RECENT DEVELOPMENTS IN THE BIOCHEMISTRY OF NUCLEOTIDE COENZYMES

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IT would be difficult, and perhaps unnecessary, to attempt a rigid definition of the term " coenzyme ". The coenzymes were originally understood to include a small group of organic compounds of relatively low molecular weight which are required in catalytic amounts in certain enzymic reactions. These coenzymes possess no enzymic properties themselves, but presumably combine with the true enzyme protein to form a complex which is able then to catalyse the overall reaction. This description sufficed for some time and it was assumed that the coenzymes, like any other catalysts, must actually participate chemically in the reactions which they catalysed. It soon became apparent, however, that this description was losing its significance. Most of the metalloporphyrin catalysts, usually known as prosthetic groups when attached to an enzyme protein, would fit the above definition. On the other hand, the nucleotide coenzymes, *e.g.*, cozymase and flavin-adenine dinucleotide, are only catalytic in their action in multienzyme systems where cyclic processes are able to regenerate the coenzyme continuously. If the enzymes responsible for the processes of coenzyme regeneration are removed or destroyed, then the coenzyme must be regarded as a substrate and will be required in stoicheiometric amounts. We now know of nucleotides which, although catalytic when accompanied by the necessary regenerating enzymes and substrates, could only be described most loosely as coenzymes. In this group are the " nucleoside-diphosphate-sugar " compounds. These include uridine diphosphate glucose, where the substrate for the reaction (glucose 1-phosphate $\rightleftharpoons$ galactose 1-phosphate) is actually a part of the coenzyme molecule. Such compounds are frequently termed " reactive intermediates ", but in many respects they must be regarded as coenzymes.

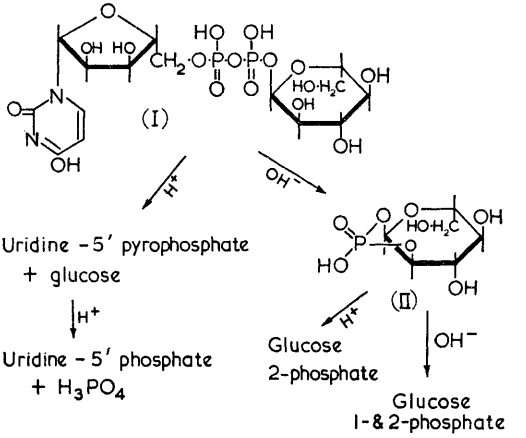
Until about 1945 four nucleotide coenzymes were known. These were the two nicotinamide compounds (DPN and TPN), the riboflavin coenzyme (flavin-adenine dinucleotide), and adenosine triphosphate. The discovery in 1945—46 of coenzyme A by Lipmann and his colleagues was soon followed by the isolation of uridine diphosphate glucose (UDPG) by Leloir. Since that time steadily increasing numbers of nucleotides of the UDPG type have been detected or isolated from animals, plants, and bacteria. Although the exact nature of the enzymic reactions in which these nucleotides participate is not always known, it is certain that they must be involved in processes similar to those already observed for UDPG. For this reason they may be classified as nucleotide coenzymes. There can be little doubt that the rapid progress in this field which has occurred in recent years is largely a result of greatly improved techniques for the isolation and

separation of nucleotide mixtures ; and of these techniques the most outstanding is that of ion-exchange chromatography, accompanied by paper chromatography.

In this Review no attempt has been made to treat the subject exhaustively. Several books and review articles which have appeared during the last few years describe both chemical and enzymic or metabolic aspects of the longer-established coenzymes such as the pyridine nucleotides, flavin-adenine dinucleotide, adenosine phosphates, etc., and also coenzyme A. For this reason, and for space considerations, these members of the group are not included in this Review. It is our purpose to emphasise the direction along which recent developments in the field have been progressing. Particular attention has been devoted here to the group of nucleotides of the uridine-diphosphate-glucose type, since this group appears to be expanding rapidly in both numbers and importance. In addition, we have included the group of nucleoside-monophosphate-X compounds, in which X is a substrate molecule (*e.g.*, acyl adenylates and nucleotide-amino-acid derivatives). " Active sulphate " is sufficiently related to this group to be included.

The Nucleoside-Pyrophosphate-Substrate Group of Compounds. Uridine Diphosphate Glucose (UDPG) and Uridine Diphosphate Galactose (UDPGal)

The first member of this group of coenzymes, uridine diphosphate glucose (I), was discovered by Leloir and his collaborators during their investigation of the conversion of α-D-galactose 1-phosphate into α-D-glucose 1-phosphate in galactose-adapted yeast.[1] The structure was established [2–4] by methods which have proved valuable in later investigations in this group. Acid-hydrolysis liberated uridine-5′ phosphate, inorganic phosphate, and D-glucose ; very mild acid hydrolysis gave uridine-5′ pyrophosphate (UDP)



[1] Caputto, Leloir, Trucco, Cardini, and Paladini, *J. Biol. Chem.*, 1949, **179**, 497.
[2] Cardini, Paladini, Caputto, and Leloir, *Nature*, 1950, **165**, 191.
[3] Caputto, Leloir, Cardini, and Paladini, *J. Biol. Chem.*, 1950, **184**, 333.
[4] Paladini and Leloir, *Biochem. J.*, 1952, **51**, 426.

and glucose. Such great lability indicated that UDPG was a derivative of glucose 1-phosphate. Treatment with ammonia gave uridine-5′ phosphate and glucose 1 : 2-hydrogen phosphate (II); further alkaline hydrolysis gave glucose 1- and 2-phosphate, while acid yielded glucose 2-phosphate. The formation of a cyclic 1 : 2-phosphate of glucose does not necessarily mean that UDPG contains α-glucose 1-phosphate; the β-anomer should also be capable of cyclisation, since the hydroxyl groups at positions 1 and 2 are then both equatorial.[5, 6] Proof of the α-configuration of UDPG, and of the pyranose ring of the hexose, comes from its enzymic [7–11] and chemical [12–14] synthesis from α-D-glucose 1-phosphate, as well as its polarimetric behaviour on acid hydrolysis.[4] Uridine diphosphate galactose (UDPGal) has also been synthesised chemically.[13] UDPG is formed enzymically [7–11] by reaction of α-D-glucose 1-phosphate and uridine triphosphate (UTP):

$$\text{Glucose 1-phosphate} + \text{UTP} \rightleftharpoons \text{UDPG} + \text{Pyrophosphate}$$

The enzyme, UDPG pyrophosphorylase, is analogous to that catalysing the reaction between diphosphopyridine nucleotide and inorganic pyrophosphate.[15]

UDPG has been shown to act as a coenzyme in the galactose–glucose transformation in the following manner: [16, 17]

$$\text{UDPG} + \alpha\text{-D-Galactose 1-phosphate} \underset{\text{transferase}}{\overset{\text{Uridyl}}{\rightleftharpoons}} \text{UDPGal} + \alpha\text{-D-Glucose 1-phosphate}$$

$$\text{UDPGal} \rightleftharpoons \text{UDPG}$$

The enzyme for the first reaction, galactose phosphate uridyl transferase,[17, 18] has been shown to be absent from patients suffering from congenital galactosæmia; [19–20] feeding of galactose leads to an accumulation of α-galactose 1-phosphate. There is evidence that in galactose-adapted *Saccharomyces fragilis* [18] and in some green plants [10] UDPGal may be formed from α-galactose 1-phosphate and UTP. The mechanism of the second

