DEPLETION AND REPLENISHMENT OF THE INORGANIC POLYPHOSPHATE POOL IN *NEUROSPORA CRASSA*¹

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ABSTRACT

HAROLD, F. M. (National Jewish Hospital, Denver, Colo.). Depletion and replenishment of the inorganic polyphosphate pool in *Neurospora crassa*. J. Bacteriol. **83**:1047-1057. 1962.—Turnover of the inorganic polyphosphate pool of *Neurospora crassa* was demonstrated in both growing and nongrowing mycelium. In nitrogen-deficient cultures, polyphosphate synthesis and degradation were in balance and no net changes occurred. When mycelium was suspended in a growth medium deficient in phosphate, polyphosphate was degraded to acid-soluble compounds, including adenosine triphosphate (ATP) and orthophosphate; net synthesis of nucleic acids and phospholipids occurred at the expense of polyphosphate. Various attempts were made to demonstrate direct formation of ATP from polyphosphate when oxidative and fermentative ATP generation were blocked. No evidence for this reaction could be obtained, suggesting that the primary product of polyphosphate degradation is not ATP but probably orthophosphate.

Addition of phosphate to phosphate-starved mycelium induced rapid replenishment of the polyphosphate pool; polyphosphate accumulation ceased abruptly when the original level had been restored. The finding that ATP accumulation within the mycelium preceded polyphosphate synthesis, together with the rapid turnover of this compound, supports the view that ATP is the metabolic precursor of polyphosphate.

The results suggest a cyclic pattern of polyphosphate metabolism. In *N. crassa* at least, polyphosphate does not appear to function as a reservoir of "high-energy" phosphate, but the polyphosphate cycle may be involved in the dissipation of excess ATP.

The inorganic polyphosphate (volutin) content of microbial cells varies within wide limits, depending upon the organism and the nutritional conditions. In general, accumulation of polyphosphate is associated with inhibition of growth while a source of metabolic energy is still available. Such conditions may be encountered in "aging" cultures (Katchman and Fetty, 1955) and during starvation for specific nutrients such as sulfur (Smith, Wilkinson, and Duguid, 1954) or essential growth factors in auxotrophic mutants (Harold, 1960). In many microbial species, rapid and extensive accumulation of polyphosphate may be induced by adding phosphate to a culture previously deprived of this nutrient (Wiame, 1949; Smith et al., 1954).

Recent studies have partly clarified the pathway of polyphosphate biosynthesis. A number of microorganisms contain an enzyme, polyphosphate kinase, which catalyzes the transfer of the terminal phosphate group of adenosine triphosphate (ATP) to polyphosphate (Yoshida and Yamataka, 1953; Kornberg, Kornberg, and Simms, 1956). In some cases at least, this reaction is reversible (Winder and Denny, 1955; Kornberg, 1957), and could thus serve to regenerate ATP from adenosine diphosphate (ADP) and polyphosphate.

These results have suggested a possible function for polyphosphate in microbial energy metabolism, and also provide a basis for speculation on the regulation of the polyphosphate level within the cell (Hoffmann-Ostenhof and Weigert, 1952; Kornberg, 1957; Huennekens and Whiteley, 1960). It is proposed that polyphosphate is in equilibrium with the adenosine nucleotide system. When the rate of ATP generation exceeds the rate of its utilization for biosynthetic or work functions, polyphosphate would accumulate. Conversely, if the rate of ATP dephosphorylation exceeds that of ATP generation, rephosphorylation of ADP would occur at the expense of

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polyphosphate. The function of polyphosphate would thus be analogous to that of the phosphagens of animals.

The present paper describes experiments on the metabolic relationships between polyphosphate and the adenosine nucleotides in intact, living *Neurospora crassa*. The results suggest the need to reconsider both the function of polyphosphate and the factors controlling its level within the cell.

MATERIALS AND METHODS

Growth experiments. The wild strain of *N. crassa*, Em5297a, was grown overnight on the synthetic medium described earlier (Harold, 1960), harvested, washed, and resuspended in deficient media or in the presence of inhibitors. Aerobic cultures were shaken in air at 120 cycles per min; anaerobiosis was induced by passing nitrogen through the cultures.

