Membrane Potential Fluctuation

in

Paramecium

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by

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Contents

Summary

In chapter I, the intracellular membrane potential fluctuation in Paramecium caudatum was studied. The fluctuation was very large and the power of fluctuation depended on the environmental salt condition, particularly on the ratio of the potassium ion concentration to the square root of the calcium ion concentration, $[K]/[Ca]^{1/2}$, in the medium. It decreased with the increase of the ratio. The fluctuation arose mainly from the local current of these ions.

In chapter II, the excitability of the membrane was examined under the same condition as fluctuation was analysed. The maximum rate of rise (MRR) of the spike evoked by applied stimuli was found to be controled by the ratio $[K]/[Ca]^{1/2}$. It increased with decreasing ratio, in proportion to the logarithm of the power of potential fluctuation. Thus, the empirical relation between the power of potential fluctuation and the membrane excitability was derived using the ratio $[K]/[Ca]^{1/2}$ as a parameter. It represented the relationship between the response of the system to an external disturbance and the magnitude of the internal fluctuation of the system in the absence of the disturbance.

In chapter III, the tactic response of the organisms for supernatant of bacterial suspension was examined by the capillary method and the effect of the environmental salt condition was studied. A maximum response appeared in the intermediate range of the ratio $[K]/[Ca]^{1/2}$. The results suggested that the relative change of the potential fluctuation affected by the environmental condition modulates the degree of membrane excitation and would be important in the mechanism of the behavioral response.

Introduction

Recent electrophysiological studies of Paramecium have given evidences for that a spikelike but graded regenerative depolarization of the membrane potential induces a reversal of orientation of cilia [1]. This ciliary reversal causes the organism to change its swimming direction. A typical example is known as an avoiding reaction [2]. When a Paramecium cell bumps to an object, the ciliary reversal occurs and the cell swims backward for a moment; then it swims again in a new direction. It was confirmed by using a Paramecium cell fixed on a glass plate that a mechanical stimulus on the cell generates a spikelike depolarization of the membrane potential [3].

A change of the swimming direction has an important role in the behavior of Paramecium [4]. Modulation of the frequency of directional changes is a basic mechanism of tactic behaviours, such as thermotaxis or chemotaxis. The cell changes the frequency of directional changes, responding to the gradient of temperature or concentration of chemicals [5, 6].

Paramecium cells change the swimming direction even in a uniform environment. The directional change occurs spontaneously without any specific stimulus from the environment.

The frequency of such directional changes is, on the average, once per ten seconds. The cause of such spontaneous directional changes should be found within the living organism itself. It is very probable that the membrane potential spontaneously fluctuates, sometimes evoking a spike, which induces the ciliary reversal.

In chapter I, it is shown that the intracellular electric potential of Paramecium fluctuates with time. The fluctuation is very large, depending on the environmental condition. The effect of potassium ions and calcium ions are particularly remarkable. The potential fluctuation is related to the fluctuation of local currents of those ions.

In chapter II, the excitability of the membrane of Paramecium is examined in various environmental conditions. Potassium and calcium ions have great influences on both the excitability and the magnitude of spontaneous potential fluctuation.

In chapter III, the chemotaxis of Paramecium to an attractant is examined in the same conditions as those for the measurement of potential fluctuation and excitability. The effect of the environmental condition on the chemotaxis is analysed by regarding the attractant as a perturbation on the potential fluctuation and the excitability.

Finally, on the basis of the results presented here, biological significance of the spontaneous fluctuation of the membrane potential is discussed.

Chapter I

POTENTIAL FLUCTUATION IN *PARAMECIUM*

I. INTRODUCTION

Many investigators have reported on the subthreshold fluctuation of the membrane potential or current in nerves and other excitable cells [7 - 10]. The potential fluctuation contains useful information about the underlying molecular mechanism and the main problem studied and discussed has been the kinetics of ion channels in the membrane. However, less attention has been paid to the biological function of the potential fluctuation. Actually, in microorganisms the fluctuation of the membrane potential may be directly related to their swimming behaviors. Discontinuous directional changes of swimming *Paramecium* cells, for example, are associated with depolarization of the membrane potential [11, 12]. Recently, Moolenaar, de Goede and Verveen [13] measured the fluctuation of the membrane potential and current in *Paramecium* cells. They obtained power density spectra of a type of 1/f (f : frequency) and suggested that potassium ions are involved in the fluctuating current.

The purpose of this chapter is to describe our results of analysis of the spontaneous fluctuation of the membrane potential in Paramecium cells. The time correlation function and the power density spectrum at various environmental conditions are shown. The effects of calcium and potassium ions on the fluctuation are investigated.

II. MATERIALS AND METHODS

A. Sample Preparation

The specimens of Paramecium caudatum supplied by Dr. Shimizu of Osaka University were cultured in a hay infusion with aerobactor at 25 °C. Cells were concentrated by filtration and washed with an equilibration solution containing 0.5 mM KCl, 1.0 mM $CaCl_2$ and 1.0 mM Tris-HCl buffer of pH 7.2. Then, the cells were incubated in the same solution for 30 min before the experiment at 25 °C.

B. Measurements and Analysis of Membrane Potential Fluctuation

Under microscopic observation, glass capillary microelectrodes filled with 0.1 M KCl were inserted to a Paramecium cell fixed on a slide glass, according to the method of Naitoh [14]. An agar bridge containing 3 M KCl and 1 % agar was used as a reference electrode in the external medium. The potential difference was recorded on an FM magnetic tape recorder. The external medium was replaced by solutions containing 1.0 mM Tris-HCl of pH 7.2 and various concentrations of KCl and $CaCl_2$. The temperature of the medium was maintained at 25 °C by circulation of temperature-controled water under the slide glass.

To detect a slow change of the resting potential, the autocorrelation function $C(\tau)$ of the membrane potential was calculated according to the equation:

$$C(\tau) = \overline{(V(t) - \overline{V})(V(t+\tau) - \overline{V})} \qquad (1)$$

where $V(t)$ is the membrane potential (the voltage) at time t and τ is the time difference. The bar means the average with respect to the time t. Calculation was carried out using the data of 600 points at 500 ms intervals for 5 min in the record.

After elimination of slow components by a high pass filter with a cut-off frequency of 0.02 Hz, the fluctuating potential was sampled at 20 Hz for 140 sec and the histogram

of potential distribution was made.

The power density spectrum (PDS) of fluctuating potential was obtained from the record by a computer in a frequency range from 0.4 Hz to 40 Hz at intervals of 0.2 Hz. Parts of the record where sharp spikelike depolarizing and hyperpolarizing fluctuation was excluded were chosen and the values of the potential were read at 102.4 Hz for 17.5 s, and the PDS was computed. The final PDS was obtained after averaging 5 to 10 spectra derived from different parts of the same record from the same cell in the same condition.

The total power of fluctuation was defined by

$$I = \sum_i I(f_i)\,\Delta f \qquad (2)$$

where $I(f_i)$ is the power density in the interval Δf which was taken to be 0.2 Hz. The summation was performed in a range from 0.4 Hz to a maximum frequency where the density almost vanished.

III. RESULTS

A. General Profile of Potential Fluctuation

1. Slow Components

The intracellular membrane potential of Paramecium caudatum was found to fluctuate with time around the resting potential which was about -30 mV under the physiological condition. Fig. 1 and 2 show examples of fluctuating potential in the presence of 0.5 mM KCl and 1.0 mM $CaCl_2$ at 25 °C. Fluctuating potential had an amplitude in the range of 0.5 mV to 5 mV from the resting potential, sometimes including sharp spikelike depolarization or hyperpolarization.

A slow and periodic change of the potential is found in the record shown in Fig. 2. To detect such a slow component of the potential change, the time correlation was analysed. Fig. 3 shows the autocorrelation function $C(\tau)$, where the ordinate is normalized by $C(0)$. $C(\tau)$ decreases gradually, and has negative values at 40 - 50 s and has positive values at 60 - 100 s. It did not tend to zero within the time range analysed. Such an oscillation of $C(\tau)$ indicates that the fluctuation contains a periodic component. The period was between 60 and 100 s, depending on the sample; it is equivalent to 0.01 to 0.016 Hz in the frequency. The amplitude of this slow oscillation of the potential was within 2.5 mV.

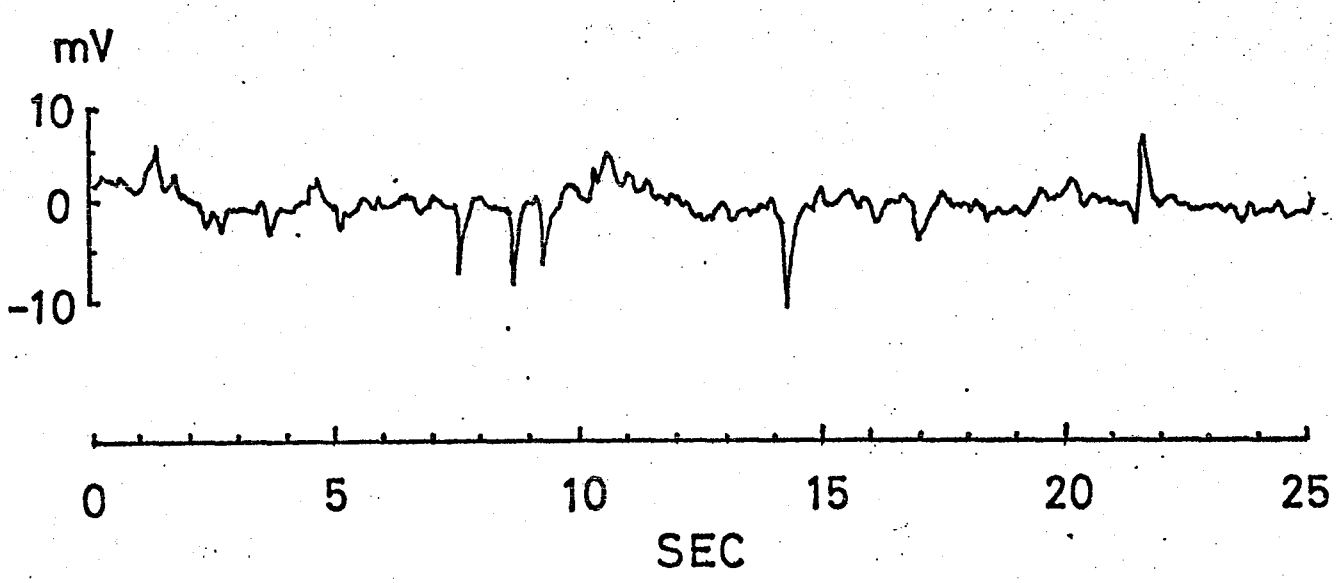


Fig. 1. Wave form of fluctuating potential in the presence of 0.5 mM KCl and 1.0 mM $CaCl_2$ at 25 °C. Spontaneous spike-like depolarization and hyperpolarization are observed.

