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5 anthers were withdrawn at random from all the media after 25 days in culture for a comparative study of pollen embryoid development.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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2 Address: Division of Genetics, Central Plantation Crops Research Institute, Kasargod, Kerala (India).
3 R.D. Iyer and S.K. Raina, Planta *104*, 146 (1972).
4 S.K. Raina and R.D. Iyer, Indian J. Genet. *34A*, 283 (1974).
5 J.P. Nitsch, Phytomorphology *19*, 389 (1969).
6 S.K. Sopory and S.C. Maheshwari, J. exp. Bot. *27*, 58 (1976).
7 B.F. Beck and D. Smedley, in: Honey and your Health, p.246. Dodd, Mead & Co., New York 1966.

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

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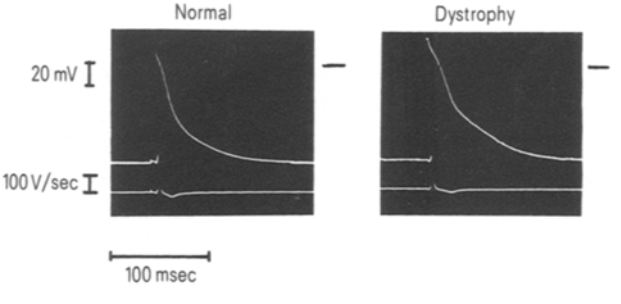
Summary. Decreased resting potential and prolonged duration of the action potential were observed in left ventricular muscles of dystrophic mice, while there was no change in myocardial potassium content.

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.

Action potential of left ventricular muscle in normal and dystrophic mice

	RP(mV)	OS(mV)	AP(mV)	dv/dt(v/sec)	APD_{50}(msec)	APD_{80}(msec)
Normal (n = 197)	81 ± 5	26 ± 4	106 ± 6	330 ± 35	15 ± 2	36 ± 5
Dystrophy (n = 198)	77 ± 4	24 ± 4	101 ± 6	303 ± 31	19 ± 3	47 ± 8
p	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001

Values in this table are expressed as mean ± SD. OS, overshoot; RP, resting potential; AP, action potential amplitude; APD_{50}, 50% duration of action potential; APD_{80}, 80% duration of action potential; dv/dt, maximal rate of depolarization.

RP, AP and dV/dt were significantly decreased in dystrophic mice.

The APD50 and APD80 were significantly longer in dystrophic mice than in normal mice. The representative recordings of action potentials are shown in the figure.

The basis for the RP of the myocardial cell can be understood in terms of the electrochemical gradient for potassium that exists across the sarcolemma. We reported that[4] both the potassium content and the RP of skeletal muscles in dystrophic mice were significantly lower than those in normal mice at the same age of 8 weeks. While there was no difference between the myocardial potassium contents in dystrophic and normal mice, the RP of LV in dystrophic mice was significantly lower than that in normal mice. This result may be explained by either an increased inward background current or a decreased outward current. It is well known that membrane conductance to potassium is significantly and rapidly altered by an alteration in the intracellular calcium ion concentration[5,6], and the plateau phase of the action potential is determined by the balance between the outward current and slow inward current[7].

The plateau of the action potential in dystrophic mice was more prolonged than that in normal mice. This finding could be explained by either an increased slow inward current or a decreased late outward current. In conclusion, we demonstrate in this study that myocardial cells of dystrophic mice have abnormal membrane permeability at the stage when changes in contents of potassium, water and fat are not developed.

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2 G. Jasmin and E. Bajusz, Nature *193*, 181 (1962).
3 N. Valentin and K.H. Olesen, Scand, J. clin. Lab. Invest. *32*, 155 (1973).
4 K. Saito, H. Ohkura, H. Tanaka, T. Kashima, H. Katanasako and T. Kanehisa, Japan. J. exp. Med. *50*, 179 (1980).
5 G. Isenberg, Nature *253*, 273 (1975).
6 J.B. Bassingthwaighte, C.H. Fryg and J.A.S. McGuigan, J. Physiol. *262*, 15 (1976).
7 G.W. Beeler and H. Reuter, J. Physiol. *268*, 177 (1977).

The size of motor units in laryngeal muscles of the rat

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Summary. The size of motor units in rat laryngeal muscles was determined by correlating the number of neurons labeled by i.m. injections of horseradish peroxidase with the number of motor end plates stained for acetylcholinesterase. The cricothyroid has a motor unit size of 8 muscle fibers per motor neuron and the posterior cricoarytenoid 4–5 muscle fibers per motor neuron.

Derived from the primitive sphincteric musculature of the pharynx, the laryngeal muscles in mammals have taken on such diverse functions as the control of air distribution in the lungs during respiration[1], protection of the pulmonary apparatus[2], and phonation or the production of sound[3]. It might be anticipated in such highly skilled musculature, that the size of motor units would be small. However, morphological support for this premise is scant and conflicting. Rüedi[4] states that there are 2–3 muscle fibers per motor unit in human laryngeal muscles but gives no details of how this figure was obtained. English and Blevins[5] estimate that there are 30 muscle fibers per motoneuron in the cricothyroid (CT) of human and 50–60 muscle fibers per motor neuron in CT of cat. They related the number of fibers (stained with silver) in muscle nerves to the number of muscle fibers (the innervation ratio) and assumed the same ratio of sensory to motor fibers as exists in spinal nerves (an untenable assumption[6]). Faaberg-Anderson[7] in a study of a newborn infant has determined that the average motor unit contains 166 fibers in CT and 116 in PCA.

Difficulties in accurately determining the size of laryngeal motor units can be related to the demonstration of multiple innervation of laryngeal muscles in some species[8–10], the great variability in the number of fibers in nerves supplying the larynx[11], variation in branching of laryngeal nerves[12] and the recent demonstration that, in the kitten, at least 2 nuclei in the brainstem provide motor innervation to laryngeal muscles[13].

We have determined the size of laryngeal motor units in the rat by correlating the number of cells labelled in the brainstem by retrograde transport of horseradish peroxidase (HRP, Boehringer) following i.m. injection of a 50% solution in 2% dimethylsulphoxide[14,15], with the number of motor end plates counted from serial longitudinal sections of muscle stained for acetylcholinesterase[16].

Under chloral hydrate anaesthesia (0.3 ml/100 g b.wt of a 12% aqueous solution) HRP was injected into the CT or PCA of 23 adult male hooded rats (Wistar strain) weighing 200–500 g, using a 1 µl Hamilton syringe or glass pipette attached to a hydraulic system.