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

the duodenum. Three mg. of histamine dihydrochloride were injected subcutaneously at the end of the operation. One hour later and every 15 minutes thereafter for the duration of the experiment, 0.3 mg. of histamine dihydrochloride was injected by the same route. Gastric secretion was collected for half-hour periods for 6 to 9 hours. When the rate of secretion became stabilized, secretions of four 30-minute periods were collected to serve as control samples. The stomach was then filled through the fistula with a 2 per cent aqueous solution of sodium dodecyl sulfate under a pressure of 4 cm. of water. This pressure was maintained for 30 minutes, after which the stomach was emptied, and the collection of samples for 30-minute periods was resumed and continued for four hours. Water and electrolytes lost in the secretion were replaced by the instillation of an isotonic sodium chloride—HCl mixture—through the duodenal catheter. Animals of similar weight and sex, treated identically except that no detergent was instilled into the stomach, served as controls for the maintenance of histamine action. Each sample of secretion was measured and analyzed for free and total acidity by titration with N/20 NaOH: total chloride, by the method of Wilson and Ball (*5*); pepsin concentration, by Nierenstein's modification (*2*) of the Mett method; and mucin, by a colorimetric method developed in our laboratory. The output of hydrochloric acid was calculated for each specimen.

The data obtained show clearly that even relatively short (30-minute) contact with the gastric mucosa of a 2 per cent solution of sodium dodecyl sulfate causes a striking and prolonged reduction of the gastric secretory response to large doses of histamine. Free acidity was actually reduced to zero for a period. The volume of secretion decreased for four hours to approximately 50 per cent (average) of that of the control period. Pepsin concentration remained unchanged, but the concentration of mucin in the secretion was increased considerably (2.5 to 5.2 times). Total chloride concentration decreased from 158–160 meq./l. in the control period to 125–130 meq./l. The latter chloride values are within the range which we previously reported (*3*) for pure mucus obtained from the dog's stomach. Our results show that parietal cell secretion in the dog, in response to the continued administration of large doses of histamine, is strikingly inhibited by 2 per cent sodium dodecyl sulfate. Under these conditions gastric secretion was represented mostly, and at times exclusively, by mucus. The effect lasted for more than four hours with only slight evidence of recovery of parietal cell activity at the end of that period. Table 1 represents the results of the rates of secretion, concentrations, and output of free acid from two representative experiments.

It is important to note that, in the numerous experiments performed in animals with the pylorus tied, we observed no noticeable change in the gastric mucosa after its exposure to a 2 per cent solution of sodium dodecyl sulfate for two hours in the dog (*3*) and for six hours in the rat (*4*). Careful histologic study of the dog's stomach sacrificed at the end of these experiments failed to show the slightest evidence of irritation.

The significance of the inhibitory effect of sodium dodecyl sulfate becomes apparent if it is considered that not only is histamine the most powerful known stimulant of gastric secretion, but also that its action on the acid-producing parietal cell is a selective one. There are reasons to believe that histamine plays an important part in the normal mechanism of gastric secretion as well as in the pathogenesis of peptic ulcer in man (*1*). A few agents are known which can inhibit the action of histamine on gastric secretion, notable among which are preparations of urogastrone and enterogastrone. However, these agents are effective only when administered parenterally. The marked inhibition of hydrochloric acid production by the alkyl sulfate through contact with the gastric mucosa is of considerable theoretical interest. By increasing mucus secretion and by the inhibition of acid (concentration and rate of secretion) and pepsin (rate of secretion only), therapy with sodium dodecyl sulfate, if conditions for its satisfactory employment for the human subject can be established, would more nearly approach the physiological requirements for the medicinal management of ulcer than any previously recommended form of treatment.

TABLE 1

Periods	1	2	3	4	5	6	7	8	9	10	11	12	13
Control, Dog #9,* M, 7.5 kg.													
Rate of secretion (ml./30 min.)	6.8	8.0	8.5	8.3	7.0	7.3	8.0	7.0	9.3	9.5	9.5	10.0	10.5
Free acid (meq./l.)	116	102	116	114	108	115	110	108	111	119	121	119	120
Output HCl (mg./30 min.)	29	30	36	35	28	31	32	28	38	41	42	43	46
Sodium dodecyl sulfate, Dog #8, M, 7.5 kg.					½ hour								
Rate of secretion (ml./30 min.)	9.3	9.2	9.3	9.9		*4.7*	*3.8*	*6.0*	*3.6*	*3.3*	*3.3*	*4.1*	*4.8*
Free acid (meq./l.)	119	118	123	129		*31*	*28*	*22*	*10*	*3*	*0*	*2*	*9*
Output HCl (mg./30 min.)	40	40	42	47		*5.3*	*3.9*	*4.8*	*1.3*	*0.4*	*0*	*0.3*	*1.6*

* Control to show sustained action of histamine upon gastric secretion for duration of experimental period. Actually, a slight rise in secretory rate without drop in concentration of acid toward the end of the experiment is seen, which is rather typical in such experiments.

† Stomach filled with 2 per cent aqueous solution of sodium dodecyl sulfate for 30 minutes. Figures in italics are the results obtained in the periods following the withdrawal of the sodium dodecyl sulfate solution from the stomach.

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