Considerable information is available on the accumulation of polyphosphates and on the enzymes metabolising them (Kulaev (1975) Rev. Physiol. Biochem. Pharmacol. 73: 131). However, very little is known about the effectors that regulate the level of polyphosphates in vivo. We (EC 3.6.1.11, polyphosphate phosphohydrolase).

Cultures were grown on synthetic medium (Mattoo et al. (1973) Indian J. Exp. Biol. 11: 511) with either 0.0025% or 8.7 mg % of phosphate respectively. Cell extracts for enzymic activity were prepared and stored as described previously (Mehta et al. (1972) Biochem. J. 130: 151). Standard reaction mixtures contained: tris-HCl buffer (pH 7.6), 100 mM; MgCl2, 2 mM; 6-mercaptoethanol, 1 mM; dialysed sodium polyphosphate (pH 7.5), 1 mg; and an appropriate aliquot of enzyme solution in a final volume of 5 ml. Incubation was carried out at 23°C for various time intervals and reactions terminated by the addition of ice-cold trichloroacetic acid (final concentration 15%). Orthophosphate released was determined by the method of Friske and Subbarow (1925, J. Biol. Chem. 66: 375). One unit of enzyme activity corresponds to the release of 1 nmole of the product per min under the assay conditions. Only those values which showed a linear relationship with enzyme concentration and time of incubation were taken into consideration. Each experiment was run in duplicate and repeated twice.

The level of intracellular polyphosphate varied with the age of the culture and was found to be dependent upon the initial phosphate level in the culture medium (Fig. 1). Cultures grown on limiting phosphate concentration (1.7 mg %) elaborated a much higher level of polyphosphate which reached a maximum by 96 hr and was 4-fold higher than the enzyme level in the cultures grown on high phosphate (8.7 mg %)-containing medium.

In order to further examine the role of inorganic phosphate in modulating the intracellular level of polyphosphate, experiments were designed to check the effect of transferring limiting phosphate-grown cultures to fresh media containing either high or limiting phosphate on the levels of polyphosphate. The results (data not shown) demonstrated a marked increase (4-fold) in the polyphosphate level within 48 hr of transferring cultures grown on limiting phosphate medium to phosphate-deficient medium. Repression of enzyme activity was evident in cultures that had grown previously in limiting phosphate medium but were transferred to the high phosphate medium. The increase in enzyme obtained with cultures grown on limiting phosphate medium was distinctive and may be related to a low intracellular level of orthophosphate in those cultures compared to those grown in high phosphate.

Inclusion of orthophosphate (0.25 mM) in the reaction assay mixture resulted in 50% inhibition of enzyme activity as was also shown by Afanasieva and Kulaev (1973). Biocim.

Mutant ser (JBM5) was isolated by filtration enrichment (V. W. Woodward, J. R. de Zeeuw and A. M. Srn (1954) PNAS 40: 42) following ultraviolet irradiation to twenty percent survival of al-2 (15300); cot-1 (C102h1) A. Preliminary crosses indicated that ser (JBM5) was on linkage group I since it showed linkage to mating type, to locate ser (JBM5) with respect to ster-3, a sterile isolate of genotype ser (JBM5); org-5, a was crossed with ster-3 (47998 A) (FOSC #1211); on Westergaard-2 (1964). Treatment of 0.2 g/l-L-serine, 0.15 g/l-D-serine, 0.1 g/l-L-arginine and 2% agar. Random spores were isolated onto small slants of appropriately supplemented Vogel's medium containing 2% sucrose. The tinge spore isolates were heat shocked at 60°C for 45 minutes and incubated at 32°C. Of 1026 spore isolates, 528 required serine alone and 498 required both serine and arginine. No serine-independent recombinants were obtained. We conclude that ser (JBM5) is allelic with ster-3.

Mitchell medium (1947, Am. J. Bot. 34: 573) containing 2% sucrose and 0.2 g/l-L-serine, 0.15 g/l-D-serine, 0.1 g/l-L-arginine and 2% agar. Random spores were isolated onto small slants of appropriately supplemented Vogel's medium containing 2% sucrose. The tinge spore isolates were heat shocked at 60°C for 45 minutes and incubated at 32°C. Of 1026 spore isolates, 528 required serine alone and 498 required both serine and arginine. No serine-independent recombinants were obtained. We conclude that ser (JBM5) is allelic with ster-3.