[5] Khorana, Tener, Wright, and Moffatt, *J. Amer. Chem. Soc.*, 1957, **79**, 430.
[6] Brown and Higson, *J.*, 1957, 2034.
[7] Trucco, *Arch. Biochem. Biophys.*, 1951, **34**, 482.
[8] Munch-Petersen, Kalckar, Cutolo, and Smith, *Nature*, 1953, **172**, 1036.
[9] Smith, Munch-Petersen, and Mills, *ibid.*, p. 1038.
[10] Neufeld, Ginsburg, Putman, Fanshier, and Hassid, *Arch. Biochem. Biophys.*, 1957, **69**, 602.
[11] Munch-Petersen, *Acta Chem. Scand.*, 1955, **9**, 1523.
[12] Kenner, Todd, and Webb, *J.*, 1954, 2843.
[13] Michelson and Todd, *J.*, 1956, 3459.
[14] Chambers, Moffatt, and Khorana, *J. Amer. Chem. Soc.*, 1957, **79**, 4240.
[15] Kornberg and Pricer, *J. Biol. Chem.*, 1951, **191**, 535.
[16] Leloir, *Arch. Biochem. Biophys.*, 1951, **33**, 186.
[17] Maxwell, Kalckar, and Burton, *Biochim. Biophys. Acta*, 1955, **18**, 444.
[18] Kalckar, Braganca, and Munch-Petersen, *Nature*, 1953, **172**, 1038.
[19] Kalckar, Anderson, and Isselbacher, *Proc. Nat. Acad. Sci. U.S.A.*, 1956, **42**, 49; *Biochim. Biophys. Acta*, 1956, **20**, 262.
[20] Kalckar, *Science*, 1957, **125**, 105.

reaction, catalysed by galactowaldenase,* has aroused much interest. At equilibrium, the ratio of the glucose to galactose nucleotide is 3 : 1, showing the increased stability of the equatorial 4-hydroxyl group in glucose over that of its axial isomer. Several mechanisms have been proposed for the transformation,[21] which requires a formal inversion of the hydroxyl group at the 4-position in the hexose, but it has now been found that the enzyme is stimulated by diphosphopyridine nucleotide (DPN) [22] and that an intermediate 4-keto-derivative is involved.[23–25]

UDPG has been shown to occur widely in plants and animals,[26–31] and it has become apparent [3] that it is involved in reactions other than the galactose–glucose interconversion; UDPGal and galactowaldenase are present in large amounts in some yeasts not adapted to galactose.[32] It was suggested [33] that compounds of the UDPG type could be concerned in transformation of sugars and their subsequent incorporation into polysaccharides. At the same time it was suggested that UDPG might be involved in sucrose biosynthesis.[33, 34] The first direct evidence for a reaction of this type was given by Leloir and Cabib [35] who showed that the trehalose phosphate of Robison and Morgan [36] was synthesised from UDPG and D-glucose 6-phosphate by yeast preparations. Leloir and Cardini [37–39] later described two enzymes occurring in plants which catalyse the reactions :

$$\text{UDPG} + \text{D-Fructose 6-phosphate} \rightleftharpoons \text{Sucrose phosphate} + \text{UDP}$$

$$\text{UDPG} + \text{D-Fructose} \rightleftharpoons \text{Sucrose} + \text{UDP}$$

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[21] Leloir, *Adv. Enzymol.*, 1953, **14,** 193.
[22] Maxwell, *J. Amer. Chem. Soc.*, 1956, **78,** 1074.
[23] Anderson, Landel, and Diedrich, *Biochim. Biophys. Acta*, 1956, **22,** 573.
[24] Kowalsky and Koshland, *ibid.*, p. 575.
[25] Kalckar and Maxwell, *ibid.*, p. 588.
[26] Ginsburg, Stumpf, and Hassid, *J. Biol. Chem.*, 1956, **223,** 977.
[27] Buchanan, Lynch, Benson, Bradley, and Calvin, *ibid.*, 1953, **203,** 935.
[28] Rutter and Hansen, *ibid.*, 1953, **202,** 323.
[29] Hurlbert and Potter, *ibid.*, 1954, **209,** 1.
[30] Hurlbert, Schmitz, Brumm, and Potter, *ibid.*, p. 23.
[31] Smith and Mills, *Biochim. Biophys. Acta*, 1954, **13,** 386.
[32] Mills, Smith, and Lochhead, *ibid.*, 1957, **25,** 521.
[33] Buchanan, Bassham, Benson, Bradley, Calvin, Daus, Goodman, Hayes, Lynch, Norris, and Wilson, " Phosphorus Metabolism ", Vol. II, Johns Hopkins Press, Baltimore, 1952, p. 440.
[34] Buchanan, *Arch. Biochem. Biophys.*, 1953, **44,** 140.
[35] Leloir and Cabib, *J. Amer. Chem. Soc.*, 1953, **75,** 5445.
[36] Robison and Morgan, *Biochem. J.*, 1928, **22,** 1277; *ibid.*, 1930, **24,** 119.
[37] Leloir and Cardini, *J. Amer. Chem. Soc.*, 1953, **75,** 6084.
[38] Leloir, Cardini, and Chiriboga, *J. Biol. Chem.*, 1955, **214,** 149.
[39] Cardini and Leloir, *ibid.*, p. 157.

* We consider that this name, originally used by Leloir to describe the overall process galactose ⟶ glucose, should be retained for the enzyme, rather than " 4-epimerase ", suggested by Kalckar (ref. 20; cf. refs. 25 & 32).

Similar results have now been obtained by Bean and Hassid,[40] who were able also to show that fructose could be replaced by D-xylulose, D-rhamnulose, or L-sorbose in the above reaction to give the appropriate disaccharides. With sugar-beet leaves, Burma and Mortimer [41] found mainly the reaction leading to sucrose phosphate. An enzyme from pea seedlings which catalyses sucrose synthesis from α-D-glucose 1-phosphate and fructose has been studied by Turner.[42] It is thought that UDPG may be an intermediate, and Cardini has independent evidence of this.[43]

Lactose synthesis has also been investigated. It has been known for some time that mammary tissue contains UDPG [17, 28, 44, 45] and that galactowaldenase is present. Bovine mammary tissue preparations will convert UDPG and α-D-glucose 1-phosphate into α-lactose 1-phosphate by the following pathway : [46]

$$\text{UDPG} \rightleftharpoons \text{UDPGal}$$

$$\text{UDPGal} + \alpha\text{-D-Glucose 1-phosphate} \xrightarrow[\text{transferase}]{\text{Galactosyl}} \text{Lactose 1-phosphate} + \text{UDP}$$

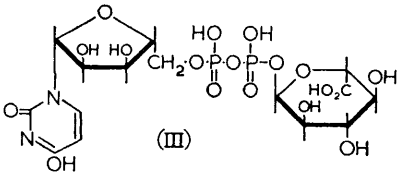
The reactions have been fully confirmed, by using both ^{32}P and ^{14}C labelling. It is not certain whether this is the only pathway by which lactose is synthesised.[47] Bean and Hassid [48] have suggested that floridoside, 2-*O*-(α-D-galactosyl)glycerol, may arise by reaction between UDPGal and α-glycerophosphate.

Glaser [49] recently described the reaction of UDPG with oligosaccharides from cellulose, using a preparation from *Acetobacter xylinum*, to give a cellulose-like polymer, and a similar system from rat liver can effect a synthesis of glycogen.[49a] The time is clearly ripe for a close investigation of well-known polysaccharide-synthesising enzymes to find whether they have a firmly bound nucleotide component.

Uridine Diphosphate Glucuronic Acid (UDPGA) and Uridine Diphosphate Galacturonic Acid (UDPGalA)

Work on the formation of glucuronides [50] in liver homogenates led to the isolation of UDPGA by Dutton and Storey.[51] Since it is formed enzymically by oxidation of UDPG by DPN there is no doubt that it has structure (III).

[40] Bean and Hassid, *J. Amer. Chem. Soc.*, 1955, **77**, 5737.
[41] Burma and Mortimer, *Arch. Biochem. Biophys.*, 1956, **62**, 16.
[42] Turner, *Nature*, 1953, **172**, 1149 ; 1954, **174**, 692 ; *Biochem. J.*, 1957, **67**, 450.
[43] Leloir, 3rd Internat. Congress Biochem., Brussels, 1955, p. 154.
[44] Caputto and Trucco, *Nature*, 1952, **169**, 1061.
[45] Smith and Mills, *Biochim. Biophys. Acta*, 1954, **13**, 587.
[46] Gander, Petersen, and Boyer, *Arch. Biochem. Biophys.*, 1956, **60**, 259 ; 1957, **69**, 85.
[47] Wood, Schambye, Peeters, and Siu, *J. Biol. Chem.*, 1957, **226**, 1023.
[48] Bean and Hassid, *ibid.*, 1955, **212**, 411.
[49] Glaser, *Biochim. Biophys. Acta*, 1957, **25**, 436.
[49a] Leloir and Cardini, *J. Amer. Chem. Soc.*, 1957, **79**, 6340.
[50] Teague, *Adv. Carbohydrate. Chem.*, 1954, **9**, 185.
[51] Dutton and Storey, *Biochem. J.*, 1953, **53**, xxxvii ; 1954, **57**, 275.



