present positive attitude toward them. This point was investigated thoroughly because Hopi tradition would allow for a religious symbol being attached to albinism.

Hopis share the legend permeating Central American Indian cultures that someday a white leader will return and lead them to a better life. Just as Cortez was welcomed by the Aztecs as their white leader, Pedro de Tovar, the first white man to visit the Hopis, was welcomed by the Hopis as their "lost white brother" (*21*). Tovar and his men quickly subdued the Hopis. The lost white brother has yet to arrive. If the birth of an albino was ever considered as a symbol or reminder of their lost white brother, it is unknown to any living Hopi. The intriguing problem remains, however, as to why albinos, especially albino children, are considered so affectionately in all villages. The admiration of their whiteness is clearly not an identification with white Americans; it represents instead an association of whiteness with cleanliness, goodness, and purity; attributes honored by traditional Hopis. Rain is essential for existence in the barren mesa country. White clouds symbolize rain and are a constant reminder of the necessity of living a pure life. "If Hopis live a clean, good and harmonious life, there will be plenty of rain and an abundance of food for the children to eat."

Conclusion

Although selection for the heterozygote or genetic drift may account for the high frequency of the albino gene in the Hopi population, the most apparent explanation is acquisition of the gene by migration and cultural selection of the type described here. A study of the Hopi people also indicates that time will soon erase albinism as a Hopi heritage on Second and Third Mesas. Paved roads now link the Hopi mesas so that only minutes are required to travel from one mesa to another by truck or automobile. These modern transportation facilities, schools on and off the reservation where members of different ethnic groups associate, and a growing population forcing young people to seek employment off the reservation all promote outbreeding, reduce the frequency of the albino gene, and decrease the probability of homozygosity for this gene. The eclipse of agriculture as a way of life negates any reproductive advantage held by albino males in past generations. The frequency of albinism among the Hopis will decrease rapidly with the decline of their culture.

References and Notes

1. T. B. Fitzpatrick, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, Eds. (McGraw-Hill, New York, 1960).
2. P. D. Trevor-Roper, *Brit. J. Ophthalmol.* **36**, 107 (1952).
3. C. J. Witkop, Jr., E. J. Van Scott, Jr., G. A. Jacoby, *Excerpta Med. Sect. 1 Anat. Anthropol. Embryol. Histol.* **F-169**, Abstr. 381 (1961).
4. K. Pearson, E. Nettleship, C. H. Usher, *A Monograph on Albinism* (Draper Company Research Memoirs, Biometric Series, Dolan, London, 1911–1913), vols. 6 and 9.
5. C. E. Keeler, *J. Hered.* **55**, 115 (1964).
6. C. M. Woolf, *Amer. J. Hum. Genet.* **17**, 23 (1965).
7. J. V. Neel, M. Kodani, R. Brewer, R. C. Anderson, *ibid.* **1**, 156 (1949).
8. A. Hrdlička, "Physiological and medical observations among the Indians of southwestern United States and northern Mexico," *Bull. Bur. Amer. Ethnol. No. 34* (1908).
9. L. L. Hargrave, *Mus. N. Ariz. Mus. Notes* **4**, No. 7 (1932).
10. E. P. Dozier, "The Hopi-Tewa of Arizona" *Univ. Calif. Publ. Amer. Archeol. Ethnol.* **44**, No. 3 (1954).
11. C. M. Woolf and R. B. Grant, *Amer. J. Hum. Genet.* **14**, 391 (1962).
12. C. M. Woolf, D. A. Dolowitz, H. E. Aldous, *Arch. Otolaryngol.* **82**, 244 (1965).
13. M. Titiev, *Old Oraibi, A Study of the Hopi Indians of Third Mesa* (Peabody Museum Papers, Harvard Univ., Cambridge, Mass., 1944), vol. 22, No. 1.
14. Sun Chief, *The Autobiography of a Hopi Indian*, L. W. Simmons, Ed. (Yale Univ. Press, New Haven, 1942), p. 68.
15. F. C. Dukepoo, thesis, Arizona State University, Tempe (1968).
16. Ching-tS'ung Li, *Population Genetics* (Univ. of Chicago Press, Chicago, Ill., 1955).
17. K. S. Brown, B. L. Hanna, A. A. Dahlberg, H. H. Strandskov, *Amer. J. Hum. Genet.* **10**, 175 (1958).
18. C. Stern, *Principles Human Genetics* (Freeman, San Francisco, ed. 2, 1960).
19. C. M. Woolf, F. E. Stephens, D. D. Mulaik, R. E. Gilbert, *Amer. J. Hum. Genet.* **8**, 236 (1956).
20. J. N. Spuhler and C. Kluckhohn, *Hum. Biol.* **25**, 295 (1953).
21. F. Waters, *Book of the Hopi* (Viking, New York, 1963).

22. Supported by AEC contracts AT(11-1)-1415 and AT(11-1)-2013-01 to Arizona State University.

A Carbon-Phosphorus Bond in Nature

A spot on a chromatogram leads to a new field of phosphorus biochemistry.

