A Tissue Derived from the Pollen of *Ginkgo biloba*

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LaRue (*1*) obtained rather remarkable results from the culture of the megagametophytes of *Zamia floridana* and has more recently shown (unpublished) that *Zamia* pollen may develop up to the sperm mother cell stage when grown on a suitable nutrient medium. In the light of this work similar studies *in vitro* were undertaken on *Ginkgo biloba* pollen.

In *Ginkgo,* concurrent with normal development in culture, several types of abnormalities arise. By far the most intriguing aberration is that in which a tissue is initiated from the mass of germinating pollen. Tissue development seems to begin with the production of extra nuclei to give three main types of plurinucleate gametophytes. One finds a coenocytic condition with many nuclei free within the gametophyte (Fig. 1), a multicellular condition at the exine area

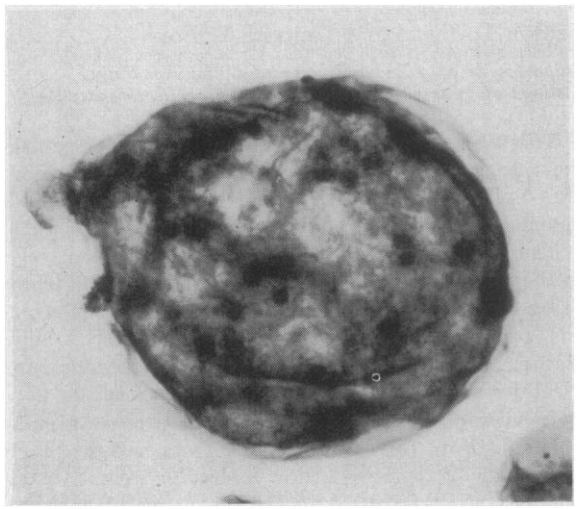

FIG. 1. Coenocytic gametophyte with more than 21 nuclei. Diameter 330 μ.

(Fig. 2), or, infrequently, a several-celled tube or haustorium (Fig. 3). Various stages in the formation of each of these types have been noted in cultures, and as many as 40 nuclei have been seen in the coenocytic gametophytes. It is not known, however, which of the abnormalities has given rise to the pollen tissue. Conceivably, of course, all the abnormalities and the cell types of the gametophyte (prothallial, generative, stalk, body, and tube) could be active in tissue formation. One would, perhaps, expect a prothallial origin, but the indications are strongly against such a hypothesis. One finds little prothallial cell activity in abnormal gametophyte development. Instead, the stalk and tube cells are the most active components. The stalk cell gives rise to the intercalary cells at the exine,

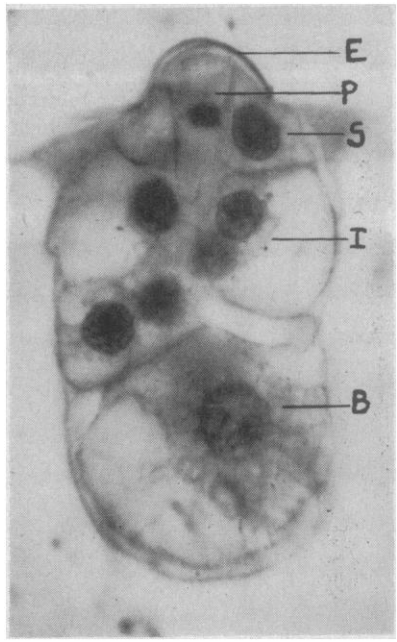

FIG. 2. Intercalary divisions between the so-called "stalk" cell and the "body" cell. E, exine layer of the pollen grain; P, prothallial cell; S, stalk cell; I, intercalated cells and nuclei; B, body cell. Exine diameter approximately 26 μ.

and the tube cell is largely responsible for the septate tubes (Fig. 3) and the coenocytic gametophytes (Fig. 1). Therefore, while both the stalk and tube cells are considered as possible origins of the pollen tissue, the more frequent occurrence of tube-derived abnormalities suggests that the tissue originated from the tube cell of the gametophyte.

The tissue, when first visible macroscopically, appears as a mass of white starch-storing cells arising from the homogeneous mass of germinating pollen. Later, the tissue grows out and may at this time be removed and subcultured. The first tissue mass was isolated on February 2, 1952, and has since passed

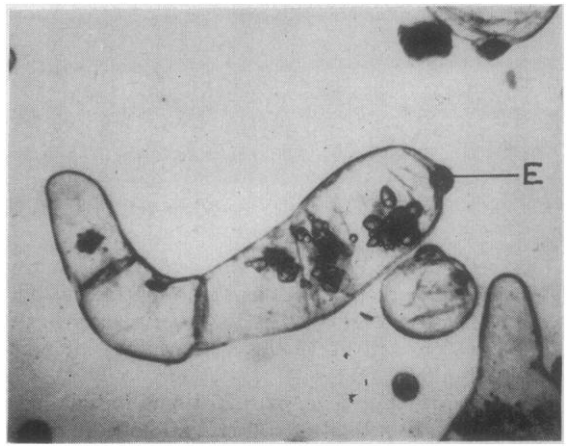

FIG. 3. A three-celled haustorium or tube. The adherent exine at the right is about 26 μ.

through 13 subcultures (Fig. 4). Growth has been most vigorous on a modified White's medium (*2*), to which 0.25% yeast extract and 1 mg IAA have been added per liter. Tissue proliferation continues either on agar or in shake culture at a rate which permits subculturing 1 : 8 in 3 weeks. Moreover, since its establishment as a tissue culture, the pollen tissue has shown no diminution of growth activity.

