Honey-induced pollen embryogenesis in anther cultures of *Datura metel*[1]

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Summary. In anther cultures of *Datura metel,* pollen-embryoids could be induced on a medium consisting of honey alone. Further studies revealed that a combination of fructose and dextrose was sufficient to trigger pollen-embryogenesis. The 2 sugars, when tried individually, did not elicit any response.

Following upon earlier studies with anther cultures of *Datura metel*[3,4], it was observed that pollen embryoids could be induced on the basal medium alone without organic supplements (Nitsch's[5] inorganic salts with 2% sucrose). A similar effect was also reported by Sopory and Maheshwari[6] for *Datura innoxia.* Mineral salts alone without the addition of sucrose did not induce any androgenesis. Subsequently, honey as a medium for anther culture was tried. Honey is known to contain 75% of fructose and dextrose, 2% sucrose, 18% water and the remaining 5% consists of minerals, dextrins etc.[7]. Media made of 3 different concentrations of honey, namely, 2%, 3% and 4% (by weight) designated as H_2, H_3 and H_4 respectively were tried. The control medium was Nitsch's[5] mineral salts with 2% sucrose.

The media were prepared by dissolving weighed quantities of honey in distilled water, adjusting the pH to 5.8 with 0.1 N NaOH (as in control) before the addition of 0.8% Difco's bacto-agar. The medium was dispensed into culture tubes plugged with cotton wool wrapped in muslin, and autoclaved at 15 psi for 15 min. Flower buds with anthers measuring 11–13 mm (at the mid-uninucleate stage, found earlier to give the highest response) from a purple pigmented variety of *Datura metel* were used. Bud sterilization, anther inoculation and cytological studies were carried out according to the procedure outlined earlier[3]. Cultures were incubated at 25° ± 1 °C under low light conditions (about 200 lux).

Anthers responded in all the 3 concentrations of honey (table 1), the maximal response being in H_3 medium. As in the control, response in the honey media was in the form of pollen-derived embryoids and/or callus which emerged out of the anthers, but there was no callusing of the somatic tissue of the anthers. Embryoids also differentiated from the calli, and most of the embryoids (direct as well as through callus) grew to different sizes and shapes. Globular and heart-shaped embryoids budded off from almost any part of the older embryoids. Cytological observations of about 20 embryoids randomly selected from each of the 4 media showed a haploid or a diploid chromosome number, the diploids predominating. However, unlike those in controls, none of the pollen-embryoids arising in honey media developed a root, although shoot-like structures were formed in a few cultures. A common feature observed in all the honey media was the complete lack of pigment synthesis in the embryoids except in 1 elongated embryoid growing in H_3 medium which developed chlorophyll as well as anthocyanin, characteristic of the purple pigmented variety used. In all others, pigment synthesis did not occur either under continuous light (approximately 4000 lux) or under alternating 12-h periods of light and dark. Embryoids at corresponding stages growing in the control medium developed both chlorophyll and anthocyanin even under low light conditions (200 lux) and developed into plantlets. Embryoids from honey media, on transfer to the control medium also synthesized pigments and developed into plantlets. Pigment synthesis also occurred in about 60% of the embryoids from anthers planted on a 3% honey medium supplemented with $MgSO_4 \cdot 7H_2O$ (185 mg/l) and Fe-citrate (10 mg/l).

Table 1. Response of *Datura metel* anthers cultured in different media

Medium	No. of anthers Inoculated	Showing embryoids/callus*	Average number of days taken for response	% response
Control Nitsh's (1969) mineral salts + 2% sucrose	54	31	30	57.4
H_2*	40	17	39	42.5
H_3	59	30	36	50.8
H_4	68	28	35	41.1
FD	41	14	58	22.9

* H_2, H_3, H_4: Honey 2%, 3% and 4% respectively. FD: Fructose & dextrose 1.5% each. ** After emergence through the anther wall.

Table 2. Comparative development of pollen embryoids on different media, after 25 days of anther culture

Medium	Pollen grains having 2–8 nuclei	More than 8 nuclei	Embryoids released from pollen	Total No. scored
Control	23	28	26	1211
H_2	11	7	3	1006
H_3	19	21	19	1112
H_4	6	4	12	1127
FD	8	–	–	1313

Since about 75% of honey is composed of fructose and dextrose, a medium consisting of only fructose and dextrose (FD) was tried, using 15 g/l of each of these sugars. After 8-9 weeks in culture the anthers gave rise to pollen-embryoids of different sizes and shapes but there was no callus formation. Budding of smaller embryoids from older embryoids was less frequent than in the honey media, but as in the latter, pigment development was absent. Subsequently, fructose and dextrose were tried individually at concentrations of 15 g/l and 30 g/l; however, the anthers did not show any response.