James S. Kittredge and Eugene Roberts

Several years ago we made a study of the easily extractable ninhydrin-reactive constituents of the tissues of a number of marine invertebrates (*1*). Two-dimensional paper chromatograms of extracts of the sea anemone *Anthopleura elegantissima* revealed an acidic ninhydrin-reactive material migrating like ethanolamine phosphate. In contrast to the latter substance, the material in anemone extracts was resistant to prolonged acid hydrolysis. Another ninhydrin-reactive substance in the extract was observed to release additional amounts of the acidic compound during hydrolysis. The acid-stable substance was isolated by ion-exchange chromatography and crystallized. The serendipitous observation that there was considerable ash left on combustion of a sample during elemental analysis led to the determination that 25 percent of the weight of the material could be accounted for as stably bonded phosphorus, and to the identification of the substance as 2-aminoethylphosphonic acid (AEP). The substance in the extract yielding AEP on hydrolysis was identified as a glyceryl ester of AEP, and the liberation of AEP on hydrolysis of the lipid extracts of the anemone suggested AEP as a possible constituent of phospholipids (*2*).

We were preparing a report on the above finding when it was brought to our attention that Horiguchi and Kan-

The authors are senior scientist and director, respectively, in the Division of Neurosciences, City of Hope Medical Center, Duarte, California.

datsu had previously isolated AEP from the hydrolysate of a "proteolipid-like" extract of ciliates from sheep rumen (*3*). This information raised the question of whether the AEP was a component of the sea anemone tissue or a constituent of the symbiotic algae contained in the anemone tissues. The isolation of phosphonic acid from the pedal discs of the anemones, tissue which does not contain algae, indicated that the phosphonic acid actually occurs in the anemone tissues; but this did not determine the site of original synthesis of the carbon-phosphorus bond. Later detection of AEP in species not known to contain algae indicated that the coelenterates are probably capable of the synthesis. The rapid incorporation of ^{32}P-labeled phosphate ion into AEP has been demonstrated with the sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* and with the hydra *Chlorohydra viridissima*. The synthesis has similarly been demonstrated with the pulmonate snail *Helix aspersa* and with the slugs *Lehmannia poineri* and *Limus flavus* (*4*). Because of the difficulty of obtaining these organisms free from symbionts, belief in their capacity for synthesizing the C-P bond rests insecurely on the rates and quantities observed. Quin isolated the AEP from the sea anemone *Metridium dianthus* and found that this phosphonic acid made up 1 percent of the total dry weight of the organism. In another anemone, *Tealia felina*, he found the C-P phosphorus to be about 50 percent of the total phosphorus (*5*). Kandatsu and Horiguchi demonstrated that the ciliate *Tetrahymena pyriformis*, in axenic culture, rapidly synthesizes AEP from ^{32}P-labeled phosphate ion and that 13 percent of the total phosphorus is fixed in this compound (*6*). Recently we have demonstrated the synthesis of several phosphonic acids by three species of dinoflagellates and two species of coccolithophorids, but at rather low levels (*7*), and Baldwin and Braven have isolated AEP from the unicellular alga *Monochrysis lutheri* (*8*).

Since the biosynthesis of many amines occurs through the decarboxylation of amino acids, we sought the likely precursor of AEP, 2-amino-3-phosphonopropionic acid. At this point, fortunately, we received a letter from A. F. Isbell of Texas A&M University informing us that he had in progress a program for synthesizing the aminophosphonic analogs of the natural amino acids (*9*). Utilizing his synthetic standard, we were able to detect the new aminophosphonic acid in extracts of the zoanthid *Zoanthus sociatus*, and to demonstrate its synthesis by *Tetrahymena* by cocrystallization of the isolated ^{32}P-labeled compound with the synthetic 2-amino-3-phosphonopropionic acid (*10*). Subsequently the *N*-methyl, *N*-dimethyl, and *N*-trimethyl derivatives of AEP were isolated from ethanolic extracts of the sea anemone *Anthopleura xanthogrammica* (*11*).

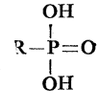
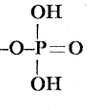
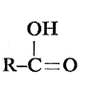
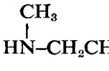
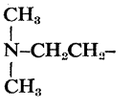
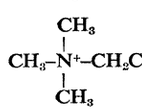
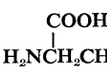
This work established the biological occurrence of the phosphonic acid analogs of ethanolamine phosphate, serine phosphate, and choline phosphate, three major constituents of phospholipids. The structures of the C-P compounds isolated to date and their relationship to other known substances are shown in Table 1. The chemical properties of AEP and its synthesis, as well as the natural distribution, are described elsewhere (*12*).

C-P Compounds as Components of Phospholipids

Shortly after AEP was discovered in anemones, we were visited by Eric Baer. It was apparent from our data (*2*) and those of Horiguchi and Kandatsu (*3*) that AEP was a constituent of lipids. This presented a challenge, and Baer responded in typical fashion by initiating a program for synthesizing phosphonic acid analogs of phospholipids; for those analogs he proposed the name phosphonolipids (*13*). A report by Rosenthal and Pousada on the synthesis of AEP-containing cephalin analogs (*14*) appeared virtually simultaneously with Baer's first paper.

After having determined that AEP is a major constituent of the lipids of the sea anemone, we proceeded with an examination of chloroform-methanol extracts of fresh anemones. Rouser of our laboratory was successful in isolating and characterizing ceramide-2-aminoethylphosphonic acid (ceramide-AEP) (*15*), elucidating its structure by degradative techniques (*16*). Meanwhile, Hori *et al.* isolated from the clam

Table 1. Phosphonic acids found in nature, and phosphate ester and carboxylic acid analogs.

