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PHOSPHATE MEDIATED BIOCHEMICAL CHANGES IN *NEUROSPORA CRASSA*

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PHOSPHATE was found to influence the morphology of *Neurospora crassa*. High phosphate was found to stimulate growth, sugar uptake and polyphosphate content of the culture. Carotenoids were found to be inhibited under high phosphate conditions. Glucose 6P dehydrogenase, mitochondrial and cytosolic malate dehydrogenase and invertase showed considerable decrease in the activity while amylase showed an increase in the activity under high phosphate conditions as compared to low phosphate conditions. These data demonstrate a significant regulatory role of phosphate in primary as well as secondary metabolism.

Though the regulation of secondary metabolism by inorganic phosphate is well documented[1,2] information on the role of inorganic phosphate in the regulation of enzymes of primary metabolism leading to the formation of secondary metabolites is meagre. Increase in the production of a variety of commercially important and useful secondary metabolites can be achieved by adjusting carefully the phosphate concentration in the growth medium. An understanding of the control of enzymes of primary metabolism by phosphate could boost the production of secondary metabolites. The present study demonstrates the increased production of invertase and carotenoids by controlling the phosphate in the growth medium, which controls several enzymes of primary metabolism.

The carotenogenic strain of *N. crassa* (wild type) was maintained on Saboraud's agar slants. The composition of the synthetic medium employed was the same as described earlier[3]. The culture was grown in 100 ml liquid medium in 250 ml Erlenmeyer flasks on a rotary shaker (180 rpm) at 30° C for 6 days. The mycelia were harvested and stored at −5° C before use. For growth measurement the mycelia were dried at 50° C to a constant weight. The growth was expressed as dry mat weight per 100 ml.

All studies were carried out using 5% glucose as carbon source. For study of amylase and invertase, 1% starch and 2.5% sucrose respectively were added as carbon sources in the growth medium.

'Low phosphate' indicates the addition of 0.01% KH_2PO_4 while 'high phosphate' means the addition of 1% KH_2PO_4 to a synthetic medium devoid of phosphate. These concentrations were selected since they were used earlier in this laboratory[4]. The pH after growth was not affected under these conditions.

Protein, carotenoids and polyphosphates were estimated according to the methods of Warburg and other workers[5-7].

For intracellular enzyme assays, a cell-free extract for cytosolic as well as mitochondrial enzyme was prepared as described earlier[8].

The assay methods used for malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) and glucose-6P dehydrogenase (D-glucose 6-phosphate: NADP oxidoreductase, EC 1.1.1.49) were the same as described by Ochoa[9] and Kornberg and Horecker[10] respectively. Units are defined as the amount of enzyme which causes 0.001 change in optical density at 30° C per min.

Amylase (1,4 α D glucan maltohydrolase, EC 3.2.1.2) was assayed according to the method of Bernfeld[11]. Invertase (B fructofuranoside fructohydrolase, EC 3.2.1.26) activity was measured by estimating the reducing sugars released by Bernfeld method[11]. Unit of amylase and invertase are defined as the amount of enzyme which cause the liberation of 1 μ mole of maltose and reducing sugars respectively at 37° C hr.

TABLE 1

Changes in growth, glucose utilization, polyphosphate, carotenoids and malate dehydrogenase from N. crassa grown under low and high phosphate conditions.

	Low Phosphate	High Phosphate
Growth dry wt. (g/100 ml)	0.48	0.57
Glucose uptake (g/100 ml)	2.5	4.23
Polyphosphate (μg/mg mat)	390	619
Carotenoids (mgs/mat)	72.05	ND
Malate dehydrogenase (units/mg protein)		
Cytosolic	31	44
Mitochondrial	44	9

ND: Not detectable

N. crassa when grown under high phosphate conditions showed an increase in the growth and sugar uptake as compared to low phosphate conditions (table 1). Earlier Liras *et al*[12] and Martin and McDaniel[13] had also shown that upon the addition of excess phosphate to the growth medium, the rate of glucose utilization and that of growth were increased.

