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# Effects of External Osmotic Pressure on the Membrane Potential of *Opalina*<sup>1)</sup>

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(With 4 Text-figures)

It has been known that the frequency of pulsation of the contractile vacuole of protozoa depends on the external osmotic pressure. In *Paramecium caudatum* and *Nyctotherus cordiformis* the interval of pulsation of the contractile vacuole was increased by increase of the external osmotic pressure (Herfs, 1922). According to Yamaguchi (1960), the change in membrane potential occurred in accordance with contraction of the contractile vacuole of *Paramecium caudatum* when the tip of microelectrode was inserted into the vacuole or near it.

On the other hand, Nakatani (1971) obtained the results which suggest the relationship between the spontaneous change in the membrane potential and the osmotic regulation in *Opalina*. But it is not clear from the results whether the effect on the change in membrane potential is of the external osmotic pressure or of the change of Na-ions concentration. In the present experiments, this problem was examined and discussed.

## Material and Methods

*Opalina*, obtained from the rectum of the frog, *Rana japonica*, was used for the experiments. The cells were washed well with the normal adaptation medium containing 110 mM NaCl, 2 mM KCl, 2 mM CaCl<sub>2</sub> and 5 mM tris buffer (pH=7.4) and kept in the medium for longer than one hour before the experiments.

The external osmotic pressure was changed by change of choline chloride concentration in the external medium. Namely, test solutions were prepared by mixture of the solution containing 30 mM NaCl, 2 mM KCl, 2 mM CaCl<sub>2</sub> and 5 mM tris buffer and the solution containing 170 mM choline chloride, 2 mM KCl, 2 mM CaCl<sub>2</sub> and 5 mM tris buffer. Further, the test solution with a constant osmotic pressure were prepared by mixing the solution containing 170 mM NaCl, 2 mM KCl, 2 mM CaCl<sub>2</sub> and 5 mM tris buffer with the solution containing 170 mM choline chloride, 2 mM KCl, 2 mM CaCl<sub>2</sub> and 5 mM tris buffer.

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Before impalement of the microelectrode, the washed animals were transferred into the test solution. The recording method of membrane potential was the same with that of the previous paper (Nakatani, 1971).

The experiments were carried out at room temperatures ranging from 18° to 20°C.

## Results

### *Effects of the external osmotic pressure on the membrane potential.*

The tonicity of the external solution were changed from 40 mM to 180 mM equivalent of NaCl solution by change in the concentration of choline chloride, but the concentration of NaCl, KCl and  $\text{CaCl}_2$  was kept constant at 30 mM, 2 mM and 2 mM, respectively. This range of tonicity is equivalent to that where a linear relationship was found between the frequency of membrane potential change and the NaCl concentration in the previous paper (Nakatani, 1971).

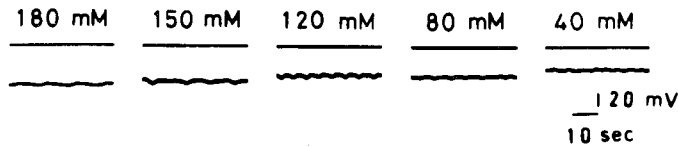


Fig. 1. Typical records of the change in membrane potential of *Opalina* in the medium at different tonicities. Numerals with mM indicate the tonicity of external medium equivalent to those of NaCl solution.

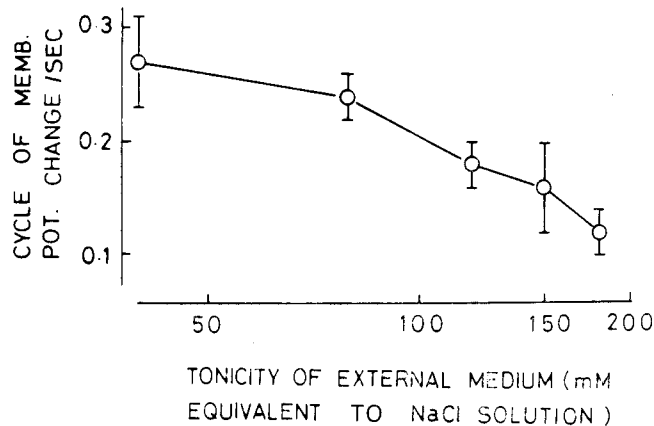


Fig. 2. Effects of change in the external osmotic pressure on the frequency of spontaneous change in membrane potential of *Opalina*. The osmotic pressure of external medium was changed by change in the concentration of choline chloride, but the concentration of NaCl, KCl and  $\text{CaCl}_2$  was kept constant at 30 mM, 2 mM and 2 mM, respectively. Each plot was obtained from mean value of 5 individuals.

After impalement of the electrode, the change in membrane potential of comparatively large amplitude occurred, then the amplitude decreased gradually and after few minutes the amplitude became small but was kept almost constant. So, the records 2 or 3 minutes after electrode impalement were adopted.

Membrane potential increased and the frequency of membrane potential change decreased accompanied with increase of choline chloride concentration in the external medium (Figs. 1 and 2). The frequencies of membrane potential change were  $0.27 \pm 0.04/\text{sec}$  and  $0.12 \pm 0.02/\text{sec}$  at the tonicities of external medium equivalent to 40 mM and 180 mM NaCl solution, respectively. And as clear from Figure 2, the frequency of membrane potential change was decreased linearly by increase of the external tonicity. This result is almost the same as that of the case where the external tonicity was changed by change of NaCl concentration (Nakatani, 1971).