R group	Phosphonic acid	Phosphate ester	Carboxylic acid
			
$H_2NCH_2CH_2$–	2-Aminoethylphosphonic acid* (AEP)	2-Aminoethyl phosphate (ethanolamine phosphate)	2-Aminoethyl carboxylic acid (β-alanine)
	2-Methylaminoethylphosphonic acid (*N*-methyl AEP)	2-Methylaminoethyl phosphate (*N*-methyl ethanolamine phosphate)	2-Methylaminoethyl carboxylic acid (*N*-methyl β-alanine)
	2-Dimethylaminoethylphosphonic acid (*N*,*N*-dimethyl AEP)	2-Dimethylaminoethyl phosphate (*N*,*N*-dimethyl ethanolamine phosphate)	2-Dimethylaminoethyl carboxylic acid (*N*,*N*-dimethyl β-alanine)
	2-Trimethylaminoethylphosphonic acid (*N*,*N*,*N*-trimethyl AEP)	2-Trimethylaminoethyl phosphate (choline phosphate)	2-Trimethylaminoethyl carboxylic acid (*N*,*N*,*N*-trimethyl β-alanine)
	2-Amino-3-phosphonopropionic acid† (phosphonoalanine)	Serine phosphate	Aspartic acid

* The naturally occurring sulfonic acid analog of AEP is taurine, $H_2NCH_2CH_2SO_3H$. † The naturally occurring sulfonic acid analog of 2-amino-3-phosphonopropionic acid is cysteic acid, $HOOCCH(NH_2)CH_2SO_3H$.

Corbicula sandai a sphingolipid which appeared to contain ethanolamine phosphate. However, subsequent studies showed this constituent to be ceramide-AEP, and Hori *et al.* have now found this phosphonolipid in a number of mollusks (*17*). De Koning demonstrated the presence of ceramide-AEP in the abalone *Haliotis midae* (*18*). Carter and Gaver isolated the ceramide-AEP from *Tetrahymena* and found that it contains C_{17} and C_{19} branched-chain sphingosines (*19*). Dawson and Kemp have reported the probable presence of both ceramide-AEP and ceramide phosphorylethanolamine in the lipids of rumen protozoa (*20*).

The earlier finding of a glyceryl ester of AEP in sea anemones clearly indicated that the occurrence of phosphatidyl-AEP was a possibility. During a visit to our laboratory, Rosenberg, working with Kittredge, found that the phosphatidylethanolamine fraction of *Tetrahymena* lipids contains AEP. Baer demonstrated that the synthetic phosphatidyl-AEP and phosphatidylethanolamine behave almost identically in many chromatographic systems (*13*). Upon his return to Australia, Rosenberg was able to show by indirect, but convincing, methods that phosphatidyl-AEP is present in *Tetrahymena*. The molar ratio of phosphatidylethanolamine to phosphonolipid was found to be approximately 13:1. Mild saponification failed to release about 20 percent of the lipid-AEP, a fact which suggested that plasmalogen or sphingolipids, or both, containing AEP were also present (*21*). Rumen protozoa also have been shown to contain phosphatidyl-AEP and plasmalogen-AEP (*20*). The presence in *Tetrahymena* of a glyceryl monoether phosphatidyl-AEP was established by Thompson, who isolated the AEP ester of chimyl alcohol from the lipid-soluble saponification products of a phospholipid fraction (*22*). Recently Chacko and Hanahan have synthesized the monoether phosphatidyl-AEP and have confirmed the presence of both the diester and the monoester phosphonolipids in the *Tetrahymena* (*22a*).

Liang and Rosenberg investigated the biosynthesis of phosphatidyl-AEP in *Tetrahymena* and were able to demonstrate the incorporation of AEP into cytidine monophosphate-AEP and the in vitro synthesis of an AEP-containing glyceryl phospholipid from cytidine monophosphate-AEP and dipalmitin (*21*).

Two-dimensional thin-layer chromatography of ^{32}P-labeled lipid extracts of *Anthopleura elegantissima* provided evidence of the presence of two new phosphonolipids, one containing *N*-methyl-AEP and the other *N*-trimethyl-AEP (*23*), but these lipids have not yet been completely characterized. Hayashi *et al.* subsequently isolated a sphingolipid containing *N*-methyl-AEP from the snail *Turbo cornutus* (*24*).

Organisms which do not synthesize AEP may readily incorporate this compound into their tissue lipids. Thus, administration of ^{32}P-labeled AEP to rats resulted in its incorporation into two liver lipids (*25*). The injection of labeled AEP into a goat also resulted in its incorporation into liver lipids, and dilution of the activity permitted estimation of the endogenous lipid-AEP (*26*). Housefly larvae (*Musca domestica*) reared on media containing AEP, *N*-dimethyl-AEP, or *N*-trimethyl-AEP incorporate these C-P compounds into phosphonolipids (*27*). It is likely that this synthesis of phosphonolipids occurs by way of the Kennedy pathway, the phosphonobases utilizing the same enzymes which transfer phosphobases from the cytidine phosphobase into phospholipids. This would account for the fact that AEP has been detected in crustacea and mammals, animals which are not known to synthesize AEP but which might assimilate phosphonic acids from the digestive tract. Free phosphonic acids or phosphonolipids have been detected in rat lipids (*24*), in goat liver lipids (*26*), in bovine brain (*28*), in bovine kidney and milk, and in human erythrocytes (*29*).