Fractionation of phosphorus compounds. Procedures for the fractionation of the phosphorus compounds of *N. crassa*, and for the estimation of some of them, have been described earlier (Harold, 1960). Briefly, mycelium was dried with acetone and then extracted with cold 0.5 N perchloric acid (PCA; twice for 15 min at 4 C), ethanol and ethanol-ether, and hot 0.5 N PCA (twice for 15 min at 70 C).

The following determinations were carried out on the combined cold PCA extracts. Orthophosphate (P_i) and total phosphate (P_{tot}) were determined by Allen's (1940) method; P_i^{32} was determined by the method of Weil-Malherbe and Green (1951). Nucleotides were removed by adsorption on Norit A (see below); any acid-labile phosphate remaining in the supernatant fluid was considered to be soluble polyphosphate. The P^{32} content of the soluble polyphosphate was determined by precipitation with Ba^{++} in the presence of carrier polyphosphate. The organic phosphorus fraction (P_o), defined as $P_{tot} - (P_i + \text{soluble polyphosphate})$, was determined by difference.

The combined hot PCA extracts contained the insoluble polyphosphate together with the nucleic acids. The latter were removed by adsorption on Norit A and determined by difference. The residual phosphorus, virtually all of which was acid-labile, was considered to be the insoluble polyphosphate. Thus, the total P^{32} content of the charcoal supernatant was taken

as an approximate measure of the P^{32} in insoluble polyphosphate. In some experiments, more precise data for the specific radioactivity of the insoluble polyphosphate were obtained by precipitation with Ba^{++} , using a modification of the method of Katchman and Fetty (1955). However, recoveries were poor, and the use of this method was therefore restricted to the determination of specific radioactivity.

Nucleotide analysis. Nucleotides in the cold PCA extract were adsorbed on Norit A. The charcoal was washed with water and eluted with 1% NH_3 in 60% acetone as described by Tsuboi and Price (1959). Recoveries of 80% or better were obtained with Norit A deactivated by treatment with secondary octanol (Threfall, 1957). The eluates were evaporated in vacuo and the nucleotides redissolved in water.

ATP, ADP, and adenosine monophosphate (AMP) were measured enzymatically by the methods of Bücher, essentially as described by Thorn et al. (1955). In some experiments, the specific radioactivity of the terminal phosphate of ATP was determined after liberation with potato adenosine triphosphatase. ATP was isolated by paper chromatography (Krebs and Hems, 1953); the spot containing the ATP was treated on the paper with the potato enzyme (Lee and Eiler, 1951), exactly as described by Whitam, Bartley, and Weber (1955). The P_i liberated was then extracted into isobutanol (Weil-Malherbe and Green, 1951) and its specific radioactivity determined. Excellent agreement was obtained between ATP values determined by the two methods.

Chemicals. Nucleotides were purchased from Sigma Chemical Co., enzymes and reagents for the determination of ATP, ADP, and AMP from Biochemica Boehringer. Norit A for use in the nucleotide analysis was purchased from Fisher Scientific Co.

RESULTS

Turnover of polyphosphate in growing and resting mycelium. Growing mycelium of *N. crassa* contains approximately 4 mg of polyphosphate P per g dry wt, half of which is extracted with cold PCA (soluble polyphosphate) and half with hot PCA (insoluble polyphosphate). This high level of polyphosphate is maintained throughout the growth of the culture (Harold, 1960). However, the constancy of the polyphosphate level con-

FIG. 1. Incorporation of P^{32} into nitrogen-deficient *Neurospora crassa*: A, phosphorus compounds; B, incorporation of P^{32} . Mycelium was suspended in nitrogen-deficient medium for 3 hr before addition of P^{32} at 0 min. Mycelial concentration, 200 mg dry wt per 100 ml. Specific activity as per cent of medium phosphate.

FIG. 2. Redistribution of phosphorus (A) and P^{32} (B) in phosphate starvation. Mycelium of *Em5297* was exposed to P^{32} for 5 min and resuspended in phosphate-deficient medium at 0 min. Mycelial dry weight per 100 ml: 100 mg at 0 min, 175 mg at 160 min.

ceals substantial turnover of the polyphosphate pool. When mycelium of Em5297 was grown overnight on medium containing P_i^{32} and then transferred to fresh, unlabeled growth medium, the amount of both soluble and insoluble polyphosphate increased threefold in 8 hr; at the same time, half of the P^{32} originally present in these two fractions was lost and transferred to other compounds.