5 anthers were withdrawn at random from all the media after 25 days in culture for a comparative study of pollen embryoid development. Among the honey media, medium H_3 which had stimulated the maximum number of anthers to produce embryoids/calli (table 1), also showed the highest number of pollen grains undergoing embryogenesis (table 2). On the other hand, anthers from FD medium showed only few pollen divisions, most of them as yet (25 days) being at the binucleate stage. In media having fructose or dextrose at either of the 2 concentrations, no additional nuclei or nuclear divisions were observed in pollen grains even after 10 weeks in culture. It would appear from the present study that sugars (fructose and dextrose) alone could stimulate pollen embryogenesis and the production of fully developed embryoids in anther cultures of *Datura metel*, but for their development into plantlets, the addition of inorganic salts would be necessary.

The honey used in this experiment was obtained from the Bee Keeping Laboratory of the Division of Entomology in this Institute. The honey collected in the bee-hives were mostly from various *Brassicae*, the major crop during the season.

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An electrophysiological study on the myocardium of dystrophic mice[1]

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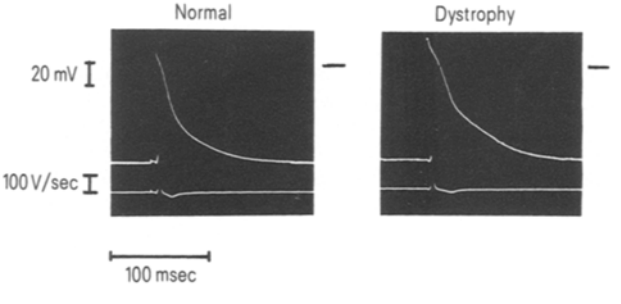
Summary. Decreased resting potential and prolonged duration of the action potential were observed in left ventricular muscles of dystrophic mice, while there was no change in myocardial potassium content.

It is well recognized that in dystrophic mice cardiac muscles are also involved[2], but there is no report on the electrophysiological features of the myocardium in dystrophic mice. In order to clarify the abnormality of cardiac muscle in dystrophic mice, we estimated the potassium content and the transmembrane action potential of the left ventricular muscles (LV) in normal and dystrophic mice.

Materials and methods. Potassium, water and fat contents in LV were determined in 8 homogeneous normal and 8 dystrophic mice of the C57BL/6JCL strain of either sex aged 8 weeks. Potassium content was measured as follows[3]; the cardiac muscle, weighing 30-80 mg, was weighed in a dry, dust-free, and preweighed glass. The samples were dried at 90 °C vacuum for 6 h. After extraction with petroleum ether and repeated drying and weighing, the amount of fat-free solid could be computed. The fat-free dry samples were extracted with 1.5 M HNO_3 overnight. The potassium level was determined on a flame photometer using lithium as internal standard. Electrophysiological investigation was made in 10 normal and 10 dystrophic mice. The left ventricular muscles, which were dissected from the mouse under sodium pentobarbital anesthesia, were placed in a tissue chamber where oxygenated Tyrode solution was continuously perfused. The Tyrode solution had the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, glucose 5.5. Temperature of the Tyrode solution was maintained at 36-37 °C.

Each preparation was initially driven at a basic cycle length of 200 msec for 30 min, and the membrane potential was measured by the conventional microelectrode technique. All microelectrodes were filled with 3M KCl and had resistances of 10 to 30 MΩ. The maximal rate of depolarization (dV/dt) was obtained by an electronic differentiator (Nihon Koden, S-4103).

Results and discussion. There were no significant differences between normal and dystrophic mice in the contents of potassium, water and fat in the LV. The overshoot (OS), resting potential (RP), action potential amplitude (AP), dV/dt, and 50% and 80% durations of action potential (APD50 and APD80) in normal and dystrophic mice are given in the table. As compared with normal mice, the OS,



Representative recordings of action potentials for normal and dystrophic mice. Prolonged duration and decreases in overshoot and maximal rate of depolarization are observed in dystrophic mice. Upper trace shows transmembrane action potentials, and lower one shows maximal rate of depolarization. Bars (−) indicate zero line.