C-P Compounds Associated with Proteins

Quin found that a major portion (72 percent) of the AEP in the sea anemone *Metridium dianthus* was present in a bound form in the lipid-free insoluble residue (*5*). AEP associated with insoluble residue also was observed by Rosenberg in *Tetrahymena* (*30*). The residue from *Tetrahymena* was resistant to the action of proteolytic enzymes, but that from the anemone was partially solubilized by pepsin and, on fractionation, yielded a peptidic material containing AEP (4.1 percent) (*12*). Dinitrophenylation of the latter material indicated that the amino group of the AEP is blocked and suggested that the amino group might be bound in peptide linkage. Quin has found similar insoluble residues containing AEP in several species of anemones, in a soft coral (*Leptogorgia virgulata*) and in a nudibranch mollusk (*Archiodoris* sp.). In the sea anemone *Anthopleura xanthogrammica* the residue was found to contain more *N*-methyl-AEP (78 percent) than AEP (22 percent) (*31*). Recently Stevenson, Gibson, and Dixon, of the University of British Columbia, have isolated the first pure proteins containing AEP (*32*). Two proteases, A and B, isolated from the sea anemone *M. senile* have been found to contain 7.4 and 3.7 molecular residues, respectively, of AEP (a molecular weight of 20,000 being assumed for each protease).

Biosynthesis of the C-P Bond

The organism of choice for study of the formation of the C-P bond is *Tetrahymena pyriformis*. It contains a large amount of AEP, and a likely precursor, phosphonoalanine, both of which are labeled when ^{32}P-labeled phosphate ion is added to the culture medium. Rosenberg observed that the lipid-AEP from *Tetrahymena* in the log phase of growth had a higher specific activity than either free or residue-bound AEP. Similar experiments with cells in the stationary phase resulted in little or no labeling of AEP, although there was considerable activity in the phospholipid phosphate (*30*). Subsequently, during a visit with us, Horiguchi extended this work to cultures of *Tetrahymena* brought into synchronous division by cyclic heat shocks. In brief successive labeling experiments the specific activity of the AEP was found to fall during the shock treament and then to rise to a maximum, which coincided exactly with the period of cell division (*33*). Chou and Scherbaum observed that a single heat shock led to the accumulation of a still uncharacterized substance containing AEP and possibly phosphonoalanine and glycerol (*34*). The foregoing results, together with Rosenberg's observation that there was no decrease in the amount of labeled AEP when log-phase cells were transferred to unlabeled medium containing excess orthophosphate (*30*), suggest that the synthesis of AEP is linked to synthetic activities occurring during cell division. The results also indicate that free AEP is not the precursor of bound AEP in *Tetrahymena*. In contrast, recent data (*4*) suggest that the free AEP is the precursor of bound AEP in the garden slugs and the garden snail. However, inability to

estimate the size or number of pools of either the free or the bound forms of AEP precludes an unequivocal statement.

A major difficulty in attempts to elucidate the biosynthesis of the C-P bond has been inability to prepare cell-free systems capable of this synthesis, although Warren has reported success with "broken cell" preparations. An attractive hypothesis was suggested by consideration of the structural analogy of AEP and phosphonoalanine to the sulfonic acids taurine and cysteic acid. These compounds arise from cysteine, which can be synthesized in some organisms by the addition of sulfide to an intermediate arising from a serine derivative. We made an extensive effort to detect reduced forms of phosphorus in *Tetrahymena*, without success, and subsequently learned that Liang and Rosenberg (*35*) also had examined this possibility. Segal proposed a phosphoramidic rearrangement of phosphatidylethanolamine to phosphatidyl-AEP and a similar rearrangement of phosphatidylserine to phosphatidylphosphonoalanine (*36*). However, three laboratories reported that ethanolamine and serine were incorporated rapidly into phospholipids but that these substances were poor precursors of the phosphonolipids (*22, 35, 37*).

