Changes in the Activities of Aldolase and of D-Glyceraldehyde-3-Phosphate Dehydrogenase during the Mitotic Cycle in Microspores of *Lilium longiflorum**‡§

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ABSTRACT

Microspores of *Lilium longiflorum* were isolated at various stages of development surrounding the mitotic interval and were analyzed for changes in the activities of D-glyceraldehyde-3-phosphate dehydrogenase and aldolase. Fructose 1,6 diphosphate was used as substrate. Activities were measured by the increase in optical density due to the reduction of diphosphopyridine nucleotide.

It was found that mitosis occurs during the minimal activity of both aldolase and D-glyceraldehyde-3-phosphate, thus indicating that heightened glycolytic capacity is not necessarily related to mitosis. It was also found that soluble-SH levels were highest when the enzymes were least active. It appeared, therefore, that the "—SH enzymes" are not necessarily activated intracellularly by high concentrations of soluble thiol. These results are discussed in connection with the theory that soluble-SH compounds stimulate glycolysis and in this way initiate mitosis.

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INTRODUCTION

The precise nature of the energy sources for mitosis is undetermined. In 1931, Rapkine (1) suggested that mitosis was initiated by heightened glycolytic activity. The main idea in this theory—that glycolysis is the principal channel of mitotic metabolism—has been current for more than twenty-five years. Warburg (2), who was the first to observe a striking relationship between cell proliferation and glycolysis, recently reiterated his belief that rapidity of cell-division results from an impairment of respiration and an increase in fermentation. In contrast to this view, others (3) hold that the energy for mitosis is anticipated by respiration during interphase. Energy thus stored is considered to be necessary for mitosis but not to be part of the triggering mechanism. The results to be reported, which describe the variations in capacities of mitotic cells to metabolize fructose 1,6 diphosphate, have a bearing on this controversy.

Materials and Methods

Plants of *Lilium longiflorum* Thunb. (var. "Croft") were raised from bulbs in a greenhouse. Flower buds of appropriate length were removed and kept on ice until anthers were excised to prepare suspensions of microspores. The use of bud length as an index of microscope development has already been fully described (4).

Groups of four anthers were placed in 2 ml. of 0.5 M sucrose, and twice slit longitudinally with glass needles to expose the microspore-containing interior. All operations were carried out at temperatures ranging from 0 to 5°C. To facilitate removal of the microspores, the anthers were then cut into three fragments by transverse section and the whole stirred with a glass rod for 15 to 20 seconds. The mixture was filtered through two layers of cheese cloth, and the filtrate containing the microspores was retained. The residue, which consisted largely of wall and adjacent tissues, was again stirred in 2 ml. of sucrose, filtered, and the two filtrates combined. The microspore suspension was made up to 6 ml. with 0.5 M sucrose and centrifuged at 154 × *g* for 4 minutes. The sedimented cells were resuspended by shaking in 6 ml. of sucrose and again centrifuged.

