THE ROLE OF PHOSPHORIC ESTERS IN BIOLOGICAL REACTIONS

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As can be seen from the recent Reviews by Atherton [1] and Topley [2] the phosphoric acids and their derivatives are of very considerable general chemical interest at the present time. It is intended here to attempt to set out some of the more important ways in which phosphorylated substances participate in living processes. Although many excellent partial reviews have been written by authorities eminent in particular aspects—to whom the present Reviewers acknowledge a great debt—the accumulation of knowledge and speculation is now so rapid that it is not easy for those who do not specialise in biological chemistry to obtain a general idea of the subject. This is unfortunate, for the last ten years' developments in particular have revolutionised our conception of the manner in which chemical reactions can provide for the needs of the living organism. In trying to provide such a broad account it is inevitable that many relevant topics must be passed over or receive scant attention and many workers' names unjustly omitted. However, reference to appropriate comprehensive surveys will, it is hoped, ensure that most of the more important work is covered.

I. The Phosphoric Esters Found in Biological Systems

Only esters of ortho-, pyro-, and tri-phosphoric acids are so far known to be of biological significance. At least one hydrogen atom is generally left unsubstituted so that the compounds exist as anions at physiological pH and are usually water-soluble. These substances all tend to be stronger acids than the phosphoric acid from which they are derived.[3]

The esters of orthophosphoric acid are more numerous than the others and some idea of their diversity can be obtained from Table I. Apart from these esters we have also had to include consideration of acyl phosphates and the substituted guanidinophosphonates, phosphocreatine and phosphoarginine, in view of their close biological relation to the actual phosphoric esters.

An important property of phosphoric esters is their varying degrees of stability to acid hydrolysis, which can be used to identify the type of ester involved in an unknown compound or mixture. Thus di- (pyro-) and tri-phosphates have a lability similar to that of the free acids,[2] while unstable orthophosphoric esters are those of enols (pyruvic acid enol) and hemiacetals (aldose-1 phosphates). An even greater instability attaches to acyl phosphates and guanidinophosphonates. Other esters are relatively stable

[1] *Quart. Reviews*, 1949, **3**, 146. [2] *Ibid.*, p. 345.

[3] See, for example, Meyerhof and Lohmann, *Biochem. Z.*, 1927, **185**, 113; Kosterlitz, *Biochem. J.*, 1943, **37**, 321.

TABLE I

Naturally occurring phosphoric esters *

Name.	References. Isolation.	References. Synthesis.	Function.
2-Aminoethyl phosphate	4	5	Phosphatide metabolism (?)
Propane-1 : 2-diol 1-phosphate	6	7	(?) Occurs in mammalian brain, liver, and kidney, and in sea-urchin eggs
Glycerol 1-phosphate	8	9	Phosphatide metabolism
Glyceraldehyde 3-phosphate (" Fischer–Baer ester ")	10	11	Glycolysis
Glyceric acid 2-phosphate (" Kiessling ester ")		12	
Glyceric acid 3-phosphate (" Nilsson–Lohmann ester ")	13	12	
3-Phosphatoglyceroyl phosphate	14		
Pyruvic acid enol (α-hydroxyacrylic acid) phosphate	15	16	
Deoxyribose-1 phosphate	17		Nucleoside metabolism
Deoxyribose-5 phosphate	18		
Ribose-1 phosphate	19		
Ribose-5 phosphate	20	21	Nucleoside metabolism, oxidation of glucose
Glucose-1 phosphate (" Cori ester ")	22	23	Glycolysis, polysaccharide synthesis
Glucose-6 phosphate † (" Robison ester ")	24	25	Glycolysis, glucose absorption
Gluconic acid-6 phosphate	26		Oxidation of glucose
Fructose-6 phosphate † (" Neuberg ester ")	27		Glycolysis
Fructose-1 : 6 diphosphate (" Harden–Young ester ")	28		
Galactose-1 phosphate	29	30	Oxidation of galactose (?)
Tagatose-6 phosphate		31	
Mannose-1 phosphate		32	Oxidation of mannose (?)
Mannose-6(?) phosphate	33		
Trehalose phosphate	34		By-product of fructose-fermenting yeast
*meso*Inositol hexaphosphate (" Phytic acid ")	35		? Widely distributed in plants

* Excluding phosphatides, nucleotides and polynucleotides, and other esters dealt with fully in the text.

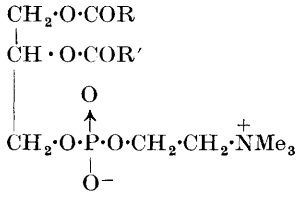
† The equilibrium mixture of glucose-6 and fructose-6 phosphates is sometimes known as " Embden ester ".

[4] Awapara, Landua, and Fuerst, *J. Biol. Chem.*, 1950, **183,** 545.
[5] Plimmer and Birch, *Biochem. J.*, 1937, **31,** 398.
[6] Lindberg, *Arkiv Kemi, Min., Geol.*, 1946, **23,** *A*, 45.
[7] Atherton, Openshaw, and Todd, *J.*, 1945, 382; Lampson and Lardy, *J. Biol. Chem.*, 1949, **181,** 697.
[8] Sotnitschewsky, *Z. physiol. Chem.*, 1880, **4,** 214.
[9] H. King and Pyman, *J.*, 1914, **105,** 1238.
[10] Meyerhof, *Bull. Soc. Chim. biol.*, 1938, **20,** 1033, 1345.
[11] Baer and H. O. L. Fischer, *J. Biol. Chem.*, 1943, **150,** 223.
[12] Neuberg, *Arch. Biochem.*, 1943, **3,** 105.
[13] Neuberg and Lustig, *ibid.*, 1942, **1,** 311.
[14] Negelein and Bröml, *Biochem. Z.*, 1939, **303,** 132.
[15] Lohmann and Meyerhof, *ibid.*, 1934, **273,** 60.
[16] Baer and H. O. L. Fischer, *J. Biol. Chem.*, 1949, **180,** 145.

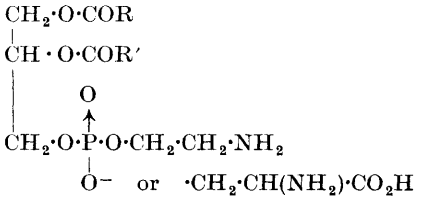
although differences can still be used to distinguish between some of them, particularly in the nucleotide field.

It has been found expedient to exclude from the main discussion two especially important groups of phosphoric esters, phosphatides and nucleic acids, although for the sake of completeness a brief account of them will be given in this introduction.

Phosphatides.—Phosphatides or phospholipids are important constituents of cells,[36] particularly of nervous tissue. They include *lecithins*, in which a fatty diglyceride is linked in the α-position through phosphate to choline, *cephalins* where the choline of lecithins is replaced by colamine (ethanolamine) or serine,[37] *plasmalogens* (or acetalphosphatides) where a fatty aldehyde [38] combined as an acetal replaces the usual two acyl groups, and *sphingomyelins*, the phosphoric esters of the unsaturated basic alcohol sphingosine. Recently, Folch [39] has isolated another component from brain cephalin fractions which does not contain nitrogen and is believed to be a derivative of inositol diphosphate (*diphosphoinositide*).



Lecithins



Cephalins

R and R′ = Alkyl or Alkenyl (C_{14}—C_{25})

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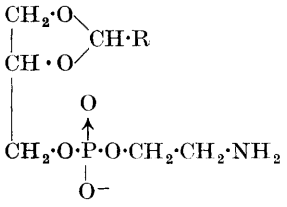
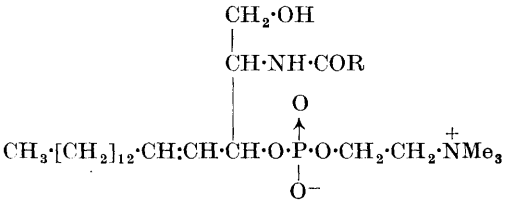
[17] Friedkin, Kalckar, and Hoff-Jørgensen, *J. Biol. Chem.*, 1949, **178**, 527.
[18] Manson and Lampen, *Fed. Proc.*, 1949, **8**, 224.
[19] Kalckar, *J. Biol. Chem.*, 1947, **167**, 477.
[20] Levene and Jacobs, *Ber.*, 1911, **44**, 748.
[21] Levene and Stiller, *J. Biol. Chem.*, 1934, **104**, 299.
[22] McCready and Hassid, *J. Amer. Chem. Soc.*, 1944, **66**, 560.
[23] C. F. Cori, Colowick, and G. T. Cori, *J. Biol. Chem.*, 1937, **121**, 465; T. Posternak, *J. Amer. Chem. Soc.*, 1950, **72**, 4824.
[24] LePage and Umbreit, *J. Biol. Chem.*, 1943, **147**, 263.
[25] Lampson and Lardy, *ibid.*, 1949, **181**, 693.
[26] Robison and E. J. King, *Biochem. J.*, 1931, **25**, 323.
[27] Neuberg, Lustig, and Rothenberg, *Arch. Biochem.*, 1943, **3**, 33.
[28] Neuberg and Lustig, *J. Amer. Chem. Soc.*, 1942, **64**, 2722.
[29] Kosterlitz, *Biochem. J.*, 1937, **31**, 2217.
[30] *Idem, ibid.*, 1939, **33**, 1087.
[31] Totton and Lardy, *J. Biol. Chem.*, 1949, **181**, 707.
[32] Colowick, *ibid.*, 1938, **124**, 557.
[33] Jephcott and Robison, *Biochem. J.*, 1934, **28**, 1843.
[34] Robison and Morgan, *ibid.*, 1928, **22**, 1277.
[35] S. Posternak, *Compt. rend.*, 1903, **137**, 202.
[36] See, for example, the discussion by Claude, *Adv. Protein Chem.*, 1949, **5**, 423.
[37] Folch, *J. Biol. Chem.*, 1948, **174**, 439.
[38] Leupold, *Z. physiol. Chem.*, 1950, **285**, 182.
[39] *J. Biol. Chem.*, 1949, **177**, 505.



Plasmalogens



Sphingomyelins

Synthesis of a naturally occurring phosphatide, L-α-dipalmitoyl-lecithin, has recently been achieved.[40] The turnover of phosphatides in various tissues as revealed by radioactive tracers has been discussed in the book by G. Hevesy.[41] Details of modern work on phosphatides can be found in two recent reviews.[42]

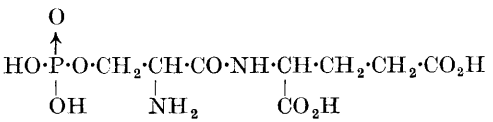
Nucleic Acids.—These very important, highly polymerised compounds occupy a position similar to that of the proteins themselves in providing the essential " bricks " of living organisms. The chemistry and biochemistry of the nucleic acids have been very well surveyed by F. Schlenk [43] and by Chargaff [44] and, in view of present limitations of space, very little will be written here. Nucleic acids consist, according to modern ideas, of *N*-ribosidyl- and *N*-deoxyribisodyl-purines and -pyrimidines linked together by phosphate groups attached to the sugar side-chains at points which are still undecided. Molecular weights of nucleic acids are very high, of the order of 500,000—2,000,000, although those of commercial preparations may be as low as 10,000 owing to degradation during isolation. The ribonucleic acids are found in the cytoplasm, whilst the deoxyribonucleic acids are found only in the cell nucleus where they are closely associated with the chromosomes and are believed to be involved in genetical functions.[45]

The bases found in nucleic acids are the purines adenine and guanine (as well as others in minor quantity) and the pyrimidines cytosine and uracil or thymine. D-Ribofuranose and 2-deoxy-D-ribofuranose are attached with β-configuration to the 9-position of purines or the 3-position of pyrimidines, giving the units known as *nucleosides*. Nucleosides phosphorylated in the sugar moiety are called *nucleotides*, compounds of great interest apart from their presence as units in nucleic acids. The term nucleotide has been extended to include the quaternary riboside phosphate of nicotinamide (" nicotinamide mononucleotide ") and even riboflavin phosphate (" flavin mononucleotide ") although there seems to be very little justification for this.

