Inorganic Polyphosphates in Biology: Structure, Metabolism, and Function

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INTRODUCTION

Twenty years ago, Jeener and Brachet (58) observed that addition of inorganic phosphate (Pi) to a suspension of yeast cells previously subjected to phosphate starvation induced massive accumulation of a basophilic substance within the cells. At the time this was thought to be ribonucleic acid (RNA), but soon afterwards Wiame (145–147) and Schmidt et al. (116) isolated the substance and identified it as inorganic polyphosphate (polyP).

This was by no means the first isolation of polyP from microorganisms. As early as 1888,

Liebermann had obtained "metaphosphate" from yeast, and further reports on this substance appeared sporadically during the next fifty years. The significance of Wiame's work lay rather in the demonstration that the cytological entities which had long been known as "metachromatic" or "volutin" granules correspond to deposits of polyP (146). These studies therefore mark the fusion of the chemical and cytological approaches, and inaugurate the modern phase of biological research on inorganic polyphosphate.

During the following decade, numerous investigators described the occurrence of polyP in a

variety of microorganisms and its accumulation under nutritional conditions unfavorable to growth ("nutrient imbalance"; 122). Structurally, the substance was found to be a polymer of orthophosphate, with phosphoanhydride linkages thermodynamically equivalent to the "energy-rich" phosphate of adenosine triphosphate (ATP) (155). With the demonstration by Yoshida and Yamataka (156) and by Kornberg et al.(68, 69) of an enzyme which catalyzes the reversible transfer of phosphate between polyP and the terminal group of ATP, the ground was prepared for the formulation of a comprehensive hypothesis to explain both the function of polyP and its accumulation under conditions unfavorable to growth. It was proposed (50–52, 69) that polyP serves as a microbial phosphagen, analogous to creatine phosphate of mammalian muscle, and that its accumulation results from ATP production in excess of cellular energy demands for work and biosynthesis. Evidence accumulated in recent years has cast doubt upon this hypothesis, and a reassessment of the entire field appears timely.

The present survey will be restricted to inorganic condensed phosphates excluding pyrophosphate. Current concepts of the metabolism and function of pyrophosphate have been discussed by Hoffmann-Ostenhof (50, 51) and by Kornberg (67). Aspects of the biology of inorganic polyphosphate have been reviewed periodically (54, 73, 115, 148, 150), recently, and with admirable thoroughness by Kuhl (71). Therefore, the present essay aims at comprehensive coverage of only the most recent literature. My purpose is rather to provide a critical appraisal of the present state of knowledge in this field, to attempt a synthesis of the available information, and to point out the numerous gaps that remain.

CHEMISTRY OF INORGANIC CONDENSED PHOSPHATES

The history of chemical studies on condensed phosphates goes back to the work of Graham in the early 19th century, but only in recent years have their structures been established, largely due to the pioneering work of van Wazer, Thilo, Ebel, and their associates. The chemistry of these compounds is sufficiently unfamiliar to require a few comments; details will be found in recent reviews (136, 137, 142). Condensed inorganic phosphates, the general term applied to all "pentavalent phosphorus compounds in which various numbers of tetrahedral PO_4 groups are linked together by oxygen bridges" (136), fall into three classes (Fig. 1).

FIG. 1. *Structures of some condensed inorganic phosphates.*

Cyclic Condensed Phosphates

These conform to the general elementary formula $M_nP_nO_{3n}$ and are properly designated metaphosphates. Only tri- and tetrametaphosphate are well known as individual compounds, and occur in certain melts. The free metaphosphoric acids have a single strong acid function per P atom. Strong alkali converts metaphosphates quantitatively to the corresponding linear polyphosphate, and they are hydrolyzed to orthophosphate (Pi) when heated in acid.

Linear Condensed Phosphates

Unbranched structures with the elementary composition $M_{n+2}P_nO_{3n+1}$ are called polyphosphates (polyP). (The term polymetaphosphate is both ambiguous and obsolete, but regrettably is still occasionally encountered in the biological literature.) The series ranges from pyrophosphate with chain length 2 to the insoluble, crystalline Kurrol's and Maddrell's salts of chain length around 10^4. Only the lowest homologues are known as individual compounds. All other polyphosphates, including "hexametaphosphate" and Graham's salt, must be regarded as mixtures containing various proportions of species of different molecular size. The average chain length is most commonly estimated by titration of the secondary acid function (pK about 7) associated with the end groups.

Analytically, polyP is stable to alkali (though the presence of divalent cations catalyzes degradation), but labile to acid. Typically, complete hydrolysis to Pi by 1 N acid requires about 15 min at 100 C. The degradation of polyP has been studied by Thilo (136, 137), who distinguishes three reactions. PolyP is split by hydrolytic removal of the terminal links; at very low pH, chain scission also occurs. Of particular interest is the degradation to trimetaphosphate, which is catalyzed by cations in general, particularly Mg^{+2}. Salts of polyP and long-chain organic amines are soluble in organic solvents; upon

heating, the polyP is degraded by ligand exchange to give trimetaphosphate in yield up to 95% (136). This reaction may be useful as a criterion for the identification of polyP (138).

Cross-Linked Condensed Phosphates

Cross-linked condensed phosphates, or "ultraphosphates," occur in certain melts. Their characteristic feature is the presence of branching points, phosphate groups in which three oxygen atoms are shared with neighboring phosphate groups. Branching points are readily hydrolyzed in water and therefore the biological occurrence of ultraphosphates would not be expected.

ANALYTICAL METHODS

Chemical Estimation of Polyphosphate

In the last analysis, all the methods in current use depend upon the hydrolysis of polyP to Pi with strong acid. Since this treatment will release some Pi even from compounds normally considered acid-stable, such as nucleic acids, the crux of the matter is the separation of polyP from other compounds before hydrolysis. By and large, the methods are derived from the general fractionation schemes of Schneider and of Schmidt and Thannhauser, and yield polyphosphates in two fractions: "soluble" polyP, which is extractable with cold 5% trichloroacetic or perchloric acid; and "insoluble" polyP, which is recovered in the nucleic acid fraction.

The polyP in the cold acid extract may be precipitated as the Ba^{+2} salt at pH 4.5, although the reaction is not quantitative for small amounts. Alternatively, nucleotides can be removed by adsorption to charcoal; the acid-labile phosphate in the supernatant fluid is often used as a measure of the polyP content, but will include other non-nucleotide, acid-labile phosphate esters. To measure the acid-insoluble polyP, it must be put into solution and separated from nucleic acids. Extraction of the nucleic acids with hot trichloroacetic or perchloric acid will solubilize the polyP but simultaneously degrade it considerably. Nucleic acids are removed by adsorption to charcoal; polyP remains in the supernatant fluid and is measured as Pi after complete hydrolysis. If necessary, a correction can be applied for Pi liberated during extraction of the nucleic acids. In the Schmidt-Thannhauser procedure, the polyP, together with the nucleic acids, is solubilized by 1 N KOH at 35 C. Upon acidification, the polyP may be partly precipitated, but a considerable fraction remains soluble. Once again, polyP and nucleic acids are separated with charcoal. Several authors have presented full descriptions of their methods (2, 35, 38, 60, 63, 74, 75, 79, 81), and Ebel has studied in detail the separation of polyphosphate from nucleic acids by charcoal and other means (17, 25, 26, 98, 143).

Methods for the isolation of polyphosphates from living cells have usually been based upon these standard fractionation procedures, followed by precipitation of polyP as the barium salt. Most investigators have been content to differentiate simply between "acid-soluble" and "acid-insoluble" polyP. However, Liss and Langen (75, 79) successively extracted from yeast four polyP fractions of increasing molecular weight (Table 1). The polyP of highest molecular weight was solubilized with sodium dodecyl sulfate, and was isolated by precipitation with sodium chloride. The standard precipitation of polyP as the barium salt is undesirable, as divalent cations catalyze degradation of polyP (79, 136, 137). In a somewhat similar approach, Harold (39) digested bacterial cells with sodium hypochlorite (151). PolyP of high molecular weight is insoluble in the hypochlorite, and was subsequently extracted from the residue with water.