Turnover of the polyphosphate pool was also demonstrable in the absence of growth and net synthesis of phosphorus compounds. Mycelium of Em5297 was harvested from an overnight culture, washed, and transferred to fresh medium lacking the nitrogen source. After 3 hr (to allow depletion of internal nitrogen reserves), P_i^{32} was added to the medium and its incorporation into various phosphorus compounds was followed. No net synthesis of any phosphorus fraction was observed, but substantial turnover of P_i , P_o , and polyphosphate took place (Fig. 1). The fact that but little P^{32} entered the nucleic acid fraction suggests that cellular turnover is minimal under these conditions. The turnover of polyphosphate thus presumably reflects the simultaneous occurrence of both synthesis and degradation, whose balance would determine the polyphosphate level.

Depletion of the polyphosphate pool during phosphate starvation. When mycelium was transferred to fresh medium lacking phosphate, growth and nucleic acid synthesis continued, but at the expense of the inorganic polyphosphate. During this time, the amount of P_i , P_o , and adenosine nucleotides per unit volume of culture remained approximately constant (Fig. 2A).

Results obtained with P^{32} are consistent with the interpretation that polyphosphate is broken down to acid-soluble products which are subsequently incorporated into nucleic acids, lipids, and other compounds. In a typical experiment, mycelium was exposed to P_i^{32} for 5 min and subsequently subjected to phosphate starvation. The fate of the P^{32} is shown in Fig. 2B: initially, most of the P^{32} was found in the P_i and P_o fractions; during subsequent growth, much of it was transferred to the lipids and nucleic acids, while the specific radioactivity of P_i and P_o decreased. In one such experiment, within 2 hr the specific activity of P_i fell to 30%, and that of the terminal phosphate of ATP to 22%, of their original value. At the same time, the P^{32} content of polyphosphate, especially that of the soluble fraction, passed through a peak, indicating the continued turnover of the polyphosphate pool. It is thus evident that polyphosphate

FIG. 3. Effect of anaerobic sucrose starvation on the levels of phosphorus compounds (A) and nucleotides (B) in Em5297. Mycelium was suspended in medium lacking phosphate and sucrose at 0 min and made anaerobic. Mycelial dry weight, 158 mg per 100 ml.

was a precursor of P_i , ATP, and other compounds, but the nature of the primary product of polyphosphate degradation remains in doubt.

Mobilization of the intracellular polyphosphate pool during phosphorus starvation was inhibited if circumstances did not permit growth. Simultaneous starvation of wild-type *N. crassa* for both phosphate and nitrogen resulted in the loss of only 35% of the insoluble and 60% of the soluble polyphosphate in 4 hr. During phosphate starvation under anaerobic conditions, 40% of the insoluble polyphosphate and none of the soluble were degraded in 6 hr. If sucrose and phosphate were both withheld, no polyphosphate degradation took place; indeed, there was some net accumulation of polyphosphate (Harold, 1960).

Is polyphosphate a storage form of energy-rich phosphate? Formation of acid-soluble phosphorus compounds, including P_i and ATP, by the degradation of polyphosphate, is in accord with the proposed role of polyphosphate as a microbial phosphagen. However, these transformations could also result from hydrolysis of the polyphosphate to P_i , which would then be metabolized further. To distinguish between these two routes, attempts were made to detect formation of ATP from polyphosphate when the fermentative and oxidative mechanisms of ATP generation were blocked.

N. crassa mycelium was harvested and resuspended in medium lacking the energy source,

sucrose, and phosphate. The culture was then made anaerobic to limit the availability of endogenous energy reserves (see below), and the changes in nucleotide and polyphosphate levels were followed. Anaerobic sucrose starvation induced slow degradation of the P_o fraction, including ATP, resulting in accumulation of P_i . Little or no nucleic acid breakdown was observed, and small losses of insoluble polyphosphate were balanced by an equivalent gain of soluble polyphosphate (Fig. 3).

More severe limitation of ATP generation was imposed by means of cyanide (0.1 M), 2,4-dinitrophenol (2.7×10^{-4} M), or iodoacetate (3×10^{-3} M, anaerobic). These concentrations were sufficient to block all incorporation of P^{32} into the mycelium. The effects of these inhibitors on mycelium suspended in medium lacking sucrose and phosphate were alike. The level of ATP dropped precipitously, with concomitant accumulation of AMP (Fig. 4B). At the same time, P_i accumulated within the mycelium, derived largely from the P_o fraction. Dinitrophenol induced slight degradation of polyphosphate (Fig. 4A); no loss of polyphosphate was seen in mycelium treated with iodoacetate or cyanide.