(III)

UDPG dehydrogenase has been found both in liver [52, 53] and in pea seedlings.[54] Attempts to trap an aldehyde intermediate in the oxidation have, at least in the case of the liver enzyme,[53] been unsuccessful. UDPGA is thought to arise solely by the action of UDPG dehydrogenase on UDPG, no UDPGA pyrophosphorylase having been detected.[31] The nucleotide has been isolated from mung-bean seedlings [55, 56] and from Type II and Type III pneumococci,[57] as well as from liver.[31, 51] It has been shown that UDPGA will transfer its glucuronic acid residue to form both ether [51, 58, 59] and ester [60] glucuronides, and is evidently the coenzyme for such reactions.

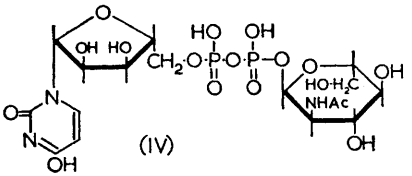
UDPGalA has been isolated from a Type I pneumococcus.[61] There is none of the glucuronic acid derivative present, although both Type II and III organisms contain it. There appears to be a correlation between the nucleotides present in Type I and III organisms and the uronic acid residues in their capsular polysaccharides. This is consistent with the polysaccharide-precursor hypothesis for nucleoside-diphosphate-sugar compounds. More direct evidence for the participation of UDPGA in polysaccharide synthesis has come from studies of ^{14}C-labelled nucleotides. An enzyme from the Rous chicken sarcoma will convert UDPGA and uridine diphosphate acetylglucosamine (UDPAG ; see below) into polymers having the properties of hyaluronic acid.[62] The UDPGA can be replaced by UDPG and DPN.

Uridine Diphosphate Acetylglucosamine (UDPAG) and Uridine Diphosphate Acetylgalactosamine (UDPAGal)

Paladini and Leloir [4] found an unidentified uridine nucleotide in their preparations of UDPG from yeast. This was later identified as UDPAG [63] (IV).

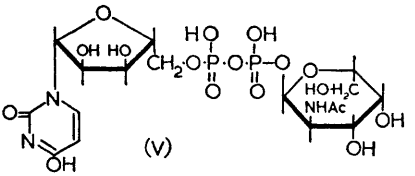
Gentle acid-treatment liberated uridine-5′ pyrophosphate and *N*-acetyl-D-glucosamine. The compound is more stable to alkali than UDPG, and the products are uridine-5′ phosphate and *N*-acetylglucosamine 1-phosphate,

[52] Strominger, Maxwell, Axelrod, and Kalckar, *J. Amer. Chem. Soc.*, 1954, **76**, 6411 ; *J. Biol. Chem.*, 1957, **224**, 79.
[53] Maxwell, Kalckar, and Strominger, *Arch. Biochem. Biophys.*, 1956, **65**, 2.
[54] Strominger and Mapson, *Biochem. J.*, 1957, **66**, 567.
[55] Solms, Feingold, and Hassid, *J. Amer. Chem. Soc.*, 1957, **79**, 2342.
[56] Solms and Hassid, *J. Biol. Chem.*, 1957, **228**, 357.
[57] Smith, Mills, and Harper, *J. Gen. Microbiol.*, 1957, **16**, 426.
[58] Storey and Dutton, *Biochem. J.*, 1955, **59**, 279.
[59] Isselbacher and Axelrod, *J. Amer. Chem. Soc.*, 1955, **77**, 1070.
[60] Dutton, *Biochem. J.*, 1956, **64**, 693.
[61] Smith, Mills, and Harper, *Biochim. Biophys. Acta*, 1957, **23**, 662.
[62] Glaser and Brown, *Proc. Nat. Acad. Sci. U.S.A.*, 1955, **41**, 253.
[63] Cabib, Leloir, and Cardini, *J. Biol. Chem.*, 1953, **203**, 1055.



(IV)

an interesting consequence of the lack of a 2-hydroxyl group. The assignment of an α-configuration depends on the enzymic synthesis of UDPAG from α-D-*N*-acetylglucosamine 1-phosphate by yeast [64] and liver preparations.[65] UDPAG has been detected in plants,[55, 56] animal tissues,[30, 31] and micro-organisms.[57, 61, 66] It has been noted that labelled hyaluronic acid is formed from labelled UDPGA and UDPAG by Rous sarcoma.[62] Glaser and Brown [67] have now found that an enzyme from *Neurospora crassa* catalyses the incorporation of the *N*-acetylglucosamine moiety of UDPAG into an insoluble polysaccharide with the properties of chitin.



(V)

The UDPAG fraction from liver contains UDPAGal [68] (V); acid-hydrolysis gives *N*-acetylgalactosamine together with *N*-acetylglucosamine. The free hexosamines have been separated by paper chromatography and yield the appropriate pentoses on degradation with ninhydrin. A waldenase enzyme exists for the equilibration of the two nucleotides.[43, 68]

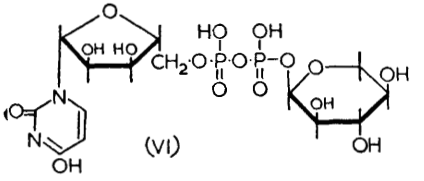
Lardy has found that both yeast and liver can convert α-D-glucosamine 1-phosphate and UTP into uridine diphosphate glucosamine. The biochemical significance of this reaction is not yet clear.

Uridine diphosphate acetylglucosamine phosphate and uridine diphosphate acetylgalactosamine sulphate have been isolated from hen's oviduct.[69]

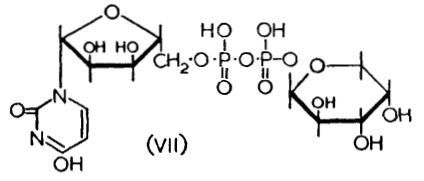
Uridine Diphosphate Pentoses

Ginsburg, Stumpf, and Hassid [26] have isolated uridine diphosphate D-xylose (UDPXy) (VI) and uridine diphosphate L-arabinose (UDPAr) (VII) from mung-bean seedlings as well as from other plant sources.[10] The nucleotides can be synthesised by reaction of α-D-xylose 1-phosphate or α-L-arabinose 1-phosphate with UTP in the presence of plant extracts.

[64] Maley, Maley, and Lardy, *J. Amer. Chem. Soc.*, 1956, **78**, 5303.
[65] Maley and Lardy, *Science*, 1956, **124**, 1207.
[66] Smith and Mills, *Biochem. J.*, 1956, **64**, 52P.
[67] Glaser and Brown, *Biochim. Biophys. Acta*, 1957, **23**, 449; *J. Biol. Chem.*, 1957, **228**, 729.
[68] Pontis, *ibid.*, 1955, **214**, 195; Cardini and Leloir, *ibid.*, 1957, **225**, 317.
[69] Strominger, *Biochim. Biophys. Acta*, 1955, **17**, 283.



(VI)



(VII)

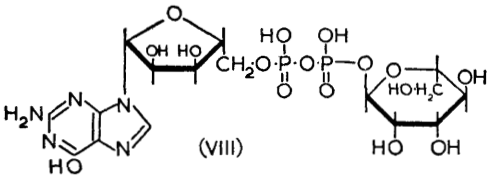
β-D-Xylose 1-phosphate is inactive, but β-L-arabinose 1-phosphate is active in this system. Plants also contain a waldenase [26, 70] which catalyses the reaction :

$$\text{UDP D-Xy} \rightleftharpoons \text{UDP L-Ar}$$

The reaction is not catalysed by yeast galactowaldenase, although a formal similarity between this reaction and that catalysed by galactowaldenase is apparent.