Four laboratories almost simultaneously reported success in demonstrating that phosphoenolpyruvate, or possibly oxaloacetate, is the likely precursor of AEP. Trebst and Geike, in a well-executed series of experiments, provided convincing evidence that this is the case. They added specifically labeled glucoses to cultures of *Tetrahymena*, isolated the AEP formed, and recrystallized it to constant specific activity. The AEP was then degraded, and the position of the label in the AEP was determined. The results proved that the 1-carbon of AEP can be derived from the 1-carbon (or 6-carbon) of glucose, and that the 2-carbon of AEP can be derived from the 2-carbon of glucose. They proposed a rearrangement of phosphoenolpyruvate to 2-keto-3-phosphonopropionate, transamination to phosphonoalanine, and decarboxylation to AEP. Their work was published in July 1967 (*38*). The following month at the Seventh International Congress of Biochemistry in Tokyo, Horiguchi, Kittredge, and Roberts (*37*), unaware of the publication of Trebst and Geike, presented the results of a series of experiments in which they had traced the origin of the carbon skeleton of AEP by following the incorporation of ^{14}C from a large variety of specifically labeled ^{14}C compounds into AEP in growing *Tetrahymena* and then determining the distribution of the ^{14}C by chemical degradation of the AEP isolated from hydrolysates of the cells. The results strongly supported the view that the most likely precursors among the known metabolic intermediates are phosphoenolpyruvate or oxaloacetate—compounds which are interconvertible by phosphoenolpyruvate carboxylase (E.C. 4.1.1.32)—and that the 1-carbon of AEP is derived from the 3-carbon of phosphoenolpyruvate or oxaloacetate and the 2-carbon of AEP is derived from the 2-carbon of these substances. At the same meeting Rosenberg independently presented similar work and suggested that a reaction might take place between phosphoenolpyruvate and phosphatidic acid in such a way that a glyceride ester of 3-phosphonopyruvic acid would be formed (see *35*). In addition, Kidder informally circulated a then unpublished manuscript by Warren containing results in agreement with those of Trebst and Geike, Horiguchi *et al.*, and Rosenberg. Warren also had isolated phosphonoalanine and demonstrated labeling from glucose-3,4-^{14}C, as well as from glucose-1- or glucose-2-^{14}C. He further showed in in vitro experiments with "broken cell" suspensions of *Tetrahymena* (*39*) that there was incorporation of ^{32}P into AEP from ^{32}P-labeled phosphoenolpyruvate and phosphonoalanine. He made, in somewhat more detail, the same proposal that Trebst and Geike had made—the proposal that intramolecular rearrangement to form 3-phosphonopyruvic acid might be the first step in the reaction sequence, in which both the phosphorus and carbon of phosphoenolpyruvate would appear in the AEP—and indicated that this possibility is being tested with phosphoenolpyruvate labeled with both ^{32}P and ^{14}C. Since lipid-bound AEP appears to be synthesized in *Tetrahymena* before free AEP is, it appears probable that the carbon precursor of the aminated forms is bound into phosphatidyl linkage in this organism prior to amination. The possible routes are indicated in Fig. 1. These differ in that the rearrangement of phosphoenolpyruvate to phosphonopyruvate may take place either in the free state, in cytidine monophosphate-phosphoenolpyruvate, or in phosphatidylphosphoenolpyruvate (*40*). Each of these postulated routes leads to phosphatidylphosphonopyruvate, which then may be aminated and decarboxylated to phosphatidyl-AEP. At the Congress in Tokyo we reported the detection of a lipid in *Anthopleura xanthogrammica* which yields phosphonoacetic acid on hydrolysis (*23*). This fragment could arise from degradation of phosphatidylphosphonopyruvate, or by the oxidation of phosphatidylphosphonoacetaldehyde during hydrolysis.

Metabolism and Transport of C-P Compounds

The aminophosphonic acids are nontoxic to mammals and are incorporated into tissue phosphonolipids (*25, 26*). The only catabolism of natural aminophosphonic acids by mammalian tissue that has been reported is the transamination between phosphonoalanine and α-ketoglutarate observed with mouse liver homogenate (*41*).

The mineralization of these stable compounds and return of the phosphorus to the circulating pool in nature can be accomplished by several species of bacteria. Zeleznick *et al.* (*42*) were the first to demonstrate the biological cleavage of the C-P bond. In media in which methyl- or ethylphosphonate was the sole source of phosphorus, *Escherichia coli* maintained the same generation time through nine serial transfers, and the total phosphorus of the bacterial pellets after a 24-hour incubation increased approximately tenfold. Mastalerz *et al.* (*43*) found that, of ten species of microorganisms examined, only *E. freundi* and *E. coli* could sustain growth on ethylphosphonate, while Harkness (*44*), utilizing media containing various aminophosphonic acids in place of phosphate, observed growth in nine of the ten species he studied. A comparison of the doubling time in each medium indicated that AEP was the most readily utilized phosphonic acid. Comparing only orthophosphate and AEP, Tanabe *et al.* observed growth on AEP with 24 of 62 species of microorganisms. Generally, the pseudomonads and coliform bacteria and some actinomycetes could utilize AEP, while lactic acid bacteria and yeasts could not (*45*).

In our laboratories, transport of the aminophosphonic acids analogous to glutamic acid, aspartic acid, alanine, and valine was studied in cultures of *Lactobacillus plantarum* by Holden (*46*). Uptake was dependent on the availability of glucose and occurred against a concentration gradient. Competition studies with phosphonoalanine indicated

that its transport in *L. plantarum* and *Streptococcus faecalis* was antagonized only by the structurally related amino acids—glutamic, aspartic, and cysteic acids. Kinetic studies with *S. faecalis* and a mutant strain lacking one of two dicarboxylic amino acid transport systems indicated that the aminophosphonic acids are accumulated by the amino acid transport systems in these bacteria.

Rosenberg (*47*) isolated a strain of *Bacillus cereus* which could use AEP as its sole source of phosphorus. This bacterium exhibited a break in its growth curve when grown in the presence of AEP and orthophosphate. Examination revealed that all of the orthophosphate was utilized before AEP was taken up. The control was shown to operate through a transport system induced by AEP and suppressed by orthophosphate, and kinetic experiments showed that the transport system has a high affinity for AEP (apparent K_m, 10^{-7}). A mutant of this bacterium was isolated which was constitutive for the transport system, and this mutant was utilized in studying the catabolism of AEP. The supernatant of a homogenate degraded AEP to orthophosphate. The rate of the reaction was increased by the addition of pyridoxal phosphate and an amino group acceptor, pyruvate. This rate increase and the results of studies with inhibitors were consistent with the occurrence of a transamination reaction. When radioactive AEP was the substrate, an intermediate could be detected which was suspected of being 2-phosphonoacetaldehyde (*48*). Positive identification resulted from an exchange of ideas between Canberra and College Station, Texas. The final successful synthesis by Isbell (*49*) was followed by comparison of the intermediate with the synthetic substance by Rosenberg (*50*). The synthetic phosphonoacetaldehyde also was degraded to phosphate and acetaldehyde by the cell-free preparation.

