ACCUMULATION OF INORGANIC POLYPHOSPHATE IN *AEROBACTER AEROGENES*

II. Environmental Control and the Role of Sulfur Compounds

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Abstract

Harold, F. M. (National Jewish Hospital, Denver, Colo.) and Susan Sylvan. Accumulation of inorganic polyphosphate in *Aerobacter aerogenes*. II. Environmental control and the role of sulfur compounds. J. Bacteriol. **86**:222–231. 1963.—The accumulation of inorganic polyphosphate in *Aerobacter aerogenes* was shown to be a function of the growth medium. In low-phosphate medium, polyphosphate accumulated whenever nucleic acid synthesis ceased due to a nutritional deficiency, regardless of its nature. In high-phosphate medium polyphosphate accumulation was induced only by sulfur starvation. Polyphosphate accumulation could thus be induced or suppressed at will by manipulation of the sulfur and phosphorus content of the medium. The specific requirement for sulfur starvation was traced to the presence of an intracellular inhibitor of polyphosphate accumulation. This was depleted during sulfur starvation and replenished when sulfate was restored. The inhibitor was identified as oxidized glutathione or a closely related compound. Suppression of polyphosphate accumulation required the simultaneous presence of a high exogenous phosphate concentration and a high intracellular glutathione level. Suppression of polyphosphate accumulation resulted in a constant polyphosphate level, due to a steady state of polyphosphate synthesis and degradation. The former continued at half the original rate while the latter was sharply accelerated. The synthetic and degradative phases of polyphosphate metabolism could be completely dissociated by inhibitors of energy generation. It is proposed that the primary effect of glutathione plus phosphate is the stimulation of polyphosphate degradation. Polyphosphate synthesis appears to be a general consequence of the inhibition of nucleic acid synthesis, but net accumulation may be obscured by concurrent degradation.

In the preceding paper (Harold, 1963), the reciprocal relationship between growth and the accumulation of inorganic polyphosphate in *Aerobacter aerogenes* was traced to a complex competition between polyphosphate and nucleic acids for intracellular phosphorus. Polyphosphate accumulation occurred only after nucleic acid synthesis had ceased and was reversed if nucleic acid synthesis was permitted to resume. One would consequently expect polyphosphate to accumulate whenever growth of *A. aerogenes* ceases, provided a source of energy, phosphate, and essential metal ions is available. This proved, in fact, to be the case in growth media of low phosphate content. Polyphosphate accumulation was observed whenever growth of the cells was halted by a nutritional deficiency, regardless of the nature of the deficiency. However, in media of high phosphate content polyphosphate accumulation could be induced only by sulfur starvation.

It thus appears that even in cells not engaged in nucleic acid synthesis the polyphosphate level is subject to control by environmental factors, mediated at least in part by sulfur compounds. The present paper deals with the metabolic basis of these phenomena and with the identification of the sulfur compound involved.

Materials and Methods

The organisms used, growth media, sampling techniques, and the methods for the fractionation and estimation of phosphorus compounds were described in the preceding paper (Harold,

1963). Methods for the estimation and identification of sulfur compounds were adapted from Roberts et al. (1957). Samples of medium or cell extract (hot water, or cold perchloric acid neutralized with KOH) were passed through Dowex 50 columns (H^+ form). The sulfur compounds were eluted with ammonium hydroxide and characterized by paper chromatography using solvents 3 and 6 of Roberts et al. (1957). Quantitative estimations of sulfate metabolism were carried out by the use of S^{35} sulfate of known specific radioactivity and the assay of various cell fractions for S^{35}.

The major component of the acid-soluble sulfur pool was identified as oxidized glutathione by paper chromatography in three solvents [3 and 6 of Roberts et al. (1957) and *n*-butanol acetic acid-water (2:1:1)]. The identification was confirmed by chromatography after oxidation of the glutathione with hydrogen peroxide, and by chromatographic identification of cysteic acid after hydrolysis of glutathione and oxidation of the products with H_2O_2. In each case the S^{35} spot coincided precisely with the position of the carrier glutathione or cysteic acid as visualized by ninhydrin.

Results

Induction of polyphosphate accumulation by nutrient imbalance in W and T media. It was shown in the preceding paper that accumulation of polyphosphate occurs when growth and nucleic acid synthesis cease as a result of a nutritional deficiency, such as sulfur starvation. When other nutritional deficiencies were investigated, it was discovered that the result was markedly dependent upon the growth medium.

In T_{10} medium [tris(hydroxymethyl)aminomethane (tris) buffer, 0.3×10^{-3} M phosphate], polyphosphate accumulation was a nonspecific response to a variety of conditions inhibitory to growth. Thus, all the auxotrophic strains accumulated polyphosphate when growth was limited by exhaustion of their specific growth factor, or of sulfate. The minimal requirements for polyphosphate accumulation under these conditions proved to be glucose, phosphate, K^+, and Mg^{++}. The presence of NH_4^+ stimulated polyphosphate accumulation threefold, and it was therefore included in all experiments. It should be noted that growth of the cells in the deficient medium was not required, since accumulation was observed in the complete absence of required amino acids or pyrimidine bases.