Although these results contradict the hypothesis of a rapid equilibrium between polyphosphate and ATP, the residual ATP found in the preceding experiments might still be derived from polyphosphate. If that were the case, the

FIG. 4. Effect of 2,4-dinitrophenol on the levels of phosphorus compounds (A) and nucleotides (B) in *Em5297*. Mycelium was suspended in medium lacking phosphate and sucrose at 0 min; 2,4-dinitrophenol (2.7×10^{-4} M) was then added. Mycelial dry wt, 136 mg per 100 ml.

TABLE 1. *etabolic Morigin of the terminal phosphate of ATP under various conditions**

Experiment	Conditions	Time	P (μmoles per g dry wt)			Specific activity†		
			P _i	Polyphosphate (total)	ATP	P _i	Polyphosphate (insoluble)	ATP

		<i>min</i>						
1	Anaerobic, sucrose starvation	0	31	—	2.1	75	5.2	92
		180	53	—	1.0	67	7.5	65
2	Anaerobic, sucrose starvation	0	43	114	3.9	228	11.7	189
		120	74	115	2.5	220	11.0	168
3	Iodoacetate (3 × 10 ⁻³ M), anaerobic	0	31	122	2.4	182	9.3	182
		20	47	110	0.5	155	8.3	155
4	2,4-Dinitrophenol (2.7 × 10 ⁻⁴ M)	0	46	97	2.2	275	10.9	260
		120	88	98	0.3	238	11.6	217
5	2,4-Dinitrophenol (2.7 × 10 ⁻⁴ M)	0	25	130	2.3	156	4.6	145
		90	85	110	0.7	120	6.1	70
6	Phosphorus starvation	0	31	100	3.1	163	14.2	155
		120	18	11.5	2.4	49	40‡	34

* Mycelium was exposed to P_i³² for 5 min and then subjected to the metabolic conditions shown. Total amounts and specific radioactivities were determined at the beginning and the end of the experiment.

† Count per min per μ g of phosphate.

‡ Approximately

terminal phosphate of the ATP should be in isotopic equilibrium with polyphosphate. To detect this reaction, 18-hr mycelium was exposed to P_i³² for 5 min, harvested, resuspended in medium lacking phosphate and sucrose, and subjected to anaerobiosis or metabolic inhibitors as before. Total amounts of P_i, ATP, and polyphosphate, as well as their specific radioactivities, were determined at various times. The results (Table 1) give no evidence for isotopic equilibrium between polyphosphate and the terminal phosphate of ATP. Only the last experiment (no. 6), describing the redistribution of P³² in mycelium growing in a phosphate-free medium, shows the expected correspondence of the final specific activities of P_i, ATP, and polyphosphate.

Replenishment of the polyphosphate pool. When P_i was added to a culture previously subjected to a period of phosphorus starvation, a dramatic resynthesis of both insoluble and soluble polyphosphate took place. Within 2 hr, the polyphosphate pool returned to its original level; net increase of the nucleic acid fraction began after a short lag of 30 to 60 min and growth resumed concurrently.

The syntheses of polyphosphate and of nucleic acid were easily dissociated. Nucleic acid synthesis could be completely suppressed by withholding the nitrogen source, without affecting either the rate of replenishment of the polyphosphate pool or the final level attained. Conversely, resumption of nucleic acid synthesis did not require prior replenishment of the polyphosphate pool. When phosphate-starved mycelium was exposed to a pulse of P_i³² and then returned to a phosphate-deficient medium, no net polyphosphate synthesis was seen; nevertheless, the P³², initially present in the P_i and P_o fractions, accumulated in the lipid and nucleic acid fractions.

The energy required for polyphosphate synthesis could be supplied either by fermentation or by respiration. This follows from the observations that, in the presence of the energy source, sucrose, replenishment of polyphosphate was observed both aerobically and anaerobically. In the absence of sucrose, it could be supported by endogenous reserve substances, but only under aerobic conditions. Both aerobic and anaerobic replenishment were blocked by iodoacetate

FIG. 5. Replenishment of the polyphosphate pool after phosphorus starvation. Mycelium of *Em5297*, phosphate starved for 3 hr, was exposed to a trace of P_i^{32} for 60 sec. It was then resuspended in complete, unlabeled medium at 0 min. A, phosphorus compounds; B, nucleotide levels. Mycelial dry wt, 138 per 100 ml.

and by 2,4-dinitrophenol. The latter observation has also been made by Windisch and Heumann (1960) in regard to yeast.