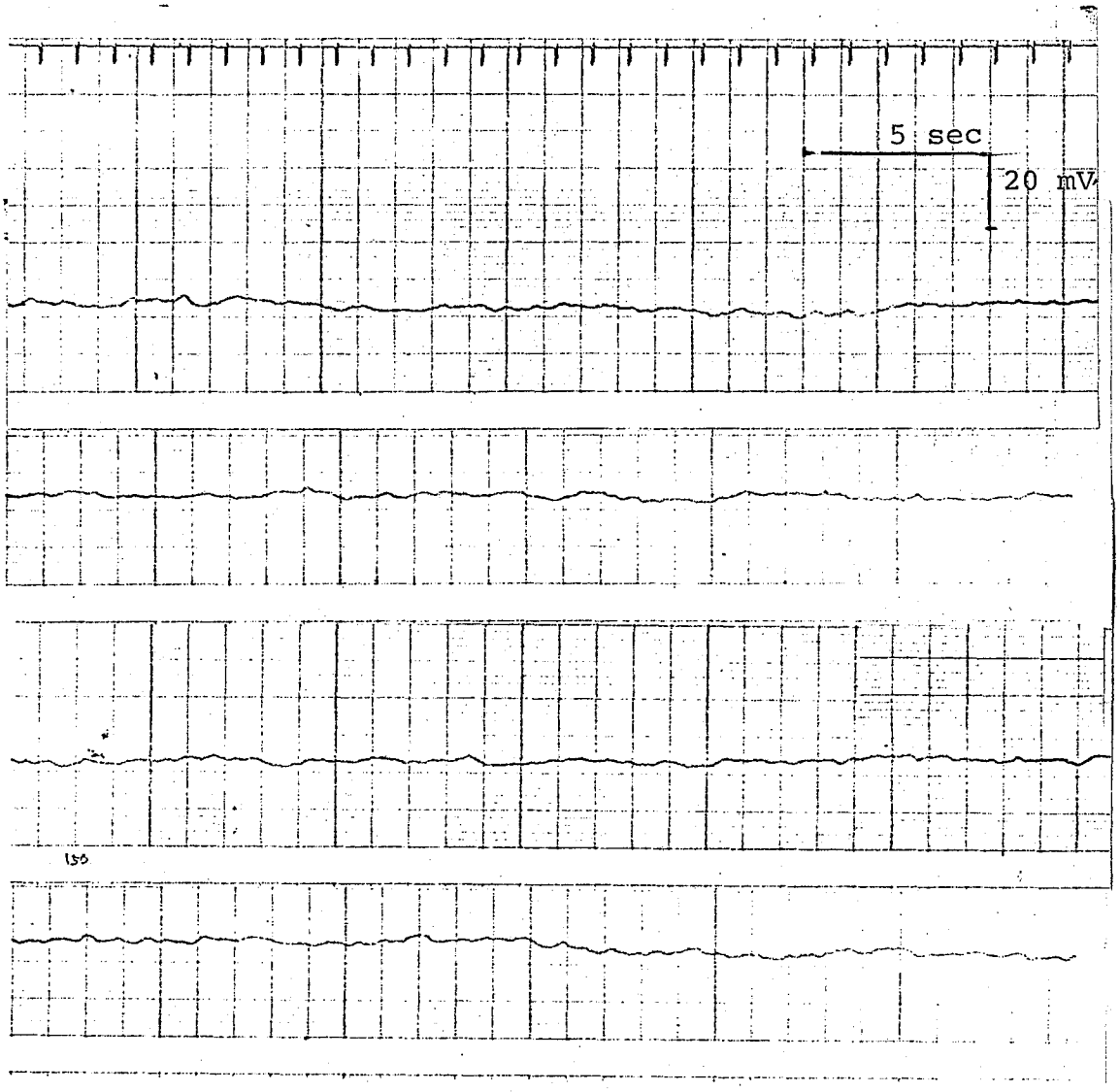


Fig. 2. Slow component of potential fluctuation.

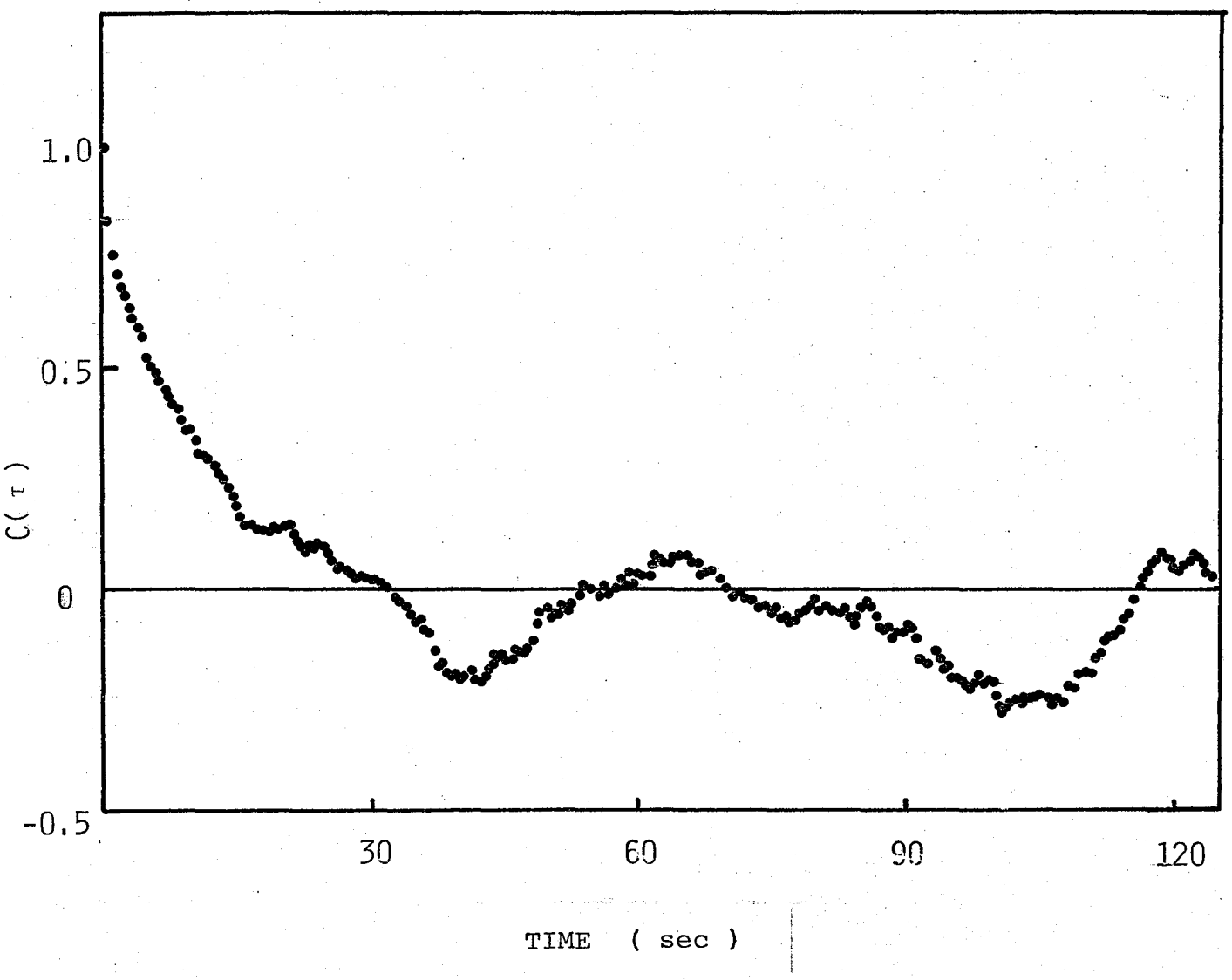


Fig. 3. Autocorrelation function C(τ) of the slow periodic change of resting potential.

2. Histogram

The histogram of fluctuating potential was obtained after omitting the slow periodic change of the resting potential by a high pass filter which had the cut-off frequency of 0.02 Hz. Fig. 4 shows the histogram of fluctuating potential in 0.5 mM-KCl and 1.0 mM $CaCl_2$ at 25 °C. Solid circles in the figure represent a Gaussian distribution of the same mean and the same standard deviation. The histogram is nearly of Gaussian. Fig. 5 shows other examles. The amplitude of the potential fluctuation changes with a change of the salt concentration in the medium. These examples show the effect of RbCl on the histogram. The fluctuation decreased with the increase of the concentration of RbCl and the histogram became sharp, but it remained nearly Gaussian.

3. Power Density Spectra

For the purpose of analysing fast components of potential fluctuation around the resting potential, the power density spectrum (PDS) was obtained from the record. Fig. 8, 10 and 12 show the PDS of fluctuating potential at various concentrations of KCl and $CaCl_2$. The absolute value of the power of fluctuation was different from cells to cells as far as examined. However, the spectrum of the same cell at the same condition was reproducible and the profile of the spectrum from different cells in the same condition was nearly reproducible. The relative magnitude of the power

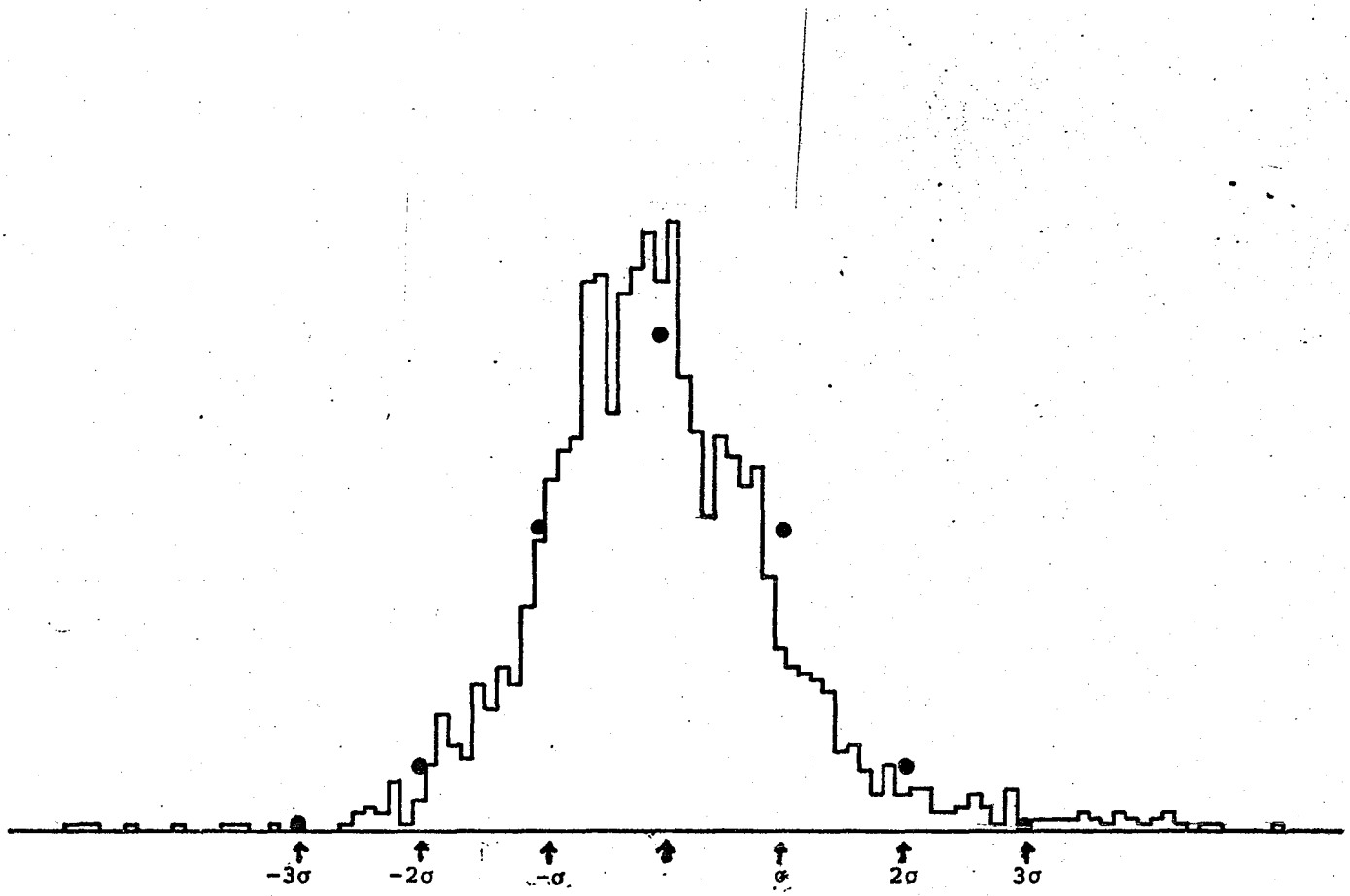


Fig. 4. Histogram of fluctuating potential at 0.5 mM-KCl and 1.0 mM-$CaCl_2$. Solid circles represent a Gaussian distribution.
σ denotes a standard deviation and is equal to 1.6 mV.

