other compounds with the loss of pyrophosphate, or other combinations with the loss of phosphate. In either case one high-energy anhydride linkage is present. As above, this represents activation of the compound over a thermodynamic barrier. In at least one case both processes take place, i.e., both acetyl phosphate and adenyl acetate are known in the same reaction, the former in microöorganisms, the latter in higher animals.

ATP may also react to form part of a compound whose relative stability and life span are greater than those of the "activated" compounds just discussed. Such are the pyridine and flavine coenzymes, etc., which will be taken up individually.

A few rather poorly understood reactions seem to require adenine nucleotides in a purely catalytic capacity. AMP is needed in catalytic amounts for a DPN-linked isocitric dehydrogenase system in yeast (147). In this case it can be recovered quantitatively at the end of the reaction, where it has catalyzed the oxidation of several times the stoichiometric amount of isocitrate. ATP catalyzes an adenylic deaminase reaction in brain, but no liberation of ortho- or pyrophosphate is detectable (216). The best-known case is that of the conversion of phosphorylase b to phosphorylase a, for which AMP is required (62). Its mode of action is not understood.

Finally, the adenine coenzymes may act in the general control of metabolism. Since ATP is necessary for so many reactions, the ratio of ATP to ADP and AMP is important in determining the rates of many cellular reactions.

Recent studies have shown that both nucleoside diphosphate and nucleoside triphosphate can be direct precursors of nucleic acids. Ochoa and his collaborators have shown that cell-free extracts of certain bacteria can accomplish the net synthesis of RNA-like polynucleotides from purine and pyrimidine nucleoside diphosphates (103, 219). The diphosphates polymerize on a primer oligonucleotide by combination with the free 3′-hydroxyl of the terminal primer nucleotide with the release of phosphate (269). Although the resulting polymer has all of the chemical and physical characteristics of natural RNA, in view of the lack of specificity of the system its exact physiological role, except possibly as a storage pool, is unknown. The system has been demonstrated in various microörganisms, in spinach, and in guinea pig liver (117).

Although the net synthesis of RNA by cell-free preparations from animal tissues has not been demonstrated, there is evidence that the 5′-nucleotides are the precursors of RNA, the exact level of phosphorylation being as yet uncertain. A number of investigators have incorporated 5′-nucleoside monophosphates into RNA in homogenates (100, 114, 115, 231, 242) and isotopic dilution experiments with 2′- and 3′-monophosphates show that these are not precursors (115). Although net synthesis was not achieved, the recovery of labeled precursor in nucleotide 2′,3′-monophosphates from alkaline hydrolysates of the RNA indicates that the newly formed phosphodiester linkages were typical of those in RNA.

Edmonds and Abrams have purified an enzyme system from ascites tumor cells which will incorporate ATP, apparently at this level of phosphorylation, into RNA (78). ADP is incorporated at a much lower rate. The incorporated ATP goes into nonterminal positions, as evidenced by recovery of the 2′,3′-monophosphates after alkaline hydrolysis. Other workers have previously found that RNA synthesis in certain microörganisms is dependent on the presence of amino acids and apparently synthesized at the same time as protein (20, 37, 101, 225). Evidence to be discussed in detail below suggests that the triphosphate, at least of adenine, is the precursor in this case also.

Kornberg has purified enzymes from *Escherichia coli* (145, 146) which polymerize deoxyribose nucleoside triphosphates irreversibly, forming a DNA-like polymer with the liberation of pyrophosphate. All four deoxyribonucleoside triphosphates are incorporated, and are necessary for maximum reaction. A primer of DNA is needed in this case also, and net synthesis has been achieved. The diphosphates are unable to replace triphosphates in a purified system. A similar system, purified from mammalian tissues by Bollum and Potter, likewise uses the triphosphates and requires a DNA primer (35, 36).

B. Inosine mono- and diphosphates

Although IMP has been known since the original work of Liebig, until recently its function, except as a deamination product of AMP, has been unknown. The work of Greenberg and of Buchanan and their associates on the *de novo* synthesis of the purine ring from smaller molecules has demonstrated that in this long chain of reactions IMP is the first product which possesses the intact purine ring. (This work has been reviewed by Carter (48).) It is then converted to AMP by one set of reactions, and to guanosine monophosphate by another (see below).

Szent-Gyorgi has found a fluorescent IDP compound in muscle. It appears to be a quadridentate complex with magnesium ion or calcium ion, the metal probably being chelated to the 6-OH and the N-7 and doubly to the phosphates (288).

C. Pyridine adenine nucleotides

The long and arduous history of the determination of the structure of the pyridine-containing coenzymes illustrates well the progress that nucleotide chemistry has achieved in the past twenty to thirty years. First discovered by Harden and Young in 1904 as a thermostable cofactor involved in alcoholic fermentation (112), DPN was not completely characterized until 1942, and the structure of the closely related compound, TPN, was not cleared up until 1950. The thirty to fifty years which elapsed between discovery and characterization spanned the formative period of nucleotide biochemistry, with the consequent evolution of both the preparative and the determinative methods which have permitted the rapid progress in this field in recent years. If, with the present methodological advantages, DPN were to be discovered today, at most only a few years would be required for its characterization. This is not to cast aspersions on Euler, Warburg, and others who performed this work, for it was truly a classical achievement, but to stress the methodological and conceptual advancements which have more recently been brought to bear on this field.

The development of the structural knowledge of these compounds has been reviewed in part by Schlenk (258), Kornberg (148), and Lutwak-Mann (188), and readers are referred to these authors for detailed references. Since, however, it is pertinent to the theme of this paper, a brief treatment will be given here. Until 1935 little was known of the structure of DPN except that Euler had demonstrated the presence of ADP in the molecule. Shortly thereafter, Warburg

and Christian discovered nicotinamide in the closely related compound, TPN. With the discovery of this second base, and the subsequent degradative work giving ratios of nicotinamide:adenine:ribose:phosphate = 1:1:2:2, the "dinucleotide" structure became apparent. The presently accepted structure (figure 8) was proposed by Euler and Schlenk. Enzymatic degradation and synthesis from the constituent parts has shown this to be, indeed, the correct structure. The glycosidic bond on the nicotinamide is in the beta configuration.

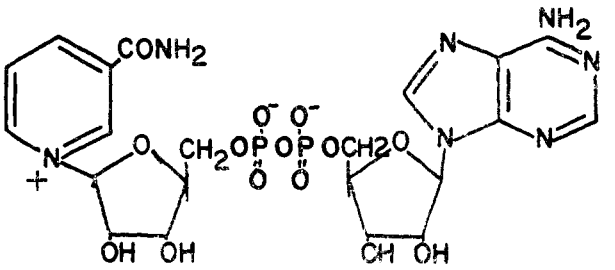


FIG. 8. Diphosphopyridine nucleotide

In connection with studies on glucose oxidation in hemolyzed red blood cells, Warburg and Christian discovered an enzyme which they called "Zwischenferment," capable of the oxidation of glucose 6-phosphate to the corresponding hexonic acid in the presence of a new coenzyme. It was in the course of the analysis of this coenzyme that nicotinamide was discovered to be a component, and the close relationship between this compound and DPN soon became apparent. Subsequent work showed that it differed only in the possession of a third phosphate group, the disposition of which was unknown. One suggestion was that nicotinamide riboside and adenosine were linked by three phosphates, not two. However, the interconversion of TPN and DPN in yeast maceration juice and the phosphorylation of DPN by ATP to form TPN led to the suggestion that the third phosphate was on the ribose moiety of the adenine ribotide. This was confirmed by the use of nucleotide pyrophosphorylase, which splits TPN to NMN and a diphosphoadenosine which was not ADP. By ion exchange and paper chromatography, and the use of a phosphatase with different activities on 2′- and 3′-phosphates, Kornberg was able to show that the third phosphate was at the 2′-position. With purified enzymes from yeast or liver Kornberg also synthesized DPN from ATP and NMN (143). The structure of TPN (in the reduced form) is shown in figure 9.