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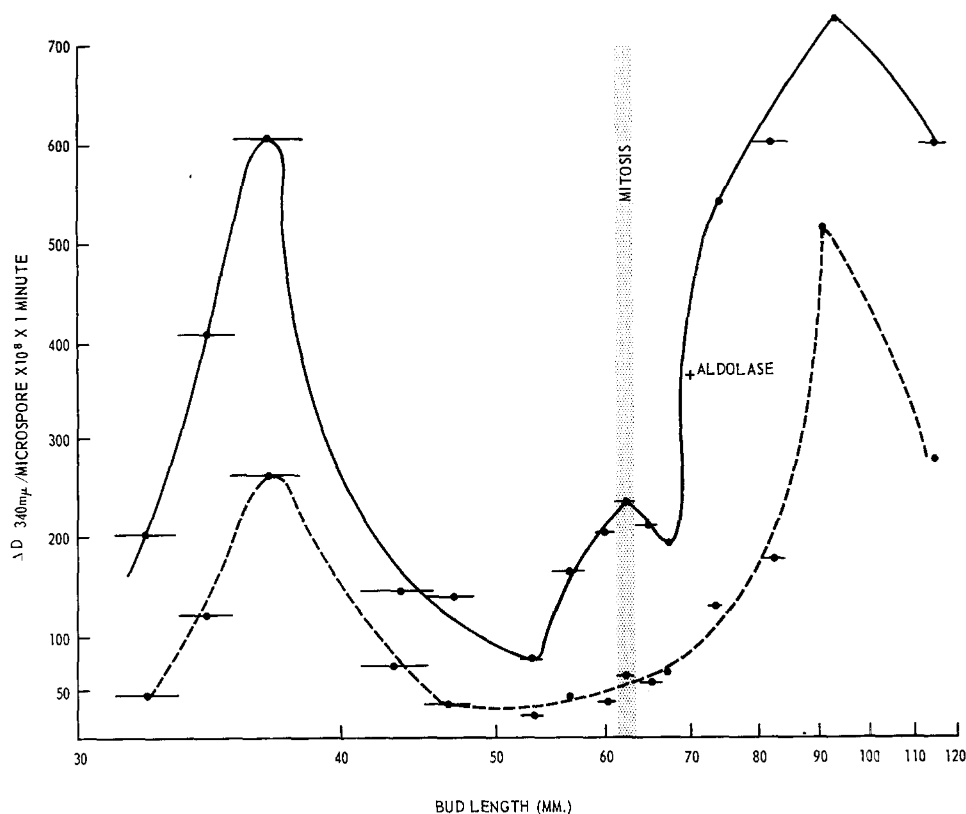

FIG. 1. Variations in aldolase and D-glyceraldehyde phosphate dehydrogenase activities during microspore development. The dotted line represents the activity of the enzyme system catalyzing the reduction of DPN by hexose diphosphate. The solid line represents activities measured in presence of an excess of crystalline aldolase. Condition of measurement described under Methods.

Microspores thus freed of most other components of the anther were suspended in 1.5 ml. of 0.5 M sucrose. Two 0.01 ml. portions were transferred to a microscope slide for counting and 0.02 to 0.05 ml. samples were taken for enzymatic assay. Parallel studies were also made with preparations of whole anthers. These were homogenized in 0.1 M Tris (hydroxy-methyl) aminomethane at pH 8.3 in the presence of 0.01 M cysteine. The final concentration of the suspension was 15 mg. fresh weight of anther per ml.

Aldolase and dehydrogenase activities were followed spectrophotometrically by measuring the changes in optical density at 340 mμ. The reactions were carried out in spectrophotometer cuvettes, a temperature of 25°C. being maintained in the cuvette chamber by means of thermospacers.

Homogenates of whole anthers were incubated in 3 ml. of medium containing the following components (in micromoles): Diphosphopyridine nucleotide (DPN), 0.5; Tris buffer (pH 8.3), 240; NaCN, 0.5; adenosinediphosphate, 0.2; inorganic phosphate, 1.0; nicotinamide, 1.0; fructose-1,6-diphosphate, 3. Microspores were incubated in a volume of 1 ml. The concentration of components in the medium was the same as for whole anthers with the exception that nicotinamide was omitted, and 10 micromoles of cysteine added. In assaying activities at different stages of microspore development, adenosinediphosphate and inorganic phosphate were replaced by sodium arsenate.

Under the conditions just described, aldolase and triose phosphate dehydrogenase would be measured together. It was found, however, that aldolase was the limiting reaction at all stages studied so that by adding crystalline aldolase in adequate amounts, the changes in activity of glyceraldehyde-3-phosphate dehydrogenase could be followed.