Chromatography

Polyphosphates up to a chain length of about 15 can be separated by anion exchange chromatography on columns (85), but the method is of limited use for longer chains. Paper chroma-

TABLE 1. *Polyphosphate fractions of yeast*[a]

Fraction no.	Extraction with	Amt of P$_2$O$_5$ per g (dry wt) of yeast	Per cent of total yeast phosphate	Avg chain length	Mol wt (as K$^+$ salt)
		mg			
1	Trichloroacetic acid	2.5	6	4	530
2	NaClO$_4$	8.0	21	20	2,400
3	pH 10	1.5	4	55	6,500
4	0.05 N NaOH or sodium dodecyl sulfate	2.5	6	260	30,700

[a] After Langen et al. (75).

tography is the method of choice for the detection of metaphosphates and of polyP up to a chain length of about 10 (reviewed by Hettler, 49). Very recently, Ohashi and van Wazer (102) modified the original solvents of Ebel to permit resolution of a mixture of polyphosphates into broad classes according to average chain length. This affords a convenient method for the rough delineation of the chain-length profile of polyphosphate preparations, and has already been applied to the polyP fraction of mammalian nuclei (29).

Cytological Identification

The cytochemical detection of polyP as "metachromatic granules" depends upon the reaction of polyP with certain basic dyes, which induces a characteristic shift in the absorption spectrum. Under optimal conditions (134), the intensity of the spectral shift is a function of the chain length, as it is apparently produced by the interaction of closely neighboring dye molecules (7). The metachromatic reaction can thus be used as a rough index of chain length, but its application is hazardous because the intensity of the shift is affected by a number of factors which are not well understood (7, 11, 134).

For cytological purposes, the most widely used procedure is that of Laybourn (76). However, it should be recognized that metachromatic staining is rather nonspecific and is therefore not a sufficient criterion for the identification of polyP. Ebel and his associates (23) have described a procedure in which polyP is precipitated in situ with lead, which is subsequently converted to black lead sulfide, and a summary of methods available for the differential staining of polyP has been provided by Widra (149). The appearance of polyP granules in electron micrographs is also diagnostic: the granules are extremely opaque and volatilize at high electron intensity leaving characteristic holes (20, 22; *but see* 140).

POLYPHOSPHATES OF BIOLOGICAL ORIGIN

Microorganisms

PolyP is widely distributed among the bacteria, blue-green algae, fungi, protozoa, and algae. A list of species in which polyP has been detected is given by Kuhl (71). To this should be added *Nitrobacter* (8), *Micrococcus denitrificans* (61); *Staphylococcus aureus* (53); *Chlamydomonas* (10); *Mucor racemosus* (57), and *Claviceps purpurea* (141). However, it remains to be seen whether polyP is a universal constituent of microbial cells. For instance, a cursory search

in this laboratory failed to find it in *Streptococcus faecalis*.

The polyphosphates obtained from microorganisms cover a wide range of molecular size. In yeast and other fungi, polyP up to a chain length of 10 have been detected on paper chromatograms of the acid-extractable fraction (75, 81, 97), together with material of higher chain length (35, 65, 75, 81, 97). Metaphosphates also occur in this fraction, but their origin is not entirely clear. As noted above, metaphosphates can readily arise from longer chains by degradation. Dirheimer and Ebel (14), in a careful study, found that the trimetaphosphate content of yeast varied with the extraction procedure employed, suggesting that it is an analytical artifact. It is also conceivable that some trimetaphosphate arises by nonenzymatic degradation of polyP in vivo. The enzyme described by Rafter (108), which catalyzes the phosphorolysis of trimetaphosphate to tetrapolyphosphate, might then serve as a scavenging mechanism.

The bulk of the polyphosphate content is not readily extracted with dilute acid, and behaves like inorganic polyphosphate of high molecular weight. The chain length can be considerable: fractions from yeast were found to have a chain length of 300 by titration (79), whereas polyP of *A. aerogenes* had a chain length of about 500 by both end-group titration and viscometry (39). Fractions of lower average chain length, in the region of 50 to 100, have been obtained from yeast and characterized by titration (75, 79) and by diffusion rates (65). Light-scattering has apparently not been applied to polyphosphates of biological origin.

It should be pointed out that, although a large body of evidence is consistent with the prevailing view that the structure of biological polyP is the same as that of the synthetic material (Fig. 1), rigorous proof of this proposition has never been presented. Indeed, very recently Correll (11a, 11b) proposed that at least part of the "polyphosphate" fraction of *Chlorella* contains both phosphoanhydride and imidodiphosphate linkages. The experimental evidence is principally the presence of an absorption band at $1,400$ cm^{-1} in the infrared spectrum of *Chlorella* "polyP," and the finding of large amounts of ammonium nitrogen after digestion with sulfuric acid (P-N ratio, 1:2). Unfortunately the published report is sketchy, particularly on criteria of purity of the fractions analyzed, and leaves me unconvinced that the results require so radical a revision of the structure accepted by the majority of investigators. Confirmation of Correll's claims would be highly desirable.

Higher Plants and Animals

Until recently, polyP was regarded as a uniquely microbial constituent, and repeated attempts to detect it in higher organisms were unsuccessful. In the past few years, however, convincing claims have been presented for the occurrence of polyP in at least some higher plants. In the case of spinach (90) and the dodder, *Cuscuta reflexa* (135), the substance was isolated by Ba^{+2} precipitation and identified by its acid lability, metachromasy, and failure to move on paper chromatograms, and was clearly differentiated from phytin. The amounts found were minute, only 2% of the total tissue phosphorus. Vagabov and Kulaev (138) detected polyP in corn roots and identified it by conversion to trimetaphosphate.

The wax moth *Galleria*, which excretes polyP of low molecular weight (99), was until recently unique in the animal world. However, in 1962, Grossman and Lang (31) reported traces of short-chain polyP in animal nuclei. During the past year, Penniall and associates (29, 30, 105) have adduced impressive evidence for the formation of polyP of high molecular weight by isolated rat liver nuclei. The criteria for identification were barium precipitation (in the presence of carrier polyP), metachromasy, rate of acid hydrolysis, and paper chromatography. The latter procedure was used to estimate chain length, which was found to range up to 500. Here again, polyP is a minor constituent: there are only 1 to 2 μg of polyP per gram of tissue, all of which may be localized in the nucleus.

In a preliminary report, Lynn and Brown (83) described the formation of polyP by mitochondria. Identification was based upon barium precipitation and both paper and anion exchange chromatography, but appears to me to fall short of complete proof. According to a personal communication from W. S. Lynn, the authors have encountered difficulties in reproducing their original results, and there is a possibility that they were misled by impurities in the Pi^{32} used. PolyP may also occur in developing frog embryos (120), but here again the chemical identification is not yet complete. The case for the occurrence of polyP in higher plants and animals would be materially strengthened by the demonstration of appropriate biosynthetic enzymes.

INTRACELLULAR STATE OF POLYPHOSPHATES

Complexes of Polyphosphate with Protein and RNA

The concept that, in the living cell, polyP occurs as a complex with proteins or nucleic acids has a long history, arising originally from the finding that much of the cellular polyP is not readily extracted with dilute acid. It soon became apparent that the "insoluble" polyP has a considerably higher chain length than does the acid-soluble fraction (65, 66, 74, 75, 79), and the suggestion arose that the former constitutes a bound form of polyP.

Pure polyP is acid-soluble, regardless of chain length. However, polyphosphates are strong polyanions and bear negative charges even at acid pH. They will therefore combine with, and precipitate, positively charged macromolecules, such as proteins. This coprecipitation depends upon the molecular weights of both polyP and protein, and under standard conditions will tend to fractionate polyP by chain length (66). Compounds other than protein may serve as acceptors: in *Neurospora crassa*, for instance, a polymer of galactosamine which occurs in the cell wall binds polyP tightly even at neutral pH (36). The amounts of "soluble" and "insoluble" polyphosphate will therefore depend also upon the availability of basic receptor. The latter may become limiting in cases where polyphosphate accumulates, resulting in accumulation of "soluble" polyphosphate of considerable chain length (36).

Thus, the formation of acid-insoluble polyP is probably an analytical artifact. Nevertheless, the possibility that, in the living cell, part of the polyP is covalently bound to protein cannot be completely excluded. Liss and Langen (79) found that extraction of polyP of high molecular weight (their fraction 4, chain length about 300; *see* Table 1) from yeast by dilute alkali was a temperature-sensitive, first-order reaction. It is conceivable that the rate-limiting step is the cleavage of a covalent polyP-protein bond, but it may also be the scission of the polyP chain itself, catalyzed by divalent metal ions.

The existence of a polyP-RNA complex in vivo has been championed particularly by Kulaev and Belozerskii (72), who isolated the complex from *Aspergillus niger* and showed that it remained homogenous upon electrophoresis. Complex formation also occurred in artificial mixtures of polyP and RNA, but these could be readily resolved by electrophoresis. It is noteworthy that the synthetic polyP employed in the artificial mixtures had a chain length of the order of 50, whereas that of *A. niger* may resemble yeast polyP which attains a chain length around 300 (79). The natural occurrence of a polyP-RNA complex has also been invoked to account for the chromatographic behavior of algal extracts (10, 11).