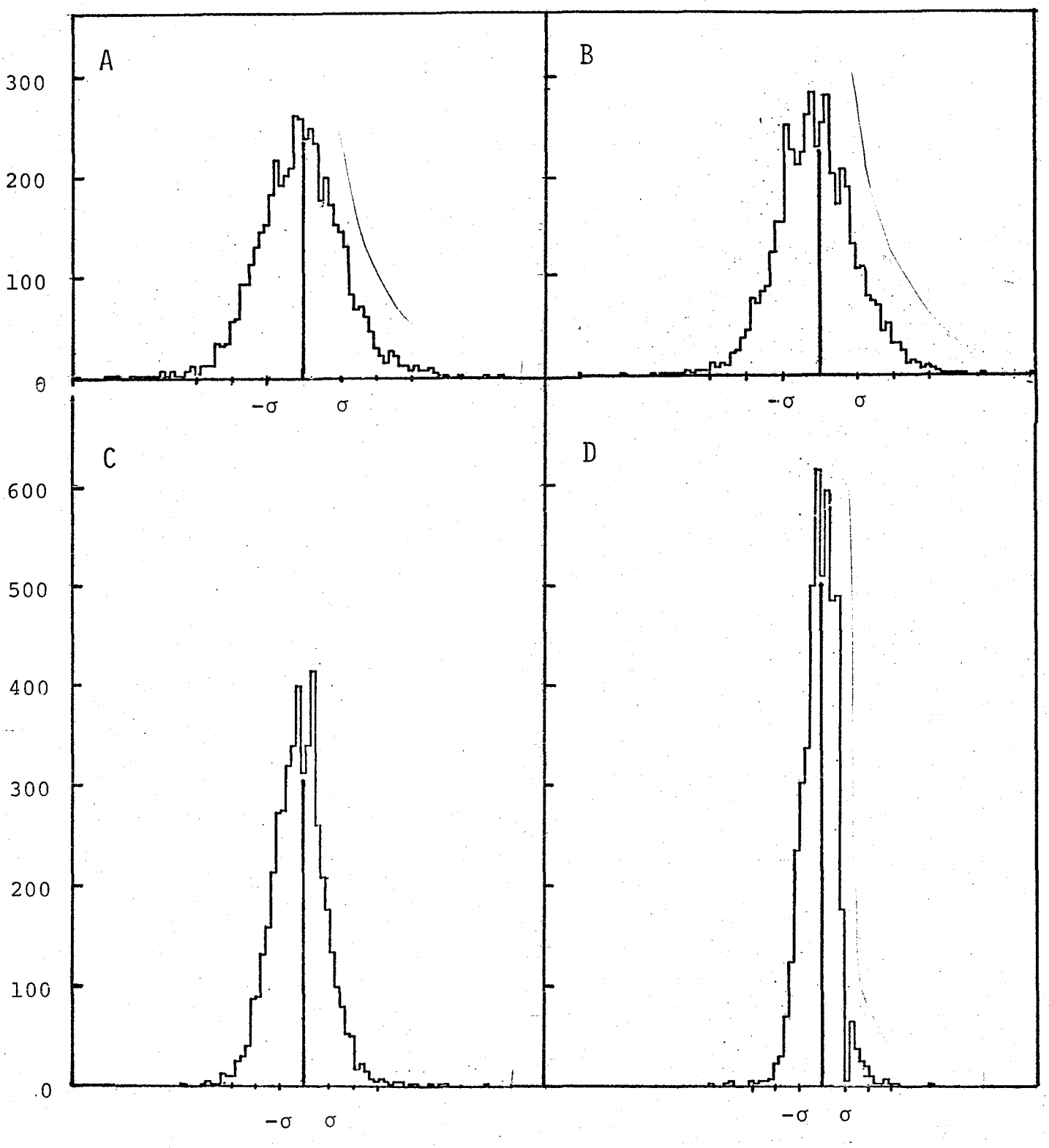


Fig. 5. Effect of RbCl on the Histogram of fluctuating potential; 0.5 mM-RbCl (A), 1.0 mM-RbCl (B), 2.0 mM-RbCl (C), 4.0 mM-RbCl (D). The concentration of $CaCl_2$ was fixed at 1.0 mM. σ is a standard deviation; 0.77 mV (A), 0.73 mV (B), 0.47 mV (C), 0.40 mV (D).

density in different conditions was similar in different cells.

The PDS changes its profile with the change of the salt condition. When the total power of fluctuation was small, the power density decreased with increasing frequency approximately in proportion to $1/f^{\alpha}$, where α was about unity or a little larger (e.g., ■ in Fig. 8; □ in Fig. 10). On the other hand, when the total power of fluctuation was large, the PDS had a different profile from the 1/f type. It had a plateau or a small slope below nearly 1 Hz, and decreased with increasing frequency in proportion to $1/f^{\alpha}$, above a few Hz. In this case, α was about 2 or larger and sometimes attained 2.3 (e.g., ● in Fig. 8). Sometimes, the PDS seemed to have a profile of the Lorentzian type, or the $1/(1 + (f/f_c)^2)$ type. The corner frequency f_c was apparently in a range from 1 to a few Hz, which corresponds to the relaxation time in a range from 150 to 50 ms. With the decrease of the total power of potential fluctuation, the corner frequency shifted toward the low frequency side, becoming undefinable, and α decreased close to unity. This suggested that the PDS was composed of the two components of the 1/f type and the Lorentzian type or the $1/f^2$ type. The latter component decreased with the decrease of the total power of fluctuation. In addition, it must be remarked that a value of α larger than 2 and a peak occasionally found in the spectrum near the corner (e.g., ● in Fig. 8; O in Fig. 10;

▲, △ in Fig. 12) suggest participation of some active processes in magnification of fluctuation [15]. Sharp spikelike depolarization or hyperpolarization in the record was removed in the spectrum analysis. Nevertheless, large values of α were obtained.

The PDS obtained was derived from the potential fluctuation under the condition that the total current was kept (nearly) zero. The power spectrum of current fluctuation can be obtained by using the data on the membrane impedance previously reported. The membrane resistance of Paramecium depended on the salt condition; however, it was in the range of 2.2 to 6.7 $k\Omega\ cm^2$ and the membrane capacitance was in the range of 3.9 to 9.3 $\mu F/cm^2$ [1]. The calculated values of the relaxation time of the membrane were in the range of 25 to 30 ms, which agreed with the results of direct measurements of the time constant of the Paramecium membrane by Saji [16]. It corresponded to the frequency of 5 to 6 Hz which was larger than the corner frequency f_c in the PDS. Therefore, the profile of the power spectrum of potential fluctuation remained in the spectrum of current fluctuation after correction by the impedance spectrum. An example is given in Fig. 6.

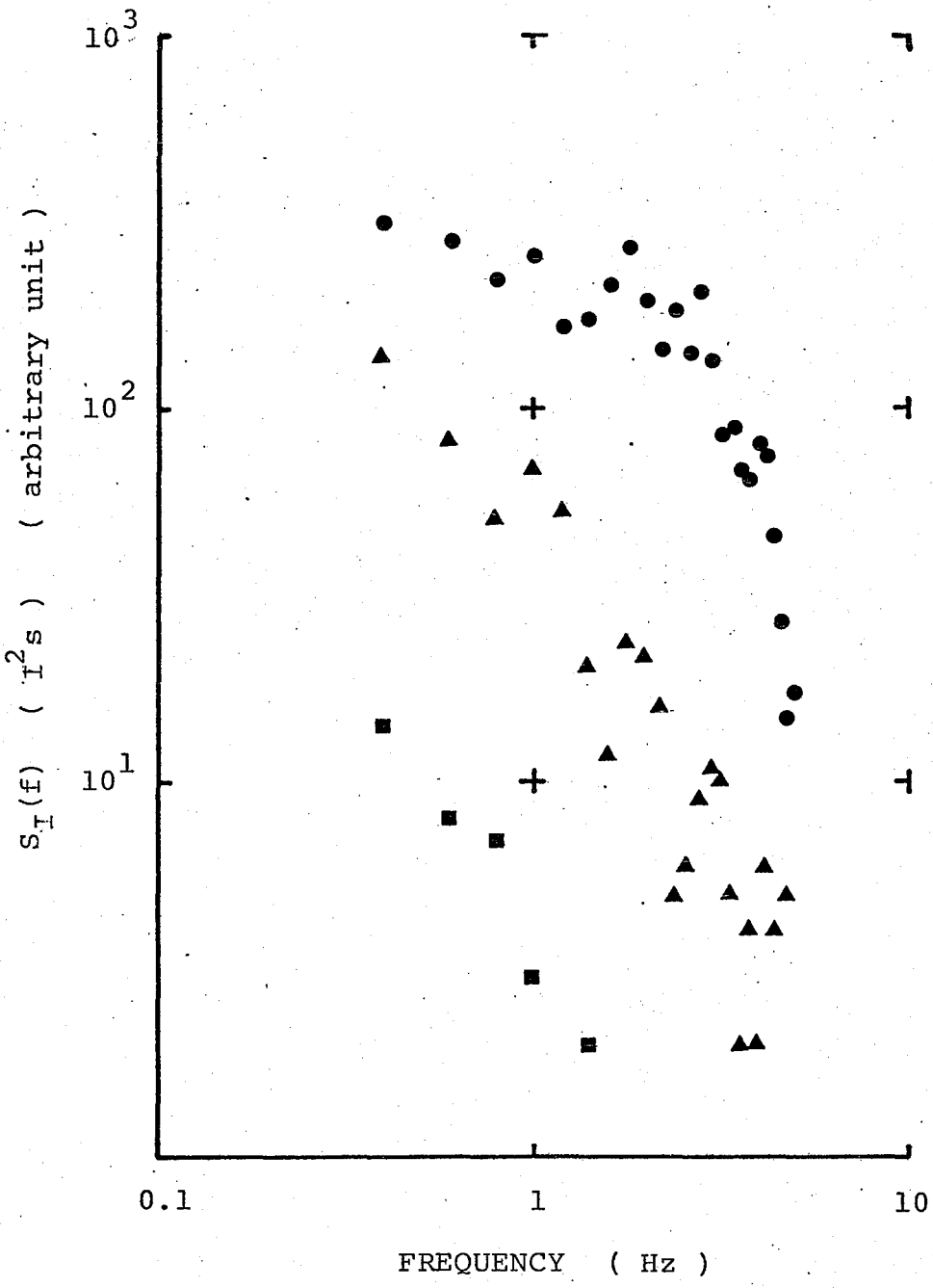


Fig. 6. Calculated power density spectrum of the current fluctuation. Abscissa; frequency: Ordinate; power density in arbitrary unit. 0.5 mM-KCl (●), 2.0 mM-KCl (▲), 8.0 mM-KCl (■). The membrane resistance; 37 MΩ at 0.5 mM KCl, 40 MΩ at 2.0 mM KCl, 32 MΩ at 8.0 mM KCl and the membrane capacitance 860 pF were used for the calculation of impedance spectrum. Compare with the potential fluctuation in Fig. 8.

B. Effect of Ions on the Potential Fluctuation

1. Effect of Potassium Ions

The potential fluctuation in Paramecium depended on the salt condition in the medium. Potassium ion and calcium ion are important for Paramecium, which contribute to produce the intracellular membrane potential [17]. Therefore, the effects of these cations on the potential fluctuation were studied.

The effect of potassium ion on the membrane potential fluctuation was examined by varying the concentration of KCl. pH was kept at 7.2 in Tris-HCl buffer of 1.0 mM and the concentration of $CaCl_2$ was fixed at 1.0 mM. Fig. 7 shows the records of the fluctuating potential at different KCl concentrations. The fluctuation decreased with the increase of the KCl concentration. This change of potential fluctuation induced by the increase of the KCl concentration was reversible. The decrease of KCl concentration caused the increase of the potential fluctuation.