The morphology of *N. crassa* was found to be affected to a considerable extent. The cells were found to be more swollen, with bigger diameter in high phosphate conditions. Tsukagoshi *et al*[14] had also shown in *Bacillus brevis* 47 that the morphology of cells varied according to the concentration of the phosphate.

High phosphate grown cells of *N. crassa* showed more intracellular granules, probably of polyphosphate in nature. When polyphosphate was estimated, it was found to be present in much higher amounts in high phosphate conditions (table 1). In *Saccharomyces cerevisiae* also, phosphate is known to be accumulated in the form of polyphosphate[15]. The carotenoid content in our studies was also found to be higher in low phosphate conditions but in high phosphate, it was not detectable. One of the factors which greatly influences carotenogenesis is the availaility of reducing power. The need for reducing power can be met with dehydrogenases. Our studies with low and high phosphate conditions showed that glucose-6P dehydrogenase and cytosolic and mitochondrial malate dehydrogenase showed lower activities under high phosphate conditions (table 1 and figure 1). This may probably decrease the generation of reducing power and could thereby be one of the factors which affects the level of carotenoids under high phosphate conditions.

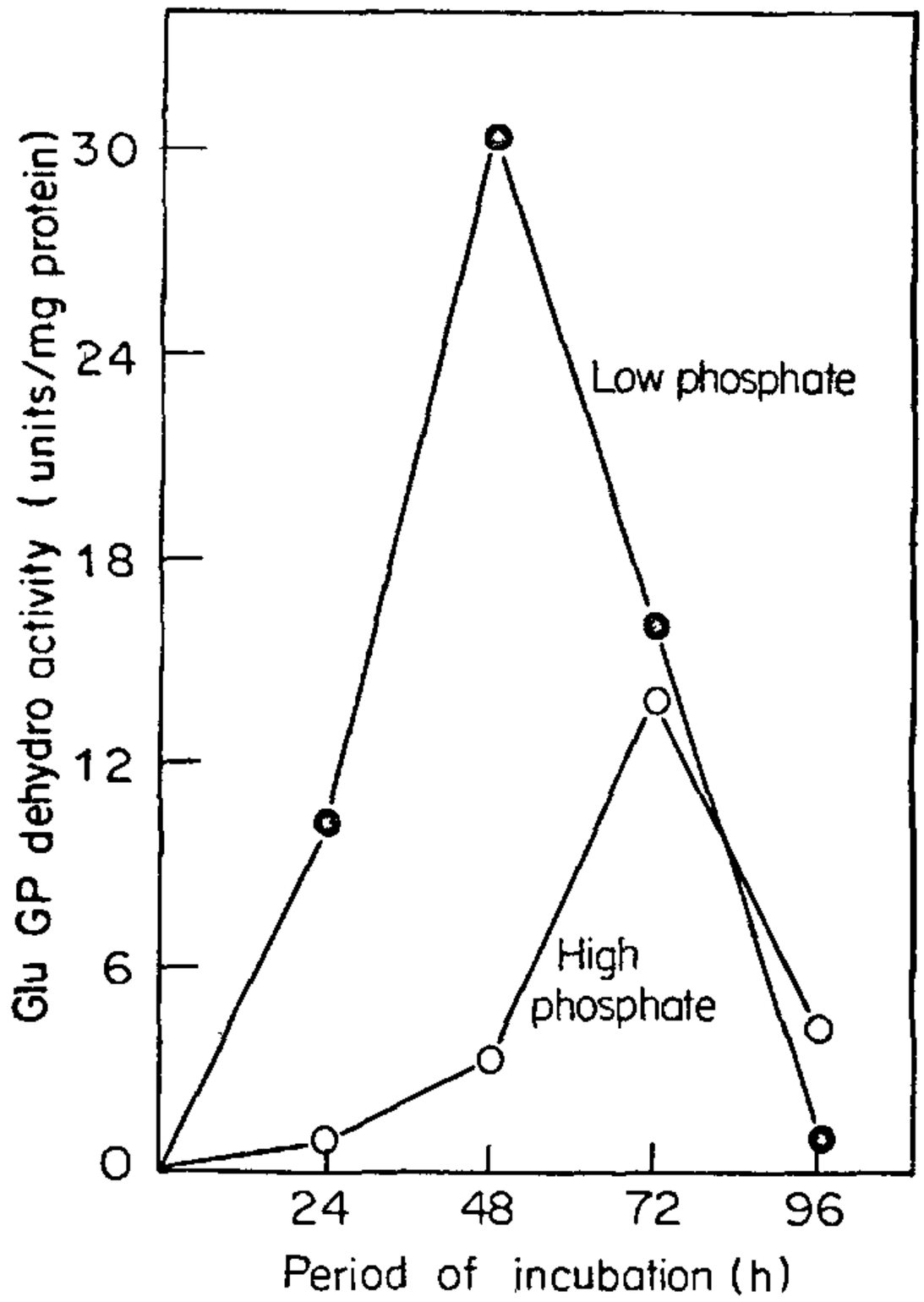

Figure 1. Effect of inorganic phosphate on glucose 6P dehydrogenase activity from *N. crassa.*

Phosphate was also found to influence the activities of extracellular enzymes. Under high phosphate conditions amylase was found to be higher (Not presented here). However, invertase was found to be significantly higher in low phosphate grown cells.

These studies on the regulatory effects of phosphate on biochemical changes will be useful in understanding the influence of enzyme levels of primary metabolism on secondary metabolism. Boosting of related primary metabolism might trigger secondary metabolism and hence the desired products of secondary metabolism can be increased by maintaining a suitable phosphate concentration in the growth medium.

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APOSPORY IN *EREMOPOGON FOVEOLATUS* (DEL.) STAPF.