Radioactive-tracer studies of normal nucleic acid turnover and of its reaction to *X*-rays (with reference to cancer research) have been reviewed by Hevesy [41] and by Marshak.[46]

[40] Baer and Kates, *Science*, 1949, **109**, 31.
[41] " Radioactive Indicators ", New York, 1948.
[42] Folch and Sperry, *Ann. Reviews Biochem.*, 1948, **17**, 147; Lovern, *ibid.*, 1949, **18**, 97.
[43] *Adv. Enzymology*, 1949, **9**, 455.
[44] *Experientia*, 1950, **6**, 201.
[45] See, for example, Danielli, " Cell Physiology and Pharmacology ", 1950, pp. 7—11, 143—146.
[46] *J. Clin. Invest.*, 1949, **28**, 1324.

Phosphoproteins.—Very little is known of phosphorus compounds forming part of proteins, although phosphorylated serylglutamic acid



has been isolated after degradation of casein.[47] Another phosphorylated protein is phosphovitin, isolated [48] from the vitellin fraction of egg yolk. Phosphoproteins have also been found in the lens of the ox and sheep eye.[49] It has been suggested [50] that phosphorylation of the serine and threonine residues of the protein myosin is responsible for the extension of the latter in " relaxed " muscle fibres.

Finally, a phosphoric ester of so far unknown composition has been found in Gram-positive bacteria but not in Gram-negative organisms.[51] Its presence is also correlated with other characteristic differences between the two classes of bacteria such as penicillin sensitivity. The compound is not a nucleic acid.

II. Phosphoric Esters as Coenzymes

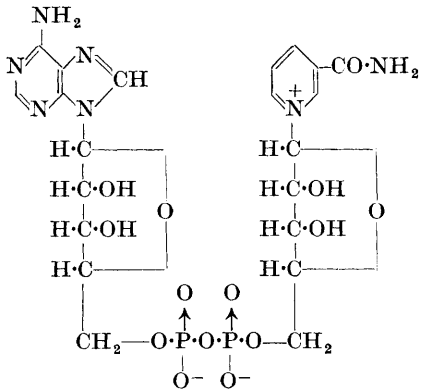
It is perhaps worthwhile at this stage to remind the general reader of the special nature of chemical reactions in biological systems. These reactions, of a striking similarity for all forms of life, are, for the most part, catalysed by specific proteins known as enzymes. Enzymic catalysis, although of infinitely greater potentialities, may be regarded as not very essentially different from the heterogeneous catalysis well-known in non-biological chemistry. That is, attachment of the reacting molecules to the protein (" adsorption ") occurs and results in their activation before reaction. Enzymes frequently require certain important substances called *coenzymes* [52] in addition to the substrate molecules. These coenzymes are, in general, relatively simple molecules distinguished by playing an essential role in the enzymic reaction, either by forming transitory compounds with the substrate or by donating or accepting groups or electrons or in some similar, reversible, and catalytic manner. Of the known coenzymes, most are phosphoric esters and some of the most important are derivatives of vitamins, being the forms in which the latter are known to be involved in physiological processes.[53]

Coenzymes of Electron Transfer.—Although it is, of course, hardly possible to speak of one reaction being more important than another with reference to living organisms whose chemistry is so completely integrated, it is fair to say that the processes of respiration are at least the most striking.

[47] T. Posternak and Pollazcek, *Helv. Chim. Acta*, 1941, **24,** 921.
[48] Mecham and Olcott, *J. Amer. Chem. Soc.*, 1949, **71,** 3670.
[49] Mandel, Nordmann, Zimmer, and Harth, *Nature*, 1949, **164,** 794.
[50] Riseman and Kirkwood, *J. Amer. Chem. Soc.*, 1948, **70,** 2820.
[51] Mitchell and Moyle, *Nature*, 1950, **166,** 218.
[52] Sometimes rather loosely termed prosthetic groups.
[53] See Rosenberg, " Chemistry and Physiology of the Vitamins ", New York, 1945.

Respiration involves, chemically speaking, the uptake of gaseous oxygen by the organism and its use, by some scheme of electron transfer, for the oxidation of organic compounds taken into the system as foods or derived by breakdown from them. As far as is known all cells make use of iron–porphyrin–protein derivatives (" cytochromes " [54]) as the direct link with atmospheric oxygen in such processes. The further electron transfer (" hydrogen transfer ") required is achieved by two important sets of coenzymes, phosphoric ester derivatives of nicotinamide and riboflavin.

H. von Euler *et al.*[55] and O. Warburg *et al.*[56] isolated, in 1935, compounds from yeast juice (" cozymase ") and red blood cells respectively. These were termed coenzymes I and II, and both were shown to be nicotinamide-adenine dinucleotides, only differing in the presence of an additional phosphate residue in the latter. The structure of coenzyme I now generally accepted was first put forward by F. Schlenk and H. von Euler [57] and confirmed by the later work of Karrer and his associates.[58] This compound is now generally given the trivial name *diphosphopyridine nucleotide* (DPN), whilst coenzyme II is referred to as *triphosphopyridine nucleotide* (TPN). The latter most probably has its additional phosphate group attached in the 2′-position of the adenosine moiety.[59] The enzymic synthesis of TPN from DPN and adenosine-5′ triphosphate has been demonstrated.[60]



Diphosphopyridine nucleotide
(*DPN, Coenzyme I, Cozymase*).

Syntheses of DPN and TPN have not so far been achieved, although Haynes and Todd [61] have been able to prepare a number of glycosidyl-3-carbamylpyridinium salts and their dihydro-derivatives, including *N*-D-ribofuranosidyl-1 : 2(or 6)-dihydronicotinamide, probably identical with the

[54] See Theorell, *Adv. Enzymology*, 1947, **7**, 265.
[55] H. von Euler, Albers, and F. Schlenk, *Z. physiol. Chem.*, 1935, **237**, 1.
[56] Warburg, Christian, and Griese, *Biochem. Z.*, 1935, **282**, 157.
[57] *Naturwiss.*, 1936, **24**, 794.
[58] Reviewed by F. Schlenk, *J. Biol. Chem.*, 1942, **146**, 619.
[59] Kornberg and Pricer, *ibid.*, 1950, **186**, 557.
[60] Kornberg, *ibid.*, 1950, **182**, 805.
[61] *J.*, 1950, 303.

pyridine nucleoside present in the natural dihydro-coenzymes. Calculation of the distribution of charge in 3-substituted pyridine derivatives by the method of molecular orbitals confirms the general belief that dihydrogenation of DPN and TPN (as in the enzymic reactions) most likely involves the 1 : 2-position.[62] The value of the redox potential for the reaction

$$\mathrm{DPN} + 2\mathrm{H}^+ \underset{-\,2e}{\overset{+\,2e}{\rightleftharpoons}} \mathrm{DPN,H_2}$$

has been calculated [63] from equilibrium data to be — 0·282 volt at 30° and pH 7·0.

The improved methods recently described for the isolation and purification of DPN and TPN from yeast [64] and liver,[65] respectively, should give new impetus to research with the pure compounds.

The type of combination of these coenzymes with enzyme proteins [66] is well illustrated by some interesting experiments by C. F. Cori *et al.*[67] A dehydrogenating enzyme from rabbit muscle was shown to contain 1 mole of DPN per 50,000 g. of protein. This ratio was maintained after dialysis or even recrystallisation from ammonium sulphate solutions. However, DPN could readily be removed from the enzyme by treatment of an aqueous solution with charcoal! Addition of DPN together with ammonium sulphate to the resulting solution caused the formation of crystals with the original DPN : protein ratio.

The oxidation of reduced DPN and TPN is generally brought about by enzymes characterised by a vivid yellow colour owing to the presence of a riboflavin derivative as coenzyme. D-Riboflavin-5′ phosphate (*flavin mononucleotide*, FMN), originally shown to be present in an artefact, the so-called " old " yellow enzyme of Warburg and Christian, and called " cytoflav " [68] before its structure was known, is now recognised as the coenzyme of the very important cytochrome *c* reductase [69] which transfers electrons from TPN to cytochrome *c*. The coenzyme chiefly involved in transporting electrons from the reduced pyridine nucleotides to cytochrome, however, is a mixed diphosphate ester of riboflavin and adenosine, *flavin-adenine dinucleotide* (FAD).

FMN was first isolated from " old " yellow enzyme in 1935 by Theorell,[70] whose conclusion that it was a flavin monophosphate was confirmed by Kuhn and Rudy [71] and by Karrer *et al.*,[72] who demonstrated it to be the 5′-phosphate of D-riboflavin. Synthesis, the first to be achieved for any

[62] Ciusa and Nebbia, *Gazzetta*, 1949, **79**, 526 ; cf. Karrer *et al.*, *Helv. Chim. Acta*, 1938, **21**, 223 and preceding papers.

[63] Borsook, *J. Biol. Chem.*, 1940, **133**, 629.

[64] Hoogeboom and Barry, *ibid.*, 1948, **176**, 935 ; Clarke, Dounce, and Stotz, *ibid.*, 1949, **181**, 459.

[65] Le Page and Mueller, *ibid.*, 1949, **180**, 975.

[66] Cf. Zerfas and Dixon, *Biochem. J.*, 1940, **34**, 371.

[67] C. F. Cori, Velick, and G. T. Cori, *Biochim. Biophys. Acta*, 1950, **4**, 160.

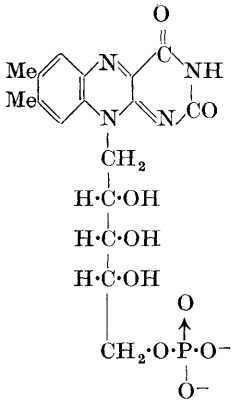
[68] Banga and Szent-Györgyi, *Biochem. Z.*, 1932, **246**, 203.

[69] Haas, Horecker, and Hogness, *J. Biol. Chem.*, 1940, **136**, 747.

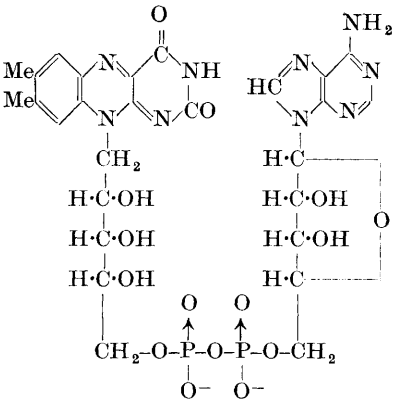
[70] *Biochem. Z.*, 1935, **275**, 344 ; see also Theorell, Karrer, Schöpp, and Frei, *Helv. Chim. Acta*, 1935, **18**, 1022.

[71] *Z. physiol. Chem.*, 1936, **239**, 47.

[72] Karrer, Frei, and Meerwein, *Helv. Chim. Acta*, 1937, **20**, 79.