We examined, in *Escherichia coli, Tetrahymena, Anthopleura,* and mouse liver, the possibility of transaminative degradation of 18 synthetic aminophosphonic acids synthesized by Isbell. *Tetrahymena* and sea anemone homogenates readily transaminated AEP with α-ketoglutarate, while the other two tissues did not. All of the tissues transaminated γ-aminopropylphosphonic acid and phosphonoalanine. The glutamic acid analog was degraded extremely rapidly by the *E. coli* extract and slowly by *Tetrahymena* and sea anemone homogenate. Only low levels of activity were observed in the case of a few of the other synthetic aminophosphonic acids (*41*).

There appears to be some diversity in the mode of catabolism of the natural aminophosphonic acids. These acids are actively assimilated by means either of amino acid transport systems or of inducible or constitutive transport systems having a high affinity for the aminophosphonic acid. The degradation occurs through transamination, but various systems employ different acceptors. We and others (*41, 51*) had previously suspected that phosphonoacetaldehyde might be unstable and might decompose spontaneously to phosphate and acetaldehyde, but the successful synthesis of the compound and Rosenberg's recent work indicate that this last step of degradation probably is catalyzed enzymatically (*50*).

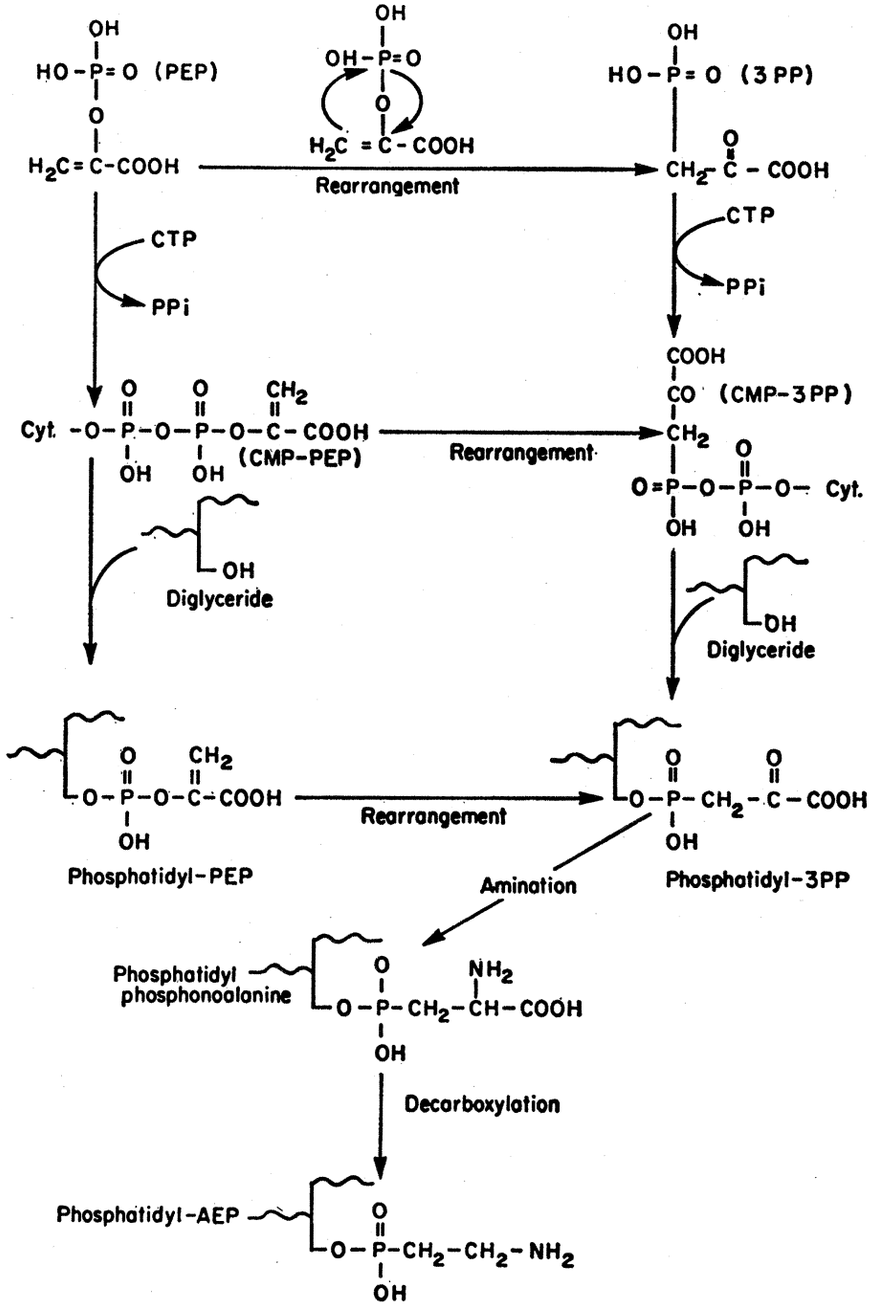


Fig. 1. Biosynthesis of 2-aminoethylphosphonic acid (AEP) from phosphoenolpyruvate (PEP) may occur through rearrangement of the PEP to 3-phosphonopyruvate (3PP). The rearrangement may take place either from PEP, from cytidine monophosphate-PEP (CMP-PEP), or from the lipid, with the activation by cytidine triphosphate (CTP), liberation of inorganic pyrophosphate (PPi), and addition to diglyceride occurring in the alternate steps shown. The phosphatidyl-3PP is then probably transaminated and decarboxylated to yield lipid AEP (*49*).