Guanosine Diphosphate Mannose (GDPM)

From among the nucleotides from yeast, Cabib and Leloir [71, 72] isolated GDPM (VIII). Mild acid-hydrolysis liberated mannose and guanosine-5′ pyrophosphate ; further hydrolysis gave inorganic phosphate and guanosine-5′ phosphate. More drastic hydrolysis gave guanine. That the



(VIII)

nucleotide is a derivative of α-D-mannose 1-phosphate follows from its enzymic synthesis from the latter and guanosine triphosphate (GTP).[73] GDPM is present in yeast [72, 74] and in hen's oviduct.[75] It is of interest that yeast contains a mannan.[76]

Pontis,[74] using an improved ion-exchange technique,[77] has discovered a

[70] Ginsburg, Neufeld, and Hassid, *Proc. Nat. Acad. Sci. U.S.A.*, 1956, **42**, 333.

[71] Leloir, " Phosphorus Metabolism ", Vol. I, Johns Hopkins Press, Baltimore, 1951, p. 75.

[72] Cabib and Leloir, *J. Biol. Chem.*, 1954, **206**, 779.

[73] Munch-Petersen, *Arch. Biochem. Biophys.*, 1955, **55**, 592.

[74] Pontis, *Biochim. Biophys. Acta*, 1957, **25**, 417.

[75] Strominger, *Fed. Proc.*, 1954, **13**, 307.

[76] Haworth, Hirst, and Isherwood, *J.*, 1937, 784 ; Haworth, Heath, and Peat, *J.*, 1941, 833.

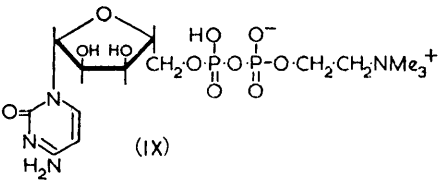
[77] Pontis, Cabib, and Leloir, *Biochim. Biophys. Acta*, 1957, **26**, 146.

new monophosphate of guanosine. The compound appears to be a phosphodiester, linking guanosine in the 3′-position with an unknown residue.

Cytidine Diphosphate Choline and Cytidine Diphosphate Ethanolamine

The discovery by Kennedy and Weiss that a cytidine derivative participates in the biosynthesis of lecithin has clarified considerably our understanding of phospholipid metabolism. Isotope studies [78, 79] have shown that choline phosphate enters the lecithin molecule as a unit. In the presence of a particulate enzyme system isolated from rat liver the incorporation of choline phosphate into lecithin required the addition of an impure sample of adenosine triphosphate (ATP). The activity of this crude nucleotide was not associated with its ATP content but was derived from cytidine-5′ triphosphate (CTP) which was present as an impurity.[80]

It was shown that both cytidine triphosphate and choline phosphate could be substituted in the multienzyme system from liver by synthetic cytidine diphosphate choline (IX). Similarly, CDP-ethanolamine (X) was shown to be an intermediate in the enzymic synthesis of phosphatidylethanolamine.[81]



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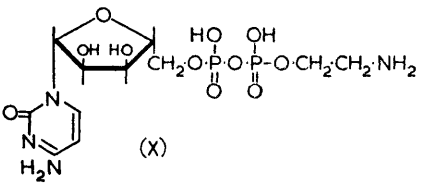


(X)

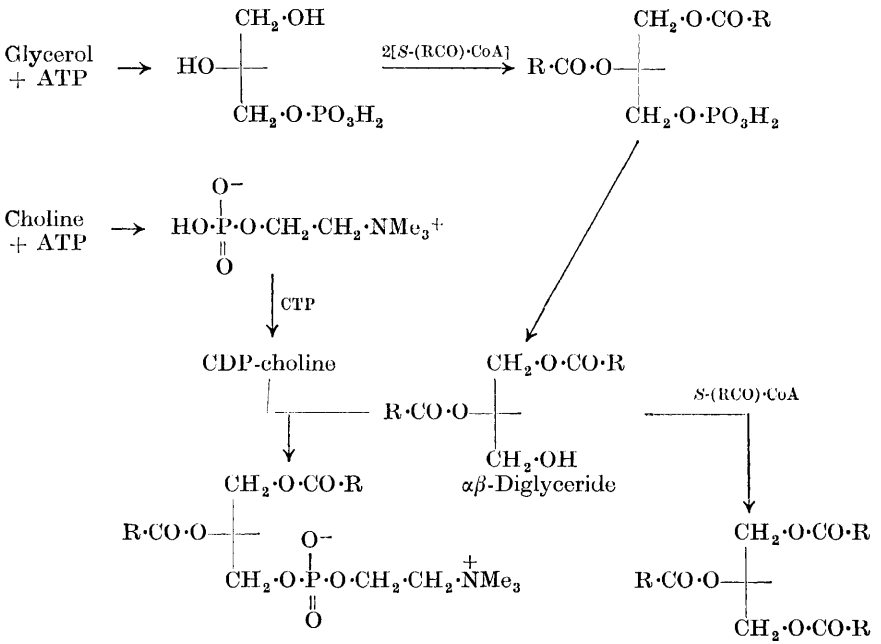
Both CDP-choline and CDP-ethanolamine readily yielded the corresponding phosphatides in the presence of particulate systems from liver. The rate of synthesis of lecithin from CDP-choline prepared from labelled choline phosphate was much higher than from CTP and choline phosphate.[80] This and other tracer experiments strongly support the view that CDP-choline is an intermediate in lecithin synthesis.

Kennedy and Weiss detected CDP-choline and CDP-ethanolamine in the livers of various animals,[80] and the crystalline sodium salt of CDP-choline has been isolated in reasonable amount from yeast.[82] Direct comparison of the nucleotide from yeast with synthetic CDP-choline proved their identity. The wide significance of the cytidine coenzymes in phospholipid

[78] Kornberg and Pricer, *Fed. Proc.*, 1952, **11**, 242.
[79] Rodbell and Hanahan, *J. Biol. Chem.*, 1955, **214**, 607.
[80] Kennedy and Weiss, *ibid.*, 1956, **222**, 193.
[81] Kennedy, *ibid.*, p. 185.
[82] Lieberman, Berger, and Giminez, *Science*, 1956, **124**, 81.

synthesis is indicated by their participation in cell-free preparations from brain [83] and seminal vesicle.[84] Furthermore, enzymes which catalyse the synthesis and utilisation of CDP-choline have been detected in liver, kidney, brain, yeast, and carrot root.[80]

According to Kennedy [85] the biosynthesis of phospholipids and fats occurs mainly through the annexed route. It is not possible to discuss here



the details of all stages in this scheme, but a brief outline of the enzymic synthesis of CDP-choline from CTP, and the subsequent formation of lecithin, is included.

The enzymic synthesis of CDP-choline occurs readily in extracts of mammalian tissues to which CTP and choline phosphate have been added. The enzyme is known as phosphorylcholine-cytidyl transferase, or PC-cytidyl transferase.[86] Reaction occurs according to the equation :

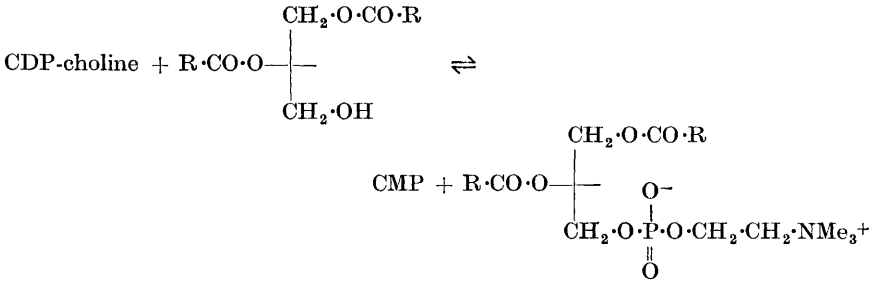
$$\text{CTP} + \text{Choline phosphate} \rightleftharpoons \text{CDP-choline} + \text{Pyrophosphate}$$

Magnesium or manganese ions are required to activate the enzyme, which is absolutely specific for CTP. Other nucleotides, *e.g.*, ATP, UTP, GTP, and inosine triphosphate, were without effect. The reaction, which is readily reversed, is an example of the general type of reaction for the synthesis of unsymmetrically substituted nucleoside pyrophosphate coenzymes from nucleoside triphosphates. A separate enzyme, phosphoryl

[83] McMurray, Berry, and Strickland, *Fed. Proc.*, 1956, **15**, 313.
[84] Williams-Ashman and Banks, *J. Biol. Chem.*, 1956, **223**, 509.
[85] Kennedy, *Ann. Rev. Biochem.*, 1957, **26**, 119.
[86] Borkenhagen and Kennedy, *J. Biol. Chem.*, 1957, **227**, 951.

ethanolamine cytidyl transferase (PE-cytidyl transferase) catalyses the synthesis of CDP-ethanolamine. In this case the reversible reaction between CTP and ethanolamine phosphate is analogous to the one described above.