A very different pattern was observed in W medium (0.075 M phosphate as P source and buffer). In this medium, polyphosphate accumulation could be induced only by sulfur starvation. Restriction of the growth of auxotrophic mutants by depriving them of guanine, uracil, histidine, tryptophan or tyrosine failed to induce polyphosphate accumulation. Surprisingly, this was true even for mutants requiring sulfide, cyst(e)ine, or methionine for growth. Inhibition of growth by 5-fluorouracil, 6-azauracil, or 5-methyl tryptophan also proved ineffective. Nitrogen deficiency did produce a small accumulation as reported by Smith, Wilkinson, and Duguid (1954). In general, polyphosphate accumulation in W medium required growth of the bacteria under sulfur starvation. Simultaneous deprivation of mutant Sl_{try-} of both sulfur and tryptophan prevented polyphosphate accumulation. Addition of chloramphenicol to A3(0) had a similar effect. However, this apparent requirement for prior protein synthesis was transient and disappeared once polyphosphate accumulation had begun.

The divergent response of the polyphosphate level to nutrient imbalance in W and T media was traced to the phosphate level. Upon addition of phosphate (0.04 M or more) to T_{10} medium, polyphosphate accumulation became specifically dependent upon sulfur starvation and was no longer induced by other deficiencies. A curious difference between mutants derived from A3(0) and A3(Sl) was noted when the effect of other salts on the specificity of the induction was studied. In mutants of A3(0), sodium or potassium phosphate alone (0.04 M) imposed the specific requirement for sulfur starvation; other salts had no effect. However, in mutants derived from A3(Sl), polyphosphate accumulation could be rendered specific to sulfur starvation by addition of KCl, NaCl, KNO_3 (0.1 M), or even by sucrose (0.2 M). These observations stand without explanation at this time.

Suppression of polyphosphate accumulation. By taking advantage of the dependence of polyphosphate accumulation both upon the nature of the nutritional deficiency and upon the extracellular phosphate level, it became possible to induce or suppress polyphosphate accumulation in nongrowing cells at will.

Mutant Sl_{u^-} was allowed to accumulate polyphosphate in sulfur-deficient W medium supplemented with uracil. After 100 min, the cells were sedimented, washed, and resuspended in W medium (no uracil); sulfate was added to part of the culture. The results are shown in Fig. 1. In the absence of both sulfur and uracil, polyphosphate accumulation continued as before. However, in the presence of sulfate polyphosphate accumulation ceased, and its level remained constant thereafter. Suppression of polyphosphate accumulation by sulfate was observed only in W medium, not in T_{10} medium.

Suppression of polyphosphate accumulation was as readily achieved by manipulating the salt content of the medium. Mutant Sl_{u^-} was induced to accumulate polyphosphate by uracil starvation in T_{10} medium (sulfate present). After 90 min, the culture was distributed among three flasks which were supplemented with sodium phosphate (0.04 M) or sodium chloride (0.1 M) and an unsupplemented control. Again

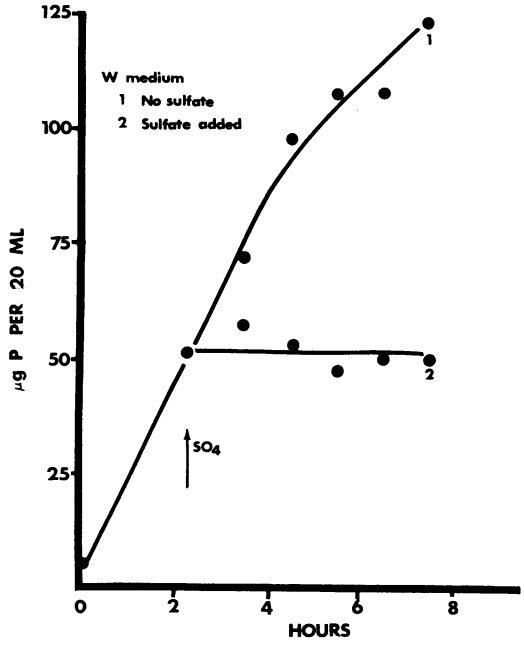

FIG. 1. *Suppression of polyphosphate accumulation by sulfate. Mutant Sl_{u^-} was subjected to sulfur starvation in W medium supplemented with uracil. After 100 min, the cells were centrifuged, resuspended in W medium containing neither sulfur nor uracil, and divided (arrow). Flask 1 received no additions; flask 2 received 0.7 × 10^{-3} M sulfate.*

polyphosphate accumulation continued in the control flask but was suppressed by the addition of phosphate or sodium chloride (Fig. 2A). It should be noted that suppression by salts was observed only in uracil-starved cultures, the effect being much less striking in sulfur-starved ones (Fig. 2B). Similar experiments were performed with the methionine-requiring mutant.