Comment

The work discussed here concerning a new chemical bond in nature, the C-P bond, originated with the observation in two laboratories of a previously unidentified spot on a chromatogram. The outlines of the biosynthetic route to the aminophosphonic acids was provided almost simultaneously by four laboratories, but the nature of the direct phosphorus precursor is still not known. The incorporation of compounds with the C-P bond into a variety of lipids has been demonstrated, and a biosynthetic route has been indicated. The existence of insoluble structural and soluble proteins containing C-P compounds seems established.

Phospholipids may be essential components of membrane structures. There may be a similar role for natural phosphonolipids. Since the C-P bond is resistant to the action of phosphatases, in some instances an important biological advantage might be conferred by the presence of phosphonolipids in membranes. In the gastrovascular cavity of anemones, into which many hydrolytic enzymes are released, cell membranes may benefit from this stability. The presence of AEP in proteolytic enzymes from sea anemones (*32*) suggests that substances with C-P bonds may provide groups which are closely similar to phosphate groups in their dissociation constants but which are more likely to be stable to the action of other enzymes in the gastrovascular cavity. Similar reasoning may be applicable in the case of surface structures of ciliates which are found in the phosphatase-rich environment of the rumen.

Since there is a ready incorporation of dietary aminophosphonic acids into lipids in mammalian organisms, determination of the sites and extent of deposition of these substances and the possible effects on membrane functions will be of considerable interest. This may be particularly important in instances in which the chief dietary sources are marine or dairy products.

It is of particular interest to marine biology that C-P compounds are major constituents in three phyla and that they are synthesized by phytoplankton, the base of the food chains in the ocean. This observation forces a reevaluation of the phosphorus cycle in the sea. Since the utilization of AEP by bacteria may be inhibited in the presence of orthophosphate (*47*), the fraction of the total phosphorus which becomes incorporated into C-P compounds may be only slowly returned to the cycle. To date, C-P compounds have been found in six species of phytoplankton (*7, 8*), in two ciliates (*6, 19*), in 25 species of coelenterates (*5, 52*), and in 33 species of mollusks (*5, 16, 17, 28*). In addition, they have been detected in nematodes, annelids, echinoderms, and crustacea (*5, 28*).

No attempt has been made here to cover the entire literature dealing with the natural occurrence of C-P compounds or with the recent use of many synthetic phosphonic acid analogs of naturally occurring substances as potential inhibitors of various cellular processes. We have discussed only those aspects of our work and of the work of others which could be woven into a meaningful pattern.

References and Notes

1. J. S. Kittredge, D. G. Simonsen, E. Roberts, B. Jelinek, in *Amino Acid Pools*, J. T. Holden, Ed. (Elsevier, Amsterdam, 1962), p. 176.
2. J. S. Kittredge, E. Roberts, D. G. Simonsen, *Biochemistry* **1**, 624 (1962).
3. M. Horiguchi and M. Kandatsu, *Nature* **184**, 901 (1959); *Bull. Agr. Chem. Soc. Japan* **24**, 565 (1960).
4. C. Liang and H. Rosenberg, *Comp. Biochem. Physiol.* **25**, 673 (1968).
5. L. D. Quin, *Science* **144**, 1133 (1964); *Biochemistry* **4**, 324 (1965).
6. M. Kandatsu and M. Horiguchi, *Agr. Biol. Chem. Tokyo* **26**, 721 (1962).
7. J. S. Kittredge, P. M. Williams, M. Horiguchi, *Comp. Biochem. Physiol.*, in press.
8. M. W. Baldwin and J. Braven, *J. Marine Biol. Ass. U.K.* **48**, 603 (1968).
9. J. R. Chambers and A. F. Isbell, *J. Org. Chem.* **29**, 832 (1964).
10. J. S. Kittredge and R. R. Hughes, *Biochemistry* **3**, 991 (1964).
11. J. S. Kittredge, A. F. Isbell, R. R. Hughes, *ibid.* **6**, 289 (1967).
12. L. D. Quin, in *Topics in Phosphorus Chemistry*, M. Grayson and E. J. Griffith, Eds. (Interscience, New York, 1967), vol. 4, p. 23; M. Horiguchi, *Tanpakushitsu-kakusan-koso* **12**, 315 (1967).
13. E. Baer and N. Z. Stanacev, *J. Biol. Chem.* **239**, 3209 (1964); ———, *ibid.* **240**, 44 (1965); ———, *J. Amer. Chem. Soc.* **87**, 679 (1965); E. Baer and G. Sarma, *Can. J. Biochem.* **43**, 1353 (1965); E. Baer and N. Z. Stanacev, *J. Biol. Chem.* **240**, 3754 (1965); ———, *Can. J. Biochem.* **44**, 893 (1966); E. Baer and K. V. J. Rao, *Lipids* **1**, 291 (1966); ———, *Can. J. Biochem.* **45**, 317 (1967); E. Baer, *Lipids* **2**, 194 (1967); ———, H. Basu, B. C. Pal, *Can. J. Biochem.* **45**, 1467 (1967); E. Baer and R. Robinson, *ibid.*, p. 1747; E. Baer and R. Sarma, *ibid.*, p. 1755; E. Baer and B. C. Pal, *ibid.*, p. 1478; E. Baer, G. R. Sarma, R. Robinson, *ibid.*, p. 1783; E. Baer and H. Basu, *ibid.* **46**, 351 (1968).
14. A. F. Rosenthal and M. Pousada, *Proc. Chem. Soc.* **1964**, 358 (1964); see also *Rec. Trav. Chim.* **84**, 833 (1965); A. F. Rosenthal, G. M. Kosolapoff, R. P. Geyer, *ibid.* **83**, 1273 (1965); A. F. Rosenthal, *J. Lipid Res.* **7**, 779 (1966).
15. G. Rouser, G. Kritchevsky, D. Heller, E. Lieber, *J. Amer. Oil Chem. Soc.* **40**, 425 (1963).
16. G. Simon and G. Rouser, *Lipids* **2**, 55 (1967).
17. T. Hori, O. Itasaka, T. Hoshimoto, H. Inoue, *J. Biochem. Tokyo* **55**, 545 (1964); T. Hori, O. Itasaka, H. Inoue, K. Yamada, *ibid.* **56**, 477 (1964); T. Hori, O. Itasaka, H. Inoue, *ibid.* **59**, 570 (1966); T. Hori, I. Arakawa, M. Sugita, *ibid.* **62**, 67 (1967); S. Higashi and T. Hori, *Biochim. Biophys. Acta* **152**, 568 (1968).
18. A. J. de Koning, *Nature* **210**, 113 (1966); *J. Sci. Food Agr.* **17**, 460 (1966).
19. H. E. Carter and R. G. Gaver, *Biochem. Biophys. Res. Commun.* **29**, 886 (1968).
20. R. M. C. Dawson and P. Kemp, *Biochem. J.* **105**, 837 (1967).
21. C. Liang and H. Rosenberg, *Biochim. Biophys. Acta* **125**, 548 (1966).
22. G. A. Thompson, Jr., *Biochemistry* **6**, 2015 (1967).