The mechanism of lecithin synthesis from CDP-choline followed from the recognition [87] that a substrate in this reaction was an $\alpha\beta$-diglyceride. It is now known that the following reaction occurs :



The enzyme required for this reaction is known as PC-glyceride transferase. An analogous enzyme, PE-glyceride transferase, catalyses a similar reaction with CDP-ethanolamine. PC-glyceride transferase requires magnesium or manganese ions for activity and is specific towards $\alpha\beta$-diglycerides ; triglycerides and phosphatidic acids are unaffected. It is also specific for CDP-choline, since synthetic UDP-choline, ADP-choline, and GDP-choline are inactive.

Liver cells, and presumably cells of other tissues, possess an enzyme which regenerates cytidine triphosphate from the monophosphate : [88]

Cytidine-5′ phosphate + ATP $\rightleftharpoons$ CTP + Adenosine-5′ phosphate

The reaction proceeds at the expense of ATP, thereby enabling lecithin synthesis to proceed in the presence of only catalytic amounts of CTP and CDP-choline.

Although the route for phospholipid synthesis outlined above probably represents the major pathway, at least in mammalian tissue, other routes may occur in certain circumstances. For example, glycerophosphate formed during glycolysis may well contribute to synthesis of phospholipid and fat in appropriate circumstances. It has also been suggested that the nucleotide cytidine diphosphate glycerol might participate in phospholipid metabolism. However, evidence for this is still lacking and other functions for this nucleotide are discussed below. On the other hand, the presence of serine and inositol in certain phospholipids suggests that the as yet unknown CDP-serine and CDP-inositol might participate in the synthesis of such compounds. Deoxycytidine diphosphate choline has been isolated from sea-urchin eggs, but its biochemical function has not yet been described.[88a]

The biosynthesis of sphingomyelin is apparently analogous to that of lecithin. Sribney and Kennedy [88b] have shown that CDP-choline and

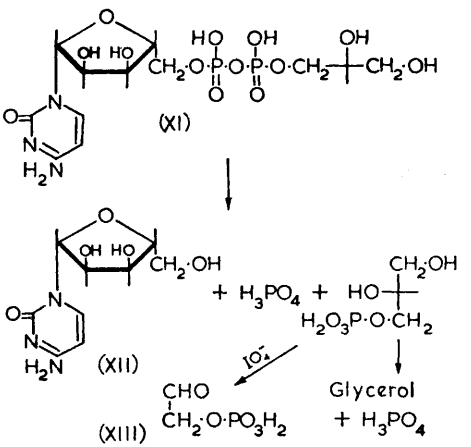
[87] Weiss, Smith, and Kennedy, *Nature*, 1956, **178**, 594.
[88] Herbert and Potter, *J. Biol. Chem.*, 1956, **222**, 453.
[88a] Sugino, *J. Amer. Chem. Soc.*, 1957, **79**, 5074.
[88b] Sribney and Kennedy, *ibid.*, p. 5325.

N-acetyl-DL-*threo-trans*-sphingosin react together in the presence of liver enzymes to give a sphingomyelin. Sphingosins containing higher fatty acid residues are less reactive.

Cytidine Diphosphate Glycerol (CDP-glycerol) and Cytidine Diphosphate Ribitol (CDP-ribitol)

These nucleotides were isolated by Baddiley and Mathias from *Lactobacillus arabinosus.*[89] They possess closely similar chemical and physical properties and refined ion-exchange methods were necessary for their separation and purification.[90] Both yielded cytidine-5′ phosphate on hydrolysis in acid and contained two phosphate groups to each cytidine residue.

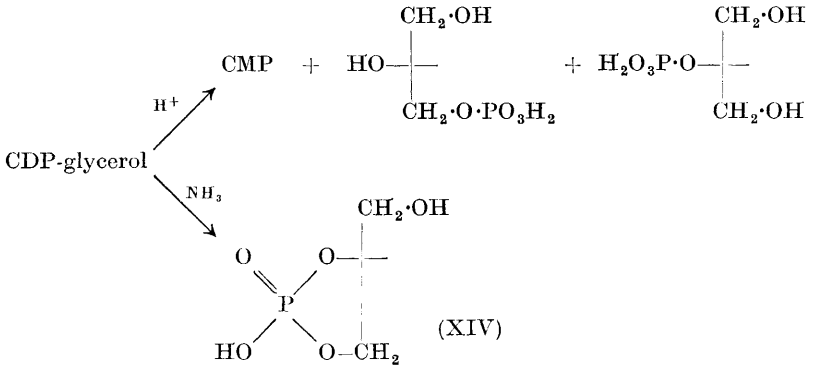
The structure of CDP-glycerol (XI) was established by the following observations : [91] The venom of the rattlesnake, *Crotalus atrox*, which contains a pyrophosphatase and a nucleoside-5′ phosphatase, hydrolysed the nucleotide to cytidine (XII), inorganic phosphate, and a phosphate of glycerol. Further hydrolysis of the glycerophosphate by the action of prostate phosphomonoesterase gave glycerol and a second mol. of inorganic phosphate. It follows that CDP-glycerol is a derivative of cytidine-5′ pyrophosphate with a glycerol residue on the terminal phosphate group. The location of the phosphate on the glycerol residue was determined by its ready oxidation with periodate to glycollaldehyde phosphate (XIII). It follows that the phosphate residue occupies the α-position.



The structure (XI) for CDP-glycerol was confirmed by chemical hydrolysis. Dilute mineral acid yielded cytidine-5′ phosphate and a mixture of α- and β-glycerophosphates (mainly α). It would be expected that acid-catalysed migration of the phosphate group on the glycerol residue would occur during the hydrolysis. Hot aqueous ammonia hydrolysed the

[89] Baddiley and Mathias, *J.*, 1954, 2723.
[90] Baddiley, Buchanan, Carss, Mathias, and Sanderson, *Biochem. J.*, 1956, **64**, 599.
[91] Baddiley, Buchanan, Mathias, and Sanderson, *J.*, 1956, 4186.

nucleotide in a manner analogous to that observed with UDPG. The products were cytidine-5 phosphate and the cyclic glycerol 1 : 2-hydrogen phosphate (XIV). The latter was stable to periodate and was hydrolysed by acid to a mixture of α- and β-glycerophosphate. With alkali it gave more β- than α-glycerophosphate, as is to be expected for a cyclic phosphate of this type.



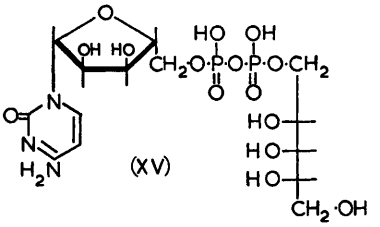
(XIV)

The configuration of the glycerophosphate residue was determined enzymically.[92] It was readily oxidised by diphosphopyridine nucleotide in the presence of glycerophosphate dehydrogenase to give dihydroxyacetone phosphate. This enzyme is known to be specific towards L-α-glycerophosphate, and so the glycerophosphate from CDP-glycerol must possess the L-α-configuration which corresponds with that present in the phospholipids. A synthesis of CDP-glycerol confirms this structure.[93]

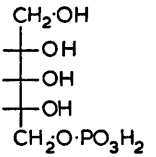
The structure of CDP-ribitol (XV) was determined by methods similar to those used for CDP-glycerol.[94] With *Crotalus atrox* venom it was hydrolysed to cytidine, inorganic phosphate, and a phosphate (XVI) of ribitol. Prostate phosphomonoesterase converted the ribitol phosphate into ribitol and inorganic phosphate. The position of the phosphate group followed from the observation that glycollaldehyde phosphate was formed by oxidation with periodate. This could only occur if the phosphate occupied a terminal (primary) position. Hydrolysis of CDP-ribitol with ammonia supports the structure (XV) : the compound is more labile than CDP-glycerol under comparable conditions, the products being cytidine-5′ phosphate and ribitol 1 : 2(4 : 5)-hydrogen phosphate (XVII). The structure of the cyclic phosphate follows from its ready oxidation with periodate and lability towards acid. A synthetic compound, prepared by the action of trifluoroacetic anhydride on ribitol 1(5)-phosphate, was indistinguishable from that obtained from the nucleotide.