The PDS obtained from the same records as shown in Fig. 7 are shown in Fig. 8. The PDS showed the $1/(1 + (f/f_c)^2)$ type at 0.5 mM KCl. A hump disappeared with the increase of KCl concentration and the PDS showed the 1/f type at 8 mM KCl. Table I gives the summary of the results. The third line of Table I shows the effect of KCl on the total power of potential fluctuation which was normalized to be 1* at 0.5 mM

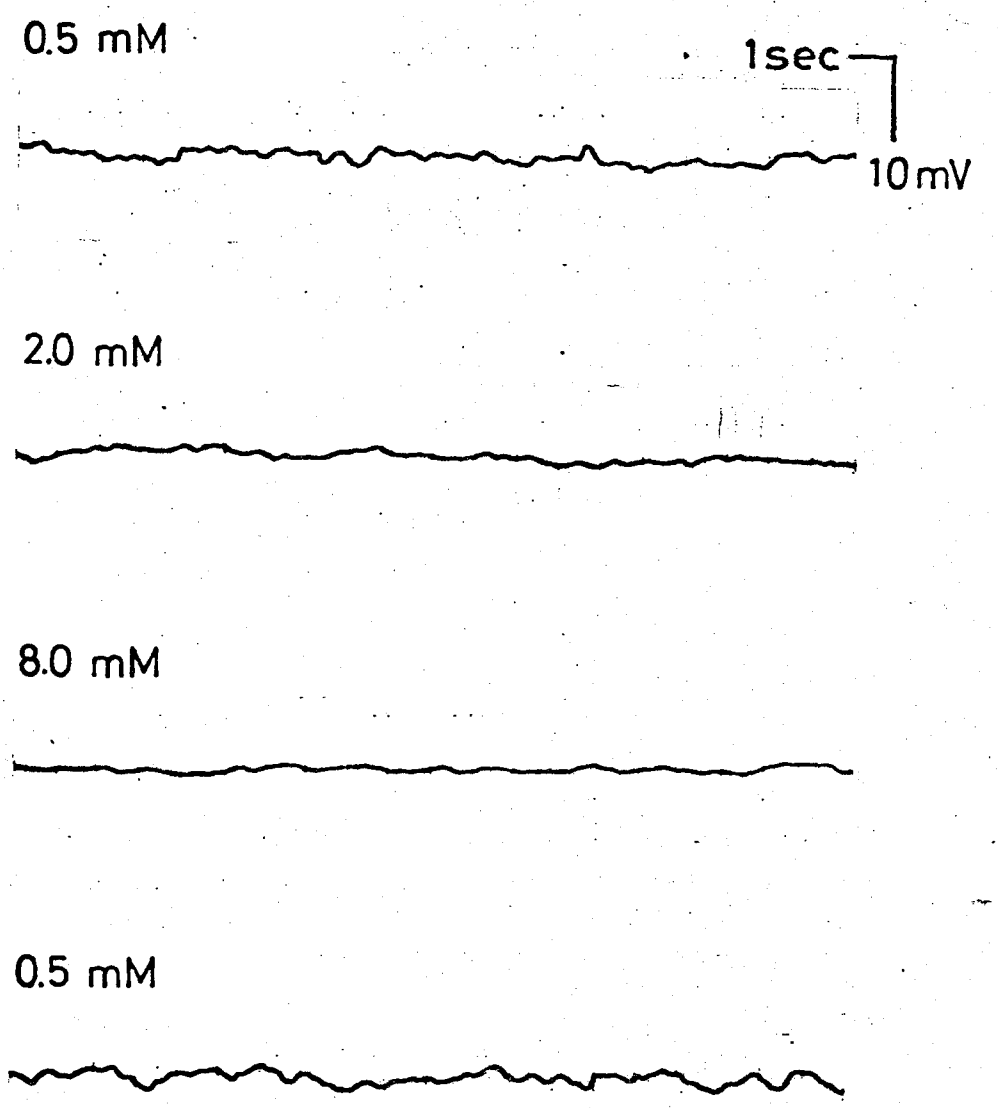


Fig. 7. Effect of KCl on the fluctuating potential in the presence of 1.0 mM $CaCl_2$ at 25 °C.

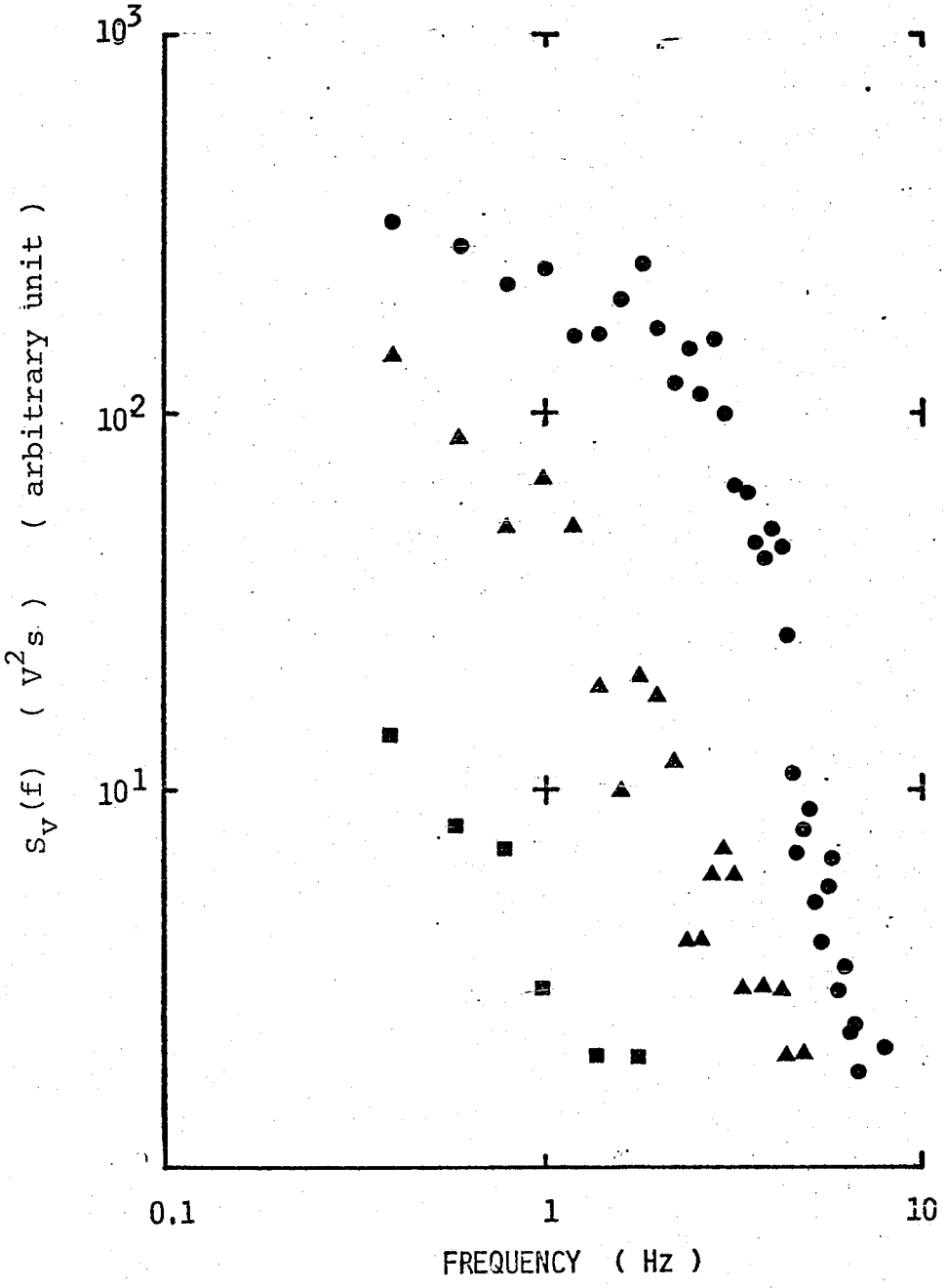


Fig. 8. Power density spectrum of potential fluctuation at 0.5 mM-KCl (●), 2.0 mM-KCl (▲), 8.0 mM-KCl (■). The PDS showed the $1/f^2$ type at 0.5 mM KCl and the 1/f type at 8.0 mM KCl.

Table I Effect of the Ratio $[K]/[Ca]^{1/2}$ on the Potential Fluctuation

Ratio $[K]/[Ca]^{1/2}$	0.5	1	2	4	8
K Series (KCl mM $CaCl_2$ = 1 mM)	0.5	1	2	4	8
Normalized Power of Fluctuation	1*	0.27	0.17	0.017	0.013
Resting Potential (mV)	-36	-33	-28	-23	-14
Ca Series ($CaCl_2$ mM KCl = 1 mM)	4	1	0.25	0.063	0.016
Normalized Power of Fluctuation	1*	0.38	0.30	0.042	0.011
Resting Potential (mV)	-18	-32	-45	-51	-56
Ratio of Normalized Power	1	0.7	0.6	0.4	1.2

* The concentration of potassium ion and calcium ion, [K] and [Ca], expressed in mM are used to calculate the value of ratio $[K]/[Ca]^{1/2}$.

KCl and 1.0 mM $CaCl_2$. The power decreased with the increase of KCl concentration. The power at 0.5 mM KCl was eighty times larger than that at 8.0 mM KCl.

2. Effect of Calcium Ions

The effect of calcium ion on the potential fluctuation was examined by varying the concentration of $CaCl_2$ in the medium. The KCl concentration was fixed at 1.0 mM. The records of fluctuating potential at different $CaCl_2$ concentrations are shown in Fig. 9. The increase of the $CaCl_2$ concentration caused the increase of potential fluctuation. The effect of increasing $CaCl_2$ was similar to the effect of decreasing KCl. The PDS obtained from the same records as shown in Fig. 9 are shown in Fig. 10. The $1/(1 + (f/f_c)^2)$ type spectrum was obtained at 4.0 mM $CaCl_2$. With the decrease of $CaCl_2$ concentration, the hump disappeared and the PDS showed the 1/f type spectrum. The sixth line of Table I shows the normalized value of the power at various concentrations of $CaCl_2$. The power increased with the increase of the $CaCl_2$ concentration. The power at 4.0 mM $CaCl_2$ was about a hundred times larger than that at 0.016 mM $CaCl_2$. The effect of $CaCl_2$ was also reversible. The decrease of the $CaCl_2$ concentration decreased the potential fluctuation.

The effect of calcium ion, however , depended on the potassium ion. When the calcium concentration in the medium

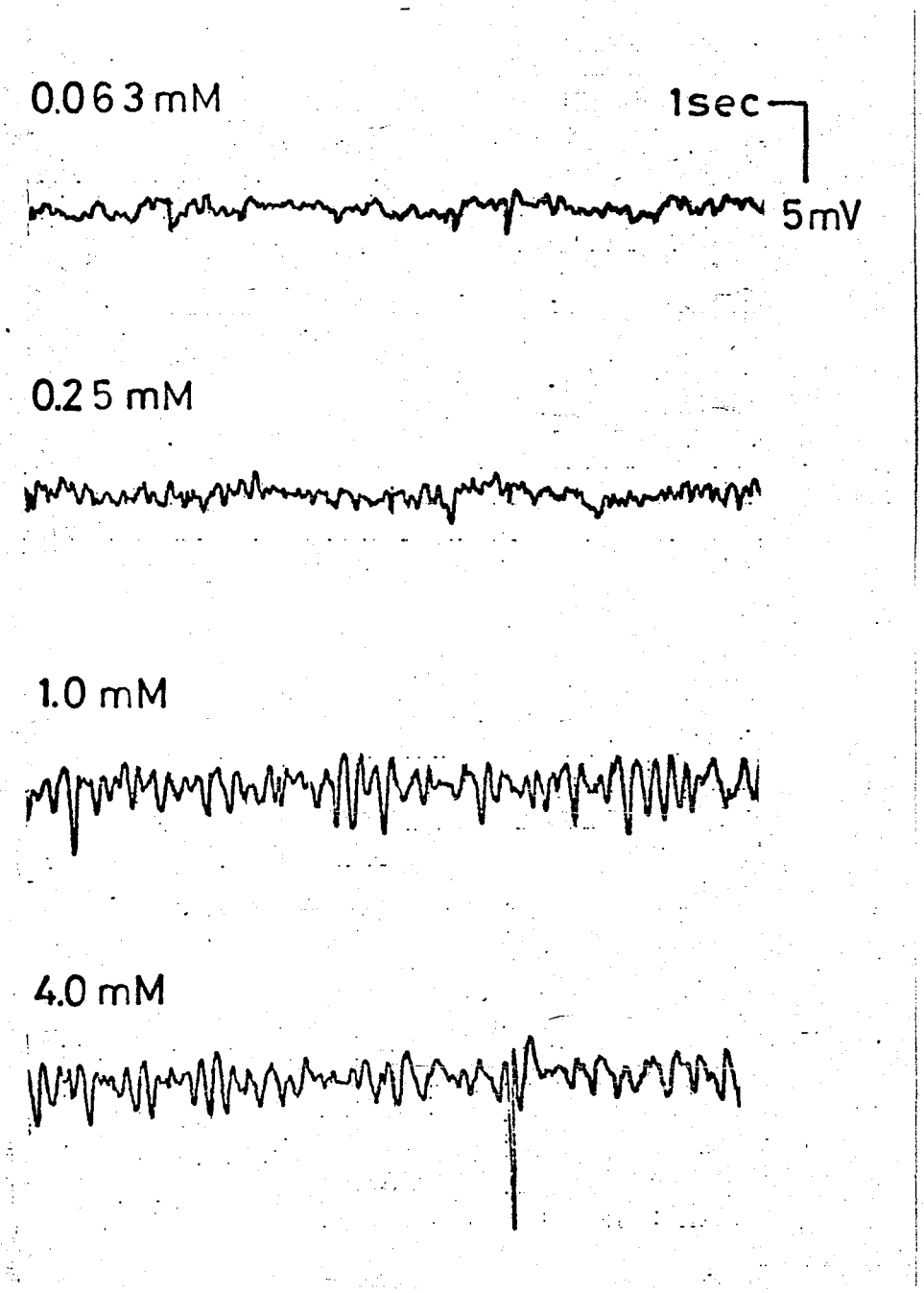


Fig. 9. Effect of $CaCl_2$ on the fluctuating potential in the presence of 1.0 mM KCl at 25 °C.