Rates of polyphosphate synthesis and degradation during suppression. The suppression of polyphosphate accumulation by addition of sulfate to sulfur-starved cells in W medium, or by the addition of phosphate to uracil-deficient cells in T_{10} medium, results in a constant polyphosphate level (Fig. 1 and 2). The experiments described below were carried out to determine which step in polyphosphate metabolism was sensitive to these manipulations.

Cells of Sl_{u^-} were subjected to uracil starvation in T_{20} medium. After 90 min, the culture was distributed among three flasks. The first received no additions; the others, 0.15 M sodium chloride. P^{32} was added to the control and to one of the experimental flasks at this time, and to the second experimental flask 1 hr later. It is clear from the results shown in Fig. 3 that despite the suppression of polyphosphate accumulation P^{32} uptake into this fraction continued. The new rate of polyphosphate biosynthesis, about half that of the control culture, was established immediately upon the addition of sodium chloride and remained constant for at least 1 hr thereafter.

The constancy of the polyphosphate level despite continued synthesis is explained by the concurrent degradation of polyphosphate. A culture of Sl_{u^-} was permitted to accumulate P^{32} polyphosphate by either uracil or sulfur starvation in P^{32}-T_{10} medium. After 90 min, the phosphate level was raised to 0.04 M, thereby diluting the P^{32} and suppressing the accumulation of polyphosphate in the uracil-deficient culture but not in the sulfur-deficient one (see also Fig. 2). At the same time, extensive degradation of P^{32} polyphosphate occurred in the former, but not in the latter, culture (Fig. 4).

The product of polyphosphate degradation was found to be inorganic phosphate, which was discharged into the medium. The degradative reaction was stimulated not only by high levels of phosphate but also [in strains derived from A3(Sl)] by sodium chloride. The stimulation of

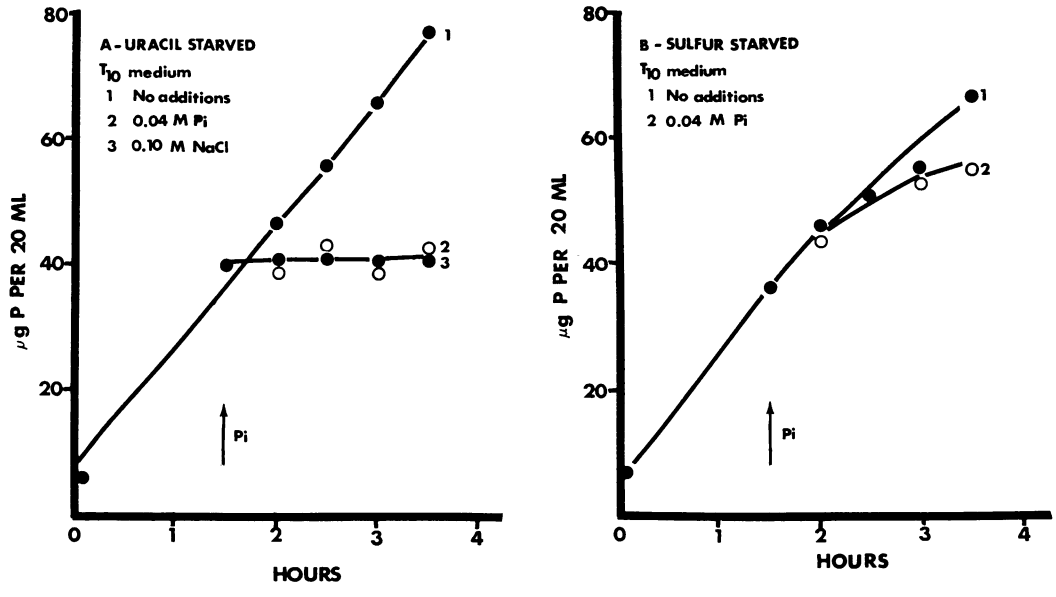

FIG. 2. *Suppression of polyphosphate accumulation by salts. (A) Mutant* Sl_{u-} *was subjected to uracil starvation in* T_{10} *medium. After 90 min (arrow), the culture was divided and supplemented: flask 1, no additions; flask 2, 0.04 M sodium phosphate; flask 3, 0.1 M NaCl. (B) Mutant* Sl_{u-} *was subjected to sulfur starvation in* T_{10} *medium containing uracil. After 90 min (arrow), the culture was divided. Flask 1 received no additions, flask 2 received 0.04 M sodium phosphate.*

polyphosphate degradation by a high salt level was demonstrable even after transfer to medium lacking phosphate and glucose, and is thus not coupled to phosphate uptake. Dissociation of polyphosphate degradation from synthesis could also be achieved with sodium azide; polyphosphate degradation was accelerated by a high exogenous salt level even in the presence of 0.04 M azide, which completely blocks the incorporation of P^{32} into polyphosphate.