The nature of both natural and artificial polyP-RNA complexes has recently been reinvestigated very thoroughly by Ebel and his associates (19,

24, 26, 125). They confirmed the observation that natural complexes of polyphosphate and RNA, obtained from yeast, could not be separated by electrophoresis or by precipitation with bases (19, 26), but showed that they were completely dissociated by charcoal (19, 26, 98). Covalent bonds are thus not involved. Complex formation is apparently due to ionic interaction between the phosphate groups and the nucleic acid bases, possibly with Mg^{+2} serving as a bridge. The tightness of the complex depends upon both chain lengths (125). On balance, the authors favor the view that the RNA-polyP complexes are formed during extraction and do not occur in vivo, but they do not regard the latter alternative as having been rigorously eliminated.

Volutin Granules

Intracellular granules that stain metachromatically with basic dyes were the first subcellular entities to be recognized in bacteria, and have been variously referred to as volutin, metachromatic, or Babes-Ernst granules. They have been identified with nuclei or sites of oxidoreduction, but are now generally regarded as distinct entities (22, 71, 149, 150). Volutin granules are certainly not fixation artifacts, since they can be seen in living bacteria by phase-contrast microscopy (149, 150).

It is generally agreed that the volutin granules contain polyP. This conclusion derives primarily from the correlation of the polyP content of microbial cells with the size and number of the volutin granules (21, 22, 145–148, 150). Moreover, mutants blocked in polyP biosynthesis also lack the granules (46). The consensus has recently been challenged by Martinez (84), who claimed that the metachromatic granules of *Spirillum volutans* are composed of poly-β-hydroxybutyric acid rather than of polyP. Unfortunately, his paper contains few details of the cytological procedures employed. It seems quite possible that not all metachromatic inclusions of microbial cells need be composed of polyP, but there *is* overwhelming evidence that polyP is deposited in granules that exhibit metachromatic staining.

Although it is well established that the cellular polyP of bacteria is found in metachromatic granules, it is not at all clear what other components are present. Widra (149) has recently summarized the available observations, and has drawn a complex picture of the volutin granule composed of polyphosphate, RNA, lipid, protein, and Mg^{+2}. Unfortunately, bacteria are simply too small for adequate resolution of anatomical features by light microscopy. Under the electron microscope, bacterial polyP granules appear as opaque bodies devoid of internal structure or limiting membrane; they may volatilize under high electron density (20, 22, 140). Photomicrographs published in recent years have shown the polyP granules in association with a number of subcellular entities. Drews (20) first suggested that polyP granules in mycobacteria appear to be deposited onto a protein matrix. In mycobacteria grown on media containing oleate, the polyP granules appear to be enclosed within what are probably lipid deposits (113). Voelz and his associates (140) have recently studied the deposition of polyP granules in *Myxococcus xanthus* under a variety of nutritional conditions. PolyP granules were found to be associated with glycogen inclusions, dispersed in the cytoplasm or embedded within the nucleoid (Fig. 2). The time-course of granule formation strongly suggested progressive precipitation of the polyP onto strands of cytoplasmic material.

In larger cells, at least some of the polyP is localized in the vacuole, presumably in solution. This is clearly shown in photomicrographs of yeast (6) and of *Chlamydomonas* (110).

To settle the composition of polyphosphate granules it is evidently necessary to isolate them by physical means. An unsuccessful attempt to do so in *Neurospora crassa* (44) led to recovery of the polyP bound to the cell wall fraction, and the results suggested that, in vivo, polyP was essentially free in solution. A similar conclusion was reached with regard to the high-molecular weight polyP of *Aerobacter aerogenes*, leading to the suggestion that volutin granules might result simply from the precipitation of polyP owing to the high ionic strength of the cytoplasm (39).

The only report of a successful isolation of polyP granules appears to be that of Hase et al. (47), who employed differential centrifugation of *Chlorella* homogenates in nonaqueous solvents. A low yield of granules was obtained, composed mostly of polyphosphate with traces of protein, deoxyribonucleic acid (DNA), and RNA (the polyP was acid-soluble, which is not surprising because of the virtual absence of protein). These findings are quite consistent with the hypothesis that volutin granules are formed by the precipitation of polyP at high ionic strength, but more extensive application of nonaqueous cell fractionation will be required before the composition of polyP granules is established.

ENZYMES OF POLYPHOSPHATE METABOLISM

Biosynthesis

Polyphosphate kinase. There appears to be only a single pathway for the biosynthesis of long-chain polyP. This is the reaction catalyzed by
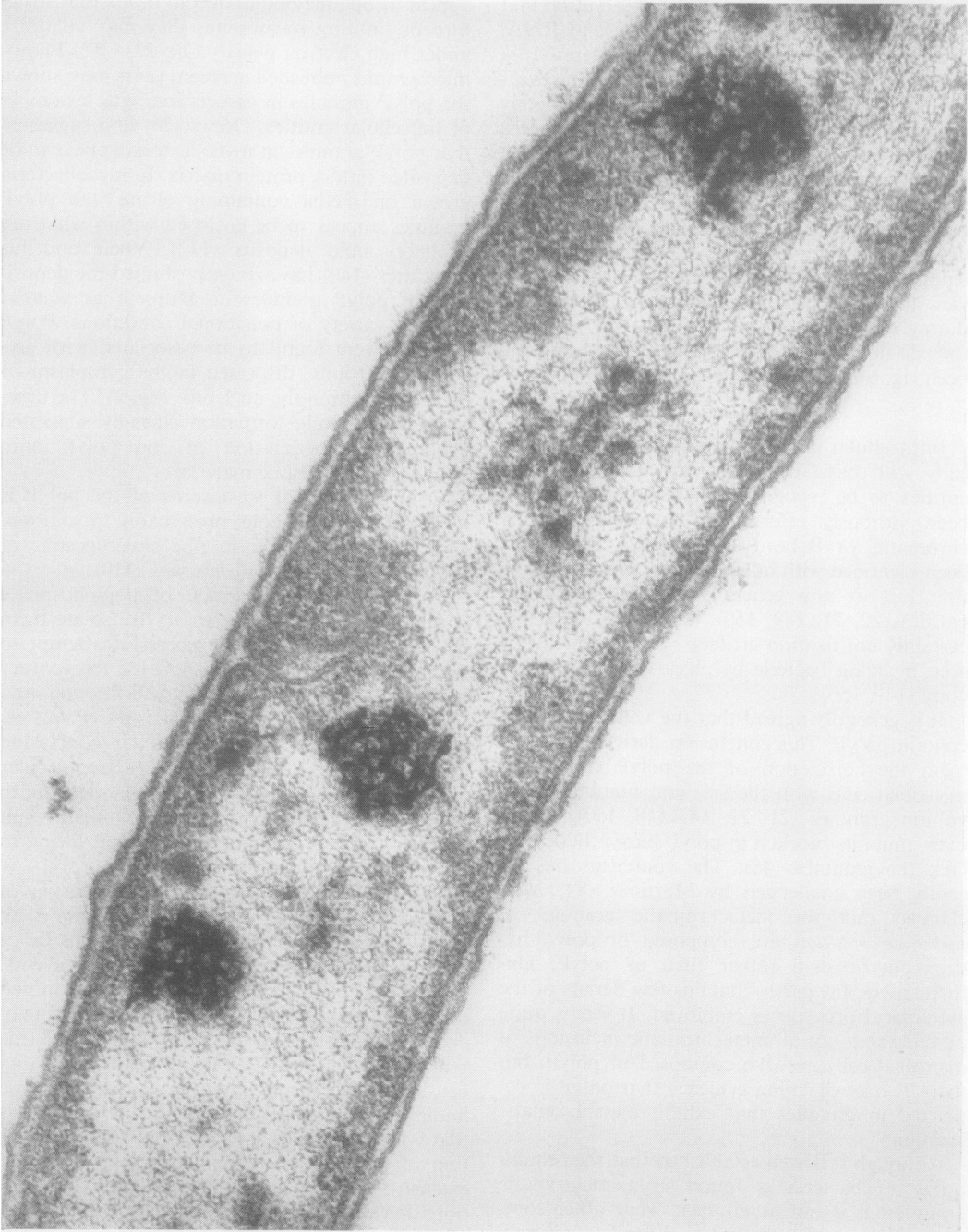
FIG. 2. *Polyphosphate granules in Myxococcus xanthus. Electronmicrograph by H. G. Voelz (unpublished data). Magnification,* × 110,000.

polyphosphate kinase, in which the terminal phosphoryl group of ATP is transferred to polyP, according to reaction (a): ATP + (Pi)n ⇌ ADP + (Pi)n + 1.

