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SHORT NOTE

PARTIAL PRESERVATION OF THE ABILITY OF ACCUMULATING ALKALI-METAL IONS IN 2 mm MUSCLE CELL SEGMENTS WITH BOTH ENDS OPEN

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A frog sartorius muscle contains about 1,000 fine, cylindrical cells, each measuring 50 to 100 μ wide and 2 to 3 cm long, all running without interruption from one end of the muscle to the other (Ling, 1984, p. 133). It has been firmly established that if a razor-blade cut is made across the muscle cells, no regeneration of the lost cell membranes occurs at the cut ends of the cells (Ling, 1978; 1984, p. 136; Cameron, 1988). A series of razor-blade cuts (2 mm apart) on a sartorius muscle produces a useful model for the direct study of cytoplasmic functions of living cells without the limitations imposed by the presence of an enclosing surface membrane barrier. Indeed, similar preparations of open-ended muscles cells have already proven useful in helping to choose between the membrane pump model of the living cell and the alternative association-induction hypothesis (Ling and Ochsenfeld, 1973; Ling and Walton, 1976; Ling, 1978).

Unfortunately, cut muscles as a rule, do not retain their normal physiological functions for long; deterioration sets in soon after the cells are cut, spreading slowly but steadily from the cut toward the intact end of the cells. This deterioration is accompanied by loss of the ability of selectively accumulating K^+ and of excluding Na^+ (Ling, 1978). Much effort has been made in the past to find the right additive(s) (e.g., ATP, creatine phosphate with or without creatine kinase) or a better composition of frog Ringer solution that can prevent, or at least delay, the deterioration of the cut muscle segments. All ended in failure.

Then on March 1,1985 I finally found such an additive: poly (ethylene glycol) (Carbowax PEG 8000, Cat. No. P156-500, Fisher Scientific Co., Springfield, N.J.).

Table I presents the original data obtained as well as the chemical composition of the various experimental solutions used. In a solution containing from 16 to 20% PEG 8000, the open-ended 2 mm long muscles segments maintained at 4°C a high intracellular K^+ for hours.

Why does PEG 8000 preserve normal functions of the open-ended muscle cell segments is not fully understood. I suggest the following tentative explanation:

PEG 8000 effectively polarizes water in multilayers (Ling and Ochsenfeld, 1983). From its enormous molecular weight (80,000 daltons) and from its propensity to polarize and to attach itself to layers of water molecules, one predicts an unusually low equilibrium distribution coefficient or q-value of this polymer in the cell water. This prediction is based upon the "size rule", according to which the q-value of a solute molecule in water existing in the dynamic structure of polarized multilayers decreases with increasing molecular size (Ling, 1970; 1986; 1987; Ling and Hu, 1988). As a result, PEG 8000

TABLE I. The Effectiveness of Various Solutions in Preserving the Selective Accumulation of K^+ in 2 mm Cut Sartorius Muscle Segments at 4°C. Sartorius muscles were cut into 2 mm long segments with both ends open and incubated in the various solutions described. After an incubation period, long enough to assure a new equilibrium, (2 hrs.), the muscle segments and aliquots of the final bathing solutions were analyzed for K^+ by atomic adsorption spectrophotometry. $[K^+]_{ex}$ and $[K^+]_{in}$ are respectively the K^+ concentration in the external medium (in millimolarity) and in the muscle cell segments (in μmoles per gram of fresh muscle segment weight).

	KCl (mM)	$MgCl_2$ (mM)	D-glucose (mM)	Sorbitol (mM)	Sucrose (mM)	PVP-10 (%)(w/v)	Dextran (%)(w/v)	PEG 8000 (%)(w/v)	$[K^+]_{ex}$ (mM)	$[K^+]_{in}$ (μmoleslg.)	$\frac{[K^+]_{in}}{[K^+]_{ex}}$
1	2.5	115.5							6.70	7.80 ± 0.45(4)	1.16
2	2.5		231						6.30	16.1 ± 0.93(4)	2.56
3	2.5			231					6.40	18.1 ± 4.9(4)	2.82
4	2.5				231				3.28	14.4 ± 19.8(4)	4.36
5	2.5					19.6			3.12	16.9; 16.5(2)	5.4
6	2.5						19.6		3.21	22.0; 22.2(2)	6.7
7	2.5							19.6	2.57	70.0 ± 3.2(4)	27.2

stays almost entirely outside the open-ended cut muscle segments and therefore does not intrude upon and disrupt the cytoplasm and its normal functions; yet by lowering the osmotic activity of the surrounding external solution (Ling, 1983), it also prevents cell swelling and damage associated with cell swelling (Ling, 1984, p. 438).

The high osmotic activity of the 16 to 20% PEG 8000 eliminates the need to include 100 mM NaCl in the bathing solution as is usual in preparing the normal Ringer solution. This elimination may be beneficial to the open-ended cells for the following reason: According to the association-induction hypothesis, the cytoplasmic protein, actin in resting voluntary muscle cells does not exist in the fibrous *F-actin* form — as it is widely taught — but in a *profilamentous* form (Ling, 1984, p. 568). However, upon contact of the exposed cytoplasm at the cut end of the muscle segment with a 100 mM NaCl present in the Ringer solution, the water-polarizing profilamentous actin in the cell rapidly transforms into the *non-water-polarizing* fibrous actin form. Depolarization of cell water and a loss of the cell's ability to exclude Na^+ (and other large molecules and hydrated ions) as well as the closely associated ability of selectively adsorbing K^+ may follow in consequence.

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