In spite of the long period which separated the discovery of these coenzymes from the final recognition of their true structures, much information as to their function had accumulated. The most important property of the pyridine nucleotides is their ability to undergo a reversible oxidation-reduction; this is the basis of their biological function as prosthetic groups of numerous dehydrogenases. The reduction was recognized early through the chemical oxidation-reduction of nicotinamide derivatives (85, 300) and involves the transfer of a pair of electrons and a hydrogen atom to the pyridine ring. Investigations by those working with

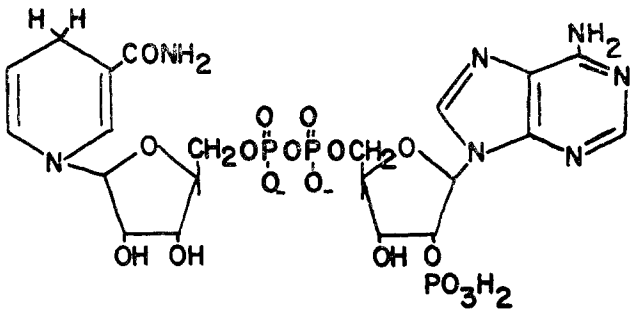


FIG. 9. Triphosphopyridine nucleotide (reduced)

Colowick (244) and with Vennesland (294) have shown that the redox reaction occurs at the 4-position of the pyridine ring (figure 9).

In a broad sense, all the reactions leading to the formation of high-energy compounds may be considered as redox processes. In addition to these reactions, numerous synthetic reactions involve redox reactions. In view of the multiplicity of metabolic processes involving hydrogen and electron transfer, the importance of the pyridine coenzymes is evident. The number of such reactions is much greater than can be listed here, but several reviews have appeared recently (245, 268). Singer and Kearney have made an important point: "Perhaps the most impressive aspect of pyridine nucleotide catalysis is the diversity of chemical structures whose oxidation-reduction is mediated by DPN and TPN. Regardless of the high degree of specialization of the dehydrogenase for its substrate, be it glucose 6-phosphate, luciferin, or molecular hydrogen, its active center is also structurally adapted to utilize one of the pyridine nucleotide coenzymes as a reaction partner—an extremely efficient aspect of cellular economy" (268, p. 113).

Besides the above forms of these coenzymes, several derivatives occur in nature, some not completely characterized. Not all the functions of these are understood, either. Kaplan, Ciotti, Stolzenbach, and Bachur (130) found that a few per cent of the DPN prepared from liver or yeast resisted the action of DPNase. Analytically identical to DPN, this component had no activity in a number of DPN-requiring enzyme systems. Finally it was noted that the optical rotation of the nicotinamide riboside of the new DPN component was $+58.2°$ compared with $-38.3°$ for normal DPN. It was concluded that the glycosidic bond was alpha in this case instead of beta. It is detectable even in crude extracts, so it would not seem to be an artifact caused by inversion during isolation.

Chaykin, Meinhart, and coworkers have discovered (54) a modified DPNH whose spectral peak is moved from 340 mμ to 290 mμ. It is produced by the action of triosephosphate dehydrogenase on DPNH. This DPNH-X will not act as a coenzyme for a number of DPNH-requiring systems, but will be irreversibly converted back to DPNH by ATP and yeast enzymes (203). There is no loss of nitrogen nor gain in orthophosphate in this conversion. By causing the trans-

formation in D_2O, one deuterium atom per molecule is introduced into the nicotinamide ring (204). This information, coupled with the fact that there is only one easily hydrogenated double bond in DPNH-X, has led these workers to the conclusion that it is a monohydroxytrihydroDPN. The exact configuration is not known, nor is the function of this compound.

The name Coenzyme III (DPN and TPN are referred to as Coenzymes I and II, respectively) has been assigned to a thermostable coenzyme from yeast found necessary for the oxidation of cysteinesulfinic acid by cell-free extracts of *Proteus vulgaris* (266, 268). This cofactor is not replaceable by any known factor, including DPN, TPN, and their components, although it has many of the spectral and chemical properties of a pyridine nucleotide (267). Analytically it is almost identical with DPN and can replace DPN in several DPN-requiring dehydrogenases. However, it is not precipitated by mercury and silver under conditions where DPN is; it is not held on Dowex 1-Cl when DPN is; and it has different K_m and V_{max} values in certain DPN–enzyme systems. Under a variety of fairly mild conditions Coenzyme III seems to decompose to form DPN. Singer and Kearney believe that it must contain some unknown substituent in a labile linkage, perhaps triply esterifying a phosphate, thus neutralizing one of its acid groups.

Two paths of biosynthesis lead to DPN, one from nicotinamide, going directly to DPN, and secondly, a system recently described by Preiss and Handler which starts from nicotinic acid in which the acid is not amidated until the last step (243). Both yeast and human erythrocytes form desamido-NMN and then make desamido-DPN on addition of ATP. Glutamine donates the amide group to form DPN. Desamido-DPN has also been identified in *Penicillium chrysogenum* (265).

D. Flavine adenine nucleotide

Riboflavine derivatives comprise another class of oxidation-reduction coenzymes, one of which may also be considered an adenine nucleotide. It was found that hydrogen was transferred from TPNH in Warburg's Zwischenferment to a yeast yellow enzyme or flavoprotein, the coenzyme for which was found to be riboflavine phosphate [6,7-dimethyl-9-(1′-D-ribityl-5′-phosphate)-isoalloxazine] (291). Some time later Das, working on D-amino acid oxidase, discovered that a new coenzyme was necessary for this reaction (65, 66). Warburg and Christian succeeded in separating the enzyme from the coenzyme, which they reported to contain flavine and adenine (299). An identical observation about the flavine nature of the coenzyme and details of its purification from heart muscle were published shortly before this by Straub (278). Warburg and Christian found on further analysis that it contained two phosphates, one adenine, and one luminoflavine (6,7,9-trimethylisolloxazine, the photodecomposition product of riboflavine in alkaline solution) (302). Abraham later found riboflavine 5-phosphate and AMP as hydrolysis products, thus completing the characterization of this coenzyme now usually called flavine adenine dinucleotide (really a misnomer) (1). Schrecker and Kornberg synthesized FAD from ATP

and riboflavine phosphate, using a purified enzyme from yeast (262). Its structure is shown in figure 10. Todd's group has synthesized it chemically, starting with 2,3-isopropylidene-5-benzylchlorophosphoronate adenosine and silver riboflavine 5-phosphate (55).

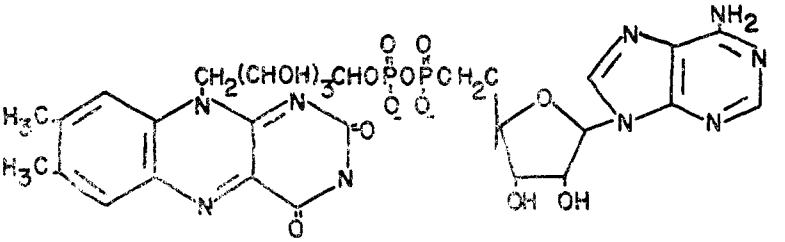


FIG. 10. Flavine adenine dinucleotide

The general function of this coenzyme is to bridge the electron transport gap between the pyridine nucleotides and the cytochrome system. In some cases, however, flavine enzymes react directly with a substrate, as in D-amino acid oxidase. The primary electron acceptor, whether pyridine or flavine, depends at least in part on the redox potential of the substrate.

Lyxoflavine [6,7-dimethyl-9-(1′-L-lyxosyl)isoalloxazine] has been reported to be present in human cardiac muscle (224), although this has not been confirmed (273). This flavine was substituted for riboflavine in the growth medium for *Lactobacillus lactis*. The culture grew well and LMN and LAD[2] were subsequently isolated from the bacteria (125).

E. Coenzyme A and derivatives

Studies on the acetylation of sulfanilamide (177) and on the action of choline acetylase (217) led to the discovery of a new heat-stable coenzyme by Lipmann and coworkers (178). (Lipmann has reviewed the development of this problem (179).) Microbiological analysis for known vitamins, after enzymatic hydrolysis of the coenzyme, led to the discovery of pantothenic acid, whose function had not previously been known. The specificity of the enzymes used for the release of this vitamin led to the conclusion that pantothenic acid was attached at one point to a phosphate and at another point to some unknown residue. In addition, sulfur had been detected and identified as a potential —SH grouping by cyanide-nitroprusside test. Also, large amounts of AMP were found in the preparation.

Further studies and the preparation of very pure samples of CoA from *Streptomyces fragilis* indicated the nucleotide nature of this Coenzyme A. Adenosine was found to be linked to the pantothenic acid at the 4-position through a pyrophosphate bridge, and the existence of a third phosphate was indicated. This was later found to be attached on the 3′-position of the adenylic acid. In the meantime Snell and his associates had isolated several growth factors for *L. bulgaricus* which contained pantothenic acid and β-mercaptoethylamine, and had shown that they were joined by a peptide linkage. The complete structure of CoA is shown in figure 11.