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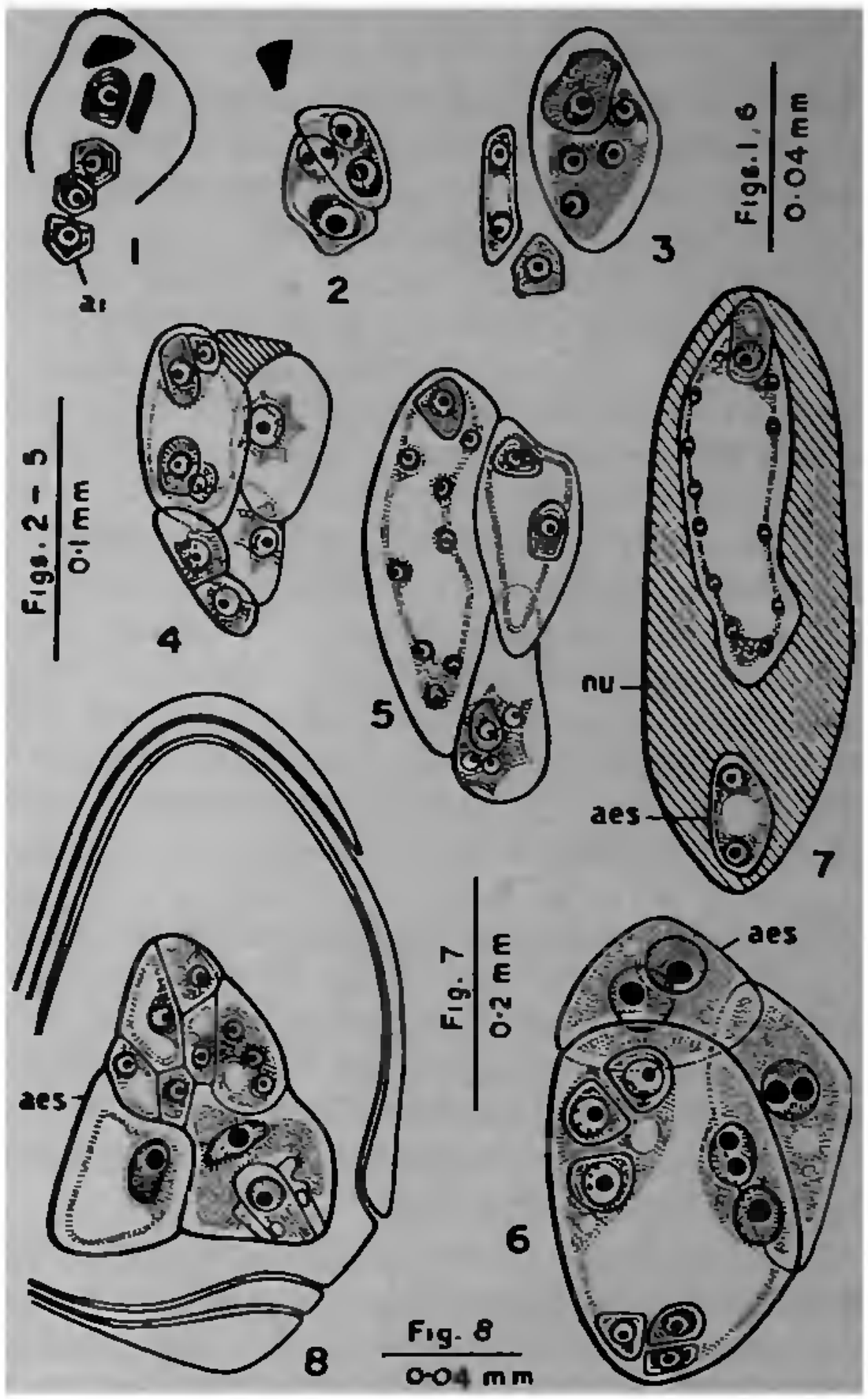
THE family Poaceae is of especial interest to the embryologists with its problems associated with apospory, polyembryony and vivipary[1-6]. The present report records the occurrence of apospory in *Eremopogon foveolatus* growing wildly in Visakhapatnam and its outskirts.

In the ovules of *E. foveolatus* meiosis of the megaspore mother cell is normal resulting in linear tetrad of megaspores. While in a few cases the megaspore mother cell degenerates without further division. Such a situation is often associated with enlargement and vacuolisation of one or more nucellar cells which form the aposporous initials, though these appear above or below or at the sides of the young sexual embryo sacs (figures 1–7). The aposporous initials enlarge and force their way to displace the normal embryo sac (figure 4). During enlargement these initials undergo two nuclear divisions mitotically forming a four nucleate embryo sac. The aposporous embryo sac is much smaller in size and differs in shape from the normal embryo sac.



Figures 1–8. 1–5. The development of aposporous embryo sacs. **6.** Multiple embryo sacs. **7.** Ovule showing a normal embryo sac at the micropylar end and an aposporous embryo sac at the chalazal end. **8.** L.s. ovule showing sexual embryo sac and aposporous 1-nucleate sac. (aes. aposporous embryo sac; ai. aposporous initial; nu. nucellus).

The organisation of the four nuclei in the mature aposporous embryo sac is also not constant. Some of these show one egg, one synergid and two polar nuclei, whereas others show one egg, two synergids and one polar nucleus depending on whether the dividing nuclei of the aposporous initial remain together or part to the poles.

Rarely there is a single aposporous embryo sac in the ovule (figure 8), but in a majority of the ovules two to five aposporous embryo sacs could be observed in the same ovule (figures 4–6). These multiple embryo sacs are crowded in the ovules and develop in close contact with each other, sometimes encroaching into one another. The position of the egg apparatus is not restricted to the micropylar end but is variable.