

Electron Microscopy of the Degeneration of Fine Structure in *Saintpaulia ionantha* Wendl.

Pollen Walls. BY H. G. EHRLICH.* ‡ (From the Department of Botany, University of Minnesota, Minneapolis.) §

It has been widely accepted that oxidation is a major cause of the degeneration of the exine layer of spore and pollen grain walls. Recently this factor has come into consideration regarding the fine structure of these walls (Afzelius, 1956). Afzelius demonstrated that oxidation may change a "granular laminated structure into an amorphous granular" one, the latter showing no additional effects of oxidation.

When *Saintpaulia ionantha* pollen is fixed for 24 hours with 1 per cent osmium tetroxide (pH 7.5) or with 10 per cent formaldehyde (for less than 12 hours; pH 8.0) followed by OsO_4 for 3 or 24 hours, the exine fine structure is uniformly granular (Fig. 1). Avoiding the oxidative catalyst (benzoyl peroxide) by substituting ultraviolet light for hardening the methacrylate has no effect on the exine. It must be remembered, however, that OsO_4 is itself a powerful oxidizing agent. When the time of formaldehyde fixation is increased to 24 hours and is followed by OsO_4 for 3 hours, a slightly amorphous appearance of the exine results (Fig. 2). When pollen is subjected to 24 hours of leaching in water between the 24 hour formaldehyde fixation and 3 hour OsO_4 fixation periods, the exine degeneration is severe (Fig. 3). No degeneration occurs if OsO_4 fixation is used first or if leaching of unfixed pollen is followed by OsO_4 fixation. Also, no degeneration occurs if the pollen is treated with saturated ammonium oxalate for 23 hours prior to formaldehyde fixation.

It appears that a reducing agent such as formaldehyde can chemically alter the exine and cause its degeneration. This serves to question the advisability of storing pollen in preservatives containing formaldehyde until such time as experiments testing the effects of various dilution levels of formaldehyde and time of exposure to them are made. Since even the ultrastructure of

fossil pollen exines can be exceedingly well preserved in nature (Ehrlich and Hall, 1959), it is suggested that factors other than oxidation as well as the conditions under which oxidation may occur receive more attention in considering problems relating to pollen preservation.

Of greater significance is the information presently reported with respect to the nature of the outer layers of the pollen grain wall. It is evident from the figures that formaldehyde affects only the exine and that the mesine layer seems unaltered. This lends further support to the mesine being a wall layer distinct from the exine (Ehrlich, 1958; Rowley, 1959), since the two must be somewhat different chemically. Neither cellulase nor pectinase, when applied for 5 hours, singly or in combination, show any effect on either the exine or mesine (cf. Ehrlich, in preparation).

Of further interest is the observation that the exine of *S. ionantha* pollen does not appear to be formaldehyde susceptible during the course of its development. However, the mature exine is susceptible, and the exines of germinating pollen seem to be even somewhat more so (Fig. 4). This suggests the possibility that, upon germination, renewed protoplasmic activity may alter the chemistry of the exine.

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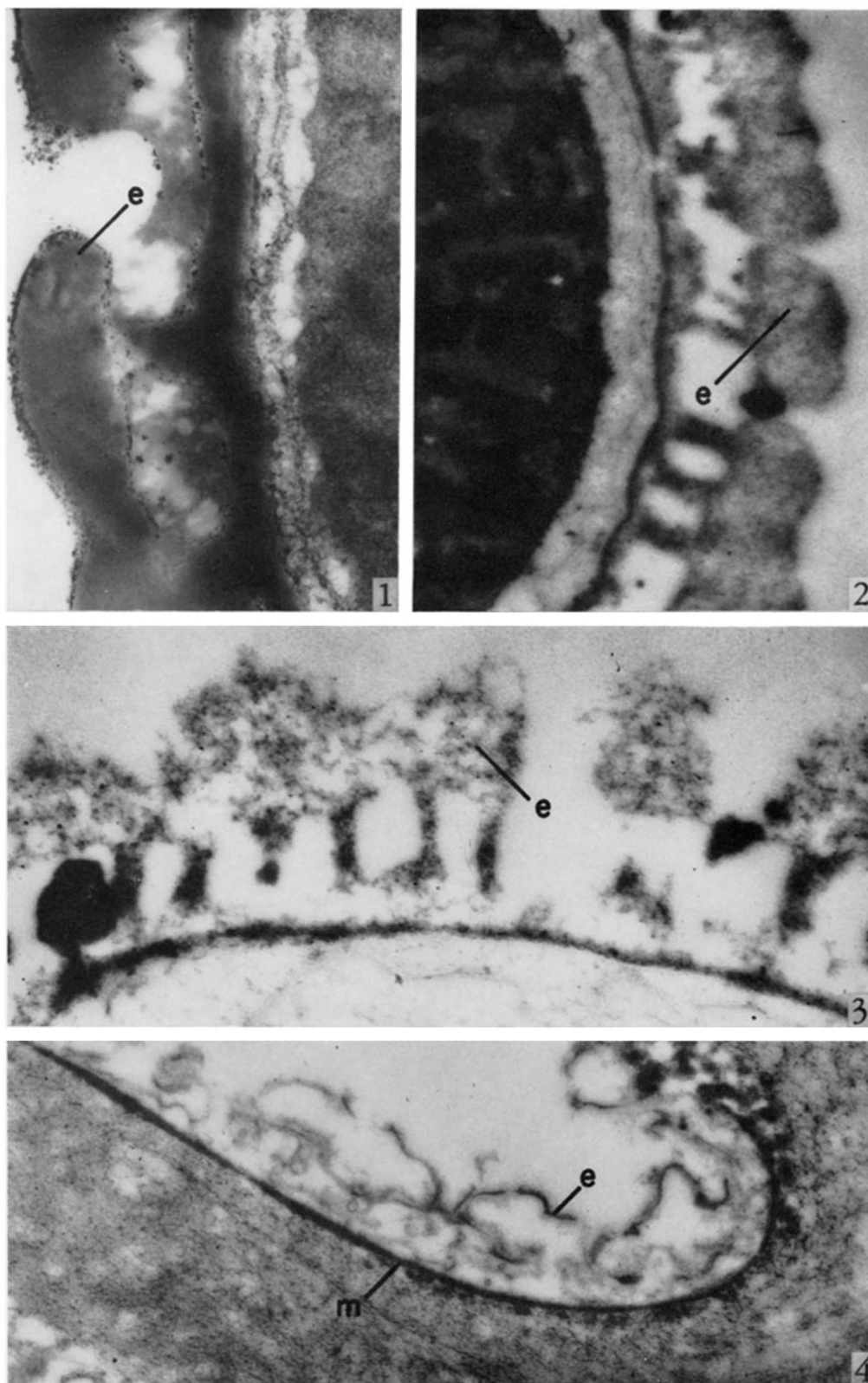
EXPLANATION OF PLATE 105

FIG. 1. Portion of non-germinating pollen grain. *e*, exine. Osmium fixation. $\times 54,600$.

FIG. 2. Portion of non-germinating pollen grain. *e*, exine. Formaldehyde and osmium fixation. $\times 34,300$.

FIG. 3. Portion of non-germinating pollen grain. *e*, exine. Formaldehyde followed by water leaching and osmium fixation. $\times 34,300$.

FIG. 4. Portion of germinating pollen grain in region where pollen tube emerges from pollen grain. *e*, remnants of exine, *m*, mesine. Formaldehyde and osmium fixation. $\times 34,300$.



(Ehrlich: Fine structure in pollen walls)