Acid-hydrolysis of CDP-ribitol was unexpectedly complex. Under mild conditions the products were cytidine-5′ phosphate, ribitol 1(5)-phosphate,

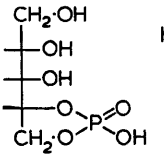
[92] Baddiley, Buchanan, and Carss, *J.*, 1957, 1869.
[93] Baddiley, Buchanan, and Sanderson, unpublished work.
[94] Baddiley, Buchanan, Carss, and Mathias, *J.*, 1956 4583.



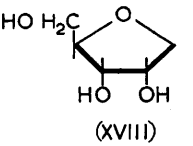
(XV)



(XVI)



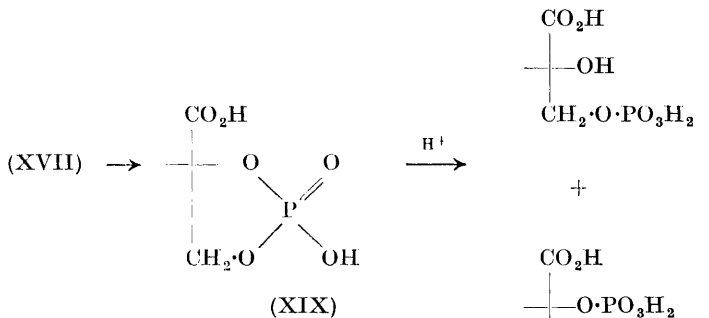
(XVII)



(XVIII)

and the isomeric ribitol phosphates which arose through acid-catalysed migration of the phosphate group from the terminal position. However, even after short periods of hydrolysis some inorganic phosphate was liberated, and more prolonged conditions effected almost complete removal of phosphate from the ribitol phosphate. The main product [95] of this reaction was 1 : 4-anhydroribitol (XVIII). No ribitol was formed under the acidic conditions. Ribitol itself gave anhydroribitol, but under more vigorous conditions. This reaction, which occurs to varying extents with all pentitols and hexitols, has been used for the characterisation of ribitol and its phosphate from CDP-ribitol.[96] The ease of reaction and nature of products were characteristic in all cases.

Straightforward methods could not be applied to the determination of the configuration of the ribitol phosphate residue in CDP-ribitol, since ribitol phosphates were hitherto unknown in Nature and consequently no enzymic method was available. By the annexed series of reactions Baddiley, Buchanan, and Carss [92] degraded a very small sample of the cyclic phosphate (XVII), obtained from the nucleotide by hydrolysis with ammonia, to known



(XIX)

[95] Baddiley, Buchanan, and Carss, *J.*, 1957, 4058. [96] *Idem*, *J.*, 1957, 4138.

compounds which could be determined enzymically. The cyclic phosphate (XIX) of glyceric acid was obtained by oxidation of the ribitol derivative first with periodate, then with bromine water. The cyclic phosphate acts as a protecting group in this oxidation and the asymmetry at position 2(4) has been retained. Acidic hydrolysis of this cyclic phosphate gave a mixture of glyceric acid 2- and 3-phosphate. The D-form of both of these esters occur as intermediates in the Embden–Meyerhof scheme of glycolysis in many tissues. A multienzyme system from rabbit muscle utilised readily and completely the glyceric acid phosphates obtained in this way from CDP-ribitol. It follows that these were derivatives of D-glyceric acid and so the ribitol phosphate must be that shown in (XVI). As this would be related to D-ribose 5-phosphate (or D-ribulose 5-phosphate) by reduction, it is referred to as D-ribitol 5-phosphate (instead of L-ribitol 1-phosphate).

Evidence relating to the mechanism of biosynthesis and the function of these nucleotides has appeared recently. An enzyme from *Lactobacillus arabinosus* catalyses both the pyrophosphorolysis of CDP-glycerol with inorganic pyrophosphate and its synthesis from CTP and α-glycerophosphate : [97]

L-α-Glycerophosphate + CTP $\rightleftharpoons$ CDP-glycerol + Inorganic pyrophosphate

CDP-ribitol is probably synthesised similarly from CTP and D-ribitol 5-phosphate.

Although the presence of L-α-glycerophosphate in one of these cytidine coenzymes has led to suggestions [91] that it may participate in phospholipid synthesis, no evidence has been obtained to support this view. On the other hand, Baddiley, Buchanan, and Greenberg [98] detected a polymeric substance in *L. arabinosus* which was extracted from the organism with trichloroacetic acid and gave on hydrolysis the products expected from a compound composed of glycerophosphate and ribitol phosphate residues joined together through phosphodiester linkages. It is not yet possible to formulate such a compound accurately, since it is not known whether the glycerophosphate and ribitol phosphate residues occur as a mixed polymer or in separate molecules. Glucose and other residues may also be present in the polymer. It is probable that CDP-glycerol and CDP-ribitol participate in the synthesis of these polymers by successive donation of polyol phosphate residues.

The cell walls of *L. arabinosus* and *Bacillus subtilis* are now known to contain appreciable amounts (20—30%) of ribitol phosphate and it is likely that this is present as a polymer.[99] Very little glycerophosphate was detected in the cell-wall preparations but its presence has been reported in larger amounts in other bacteria in macromolecular structures of uncertain composition.[100]

The above observations suggest a similarity in the function of the cytidine compounds and the uridine derivatives isolated by Park from

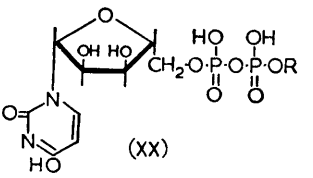
[97] Shaw, *Biochem. J.*, 1957, **66**, 56P.
[98] Baddiley, Buchanan, and Greenberg, *ibid.*, p. 51P.
[99] Baddiley, Buchanan, and Carss, *Biochim. Biophys. Acta*, 1958, **27**, 220.
[100] Mitchell and Moyle, *J. Gen. Microbiol.*, 1951, **5**, 981.

Staphylococci (see below). Both types appear to be involved in cell-wall synthesis.

Uridine Diphosphate Acetylmuramic Acid and Related Compounds

When *Staphylococcus aureus* was grown in the presence of penicillin, acid-labile phosphoric esters accumulated.[101] These esters were later shown by Park to be uracil derivatives containing two phosphate groups, a pentose, and an unidentified sugar.[102] At least three compounds were detected, and two of these contained amino-acids. In later work Park used partition chromatography for their purification.[103]

Cautious acid-hydrolysis liberated a uridine diphosphate from the three nucleotides.[104] Further hydrolysis yielded uridine-5′ phosphate. The ready hydrolysis of one of the phosphate groups in these compounds suggested the presence of a pyrophosphate group. This was confirmed by electrometric titration of the diphosphate before and after hydrolysis. It was found that the diphosphate, like that from UDPG, had two primary and one secondary phosphate acidic group. After hydrolysis an additional secondary phosphate acidic group was liberated. It was also shown that one of the unhydrolysed nucleotides (later identified as UDP-acetylmuramic acid) contained only two primary and no secondary phosphoric acid groups. This suggested that it must bear a substituent on the terminal phosphate of the UDP residue as in (XX).



(XX)

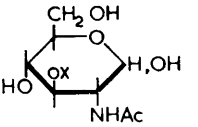
The nucleotide pyrophosphatase from potato hydrolysed UDP-acetylmuramic acid to uridine-5 phosphate, thus confirming the presence of a pyrophosphate group in the molecule.

The nature of the group R was not established until later. It was known, however, that, although the nucleotide was non-reducing, acid-hydrolysis under conditions which removed the group R caused the appearance of a reducing substance. The reducing substance showed reactions characteristic of an *N*-acetylhexosamine containing a carboxyl group.