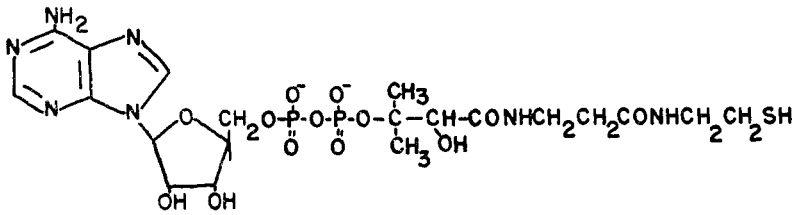


FIG. 11. Coenzyme A

Parts of this structure have been confirmed by analysis and synthesis, and Novelli has synthesized dephospho-CoA enzymatically from pantotheine 4-phosphate and ATP. CoA has been found in all living cells,—animal, plant and microbial. Furthermore, it was found that all cellular pantothenic acid could be accounted for by CoA, thus indicating that CoA represents the only functional form of this vitamin.

A dependence on CoA for the formation of acetoacetate, for the synthesis of citrate, and the acetylation of choline led to the conclusion that there exists in cellular metabolism an acetyl distribution system centering around CoA as the acetyl carrier. Careful fractionations led to the separation of the acetyl donor from the acetyl acceptor systems. The reactions were formulated as follows:

$$\text{ATP} + \text{CoA} + \text{acetate} \rightleftharpoons \text{acetyl-CoA} + \text{AMP} + \text{PP}$$

$$\text{Acetyl CoA} + \text{acceptor} \rightleftharpoons \text{acetyl-acceptor} + \text{CoA}$$

Lynen chemically identified acetyl-CoA as the thioester of CoA, the thioester linkage being a new type of high-energy bond (III) (191).

$$\text{CoA—S—CO—R}$$

III

Not only does CoA act in acetylation reactions, linking acetate to various amines, such as glucosamine, or acids, as in citrate synthesis, and in acetyl dimerization in acetoacetate formation, but this coenzyme is a catalyst of acyl activation in general. Thus benzoyl-CoA is intermediary in hippurate formation, and cholyl-CoA conjugates with taurine to form taurocholic acid. The well-known combinations of acetate groups to form fatty acids, and their intermediates, the β-keto, β-hydroxy, and β-unsaturated derivatives all occur in combination with CoA. Likewise, the synthesis of isoprene and of steroids appears to take place through appropriate acyl CoA combinations. Succinyl-CoA is also a donor of CoA of considerable importance in acetoacetate metabolism in extrahepatic tissues. A number of deaminated derivatives of amino acids, concerned in their synthesis and degradation, as well as some of the amino acids themselves (33), occur as CoA derivatives. The full description of these and related compounds is too long to relate here, but those acyl compounds which are linked to CoA are listed in table 4. Reactions involving CoA have been reviewed by Lynen (189), Lynen and Ochoa (190), Lipmann (179), and Kennedy (135).

Succinate activation has been found to be mediated by *S*-phosphoryl-CoA

(272). The phosphorylation of CoA by ATP was found in brain by Wallerman and Feuer (296, 297), and others have found evidence that it is this compound which transfers its CoA moiety to succinate with the release of phosphate. In heart muscle the phosphorylating agent is guanosine triphosphate (255); in other tissues it is ATP (131). The generality of this reaction has not yet been investigated, but could lead to interesting developments.

On the other hand, Cooper, Martin, and Korkes (61) have isolated from guinea pig and hog kidney a compound tentatively identified as desamino CoA, which was required for succinate oxidation. It had no acetylating activity.

Leuschner (165) has found five combinations of CoA with sulfur-containing substances in disulfide linkage in guinea pig liver.

F. Pseudovitamin B_{12}'s

A number of compounds have been isolated which have vitamin B_{12} activity in microörganisms but not in animals. These "pseudovitamins" are produced by intestinal microörganisms and have been isolated from intestinal contents and feces. While B_{12} itself contains dimethylbenzimidazole-1-riboside-3′-phosphate in an α-glycosidic bond, the pseudovitamins contain other bases, some of them purines. Only this latter class of compounds will be discussed. Pseudovitamins B_{12} (72) and B_{12b} (234) both contain adenine and are apparently identical chemically. However, they differ crystallographically in optical indices of refraction. The structural basis for this finding is not clear. The same difference exists between B_{12b} (71) and B_{12f} (233), which contain 2-methyladenine. This is the first time this base has been found in nucleotide linkage. So-called Factor G (39), isolated from pig manure, contains hypoxanthine, and Factor H (39) contains 2-methylhypoxanthine. Recently a guanine-containing analog of vitamin B_{12} has been isolated from *Nocardia* sp. (15). The metabolic and evolutionary significance of these compounds remains an interesting problem in comparative biochemistry. Since several other B_{12}-like compounds remain unanalyzed, new developments in this field may be expected. (See 89 and 90 for reviews.)

G. Other adenine nucleotides

In this section will be discussed those adenine nucleotide complexes which have been isolated and so exist as free, stable compounds. The following section will consider those for which there is convincing but indirect evidence, but which have not been isolated.

The conversion of IMP to AMP involves the formation of an addition compound with aspartic acid at the 6-position of the purine ring (49, 50, 171). This nucleotide, adenylosuccinic acid (figure 12), is subsequently cleaved with the release of fumaric acid, leaving the aspartate amino group to form the 6-amino group of adenine. This 6-amino group does not seem to be formed by any other mechanism, such as the direct amination by glutamine used for nitrogens 3 and 9. Storey and Love have isolated a nucleotide from cod liver which is related to adenylsuccinate, but whose 6-substituent is not succinate, but some other, still unidentified, component (277). A derivative containing sulfate and a peptide

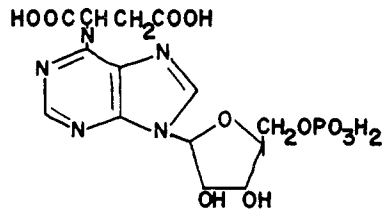


FIG. 12. Adenylosuccinic acid

has been isolated from salmon liver by Tsuyuki and Idler. It has the structure shown below (IV) (292):

Adenylosuccinate-5′-phosphosulfate-glutamic-serine

IV

Its function is unknown. There is also some evidence for an intermediate, 6-phosphoryl IMP, in the conversion of IMP to adenylosuccinate (171).

One report of ADP-amino acid conjugates has appeared (110). Hansen and Hageman reported the isolation of ADP-glutamate and ADP-aspartate from liver and lactating mammary glands. The exact position of the bonding was not ascertained, but on the basis of evidence that O^{18} is transferred from carboxyl to phosphate during glutamine formation (38, 149), the ADP compound might be formed at the ω-carboxyl. This remains to be explored.

A degradation product of DPN, ADP-ribose, has been isolated from liver extracts by Hansen, Hageman, Freedland, and Wilkie (111).

To stray just a little afield, in 1953 Cantoni and his group found an intermediate in the transfer of the methyl group from methionine which was identified as *S*-adenosylmethionine (44) (figure 13). In this case the sulfonium linkage

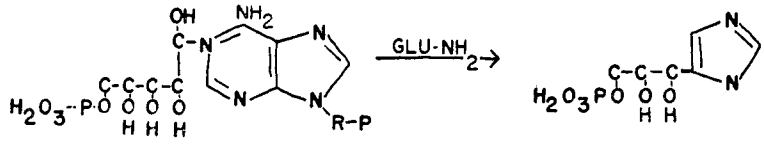


FIG. 13. Conversion of the N-1, C-2 of adenosine monophosphate to the N-1, C-2 of imidazole.

apparently is of the high-energy type. This discovery recalls the much earlier identification of thiopentose adenosine in 1912–24 (166, p. 158). The formation of *S*-adenosylmethionine is quite interesting, as it apparently involves a two-step dephosphorylation of ATP, first pyrophosphate and then orthophosphate being released (45). The mechanism is not known. After methyl transfer, *S*-adenosylhomocysteine is formed. This compound is also formed independently from adenosine and homocysteine (106). In the formation of spermidine from methionine and putrescine, the methionine moiety is decarboxylated to form *S*-adenosylmethylpropylamine (289). Since selenomethionine can function in this type of transmethylation reaction (212), presumably selenium analogs of these compounds can also exist.