D-*Riboflavin-5′ phosphate*
(*Flavin mononucleotide, FMN*)



Flavin-adenine dinucleotide
(*FAD*)

known coenzyme, was reported in the following year,[73] being carried out by conversion of riboflavin into the trityl ether, acetylation, removal of the ether group, and phosphorylation of the exposed 5′-hydroxyl group with phosphorus oxychloride. Hydrolysis then gave the desired 5′-phosphate identical in every respect with the active substance from yellow enzyme.[74]

FAD was originally isolated by Warburg and Christian [75] as the coenzyme involved in the oxidations of the unnatural D-amino-acids and of the purine xanthine. In these reactions one molecule of FAD can transfer 5760 electrons/minute directly from substrate to gaseous oxygen. The structure of FAD cannot be regarded as definitely proved in the absence of a chemical synthesis, but is very probably that shown by virtue of its enzymic synthesis [76, 77] from adenosine-5′ diphosphate and D-riboflavin. On hydrolysis, adenosine-5′ phosphate and D-riboflavin-5′ phosphate are produced.

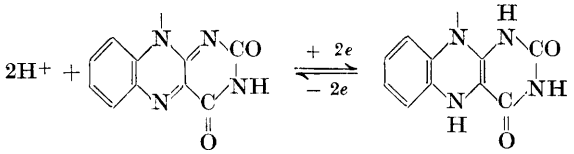
All the riboflavin in animal brain, heart, liver, and kidney is present in the form of either FMN or FAD. Spleen, on the other hand, contains an enzyme rapidly degrading these phosphates to free riboflavin.[78] Synthesis of FAD has been demonstrated in blood cells,[79] as well as in liver, heart,[80] brain, kidney, intestine,[76] and muscle.[77]

Kuhn and Boulanger [81] remarked on the lack of fluorescence of the "old" yellow enzyme as compared with free riboflavin-5′ phosphate, suggesting involvement of the alloxazine nucleus as well as the phosphate group in attachment to the protein. Recently Weber [82] has suggested that the relatively

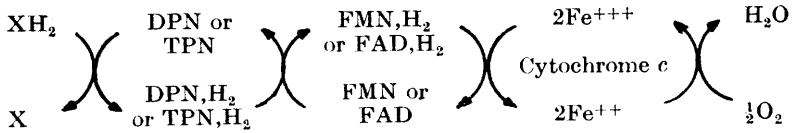
[73] Kuhn, Rudy, and Weygand, *Ber.*, 1936, **69,** 1543; see also Forest and Todd, *J.*, 1950, 3295.
[74] Kuhn and Rudy, *Ber.*, 1936, **69,** 1974.
[75] *Biochem. Z.*, 1938, **298,** 150.
[76] Trufanov, *Biokhim.*, 1941, **6,** 301.
[77] *Idem, ibid.*, 1942, **7,** 188.
[78] Crammer, *Nature*, 1948, **161,** 349; Comline and Whatley, *ibid.*, p. 350.
[79] Klein and Kohn, *J. Biol. Chem.*, 1940, **136,** 177.
[80] Ochoa and Rossiter, *Biochem. J.*, 1939, **33,** 2008.
[81] Kuhn and Boulanger, *Ber.*, 1936, **69,** 1557.
[82] *Biochem. J.*, 1950, **47,** 114.

weak fluorescence of FAD may be caused by internal quenching by interaction of alloxazine and adenine portions of the molecule. This is borne out by the absorption spectrum. Disappearance of fluorescence on combination with certain proteins could be explained in a similar way. Combination with the enzyme protein has also been shown to raise the redox potential from − 0·19 volt for FMN to − 0·06 volt for " old " yellow enzyme.[81] Riboflavin coenzymes are dissociated from their enzymes by dialysis against 0·01N-hydrochloric acid.[83]

The redox system of the riboflavin coenzymes effects the transfer of two electrons as follows :



It is probable that here and in the case of the pyridine nucleotides, a semiquinone intermediate is involved.[84] This would be of great importance in functioning between a compulsory monoelectron-transferring system like ferro-ferri-cytochrome and systems which can only part with two electrons simultaneously (*i.e.*, negligibly low semiquinone concentrations) in avoiding the need for termolecular reaction.[85] The chain of enzymic oxidation of a substance XH_2, then, may be represented as



Kornberg [86, 87] has recently shown that the reversible enzymic synthesis of DPN and FAD can occur by a common route from adenosine-5′ triphosphate (ATP) which may be of considerable significance in all cells :

Nicotinamide mononucleotide (NMN) + ATP ⇌ DPN + inorg. pyrophosphate
FMN + ATP ⇌ FAD + inorg. pyrophosphate

Decarboxylation.—The previous discussion has shown how electrons are transferred from metabolites to gaseous oxygen. Another very important coenzyme, aneurin diphosphate (*cocarboxylase*), is involved in the decarboxylation of α-keto-acids leading to the production of gaseous carbon dioxide. The intervention of cocarboxylase-requiring enzymes will be indicated in the subsequent sections.

Cocarboxylase was first isolated in a pure state from yeast and its

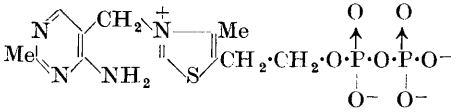
[83] Warburg and Christian, *Biochem. Z.*, 1938, **298**, 368.

[84] Michaelis, Schubert, and Smythe, *J. Biol. Chem.*, 1936, **116**, 587 ; see also Waters, *J.*, 1946, 409.

[85] Cf. Goddard in " The Physical Chemistry of Cells and Tissues ", Ed. by R. Höber, p. 395, London, 1945.

[86] *J. Biol. Chem.*, 1950, **182**, 779. [87] Schrecker and Kornberg, *ibid.*, p. 795.

structure was determined by Lohmann and Schuster in 1937.[88] Synthesis was accomplished in rather low yield by the action of pyrophosphoric acid on aneurin.[89] Phosphorus oxychloride had previously been shown to produce some cocarboxylase, but side reactions make the product difficult to isolate.[90] In spite of further work on the synthesis of cocarboxylase,[91] as

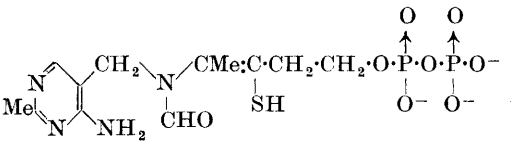


Cocarboxylase

well as on the probably non-physiological aneurin triphosphate,[92] it cannot yet be said that the chemistry of phosphoric esters of aneurin has been completely clarified or that a reliable published method of preparation is available.

It is suggested by Kalckar [93] on the basis of inhibition studies with structural analogues [94] that the linkage of aneurin diphosphate to the carboxylase enzyme of yeast involves the amino- and diphosphate groups. The purified yeast enzyme was shown by Green and his co-workers [95] to contain equimolecular proportions of aneurin diphosphate and protein together with five atomic proportions of magnesium. It was suggested that the metal, which is essential for enzymic activity, plays a role in binding the cocarboxylase to the protein. Dissociation from the protein occurs readily outside a pH range of 4·6—7·8.[96]

The older belief that cocarboxylase could act in oxidative decarboxylations through a redox system involving hydrogenation of the thiazole ring at the quaternary nitrogen atom [97] has been rendered most unlikely by the chemical investigations of Karrer and his associates.[98] Another possibility that the thiol form of cocarboxylase



[88] *Biochem. Z.*, 1937, **294**, 188.

[89] Weijlard and Tauber, *J. Amer. Chem. Soc.*, 1938, **60**, 2263.

[90] Stern and Hofer, *Science*, 1937, **85**, 483; cf. Roux *et al.*, *Compt. rend. Soc. Biol.*, 1948, **142**, 368, 370; 1949, **143**, 524.

[91] Weil-Malherbe, *Biochem. J.*, 1940, **34**, 980; Karrer and Viscontini, *Helv. Chim. Acta*, 1946, **29**, 711; Viscontini, Bonetti, and Karrer, *ibid.*, 1949, **32**, 1478.

[92] Velluz, Amiard, and Bartos, *Compt. rend.*, 1948, **226**, 735.

[93] *Chem. Reviews*, 1941, **28**, 111.

[94] Buchmann, Heergaard, and Bonner, *Proc. Nat. Acad. Sci., U.S.A.*, 1940, **26**, 561; Stumpf, *J. Biol. Chem.*, 1945, **159**, 529.

[95] D. E. Green, Herbert, and Subrahmanyan, *ibid.*, 1940, **135**, 795.

[96] Stumpf, Zarudnaya, and D. E. Green, *ibid.*, 1947, **167**, 817.

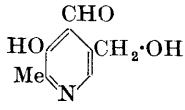
[97] Lipmann and Perlmann, *J. Amer. Chem. Soc.*, 1938, **60**, 2574.

[98] Karrer, Graf, and Schukri, *Helv. Chim. Acta*, 1945, **28**, 1523; Karrer and Krishna, *ibid.*, 1950, **33**, 555.

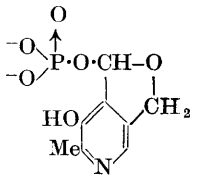
is involved through a reversible oxidation–reduction with its disulphide (cf. cysteine–cystine) has similarly been effectively disposed of by the Swiss workers.[99] It can therefore be assumed that aneurin diphosphate plays no direct role in electron transfer as do the pyridine and flavin nucleotides.

Pyridoxal Phosphate.—Another coenzyme involved in decarboxylations is the phosphorylated pyridoxal derivative, *codecarboxylase*. This was first described by Gale [100] in relation to the compound isolated from bacteria which, in the presence of the appropriate enzymes, catalysed the decarboxylation of a number of α-amino-acids. The general significance of codecarboxylase in amino-acid decarboxylation, as well as in important transamination reactions, in animal tissues was soon realised and has been reviewed by Blaschko [101] and by Gunsalus.[102]

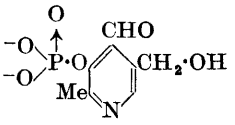
The situation regarding the chemistry of the pyridoxal phosphate, $C_8H_{10}O_6NP$, known to be the coenzyme of these reactions is far from satisfactory. A pure oxime has been prepared [103] from the natural material, from which, it has been claimed, the coenzyme may be regenerated with nitrous acid, thus apparently ruling out structures involving participation



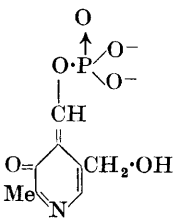
Pyridoxal



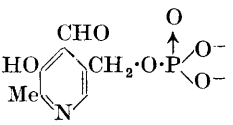
(I.)



(II.)



(III.)



(IV.)

of the aldehyde group in the phosphoric ester group (I, III), and, in addition, structure (II) has been ruled out by its synthesis [103, 104] and comparison with the true coenzyme ; [105] yet none of the workers in this field seems inclined to accept unreservedly the only remaining alternative, (IV). Gunsalus *et al.*[106] claim to have synthesised the coenzyme by phosphorylation

[99] Karrer and Viscontini, *Helv. Chim. Acta*, 1946, **29**, 711.

[100] See Gale, *Adv. Enzymology*, 1946, **6**, 1.

[101] *Ibid.*, 1945, **5**, 67.

[102] *Fed. Proc.*, 1950, **9**, 556.

[103] Heyl, Harris, and Folkers, 110th Meeting Amer. Chem. Soc., Sept. 1946, Abstracts of Papers, 35B.

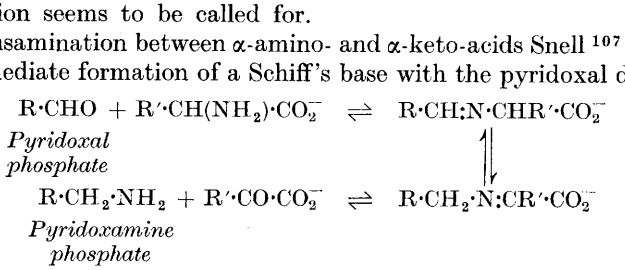
[104] Karrer, Viscontini, and Forster, *Helv. Chim. Acta*, 1948, **31**, 1004.