More detailed analysis of the sequence of events in replenishment of the polyphosphate pool was carried out in the absence of a nitrogen source, to minimize complications due to resumption of growth. Phosphate-starved mycelium was exposed to P_i^{32} for 1 min and then transferred to nitrogen-deficient medium containing unlabeled phosphate. The onset of polyphosphate accumulation was preceded by transient accumulations of P_i and ATP, both of which exhibited a pronounced "overshoot" (Fig. 5). During the first 30 min of replenishment, insoluble polyphosphate alone was made; after this ceased abruptly, accumulation of soluble polyphosphate began.

The fate of P^{32} under these conditions is shown in Fig. 6. Initially, the bulk of the P^{32} was found in P_i , nucleotides (including ATP, which was identified by paper chromatography), and in a

number of unidentified components of the P_o fraction, presumably intermediates of carbohydrate metabolism. In some experiments, the unidentified components accounted for 80% of the P^{32} present after a brief incubation. With the uptake of unlabeled phosphate from the medium, there was a pronounced flow of P^{32} from all these fractions to polyphosphate. This redistribution is consistent with the proposition that P_i and ATP are intermediates in polyphosphate synthesis. In the experiment shown in Fig. 6, for example, the P^{32} content of the ATP fell to 12% of its initial value within 40 min.

Essentially the same pattern was obtained when replenishment was carried out under nitrogen, but completion of the process required twice as long as in the presence of air. Also, the overshoot of P_i and ATP levels was much less pronounced.

With the completion of replenishment, the pattern of polyphosphate metabolism character-

FIG. 6. Redistribution of intracellular P^{32} during replenishment of the polyphosphate pool. Mycelium of *Em5297*, phosphate starved for 3 hr, was exposed to a trace of P_i^{32} for 60 sec and then resuspended in unlabeled, complete medium at 0 min. P^{32} content is referred to as the total amount in the mycelium at 0 min. Small amounts of P^{32} in lipids and nucleic acids omitted for clarity.

istic of normal cultures (Fig. 1) was re-established. Addition of P_i^{32} to a culture which had been allowed to replenish (aerobically) for 2 hr with unlabeled phosphate was followed by slow incorporation of P^{32} into the P_i and polyphosphate pools. Within 50 min, the specific activity of the P_i had reached 21% of that of the medium, while that of the insoluble polyphosphate was 3.7%. It should be stressed that cessation of replenishment was not accompanied by efflux of phosphate from the mycelium (Fig. 6), but apparently reflects equilibration of synthesis and breakdown within the mycelium.

DISCUSSION

When *N. crassa* mycelium was incubated in a medium containing P_i^{32} , the P^{32} was incorporated into various phosphorus fractions even in the absence of net synthesis. P_i and P_o exhibited the highest turnover, followed by the insoluble polyphosphate; incorporation of P^{32} into the soluble polyphosphate was considerably slower. In the case of nitrogen-starved mycelium, no net changes occurred in any fraction, and hence polyphosphate synthesis was presumably compensated for by concurrent degradation. In

mycelium growing in phosphate-deficient medium, degradation predominated over synthesis, though some incorporation of P^{32} into polyphosphate continued to take place. Conversely, when P_i was added to phosphate-starved mycelium, the original level of polyphosphate was quickly restored; concurrent degradation probably occurred but was not unequivocally demonstrated.

The results obtained in the present experiments are consistent with the current concept that ATP, generated either by fermentation or by respiration, is the immediate precursor of polyphosphate. The demonstration of net synthesis and rapid turnover of ATP during replenishment of the polyphosphate pool supplement the extensive evidence summarized by Huennekens and Whiteley (1960).