The existence of an ATP-activated sulfate has been reported. Its structure has been determined to be adenosine 3′-phosphate-5′-phosphosulfate (118, 249, 264). It is synthesized through these reactions (14, 250):

$$ATP + SO_4 \rightleftharpoons \text{adenosine 5′-phosphosulfate (APS)}$$

$$APS + ATP \rightleftharpoons \text{adenosine 3′-phosphate-5′-phosphosulfate}$$

The selenate analog has been prepared *in vitro* and may be physiological under conditions when selenium is metabolically active (308).

In the biosynthesis of branched-chain acids it was discovered that carbon dioxide is fixed and that ATP is needed for this reaction. An active CO_2 compound has been found, and while identification is not complete, Coon proposes that this compound is AMP-CO_2 (8, 59, 60).

It has been known for some time that the C-2 of the purine ring can be transformed into the C-2 of the imidazole of histidine (193, 209). Moyed and Magasanik find that ribose 5-phosphate (or more probably, phosphoribosylpyrophosphate) attaches to the N-1 of AMP and then leaves, taking N-1 and C-2 of AMP to form the imidazole ring. Aminoimidazolecarboxamide is left as the remnant of AMP (211). The main reaction is given in figure 14.

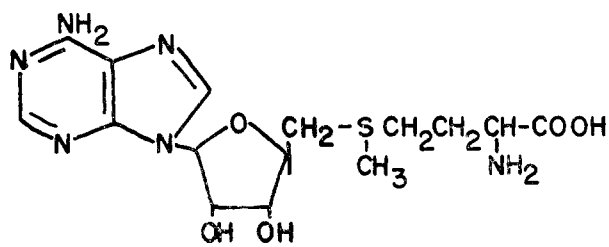


FIG. 14. *S*-Adenosylmethionine

2-Methoxyadenosine has been isolated from sponges; since they were allowed to undergo autolysis before analysis, this compound may well have existed as the ribotide *in vivo* (30). This is the first methoxy derivative of a purine found in nature.

H. "Activation" reactions involving adenine nucleotide intermediates

Reactions have been known for years in which ATP is a necessary cofactor, but for which no direct phosphorylating role was known. It was known that the ATP was split to AMP + PP, and it was assumed that its function was to furnish energy for the reaction. How this energy was transferred from ATP to the substrate was not known. In recent years evidence has been accumulating which indicates that "active" complexes of AMP and substrate are formed, usually anhydrides (V), where the substrates are acids. The "activated" substrate is

$$\text{Adenosine—}O_3HP\text{—O—COR}$$

V

then transferred to some acceptor, with the release of AMP.

The development of this concept may be briefly outlined as follows: Lipmann discovered that the pyrophosphorolysis of ATP was necessary for the formation of acetyl-CoA from CoA and acetic acid (179). Later the synthesis of pantothenate from β-alanine and pantoic acid by extracts of *E. coli* was revealed to involve this reaction (192), with no requirement of Coenzyme A. The synthesis of hippurate from benzoate and glycine also requires this type of participation by ATP (257).

It was known, however, that a split of orthophosphate from ATP occurs in some bacteria during acetyl-CoA formation, according to the following reactions (298):

$$\text{ATP} + \text{acetate} \rightleftharpoons \text{acetyl-P} + \text{ADP}$$

$$\text{Acetyl-P} + \text{CoA} \rightleftharpoons \text{acetyl-CoA} + \text{P}$$

These and other considerations led Jencks to suggest that there are two different acid activation reactions: one with CoA as an obligatory component, the other where presumably reaction of ATP and acid occur at the enzyme surface preliminary to the transfer of the activated acyl groups to CoA (128).

Shortly thereafter Lipmann (180) suggested the formation of an AMP-enzyme complex which then reacted with CoA to give a CoA-enzyme complex which in turn reacted with the acyl group, as follows:

$$\text{E} + \text{ATP} \rightleftharpoons \text{E-AMP} + \text{PP}$$

$$\text{E-AMP} + \text{CoA} \rightleftharpoons \text{E-CoA} + \text{AMP}$$

$$\text{E-CoA} + \text{acetate} \rightleftharpoons \text{acetyl-CoA} + \text{E}$$

Berg, however, has found that acetate must be present for the exchange of pyrophosphate with ATP (21, 22, 23, 25). Thus the reactions must be:

$$\text{ATP} + \text{acetate} \rightleftharpoons \text{AMP-acetate} + \text{PP}$$

$$\text{AMP-acetate} + \text{CoA} \rightleftharpoons \text{acetyl-CoA} + \text{AMP}$$

Berg also synthesized AMP-acetate chemically and showed that it would serve as substrate in the second reaction, with no ATP present.

Further evidence for anhydride-type intermediates has been presented by Boyer on the basis of O^{18} experiments (38). He has found that the formation of acetyl-CoA from ATP, CoA, and acetate by an enzyme preparation from heart muscle results in the transfer of O^{18} from the acetate to the AMP formed.

Mahler (194) has found enzymes which convert fatty acids to the CoA derivatives as the initial reaction in the mitochondrial oxidation of these substances, with the concomitant release of pyrophosphate from ATP. Later, using these enzymes and introducing butyryl adenylate, synthesized chemically, Talbert and Huennekens (290) and Peng (232) found a stoichiometric pyrophosphate exchange with ATP, indicating that this compound could act as an intermediate in the activation reaction.

Two further investigations complete the main lines of evidence for the presence of such intermediates. In a protein-synthesizing system from *E. coli*, De Moss,

Genuth, and Novelli (69, 70) were able to incorporate leucine into protein without the ATP-amino acid activating system, by introducing synthetic leucyl-AMP. Specificity for the L-isomer was shown. Finally, Hoagland *et al.* have found O^{18} transfer from the carboxyl of tryptophan to the phosphate of AMP in a purified tryptophan-activating system (121).

This evidence seems to indicate the existence of such acyl-adenylates as biochemical intermediates. Such compounds have not been isolated, however, and may be bound to the enzymes, or may have very brief existence under physiological conditions. Sweeping generalizations must be avoided in the consideration of these compounds. Such intermediates are often deduced from the formation of acyl hydroxamates after the addition of concentrated hydroxylamine. However, hydroxamates could be produced under such circumstances from acyl phosphates or from acyl thioesters, such as CoA. Very often the amount of hydroxamate formed is very irregular and not stoichiometric in relation to pyrophosphate formation. Thus, exchange of pyrophosphate with ATP in such systems is a much better indication.

A brief survey of other possible acyl-adenylates follows. As in fatty acid synthesis, there is evidence that AMP complexes are made during bile acid conjugation prior to acyl-CoA formation. Elliott found that a hydroxamate of cholic acid was formed during the conjugation of glycine or taurine in the presence of ATP. This may be the precursor of cholyl-CoA (79, 80).

In an investigation of pyruvate dismutation, Reed and associates have found a system which activates lipoic acid, forming an intermediate which can be replaced by synthetic lipoyl adenylate. After activation this compound is then incorporated into a nondialyzable form which participates in the dismutation (247).

In the conversion of citrulline to argininosuccinic acid it was found by Ratner and Petrack that the reversible formation of the amidine bond of argininosuccinate is definitely associated with the reversible cleavage of one adenyl pyrophosphate bond. This would suggest the presence of adenyl-citrulline (246).

A somewhat unusual compound for which there is evidence is AMP-luciferin. The structure of firefly luciferin is as yet unknown, so that absolute characterization is not possible. But on the basis of pyrophosphate exchange with ATP and other evidence, McElroy has postulated this combination, with both the reduced and the oxidized luciferin, as well as with the intermediate light-activated form (201, 202).

Recent work on the mechanism of protein synthesis has brought forth a number of cases in which there is presumptive evidence for amino acid adenylates. Hoagland found that pyrophosphate exchange with ATP in rat liver fractions was enhanced by the addition of amino acids (119). Later he found that only five amino acids would react in this system (120). De Moss and Novelli found a similar system in microörganisms, but here eight amino acids were active (70). Carboxyl activation was demonstrated by forming hydroxamates of the amino acids, as well as by pyrophosphate exchange (69, 119), and, as mentioned above, by using synthetic leucyl-AMP in the system. McCorquodale and Mueller have

TABLE 6

Uracil nucleotides and derivatives

Uridine monophosphate	UDP *N*-acetyl-3-*O*-carboxyethyleneglucosamine
Uridine triphosphate	UDP glucosamine
UDP glucose	UDP *N*-acetylglucosamine
UDP galactose	UDP *N*-acetylgalactosamine
UDP xylose	UDP *N*-acetylgalactosamine sulfate
UDP arabinose	UDP *N*-acetylglucosamine phosphate
UDP glucuronic acid	UDP muramic acid-ala-glu-lys-ala-ala (and intermediates)
UDP galacturonic acid	UMP aspartic acid
UDP *N*-acetyl-3-*O*-carboxyethylglucosamine	Orotidine monophosphate

obtained the activation of eight amino acids in rat uterine homogenates (200). Most recently, Berg's group has been able to activate eighteen amino acids in an *E. coli* preparation (218). Separate activating systems have been purified for methionine (24), alanine (123), tyrosine (263), and tryptophan (67). The amino acids thus activated in the various systems are listed in table 5. There is evidence that the amino acid is transferred from the adenylate to at least two RNA's in turn and then polymerized into protein (122).

