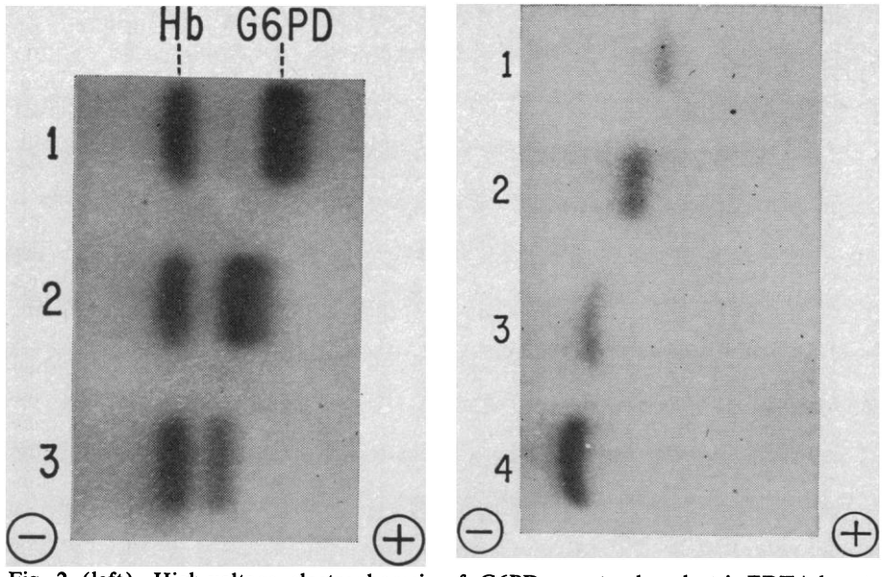

Fig. 2 (left). High-voltage electrophoresis of G6PD on starch gel; tris-EDTA-borate buffer, *p*H 8.6. *1*, G6PD A; *2*, G6PD B; *3*, G6PD Athens. The horizontal electrophoresis was carried out, on gels 2 mm thick, for 4 hours at 2° to 4°C; voltage, 10 volt/cm. For the buffer solution and staining conditions, see the legend to Fig. 1. Fig. 3 (right). Electrophoresis of G6PD on starch gels, phosphate buffer, *p*H 7.0. *1*, G6PD A; *2*, G6PD B; *3*, G6PD Athens; *4*, G6PD Seattle. Horizontal electrophoresis, on gel 8 mm thick, for 6 hours at 2° to 4°C; voltage, 4 volt/cm. Gels were prepared with 0.01*M* phosphate buffer, and 0.1*M* buffer was used for the bridge solution. Added to the molten gels, as well as to the cathode compartment near the gel, was 2.5 mg of NADP. For the staining conditions, see the legend to Fig. 1.

a mobility of 90 percent relative to normal B+ enzyme.

Enzyme G6PD D− is supposed to be identical with G6PD Seattle (*11*). Our electrophoretic findings show that G6PD Athens differs from G6PD Seattle. The K_m's for G6P and NADP, as well as for the 2-deoxy-G6P of G6PD Seattle, are very similar to those of G6PD Athens; however, utilization of deamino-NADP is definitely different: Athens, 126 percent of NADP; Seattle, 57 percent of NADP. G6PD Athens can also be distinguished from G6PD Austin II by its faster electrophoretic mobility, low activity in erythrocytes, low K_m for G6P, and high utilization of 2-deoxy-G6P and deamino-NADP. Distinction from G6PD West Bengal could be achieved on the basis of K_m for NADP, utilization of 2-deoxy-G6P, and difference in electrophoretic mobility.

The findings from the study of G6PD Athens have some bearing on further investigations of electrophoretic variation in G6PD. Mutants associated with red-cell deficiency in G6PD and normal electrophoretic mobility under routine electrophoretic conditions (especially when the widely accepted tris-EDTA-borate buffer system, *p*H 8.6, is applied) need to be studied by various electrophoretic techniques before one may consider that they do not differ from the normal enzyme (B+). Comparison of difference in electrophoretic migration between G6PD Athens and normal G6PD (B+) in phosphate buffer at *p*H 7.0 with their close similarity in mobility in tris-EDTA-borate buffer at *p*H 8.6 is particularly instructive on that point. It seems that the experience gleaned from abnormal hemoglobins applies also in the study of electrophoretic variants of G6PD. Electrophoresis of G6PD with different buffer systems and in different media will facilitate the process of distinction and characterization of new mutants.

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14. We thank E. Giblett and R. Murray for information on phosphate buffer and high-voltage electrophoresis of G6PD, and Mrs. L. Steinmann and Miss A. Barker for technical assistance.
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Oxygen Tension as a Control Mechanism in Pollen Tube Rupture

Abstract. *The decrease in stylar* pO_2 *encountered by pollen as it approaches the ovary can induce bursting of pollen tube tips. Anaerobic conditions induced a high percentage of tube tip bursting in most pollen germinated in vitro. Changes in tube tip metabolism with decreased oxygen probably sets up cell wall stress resulting in pollen tube rupture.*

In angiosperm fertilization the general growth pattern of the pollen tube down the style culminates in the rupture of the tube tip and deposition of the pollen contents into one of the synergid cells (*1*). This allows fusion of one male cell with the egg cell, and the other with the polar nuclei, forming a primary endosperm nucleus. An oxygen gradient exists in the style. Oxygen pressure, pO_2, is high in the stigma and style but suddenly decreases at the base of the style, approaching zero values in the ovary (*2*). This report evaluates the hypothesis that the decrease in style pO_2, which the pollen

encounters at the ovary, is a major reason for tube tip bursting.