Polyphosphate kinase was first detected in yeast (156) and subsequently purified extensively from *Escherichia coli* (68). The purified enzyme required Mg^{+2} and was strongly inhibited by

fluoride. The stoichiometry of the reaction is described by equation (a), and the product was identified as polyphosphate of high molecular weight. Only the terminal phosphoryl group was incorporated; the second group, as well as the adenosine moiety, did not participate. The preparation did catalyze the incorporation of pyrophosphate into polyP, but pyrophosphate was not an obligatory intermediate in polyP biosynthesis. No evidence was obtained for the formation of short-chain intermediates, and it was proposed that the phosphoryl group is attached to a pre-existing primer molecule.

The biosynthesis of polyP was strongly inhibited by adenosine diphosphate (ADP). This was subsequently (69) shown to be due to reversal of the reaction, i.e., the phosphorylation of ADP by polyP. In fact, the K_m for ADP was found to be 4.7×10^{-5} M, and that for polyP, 2.6×10^{-5} M; whereas the K_m for ATP was much higher, 1.4×10^{-3} M. This observation suggested that, in vivo, reaction (a) would be readily reversed at low ATP-ADP ratios.

More recently, Muhammed (95) purified polyP kinase from *Corynebacterium xerosis*. Once again, no short-chain intermediates were formed, nor were there measurable amounts of polyphosphate primer in the preparation. Curiously, the enzyme from *C. xerosis* was not readily reversible (16, 95). The reason for this is not entirely clear, particularly as polyP kinase from the related *C. diphtheriae* is reversible (69). It may be relevant that Kornberg (69) found that different preparations of polyP varied in their suitability as a substrate for this reaction.

PolyP kinase is widely distributed among microorganisms. In *Aerobacter aerogenes*, it was possible to show by means of mutants (40, 46) that this is the only pathway for polyP biosynthesis. In addition, the enzyme occurs in yeast (51, 156), *Aspergillus niger* (100), *Mycobacterium smegmatis* (152), and probably in *Chlorobium* (9, 55) and *Clostridium* (129); none of these enzymes has been purified. No information is available concerning polyP biosynthesis in higher plants and animals. It should also be pointed out that in no case is the mechanism of polyP synthesis understood in detail; participation of a primer seems likely but has certainly not been established.

As will be discussed below, short-chain polyP arise primarily by degradation of large molecules. Tripolyphosphate can also arise by other reactions (70, 77, 93), whose contribution to the total tripolyP pool cannot be assessed. Enzymatic formation of the cyclic metaphosphates has apparently not been described.

Degradation

A considerable number of enzymes have been found to catalyze the degradation of polyphosphates, raising serious problems as to the contribution of each to the total rate of polyP breakdown in the intact cell.

Polyphosphate kinase. Although reaction (a) is probably the biosynthetic route, the enzyme is generally reversible and may thus participate in polyP degradation as well. Indeed, the high affinity of the *E. coli* enzyme for ADP suggested (69) that the polyP level would be directly controlled by the cellular ATP-ADP ratio.

Polyphosphate-adenosine monophosphate (AMP)-phosphotransferase. Winder and Denneny (153) first reported the transfer of phosphate from polyP to AMP in crude extracts of *M. smegmatis*, and this finding was confirmed by Szymona (130). The enzyme was purified 60-fold by Dirheimer and Ebel (18) from *C. xerosis*, and was shown to be distinct from polyphosphate kinase, polyP glucokinase, and adenylate kinase. The enzyme required Mg^{+2} and was specific for AMP and polyP of fairly high molecular weight. It is curious that the K_m for AMP was very high, 2×10^{-2} M, which calls into question the physiological role of this enzyme. As yet, the reaction [equation (b)] has only been studied in the forward direction: $(Pi)n + AMP \rightarrow ADP + (Pi)n - 1$.

Polyphosphate glucokinase. Szymona (130, 132) discovered an enzyme in *M. phlei* which catalyzes the phosphorylation of glucose by polyP according to reaction (c): $(Pi)n + glucose \rightarrow glucose$-6-phosphate $+ (Pi)n - 1$. The enzyme was found in many mycobacteria and in *C. diphtheriae*, but was not detected in *A. niger*, *E. coli*, and *A. aerogenes* (132). It phosphorylated glucosamine as well as glucose, but not mannose or fructose. Szymona and Ostrowski (131) have recently purified the enzyme somewhat, and attempted to separate it from the ATP-specific glucokinase which was also present in the extracts. Although they were not entirely successful, the evidence strongly suggests that distinct enzymes are involved and that phosphorylation of glucose by polyP occurs directly, and does not involve the prior formation of ATP via polyP kinase. PolyP glucokinase required Mg^{+2} and a high concentration of neutral salts (e.g., 0.3 M KCl) for optimal activity; the K_m for polyP was 1.75×10^{-4} M.

A very similar enzyme was purified 10-fold by Dirheimer and Ebel (15) from *C. xerosis* extracts. Again, the enzyme phosphorylated both glucose and glucosamine but was inactive towards other hexoses, pentoses, and nucleosides.

Metaphosphates and short-chain condensed phosphates were inactive as phosphoryl donors, but the enzyme had a high affinity for polyP (K_m = 0.7×10^{-4} M). Clear evidence was presented to show that the phosphorylation occurs directly and does not involve ATP as an intermediate.

Polyphosphate fructokinase. Extracts of *M. phlei* grown on fructose can utilize both ATP and polyP for the phosphorylation of fructose to fructose-6-phosphate (133). Both fructokinase activities were absent from extracts of glucose-grown organisms. By filtration through Sephadex-G-100, the fructokinase activities were clearly separated from the glucokinases discussed above, but attempts to separate ATP-dependent fructokinase from polyP-dependent enzyme resulted in loss of the latter activity. The evidence presented is consistent with the author's conclusion that *M. phlei* has two distinct inducible fructokinases, dependent upon ATP and polyP respectively (133), but falls short of rigorous proof of the existence of a specific polyP-fructokinase.

Polyphosphatases. Enzymes catalyzing the hydrolysis of polyP to Pi have been detected in various biological materials, including some which apparently do not contain polyP. Enzymes of yeast specific for short-chain polyphosphates and metaphosphates were investigated in some detail by Mattenheimer (86–88), who presented evidence that distinct enzymes catalyze the hydrolysis of pyrophosphate, tripolyphosphate, tetrapolyphosphate, and metaphosphates. Enzymes of this sort also occur in bacteria and in animal nuclei (31) but have been little studied.

The hydrolysis of polyP of high molecular weight has, on the other hand, been investigated primarily in the bacteria. Muhammed et al. (96) purified the polyphosphatase of *C. xerosis* some 100-fold. The enzyme rapidly attacked Graham's salt (with a K_m of 7.7×10^{-4} M), but was inactive towards pyrophosphate, metaphosphate, and ATP. No short-chain intermediates were detected during the hydrolysis. Curiously, this enzyme was inhibited by Mg^{+2}. A similar enzyme in *A. aerogenes* (42) required both Mg^{+2} and a high salt concentration (about 0.15 M). It was somewhat purified and separated from alkaline phosphatase and tripolyphosphatase. Once again, intermediates of short-chain length were not detected, suggesting that these enzymes attack the polyP molecule from the end groups.

Many years ago, Malmgren and his associates studied an enzyme from *A. niger* which apparently cleaves the polyP chains to smaller fragments but does not liberate Pi (for reviews, *see* 54, 115, 148, 150). PolyP depolymerase may also be present in *A. niger* spores (100), but apart from

this there appears to have been no recent work on this interesting enzyme.

ACCUMULATION AND UTILIZATION OF POLYPHOSPHATE

The most striking aspect of polyP metabolism is the variability of the polyP content. In phosphate-starved cells, polyP cannot usually be detected; at the other extreme, it may accumulate to the point where polyP is the most abundant cellular phosphorus compound, i.e., yeast may accumulate up to 20% of its dry weight as polyP (80). It has long been recognized that the polyP content is low during rapid growth and increases under conditions of nutritional imbalance unfavorable for growth, and an antagonistic relationship between polyP and nucleic acid metabolism was postulated (94, 122, 150). The purpose of the present section is not so much to describe the available information, but rather to attempt its interpretation in terms of regulatory mechanisms. I shall therefore stress those microorganisms which have been investigated systematically enough to make this attempt profitable. Virtually nothing is as yet known concerning polyP metabolism in higher plants and animals.