V. URACIL NUCLEOTIDES AND DERIVATIVES[3] (TABLE 6)

A. Uridine mono-, di-, and triphosphates

As is the case with adenine, the mono-, di-, and triphosphates of uridine have been found in the acid-soluble fractions of tissues. The monophosphate (figure 15a) (223) and triphosphate (215) were found in connection with various studies on the coenzyme uridine diphosphoglucose which will be discussed below. Later all three forms were isolated from rat tissues by Schmitz, Hurlbert, and Potter (259), and then by Smith and Mills (270). These compounds were characterized by ratios of base, pentose, total phosphate, acid-labile phosphate, paper chromatography of the base after hydrolysis, periodate reaction, copper complex formation, and 5′-nucleotidase action. On the basis of these and other analyses, it was established that the pentose was D-ribose, and that the phosphates were esterified at the 5′-position. The position of the second and third phosphates was established by the action of nucleoside pyrophosphatase, by acid lability, and by ultraviolet spectrum, which eliminated the possibility that these might be attached to the ring N-1. These compounds have subsequently been found in almost every tissue examined. Mono- and diphosphokinases which interconvert these forms are widely distributed. Some tissues and organisms are able to convert the free base, uracil, to UMP directly; others cannot (63). In all cases, however, there is capacity to synthesize UMP *de novo*.

Michelson and Todd have synthesized UMP from the isopropylidene derivative of uridine and dibenzylphosphorochloridate (207), and Hall and Khorana have made the di- and triphosphates by reaction of UMP with 85 per cent phosphoric acid and dicyclohexylcarbodiimide (109).

[3] UMP = uridine monophosphate.
UDP = uridine diphosphate.
UTP = uridine triphosphate.
OMP = orotidine monophosphate.

The uridine nucleotides function directly as precursors of RNA (115, 242) as either di- or triphosphates or both, but only indirectly in the formation of DNA, as uracil does not occur in this polymer. Ochoa's (103, 219) polynucleotide phosphorylase will incorporate uracil into RNA-like polymers, either with other bases or alone. Lieberman has shown that UTP is converted to cytidine triphosphate by direct amination with ammonia in certain bacteria (170). Herbert, Potter, and Hecht have traced the incorporation of the uridine nucleotides into RNA in rat liver, and have shown that the incorporation of the 5′-nucleotides is not diluted by mixed 2′- and 3′-nucleotides (115). Although deoxyuridine nucleotides have not been found in tissues, *in vitro* conversion of deoxyuridine to thymidine has been shown (34, 91). The nucleoside deoxyuridine has been found *in vivo* (261).

Unidentified uracil derivatives have been detected in RNA by Cohn (58) and by Davis and Allen (68). The compounds identified by these two groups might be identical, but not enough information is presently available to decide this. At any rate, the appropriate acid-soluble forms of these compounds would be expected to exist.

B. Orotidine monophosphate

The *de novo* synthesis of the pyrimidine nucleotides occurs through the condensation, cyclization, and dehydrogenation of aspartic acid and carbamyl phosphate to form orotic acid (uracil-6-carboxylic acid), which had long been suspected to be a precursor of a nucleic acid pyrimidine (126, 310). Orotic acid then reacts with PRPP[2] to form the nucleotide OMP (figure 15b) (173), and

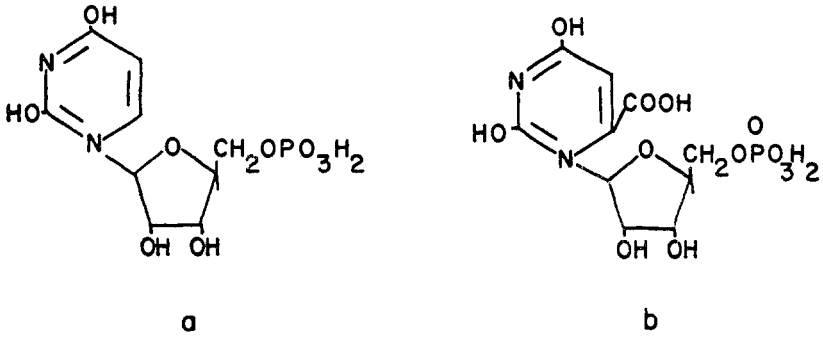


FIG. 15. (a) Uridine monophosphate; (b) orotidine monophosphate

this compound is decarboxylated to UMP. Microörganisms as well as mammals have been shown to perform these reactions. It is interesting to contrast this process with the *de novo* synthesis of the purines. In the latter process the phosphoribosidation occurs in the first step, while in the case of the pyrimidines it is almost the last reaction.

C. Uridylic acid-sugar coenzymes

The uridine coenzymes seem almost uniquely concerned with the interconversion of sugars and the synthesis of polysaccharides of various kinds. While only a few monosaccharides are well known to biochemists as ubiquitous in energy metabolism and in nucleic acid synthesis, over forty different mono-

saccharides are known in nature, and little information is available concerning the synthesis and interconversion of these substances. Not only do these sugars exist in the free state in organisms, but rather, the more common state is polymeric. Thus chitin is made up of acetylglucosamine, and glucuronic acid is present in hyaluronic acid, chondroitin sulfate, heparin, bacterial polysaccharides, and plant gums. Galacturonic acid is present in pectins and flaxseed mucilage, and mannuronic acid is the sole constituent of seaweed alginic acid. Xylans, arabans, galactans, and of course cellulose, starch, and glycogen are of common occurrence in nature. While the uridine coenzymes have not been implicated in the metabolism of all of these substances, evidence has been presented for their participation in the biochemistry of many, as can be seen in table 6, and this will be discussed below.

In 1950 the first non-adenine coenzyme was discovered by Leloir and his associates (46, 47). This was UDP glucose, whose structure (figure 16) was as-

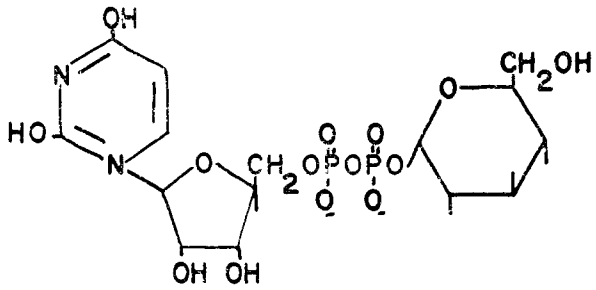


FIG. 16. Uridine diphosphate glucose

signed on the basis of evidence of the following type (223). On mild acid hydrolysis of Leloir's compound glucose is liberated, its reducing power unmasked, and a secondary acid group of phosphoric acid is liberated. Further acid hydrolysis liberates one mole of phosphoric acid, and another secondary group of phosphoric acid appears. The substance remaining after splitting off glucose and one phosphate was identified as 5′-UMP by comparison with an authentic sample. Action of nucleoside pyrophosphatase formed glucose 1-phosphate and UMP. This is also the general procedure, with some additions and deletions, that has been followed in the characterization of many of the other nucleoside diphosphate-X coenzyme types.

UDP glucose was found in the course of an investigation into the interconversion of glucose and galactose in yeast, and the reactions of this process were subsequently worked out and shown to be the following:

$$\text{Galactose 1-phosphate} + \text{UDP glucose} \rightleftharpoons \text{glucose 1-phosphate} + \text{UDP galactose}$$

$$\text{UDP galactose} \rightleftharpoons \text{UDP glucose (154)}$$

UDP galactose has been isolated, and its epimerization to glucose at the 4-position has been shown to involve DPN, indicating a redox reaction rather than a Walden inversion mechanism. The DPN is believed to oxidize the 4-hydroxyl to the symmetric keto form, followed by an asymmetric reduction and epimerization (198).