22a. G. H. Chacko and D. J. Hanahan, *Biochim. Biophys. Acta* **176**, 190 (1969).

23. J. S. Kittredge, in "Intern. Congr. Biochem., 7th, Tokyo, Colloq." (1967), p. 453.
24. A. Hayashi, F. Matsuura, T. Matsubara, *Biochim. Biophys. Acta* **176**, 208 (1969).
25. M. Kandatsu and M. Horiguchi, *Agr. Biol. Chem. Tokyo* **29**, 779 (1965).
26. ———, *ibid.*, p. 781.
27. R. G. Bridges and J. Ricketts, *Nature* **211**, 199 (1966); L. L. Bieber, *Biochim. Biophys. Acta* **152**, 778 (1968).
28. H. Shimizu, Y. Kakimoto, T. Nakajima, A. Kanazawa, I. Sano, *Nature* **207**, 1197 (1965).
29. T. Hori, O. Itasaka, I. Arakawa, in "Intern. Congr. Biochem., 7th, Tokyo" (1967), p. 834; M. Kandatsu, M. Horiguchi, M. Tamari, in "Intern. Congr. Biochem., 7th, Tokyo, Colloq." (1967), p. 449.
30. H. Rosenberg, *Nature* **203**, 299 (1964).
31. F. A. Shelburne and L. D. Quin, *Biochim. Biophys. Acta* **148**, 597 (1967).
32. K. J. Stevenson, D. Gibson, G. H. Dixon, personal communication.
33. M. Horiguchi, in preparation.
34. S. C. Chou and O. H. Scherbaum, *Exp. Cell Res.* **45**, 31 (1966).
35. C. Liang and H. Rosenberg, *Biochim. Biophys. Acta* **156**, 437 (1968).
36. W. Segal, *Nature* **208**, 1284 (1965).
37. H. Horiguchi, J. S. Kittredge, E. Roberts, *Biochim. Biophys. Acta* **165**, 164 (1968).
38. A. Trebst and F. Geike, *Z. Naturforsch.* **22b**, 989 (1967).
39. W. A. Warren, *Biochim. Biophys. Acta* **156**, 340 (1968).
40. This summary is the synthesis of the rearrangement suggested by Trebst and Geike (*38*) and Warren (*39*) with data of Liang and Rosenberg (*35*) and our data. Rosenberg, in a personal communication, first proposed the alternative rearrangements.
41. E. Roberts, D. G. Simonsen, M. Horiguchi, J. S. Kittredge, *Science* **159**, 886 (1968).
42. L. D. Zeleznick, T. C. Myers, E. B. Titchener, *Biochim. Biophys. Acta* **78**, 564 (1963).
43. P. Mastalerz, Z. Wieczorek, M. Kochman, *Acta Biochim. Polon.* **12**, 151 (1965).
44. R. D. Harkness, *J. Bacteriol.* **92**, 623 (1966).
45. I. Tanabe, T. Misono, S. Schichiji, M. Kandatsu, *Agr. Biol. Chem. Tokyo*, in press.
46. J. T. Holden, J. N. A. Van Balgooy, J. S. Kittredge, *J. Bacteriol.* **96**, 950 (1968).
47. H. Rosenberg and J. M. La Nauze, *Biochim. Biophys. Acta* **141**, 79 (1967).
48. J. M. La Nauze and H. Rosenberg, *ibid.* **148**, 811 (1967).
49. A. F. Isbell, L. F. Englert, H. Rosenberg, *J. Org. Chem.*, in press.
50. J. M. La Nauze and H. Rosenberg, *Biochim. Biophys. Acta* **165**, 438 (1968).
51. A. J. de Koning, *ibid.* **130**, 521 (1966).
52. J. S. Kittredge, thesis, University of California (1964).

53. This work was supported by grants from the Office of Naval Research (Task No. NR 108-458) and the National Institutes of Health (NIH C-2568).