tional folds are randomly disposed with respect to the long axis of the muscle fibre. The complexity in the shape and arrangement of these folds is fully displayed in this type of preparation (Fig. 2). Elevations of the muscle fibre which show a smooth surface and lack any cross striation are seen in and around the synaptic depression. The elevations undoubtedly correspond to the "terminal cone or eminence" of Doyére,[4] which are known to contain muscle (or fundamental) nuclei, an accumulation of mitochondria and sarcoplasmic reticulum.

The present study extends the previous light and transmission electron microscopical findings, adding new information to the morphology of NMJs which may allow quantitative study of the postsynaptic organization of the NMJs. Scanning microscopic study of muscle spindles is also in progress in our laboratory.

References

1) Evan, A. P., Dail, W. G., Dammrose, D. and Palmer, C., Scanning electron microscopy of cell surfaces following removal of extracellular material. *Anat. Rec.*, **185**, 433–446, 1976.
2) Uehara, Y. and Suyama, K., Visualization of the adventitial aspect of the vascular smooth muscle cells under the scanning electron microscope. *J. Electron Micros.*, **27**, 157–159, 1978.
3) Couteaux, R., Motor end-plate structure. *In: The structure and Function of Muscle (edited by Bourne, G. H.)* Vol. **1**, 337–380 Academic Press; New York, 1960.
4) Doyére, L., Memorire sur de les tardigrades. *Annals de Sciences Naturelles Zoologie,* **14**, 269–361, 1840.

High potassium effect on the mobility of sea urchin sperm

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Immobilization of sea urchin sperm by high concentration potassium cation was first described by Gray[1] and has been examined by various authors.[2,3] It would have been selected as a typical example for electrophysiological study of flagellum motility if the sperm was not so small. Recently, new techniques for measurement of membrane potential and intracellular pH were developed for such small cells using lipid soluble radioactive cation[4] and ^{31}P Nuclear Magnetic Resonance spectrometer.[5] In this report the membrane potential and intracellular pH of sea urchin sperm are described in connection with the sperm motility under high potassium concentration.

Sea urchin, *pseudocentrotus depressus,* was obtained from Misaki Marine Biological Station and its sperm was shed by conventional method. The preparations were made by dilution of the sperm into the artificial sea water of the various concentration of potassium with 10 % of volume concentration.

Tritiated TPP (Tri-phenyl-methyl-phosphonium) cation was purchased from New England Nuclear and the ethanol containing original solution was diluted 1000 times when it was used. The membrane potential was calculated from the following equations,

$$= 2.3 \frac{RT}{F} \log \frac{C_{sol}}{C_{cell}} \qquad (1)$$

$$= 2.3 \frac{RT}{F} \log \frac{v}{V} + 2.3 \frac{RT}{F} \log \frac{C_{sol}}{C^*_{sol} - C_{sol}} \quad (2),$$

where R is gas constant, F is Faraday constant, C_{sol} and C_{cell} means radioactivity of incubation solution and cells, respectively. V and v are volume of the incubation solution and cells. C* represents the radioactivity of the batching solution before incubation procedure starts.

The intracellular pH of the sperm was determined by measurement of the chemical shift of the resonance peak of inorganic phosphate using ^{31}P-NMR spectrometer (JEOL FX-60). About 1.5 ml of sample was packed in a NMR sample tube (8ϕ × 200) with 2 cm thickness. In order to determine the chemical shift value within an accuracy of 10 %, it took 10 min for measurement of each sample.

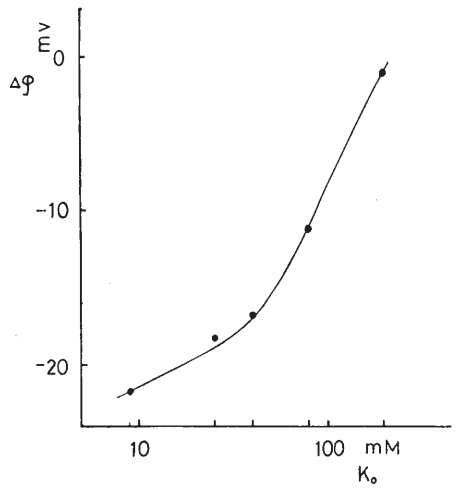

Fig. 1. Relation between membrane potential and potassium concentration of external solution. Membrane potential was calculated using eq. (2).

