Undifferentiated Growth of Orchid Embryos on Media Containing Barbiturates[1]

JOHN T. CURTIS

Department of Botany, University of Wisconsin

The embryos of orchid seeds are not accompanied by an endosperm or other storage tissue, but are suspended alone in the center of a thin, membranous seed coat. They therefore offer very favorable material for the *in vitro* study of early growth and differentiation. In the course of investigations of the micronutrilite requirements of such orchid embryos in pure culture, certain nutrient media containing barbituric acid derivatives were employed. Many of the embryos on these media produced large, undifferentiated cell masses instead of normally organized seedlings. A preliminary account of the appearance and growth of these masses is presented here.

Mature seeds of *Vanda tricolor* were used as embryo sources. The embryos averaged 77μ x 197μ in size and contained a total of about 120 cells. There was little evidence of tissue differentiation other than a slightly larger cell size at the basal end. The embryos, with their surrounding testae, were surface-sterilized with a calcium hypochlorite solution and were planted on the surface of solid media in screw-cap containers. Three barbiturates were used: sodium ethyl-(1-methyl-butyl) barbiturate, sodium cyclopentenyl-allyl barbiturate, and phenyl ethyl barbituric acid, each at a concentration of 10 ppm. These were added separately to a basal medium of mineral salts and sucrose, previously reported as favorable for the development of orchid embryos (*2*). The cultures were maintained in an incubator at 30° C. with a day length of 12 hours provided by Cooper-Hewitt fluorescent lights.

Upon examination after a two-year growth period, it was noted that all of the cultures containing barbiturates showed large numbers of undifferentiated cell masses among the normal plants, while the control cultures with no growth-factor additions showed mostly normal seedlings, with only an occasional embryo with undifferentiated proliferation. The cell masses in the barbiturate cultures were considerably larger than those in the control bottles. The maximum number of cell masses occurred in the cultures with phenyl ethyl barbituric acid. In superficial appearance they resembled the callus tissue of tobacco, described by White (*4*). The larger masses had a volume of 1.5 cc.; their surface was undulate or pebbled; and they were dark green, pale yellow green, or opaque white in color. There was no external evidence of growing points or other organized meristems. Internal examination revealed a more or less homogeneous cell mass, with no trace of vascular elements or other cell differentiation, except for subsurface patches of meristematic cells. Any given region of meristem apparently functioned only for a brief period, after which it degenerated and was followed by two new meristems, one on each side of the old, thus giving rise to an irregular type of dichotomy. A few of the masses developed absorbing hairs of the sort commonly seen on orchid protocorms. The non-meristematic internal cells presented the same appearance in cross-section as those illustrated by White (*4*) for hybrid tobacco callus, but cell-to-cell differences in size were not as great in the orchid. The nuclei of these cells were very much larger than those in the growing regions and were stained less intensely.

Other instances of abnormal growth of orchid embryos have been reported by Bernard (*1*) and Knudson (*3*). In each case the plants showed a marked proliferation of the embryo axis, with several or many growing points instead of the usual single one. They differed sharply from the callus-like masses here reported for barbiturate cultures, in that the multiple growing points soon gave rise to nearly normal stems and leaves and did not continue nonorganized growth.

Attempts to subculture the chlorophyll-containing cell masses of *Vanda* derived from the barbiturate treatment were successful. To date the cultures have gone through five passages of two months each. The rate of growth has remained about constant, with an 8-fold volume increase at each passage, or a total potential increase of 33,000-fold. They thus appear to have an unlimited capacity for proliferation. Preliminary results indicate that the nondifferentiated growth may be maintained on media free from barbiturates.

The abundance of meristematic regions and the occasional presence of a pseudoepidermis with absorbing hairs prohibit the use of the term "tissue-culture" (*sensu strictu*) in connection with these embryo masses, while the lack of organized internal tissue differentiation precludes the use of "organ-culture." In view of their origin, it may be appropriate to designate them as proliferating embryo meristem cultures. The exact relation of the barbiturates to the initiation and maintenance of these cultures is being investigated at present.

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The Inhibitory Effect of Sodium Dodecyl Sulfate Upon the Gastric Secretory Response to Histamine

HARRY SHAY, S. A. KOMAROV, and HERMAN SIPLET

Fels Research Institute,
Temple University School of Medicine, Philadelphia

We recently reported (*4*) that sodium dodecyl sulfate in concentrations of 0.1 or 0.5 per cent, introduced into the stomach of the rat, stimulates secretion of the three main components of gastric juice—acid, pepsin, and mucin. In 2 per cent concentration, the alkyl sulfate causes a profuse secretion of mucus only. The selective mucigogue effect of the 2 per cent solution was also observed in the dog (*3*). To determine whether the 2 per cent solution of sodium dodecyl sulfate failed to stimulate the parietal cells or if it actually inhibited their activity, the effect of this agent on the gastric secretory response to histamine in sacrifice experiments in dogs was studied.

In animals under pentobarbital sodium anesthesia, tracheotomy was performed, the esophagus was tied in the neck, and the pylorus was ligated. A gastric fistula was made, and a catheter sutured into the distal portion of the upper third of