Relationship between sulfur metabolism and polyphosphate accumulation. The observation that, in media high in phosphate (or other salts), polyphosphate accumulation was a specific consequence of sulfur starvation pointed to the participation of sulfur compounds in the control of the polyphosphate level. This relationship was investigated by growth experiments in W medium, with the aim of identifying the sulfur compound involved.

The suppression of polyphosphate accumulation in W medium by addition of sulfate was described in a previous section. By use of mutants Sl_{u-}, O_{met-}, and Sl_{try-}, it was possible to inquire which sulfur compounds could suppress polyphosphate accumulation under the experimental conditions of Fig. 1. The results were clear. Sulfate, sulfite, sulfide, cyst(e)ine, and oxidized as well as reduced glutathione (0.7×10^{-3} M sulfur) suppressed. The others, including methionine, homocyst(e)ine, cystathionine, cysteic acid, and cysteamine (0.7×10^{-3} M); lipoic acid, thiamine, biotin, and pantethine (0.3×10^{-3} M); and coenzyme A (0.15×10^{-3} M) did not suppress polyphosphate accumulation.

The suppression of polyphosphate accumulation by sulfur compounds was a reversible process. A methionineless mutant ($O_{met-tyr-}$) was allowed to accumulate polyphosphate in W medium lacking both sulfate and methionine. This accumulation was completely suppressed by the addition of 0.7×10^{-3} M sulfate and transiently suppressed by 1.2×10^{-4} M sulfate (Fig. 5). The transient suppression of polyphosphate accumulation by the lower concentration of sulfate was undoubtedly due to its conversion to cystathionine which is excreted by this mutant (Harold, 1962*a*); as the sulfate disappeared from the medium, polyphosphate accumulation gradually resumed (Fig. 5).

This experiment is open to two interpretations. Polyphosphate accumulation and sulfur metabo-

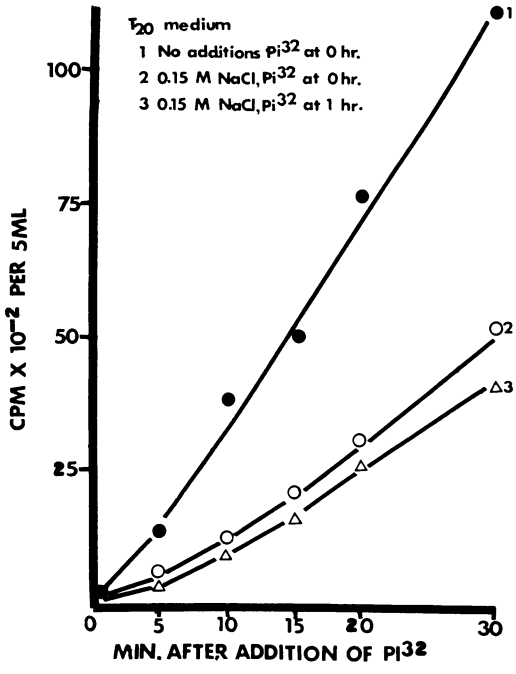

FIG. 3. *Incorporation of P^{32} into polyphosphate in accumulating and suppressed cultures. Mutant Sl_{u-} was subjected to uracil starvation in T_{20} medium. After 90 min, the culture was divided and additions were made: flask 1, P^{32} only; flask 2, 0.15 M NaCl and P^{32}; flask 3, 0.15 M NaCl, P^{32} added 1 hr later.*

lism may constitute competing pathways, so that accumulation would be suppressed only while the added sulfate is being *metabolized* by the cells. Alternatively, suppression might result from the *presence* within the cells of a sufficient level of some sulfur compound which disappears upon sulfur starvation. It is possible to discriminate between these two hypotheses, since only the former implies a stoichiometric relationship between sulfur metabolism and the diversion of phosphate. Several lines of evidence support the view that polyphosphate accumulation is suppressed, in a nonstoichiometric manner, by a constituent of the pool of sulfur compounds. In one group of experiments, we compared the ratio of sulfate metabolized to phosphate diverted in two mutants, O_{met-} and Sl_{u-}. As was shown above, polyphosphate accumulation was suppressed by sulfate in both; yet Sl_{u-} metabolized less than one-tenth as much $S^{35}O_4^=$ as did O_{met-}. A more direct experiment was performed with the double auxotroph, $O_{met\text{-}tyr-}$. The addition of methionine to a culture of this mutant completely blocks the conversion of sulfate to cystathionine (Harold, 1962*a*). Nevertheless, with tyrosine absent throughout to prevent growth, polyphosphate accumulation was suppressed both by sulfate alone and by sulfate plus methionine.