Cytological procedures: Lily microspores, because of their thick walls, are difficult to observe cytologically by commonly used techniques. The following method was, therefore, adopted to identify mitotic stages. Segments of anthers were fixed overnight or longer in a mixture of three parts absolute ethanol to one part glacial acetic acid. These were then placed on a microscope slide, the microspores teased out, and a drop or two of glycerin mixed with the fixative and microspores. The slide was warmed over an alcohol lamp until all

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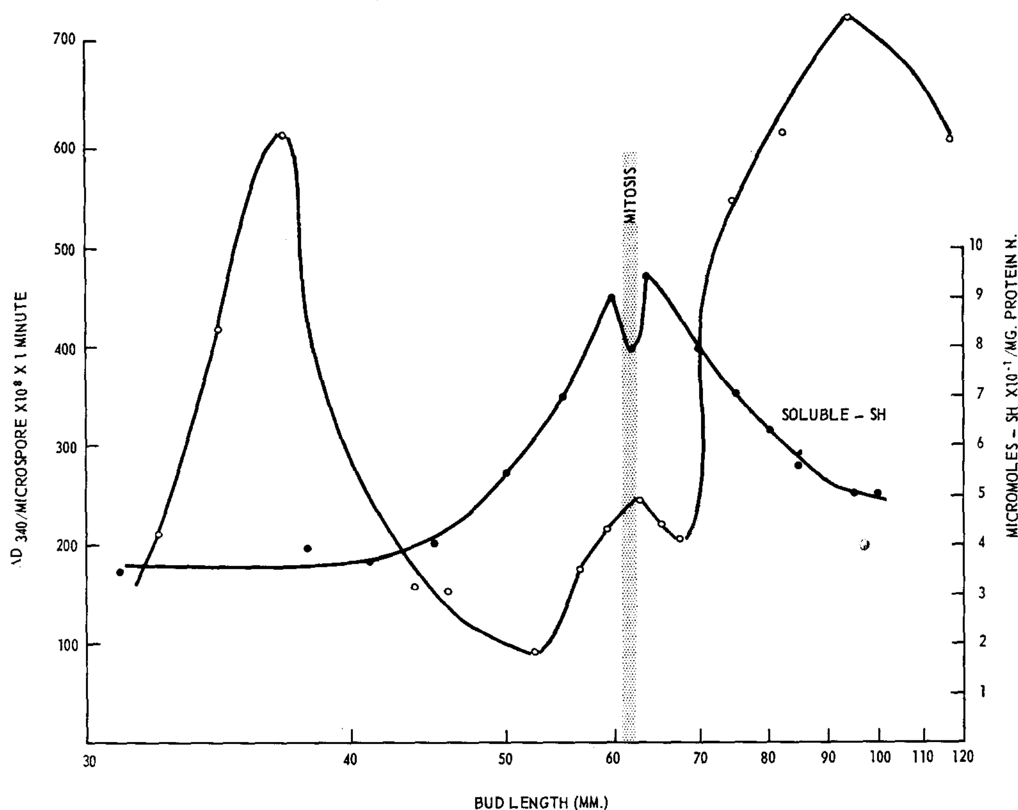

FIG. 2. A comparison of soluble sulfhydryl changes in anther tissues and the dehydrogenase activities of isolated microspores. For reasons discussed elsewhere (9), it may be presumed that the changes plotted in sulfhydryl concentration reflect the changes occurring within the sap of the anther containing the microspores.

the fixative evaporated and iron-acetocarmine was then added to the glycerin. The microspores were again warmed until sufficiently stained, and a coverslip added. At some stages of development the protoplasts are in this way freed of the cell wall; at others it is necessary to exert gentle pressure on the coverslip. The success of the method is mainly due to the fact that glycerin keeps the protoplasts from dispersing when squeezed out of the cells.