More recently, Park and Strominger [105] have shown that the *N*-acetylhexosamine present in this and the other two uridine derivatives from *S. aureus* is identical with 3-*O*-1′-carboxyethyl-2-acetamido-2-deoxyglucose (XXI), *i.e.*, the *N*-acetyl derivative of muramic acid. The structure suggested by Strange [106] for muramic acid has been confirmed by synthesis.[107]

From the above evidence it is clear that all these uridine derivatives possess the general structure (XX ; R = *N*-acetylmuramic acid), in which the muramic acid residue is attached to the pyrophosphate residue through its reducing group. The configuration of the linkage between amino-sugar and phosphate is not known.

[101] Park and Johnson, *J. Biol. Chem.*, 1949, **179**, 585.
[102] Park, *Fed. Proc.*, 1950, **9**, 213.
[103] *Idem*, *J. Biol. Chem.*, 1952, **194**, 877. [104] *Idem*, *ibid.*, p. 885.
[105] Park and Strominger, *Science*, 1957, **125**, 99.
[106] Strange, *Biochem. J.*, 1956, **64**, 23P. [107] Kent, *ibid.*, 1957, **67**, 5P.



(XXI)

The other uridine derivatives from *S. aureus* yield on hydrolysis amino-acids, in addition to the products already discussed.[108] One nucleotide contains a single L-alanine residue, and the other contains a peptide composed of L-lysine, D-glutamic acid, and three alanine residues. The alanine obtained from this peptide consisted of approximately equal amounts of the D- and the L-form (estimated microbiologically).

The presence of all three uridine derivatives in small amount in cells which had not been treated with penicillin indicated that they have a function in normal metabolic processes. Although their exact rôle is still not clearly understood, it is likely that they are coenzymes concerned with the metabolism of muramic acid. Muramic acid was first characterised as a component of certain peptides from bacterial spores,[109] and occurred in bacterial cell walls.[110] Moreover, both D- and L-alanine, D-glutamic acid, and L-lysine are also found in considerable amounts in many bacterial cell walls.[111] It seems likely then that these nucleotides are concerned with the synthesis of cell-wall material.[105] It is possible to visualise their action as somewhat similar to that of UDPG in glycoside and oligosaccharide synthesis, where the nucleotide donates a sugar or, in this case, more complex sugar derivative to a hydroyxl group in another molecule. It is interesting that at least one other nucleotide of this general type, CDP-ribitol, is also most probably involved in cell-wall synthesis.

Strong evidence for the view that Park's uridine compounds are concerned in cell-wall synthesis comes from the known effects of penicillin on bacteria.[105, 111] The antibiotic seriously affects the walls during the very early stages of its action. This would be consistent with the observation that the accumulation of the uridine compounds is also a primary effect of penicillin action.[112] Further studies on the mechanism of the enzymic processes involving these uridine derivatives should considerably assist our understanding of penicillin action.

The Adenosine-5′ Phosphate-X Group of Compounds. Acyl Adenylates

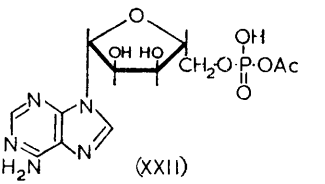
It is now clear that in several enzymic syntheses acid anhydrides of adenosine-5′ phosphate (AMP) are involved. The first of these to be fully authenticated [113, 114] occurs during the synthesis of *S*-acetyl-coenzyme A

[108] Park, *J. Biol. Chem.*, 1952, **194**, 897.
[109] Strange and Powell, *Biochem. J.*, 1954, **58**, 80.
[110] Cummins and Harris, *J. Gen. Microbiol.*, 1956, **14**, 583.
[111] Cf. Work, *Nature*, 1957, **179**, 841.
[112] Strominger, *J. Biol. Chem.*, 1957, **224**, 509.
[113] Berg, *J. Amer. Chem. Soc.*, 1955, **77**, 3163.
[114] *Idem*, *J. Biol. Chem.*, 1956, **222**, 991.

from acetate, ATP, and coenzyme A (CoA). Contrary to earlier work,[115] Berg showed that the reaction sequence was as follows :

$$\text{ATP} + \text{Acetate} \rightleftharpoons \text{Acetyl-AMP} + \text{Pyrophosphate}$$
$$\text{Acetyl-AMP} + \text{CoA} \rightleftharpoons \text{Acetyl-CoA} + \text{AMP}$$

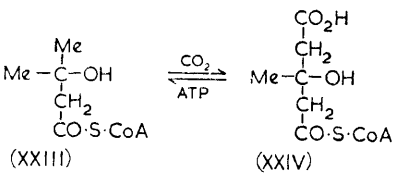
Acetyl-AMP (XXII) has been synthesised.[113, 116–120] The synthetic compound was converted enzymically into acetyl-CoA.[113, 114, 121] Experiments designed to trap acetyl-AMP, produced in the above reaction sequence, were unsuccessful, and it appears that all the acyl-AMP intermediates so far described are very tightly bound to their enzymes ; acetylhydroxamic acid could, however, be isolated by the addition of hydroxylamine to reactions in which CoA was omitted. The formation of hydroxamic acids under these conditions is generally regarded as indicating the presence of acyl anhydrides.



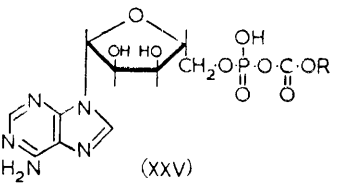
(XXII)

n-Butyryl adenylate has been synthesised [120, 122] and shown to be an intermediate in formation of butyryl-CoA by the fatty-acid-oxidising enzyme system of liver ; similarly, hexanoyl and octanoyl adenylate are oxidised in liver systems [121, 123] without the addition of ATP.

An intermediate in leucine metabolism, β-hydroxy*iso*valeryl-CoA (XXIII) undergoes carboxylation to β-hydroxy-β-methylglutaryl-CoA (XXIV) in the presence of bicarbonate and ATP.[124]



(XXIII) (XXIV)

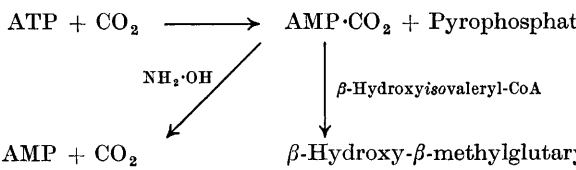


(XXV)

It has been suggested [125] that an " activated " form of carbon dioxide is concerned in this reaction, and structure (XXV ; R = H) was proposed for it. When silver adenylate was treated with ethyl chloroformate among the products were (XXV ; R = Et) and (XXV ; R = H).[125] Experiments

[115] Jones, Lipmann, Hilz, and Lynen, *J. Amer. Chem. Soc.*, 1953, **75**, 3285.
[116] Berg, *J. Biol. Chem.*, 1956, **222**, 1015.
[117] Avison, *J.*, 1955, 732.
[118] Stadtman and White, *J. Amer. Chem. Soc.*, 1953, **75**, 2022.
[119] Baddiley, Buchanan, and Letters, unpublished work.
[120] Talbert and Huennekens, *J. Amer. Chem. Soc.*, 1956, **78**, 4671.
[121] Whitehouse, Moeksi, and Gurin, *J. Biol. Chem.*, 1957, **226**, 813.
[122] Peng, *Biochim. Biophys. Acta*, 1956, **22**, 42.
[123] Jencks and Lipmann, *J. Biol. Chem.*, 1957, **225**, 207.
[124] Bachhawat, Robinson, and Coon, *J. Amer. Chem. Soc.*, 1954, **76**, 3098 ; *J. Biol. Chem.*, 1956, **219**, 539.
[125] Coon, *Fed. Proc.*, 1955, **14**, 762. Bachhawat, Woessner, and Coon, *ibid.*, 1956, **15**, 214.

with synthetic carbonatoadenylate (AMP·CO_2) (XXV ; R = H)[125,126] have shown the following reactions to occur :



It is believed that an activated form of carbon dioxide is also an intermediate in propionate metabolism.[127, 128]