Independent support for the concept of a sulfur-containing regulator of polyphosphate metabolism, and a clue to its identity, came from experiments with the cystineless mutant O_{cys-}. Unlike all the other mutants used in these studies, three independent cystineless mutants failed to accumulate polyphosphate when subjected to sulfur or cystine starvation in W medium. Polyphosphate accumulation was, however, readily induced by either deficiency in T_{10} medium. This puzzling contradiction was resolved in the course of experiments on the fate of the acid-soluble pool during sulfur starvation. When cells of A3(0), previously labeled by growth in W medium supplemented with S^{35} sulfate or S^{35} cystine, were transferred to sulfur deficient medium, S^{35} was rapidly lost from the pool. In O_{cys-} previously grown on S^{35} cystine, no loss of S^{35} from the acid-soluble pool occurred upon sulfur starvation (Fig. 6).

The main constitutent of the acid-soluble sulfur pool in *A. aerogenes*, as in *Escherichia coli* (Roberts et al., 1957), was identified as oxidized glutathione by the criteria listed in the experimental section. This substance could serve as sole source of sulfur for the growth of all the strains used here, except for O_{cys-}. Cystineless mutants alone were unable to utilize oxidized glutathione, either intracellular or extracellular, but required cyst(e)ine or reduced glutathione. While the failure of O_{cys-} to metabolize oxidized glutathione is at present not understood, the correlation with the failure of these mutants to accumulate polyphosphate implicates oxidized glutathione in the regulation of the polyphosphate level.

Quantitative relationship between polyphosphate accumulation and adenosine triphosphate (ATP) utilization. In the course of studies on phosphate uptake in *Staphylococcus*, Hotchkiss (1956) demonstrated the suppression of polyphosphate accumulation by the addition of amino acids and obtained evidence for competition between polyphosphate synthesis and amino acid activation for cellular ATP. In the experiments de-

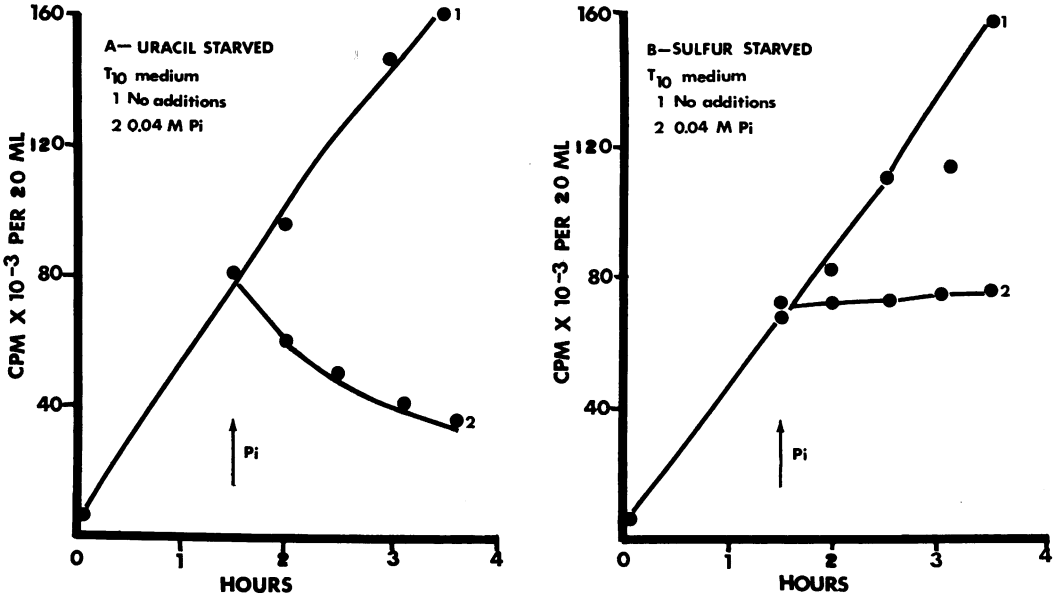

FIG. 4. *Loss of P^{32} from polyphosphate during suppression of accumulation. (A) Mutant Sl_{u-} was subjected to uracil starvation in T_{10}-P^{32} medium. After 90 min (arrow), 0.04 M unlabeled orthophosphate (Pi) was added to half the culture. (B) Mutant Sl_{u-} was subjected to sulfur starvation in T_{10} medium containing uracil and P^{32}. After 90 min, 0.04 M unlabeled Pi was added to half the culture. For the corresponding polyphosphate levels see Fig. 2.*