RESULTS

Initial experiments were carried out with homogenates of whole anthers and with isolated microspores to establish the identity of the enzyme system studied. The following properties of the system permitted the conclusion that the activities followed spectrophotometrically were those of the initial phase of glycolysis. In the presence of adenosinediphosphate and inorganic phosphate the rate of DPN reduction was five times that in their absence. Addition of arsenate to the system removed the need for these phosphates. Iodoacetate (2×10^{-3} M) inhibited the reaction, whereas cysteine (1×10^{-3} M) stimulated it. Cyanide (5×10^{-4} M) increased the apparent rate of coenzyme reduction.

The variation in enzymatic activities of developing microspores is plotted in Fig. 1. It was established that both substrate and DPN were present in excess at all stages examined. The variations observed did not arise from corresponding changes in sulfhydryl content of the tissue. Parallel assays run without cysteine in the test medium showed the same pattern of change except that the level of activity was much lower. No evidence was found for a variable stability of the enzyme system; at each developmental stage DPN reduction was followed for a period of 15 minutes and the linearity of the reaction was much the same throughout. It was, therefore, concluded that the variations observed reflected real variations in the capacity of the cells to reduce DPN via the initial steps of glycolysis.

The most striking feature of the developmental

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metabolic pattern is the U-shaped character of the curve enclosing the mitotic interval. Where aldolase is limiting (dotted line in Fig. 1) the minimal capacity to metabolize fructose 1,6 diphosphate includes the time of mitosis. When the limiting effect of aldolase is removed (solid line in Fig. 1), the over-all shape of the curve is preserved except for the appearance of a secondary rise in DPN-reducing capacity at the time of mitosis. It would appear that although the activities of these two primary glycolytic enzymes follow a similar course, they do not follow an identical one, mitosis being one event which stimulates in the microspores a greater capacity for dehydrogenase than for aldolase activity. It is clear, however, that even with respect to dehydrogenase, it is not mitosis but other phases of cellular development which are associated with high capacities for at least partial glycolytic activity. One of these phases occurs about a week before mitosis, shortly after the tetrads of meiosis separate into free microspores. It has been suggested to us (D. F. LaCour, private communication) that this premitotic peak may be associated with microspore vacuolation, but undoubtedly other correlations could be found. The second of these phases occurs 4 days after mitosis, at which time there is an intense production of deoxynucleosidic compounds (5). Here, too, however, there is no necessary connection as an earlier equally intense interval of deoxynucleoside formation (59 mm. bud length) occurs during a trough in initial glycolytic capacity (5).

DISCUSSION

Since the experiments reported here deal with the capacity of cells to perform the initial steps of glycolysis, no direct conclusions can be drawn respecting the metabolic performance of the cells *in vivo*. On the other hand, the fact that such capacities vary markedly in a synchronously developing population of mitotic cells, provides informative pointers on the importance of glycolysis to the mitotic process. Unless one assumes that the striking variations noted in enzymatic capacity bear no relationship to enzymatic performance, it is difficult to escape the conclusion that glycolysis, *per se*, does not initiate the mitotic process, as Rapkine and Warburg supposed (1, 2). Furthermore, the long held view of Rapkine (1) that the —SH enzymes of glycolysis are activated *"in vivo"* by glutathione is not supported by the behaviour of isolated lily microspores. In Fig. 2 the changes in soluble sulfhydryls and in dehydrogenase activity are plotted together. It can be seen that the two curves follow nearly opposite courses. Thus, although there is a high concentration of soluble thiols during the mitotic interval as Rapkine had supposed, their role appears to be directed elsewhere than towards the stimulation of glycolytic enzymes.

Studies of whole anthers (6, 7) and of isolated microspores (8) indicate a temporary fall in respiration during active mitosis. This fall appears to be a consequence rather than a cause of mitosis. If so, a relative increase in glycolysis over respiration would be expected in proliferating tissues, the magnitude of the increase being a function of the proportion of cells in division and the intensity of interphase respiration. No distinctive source of mitotic energy has been found in these experiments; but clearly, activation of at least some of the glycolytic enzymes has a more immediate bearing on morphogenetic processes other than mitosis.

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