[105] Umbreit and Gunsalus, *J. Biol. Chem.*, 1949, **179**, 279.

[106] Umbreit, Bellamy, and Gunsalus, *Arch. Biochem.*, 1945, **7**, 185 ; Gunsalus, Umbreit, Bellamy, and Foust, *J. Biol. Chem.*, 1945, **161**, 743.

of pyridoxal with phosphorus oxychloride in the presence of aqueous alkali and fractionation of the barium salts of the mixture of products so obtained. This synthetic material had 2000—3000 times the activity of the synthetic 3-phosphate (II) in activating a bacterial decarboxylase.[105] In view of the paucity of detail in the published chemical work, some further investigation seems to be called for.

In transamination between α-amino- and α-keto-acids Snell [107] postulated the intermediate formation of a Schiff's base with the pyridoxal derivative :



Blaschko [108] has suggested that since *N*-methylamino-acids (which cannot form Schiff's bases) are not substrates for amino-acid decarboxylases, a similar mechanism may be involved in decarboxylation.

Miscellaneous Coenzymes.—Yet another vitamin whose phosphorylated derivative is known to play a very important role in metabolism is pantothenic acid. Lipmann *et al.*[109] have shown that a widely dispersed coenzyme is necessary for all biological reactions of the " active acetate " (see later) obtained from the decarboxylation of pyruvate. This has been termed *coenzyme A* or *co-acetylase* and apparently contains pantothenic acid linked through a phosphate bridge to adenylic acid. A second link to pantothenic acid, possibly an amide link with 2-aminoethanethiol, is also indicated.[110] However, since isolation of a completely pure compound has not yet been achieved, further discussion here is out of place.

$$HO{\cdot}CH_2{\cdot}CMe_2{\cdot}CH(OH){\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$$

Pantothenic acid

The anti-pernicious anæmia factor, vitamin B_{12},[111] which is a phosphoric ester, has been shown to be closely involved in nucleoside formation and may be concerned in the glycosidation of thymine with deoxyribose to give thymidine, an important nucleic acid constituent.[112] Great interest attaches to the isolation, by Todd and his co-workers,[113] of 5 : 6-dimethyl-1-benziminazolyl α-D-ribofuranoside-2′ or -3′ phosphate from the hydrolytic degradation of vitamin B_{12c} in view of its structural resemblance to purine nucleotides.

[107] *J. Amer. Chem. Soc.*, 1945, **67**, 194.

[108] *Biochim. Biophys. Acta*, 1950, **4**, 130.

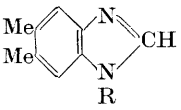
[109] Lipmann, Kaplan, Novelli, Tuttle, and Guirard, *J. Biol. Chem.*, 1947, **167**, 869.

[110] Lipmann, Kaplan, Novelli, and Tuttle, *ibid.*, 1950, **186**, 235 ; Lipmann *et al.*, *J. Amer. Chem. Soc.*, 1950, **72**, 4838 ; Snell *et al.*, *ibid.*, p. 5349.

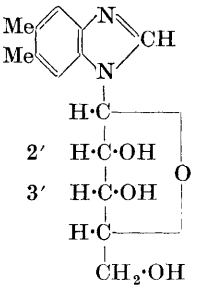
[111] Smith *et al.*, *Proc. Roy. Soc.*, 1949, *B*, **136**, 592 ; Brink, Folkers, *et al.*, *J. Amer. Chem. Soc.*, 1950, **72**, 1866 ; Ellis, Petrow, *et al.*, *J. Pharm. Pharmacol.*, 1950, **2**, 579 ; Kuehl, Folkers, *et al.*, *Science*, 1950, **112**, 354.

[112] See, *inter alia.*, Tomarelli, Norris, and György, *J. Biol. Chem.*, 1949, **179**, 485, and references cited therein.

[113] Buchanan, Johnson, Mills, and Todd, *Chem. and Ind.*, 1950, 426 ; *J.*, 1950, 2845.

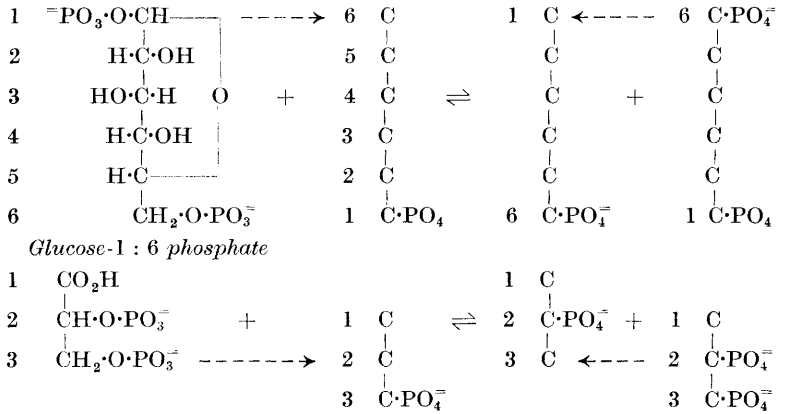


Vitamin B_{12} (R = $C_{53-54}H_{77-81}O_{13}N_{12}PCo$)
Vitamin B_{12c} (R = $C_{54}H_{88}O_{22}N_{12}PCo$)



Dimethylbenziminazole riboside

Glucose-1 : 6 diphosphate [114, 115] and glyceric acid 2 : 3-diphosphate [116] are two coenzymes of simpler structure concerned in two important enzymes of the glycolysis sequence (phosphoglucomutase and phosphoglyceric mutase), catalysing respectively the migration of the phosphate group between the 1- and the 6-position of glucose and between the 2- and the 3-position of glyceric acid. The proposed mechanisms [115, 116] may be represented as follows :



Glucose-1 : 6 phosphate

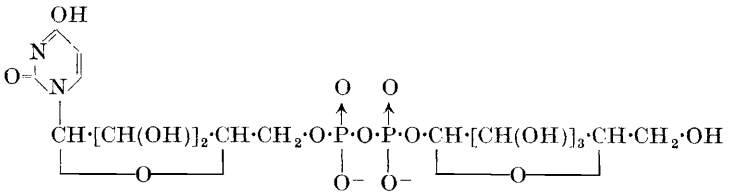
Glyceric acid 2 : 3-diphosphate

T. Posternak [117] synthesised α-D-glucose-1 : 6 diphosphate by the action of silver diphenyl phosphate on 2 : 3 : 4-triacetyl 1-bromo-1-deoxy-α-D-glucose-6 diphenyl phosphate, followed by hydrogenolysis and hydrolysis of the protecting groups. D-Glyceric acid 2 : 3-diphosphate has been synthesised by Baer [118] by phosphorylation of methyl D-glycerate.

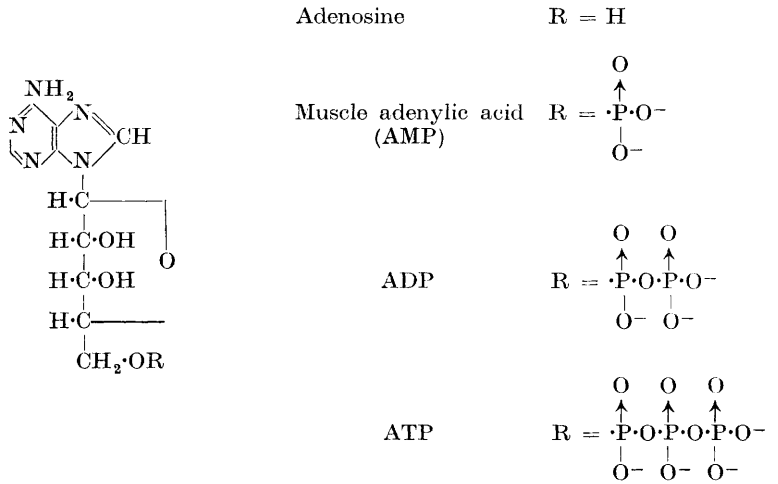
A very recently identified coenzyme is that required by the enzyme isomerising galactose-1 phosphate to glucose-1 phosphate, thus enabling

[114] Cardini, Paladini, Caputto, and Leloir, *Arch. Biochem.*, 1949, **22**, 87.
[115] Sutherland, Cohn, T. Posternak, and C. F. Cori, *J. Biol. Chem.*, 1949, **180**, 1285.
[116] Sutherland, T. Posternak, and C. F. Cori, *ibid.*, 1949, **181**, 153.
[117] *Ibid.*, 1949, **180**, 1269.
[118] *Ibid.*, 1950, **185**, 763.

the former to enter the glycolysis scheme and become oxidised. It contains uridine and glucose linked together by a diphosphate group in the following probable manner : [119]



The Adenine Nucleotides.—The realisation that adenine nucleotides might have some other significance than being mere constituents of nucleic acids may be said to date from the isolation of *adenosine-5′ triphosphate* (ATP) from muscle by Lohmann in 1929.[120] Since that time it has become apparent that these comprise a very important group of coenzymes engaged in transphosphorylations. In the following sections we will endeavour to show how they are employed by the living cell as readily available stores of energy obtained from the oxidative processes referred to earlier.



Adenine nucleotides

Adenosine-5′ phosphate (muscle adenylic acid, AMP) had been isolated in 1927 from rabbit muscle [121] and it was later shown by Lohmann also to arise from the hydrolysis of ATP. The action of a washed lobster-muscle preparation on ATP gave rise [122] to *adenosine-5′ diphosphate* (ADP) and it

[119] Caputto, Leloir, Cardini, and Paladini, *J. Biol. Chem.*, 1950, **184**, 333; *Nature*, 1950, **165**, 191.

[120] *Naturwiss.*, 1929, **17**, 624. For more recent preparative methods see Dounce *et al.*, *J. Biol. Chem.*, 1948, **174**, 361, or Bielschowsky, *Biochem. J.*, 1950, **47**, 105.

[121] Embden and Zimmermann, *Z. physiol. Chem.*, 1927, **167**, 137.

[122] Lohmann, *Biochem. Z.*, 1935, **282**, 109.

is now known that this reaction constitutes a very significant step in the complex process of muscular contraction. The structures, first suggested by Lohmann, have been confirmed by later workers [123] and finally proved by the interesting synthetical studies by Todd and his colleagues which led to the unequivocal total synthesis of muscle adenylic acid and ADP in 1947 [124] and of ATP in 1949.[125] Apart from its extremely important biochemical role, ATP is of interest as the only known naturally occurring ester of triphosphoric acid.

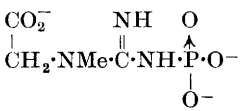
These substances take part in transphosphorylations catalysed by enzymes known as *phosphokinases* in the following manner :

$$\text{ATP (or ADP)} + \text{X·OH} \longrightarrow \text{ADP (or AMP)} + \text{X·O·PO}_3^{=}$$

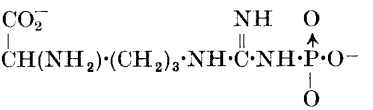
The resynthesis of the ATP (or ADP) by reversal of this reaction is discussed in later sections.

One way in which the adenosine-5′ phosphates may originate from nucleic acids is that postulated by Ostern *et al.*,[126] *viz.*, nucleic acid → adenosine-3′ phosphate [127] → adenosine → adenosine-5′ phosphate.