Aerobacter aerogenes

Early studies by Wilkinson, Duguid, and their associates (122, 150) provided the basic data on variation of the polyP content as a function of growth phase and nutritional conditions. It was demonstrated that little or no polyP is present during exponential growth, but the material accumulated when growth was inhibited by low pH of the culture fluid, sulfur starvation, and also when Pi was added to cells previously subjected to phosphate starvation. Potassium, magnesium, phosphate, and a source of energy were required. If growth was allowed to resume, the polyP previously accumulated was degraded.

A deliberate study to interpret the effect of nutritional conditions on the polyP level in terms of the amounts and activities of the enzymes involved was subsequently undertaken in the author's laboratory. It was soon realized that polyP accumulation under conditions of nutrient imbalance fell into two distinct patterns (Fig. 3). (i) One pattern involved the cessation of nucleic acid synthesis owing to exhaustion of an essential metabolite. When growth and nucleic acid synthesis were blocked by depriving the organisms of sulfate, assimilation of Pi from the medium continued resulting in slow accumulation of polyP. A similar pattern was obtained with auxotrophic mutants requiring uracil or amino acids when deprived of their required nutrilite.
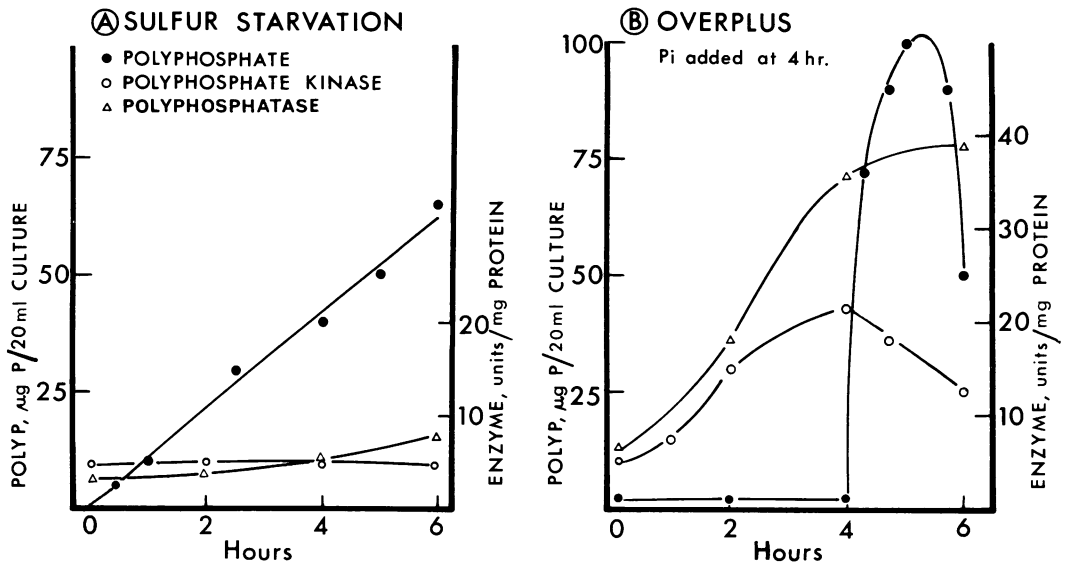
FIG. 3. *Patterns of polyphosphate accumulation in Aerobacter aerogenes. (A) Nutrient deprivation: cells were placed in medium devoid of sulfur at 0 hr. (B) "Overplus": cells were placed in medium devoid of phosphate at 0 hr; phosphate was restored at 4 hr. Initial cell density, 10^9 cells per milliliter in both. After Harold (38, 40).*

The accumulation was reversed if growth was allowed to resume: polyP was rapidly degraded and the phosphate was quantitatively transferred to the nucleic acid fraction. By the use of chloramphenicol to dissociate cell growth from nucleic acid synthesis, evidence was obtained for a dual competitive relationship between the accumulation of polyP and of nucleic acid: resumption of nucleic acid synthesis reduced the rate of concurrent polyP synthesis and at the same time stimulated its degradation (38, 41, 42). Induction of polyP accumulation by nutrient deficiency was a general pattern, but its specificity was a function of environmental factors. For example, in certain growth media, polyP accumulation could be induced only by sulfur starvation, and detailed analysis indicated an obscure relationship between polyP metabolism and intracellular glutathione (45). A similar finding was reported in *E. coli* (106). (ii) Addition of Pi to cells previously subjected to phosphate starvation induced rapid and extensive accumulation of polyP, such as had previously been described in other microorganisms (see below). This phenomenon was designated "Polyphosphat Überkompensation" by Liss and Langen (80), which was translated as "polyphosphate overplus" (40, 41, 46). Upon resumption of growth and nucleic acid synthesis, the polyP was gradually degraded by conversion to nucleic acids (40, 41). The two patterns of polyP accumulation are contrasted in Fig. 3.

As a prerequisite to studies on regulatory mechanisms, the pathway of polyP metabolism was investigated by means of mutants (46) blocked in these reactions (Table 2). Of these, mutant Pn-2 was found to be totally blocked in polyP accumulation under all nutritional conditions and to lack polyphosphate kinase (40). In mutant Pn-4, polyP accumulation was normal but its degradation was severely inhibited; this mutant was devoid of polyphosphatase (42). It was therefore concluded that polyP kinase mediates the only route of biosynthesis, whereas the primary route of polyP degradation is the hydrolytic one, regenerating Pi (Fig. 4). No evidence was obtained that the polyphosphatase catalyzes the transfer of phosphate from polyP to any acceptor other than water. Nontheless this possibility remains open, the more so as no convincing mechanism for the control of polyphosphatase activity has yet been found₃

The polyP content at any time must reflect the balance between the rates of synthesis and degradation, and each phase of metabolism is apparently subject to control at both the levels of enzyme synthesis and of enzyme activity. *A. aerogenes* harvested during the exponential phase contains low levels of polyP kinase, polyphosphatase, and alkaline phosphatase. If the cells are subjected to phosphate starvation, differential synthesis of these three enzymes is observed (Fig. 3). Two mutants were isolated which carry defects in the regulation of enzyme synthesis (Table 2). In strain Pn-1, all three enzymes

TABLE 2. *Mutants of Aerobacter aerogenes deficient in polyphosphate metabolism* (40, 41, 42, 46)

Mutant	Physiological characteristics	Enzymes			Control
		Kinase	Polypho-sphatase	Alkaline phosphatase	
Wild	PolyP undetectable in growing cells, accumulates both by nutrient deprivation and "overplus"	+	+	+	All enzymes derepressed by phosphate starvation
Pn-1	No "overplus," but does accumulate polyP upon nutrient deprivation	+	+	+	Enzymes present but not derepressed by phosphate starvation
Pn-2	Does not accumulate polyP under any conditions	−	+	+	Kinase absent, others derepressed by phosphate starvation
Pn-3	Transient accumulation of polyP in growing cells	+	+	+	All constitutively elevated
Pn-4	Blocked in polyP degradation	+	−	+	Polyphosphatase absent, others derepressed by phosphate starvation
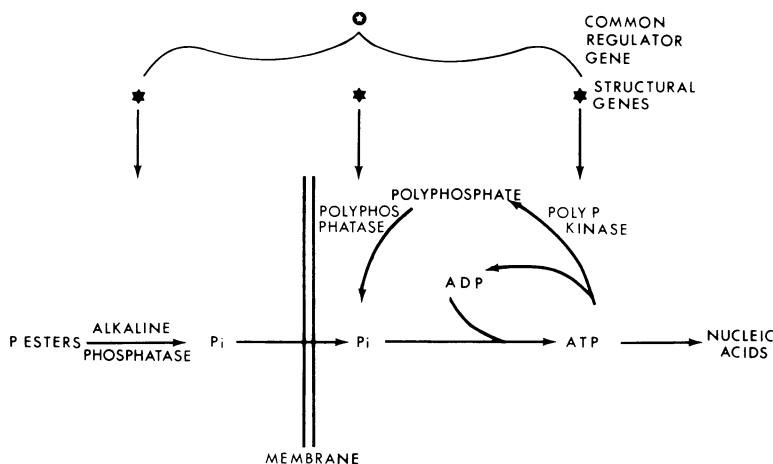
FIG. 4. *Polyphosphate cycle in Aerobacter aerogenes and its genetic control* (42)

are present, but repression of their synthesis is not released by phosphate starvation; this mutant accumulates polyP when nucleic acid synthesis is blocked by nutrient deficiency, but does not exhibit the "polyP overplus" (40). In mutant Pn-3 all three enzymes are constitutively derepressed, and this is correlated with transient accumulation of polyP during the exponential phase of growth (42). Several lines of evidence suggest that polyP kinase, polyphosphatase, and alkaline phosphatase share a common regulator gene but do not fall into a single operon. The pathway of polyP metabolism and its genetic control as currently envisaged are shown schematically in Fig. 4.

