high concentrations of 2,4-D and NAA, which inhibited growth, the amount of ethylene produced was proportionately lower. The growth which occurred when no growth regulator was added may be a consequence of the presence of adequate amounts of growth regulator in the cells of the inoculum. Both the 2,4-D and NAA accumulate in the cells and are not metabolized (unpublished results of K. Ojima and O. L. G.). The results indicate that ethylene is a normal product of cultured plant cells and its formation is not directly affected by growth regulators.

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Anther Carotenoids and the Synthesis of Sporopollenin

SHAW *et al.*[1-3] are at present carrying out valuable new work on the chemistry of resistant spore wall materials of the general class of sporopollenins following the pioneer studies of Zetzsche's group in the 1930s[4]. Shaw and Yeadon[1,2] have concluded, from observations on the pollen of *Pinus sylvestris* and *Lycopodium clavatum*, that sporopollenin contains a lignin-like fraction of 10–25 per cent and a lipid fraction of 75–90 per cent. They have explained the lack of reactivity of the lignin component by the suggestion that it is masked by the lipid moiety, or present in some unusual combination. Brooks and Shaw[3] have proposed that the precursors of sporopollenin in the anthers of *Lilium henryi* are carotenoids, and that the exine is formed by oxidative polymerization of the mixture of carotenoids and carotenoid esters contained in the anther material.

This is an interesting hypothesis, and one with wide implications, but it needs to be viewed in the light of the general pattern of development in the spore walls and in the surrounding nutritional tissue, the anther tapetum, because the principal synthesis of carotenoids absorbing at 450 nm in the anther of *Lilium longiflorum* begins after exine formation is virtually complete[5]. The sequence of events in *L. henryi* corresponds closely with that in *L. longiflorum*, and is essentially as follows.

Synthesis of a material with the resistance to acetolysis of sporopollenin but with greater reactivity ("protosporopollenin") begins in the young spore tetrad, in association with lamellae of unit membrane dimensions[6,7]. The spores at this time are effectively isolated from the tapetum by the investing callose wall, which can evidently restrain the passage of quite small molecules[8]. At about the same time particles of sporopollenin (Ubisch bodies) begin to appear on the inner locular faces of the tapetal cells, within the gelatinizing primary wall. The formation of these follows the same pattern in *Lilium* as has been described by Carniel[9] for *Oxalis* spp. and by Echlin and Godwin[10] for *Helleborus foetidus*. Osmiophilic globuli congregate within the cytoplasm of the tapetal cells, possibly taking their origin in embayments of the endoplasmic reticulum according to Echlin and Godwin, much in the manner of spherosomes as characterized by Frey-Wyssling *et al.*[11]. These globuli are unpigmented throughout their life; they are removed by lipid solvents, but their stainability suggests that they contain a lipoprotein component. They are ultimately extruded from the cells completely (*Helleborus*) or on evaginations of the plasmalemma (*Lilium*). Only with the movement into the thecal fluid does accretion of sporopollenin begin, but thereafter the coating on the surface of each globulus thickens rapidly, giving the characteristic Ubisch body.

The chief deposition of sporopollenin in the exine of the spores begins with the break-up of the tetrads; during the next 2–4 days both spore exines and Ubisch bodies continue active growth. In this interval a new population of globuli develops in the tapetal cells. These are generally similar in electron-optical appearance to the previous ones, although they gain less density with uranyl acetate staining. It is within these bodies that the carotenoid pigments accumulate. In *L. longiflorum* the principal pigment is α-carotene 5,6-epoxide; pigments present in other *Lilium* species include β-carotene, violaxanthin, capsanthin, capsorubin and antheraxanthin[13]. The pigmented lipid globuli ultimately coalesce, and the whole content is subsequently released into the loculus through the persistent curtain of Ubisch bodies. There it diffuses among the spore mass, becoming associated with the fibrillar material persisting in the cavities of the exines. The oleaginous, carotenoid-pigmented material synthesized during the last phase of the life of the tapetum forms the *Pollenkitt* of German authors[12], a name referring to its function of cementing grains together during dispersal in some entomophilous species. Various biological functions have been attributed to the pigmentation, which may be important as an insect attractant or as a protection against damaging effects of radiation[13].

In *Lilium*, then, it cannot really be supposed that the carotenoid pigments formed during the final phase of tapetal activity participate in sporopollenin synthesis, because this is largely completed before the cytoplasmic organelles responsible for the production of the pigment become active. As the studies of Echlin and Godwin[10] and others have shown, the key to the origin of sporopollenin must lie in the precursor bodies (pro-Ubisch bodies) and the chemistry of the thecal fluid. Whatever the chemical precursors of sporopollenin may be, the well established developmental facts show that they are not coloured carotenoids, unless it be supposed that these never accumulate to detectable amounts during the actual synthesis. It is not, of course, excluded that the true precursors are colourless compounds of related structure.

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