Accompanying the adenine nucleotides in muscle there is a further substance present, a so-called phosphagen which (though not an ester) must be considered briefly here. Lohmann [128] found ATP to be associated in equilibrium with a substituted guanidinophosphonate, phosphocreatine.



Phosphocreatine



Phosphoarginine

This, the so-called Lohmann reaction, is true for all vertebrate muscle whilst for most invertebrates the phosphagen is phosphoarginine : [129]

$$\text{ATP} + \underset{\text{(Arginine)}}{\text{Creatine}} \rightleftharpoons \text{ADP} + \underset{\text{(Phosphoarginine)}}{\text{Phosphocreatine}}$$

The phosphagens are thus able to act as reservoirs of the labile phosphate group of ATP.

III. Some Energy Relationships

The implication of ATP in such energy-requiring processes as muscular contraction led rapidly [130, 131] to an examination of the free-energy changes involved in the making or breaking of phosphate ester linkages. Lipmann [130]

[123] Gulland and Walsh, *J.*, 1945, 169 ; Lythgoe and Todd, *Nature*, 1945, **155,** 695.
[124] Baddiley and Todd, *J.*, 1947, 648.
[125] Baddiley, Michelson, and Todd, *J.*, 1949, 582.
[126] Ostern, Baranowski, and Terszakoweć, *Z. physiol. Chem.*, 1938, **251,** 258.
[127] " Yeast adenylic acid ", recently shown to be actually a mixture of 2′- and 3′-phosphates (Carter and Cohn, *Fed. Proc.*, 1949, **8,** 190).
[128] *Biochem. Z.*, 1934, **271,** 264.
[129] Meyerhof and Lohmann, *ibid.*, 1928, **196,** 49. At least one other phosphagen of unknown structure occurs : see Baldwin and Yudkin, *Proc. Roy. Soc.*, 1950, *B*, **136,** 614.
[130] Lipmann, *Adv. Enzymology*, 1941, **1,** 99.
[131] Kalckar, *Chem. Reviews*, 1941, **28,** 111.

in 1941 concluded that there were two types of phosphate linkage with free energies of hydrolysis in the regions of — 3000 calories (" energy-poor ") and — 10,000 calories (" energy-rich ") respectively. This concept is still useful, even though it is now recognised that there are relatively large variations within each class.

Of the available means by which free energies can be experimentally determined [132] only one is immediately relevant to our problem. Measurement of the equilibrium constant for the hydrolysis, where possible, gives a direct value for $\Delta F = -RT \log_e K$. However, reactions which go almost to completion ($-\Delta F$, $> ca.$ 5000 calories) place obvious analytical difficulties in the way of determining the equilibrium with a reasonable degree of accuracy. Resort must then be had to indirect calculation from thermochemical measurements provided that information on entropy changes is available ($\Delta F = \Delta H - T\Delta S$) or from measurable equilibria with compounds of known free energy of hydrolysis or other change. The first of these indirect methods was used by Lipmann to calculate approximately the release of free energy by hydrolysis of pyruvic acid enol phosphate (" phosphoenol pyruvic acid ", an intermediate product of the break-down of glucose), whence he obtained a value of — 16,000 calories.[130, 133, 134] The second method has been employed by Meyerhof [134, 135] to calculate a value of — 11,500 calories for the terminal group of ATP from the equilibrium :

$$\text{Glyceraldehyde 3-phosphate} + \text{DPN} + \text{ADP} + \text{HPO}_4^{=} \rightleftharpoons \text{Glyceric acid 3-phosphate} + \text{DPN,H}_2 + \text{ATP}$$

There are so far no experimental data regarding the free energies associated with diphosphate bonds such as those in ADP, cocarboxylase, or dinucleotides, but they are generally assumed to be about the same as for the terminal group of ATP. Another important anhydride type of phosphate bond is that in the acyl phosphates. Lipmann [136] calculated ΔF for the hydrolysis of acetyl phosphate to be $ca.$ — 15,000 calories, while from Bücher's data [137] that for 3-phosphatoglyceroyl phosphate (" 1 : 3-diphosphoglyceric acid ") is found to be — 16,300 calories.

The values of ΔF for the hydrolysis of ordinary phosphate esters are more readily determined, and Meyerhof and H. Green [138] have carried this out by enzymic-equilibria measurements for several of the commoner sugar phosphates all of which belong to the " energy-poor " class. The available figures are given in Table II.

A most useful recent contribution to this problem has been Oesper's theoretical discussion.[139] He considers that, in the case of pyruvate enol phosphate, the high free energy may be attributed entirely to the great difference in stability between the barely detectable enol form of pyruvic

[132] Parks and Huffmann, " The Free Energies of Some Organic Compounds ", New York, 1932.
[133] *Adv. Enzymology*, 1946, **6**, 265.
[134] Cf. Meyerhof and Oesper, *J. Biol. Chem.*, 1949, **179**, 1371.
[135] *Ann. New York Acad. Sci.*, 1944, **45**, 377.
[136] *J. Biol. Chem.*, 1944, **155**, 55.
[137] *Biochim. Biophys. Acta*, 1947, **1**, 292.
[138] *J. Biol. Chem.*, 1949, **178**, 655.
[139] *Arch. Biochem.*, 1950, **27**, 255.

TABLE II

Free energies of hydrolysis of some phosphoric acid derivatives

Compound.	ΔF (cals.).	pH.	Temp.	Method of determn.	Refs.
Glycerol 1-phosphate	— 2,200	8·5	38°	Equil. measurements of enzymic hydrolysis	138
Glucose-6 phosphate .	— 3,000	8·5	38		
Fructose-6 phosphate	— 3,350	8·5	38		
	— 3,500	8·5	38	Equil. with glucose-6 phosphate	155
Glucose-1 phosphate .	— 4,750	8·5	38		
ATP (terminal group)	— 11,500	7·5	20	Calcn.	135
3-Phosphatoglycreyol phosphate	— 16,250*	6·9	25	Enzymic equil. with ATP	137
Acetyl phosphate .	— 14,500*	6·3	37		136
Pyruvic acid enol phosphate . . .	— 15,900	?	20		134
	— 15,850	?	20	Calcn.	130, 133
Phosphocreatine . .	— 13,000*	7·7	20	Enzymic equil. with ATP	141
Phosphoarginine . .	— 11,800*	7·7	20		141

* Authors' calculations for 20°, by assuming negligible temperature coefficients of the equilibrium constants and neglecting pH differences.

acid and the keto-form. In other words the large free-energy release arises chiefly from the keto–enol transformation rather than from the hydrolysis *per se*. In the case of the anhydride types of phosphate bond the concept of " opposing resonance " is called upon. That is, formal combination of the carboxyl group and phosphate ion or of two phosphate ions results in a net loss of 13 and 55 contributing structures, respectively, chiefly on the basis of the " adjacent charge rule ".[140] The resulting hybrid structures are thereby robbed of a large amount of resonance energy and become thermodynamically less stable than the hydrolysis products. By taking into account also the free energy released on ionisation of the latter it is possible to account for the abnormally high free-energy change in the hydrolysis of acyl phosphates and diphosphates. In the case of disubstituted diphosphates such as DPN, TPN, and FAD, the net loss of canonical structures is 89, which is also the case for the second anhydride link of ATP.

Since the reversible Lohmann reaction between ATP and creatine or arginine involves very little change of energy, the guanidinophosphonates are also placed in the " energy-rich " category, ΔF of hydrolysis being — 13,000 and — 11,800 calories, respectively.[141] The source of the high free energy here is partly attributable to neutralisation of the strong base liberated on hydrolysis, the energy differences between the two phosphagens being about what might be expected on consideration of the greater possibilities of resonance in the case of arginine.

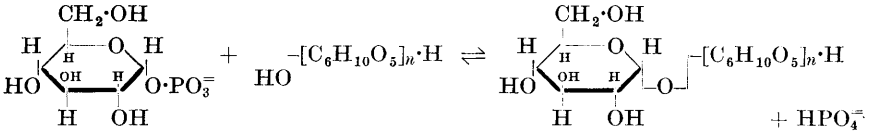
In view of the appreciable contribution to the net free energies of hydrolysis by the ionisation of the hydrolysis products, it is obvious that pH of the environment will be an important factor. Thus, for ATP a pH shift from 7·0 to 8·0 produces an additional decrease in ΔF for

[140] Pauling, " The Nature of the Chemical Bond ", 2nd Edn., pp. 199, 209—210.
[141] Calc. from the data of Lehmann, *Biochem. Z.*, 1936, **286**, 336.

hydrolysis to ADP of *ca.* 1400 calories at 38°.[142] The reverse effect operates in the case of phosphagen which phosphorylates ADP to a greater extent as the pH drops owing to the ΔF of neutralisation of guanidine derivatives becoming larger. It is possible that this sort of variation may be of great significance for it has recently been shown [143] that in the living yeast cell there is a considerable difference between the pH of the centre and of the periphery. Indeed, when one considers the situation at the surface of the protein where enzymic reactions are taking place, the pH—if that term still has its normal meaning—may be such as to cause profound changes in the properties of reactants.[144]

IV. The Oxidation of Carbohydrate

There are two main reserve carbohydrates, starch in the vegetable kingdom and glycogen in the animal kingdom. Starch consists of a mixture of amylose and amylopectin, the former a chain of 1 : 4-α-glucose units and the latter having 1 : 4-α-glucose chains branched in places by cross-linkage in the 1 : 6-position. Glycogen is similar in structure to amylopectin with the possibility of some 1 : 3-linkages.[145] These polysaccharides can be broken down by a simple enzymic hydrolysis catalysed by widely distributed enzymes in both plants and animals. However, this reaction is not reversible (the loss of free energy on hydrolysis is *ca.* 4000 calories per glucose residue [146]) and the polysaccharides are synthesised by the reversal of a phosphorolytic reaction ($n = \not< 3$) : [147]



The enzyme which catalyses this reaction is phosphorylase, and has been obtained from vertebrate muscle in crystalline form by A. A. Green and G. T. Cori.[148] Bernfeld and Meutémédian [149] have isolated from potato tubers, a similar enzyme, isophosphorylase, which catalyses the formation of 1 : 6-linkages. The latter workers have also succeeded in synthesising [150] polysaccharides with different degrees of branching by allowing varied amounts of the two enzymes to act on glucose-1 phosphate.

Bourne, Peat, and their collaborators [151] have demonstrated another way of forming 1 : 6-linkages. This is brought about by the Q enzyme, isolated from potatoes, which appears to act as a transglucosidase. The

[142] Dixon, " Multi-Enzyme Systems ", Cambridge, 1949, p. 98.
[143] Conway and Downey, *Biochem. J.*, 1950, **47**, 355.
[144] Cf. Danielli, " Cell Physiology and Pharmacology ", 1950, pp. 15—17.
[145] Bell, *Ann. Reports*, 1947, **44**, 217.
[146] Calc. from Table II of this review and from data given by Colowick and Sutherland, *J. Biol. Chem.*, 1942, **144**, 423.
[147] Bailey, Whelan, and Peat, *J.*, 1950, 3692.
[148] *J. Biol. Chem.*, 1943, **151**, 21. [149] *Helv. Chim. Acta*, 1948, **31**, 1724.
[150] *Ibid.*, p. 1735.
[151] Reviewed by Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 93.

two mechanisms for polysaccharide formation appear to operate side by side in the potato, and also in the liver from which organ C. F. and G. T. Cori [152] have obtained a " branching factor " which seems to act in the same way as the Q enzyme.