Besides interconversion, UDP glucose is also involved in the synthesis of di- and polysaccharides. Two paths of sucrose synthesis have been shown to exist in wheat germ. In one case UDP glucose reacts with fructose to give sucrose + UDP (157), and in the other fructose 6-phosphate is involved, giving rise to sucrose phosphate (158). Trehalose phosphate likewise is synthesized through the mediation of UDP glucose (156). Gander has produced evidence that synthesis of lactose in the mammary gland occurs by the transfer of a galactosyl residue from UDP galactose to glucose 1-phosphate, giving lactose 1-phosphate (92). However, other pathways may also exist (309).

The synthesis of cellulose in a cell-free system has now been achieved and shown to require UDP glucose as the sugar-donating unit (96). Several investigators have suggested a role for this coenzyme in glycogen synthesis. Buell has reported that UMP may be obtained after the acid hydrolysis of crystalline phosphorylase (41), and Korkes proposed a scheme for its action as a coenzyme of phosphorylase (142). Recently Leloir reported the realization of these hypotheses (159). In a liver system free of amylase he found a glycogen increase corresponding to the loss of glucose from UDP glucose, when glucose 1-phosphate itself was not active. Further developments in this field are awaited with interest.

Besides UDP galactose, found in a number of organisms, UDP xylose and UDP arabinose (95) have been found in mung bean seedlings and, by analogy, may be presumed to be precursors of plant polysaccharides. These two sugars are interconverted by epimerization in the coenzyme form in reactions similar to those for UDP glucose and UDP galactose (94).

In the course of the investigation of glucuronide synthesis, Dutton and Storey (77, 276), and later others (285), found UDP glucuronic acid, which transfers its glucuronic acid group to both aliphatic and steroidal alcohols by a transglycosidation reaction, thus (76):

$$\text{UDP glucuronic acid} + \text{ROH} \rightleftharpoons \text{RO glucuronic acid} + \text{UDP}$$

Further work has shown that the oxidation of glucose by DPN to give the uronic acid takes place as the UDP coenzyme (284, 285). In this oxidation there is no indication that more than one enzyme is involved, and no intermediate aldehyde has been detected (51). It may also be formed by the reaction:

$$\text{UTP} + \text{glucuronic acid} \rightleftharpoons \text{UDP glucuronic acid} + \text{PP}$$

UDP galacturonic acid has been found in a Type I pneumococcus (271) and presumably participates in the formation of the specific capsular polysaccharide of that organism. Whether this arises from the oxidation of UDP galactose, the epimerization of UDP glucuronic acid, or the reaction of galacturonic acid with UTP is not known.

One other sugar acid coenzyme has been described. Park (229) and more recently Strominger (282) have found in normal and especially in penicillin-treated bacterial cells a compound identified as UDP-*N*-acetyl-3-*O*-carboxyethylglucosamine (figure 17) (230). The sugar portion has also been called mu-

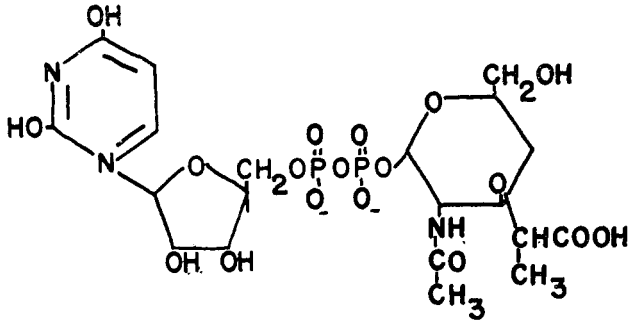


FIG. 17. Uridine diphosphate *N*-acetyl-3-*O*-carboxyethylglucosamine

ramic acid and is a component of some bacterial cell walls. It may be regarded as the 3-ether of glucosamine with lactic acid. Strominger has also identified the pyruvate derivate, UDP-*N*-acetyl-3-*O*-carboxyethyleneglucosamine (283), which may arise from the phosphorolytic combination of UDP glucosamine with phosphoenolpyruvate, and which is a precursor of UDP muramic acid. The role of these compounds in the formation of bacterial cell walls will be discussed further below.

The 2-amino derivatives of the UDP sugars have been isolated from many sources, both as such and in their *N*-acetylated forms. Yeast and rat liver will catalyze the formation of UDP glucosamine from glucosamine and UTP (195). This compound may then be acetylated (43, 274) and subsequently epimerized to UDP-*N*-acetylgalactosamine by a reaction similar to the epimerization described above for UDP glucose (155). The glucosamine compound has been isolated from mung bean seedlings, and the galactosamine analog has been found in bovine liver and yeast (235).

Glaser and Brown have reported the synthesis of a hyaluronic acid-like polymer upon incubation of UDP glucose and either *N*-acetyl-C^{14}-glucosamine and UTP or UDP *N*-acetyl-C^{14}-glucosamine with homogenates of Rous sarcoma (98). The source of the glucuronic acid was presumed to be UDP glucuronic acid formed by the oxidation of UDP glucose with DPN. The yield was of low magnitude, and it has been pointed out that exchange of the acetyl groups with preëxisting hyaluronic acid or its precursors was not precluded (142). However, the report is quite suggestive. These authors have used extracts of *Neurospora crassa* to synthesize chitin, given UDP *N*-acetylglucosamine as glycosyl donor. A primer was needed (97).

UDP-*N*-acetylgalactosamine 4-sulfate, necessary for the synthesis of chondroitin sulfate, has been obtained from hen oviduct, together with UDP-*N*-acetyl glucosamine 6-phosphate. The possible function of the latter compound is unknown (281).

Peptide forms of the UDP sugars have been implicated in the biosynthesis of bacterial cell walls. Park (226–229), in 1949, found several nucleotide peptides in normal *Staphylococcus aureus* cells, which were accumulated in large amounts when the cells were grown in the presence of penicillin. These are all derivatives

TABLE 7

Guanine nucleotides and derivatives

Guanosine monophosphate	Guanosine triphosphate	Guanosine 3′-phosphate
Guanosine diphosphate	GDP mannose	Xanthosine monophosphate

of UDP muramic acid, described above (227). He originally described one compound in which L-alanine was linked to the muramic acid by a peptide bond (228), and another which contained L-lysine, D-glutamic acid, and three alanine residues. Further work has shown the sequence to be: UDP muramic acid-L-ala-D-glu-L-lys-D-ala-DL-ala (230). The terminal alanine analyzes for an equimolar mixture of the D and L forms, suggesting that there are probably two such compounds, one having each configuration. One intermediate between the one and five amino acid forms has recently been identified, i.e., -ala-gly (283). Similar compounds have been found in penicillin-inhibited *Lactobacillus helveticus* and *Streptococcus hemolyticus* (279). A note has appeared describing a similar nucleotide containing gly, lys, and 4 ala, one more than Park's compounds, and in which the only free amino group is the epsilon group of lysine (199).

D. Other uracil nucleotides

In a study of the early intermediates in protein synthesis in Ehrlich ascites cells, Reith has found evidence for the formation of UMP aspartic acid, which is analogous to the AMP-amino acids described above. If indeed nucleotide amino acid compounds exist with bases other than adenine, the consequences for mechanisms and theories of protein synthesis will be great (248).

VI. GUANINE NUCLEOTIDES AND DERIVATIVES[4] (TABLE 7)

A. Guanosine mono-, di-, and triphosphates

Guanine was the third nucleic acid base to be found as a low-molecular-weight compound in the acid-soluble extracts of tissues. In 1951 Leloir reported briefly that a nucleotide containing guanine, phosphate, and mannose was active in the isomerization of mannose phosphates in yeast (153), and in the following year uncharacterized guanine compounds were found in mouse tumor and liver (161).

The first complete characterizations of such compounds were reported independently and almost simultaneously by Schmitz, Potter, Hurlbert, and White in the United States (260) and by Bergkvist and Deutsch in Sweden (26, 27). The latter isolated GTP from rabbit muscle and characterized this compound by phosphate and ribose analyses, ultraviolet spectrum, paper chromatography, periodate reaction, and copper complex formation. The Potter group, applying their gradient-elution, ion-exchange, chromatographic method, found the whole spectrum of guanosine phosphates—GMP (figure 18a), GDP, and GTP—in rat tumor, brain, liver, and muscle and characterized them in a similar manner.

[4] GMP = guanosine monophosphate.
GDP = guanosine diphosphate.
GTP = guanosine triphosphate.
XMP = xanthosine monophosphate.