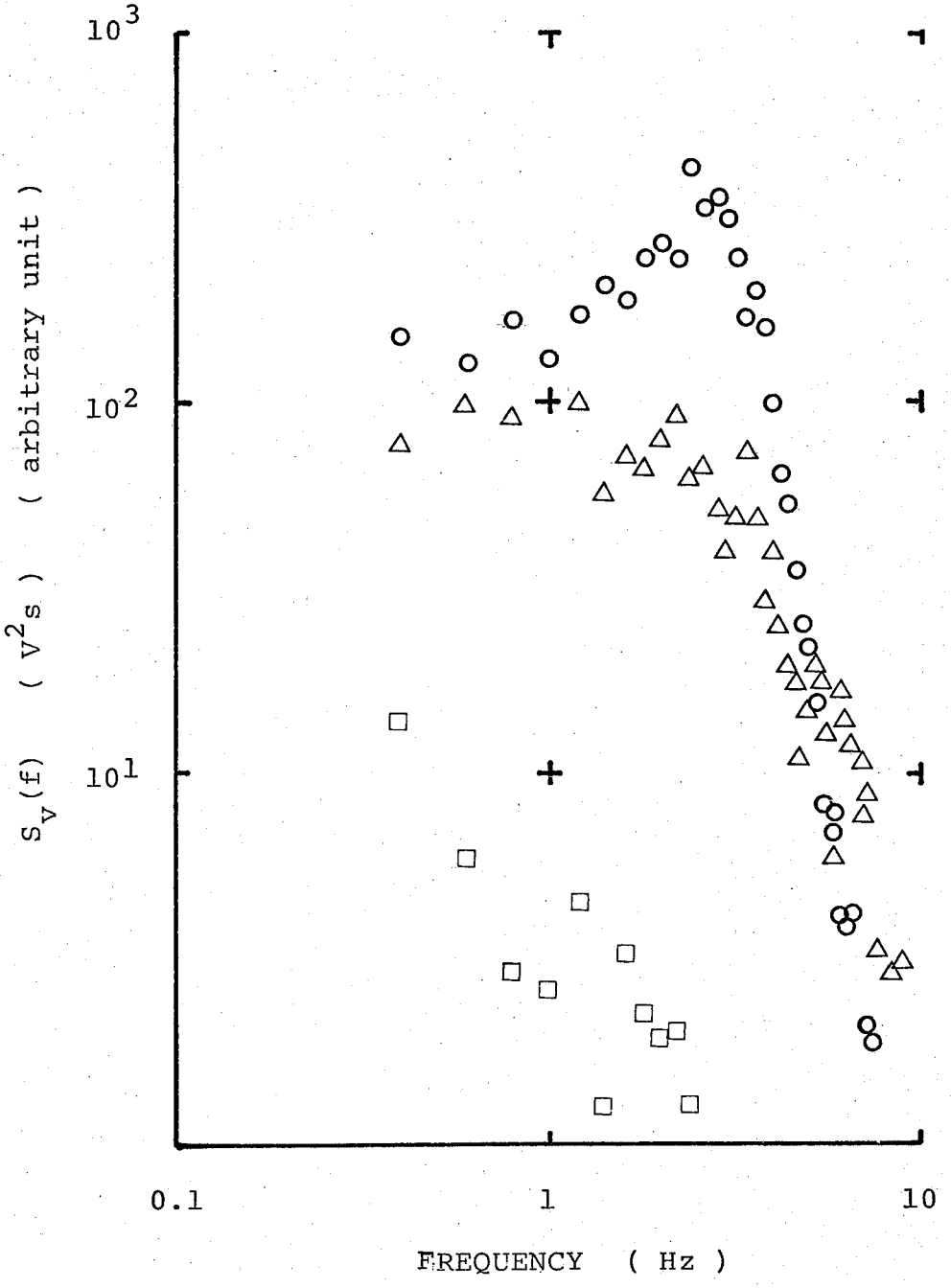


Fig. 10. Power density spectrum of potential fluctuation at 4.0 mM-$CaCl_2$ (○), 0.25 mM-$CaCl_2$ (△), 0.016 mM-$CaCl_2$ (□).

was increased in the absence of potassium ion, the power of potential fluctuation decreased with the increase of the $CaCl_2$ concentration. The PDS are shown in Fig. 11. The power normalized by the value at 1.0 mM KCl and 1.0 mM $CaCl_2$ is shown in Table II. This effect of calcium ion in the absence of potassium ion was opposite to that observed in its presence.

3. Effect of the Ratio $[K]/[Ca]^{1/2}$

In Table I, the total power of fluctuation is shown in both cases where the KCl concentration was changed at a fixed $CaCl_2$ concentration (the K series) and the $CaCl_2$ concentration was changed at a fixed KCl concentration (the Ca series). The eighth line of Table I gives the ratio of the normalized power in the K series and in the Ca series at the same value of the ratio $[K]/[Ca]^{1/2}$. The ratio was kept of the order of unity, while the total power was changed in two orders of magnitude. This means that the same value of the ratio $[K]/[Ca]^{1/2}$ gives the same order of the power of potential fluctuation for different series of experiments. To confirm the relationship between the power of potential fluctuation and the ratio $[K]/[Ca]^{1/2}$, the potential fluctuation was measured by changing the concentration of KCl and $CaCl_2$ at a constant value of the ratio $[K]/[Ca]^{1/2}$.

In this experiment, the concentrations of KCl and $CaCl_2$ were changed, satisfying the relation $[K]/[Ca]^{1/2} = 0.5$.

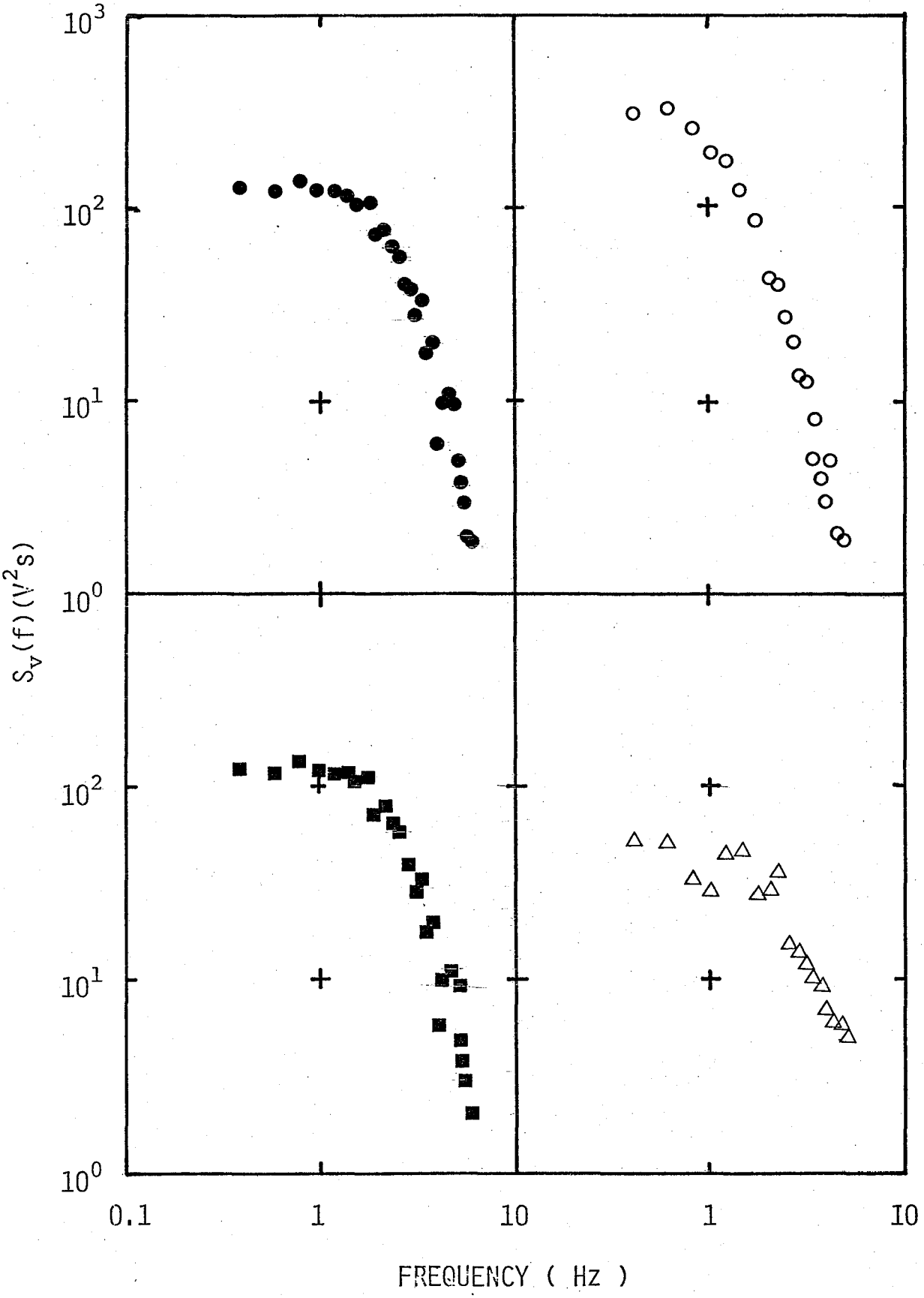


Fig. 11. Power density spectrum of potential fluctuation in the absence of KCl at 0.063 mM-$CaCl_2$ (○), 0.25 mM-$CaCl_2$ (■) and 1.0 mM-$CaCl_2$ (△). Control at 1.0 mM-KCl and 1.0 mM-$CaCl_2$ (●).

Table II Effect of $CaCl_2$ on the Potential Fluctuation in the Absene of KCl

$CaCl_2$ concentration (mM)	1.0	0.063	0.25	1.0
KCl concentration (mM)	1.0	0	0	0
Normalized Power of Fluctuation	1*	1.19	0.42	0.21
Resting potential (mV)	-33	-59	-51	-35

The PDS obtained are shown in Fig. 12. The profile of the PDS was nearly maintained during the change of the concentrations of KCl and $CaCl_2$. The slope of PDS, however, became a little steeper and the corner frequency shifted toward the high frequency side with the increase of $CaCl_2$ at a constant ratio $[K]/[Ca]^{1/2}$. Table III shows the normalized power, which was about unity. Thus, the power of potential fluctuation is nearly constant as far as the ratio $[K]/[Ca]^{1/2}$ is kept constant. The increase of the ratio decreases the power.

4. Effect of Other Ions

a. Effect of Rubidium Ions. The effect of rubidium ions resemble that of potessium ions. Therefore, the effect of rubidium ions on the potential fluctuation was examined by replacing potassium ions in the medium with rubidium ions. With increasing RbCl concentration, the magnitude of fluctuation decreased. It was thirteen times larger at 0.5 mM RbCl than at 8.0 mM RbCl. The results are summarized in Table IV. The resting potential depolarized 8 mV per ten times change of the RbCl concentration below 2.0 mM RbCl and 30 mV per ten times change above 4.0 mM RbCl. The effect of rubidium ions resemble that of potassium ions but weaker. The PDS are shown in Fig. 13. At 0.5 mM RbCl, the PDS showed the $1/(1 + (f/f_c)^2)$ type and a hump in the spectrum disappeared with the increase of the RbCl concentration. The sample was the same as the histogram shown in Fig. 5.