Sucrose and other disaccharides may be synthesised in similar ways.[153]

Glucose must be phosphorylated before it can polymerise to form glycogen or starch, and, as far as is known, it is only enzymically phosphorylated in the 6-position. This reaction takes place by the transfer of the terminal phosphate group from ATP in the presence of hexokinase [154] and is strongly exergonic, so that it proceeds practically to completion. The glucose-6 phosphate may then be converted into the 1 phosphate by phosphoglucomutase [155] to provide α-glucoside units for the synthesis of starch and glycogen.

Glycolysis.—It is well known that carbohydrate is one of the chief sources of energy for living organisms, particularly for animals which cannot utilise solar energy directly as plants can. The free energy available on oxidation of one mole of glucose to carbon dioxide and water is considerable, amounting to 688,000 calories.[156] However, this energy must be transformed into some form in which it can be directly utilised by the organism concerned. As far as is known, the only direct source of energy for the manifold activities of living cells is the free energy of energy-rich phosphate bonds, particularly that from the terminal group of ATP.

The mechanism of the first part of the breakdown of carbohydrate, which is known as glycolysis, has largely been elucidated by the work of Embden, Warburg, Parnas, Meyerhof, and others, and a brief account of this process, as it is now understood, will now be given.

Glucose is first phosphorylated by hexokinase (or, in animals, glycogen may be split by phosphorolysis to glucose-1 phosphate, which is isomerised to glucose-6 phosphate) and the glucose-6 phosphate thus formed is isomerised to fructose-6 phosphate, which is phosphorylated again at the expense of ATP to give fructofuranose-1 : 6 diphosphate. So far 23,000 calories have been lost by the breakdown of two molecules of ATP, while the two energy-poor phosphate bonds in fructose diphosphate amount to a gain of 4000 calories.[157] The net loss to the system is therefore about 19,000 calories. These initial " priming " reactions, resulting in a gain of free energy, seem to be essential for the energy of the hexose molecule to become biologically available.

Cleavage of fructose diphosphate gives two molecules of " triose phosphate ", an equilibrium mixture of dihydroxyacetone phosphate 95% and glyceraldehyde 3-phosphate (5%), followed by a simultaneous phosphorylation and dehydrogenation of the latter in the presence of DPN catalysed by the

[152] *J. Biol. Chem.*, 1943, **151,** 57.

[153] Hassid and Doudoroff, *Adv. Enzymology*, 1950, **10,** 123.

[154] Bailey and Webb, *Biochem. J.*, 1948, **42,** 60 ; Berger, Slein, Colowick, and C. F. Cori, *J. Gen. Physiol.*, 1945, **29,** 379.

[155] Colowick and Sutherland, *J. Biol. Chem.*, 1942, **144,** 423.

[156] Ball, *Ann. New York Acad. Sci.*, 1944, **45,** 363.

[157] Calc. from the data of Meyerhof and H. Green, *J. Biol. Chem.*, 1949, **178,** 655.

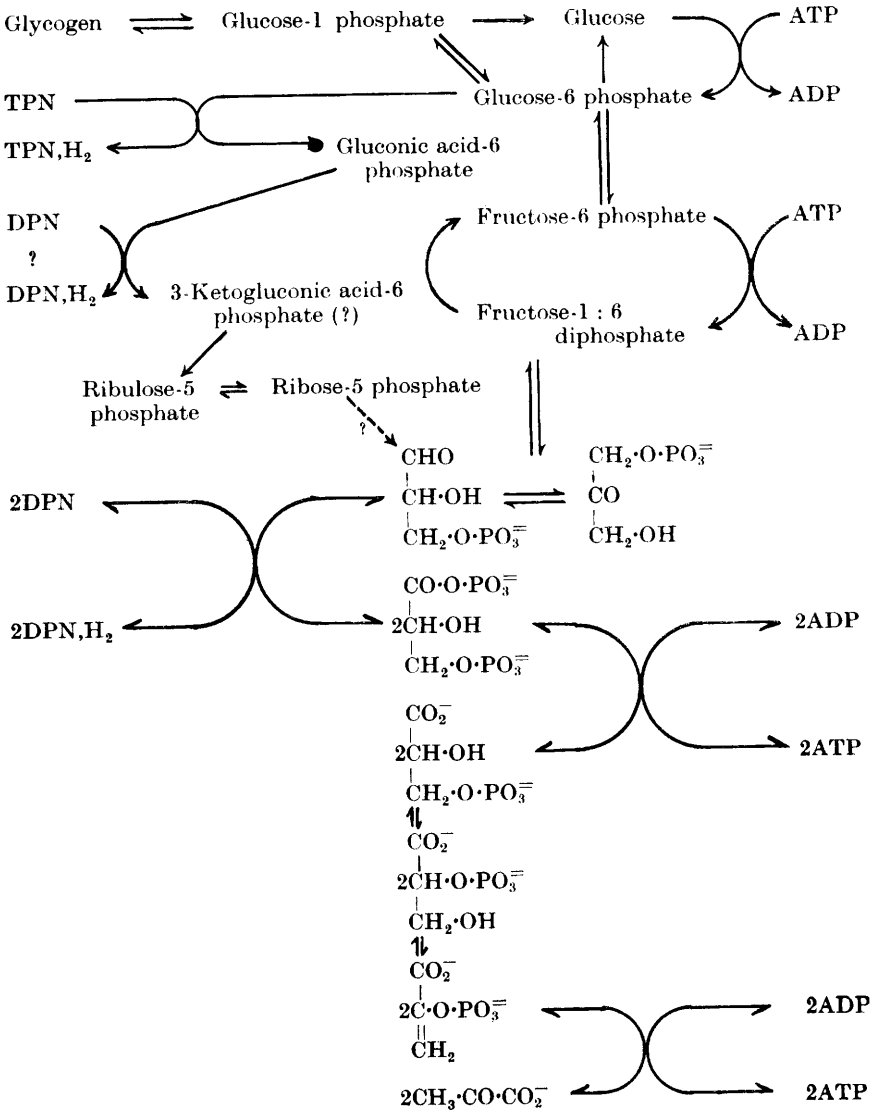


FIG. 1

The oxidation of glucose to pyruvic acid.

enzyme glyceraldehyde 3-phosphate dehydrogenase. The mechanism of this interesting reaction was clarified by Meyerhof and Junowicz-Kocholaty.[158] The 3-phosphatoglyceroyl phosphate thus formed contains a bond of even higher energy than those in ATP and in the presence of a specific phosphokinase [159] transfers this to ADP, forming glyceric acid 3-phosphate

[158] *J. Biol. Chem.*, 1943, **149**, 71. [159] Bücher, *Biochim. Biophys. Acta*, 1947, **1**, 292.

which then isomerises,[160] in a manner analogous to the phosphoglucomutase reaction, to give glyceric acid 2-phosphate. This compound is dehydrated by enolase [161] with a decrease in free energy of only *ca.* 600 calories,[162] giving rise to pyruvic acid enol phosphate containing a further high-energy phosphate bond which is transferred to ADP with another specific phosphokinase.[162] The enolase reaction is of particular interest since it involves the conversion of an energy-poor into an energy-rich bond.

It is thus apparent that during the oxidation of one molecule of glucose to two molecules of pyruvate, the energy of two 11,500-calorie bonds has been used up, while four 16,000-calorie bonds have been generated. These latter appear eventually as four 11,500-calorie bonds in ATP. However, the greater part (*ca.* 80%) of the energy of the glucose molecule is still present in the two molecules of pyruvate.

The yeasts in which many of these reactions were first studied decarboxylate pyruvate to acetaldehyde, a reaction in which aneurin diphosphate is the coenzyme. In the presence of alcohol dehyrogenase, acetaldehyde is reduced to ethanol, which accumulates as an end product. The two electrons for this reaction come from the coenzyme DPN,H_2, which was formed by the earlier reduction of DPN by glyceraldehyde 3-phosphate. It will be noted that the overall glycolytic reaction does not require the participation of oxygen, as is shown by the equation:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5{\cdot}OH + 2CO_2$$

In animal tissues, under anaerobic conditions (such as may obtain partially during severe muscular exercise), pyruvate is reduced to lactate by DPN,H_2 generated during the oxidation of glyceraldehyde 3-phosphate, in a manner analogous to the reduction of acetaldehyde in yeast. Under aerobic conditions the oxidation of glyceraldehyde 3-phosphate takes place through the complete electron-transferring system, DPN, flavin, and cytochrome *c* to oxygen. Consequently, there are now no electrons available to reduce the pyruvate and it is oxidised further, as is discussed in the following section.

It should be noted that all the glycolytic reactions are reversible, with the exception of the phosphorylations by ATP. However, sugar phosphates can be hydrolysed by the widely occurring enzymes known as phosphatases, and so this reaction scheme accounts for both the breakdown and the synthesis of carbohydrate. This is borne out by isotope experiments in which glucose labelled in the expected position has been isolated after feeding various labelled precursors.[163]

An alternative way in which glucose can be broken down ought to be mentioned here. Warburg and Christian,[164] and Dickens,[165] showed some time ago that yeast extracts could oxidise glucose-6 phosphate to gluconic

[160] Sutherland, T. Posternak, and C. F. Cori, *J. Biol. Chem.*, 1949, **181**, 153.
[161] Warburg and Christian, *Biochem. Z.*, 1941, **310**, 384.
[162] Meyerhof and Oesper, *J. Biol. Chem.*, 1949, **179**, 1371.
[163] See Hevesy, "Radioactive Indicators", 1948, p. 380.
[164] *Biochem. Z.*, 1937, **292**, 287. [165] *Biochem. J.*, 1938, **32**, 1626, 1645.

acid and thence to a pentose phosphate. Recent work [166] has confirmed that this is ribose-5 phosphate, and some indication of the nature of the process has been obtained. Gluconic acid-6 phosphate is probably oxidised to 3-ketogluconic acid-6 phosphate [167] which is then decarboxylated to ribulose-5 phosphate. The latter (25%) is in equilibrium with ribose-5 phosphate (75%). Dickens and Glock have recently prepared extracts [168]

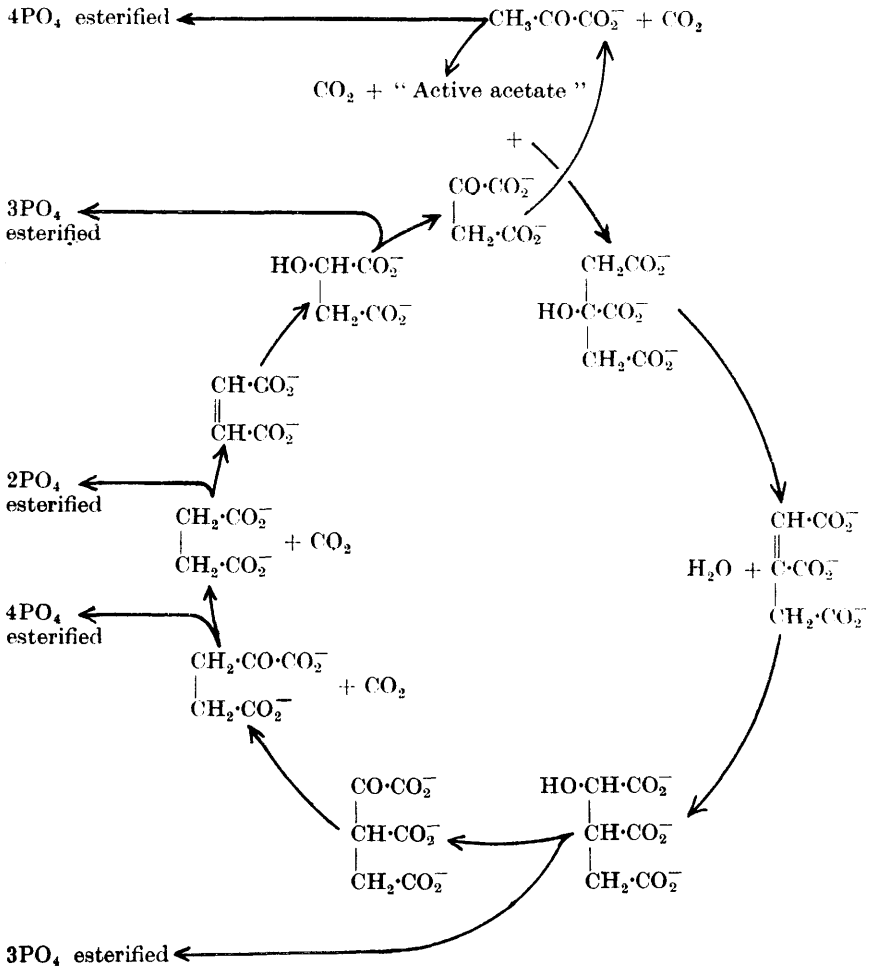


FIG. 2

The tricarboxylic acid cycle.

