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Germination of Lily Pollen: Respiration and Tube Growth

Abstract. *Germinating pollen of* Lilium longiflorum *(cv. Ace) briefly exhibited a high rate of respiration before pollen tubes began to grow. A second period of high respiration occurred while tubes were growing. Between these periods respiration proceeded at a lower rate. Respiration was stimulated by 2,4-dinitrophenol to occur at approximately the same rate in all three periods.*

Studies of respiration in germinating pollen may reveal the timing of metabolic events important to germination. In conjunction with the studies it is necessary to establish the time of pollen tube initiation. Also, tube initiation must be reasonably well synchronized in the population of germinating pollen grains.

Tupy measured growth and oxygen uptake of apple pollen germinating in several sugars (*1*). The rate of respiration increased during the first 2 hours and then remained constant until 6 hours after pollen was placed in a sucrose solution. Tupy reported that tube elongation continued at a constant rate during the 6-hour period. The time of tube initiation was not indicated, however. The initial period of germination was not studied since measurements of respiration and tube length began 30 minutes and 3 hours, respectively, after pollen was placed in the culture medium. Brewbaker and Kwack also reported that pollen tubes elongate at a constant rate (*2*).

The patterns of respiration and tube growth in germinating lily pollen [*Lilium longiflorum* (cv. Ace)] are reported here. Anthers were removed each morning from freshly opened flowers and placed in a desiccator at about 24°C. At the end of the day anthers were removed from the desiccator and stored at 1° to 3°C. The pollen was used within a week. Oxygen uptake was measured manometrically at 30°C, and air was the gas phase. The standard culture medium was a modification of media used by others (*3, 4*). It contained, per liter of deionized water: sucrose, 290 mmole; $Ca(NO_3)_2$, 1.27 mmole; H_3BO_3, 0.162 mmole; KNO_3, 0.990 mmole; KH_2PO_4, 3.0 mmole; and tetracycline, 10 mg. The *p*H of the culture medium was 5.2.

Data for percentage germination and average lengths of pollen tubes were obtained from photomicrographs. Samples of pollen were removed from shaking flasks at 15- or 30-minute intervals, and photomicrographs of three fields were taken at each removal. The total numbers of grains and of pollen tubes at each time were used to calculate percentage germination. Data for average tube length was obtained from a map measure; lengths of individual tubes were not recorded. The average number of pollen grains per Warburg flask was calculated after the grains in replicate 0.005-ml portions of culture medium had been counted.

Most tubes began to grow between 30 and 75 minutes after pollen grains had been placed in the medium (Fig. 1). Germination increased from 2 to about 75 percent during this period; a small percentage increase may have occurred between 75 and 120 minutes. The average rate of pollen tube elongation was constant in the period after 75 minutes, but for the period of growth before 75 minutes, while percent germination was increasing rapidly, the average rate was lower. The lower average growth rates may have resulted from the continuous appearance of short, newly initiated pollen tubes and not from variations in growth rates of individual tubes.

Pollen respiration remained approximately zero until the culture medium was tipped in from the sidearms of Warburg flasks. Respiration after tipping was characterized by three distinct phases (Fig. 2), which were always observed in similar experiments.

Phase 1. Respiration increased rapidly until about 10 minutes after tipping, and the rate remained high until about 30 minutes after tipping. Very little pollen tube initiation occurred during this phase.

Phase 2. After 30 minutes respiration decreased 38 percent and remained relatively low until about 60 minutes. Considerable pollen tube initiation occurred during this phase.

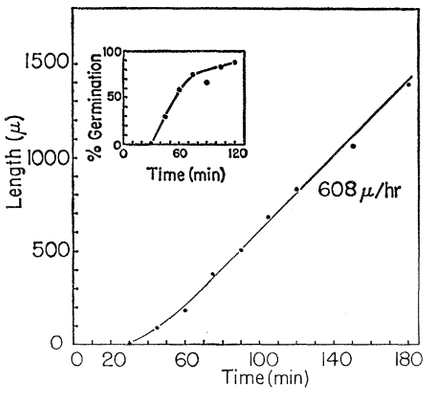

Fig. 1. Percentage germination (inset) and average tube lengths of germinating lily pollen. Data is from photomicrographs taken at the times indicated. Each value for percentage germination was calculated from counts of 133 to 256 pollen grains. Each estimate of tube length represents an average from 44 to 148 pollen tubes, except the estimate at 30 minutes, when only 3 tubes were observed. Experimental conditions were similar to those described for Fig. 2.

Phase 3. Respiration increased in the period from 60 to about 120 minutes and remained high thereafter. Some tube initiation occurred early in this phase, while respiration was increasing. Tube growth continued throughout phase 3.