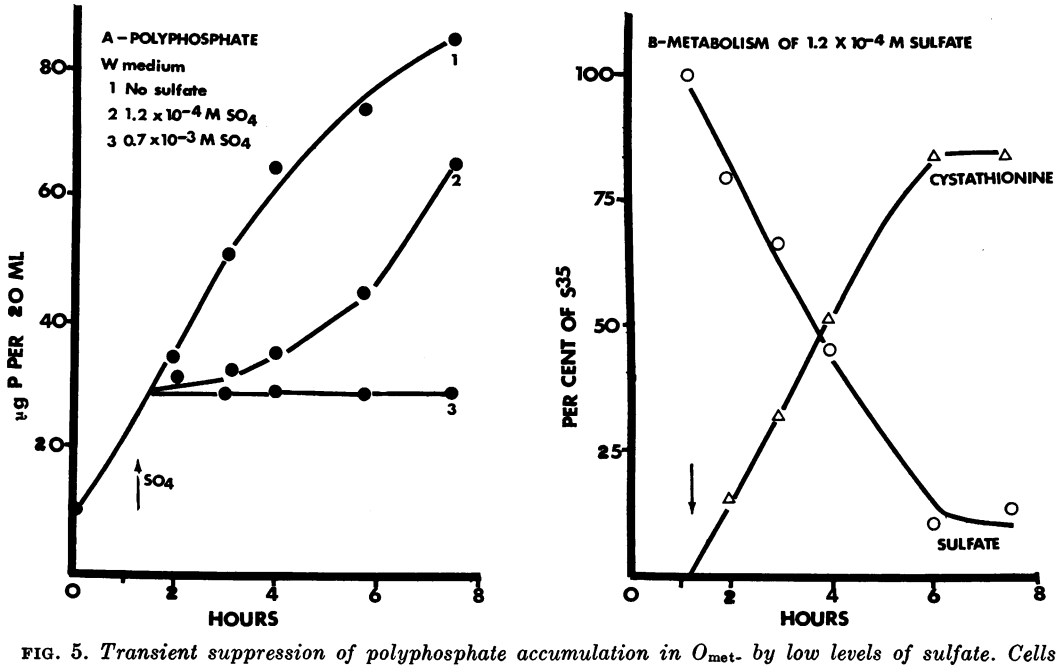

FIG. 5. *Transient suppression of polyphosphate accumulation in O_{met-} by low levels of sulfate. Cells were suspended in W medium lacking both sulfate and methionine. After 75 min (arrow), the culture was divided. Flask 1 received no additions, 2 received 1.2 × 10^{-4} M S^{35} sulfate and 3 received 0.7 × 10^{-3} M sulfate. (A) Polyphosphate accumulation in all flasks. (B) Cystathionine production in flask 2 only (1.2 × 10^{-4} M S^{35} sulfate).*

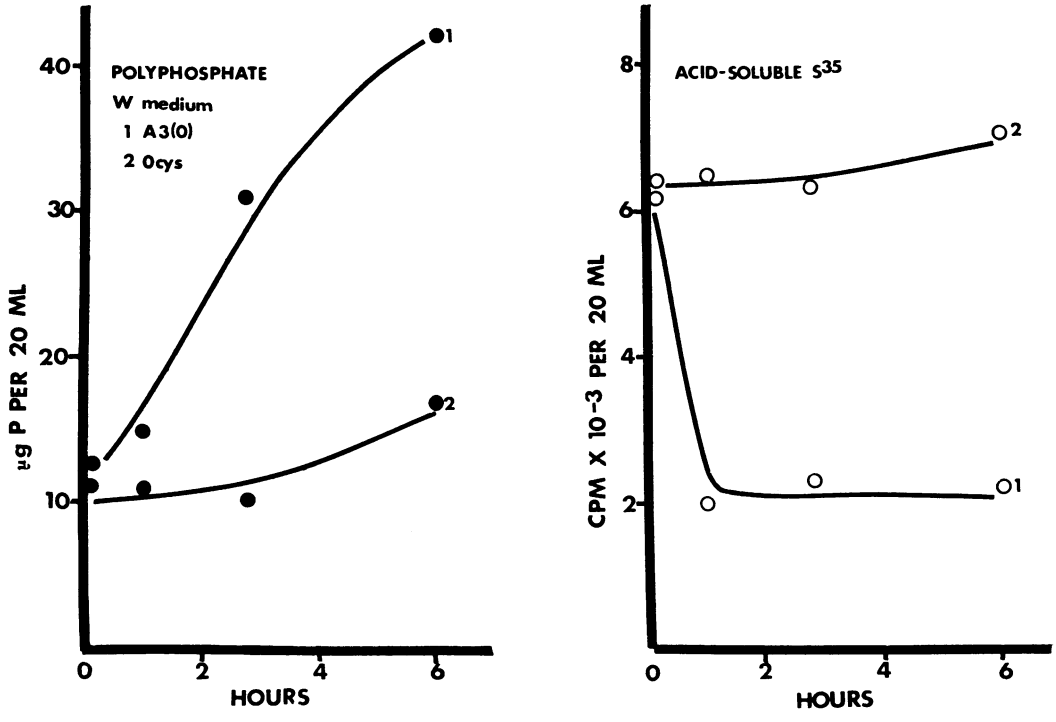

FIG. 6. *Loss of the acid-soluble sulfur pool and accumulation of polyphosphate in A3(0) and* O_{cys-} *. Cells were harvested from logarithmic cultures in W medium supplemented with* S^{35} *cystine and transferred at 0 hr to W medium containing neither sulfate nor cystine.*