From metabolic experiments with wild-type and mutant organisms, it appears that the rate of polyP synthesis is proportional to the specific activity of polyP kinase. In addition, of course, activity of this enzyme depends upon availability of ATP, and inhibition of polyP synthesis by concurrent nucleic acid synthesis may reflect competition for ATP. The rate of polyP degradation is probably likewise a function of the level of polyphosphatase and is markedly stimulated by concurrent nucleic acid synthesis. Unfortunately, the nature of the coupling between these two processes has remained elusive.

The interactions between the regulatory elements discussed above are sufficient to account in outline for the effects of nutritional conditions on the polyP content of *A. aerogenes* (42). In

growing cells, the synthesis of nucleic acids inhibits polyP synthesis and stimulates polyP degradation; consequently, little or no polyP is deposited. If growth and nucleic acid synthesis cease because of the exhaustion of an essential nutrient, polyP degradation is inhibited. At the same time the competition for ATP (?) is relieved, and polyP then accumulates at a rate determined by the level of polyP kinase. Finally, cells subjected to prior phosphate starvation contain elevated levels of the kinase and are thus capable of rapid polyP synthesis when Pi is provided; this is the basis of the "overplus" phenomenon (Fig. 3). It must be stressed, however, that this is no more than the bare bones. It has not been established at what point the competition between nucleic acid and polyP synthesis takes place, and the coupling between nucleic acid synthesis and polyP degradation requires clarification at the molecular level.

Corynebacteria and Mycobacteria

The metabolism of polyP in these two genera appears, at this time, similar enough to warrant their joint discussion. They are of particular interest in that all four known pathways of polyP utilization have been demonstrated in extracts, but there is little information which can be applied with confidence to the intact cell.

The polyP content of C. xerosis is a function of the growth phase. When cells were transferred to fresh medium, Hughes and Muhammed (56) observed accumulation of polyP during the lag phase preceding the initiation of rapid growth. The polyP was consumed during the exponential phase, to accumulate again in the stationary phase. PolyP also accumulated in cells subjected to nitrogen starvation. Similar results had been obtained earlier with various mycobacteria. Several investigators (21, 94, 153) showed that the polyP content was lowest in rapidly growing cells, and rose in the stationary phase. PolyP accumulation could also be induced by nitrogen deficiency (111) or by metabolite analogues such as azaserine (94) and ethionine (21), and by growth in a zinc-deficient medium (154). If growth was allowed to resume, the polyP was degraded and served as a source of phosphorus for the synthesis of nucleic acids and lipids (94, 153). Curiously, the polyP "overplus" has apparently not been reported in the mycobacteria and corynebacteria, probably because it has not been looked for.

In general, these results can be regarded as a reflection of the competitive relationship between the metabolism of polyP and of nucleic acids. One result that is not readily explained in this way is the observation that tetrahydrofurfuryl alcohol stimulates polyP accumulation in M. smegmatis (153) without concomitant reduction in nucleic acid synthesis. Finally, Sall and his associates (112) exposed C. diphtheriae to temperature shock; during subsequent synchronized cycles of cell division the polyP content of the culture exhibited striking fluctuations, with maxima just prior to cell division. By contrast, the RNA and DNA contents increased steadily. Here again, the regulatory mechanism involved in this most interesting pattern cannot be specified.

Accumulation of polyP is an energy-dependent process, and there is little doubt that it is mediated by polyP kinase (56, 95, 152). The degradative pathway, however, presents an intriguing problem. To the extent that polyP kinase is reversible, it may be one possible route of utilization. Hughes and Muhammed (56) claim that during the growth of C. xerosis the polyphosphatase (96) level parallels that of polyP itself, and suggest that this enzyme represents the main pathway of degradation; unfortunately, their paper has few details and there is no evidence that the fluctuations in enzyme activity correspond to a change in the amount of enzyme protein. Of great interest is the demonstration, in both mycobacteria (130–132) and corynebacteria (15), of polyphosphate glucokinase. According to Dirheimer and Ebel (16), the affinity of the enzyme for polyP is high enough that extracts of C. xerosis will utilize polyP by this pathway even in the presence of polyphosphatase. Physiological evidence in support of the thesis that polyP is utilized in vivo for the phosphorylation of glucose is found in the earlier observations that, in both mycobacteria (94) and corynebacteria (111), polyP accumulation in resting cells is favored by substrates such as malate but is suppressed by glucose. In addition, organisms grown on fructose may contain an inducible polyP-dependent fructokinase (133); if this finding is confirmed, it would strongly support the concept that polyP is utilized for the direct phosphorylation of hexoses. Finally, polyP-AMP-phosphotransferase has been proposed as a route for ADP synthesis independent of ATP (16, 18). There are thus no fewer than four possible routes for the utilization of polyP in myco- and corynebacteria, and it is unfortunate that evidence is not available which would permit us to assess the relative contribution of each pathway.

Other Bacteria

PolyP metabolism has, of course, been investigated in other bacterial genera, but the results are

too limited to warrant discussion in terms of the control of polyP metabolism in the intact cell. Kaltwasser (59, 60) has demonstrated the polyP-"overplus" in *Hydrogenomonas*; this work will be discussed below as it bears upon the function of polyP. Hughes et al. (9, 55) have studied polyP metabolism in the photosynthetic bacterium *Chlorobium*. Polyphosphate degradation in extracts of this organism requires ADP and appears to be mediated by polyP kinase and adenosine triphosphatase acting successively; polyphosphatase is believed to be absent from this organism, but direct evidence for the participation of polyP kinase in the degradative pathway in vivo has yet to be supplied. Finally, a series of papers by Zaitseva and her associates (157–160) describes polyP accumulation in *Azotobacter* species, both during nitrogen starvation and after phosphate starvation. Of particular interest is the observation (158) that the "overplus" was prevented by the absence of Ca^{+2}, whereas nucleic acid synthesis was undiminished; if Ca^{+2} was later added, polyP promptly accumulated. It is noteworthy that the ATP-ADP ratio was lowered in calcium-deficient cells, which may account for the depression of polyP synthesis, but it is difficult to see why nucleic acid metabolism should not also be reduced.

Yeast and Filamentous Fungi

Yeast is the classic organism for studies on polyP metabolism, and the "overplus" was first observed in it (58, 116, 145, 147). Later workers have continued to focus their efforts on phosphate-starved yeast, and little information on other conditions of nutrient imbalance is available. Katchman and Fetty (63) found that the polyP content is lowest during the exponential phase, followed by accumulation as growth slows down. Accumulation can also be induced by irradiation (64, 123).

The biosynthesis of polyP in yeast was clarified by Langen and his collaborators (74, 75). By differential extraction, they obtained four distinct polyP fractions of increasing chain length (Table 1). When Pi^{32} was added to resting yeast and fermentation initiated by addition of glucose, P^{32} was most rapidly incorporated into the high molecular weight fraction 4, incorporation into fractions 3, 2, and 1 being progressively slower. In another experiment, yeast was labeled by a brief exposure to Pi^{32}, and fermentation was then blocked (e.g., with iodoacetate). This induced breakdown of polyP to an equivalent amount of Pi: fraction 4 was the first to go, but the Pi released was unlabeled, the P^{32} being trapped in fractions 3, 2, and 1. It was concluded that polyP
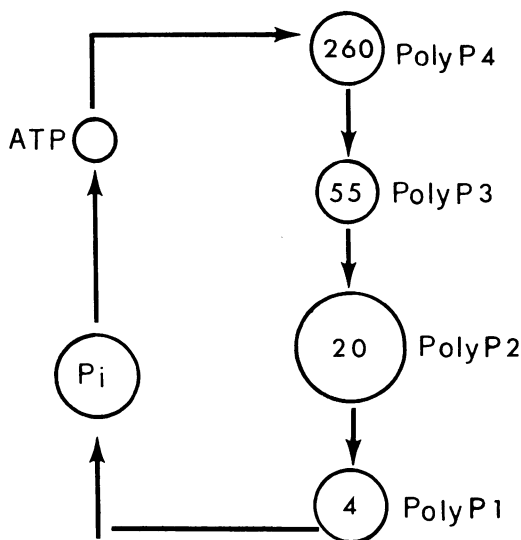

FIG. 5. *Polyphosphate cycle in yeast. The numbers denote the average chain of polyP fractions 1 to 4. The area of each circle is proportional to the amount of phosphorus in that fraction. After Langen et al. (75).*

biosynthesis results in formation of long chains which are subsequently cleaved to shorter ones. This process was designated as the "polyphosphate cycle," illustrated in Fig. 5. These results also explain the early observation that the "turnover" of the "soluble" polyP (containing polyP of low molecular weight) is much slower than that of the "insoluble" polyP. In fact, the rates of turnover of the two pools are the same, but they stand in a precursor-product relationship.