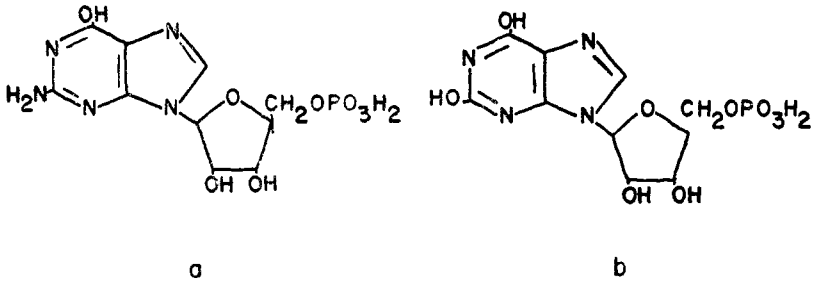


FIG. 18. (a) Guanosine monophosphate; (b) xanthosine monophosphate

GMP has been synthesized chemically from isopropylideneguanosine with either phosphoryl chloride (207) or *p*-nitrophenyl pyrophosphate (53) as phosphorylating agent. GDP and GTP can be prepared from GMP by reaction with 85 per cent phosphoric acid and dicyclohexylcarbodiimide (52).

As with the other nucleic acid bases, an important function of the guanine nucleotides is as nucleic acid precursors. The polymerizing enzyme systems of Ochoa (103, 219), Kornberg (145, 146), and Bollum and Potter (36) require GDP in the first instance and deoxy-GTP in the latter two cases. *In vivo* incorporation of GMP-2′,3′ has been demonstrated by Roll (251, 252) and Weinfeld and coworkers (303, 304), but after dephosphorylation and some transpurination (i.e., transfer of a purine from one ribose phosphate to another). Williams and LePage have shown, both *in vitro* (305) and *in vivo* (306) with ascites tumor cells, that GMP-5′ is precursor for both RNA and DNA.

Besides the function of guanosine nucleotides in nucleic acid synthesis, GTP acts as a phosphorylating agent in three reactions. The succinothiokinase reaction:

$$\text{Succinyl-CoA} + \text{ZDP} + \text{P} \rightleftharpoons \text{succinate} + \text{ZTP} + \text{CoA}$$

has been shown to occur in many tissues. In yeast and heart muscle Z = guanine (7, 255, 256); in *E. coli* and spinach Z = adenine (107). Where GTP is formed, the diphosphate is regenerated by transfer of orthophosphate to ADP via the nucleoside diphosphokinase reaction. The *E. coli* system has recently been broken down into two reactions:

$$\text{ATP} + \text{CoA} \rightleftharpoons \text{CoA-P}$$

$$\text{CoA-P} + \text{succinate} \rightleftharpoons \text{P} + \text{succinyl-CoA}$$

and it is likely that in the other tissues a similar reaction occurs, so that direct phosphorylation of CoA by GTP occurs in yeast and heart.

GTP is also required for the formation of adenylosuccinate from IMP and aspartate (see above). In this case O^{18} is transferred from the 6-position of IMP to orthophosphate derived from the breakdown of GTP to GDP, suggesting a direct phosphorylation of IMP by GTP. This product, however, has not been isolated (169, 171).

Guanosine triphosphate is also required in the oxalacetic carboxylase reaction (150):

$$\text{Oxalacetate} + \text{GTP} \rightleftharpoons \text{phosphoenolpyruvate} + CO_2 + \text{GDP}$$

in a chicken liver mitochondrial system. ITP also works well in the reaction.

Finally, GDP or GTP is needed for protein synthesis (132, 133). The transfer of activated amino acids from soluble RNA to microsomal RNA-protein requires this nucleotide, but its functions in such transfers are obscure. It appears likely that many more reactions involving guanosine nucleotides will be found.

B. Xanthosine monophosphate

The synthesis of GMP from IMP has been accomplished independently by Abrams and Bentley (2, 3) in a bone marrow enzyme system and by Lagerkvist (151), who used pigeon liver acetone powders. This work, together with that of Gehrig and Magasanik (93), shows that a requisite preliminary step is a DPN-dependent oxidation of IMP to xanthosine monophosphate (figure 18b). XMP is then aminated by the glutamine amide group to form GMP. This compound has been detected in tumor extracts by Williams and LePage (306).

C. Other guanine nucleotides

The characterization of the above-mentioned mannose nucleotide showed that it was GDP mannose (42), and it proved to be similar to the UDP sugar compounds in configuration, the mannose being attached to the phosphate at the 1-position, thus becoming nonreducing. GDP mannose has been found in yeast (42), hen oviduct (281), and plants (40), and it probably functions in the synthesis of mannans in the latter source. Much remains to be learned about the functions of this compound, however. GDP mannose is formed by this process (214, 215):

$$\text{GTP} + \text{mannose 1-phosphate} \rightleftharpoons \text{GDP mannose} + \text{PP}$$

and enzymes for this reaction have been purified from yeast.

The only other guanine nucleotides known are the guanine-containing analog of vitamin B_{12} (see above) (15), isolated from *Nocardia* sp., and a guanine nucleotide present in yeast, in which an unidentified reducing substituent is attached to the 3′-phosphate (236).

No deoxyribotide of guanine has yet been isolated from a natural source, although the deoxynucleoside has been found (129).

VII. CYTOSINE NUCLEOTIDES AND DERIVATIVES[5] (TABLE 8)

A. Cytidine mono-, di-, and triphosphates

Cytidine nucleotides exist which are both precursors of RNA and coenzymes in various metabolic reactions, especially in lipide synthesis. The monophosphate

[5] CMP = cytidine monophosphate. CTP = cytidine triphosphate.
CDP = cytidine diphosphate.

TABLE 8

Cytosine nucleotides and derivatives

Cytidine monophosphate	Deoxyribocytidine triphosphate	CDP ethanolamine
Cytidine diphosphate	5,6-Dihydro-CMP	CDP ribitol
Cytidine triphosphate	5-Methyl-CMP	CDP glycerol
Deoxyribocytidine monophosphate	CDP choline	Deoxy-DCP choline
Deoxyribocytidine diphosphate		

was found in certain bacteria (279), and this was followed by the discovery of the triphosphate (27) in rabbit muscle, and then all three forms in tumors and various rat tissues (259, 260). They have been found subsequently in almost every tissue or organism examined. These compounds were identified, as usual, on the basis of ultraviolet spectra, phosphate content, reactivity, enzymatic digestion, etc. The structure of CMP is shown in figure 19.

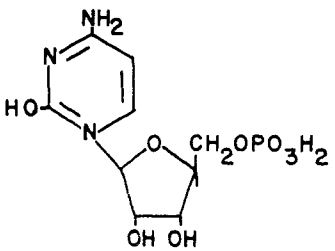


FIG. 19. Cytidine monophosphate

The synthesis of CMP has been accomplished by phosphorylation of the isopropylidenecytidine with dibenzylphosphorochloridate by Michelson and Todd (207).

Acid-soluble extracts of thymus have shown the presence of the corresponding deoxyribose compounds (240) at all three levels of phosphorylation. These, presumably, are the immediate precursors of DNA, probably at the triphosphate level. The exact details of formation of these, and of all deoxyribose compounds, is not yet fully understood.

There is one report of the identification of nucleotide-like methyl-CMP in rat tissues (64), and more recently Cohen has achieved the conversion of deoxy-CMP to 5-hydroxymethyldeoxy-CMP in *E. coli* extracts, suggesting its possible physiological occurrence (56, 88). One report exists of the isolation of 5,6-dihydro-CMP from rat liver slices after incubation with cytidine-4-C^{14} (102). Whether this is an artifact or really a metabolite is not known, although the base, 5,6-dihydrocytosine, is a degradation product of cytosine.

B. Other cytosine nucleotides

In the course of the investigation of phospholipide synthesis, a requirement for CTP was noted by Kennedy's group, and this soon led to the discovery of two cytosine coenzymes which are involved in this process. Phosphorylcholine was found to react with CTP to form CDP choline, with the release of pyrophosphate, and a corresponding reaction exists with ethanolamine, forming CDP

TABLE 9

Thymine nucleotides

Thymidine monophosphate	Thymidine diphosphate	Thymidine triphosphate

ethanolamine (16, 136, 137). These coenzymes can then combine with D-α,β-diglycerides to give CMP plus lecithin or phosphatidylethanolamine. The coenzymes have been found in rat and chicken liver, seminal vesicles (307), and yeast (172), and CDP choline (figure 20) has been crystallized from the latter source.