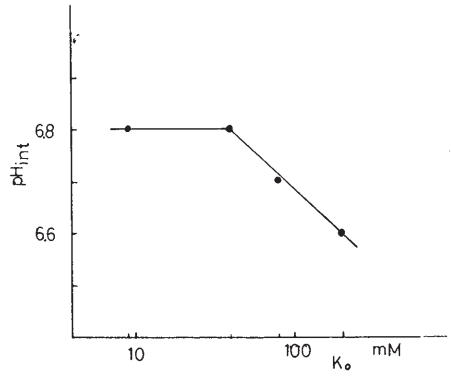

Fig. 2. Intracellular pH (pH_{int}) changes with the concentration of potassium in the incubation solution. The pH_{int} was determined by the chemical shift of resonance peak of inorganic phosphate.

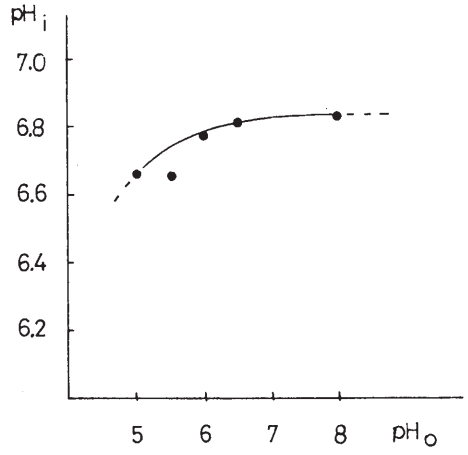

Fig. 3. Changes of intracellular pH against the external pH. External pH was adjusted by the titration of 0.1 N HCl and 0.1 N NaOH.

Cell motility was measured by the degree of the fluctuation of the light intensity which pass through optical microscope (× 150) observing systems. The photodiode was mounted on the eye-piece lense and its output was fed into the operational amplifier and finally connected to memory oscilloscope. The degree of cell motility was determined by the average amplitude of the intensity fluctuation caused by sperm moving.

In Fig. 1, the membrane potential ($\Delta\varphi$) of the sperm was plotted against the various concentration of potassium. Under the normal condition of incubation solution, the membrane potential of the sperm was about -20 mV, and it was decreased linearly with potassium concentration. Finally, it became nearly 0 when potassium concentration reached 200 mM.

Under the same range of potassium concentrations, the intracellular pH was measured by using ^{31}P-NMR. The results are shown in Fig. 2. From 10 mM to 40 mM of potassium concentration, the pH_{int} was constant (=6.8) and then decreased linearly with K^+ concentration. The same kind of decrease in pH_{int} was also obtained when the pH of the incubation solution was changed to acidic (Fig. 3). In both cases, high K^+ concentration (=200 mM) or low pH (=5.0) of external solution, pH_{int} was decreased about 0.2 unit and surprisingly, cell motility was inhibited absolutely.

From these results, we can conclude that external high potassium concentration led to the acidification of pH_{int} and reached to the immobilization of sperm. The mechanism why high potassium resulted in low pH_{int} and why pH_{int} stops flagellum motion is not clear at present but it might be very convenient to assume the existence of H^+-K^+ exchange mechanism.

Considering the simillarity in the structure of sperm flagellum and hair cell cillia,[6] these results suggest that high potassium content in endolymph of cochlea might be necessary to inhibit self moving of the hair cell cilia and increase the sensitivity to the mechanical stimulation on it..

References

1) J. Gray, *J. Exptl. Biol.*, **5**, 337 (1928).
2) Lor, Rotschild, *ibid.*, **25**, 344 (1948).
3) H. Mohri, *ibid.*, **33**, 73 (1956).
4) H. Hirata, K. Altendorf and F. Harold, *Proc. Natl. Acad. Sci.*, **70**, 1804 (1973).
5) R. B. Moon and J. H. Richard, *J. Biol. Chem.*, **248**, 7276 (1973).
6) M. A. Sleigh, *'The Biology of Cilia and Flagella'*, Pergamon, Oxford (1962).

Inhibition on cochlear nerve fibers by the sound-activated olivocochlear bundle

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The olivocochlear bundle (OCB) described by Rasmussen descends from the ascending auditory pathway, the superior olivary complex, to the receptor site in the cochlea. In addition to 500 myelinated OCB fibers estimated by Rasmussen, numerous unmyelinated