Further experiments were conducted to determine the basis of this respiratory pattern. Pollen was incubated in the standard culture medium, and 2,4-

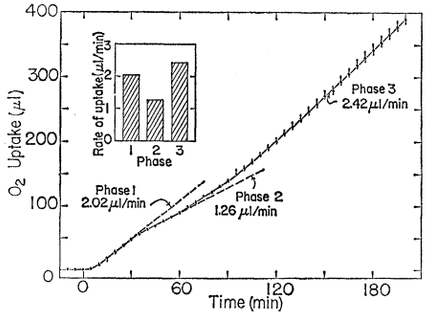

Fig. 2. Oxygen uptake of germinating lily pollen. Each point is the average of four replicate Warburg flasks, and vertical bars show extent of variation among replicates. Each flask contained 10 mg (fresh weight) pollen, representing 44,900 ± 2400 ($\bar{X} \pm S_{\bar{x}}$) pollen grains and 1.7 ml of culture medium. Time is expressed in minutes before or after addition of culture medium to pollen. The temperature was 30°C.

dinitrophenol (DNP) was added during phase 1, 2, or 3. Oxygen uptake in each phase was stimulated by DNP. Maximal stimulation was obtained with $10\mu M$ DNP during phases 1 and 2, while $5\mu M$ DNP gave maximal stimulation during phase 3. The rate of respiration was approximately the same in all phases after the addition of DNP. In a typical experiment the rates of O_2 uptake were 1.5, 0.9, and 1.7 μl/min in phases 1, 2, and 3, respectively. The DNP-stimulated rates for the three phases were between 2.6 and 2.7 μl/min. Thus, respiration during germination was probably limited by availability of adenosine diphosphate or endogenous inorganic phosphate, and the observed shifts in respiration may have resulted from altered concentrations of these compounds within the cells.

The higher respiration rates during phases 1 and 3 may be caused by increased utilization of adenosine triphosphate (ATP) in synthetic reactions. RNA (*5*), protein (*6*), and membranes (*4, 7, 8*) are formed in germinating pollen. Respiration during phase 1 may be activated by rapid synthesis of these materials in preparation for tube growth. Endogenous sucrose is hydrolyzed early in germination of lily pollen (*9*) so rapid phosphorylation of hexoses may stimulate respiration in phase 1. Adding glucose to ascites tumor cells stimulates respiration briefly because the glucose is rapidly phosphorylated (*10*).

Turnover of ATP may decrease before tube initiation, causing the low respiration of phase 2. Decreased ATP turnover could result from lowered rates of protein or membrane synthesis. Alternatively, sugar phosphates may accumulate during phase 1, depleting endogenous supplies of inorganic phosphate or free hexoses, which become limiting during phase 2.

Phosphorylations associated with growth of pollen tubes may stimulate respiration during phase 3. Cellulose, callose, and pectin are synthesized during tube growth (*7, 11*). Isolated plant enzymes form cellulose and callose from phosphorylated precursors (*12*), and the existence of phosphorylated intermediates in pectin synthesis has been postulated (*13*).

Germinating seeds and spores also undergo a transition from low to high rates of respiration, and the increased respiration is associated with the onset of various metabolic activities. However, the respiratory pattern of germinating pollen is different from the patterns exhibited by seeds or spores. For example, germinating pea and bean seeds exhibit initial increases in respiration which occur as the seeds absorb water (*14*). Thereafter, respiration remains constant or decreases slightly until the radicle emerges, when further increases in respiration occur. Respiration of pea seeds seems to be limited by availability of oxygen before the radicle splits the seedcoat. Respiration of germinating pollen does not seem to have this limitation since DNP stimulates respiration. Spores from some fungi, including *Neurospora, Puccinia,* and *Ustilago*, absorb oxygen at increasing rates during germination (*15*). In contrast, the respiration of rapidly elongating pollen tubes is only slightly greater than the respiration of pollen during phase 1 before tubes have appeared.

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"Negative" Crystals in Ice: A Method for Growth

Abstract. *Holes bounded by crystallographic faces, or "negative" crystals, may be made in ice by inserting a hypodermic needle and connecting it to a vacuum. The evaporation habit changes as a function of temperature and growth rate. This procedure is a simple way of producing mirror-smooth, uncontaminated, crystallographic surfaces in compounds which have high vapor pressure.*

Negative crystals occur naturally and can be produced in several ways, but there has been no method of growing them.

Evaporation of ice crystals has received little study, with the exception of two short reports (*1*), several papers on thermal etching, and others on the condensation coefficient of ice at about −50°C (*2*). The standard etching technique for ice (*3*), however, is little more than a method of growing negative crystals at ice surfaces (*4*). Nakaya (*5*) has studied extensively the migration of naturally occurring negative crystals in ice in temperature gradients, but the migration is caused by both evaporation and condensation and is therefore difficult to interpret in terms of different crystal faces. The purpose of growing negative crystals was to determine relative evaporation rates on ice-crystal faces, but the ultimate achievement of this aim is doubtful. The method may, however, be useful as a simple way to produce large, pure, rather perfect faces on crystals which have high vapor pressure.

The only major equipment needed for growing negative crystals in ice is a walk-in cold room, a vacuum pump, and a supply of single crystals of ice. Large, exceptionally perfect (dislocation density 10^3 to 10^4 cm^{-2}) ice crystals from the Mendenhall Glacier in Alaska were used (*6*). A hole about 1 cm long, with a diameter slightly smaller than the outside diameter of the needle to be used, is drilled into a small block cut from a crystal by twirling the drill bit between a bare thumb and forefinger. (If a mechanical drill is used, it is difficult to avoid cracking the block.) The small end of a hypodermic needle that has been ground square and deburred is rested against the opening of the hole and