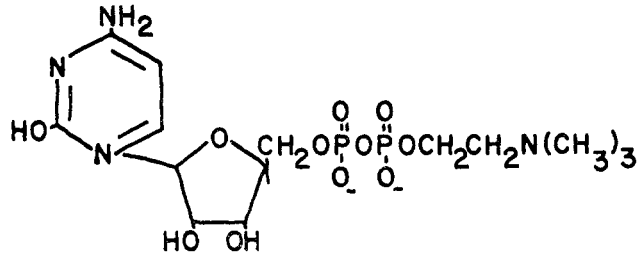


FIG. 20. Cytidine diphosphate choline

Kennedy has synthesized these compounds chemically by the reaction of CMP, phosphorylcholine, and dicyclohexylcarbodiimide (134).

A corresponding deoxyribo-CDP choline has been identified in sea urchin eggs (286) and probably in thymus (238, 241). The deoxyribo-CDP ethanolamine may likewise exist. The significance of these compounds is not understood at present.

Baddiley and associates have isolated two further CDP coenzymes from *Lactobacillus arabinosus*, CDP alpha glycerol (11, 12) and CDP ribitol (10, 11). Their existence is suggestive of some possible role in glyceride and riboflavine synthesis, but no evidence as to their function exists to date.

VIII. THYMINE NUCLEOTIDES[6] (TABLE 9)

A. Thymidine mono-, di-, and triphosphates

Thymus extracts have been found to contain the three phosphates of (deoxy) thymidine, TMP (237), TDP, and TTP (240, 241). These have also been found in rat liver and thymus (64, 239). *E. coli* also contains the triphosphate, which participates in the formation of DNA in the systems of Kornberg and of Bollum and Potter (see above).

No coenzyme function of the thymidine nucleotides has been found.

Michelson and Todd have synthesized TMP (figure 21) from 3′-acetylthymidine and dibenzylphosphorochloridate (208).

[6] TMP = thymidine monophosphate. TTP = thymidine triphosphate.
TDP = thymidine diphosphate.

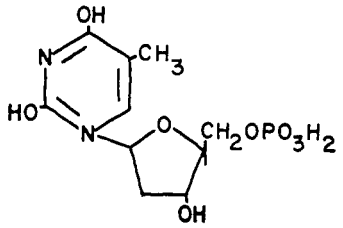


FIG. 21. Thymidine monophosphate

TABLE 10

Compound types of the acid-soluble nucleotides

Substituent on Nucleoside	Adenosine	Guanosine	Cytosine	Uridine	Thymidine
PO_4	AMP	GMP	CMP	UMP	TMP
$(PO_4)_2$	ADP	GDP	CDP	UDP	TDP
$(PO_4)_3$	ATP	GTP	CTP	UTP	TTP
$(PO_4)_4$	APPPP	—	—	—	—
$(PO_4)_5$	APPPPP	—	—	—	—
PO_4-X	Acyl-adenylates	—	—	UMP-aspartate	—
$(PO_4)_2$-X	ADP-asparate	GDP mannose	CDP choline	UDP-sugars	—
$(PO_4)_2$-X-PO_4	CoA, TPN	—	—	UDP-sugar-PO_4	—
PO_4-X-Y	AMP-SO_4-peptide	—	—	—	—
$(PO_4)_2$-X-Y	—	—	—	UDP-sugar-peptide	—

IX. NUCLEOTIDES CONTAINING ARABINOSE

A very unusual type of compound is that with arabinose as the sugar, investigated by Bergmann. These are the only exceptions found to the rule that ribose or 2-deoxyribose is the sugar in this class of compounds. Of seventeen different sponges, only one, *Cryptotethia crypta*, contains the arabanosides. Arabanothymidine and arabanouridine (29, 31) have been isolated, and Bergmann has reason to believe that a highly unstable arabanonucleic acid, "ANA," may be present in this sponge (28, 32). This organism also contains ribosides and DNA. Since the sponge was allowed to autolyze before analysis, it seems quite probable that these compounds may have existed in the nucleotide state *in vivo*.

X. DISCUSSION AND CONCLUSIONS

What acid-soluble nucleotides may we expect to be found in the next few years? Several nucleic acid components remain undiscovered in their acid-soluble forms. *N*-Methyladenine, 5-methylcytosine, 5-hydroxymethylcytosine, and the unidentified pyrimidines mentioned above should have their acid-soluble counterparts. Glucose is combined with hydroxymethylcytosine in some phage nucleic acids, and possibly this may be attached at the acid-soluble level. Then there are the compounds which may be intermediates between various known nucleotides. For instance, the conversion of uracil to thymine requires reduction as well as methylation, and an intermediate may be expected.

Another way of looking at this question is to tabulate the *types* of compounds which already exist, as has been done in table 10. Thus, there seem to be amino

acid anhydrides with adenylic and uridylic acids, perhaps with guanylic and cytidylic as well. The variety of types encountered in the case of the adenine nucleotides may be because they are unique, or because analogous forms of the other bases are present in such small quantities that they have not yet been detected. Another problem is that many of these possible nucleotides have not been looked for with the best new techniques.

A number of new UDP sugars may also be expected. This will depend in part on the examination of favorable material, and should provide a fruitful field of investigation for students of plant biochemistry. The field of interconversions and polymerization of the monosaccharides is a wide and comparatively little explored field, and seems to be closely related to the uridine nucleotides. Comparison of nucleotide-dependent and nucleotide-independent reactions in this field should be illuminating with regard to alternative metabolic pathways and their control.

As detailed investigations of protein and fatty acid synthesis are continued, more instances of acyl activation by adenylate formation may be expected to come to light.

One large group of compounds which has not yet been shown to combine with nucleotides is the isoprene derivatives, the steroids, sterols, carotenoids, etc. Workers in this area might be alert to the possibility of encountering some such compounds.

While, owing to the present status of this field, this review may seem at points to be little more than a bestiary—a catalog of strange and unusual compounds—it is hoped that some few generalizations (and many more questions) may be gathered at this point.

The whole problem of distribution of functions among the nucleotides remains, but the nucleotide with which compounds combine may be expected to involve specificities of great significance. Considering just the coenzymes, cytosine combines with alcohols (choline, ethanolamine), uracil with aldehydes (the sugars), and adenylic acid with acids (amino acids, acetic, sulfuric). Only adenine seems to react with nonphosphorylated compounds. Is there any structural or thermodynamic basis for this division of labor?

Secondly, in all these coenzymes only one high-energy anhydride bond is present. Most reactions begin with the nucleoside triphosphate. ATP loses two phosphates (as pyrophosphate) when it combines with an acid; the others lose only one when combining with compounds of lower oxidation levels.

Another very prominent point is that adenine is the principal agent for the transfer of phosphate bond energy in the cells. It is present in greater amounts than are the others, but this does not answer anything. GTP acts in a few cases, but the pyrimidines have not been shown to participate in such reactions at all (except transphosphorylations). When phosphorylations do occur, only the terminal phosphate is donated. The ADP thus formed is then either hydrolyzed to AMP or rephosphorylated to ATP, or it phosphorylates another ADP by the myokinase reaction. Only in the case of the myokinase reaction does the second high-energy bond get used for phosphorylation. In the case of the pyrophos-

phorolytic cleavage of ATP, this P^1—P^2 bond is split, but here the P^2—P^3 bond is not gainfully utilized. How is this to be rationalized?

The interconversion of high-energy forms, and the use of several different high-energy compounds in consecutive steps of a single reaction, are interesting points. For instance, acyl-adenylates go to acyl thioesters (CoA), finally to acceptors, or phosphoryl-CoA as a precursor to succinyl-CoA. Again, microörganisms use acetyl phosphate where higher forms use acetyl adenylate.

All these problems, and more, seem to be pertinent to those who are interested in the *control* of metabolism, and the group of acid-soluble nucleotides seems unlikely to be barren in this respect.

While the answers to many of our most fundamental questions seem beyond the grasp of the biochemists' tools, work on the interrelationships of the nucleotide coenzymes can be expected to give rich returns. It is perhaps to be regretted that most of the workers in this field attack it from the periphery—i.e., they are primarily interested in amino acids, or lipides, etc. Few are attempting to connect the many disjointed observations and isolated reports which fill the literature.

The authors wish to thank Dr. V. R. Potter, who encouraged them to write this review.

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