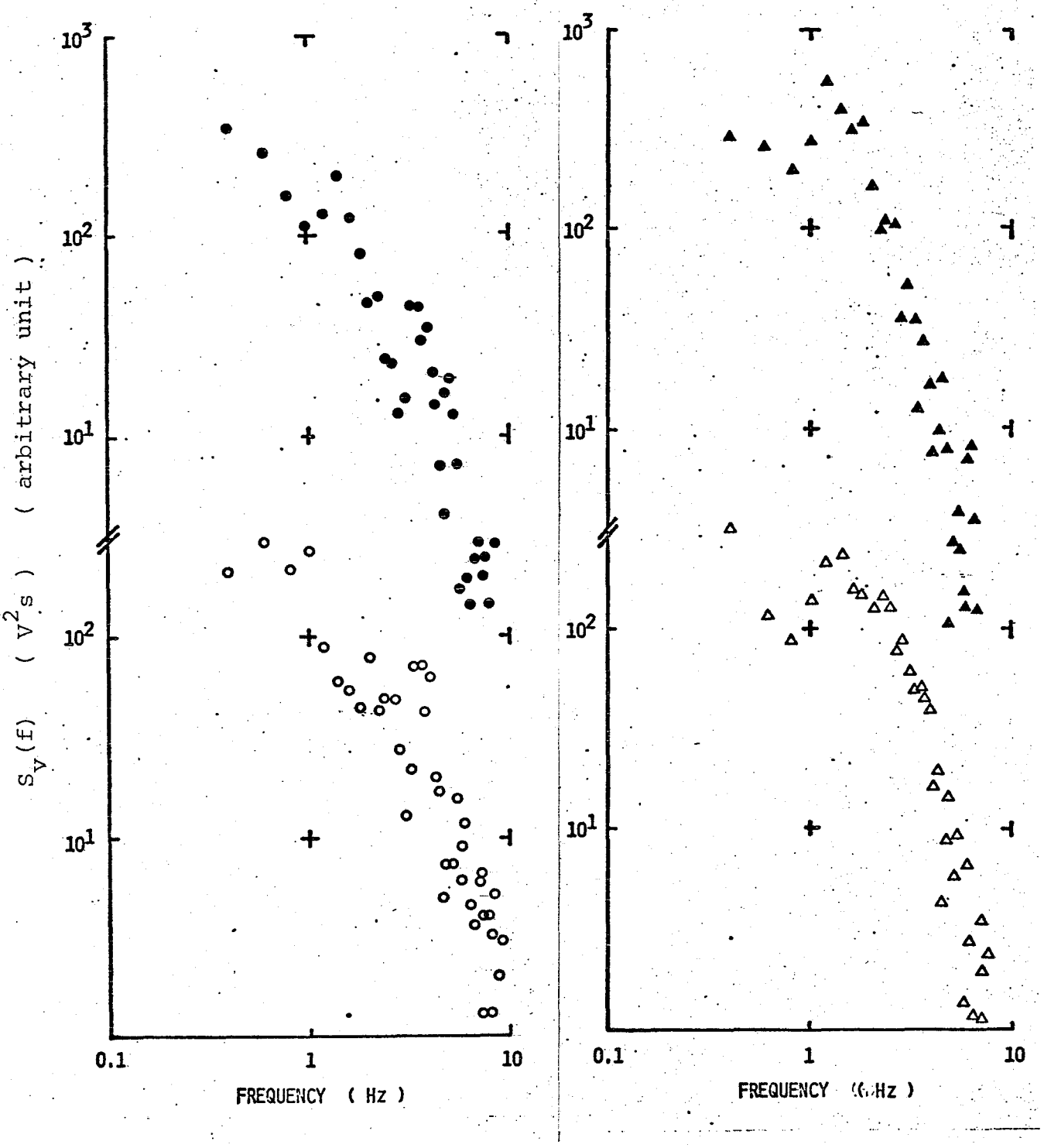


Fig. 12. Power density spectrum of potential fluctuation at the constant value of the ratio $[K]/[Ca]^{1/2} = 0.5$. 0.25 mM-KCl and 0.25 mM-$CaCl_2$ (●), 0.5 mM-KCl and 1.0 mM-$CaCl_2$ (○), 1.0 mM-KCl and 4.0 mM-$CaCl_2$ (▲), 2.0 mM-KCl and 16 mM-$CaCl_2$ (△).

Table III Effect of Constant Ratio $[K]/[Ca]^{1/2}$ on the Potential Fluctuation

Ratio $[K]/[Ca]^{1/2}$		0.5	0.5	0.5	0.5
KCl	Concentration (mM)	0.25	0.5	1	2
$CaCl_2$	Concentration (mM)	0.25	1	4	16
Normalized Power of Fluctuation		0.75	1*	1.1	0.9
Resting Potential	(mV)	-49	-35	-20	-8

Table IV Effect of RbCl on the Potential Fluctuation

Ratio $[Rb]/[Ca]^{1/2}$	0.5	1	2	4
RbCl Concentration (mM)	0.5	1.0	2.0	4.0
Normalized Power of Fluctuation	1*	0.56	0.034	0.003
Resting Potential (mV)	-36	-35	-34	-30

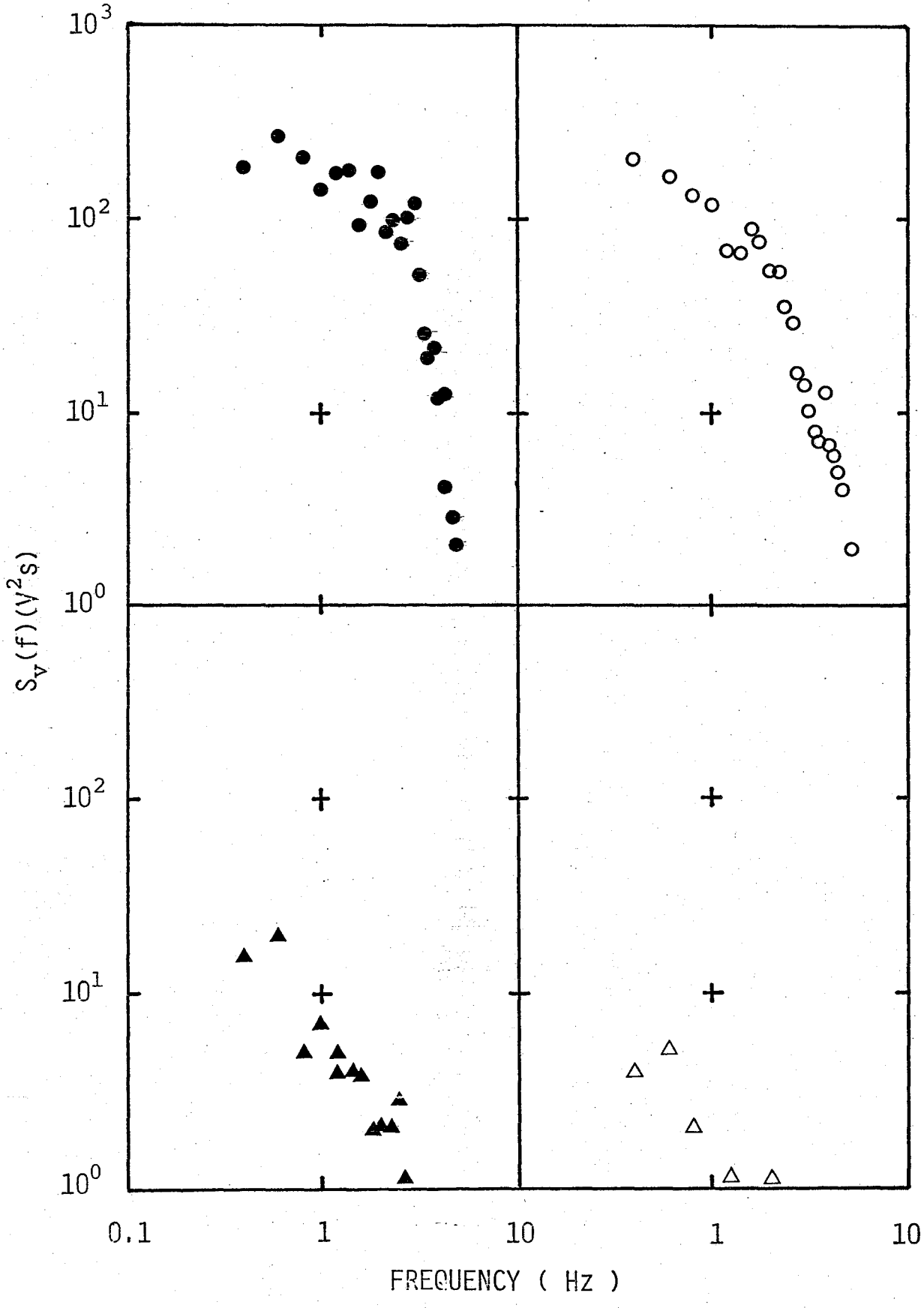


Fig. 13. Power density spectrum of potential fluctuation at 0.5 mM-RbCl (●), 1.0 mM-RbCl (○), 2.0 mM-RbCl (▲) and 4.0 mM-RbCl (△). The data are the same as shown in Fig. 5.

b. Effect of Tetraethylammonium ions. Friedman and Eckert reported that tetraethylammonium ions (TEA^+) blocked potassium conductance and thus reduced the short-curcuit leakage and the repolarizing rate in Paramecium [18]. Here, the effect of TEA on the potential fluctuation was examined. Fig. 14 shows the effect of TEA on the wave form of fluctuating potential. The external application of 1.0 mM TEA to 0.5 mM KCl and 1.0 mM $CaCl_2$ reduced the magnitude of potential fluctuation from unity to 0.16 where normalization of the power of potential fluctuation was performed at 0.5 mM KCl and 1.0 mM $CaCl_2$. The effect of TEA on the PDS is shown in Fig.15. The PDS changed its profile from the $1/(1 + (f/f_c)^2)$ type to the 1/f type by the application of 1.0 mM TEA. Histograms of fluctuating potential obtained from the same records as the PDS were examined are shown in Fig. 16. It shows an asymmetrical distribution after the application of TEA. The resting potential depolarized from -36 mV to -34 mV by the application of 1.0 mM TEA. The effect of TEA was reversible as shown in Fig. 14.

c. Effect of Lanthanium ions. Lanthanium ions inhibit the inward current of barnacle muscle fiber [18] and nerve endings [20], and are known as a calcium blocking agent. The effect of lanthanium ions on the magnitude of potential fluctuation was examined by application of 10^{-4} M $LaCl_3$ to