(*Each oxidative step involves the transfer of two electrons to appropriate acceptors, irrespective of the amount of inorganic phosphate esterified.*)

[166] Cohen and McNair Scott, *Science*, 1950, **111**, 543.
[167] Horecker and Smyrniotis, *Arch. Biochem.*, 1950, **29**, 232.
[168] *Nature*, 1950, **166**, 33.

from several animal tissues which possess specific dehydrogenases for glucose-6 phosphate and gluconic acid-6 phosphate and will also oxidise ribose-5 phosphate. F. Schlenk and Waldvogel [169] have described reactions taking place in liver homogenates which transform the ribose moiety of nucleotides into fructose diphosphate, probably through triose phosphate.

These reactions may well be the source of ribose required as a constituent of nucleosides and their derivatives. The early observation [165] that some of these reactions are inhibited by phosphate may possibly be of significance in determining whether glucose breaks down by this route or by glycolysis.

Oxidation of Pyruvate.—In contrast to the glycolytic enzymes which are all soluble in water and can therefore be studied in tissue extracts, the enzymes catalysing the oxidation of pyruvate are insoluble. This oxidation occurs through a mechanism known as the tricarboxylic acid cycle, first proposed by Krebs and Johnson [170] and since amended by Krebs.[171] Recent work [172] seems to favour the earlier scheme which is shown in Fig. 2. It will be seen that pyruvate first takes part in an oxidative decarboxylation requiring cocarboxylase in which a two-carbon fragment (" active acetate ") is formed, whose exact nature is at present unknown. Although Lipmann [173] has shown that acetyl phosphate is formed during the oxidative decarboxylation of pyruvate by certain bacteria, this does not seem to be the case in those animal tissues which can perform the tricarboxylic acid cycle. Stern and Ochoa [174] have shown that " active acetate " can be formed from ATP and acetate in the presence of extracts of acetone-dried powder from pigeon liver, and it has even been suggested that it is a compound of ATP + acetate.[175] It is known to be involved in biological acetylations [176] such as the physiologically very important formation of acetylcholine, as well as in the tricarboxylic acid cycle. In this cycle active acetate reacts with oxaloacetate to give citrate [177] which, through the widely distributed enzyme aconitase, is in equilibrium with *iso*citrate. Then a series of dehydrogenations, decarboxylations (either simple or oxidative), and a hydration leading through oxalosuccinate, α-ketoglutarate, succinate, fumarate, and malate finally regenerates oxaloacetate. This compound can either be decarboxylated to pyruvate or can react with another molecule of " active acetate ". This scheme accounts for the origin of carbon dioxide and, together with aerobic glycolysis, for the consumption of oxygen during the oxidation of carbohydrate :

$C_6H_{12}O_6 + O_2$	$\longrightarrow$	$2C_3H_4O_3 + 2H_2O$	aerobic glycolysis
$2C_3H_4O_3 + 5O_2$	$\longrightarrow$	$6CO_2 + 4H_2O$	tricarboxylic acid cycle
$C_6H_{12}O_6 + 6O_2$	$\longrightarrow$	$6CO_2 + 6H_2O$	

[169] *Arch. Biochem.*, 1947, **22**, 181. [170] *Enzymologia*, 1937, **4**, 148.
[171] *Adv. Enzymology*, 1943, **3**, 191.
[172] Martius and Lynen, *ibid.*, 1950, **10**, 167 ; Stern, Shapiro, and Ochoa, *Nature*, 1950, **166**, 403.
[173] *J. Biol. Chem.*, 1940, **134**, 463. [174] *Ibid.*, 1949, **179**, 491.
[175] Rudolph and Barron, *Biochim. Biophys. Acta*, 1950, **5**, 59.
[176] See, for example, Nachmansohn and John, *J. Biol. Chem.*, 1945, **158**, 157.
[177] Lorber, Utter, Rudney, and Cook, *ibid.*, 1950, **185**, 689.

In all the early work on the tricarboxylic acid cycle phosphate buffers, with various additions, were used as the suspending media in which the experiments were performed. Although these early workers may not have appreciated the importance of this, it has now been shown that inorganic phosphate is essential to the reactions in this cycle. Some workers [178] had actually demonstrated that inorganic phosphate is esterified during the oxidation of the dicarboxylic acids which are members of the cycle.

Although it has been known for some time that considerable amounts of utilisable energy (probably in the form of high-energy phosphate bonds) must be generated through this cycle, it is only recently that any accurate information has been obtained about this most important aspect. It may be several years before we shall have a detailed picture of the processes involved.

One approach to discovering the various reactions which lead to these phosphorylations is to determine the total amount of phosphate esterified during the catabolism of one mole of pyruvate through the cycle. It is conventional to express this as a P/O ratio (*i.e.*, moles of phosphate esterified per g.-atom of oxygen taken up); the phosphate esterified per cycle will be five times as great. The earliest measurements of P/O ratios [178] were almost certainly too low because of uncontrolled side reactions which rapidly regenerated inorganic phosphate from newly esterified phosphate.

D. E. Green and his co-workers are in the process of publishing a series of papers on an enzyme complex, *cyclophorase* [179] which consists mainly of washed mitochondria from the liver or kidney. Mitochondria are small particles present in the cytoplasm which have been known to cytologists for many years because of their ability to take up certain dyes in the living cell. It is possible to obtain them in a relatively pure state by differential centrifugation and washing,[180] and studies on the purified particles have shown that they are the site of many important metabolic reactions.[181] The cyclophorase system is capable of oxidising all members of the tricarboxylic acid cycle completely, to carbon dioxide and water, requiring, in addition, only inorganic phosphate, magnesium ions, and adenylic acid.

Using this preparation, Cross *et al.*[182] have measured P/O ratios during the oxidation of several substrates of the tricarboxylic acid cycle. Unfortunately, these experiments are not very informative because in no case was the substrate involved in either complete or single-step oxidation. Consequently, it is not possible to interpret the figures unambiguously, for no information about the number and kind of oxidative steps undergone by each substrate is available from them. Perhaps the most significant data are those recently obtained by Hunter and by Lehninger and their collabor-

[178] Kalckar, *Biochem. J.*, 1939, **33**, 631; Belitzer and Tsybakova, *Biokhimiya*, 1939, **4**, 516; Colowick, Kalckar, and C. F. Cori, *J. Biol. Chem.*, 1941, **137**, 343; Ochoa, *ibid.*, 1941, **138**, 751.

[179] D. E. Green, Loomis, and Auerbach, *ibid.*, 1948, **172**, 389.

[180] Schneider, *ibid.*, 1948, **176**, 259.

[181] For references see Keilley and Schneider, *ibid.*, 1950, **185**, 869.

[182] Cross, Taggart, Covo, and D. E. Green, *ibid.*, 1949, **177**, 655.

ators. Hunter and Hixon,[183] using an enzyme preparation similar to the cyclophorase system, have studied the P/O ratio for the one-step oxidation of α-ketoglutarate to succinate. Making careful corrections for side reactions, or showing them to be absent, they have found a P/O ratio of 3·4. They therefore conclude that four moles of phosphate are esterified during the oxidation of one mole of α-ketoglutarate to succinate.

Lehninger and Smith,[184] also using a similar enzyme preparation, measured the P/O ratio during the oxidation of the DPN-coupled oxidation of β-hydroxybutyrate to acetoacetate. After careful corrections for losses due to dephosphorylation, the P/O ratio was between 2 and 3 over short periods of time. They conclude that this phosphorylation occurs during electron transport from DPN to oxygen, for it is extremely unlikely that there is any during the electron transfer from β-hydroxybutyrate to DPN, as the free energies of these two redox systems are within 1000 calories of each other, a difference far too small to account for the generation of one energy-rich phosphate bond of at least 11,500 calories. In support of this claim, Friedkin and Lehninger [185] had previously shown incorporation of $^{32}PO_4^{3-}$ into the labile (*i.e.*, high-energy) groups of ATP when the same system was oxidising DPN,H_2. Thus it seems that during each oxidation in which DPN is involved (and we have no evidence that TPN behaves differently) three energy-rich phosphate bonds are formed from inorganic phosphate during the passage of one pair of electrons from DPN to oxygen. Although the exact mechanism is not yet known, additional evidence of a different kind is provided by data on the free-energy changes in the known electron-transport reactions, which are as follows: DPN, 4000 calories; flavins, 15,000 calories; cytochrome *c*, 31,000 calories; $\frac{1}{2}O_2$, 56,500 calories.[186]

These figures (except in the case of oxygen) are provided by potential measurements on the respective redox system, and cannot be considered to be very accurate because of the difficulties of obtaining these compounds in a pure state. However, they show that there is ample energy available for three energy-rich phosphate bonds to be created during the passage of two electrons from DPN to oxygen (*ca.* 50,000 calories $>$ 3 $\times$ 12,000 calories). It is tempting to assume that one such bond is generated at each step of the electron transfer, for the free-energy differences between each pair of electron transporters are sufficiently great (DPN—flavin, *ca.* 11,000 calories; flavin—cytochrome *c*, *ca.* 16,000 calories; cytochrome *c*—oxygen, *ca.* 25,000 calories).

We should therefore expect that during the oxidations of such substances as *iso*citrate or malate (which react with one or other of the pyridine nucleotides in the presence of their specific dehydrogenases), three energy-rich phosphate bonds should be generated during the uptake of one atom of oxygen. In the case of succinate, in which the transfer of electrons does

[183] *J. Biol. Chem.*, 1949, **181**, 73. [184] *Ibid.*, p. 415.
[185] *Ibid.*, 1949, **178**, 611.
[186] Calc. for two-electron transfers. In the case of the flavins the value refers to the enzymically bound form. (Taken from Dixon, "Multi-Enzyme Systems", Cambridge, 1949, p. 73.)

not involve participation of the pyridine nucleotides,[187] only two such bonds would be formed.

The free energy change in the system, Pyruvate + H_2O → Acetate + CO_2 + 2*e*, is − 15,000 calories,[188] so that during the passage of one pair of electrons from pyruvate or α-ketoglutarate [189] to oxygen four energy-rich phosphate bonds could be generated, one during the reaction with pyridine nucleotide (the oxidation of α-ketoglutarate has been shown to be TPN-linked [190]), and the remaining three as postulated above.

If the above estimates are correct, calculations of the phosphorylations during the complete oxidations of members of the tricarboxylic acid cycle can be made (see Table III).