scribed in this section, an attempt was made to estimate the fraction of available ATP consumed in polyphosphate accumulation in *A. aerogenes* and to inquire whether the suppression of this accumulation by salts or sulfur compounds could be attributed to a reduction in the supply of ATP.

Cells of the methionineless mutant O_{met-} were placed in T_{10} medium containing neither sulfate nor methionine, and the suspension was divided. One flask received no additions; the other received 0.5×10^{-3} M S^{35} sulfate. From the results presented in Fig. 7, it is clear that polyphosphate accumulation was not reduced by the simultaneous excretion of cystathionine. The pathway of cystathionine biosynthesis is moderately well understood, and the ATP expenditure can be calculated to be 3 moles per mole of cystathionine; 2 moles are consumed in the reduction of sulfate (Peck, 1962), and a third is apparently required in the formation of cystathionine from cysteine (Rowbury, 1961). In polyphosphate biosynthesis, 1 mole of ATP is consumed per phosphate group incorporated. Since the net amounts of polyphosphate and cystathionine produced in flask 2 were nearly equal and there is little polyphosphate degradation in T_{10} medium, it is evident that polyphosphate metabolism can account for no more than 25% of the ATP available to the cells. Possible consumption of ATP during sulfate or phosphate transport is neglected in this estimate.

Since polyphosphate synthesis requires but a fraction of the total ATP available to the cells, reduction in the availability of ATP does not seem a probable explanation for the suppression of polyphosphate accumulation by sulfur compounds and salts. This conclusion is supported by comparison of the rate of chloramphenicol-induced ribonucleic acid synthesis in T_{10} and W media. The rates of P^{32} incorporation into nucleic acids were the same, and about three times as rapid as the maximal rate of polyphosphate synthesis. Since the ATP expenditure for nucleic acid synthesis is far higher than that for an equivalent amount of polyphosphate, it can again be concluded that polyphosphate synthesis requires but a small fraction of the available

ATP, and that suppression of polyphosphate accumulation cannot be due to a lack of ATP.

Discussion

From the results presented in this and the preceding paper, it is apparent that cessation of nucleic acid synthesis is a necessary condition for the accumulation of polyphosphate but not always a sufficient one. The outcome depends upon the growth medium: in T_{10} medium polyphosphate accumulates whenever nucleic acid synthesis is halted in the presence of an energy source, phosphate, K^+, Mg^{++}, and NH_4^+; in W medium, sulfur starvation alone induces polyphosphate accumulation.

The specific requirement for sulfur starvation as a prerequisite for polyphosphate accumulation in W medium is best attributed to the presence within the cells of an inhibitor, presumably a sulfur compound. Depletion of the sulfur pool by sulfur starvation releases the inhibition, and polyphosphate then accumulates; conversely, replenishment of the pool upon addition of sulfate halts the accumulation. The finding that the effect of sulfate was not stoichiometric indicates that the presence of some sulfur compound, rather than the metabolism of the added sulfate, suppresses polyphosphate accumulation. Since the inhibitor is formed from sulfate in the absence of protein synthesis, a substance of low molecular weight was implicated. Two independent lines of evidence point to (oxidized) glutathione, or a closely related compound, as the hypothetical inhibitor. (i) Polyphosphate accumulation in sulfur-starved cells is suppressed by addition of cystine, glutathione, and their precursors, but not by methionine and its precursors nor by the sulfur-containing vitamins. (ii) Cystine-requiring mutants, unlike the wild type, cannot utilize oxidized glutathione as sole source of sulfur. Consequently, these mutants do not deplete their intracellular glutathione pool during sulfur starvation, and also fail to accumulate polyphosphate in W medium. The conclusion that intracellular glutathione regulates the polyphosphate level has also been reached by Pine (1962) on the basis of experiments with *E. coli*. It should be remembered that polyphosphate accumulation is specifically dependent upon sulfur starvation only in a phosphate-rich medium. We must therefore conclude that polyphosphate accumulation is suppressed by the *combination* of a high intracellular glutathione level and a high extracellular phosphate level. Either, by itself, permits polyphosphate to accumulate when nucleic acid synthesis ceases.