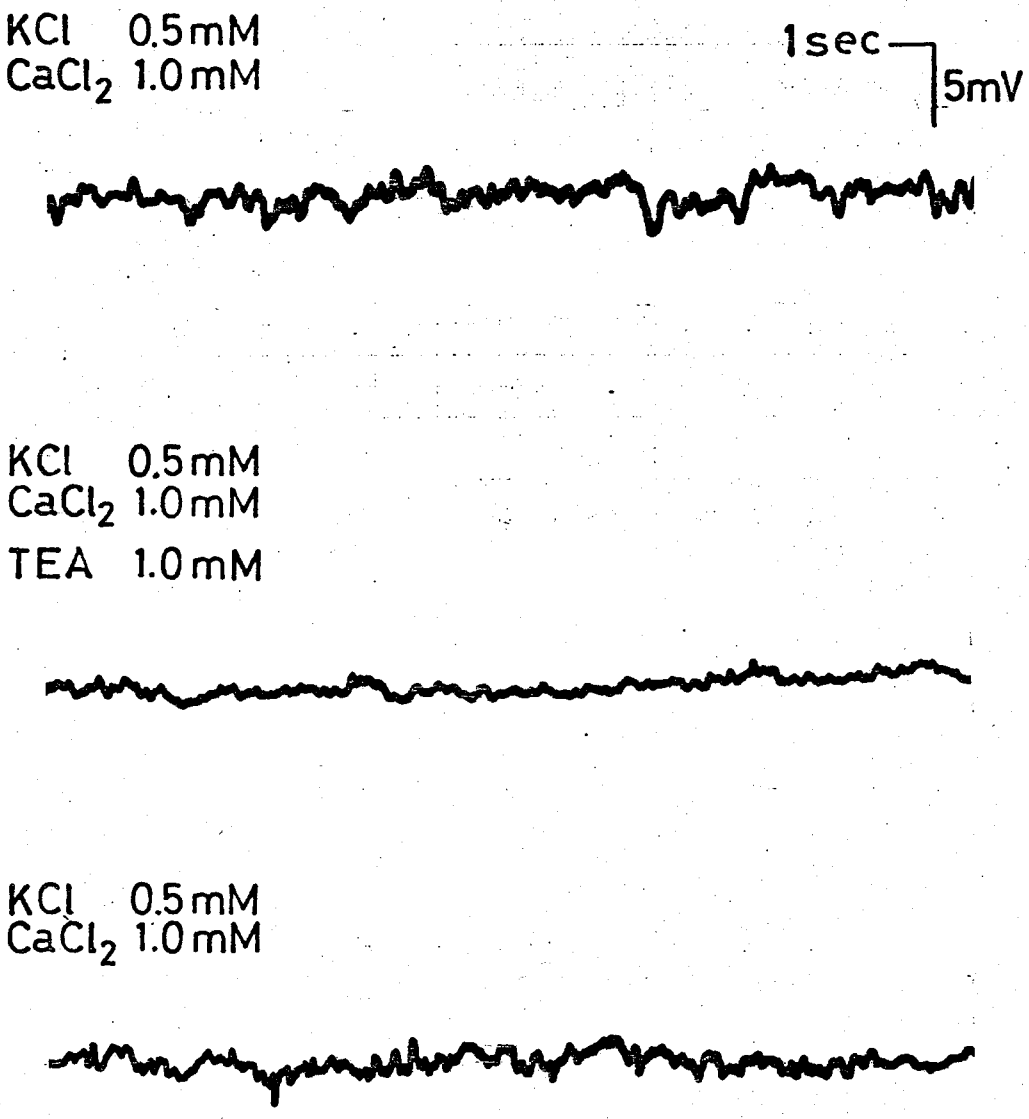


Fig. 14. Effect of tetraethylammonium (TEA) on the fluctuating potential.

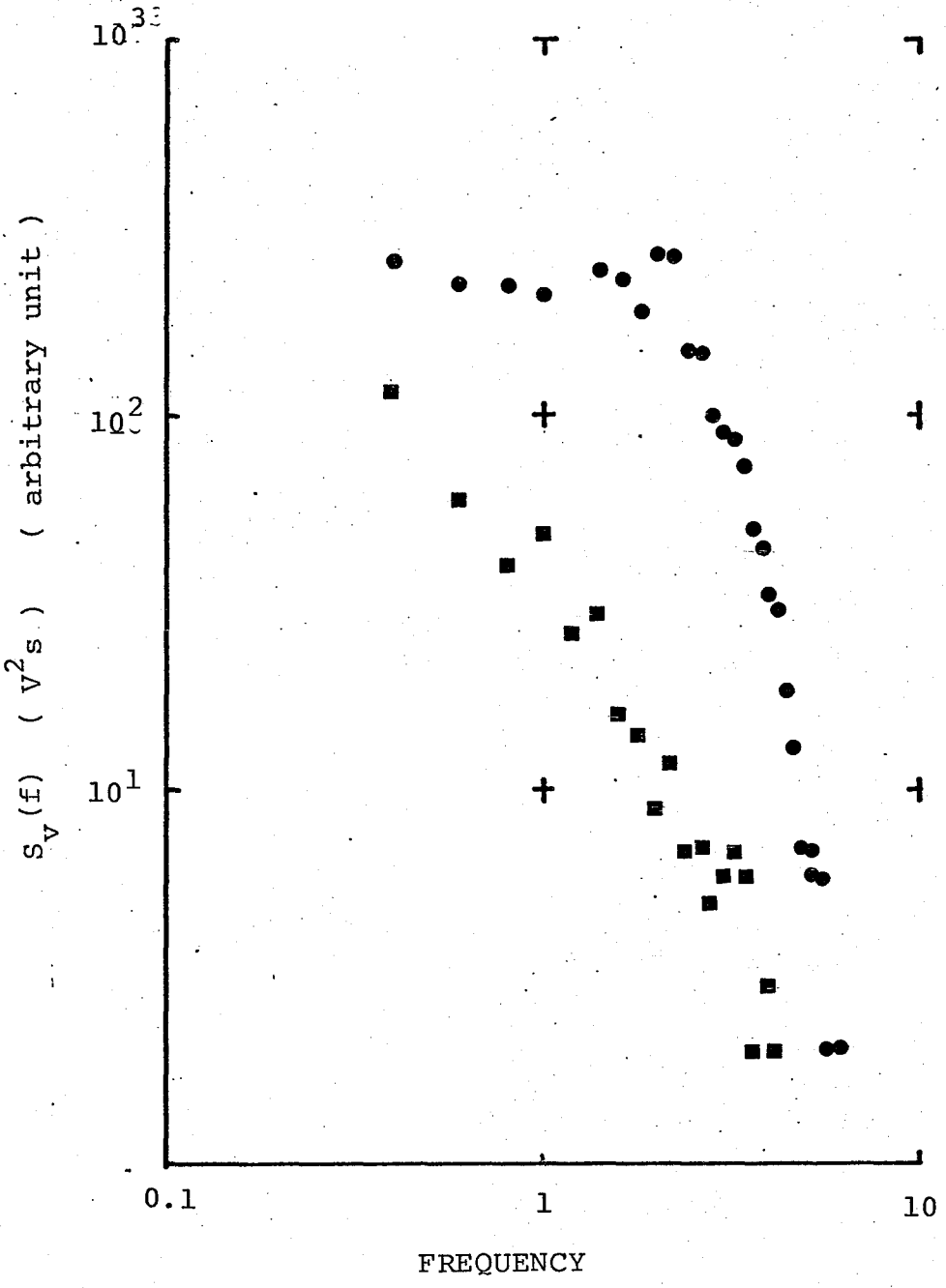


Fig. 15. Effect of TEA on the power density spectrum. 1.0 mM-TEA, 0.5 mM-KCl, 1.0 mM-$CaCl_2$ (■); 0.5 mM-KCl, 1.0 mM-$CaCl_2$ (●). PDS shows the 1/f type in the presence of TEA.

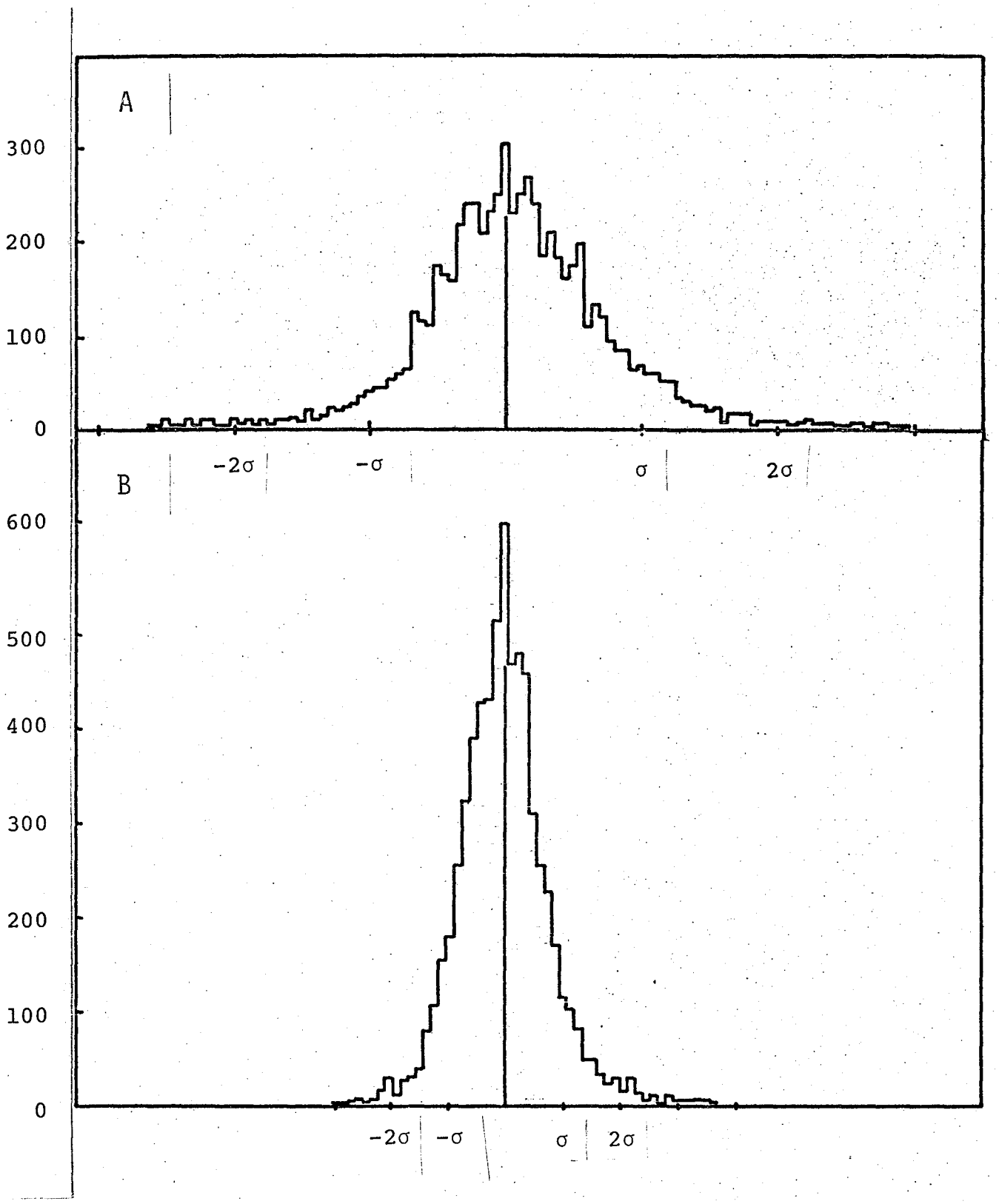


Fig. 16. Histogram of fluctuating potential in the absence of TEA (A) and in the presence of 1.0 mM-TEA (B). σ denotes a standard deviation; 1.45 mV (A), 0.63 mV (B). Ordinate; number of occurrence. Data are the same as shown in Fig. 15.

0.5 mM KCl and 1.0 mM $CaCl_2$. The magnitude of potential fluctuation was decreased by application of $LaCl_3$ as shown in Fig. 17. The resting potential hyperpolarized 10 or 15 mV from the initial potential. In the presence of 10^{-4}M $LaCl_3$, the membrane lost the excitability. The magnitude of potential fluctuation in the presence of 10^{-4} M $LaCl_3$ was a hundred times smaller than that in the absence of $LaCl_3$.

C. Effect of Ions on the Resting Potential of Paramecium

The effect of potassium ions on the resting potential in the presence of 1.0 mM $CaCl_2$ is shown in the fourth line of Table I and is also shown in Fig. 18. In the K series, the resting potential depolarized with the increase of KCl. At 25 °C, in a range of the concentration of KCl, above 4.0 mM KCl and below 16 mM KCl, the resting potential changed by 31 mV per ten times change of the concentration of KCl and below 4.0 mM KCl it changed by 15 mV per ten times change of the concentration of KCl.