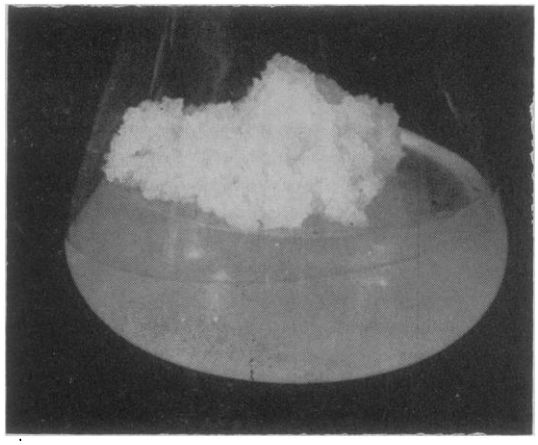

FIG. 4. *Ginkgo* pollen-derived tissue: this is one month's growth in an 125-ml Erlenmeyer flask; the original inoculum was 5 mm cube. (Natural size.)

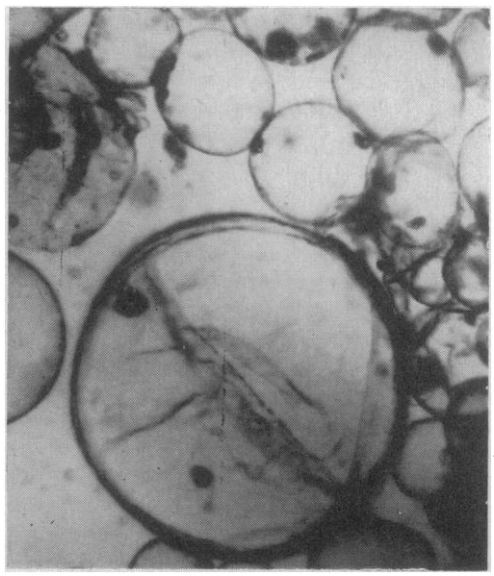

FIG. 5. Vacuolate tissue cells: the giant cell is 483 μ in diameter with clusters of 7 and 3 nuclei.

Ginkgo pollen tissue may be characterized as an undifferentiated, parenchymatous, and often multinucleate cell mass (Fig. 5). The tissue originally has a haploid complement of 12 chromosomes but later becomes polyploid.

Up to the present time, over 25 tissue initials have been observed in a total of 634 culture bottles; this is an incidence of tissue formation of about 4%. Subculturing of many of these initials has resulted in tissue proliferation, although only three such subcultures have been carried on as continuous clones. *Ginkgo* pollen tissue has thus demonstrated, repeatedly, a capacity for potentially unlimited explanation.

Growth *in vitro* of the female gametophyte of *Ginkgo* has also been obtained. In this case, marginal meristems are formed and they produce usually nodular outgrowths. And, although the initial inoculum of the female gametophyte tissue may increase sixfold in volume through marginal proliferation, excision and subculture of the outgrowths have, thus far, not been successful. When grown in light, the tissue not only retains its chlorophyllous nature, but also exhibits a marked increase in the intensity of pigmentation.

References

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A Report on the Waxy Constituents of Spanish Moss, *Tillandsia usneoides* L.

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Tillandsia usneoides L., commonly called Spanish moss, long moss, crape moss, and Florida moss, is a true epiphyte which festoons trees of swamps and hammocks of the southern coastal states.

The hard, resilient inner fibers are used extensively in the upholstery industry, the remainder of the plant being discarded or utilized as compost. Webber *et al.* (*1*) reported the presence of an antibacterial substance. Mayo Clinic studied the use of moss as a surgical dressing as it is more absorbent than cotton (*2*).

In view of the recent endeavors to procure a suitable substitute for carnauba wax from natural plant sources or by synthesis of low molecular weight waxes (*3*), attention is called to the wax present in commercial quantities in Spanish moss.

In his study on the carbohydrate constituents of Spanish moss, Schroger (*4*) reported the presence of a green-colored wax melting at 79 to 80° C. The presence of this wax was confirmed, and a constituent exhibiting steroidal characteristics was extracted and shown to possess estrogenic activity (*5*). The freshly gathered moss contains approximately 5% wax. The iodine number of this wax is 33.0, the saponification number 120.4, the acid number 25.0, the ester number 95.0, and the melting point 79–80° C.

This wax is soluble in various organic solvents, easily purified, and imparts a hard glossy finish to woodwork and leather, comparable to commercial waxes.

In view of the abundance of Spanish moss, the proximity of the supply and the economy of utilizing