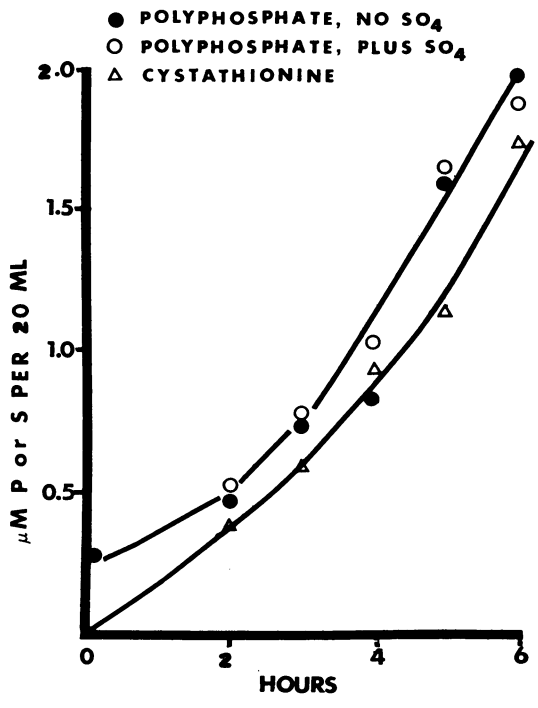

FIG. 7. *Effect of concurrent cystathionine excretion on the accumulation of polyphosphate by* O_{met-}. *Cells were suspended in* T_{10} *medium containing no sulfur (flask 1) or* 0.5×10^{-3} *M sulfate (flask 2). Note that both polyphosphate and cystathionine accumulation are given in μmoles.*

Suppression of polyphosphate accumulation by manipulation of the glutathione or phosphate concentration resulted in a constant polyphosphate level for several hours. This proved to be due to a steady state of polyphosphate synthesis and degradation: incorporation of P^{32} into polyphosphate continued at half the rate found in cells accumulating polyphosphate, while polyphosphate degradation was stimulated at least fivefold. Since the latter effect was demonstrable even when polyphosphate synthesis was blocked by inhibitors of ATP synthesis, it is our opinion that the primary effect of glutathione plus high phosphate is to accelerate polyphosphate degradation, the reduction in the rate of P^{32} incorporation being a secondary consequence thereof. It also appears that the constancy of the polyphosphate level is fortuitous, since there was no obligatory coupling

between the uptake and discharge of phosphate. In the absence of reliable information concerning the pathway of polyphosphate degradation in *A. aerogenes*, speculations about the mechanism by which the rate of this reaction is controlled by glutathione would be premature. It should, however, be pointed out that the present experiments were carried out under conditions inhibitory to protein synthesis and thus presumably reflect varying reaction rates at constant enzyme levels.

Some of the factors governing the polyphosphate level in *A. aerogenes* are thus beginning to emerge. The polyphosphate level at any time must be a function of the rates of polyphosphate synthesis and degradation. So long as nucleic acid synthesis proceeds, accumulation of a large polyphosphate pool is prevented by the high rate of polyphosphate degradation and perhaps by competition for ATP as well (Harold, 1963). When nucleic acid synthesis is blocked in the presence of a source of energy and metal ions, phosphate from the medium flows into the polyphosphate pool at a fixed rate which should be determined primarily by the amount and activity of the enzyme polyphosphate kinase. Phosphate uptake, ATP generation, and other pathways of ATP utilization are clearly not limiting factors in polyphosphate synthesis under these conditions (Fig. 7). The polyphosphate level will then depend upon the rate of concurrent polyphosphate degradation, which is in turn a function of the intracellular glutathione and extracellular phosphate levels and may be high enough to prevent net accumulation altogether.

A reappraisal of current views on the physiological significance of polyphosphate accumulation would thus seem to be in order. Since this is an ATP-dependent process resulting in the accumulation of an "energy-rich" compound (Yoshida, 1955), the formation of polyphosphate when energy generation is uncoupled from growth is generally related to the regulation of ATP metabolism—either as a microbial phosphagen (Hoffmann-Ostenhof and Slechta, 1958; Hoffmann-Ostenhof, 1962) or as a device for the dissipation of ATP (Harold, 1962*b*). It now appears, however, that polyphosphate synthesis accounts for but a small fraction of the available ATP (*see also* Liss and Langen, 1962; Kaltwasser, 1962), and that polyphosphate degradation is not necessarily associated with a high rate of ATP utilization. We are therefore inclined to discount the role of polyphosphate in energy metabolism. Polyphosphate accumulation is a problem not in bacterial energetics but in phosphorus balance, and bears more analogy to the formation of expanded pools of amino acids or monosaccharides than to the deposition of creatine phosphate in muscle. The phosphate thus accumulated within the cells is available, as orthophosphate if not as ATP, for future use. Polyphosphate is undoubtedly a storage form of phosphorus, but its formation may also be of significance in the maintenance of a low intracellular orthophosphate level (Kaltwasser, 1962).

ACKNOWLEDGMENTS

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