Experimental results indicate that differences occur in pollen response in vitro to varying levels of oxygen. Figure 1 shows the results of an experiment in which pear pollen grains (*Pyrus communis* var. Winter Nelis) were germinated in vitro for 3 hours and then transferred to various levels of oxygen. After 2 hours of additional growth, the pollen in the lowest pO_2 (99 percent N_2) showed the highest number of burst tubes. Lily and *Hippeastrum* pollen (Table 1) followed the same pattern of tube bursting when grown under varying levels of oxygen. However, *Petunia* and *Atropa* pollen (Table 1) did not show the substantial increase in tube bursting noted with pear and lily pollen.

The differences in tube tip bursting can be correlated with the anatomical structure of the styles through which the pollen must grow. In *Pyrus*, *Hippeastrum*, and *Lilium* flowers the style contains a hollow canal; in *Petunia* and *Atropa* the style has relatively solid conducting tissue which is rich in chlorophyll-containing cells (*3*).

The tube nucleus in some pollens disintegrates as the pollen tube approaches the egg cell but before the tube bursts. This indicates that the pollen tip metabolism changes when it approaches the top of the egg apparatus. Such a change probably sets up a strain in the wall at the tube tip. Just as the tube nucleus does not always disintegrate, so tip bursting may not always result from strain on the tip wall and lowered pO_2 (Table 1). In some cases contact of the tip with the fusiform apparatus may precede bursting. However, in the case of hollow styles such as *Lilium tigrinum*, changes in pollen tube bursting, presumably due to pollen tip stress, may be induced by artificially modifying the pO_2 level of the stylar canal.

We tested this hypothesis by streaming nitrogen gas (99 percent) through the stylar canal of *L. tigrinum* for 1 hour and compared the tube bursting with bursting in control plants with oxygen (100 percent) streaming through the styles. Nearly 100 percent breakage of tubes occurred in the nitrogen-perfused styles. No tubes broke under the pure oxygen stream.

These findings of pollen tube tip bursting are in agreement with observations on tip growth of root hairs (*4*). When the pO_2 is lowered, streaming ceases and cell tip bursting occurs in the root hairs and certain pollen tubes, followed by extrusion of the protoplasm.

Bursting of pollen tubes and of root hair tips in low levels of oxygen may be caused by similar mechanisms. The conspicuous tip lysing is probably due to wall softening by enzymes such as cellulase (*5*). These degradative enzymes are less sensitive to decreased oxygen than the enzymes synthesizing new pectin and microfibrils necessary for growth of the softened wall.

The physiological effect of decreased pO_2 can be best interpreted at the enzyme-metabolic activity level. The capacity of the egg to develop and remain quiescent prior to fertilization is probably a function of the pO_2 of the surrounding ovary tissue. Foreign nongenetically adapted pollens, as occur in certain self-incompatible pollinations, or cross sterile plants, are probably unable to metabolize effectively and use the available substrates under the pO_2 conditions of these ovaries (*6*). Differential metabolism of pollen under varying oxygen levels is also indicated by the fact that not all tubes burst at low pO_2.

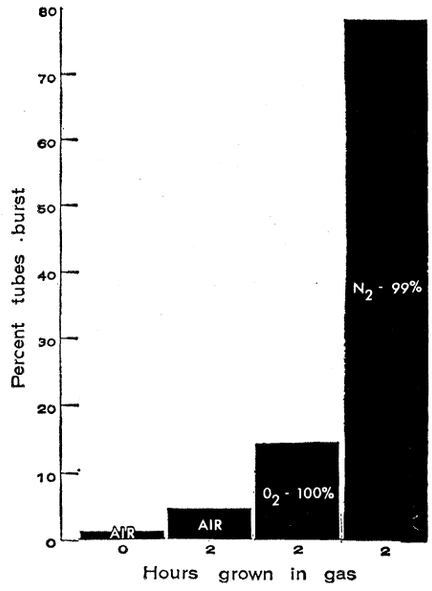

Fig. 1. Effect of oxygen level on pollen tube bursting. Pollen of *Pyrus communis* (0.5 mg) grown 3 hours at 30°C in 50 μl of 0.4*M* raffinose plus 30 parts per million of boron (H_3BO_3) in calcium phosphate, 0.015*M*, *p*H 6.0. Evacuation to 28.5 mm-Hg for 10 minutes. Resaturated with gases and allowed to grow an additional 2 hours at 30°C in controlled atmosphere indicated on the bar graph.

Table 1. Effect of oxygen level on pollen tube bursting.

Species	Germination conditions before gas treatment	Percent of tubes bursting with gas treatment indicated		
		Air	Oxygen (100%)	Nitrogen (99%)
Lilium longiflorum var. Ace	10 hours at 22°C in 0.15*M* sucrose + 0.01 percent H_3BO_3	78	6	94
Lilium longiflorum var. Nelli White	10 hours at 22°C in 0.15*M* sucrose + 0.01 percent H_3BO_3	51	10	89
Hippeastrum hybridum, var. Royal Dutch	14 hours at 24°C in 0.2*M* sucrose + 0.01 percent H_3BO_3	67	14	90
Petunia hybrida	4 hours at 24°C in 0.28*M* sucrose + 0.01 percent H_3BO_3	4	6	8*
Atropa belladonna	8 hours at 24°C in 0.2*M* sucrose + 0.01 percent H_3BO_3	12	9	15

* Approximately 5 percent of the pollen tubes formed a bulb at the tip.

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