In the present experiments, the amplitude and the duration of membrane potential change in relation to the change in osmotic pressure of the external medium were not measured, because the amplitudes of membrane potential change in each test solution were comparatively small.

*Effects of Na-ions concentration on the membrane potential under a constant osmotic pressure.*

The tonicity of external medium was kept constant at an equivalent tonicity to 180 mM NaCl solution by addition of choline chloride. Membrane potential was decreased by increase of external Na-ions concentration (Fig. 3).

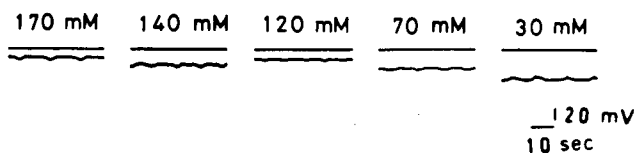


Fig. 3. Typical records of the change in membrane potential of *Opalina* in the medium with different NaCl concentrations under a constant tonicity of 180 mM equivalent to that of NaCl solution.

On the other hand, the value of effects of Na-ions concentration on the frequency of membrane potential change was within the standard error. For the change of Na-ions concentration from 30 mM to 170 mM, the difference of frequency of the membrane potential change was from 0.10/sec to 0.13/sec (Fig. 4). Further, the values of frequencies of the membrane potential change at each concentration of Na-ions as far as used in the present experiments, were included within the standard errors in Figure 2.

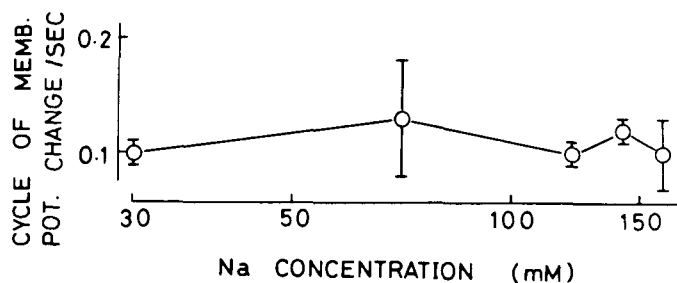


Fig. 4. Effects of change in the external NaCl concentration, under a constant tonicity equivalent to that of 180 mM NaCl solution, on the frequency of spontaneous change in membrane potential of *Opalina*. The constant tonicity of the external medium was kept by change in the concentration of choline chloride. Each plot was obtained from mean value of 5 individuals.

### Discussion

Herfs (1922) has examined the relationship between the external osmotic pressures and the activity of contractile vacuole of *Paramecium caudatum* and *Nyctotherus cordiformis*, and it was found that the interval of pulsation of the contractile vacuole is increased with increase of external osmotic pressure. Yamaguchi (1960) reported the change in membrane potential occurred accompanied with pulsation of the contractile vacuole, when the tip of recording electrode was inserted into the vacuole of *Paramecium caudatum*. Those facts may show that membrane potential change occurs accompanied with pumping out the water from the inside of protozoan cell.

*Opalina* used in the present experiments has no contractile vacuole or excretory organella. It may be thought that the internal osmotic pressure was actively regulated through the cell membrane, because the animals live in the frog rectum and the osmotic pressure may change usually in the rectum.

From this reason, in the previous paper (Nakatani, 1971) the relationship between the external Na-ions concentration and the frequency of membrane potential change was examined, and it was found that there is a linear relationship between them. But, from the results, it is not clear whether the effects on the membrane potential were due to external osmotic pressure or to Na-ions concentration. The results in Figures 2 and 4 of the present experiments may show the former is appropriate. Namely, there was a linear relationship between the tonicity of external medium and the frequency of membrane potential change, but there was no effect of Na-ions concentration on the frequency under a constant tonicity. From those facts, it is clear that the external osmotic pressure has an effect on the frequency of membrane potential change of *Opalina* but Na-ions concentration itself has no direct effect on the frequency. So, it is possible that the spontaneous and repeated change in membrane potential of *Opalina* occurs accompanied with osmotic regulation of this animal.

According to Ueda (1961), the inside negativity of *Opalina* is increased by decrease of external Na-ions concentration and it is greater in the choline chloride-Ringer's solution than that in the sucrose-Ringer's solution. Further, it is known that the membrane potential of longitudinal muscle of lobworm (*Tylorrrynchus heterochaetus*) is increased in the Na-free solution (Ito and Tashiro, 1970). In the present experiments, the membrane potential was increased when the tonicity of external medium was increased by increase of choline chloride concentration (Fig. 1) or when Na-ions concentration was decreased under a constant tonicity (Fig. 3). As it was pointed out by Ueda (1961), those facts seem to indicate that the membrane of *Opalina* is permeable to Cl-ions to some extent.

### Summary

1) Effects of tonicity of the external medium on the membrane potential of *Opalina* were examined.

2) The frequency of spontaneous and repeated change in the membrane potential was decreased linearly by increase of the external osmotic pressure.

3) The membrane potential was decreased but the frequency of the membrane potential change was not affected by increase of Na-ions concentration under a constant tonicity equivalent to 180 mM NaCl solution by change in the concentration of choline chloride.

4) The frequency of spontaneous and repeated change in the membrane potential of *Opalina* was affected directly by the external osmotic pressure and not by Na-ions concentration, so it seems that the membrane potential change occurs accompanied with regulation of the osmotic pressure.

5) The membrane of *Opalina* is permeable to Cl-ions to some extent, because the inside negativity was increased by increase of the external choline chloride concentration.

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