Interpretation of the results with regard to the pathway of polyphosphate degradation is both more novel and more hazardous. The finding that ATP, P_i , and subsequently lipids and nucleic acids are formed from polyphosphate during phosphorus starvation can be interpreted by the widely accepted hypothesis that polyphosphate is a storage form of "high-energy" phosphate and that ATP is the primary product of polyphosphate utilization (Nishi, 1961). The conclusion that this view is incorrect rests upon two lines of evidence. Inhibition of oxidative and fermentative ATP generation drastically lowered the ATP level, yet little or no degradation of polyphosphate was observed. This argues against the existence of a rapid equilibrium

FIG. 7. The polyphosphate cycle.

between polyphosphate and the adenosine nucleotides, and stands in striking contrast to the speedy loss of creatine phosphate from mammalian muscle poisoned with 3,5-dinitroresol (Parker, 1954). Furthermore, attempts to demonstrate transfer of phosphate from polyphosphate to the terminal group of ATP in the presence of inhibitors of ATP generation were not successful. The formation of ATP from polyphosphate thus appears to be an energy-dependent process.

It must be admitted that neither argument is entirely conclusive. Reservations include the possibilities that direct formation of ATP from polyphosphate might be a slow process (the localization of polyphosphate within a vacuole or granule might constitute a barrier to metabolic exchange), or inoperative under conditions that prevent growth. Also, the polyphosphate pool is undoubtedly metabolically heterogeneous (Langen and Liss, 1958); isotopic equilibration between ATP and the ends of polyphosphate chains might be obscured by analysis of the total pool, as was necessarily done in Table 1. Finally, Lynen (1958), as well as Langen and Liss (1958), described degradation of polyphosphate in yeast poisoned with dinitrophenol and iodoacetate, and attributed this to phosphorylation of ADP. Nevertheless, formation of ATP seems to be excluded as the *major* route of polyphosphate utilization in *N. crassa*.

A more likely pathway for the mobilization of polyphosphate appears to be a stepwise, hydrolytic cleavage to progressively smaller fragments, with the ultimate formation of P_i . Considerable support for the occurrence of this reaction sequence is available in the literature. Enzymes catalyzing the depolymerization and hydrolysis of polyphosphates are widely distributed among microorganisms (Ingelman, 1950; Mattenheimer, 1956). The specific activity of these enzymes may increase during a period of polyphosphate degradation (Nishi, 1960). Langen and Liss (1958) clearly demonstrated the degradation of high molecular weight polyphosphate to smaller fragments in intact yeast cells. The present results are consistent with the formation of low molecular weight, soluble polyphosphate from high molecular weight, insoluble polyphosphate (Bukhovich and Belozerskii, 1958; Belozerskii and Kulaev, 1958).

The over-all pattern of polyphosphate metabolism that emerges from these considerations is a

cyclic one, for which the term polyphosphate cycle was proposed by Langen and Liss (1958). A schematic representation of the polyphosphate cycle and its probable relationship to nucleic acid degradation is shown in Fig. 7.

If the present conclusions are correct, a revision of current concepts of the metabolic function of inorganic polyphosphate is required. It is clear that polyphosphate serves as a storage form of phosphorus, but it cannot be regarded as a microbial phosphagen, at least in *Neurospora*. In this connection it should be pointed out that the evidence for polyphosphate as a microbial phosphagen rests almost entirely upon work with cell-free extracts (Winder and Denny, 1955; Kornberg, 1957; Nishi, 1960). Evidence from work with intact cells is scant (Lynen, 1958; Langen and Liss, 1958; Widra, 1959; Shaposhnikov and Fedorov, 1960) and may be open to other interpretations. The polyphosphate cycle as depicted above suggests an alternative role for polyphosphate in microbial energy metabolism: the dissipation of excess ATP with separate regeneration of P_i and ADP. The utility of this apparently wasteful reaction, when the environment favors metabolism but restricts growth, has been stressed by Gunsalus and Shuster (1961).

The polyphosphate cycle also invites speculation on the replenishment and maintenance of a large polyphosphate pool. These are clearly dispensable functions, contingent upon the presence of excess P_i in the medium. Polyphosphate degradation would seem to be the rate-limiting step in polyphosphate metabolism, but there clearly exists a governor mechanism which sets an upper limit to the polyphosphate level, except in the case of auxotrophic mutants growing on a limiting supply of growth factor (Harold, 1960). By virtue of the interplay of phosphate uptake, polyphosphate synthesis and its degradation, the polyphosphate cycle may perform this function. As the polyphosphate pool expands, P_i formed via the cycle may restrict the entry of P_i from the medium, replacing net accumulation of polyphosphate with turnover of the pool. The occurrence of an early, transient "overshoot" of the P_i level (Fig. 7), which has also been noted in yeast (Ehrenberg, 1961), supports this interpretation.

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