If the maximum value for one energy-rich phosphate bond is assumed to be − 16,000 calories, the values in the last column are not unreasonable in the cases of pyruvate, succinate, and malate, the only three compounds for which data are available on the free energies released on oxidation :

$$CH_3{\cdot}CO{\cdot}CO_2H + 5O \longrightarrow 3CO_2 + 2H_2O - 274{,}000 \text{ cals.}^{191}$$
$$(= 17 \times 16{,}000)$$

$$HO_2C{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H + 7O \longrightarrow 4CO_2 + 3H_2O - 381{,}000 \text{ cals.}^{192}$$
$$(= 23 \times 16{,}000)$$

$$HO_2C{\cdot}CH(OH){\cdot}CH_2{\cdot}CO_2H + 6O \longrightarrow 4CO_2 + 3H_2O - 344{,}000 \text{ cals.}^{192}$$
$$(= 21{\cdot}5 \times 16{,}000)$$

TABLE III

Reaction.	CO_2 produced, mole.	O taken up, g.-atom.	O (g.-atoms) required for complete oxidation of one mole of first substance.	PO esterified per step, moles.	PO_4* (moles) esterified/complete oxidation of one mole of first-mentioned substance.
Pyruvate → active acetate	1	1	5	4	16
Active acetate + oxaloacetate → citrate	0	0	4	0	12
Citrate → *cis*-aconitate → *iso*citrate	0	0	9	0	27—28
*iso*Citrate → oxalosuccinate	0	1	9	3	27—28
Oxalosuccinate → α-keto-glutarate	1	0	8	0	24—25
α-Ketoglutarate → succinate	1	1	8	4	24—25
Succinate → fumarate	0	1	7	2	20—21
Fumarate → malate	0	0	6	0	18—19
Malate → oxaloacetate	0	1	6	3	18—19
Oxaloacetate → pyruvate	1	0	5	0	15—16

* The sum of phosphate esterified during the steps necessary to account for the oxygen uptake in column 4. The limits allow for the possibility of either further reaction of oxaloacetate with active acetate or decarboxylation to pyruvate.

[187] Slater, *Nature*, 1950, **165**, 674.

[188] Lipmann and Tuttle, *J. Biol. Chem.*, 1944, **154**, 725.

[189] Their oxidative decarboxylations are clearly analogous, being catalysed by closely similar enzymes. See Stumpf, Zarudnaya, and D. E. Green, *ibid.*, 1947, **167**, 817.

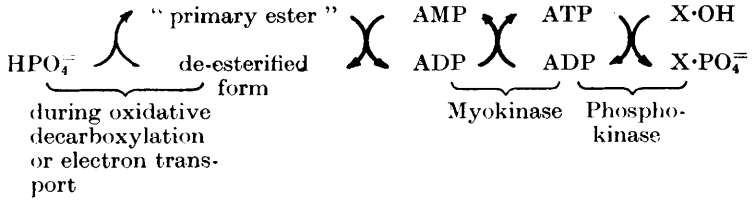
[190] Huennekens and D. E. Green, *Arch. Biochem.*, 1950, **27**, 428.

[191] Ogston and Smithies, *Physiol. Reviews*, 1948, **28**, 283.

[192] Calc. from Parks and Huffmann, " The Free Energies of Some Organic Compounds ", New York, 1932.

Because the differences between the figures of P/O ratios for the complete oxidation of the various tricarboxylic acid cycle substrates are so small as not to be measurable by present experimental methods, it is to be hoped that work in this field will be concentrated on obtaining either P/O ratios for individual steps, or values of phosphate esterified per mole of substrate completely oxidised.

Although we have extremely little knowledge of the detailed mechanisms of the synthesis of these energy-rich phosphate bonds, work on the cyclophorase system has revealed something of the nature of the primary phosphorylated product. Cross *et al.*[182] have shown that adenylic acid acts catalytically as a secondary phosphate acceptor. They therefore postulate that inorganic phosphate is first taken up in the form of an extremely labile phosphorus compound (" primary ester " or " gel P " [193]) and thus initiates a phosphate-transferring system, analogous in some ways to the electron-transferring system mentioned in the section of coenzymes.



The nature of the " primary ester " is quite unknown ; it does not appear to be an acyl phosphate.[182] In contrast to inorganic phosphate, it cannot be removed from the cyclophorase gel by washing with cold salt solutions [193] although it is estimated as inorganic phosphate in the Lowry and Lopez method [194] for determining inorganic phosphate in the presence of very labile phosphorus compounds.

The requirement of the system for inorganic phosphate is absolute,[195, 182] the previous report by Loomis and Lipmann [196] that 2 : 4-dinitrophenol could replace inorganic phosphate being an incorrect interpretation of the experimental data. It is therefore concluded that in this system, in contrast to the various isolated dehydrogenases, oxidation can only occur concomitantly with the esterification of inorganic phosphate.

Cross *et al.*[182] noted that in the absence of adenylic acid, inorganic pyrophosphate is rapidly formed during the oxidations carried out by cyclophorase. As mentioned earlier, Kornberg [197] has discovered two reactions in which inorganic pyrophosphate is produced, the phosphorylations of nicotinamide mononucleotide and flavin mononucleotide by ATP. Whether these two phenomena are connected is a matter for speculation at present,

[193] Green, Atchley, Nordmann, and Teply, *Arch. Biochem.*, 1949, **24**, 359.
[194] *J. Biol. Chem.*, 1946, **162**, 421. [195] Tepley, *Arch. Biochem.*, 1949, **24**, 383.
[196] *J. Biol. Chem.*, 1949, **179**, 503.
[197] *Ibid.*, 1950, **182**, 779 ; Schrecker and Kornberg, *ibid.*, p. 795.

but it should be noted that D. E. Green [198] has reported incorporation of $^{32}PO_4^{3-}$ into pyridine nucleotides during oxidations by cyclophorase.

The cyclophorase system is also capable of oxidising fatty acids [199] provided that a catalytic amount of a member of the tricarboxylic acid cycle is present, although it has only recently been possible to demonstrate a concomitant esterification of inorganic phosphate with a P/O ratio of 1·0—1·7.[200]

In addition the cyclophorase system will oxidise to completion a few amino-acids.[201] These reactions may be of significance in the catabolism of proteins and also for the formation of amino-acids, which can be synthesised from the appropriate keto-acid and ammonia. Pyridoxal phosphate will presumably be needed for any transaminations involving these acids.

The Utilisation of Energy-rich Phosphates.—The energy which has become concentrated into such esters as ATP in the ways described is used by living organisms to perform chemical reactions which may involve the liberation of heat, to do osmotic and mechanical work, and even to produce light [202] and electricity.[203] Among chemical activities, protein synthesis is an obvious necessity and the implication of energy-rich phosphates has been discussed recently in an excellent review by Lipmann.[204] The enzymic incorporation of [^{14}C]glycine and [^{15}N]glutamate into glutathione has been shown by Bloch [205] to require ATP. This is particularly interesting in view of the recent demonstration [206] of transpeptidation reactions between glutathione and amino-acids in the presence of kidney extracts which suggest that this tripeptide may be a key substance in the synthesis of proteins. The chief end-product in the catabolism of proteins by higher animals is urea and it is now known [207] that ATP participates in three of the reactions of the ornithine cycle [208] through which it is formed.

These represent only a very few of the ways in which the free energy stored in phosphoric esters may be used for chemical work. Chemical and mechanical work are universally linked in the process known as active transfer, in which substances are transported across cell membranes against concentration gradients. For instance, it appears probable [209] that sugars must be phosphorylated in order to pass through the intestinal wall. Many

[198] 116th Meeting Amer. Chem. Soc., Sept. 1949, Abstracts of Papers, 68C.
[199] Grafflin and Green, *J. Biol. Chem.*, 1948, **176**, 95.
[200] Johnson and Lardy, *ibid.*, 1950, **184**, 235.
[201] Taggart and Krakaur, *ibid.*, 1949, **177**, 641; Still, Buell, and Green, *Arch. Biochem.*, 1950, **26**, 406, 413; Still, Buell, Knox, and Green, *J. Biol. Chem.*, 1949, **179**, 831.
[202] McElroy and Strehler, *Arch. Biochem.*, 1949, **22**, 420.
[203] Nachmansohn, Cox, Coates, and Machado, *J. Neurophysiol.*, 1943, **6**, 383.
[204] *Fed. Proc.*, 1949, **8**, 597.
[205] *J. Biol. Chem.*, 1949, **179**, 1245; cf. also Borsook, Deasy, Haagen-Smit, Keighley, and Lowry, *Fed. Proc.*, 1949, **8**, 589.
[206] Hanes, Hird, and Isherwood, *Nature*, 1950, **166**, 288; cf. Fruton, *Yale J. Biol. Med.*, 1950, **22**, 263.
[207] Ratner, *Fed. Proc.*, 1949, **8**, 603.
[208] Krebs and Henseleit, *Z. physiol. Chem.*, 1932, **210**, 33.
[209] C. F. and G. T. Cori, *J. Biol. Chem.*, 1925, **66**, 691; Fisher and Parsons, *J. Physiol.*, 1949, **110**, 281; see also Hele, *Nature*, 1950, **166**, 786.

cases of active transfer in which carbohydrate metabolism is the source of energy are discussed by Höber.[210]

We have already referred to the synthesis of the important chemical mediator of nerve transmission, acetylcholine, from choline and " active acetate " which is believed to possess energy-rich phosphate groups. The result of the liberation of a minute amount of acetylcholine at the end of a motor nerve is, of course, muscular contraction, and the energy for this mechanical work is now widely attributed to degradation of ATP. The foundations for the present degree of understanding of this very complex process are due largely to the pioneer work of A. V. Hill, O. Meyerhof, E. Lundsgaard, A. Szent-Györgyi, and many others. Myosin, one of the chief proteins of muscle, is very closely associated, if not identical, with the enzyme which hydrolyses specifically the terminal phosphate group of ATP.[211] The action of ATP on threads of actomyosin, another muscle protein, on myofibrils, and even on isolated muscle fibres, has been shown [212, 213] to result in contraction in the same way as ATP causes contraction of whole muscle *in vivo*. Although the nature of the contractile process is still somewhat obscure, the close association with ATP breakdown seems to have been established. In view of the large amounts of ATP which could be synthesised during normal metabolic processes, and its high concentration in muscle, it is difficult not to assume that it is the direct source of energy for muscular effort. A useful summary of some modern views is available in the Meyerhof anniversary volume, " Metabolism and Function ".[213]

In conclusion, it is to be hoped that improved synthetic and purification methods will soon make these phosphoric esters readily available so that further elucidation of their role may be carried out. The possible significance for therapeutic purposes of this work has been discussed elsewhere.[214] At all events, as we have tried to indicate, these substances are concerned directly or indirectly with practically every aspect of living processes.

The authors are grateful to Professors E. Baldwin and A. R. Todd and to Dr. F. Bergel and other colleagues who have read the manuscript and made many helpful criticisms.

[210] " The Physical Chemistry of Cells and Tissues ", London, 1945, p. 524.

[211] Engelhardt, *Adv. Enzymology*, 1946, **6**, 147.

[212] Perry, *Biochem. J.*, 1950, **47**, xxxviii.

[213] See the articles by A. V. Hill, Weber, Dubuisson, Szent-Györgyi, Needham, Mommaerts, and Korey, *Biochim. Biophys. Acta*, 1950, **4**, 4—67.

[214] Cf. Bergel, *J. Applied Chem.*, in the press; H. N. Green and Stoner, " The Biological Actions of the Adenine Nucleotides ", London, 1950.