This concept was confirmed in a subsequent study by Liss and Langen (80) on the accumulation of polyP during the "overplus" (Überkompensation). Once again, the largest molecules were formed first, and served as precursors to those of lower molecular weight. It is noteworthy that the authors were the first to demonstrate that phosphate starvation resulted in a marked increase in the *rate* of subsequent polyP synthesis. This presumably reflects derepression of polyP kinase, as discussed for *Aerobacter* above. Phosphate starvation also leads to derepression of acid phosphatase in yeast, but synthesis of the enzyme could be readily dissociated from the synthesis and maintenance of the polyP pool, and there appeared to be no direct relationship between the two phenomena (144). As would be expected, significant accumulation of polyP during "overplus" occurred only if relatively large amounts of Pi were provided (27).

Upon resumption of growth polyP degrada-

tion occurs via the cycle, with ultimate transfer of the phosphorus to both nucleic acids and phospholipids. All classes of RNA acquire Pi^{32} from polyP, and the specific activities of the four nucleotides are the same. PolyP thus appears to feed into the general precursor pools of phosphorus metabolism, Pi, and ATP (124, 126).

It is not at all clear which enzymes mediate the utilization of polyP. Reversibility of polyP kinase in yeast has been demonstrated (50, 51), but Langen (73) was unable to detect direct conversion of polyP to ATP in vivo. From this and also from the tracer studies (74, 75), it seems more likely that stepwise hydrolysis by polyphosphatases as described by Mattenheimer (86–88) accounts for the formation of low-molecular weight polyP, with Pi the ultimate product.

The data available for filamentous fungi fit the pattern observed in yeast as far as they, and it, go. Earlier studies with *Aspergillus niger* have been reviewed by Kuhl (71). More recently, Nishi has investigated the utilization of polyP by germinating spores of this fungus. PolyP was shown to serve as a precursor for both nucleic acids and phospholipids (101). During germination, the specific activities of polyphosphate depolymerase and polyphosphatase increased significantly, suggesting that these enzymes play a role in the mobilization of the polyP (100).

In 1949, Houlahan and Mitchell had observed massive accumulation of polyP by various mutants of *Neurospora crassa*. The reason for this effect was later explored by Harold (35). The fungus contains substantial quantities of polyP even during the exponential phase of growth. If growth was halted by exhaustion of a nutrient (e.g., histidine in a histidine-requiring auxotroph), RNA was degraded and served as a source of phosphorus for the accumulation of polyP. The product was mostly acid-soluble, with an average chain length of about 20. By means of various manipulations it was shown that RNA degradation was a sufficient condition for polyP accumulation, but not a necessary one. If Pi was restored to *Neurospora* previously subjected to phosphate starvation, the usual "overplus" was observed, and the results suggested that ATP was a precursor. During subsequent phosphorus deficiency, the polyP was mobilized for RNA synthesis, but ATP was not an intermediate in the degradation of polyP (37). Polyphosphatase was suggested as a more likely catalyst, as with yeast.

Photosynthetic Algae

Considerable quantities of polyP occur in *Chlorella* and *Euglena* under normal growth conditions. Early investigations, reviewed by Kuhl (71), established a relationship between light and polyP, in that resting algae under photosynthetic conditions assimilate Pi into polyP. Similar results were recently reported by Kanai, Miyachi, and Miyachi (62), who found rapid uptake of Pi^{32} into polyP in the light; in the dark, incorporation was depressed but not abolished. Thus, formation of polyP is stimulated by light, but not totally dependent on it—a conclusion supported by other data as well. Overbeck (103) demonstrated the "overplus" in *Scenedesmus* in the dark, and Smillie and Krotkov (121) showed that *Euglena* grown heterotropically, or after streptomycin-bleaching, contains polyP.

As in other microorganisms, the polyP content is a function of the growth phase: lowest in the exponential phase, highest in older cultures, and a reciprocal relationship between the RNA and polyP contents appears to exist (121). Miyachi and colleagues have presented a somewhat confusing series of experiments on the relationship between nucleic acids and four different polyP fractions (62, 91, 92). According to their most recent report (91), which partly supersedes earlier ones, the relationship is quite complex. *Chlorella* was grown photosynthetically on P^{32} medium, and the cells, totally labeled, were then transferred to cold medium. In the dark (both in the presence and absence of exogenous Pi), all the polyP fractions remained constant, and there was no synthesis of DNA and RNA. In the light, with Pi absent, polyP was broken down and served as source of phosphorus for synthesis of both DNA and RNA. Surprisingly, however, in the light with Pi present, lipid-P and RNA-P were synthesized from Pi in the medium, whereas DNA-P was derived from certain fractions of the polyP pool (91, 92). These data seem difficult to interpret, since the starting cells are fully labeled, and, therefore, the various shifts would at best prove only that a certain fraction of the DNA is derived from intracellular phosphorus. Moreover, according to Schmidt (117), the analytical methods employed by the Japanese workers are open to question. Nonetheless, it is not impossible that, as suggested by the authors, the various polyP fractions differ in their intracellular locations, and that their metabolism reflects this compartmentization.

In studies on the effect of synchronized growth on the polyP level, Schmidt et al. reported that the polyP content of *Chlorella*, expressed as a percentage of the total phosphorus content, fluctuates, with a maximum just prior to nuclear division (2, 3). However, the polyP content per cell rises continuously until cell division. This

apparent paradox is explained as being due to linear polyP synthesis while the total phosphorus content rises exponentially (4, 48). The suggestion that the polyP content reflects the ATP-ADP ratio was contradicted by the finding that this ratio remained about unity during the cycle of synchronous cell division (12, 13). PolyP was not utilized at all as long as exogenous Pi was provided, but was available to phosphate-starved cells (5). Schmidt (117) has recently summarized the studies from his laboratory on polyP metabolism in algae during synchronized growth.

Nothing is yet known about the enzymes of polyP metabolism in these cells, and the relationship between photosynthesis and polyP still requires considerable illumination.

FUNCTIONS OF POLYPHOSPHATES

The concept of function implies that conditions exist under which the capacity to synthesize polyP confers a selective advantage upon the organisms. In this teleonomic sense, the function of polyP is not known, and constitutes perhaps the most challenging research problem in this area. As yet no experiments have been reported in which cells capable of polyP synthesis were compared with polyphosphate-deficient cells of similar physiological history, and attempts in this laboratory to employ the polyphosphateless mutants of *A. aerogenes* gave inconclusive results. The fact that the mutants did not exhibit gross defects in growth is clear evidence that polyP is a dispensable constituent (41, 46). On the other hand, it seems unlikely that so widespread a substance would serve no function at all, as it would surely have been eliminated in the course of evolution. Perhaps the capacity to synthesize polyP confers a small selective advantage, sufficient to ensure preservation of the enzymes even though their role is not readily detected physiologically.

Phosphagen Hypothesis

In a recent survey (50), Hoffmann-Ostenhof summarized the arguments in support of the thesis that polyP serves as a microbial phosphagen. Enzymes exist which catalyze the reversible transfer of phosphate from polyP to ADP; the polyphosphates are thermodynamically "high energy" phosphate compounds; polyP accumulates under conditions in which the ATP supply exceeds cellular demands for work and biosynthesis, and is consumed if growth (and energy demands) resumes; finally, in an argument from comparative biochemistry, polyphosphates occur only in organisms lacking the more conventional phosphagens, such as guanidinophosphates or

phytin (this argument, always rather negative, may have to be dropped if polyP should prove to be at all widespread among higher plants and animals).