In 1941, Lipmann suggested that protein synthesis from amino-acids might involve carboxyl-phosphate intermediates.[129] Evidence is now accumulating that aminoacyl adenylates are concerned with the formation of several amide linkages. In this connection the enzymic synthesis of hippuric acid and its related *p*-amino-compound have been investigated. Cohen and McGilvery showed that ATP was required for *p*-aminohippurate synthesis [130] and it was found that benzoyl-CoA was an intermediate in hippurate synthesis.[131, 132] Benzoyl phosphate was inactive [131] but the overall reaction,

$$\text{Benzoate} + \text{Glycine} + \text{ATP} \xrightarrow{\text{CoA}} \text{Hippurate} + \text{AMP} + \text{Pyrophosphate}$$

has now been demonstrated,[133] indicating the intermediate formation of benzoyl adenylate. Pantothenic acid synthesis has been shown to occur in two stages : [134, 135]

$$\text{ATP} + \text{Pantoate} \rightleftharpoons \text{Pantoyl-AMP} + \text{Pyrophosphate}$$
$$\text{Pantoyl-AMP} + \beta\text{-Alanine} \longrightarrow \text{Pantothenate} + \text{AMP}$$

More directly related to protein synthesis are observations of the " activation " of a number of amino-acids by ATP in the presence of enzymes from widely different sources. It was shown [136, 137] that enzymes from rat liver would catalyse the reaction :

$$\text{L-Amino-acid} + \text{ATP} \rightleftharpoons \text{L-Aminoacyl-AMP} + \text{Pyrophosphate}$$

Hydroxamic acids could be formed, by the action of hydroxylamine, from a number of L-amino-acids. There was evidence that several enzymes were

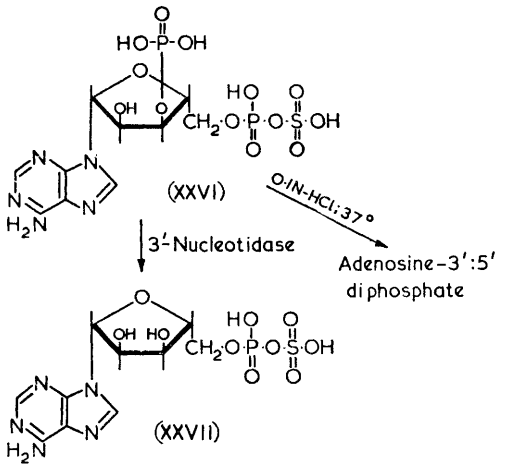
[126] Bachhawat and Coon, *J. Amer. Chem. Soc.*, 1957, **79**, 1505.
[127] Flavin, Ortiz, and Ochoa, *Nature*, 1955, **176**, 823.
[128] Flavin, Castro-Mendoza, and Ochoa, *Biochim. Biophys. Acta*, 1956, **20**, 591.
[129] Lipmann, *Adv. Enzymol.*, 1941, **1**, 99.
[130] Cohen and McGilvery, *J. Biol. Chem.*, 1947, **171**, 121.
[131] Chantrenne, *ibid.*, 1951, **189**, 227.
[132] Schachter and Taggart, *ibid.*, 1953, **203**, 925.
[133] *Idem, ibid.*, 1954, **208**, 263.
[134] Maas and Novelli, *Arch. Biochem. Biophys.*, 1953, **43**, 236.
[135] Maas, 3rd Internat. Congress Biochem., Brussels, 1955, p. 32.
[136] Hoagland, *Biochim. Biophys. Acta*, 1955, **16**, 288.
[137] Hoagland, Keller, and Zamecnik, *J. Biol. Chem.*, 1956, **218**, 345.

present, each being responsible for the activation of separate amino-acids ; that utilising L-methionine has been purified. A similar enzyme was discovered in yeast by Berg.[113, 138] A range of activating enzymes exists in pancreas and an enzyme specific towards L-tryptophan has been purified considerably.[139] Similar enzymes are present in micro-organisms [140–142] and green plants.[143]

Some aminoacyl adenylates have been synthesised chemically and their properties examined.[141, 142, 144] The synthetic compounds exhibit a maximum stability at a slightly acid pH, and are readily decomposed by mineral acid or at a pH greater than 7. The enzymic properties of the compounds, giving ATP on the addition of inorganic pyrophosphate, together with their behaviour with hydroxylamine, leave little doubt that they are the products of " activation ". Their precise rôle in protein synthesis remains to be discovered.

Adenosine-3′ Phosphate 5′-Sulphatophosphate (" Active Sulphate ", PAPS)

The enzymic synthesis of sulphuric esters of phenols [145–147] has been shown to take place in two stages ; [148–151] the first involves reaction between



[138] Berg, *J. Biol. Chem.*, 1956, **222**, 1025.
[139] Davie, Koningsberger, and Lipmann, *Arch. Biochem. Biophys.*, 1956, **65**, 21.
[140] De Moss and Novelli, *Bact. Proc.*, 1955, 125 ; *Biochim. Biophys. Acta*, 1955, **18**, 592.
[141] *Idem, ibid.*, 1956, **22**, 49.
[142] De Moss, Genuth, and Novelli, *Proc. Nat. Acad. Sci. U.S.A.*, 1956, **42**, 325.
[143] Davis, Best, and Novelli, *Fed. Proc.*, 1957, **16**, 170.
[144] Berg, *ibid.*, 152.
[145] De Meio and Tkacz, *J. Biol. Chem.*, 1952, **195**, 175.
[146] De Meio, Wizerkaniuk, and Fabiani, *ibid.*, 1953, **203**, 257.
[147] Bernstein and McGilvery, *ibid.*, 1952, **198**, 195.
[148] *Idem, ibid.*, 1952, **199**, 745.
[149] De Meio, Wizerkaniuk, and Shreibman, *ibid.*, 1955, **213**, 439.
[150] Segal, *ibid.*, p. 161.
[151] Hilz and Lipmann, *Proc. Nat. Acad. Sci. U.S.A.*, 1955, **41**, 880.

inorganic sulphate and adenosine triphosphate (ATP) to give an intermediate ("active sulphate") which transfers its sulphate group to a suitable substrate in the second stage. Active sulphate has been shown by Robbins and Lipmann [152] to be (XXVI). Rye-grass 3′-nucleotidase [153] gives adenosine-5′ sulphatophosphate (APS) (XXVII) which has been compared with a synthetic sample; [154] mild acid-hydrolysis yielded adenosine-3′ : 5′ diphosphate. Adenosine-5′ sulphatophosphate [154, 155] and active sulphate [156] have both been synthesised chemically.

The enzymic synthesis of active sulphate has now been more closely investigated [157–160] and shown to consist of two separable reactions.[159, 160] APS is an intermediate, and can be detected when inorganic pyrophosphatase has been added to the appropriate enzyme fraction: [160]

$$\text{ATP} + \text{Sulphate} \rightleftharpoons \text{APS} + \text{Pyrophosphate} \downarrow \text{Orthophosphate}$$

The enzyme, ATP sulphurylase, is active with a number of inorganic anions [159] (selenate,[158] sulphite, chromate, tungstate, and molybdate). In these cases unstable anhydrides are formed, and decomposition of ATP to AMP, competitively inhibited by sulphate, is noted.

Active sulphate arises through the action of APS-kinase: [159, 160]

$$\text{APS} + \text{ATP} \longrightarrow \text{PAPS} + \text{ADP}$$

The reactions described have been found to take place in liver, yeast, and *Neurospora*. Active sulphate will transfer its sulphate group to a number of substrates in the presence of sulphokinases.[161] Phenols have been used as substrates in the liver system [146, 148, 150, 151, 161] but it is now known that a number of steroids will act as substrates.[161, 162] The enzymic formation of active sulphate and its conversion into chondroitin sulphate in chick-embryo cartilage has recently been reported.[163]

Salmon liver is stated to contain an unstable nucleotide bearing a sulphatophosphate group.[164] In view of the remarkable partial structure suggested for this compound, further evidence of its homogeneity is desirable.

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[160] Robbins and Lipmann, *ibid.*, p. 6409.
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[162] Schneider and Lewbart, *J. Biol. Chem.*, 1956, **222**, 787.
[163] D'Abramo and Lipmann, *Biochim. Biophys. Acta*, 1957, **25**, 211.
[164] Tsuyaki and Idler, *J. Amer. Chem. Soc.*, 1957, **79**, 1771.