The term "phosphagen" is defined by Ennor and Morrison (28) as "those naturally-occurring phosphorylated guanidine compounds which function as stores of phosphate-bond energy from which phosphoryl groups may be transferred to ADP to form ATP as a result of enzymatic catalysis." If this definition is broadened by deletion of the word "guanidine," the essential point is the direct formation of ATP at the expense of the phosphagen. Two lines of evidence contradict the interpretation of polyP metabolism demanded by this definition. (i) In intact cells, polyP does not break down if energy generation is limited or blocked (37, 59); moreover, under these conditions, maintenance of the ATP pool at the expense of polyP is not observed (37). It should be mentioned that polyP breakdown does occur in yeast poisoned with iodoacetate, but apparently not by conversion to ATP (73, 74). In *Chlorobium*, incubation of the cells with CO_2 in the dark induced slight degradation of polyP which was interpreted as supporting the role of polyP as an energy store (119), but the mobilization of polyP amounted only to one-tenth of the total pool. (ii) In *A. aerogenes*, there is direct evidence (42) that polyP degradation is hydrolytic and results in dissipation of the energy-rich bond. Incidentally, even conservation of the phosphate transfer potential by the phosphorylation of glucose, which may occur in corynebacteria and mycobacteria (15, 16, 130–133), would still not be in accordance with the definition of a phosphagen.

The hypothesis that polyP serves as a microbial phosphagen in the strict sense thus appears to be no longer tenable. More generally, the results cited above, together with the observation (45, 59, 80) that polyP accumulation accounts for but a small fraction of the ATP generated by the cells, leads one to doubt that energy storage is the principal function of polyP.

Polyphosphate as a Phosphorus Reserve

Many investigators have shown that polyP can serve as a source of phosphorus for the biosynthesis of nucleic acids and phospholipids under conditions of phosphorus starvation (5, 37, 38, 42, 91, 94, 124, 126, 153). The same appears to be true in the early stages of spore germination in *A. niger* (101); the role of polyP in the germination of bacterial spores deserves further exploration. The large amounts of polyP that accumulate under appropriate conditions may suffice for

several doublings of the cell mass during subsequent phosphate starvation. Possibly of greater significance, polyP could constitute an accessible reserve for the synthesis of messenger RNA, ribosomes, and metabolic intermediates to facilitate the *initiation* of growth.

On this hypothesis, the energy content of polyP would be largely irrelevant to its physiological role. However, its structure makes it an attractive compound for phosphate storage, since accumulation of polyP (rather than of Pi itself) would minimize disturbance of the osmotic equilibrium and of the concentrations of the critical intermediates, Pi, and adenine nucleotides. It may also be recalled that the phosphate content of natural environments tends to be low, due to the insolubility of calcium phosphate (32). It seems intuitively reasonable that free-living organisms should have evolved means to accumulate a phosphorus reserve, and that phosphate starvation should derepress the enzymes concerned in this process.

Polyphosphate and Regulation of Phosphorus Economy

The phosphorus economy of all cells is dominated by the dual roles of the adenine nucleotides. On the one hand, the cyclic generation of ATP and its participation in innumerable reactions to regenerate ADP and Pi is the "metabolic wheel" by which metabolic energy is produced and utilized. On the other hand, in growing cells, phosphorus at the level of ATP is continuously withdrawn into "sinks"—the nucleic acids and phospholipids. At the same time, it appears that cells must control quite closely the steady-state concentrations of Pi, ADP, and ATP, for several reasons. One is that the efficiency of energy utilization depends upon the "phosphate transfer potential" of ATP, which will be maximal if Pi, ADP, and H^+ concentrations are kept low. Another factor is that Pi and the adenine nucleotides serve not only as substrates but also as allosteric effectors of numerous enzymatic reactions (1). A pertinent example is the role of these metabolites in the Pasteur effect, whether by Lynen's (82) mechanism, the modulation of phosphofructokinase (104), or the control of hexokinase (109). It is attractive to postulate that the physiological role of polyP is related to the regulation of Pi, ATP, and ADP levels, and that the polyP cycle serves in effect as a metabolic buffer. Unfortunately, the control of nucleotide and phosphate levels, particularly in nongrowing cells, has received little attention, and many fundamental questions remain to be answered (1,33).

Some years ago, Hoffmann-Ostenhof and Weigert (52) suggested that polyP might serve to bypass the Harden-Young effect in living cells. This is the cessation of glycolysis in yeast extracts owing to depletion of Pi. Apparently yeast extracts do not contain adenosine triphosphatase, and, consequently, the ATP formed by glycolysis is trapped as hexosediphosphate, depleting Pi to the point where glyceraldehyde phosphate dehydrogenase becomes limiting (for review, *see* 33). Hoffmann-Ostenhof and Slechta (51) found that such extracts resumed glycolysis if supplemented with polyP plus polyP kinase, and proposed that a similar bypass occurs in intact cells. In an extension of this hypothesis, Harold (37) considered the polyphosphate cycle as an adenosine triphosphatase, regenerating both ADP and Pi in nongrowing cells supplied with substrate. However, findings from several laboratories (45, 59, 80) indicate that polyP metabolism accounts for but a small fraction of the ATP generated; the polyP cycle thus appears to be of little quantitative significance as an adenosine triphosphatase.

More appealing is Kaltwasser's (59) suggestion that the polyP cycle is involved in the control of the Pi level. It is surely significant that the polyP content of microbial cells is a function of the cellular phosphate balance rather than of energy supply. PolyP accumulation is associated with nutritional conditions in which growth, and thus nucleic acid and phospholipid synthesis, are inhibited, but assimilation of Pi from the medium continues. Similarly, intracellular release of phosphate (or nucleotides) by degradation of nucleic acids is balanced by polyP accumulation (35). The polyP "overplus" can also be regarded as a mechanism for the regulation of the intracellular Pi level. It is well known that phosphate starvation leads to derepression of phosphatases located at the cell surface, which make available organic phosphate esters. Pi thus released and accumulated by the cells would be sequestered as polyP to keep both Pi and H^+ concentrations low. It certainly appears to be true that the Pi level rapidly stabilizes during "overplus" (27, 37, 59), and that polyP is formed only if sufficient quantities of Pi are provided (27, 37). In this connection, the effect of phosphate starvation upon the capacity for phosphate transport deserves investigation. It seems likely that Pi is transported by means of a "permease" and then enters metabolic cycles without prior equilibration with the Pi pool. In *S. faecalis*, phosphate uptake appears to be under "feedback" control of some sort (43), but unfortunately this organism does not accumulate polyP.

If the speculation that polyP is concerned with regulation of the Pi level should prove correct, polyP itself would be "merely" a shunt product. Although this view does not exclude a role for polyP in phosphate storage, it puts the emphasis on the cycle rather than on the size of the pool.

Other Functions

Several investigators (64, 112, 114, 123) have proposed a somewhat ill-defined relationship between polyP and cell division, the polyP serving either as an energy pool (114) or as a regulator of the cation content (64). In my opinion, the evidence currently available does not support this hypothesis, particularly since mutants devoid of polyP (41, 46) exhibited no obvious abnormality in cell division.

In recent experiments, Van Steveninck found a relationship between polyP and glucose uptake in yeast (127) and later suggested that it might serve to phosphorylate a carrier for the active transport of glucose (128). It is interesting that Weimberg and Orton (144) also noted the apparent localization of a fraction of the polyP pool outside the permeability barrier. However, direct participation of polyP in the transport step has yet to be unequivocally demonstrated.

Polyphosphate and Origin of Life

Phosphorus plays so central a role in biology that the evolution of living things must have required this element from the start. The oxidation state was probably always that of phosphate, because even under a reducing atmosphere lower oxidation states, such as phosphite (32), would have been unstable (89). A mechanism for phosphate accumulation would thus have been useful very early.

ATP is the universal energy carrier in contemporary organisms, but it has been suggested that the earliest organisms used polyP or pyrophosphate (78). The condensed inorganic phosphates could have arisen by a variety of abiogenic processes, including the condensation of Pi at high temperatures and the reaction of cyanate with calcium phosphate (89), and would thus have been constituents of the "primordial soup."

Of greatest interest is the finding that polyP and certain derivatives promote a variety of condensation and polymerization reactions in vitro. Esters of "metaphosphate," of somewhat undefined structure, promote formation of polynucleotides, polypeptides, and polysaccharides from the corresponding monomers under mild conditions (118), and also the formation of ATP from adenine, ribose, and Pi (107). According to

Schramm (118), esters of this sort could have arisen on the primitive earth by esterification of polyphosphoric acid. Moreover, polyphosphoric acid itself will, under certain conditions, promote condensation of amino acids to proteinoids (34).

There is thus the intriguing possibility that we have in polyphosphates a metabolic fossil which in the course of the millenia has lost its original role in polymer formation, presumably to assume new functions which still elude us.

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