The effect of calcium ions on the resting potential in the presence of 1.0 mM KCl is shown in the seventh line of Table I and is also shown in Fig. 19. In the Ca series the resting potential depolarized with the increase of $CaCl_2$. In a range of the concentration of $CaCl_2$, above 0.25 mM and below 4.0 mM, the resting potential changed by 22.5 mV per

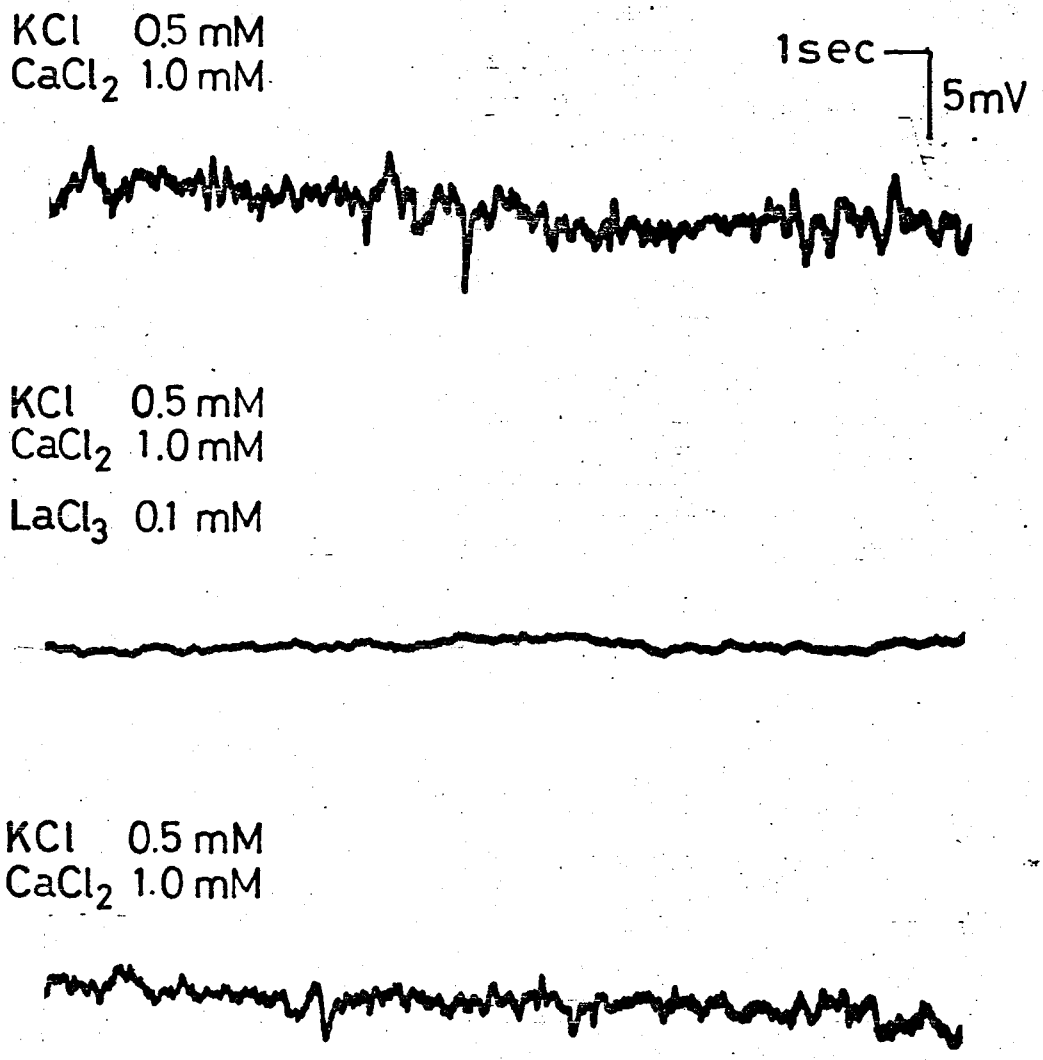


Fig. 17. Effect of $LaCl_3$ on the fluctuating potential.

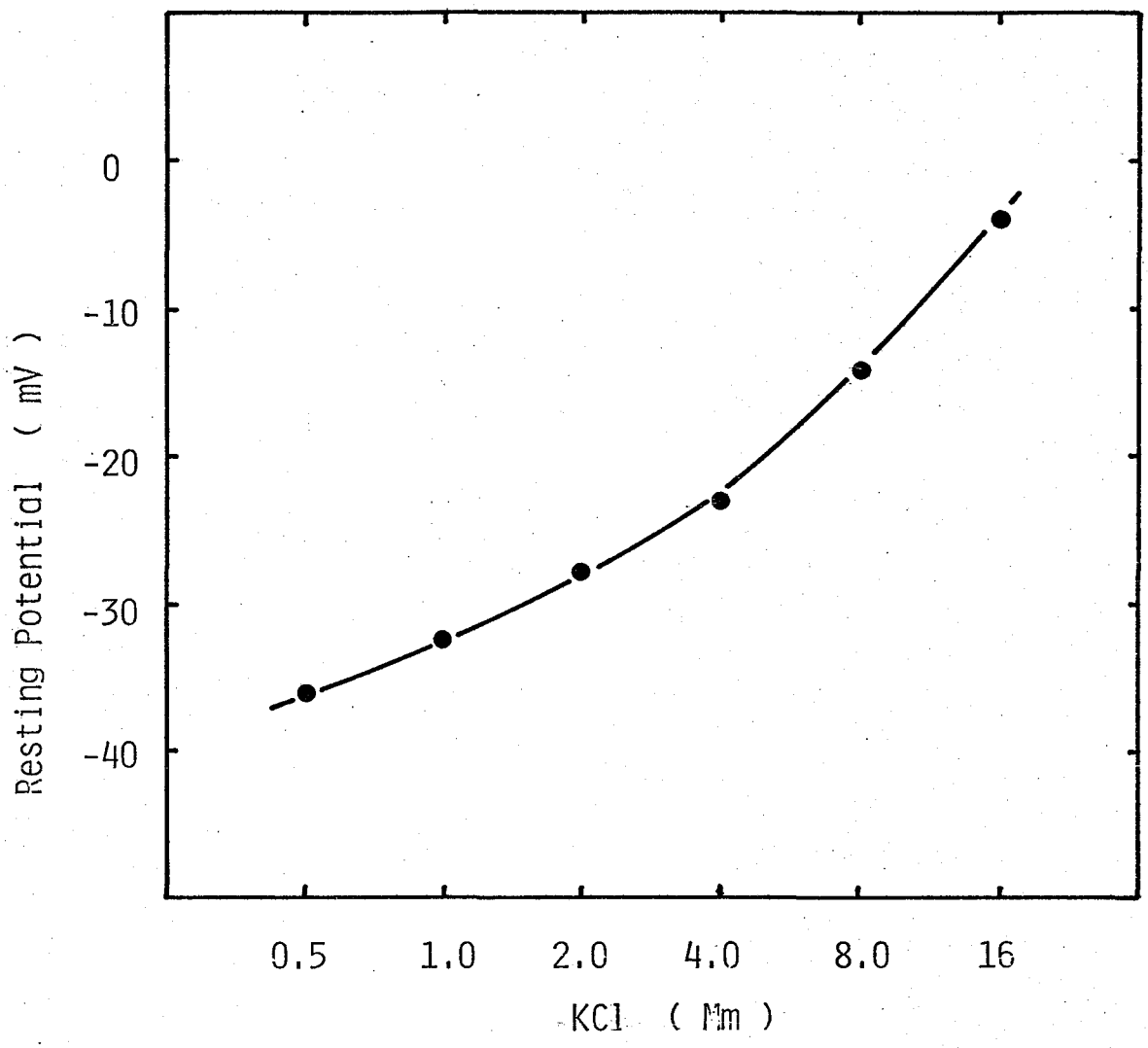


Fig. 18. Effect of the KCl concentration on the resting potential in the presence of 1.0 mM $CaCl_2$ at 25 °C.

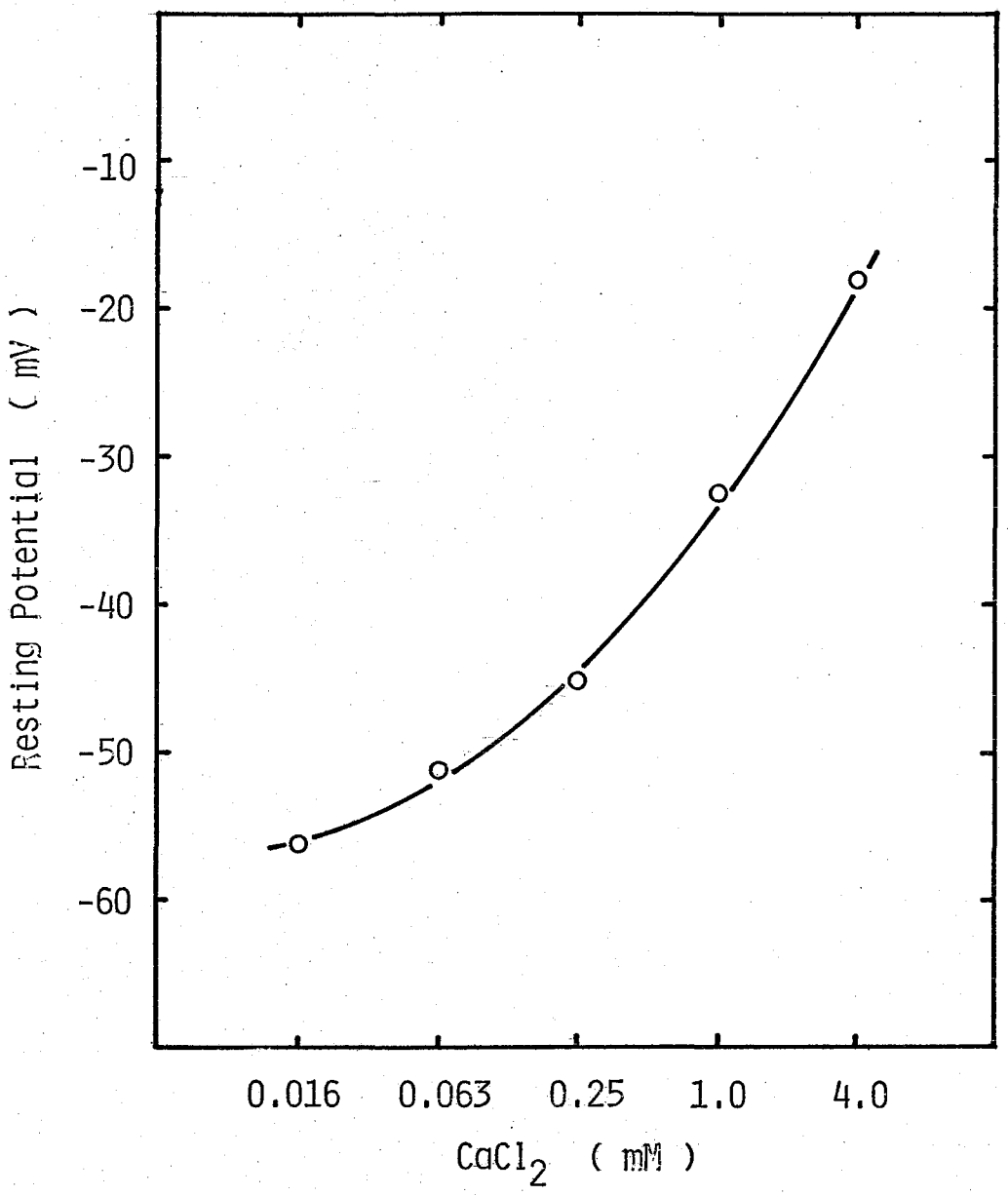


Fig. 19. Effect of the $CaCl_2$ concentration on the resting potential in the presence of 1.0 mM KCl.

ten times change of the concentration of $CaCl_2$ and below 0.25 mM it changed by 9 mV per ten times change of the concentration of $CaCl_2$ at 25 °C. The effect of KCl or $CaCl_2$ on the resting potential was reversible. The decrease of KCl concentration or the decrease of $CaCl_2$ concentration caused the recovery of the resting potential.

The fourth line of Table III and Fig. 20 shows the change of the resting potential when the value of the ratio $[K]/[Ca]^{1/2}$ was kept constant at 0.5. Under this condition, the resting potential depolarized in proportion to the logarithm of the total ion concentration. It changed by 45 mV per ten times change of the concentration of KCl or 22.5 mV per ten times change of $CaCl_2$ at 25 °C.

Fig. 21 shows the effect of calcium ions on the resting potential in the absence of potassium ions. In this case, the resting potential depolarized with the increase of the concentration of $CaCl_2$. It changed 25 mV per ten times change of $CaCl_2$ in a concentration range, above 0.25 mM and below 16 mM at 25 °C. The relation rolled off to lower values below 0.25 mM $CaCl_2$. The effect of calcium ions in the absence of potassium ions on the resting potential was similar to that in its presence, while the effect of calcium ions on the power of potential fluctuation was opposite with each other.

Summarizing the above results, in K series the depolarization of resting potential was associated with the decrease

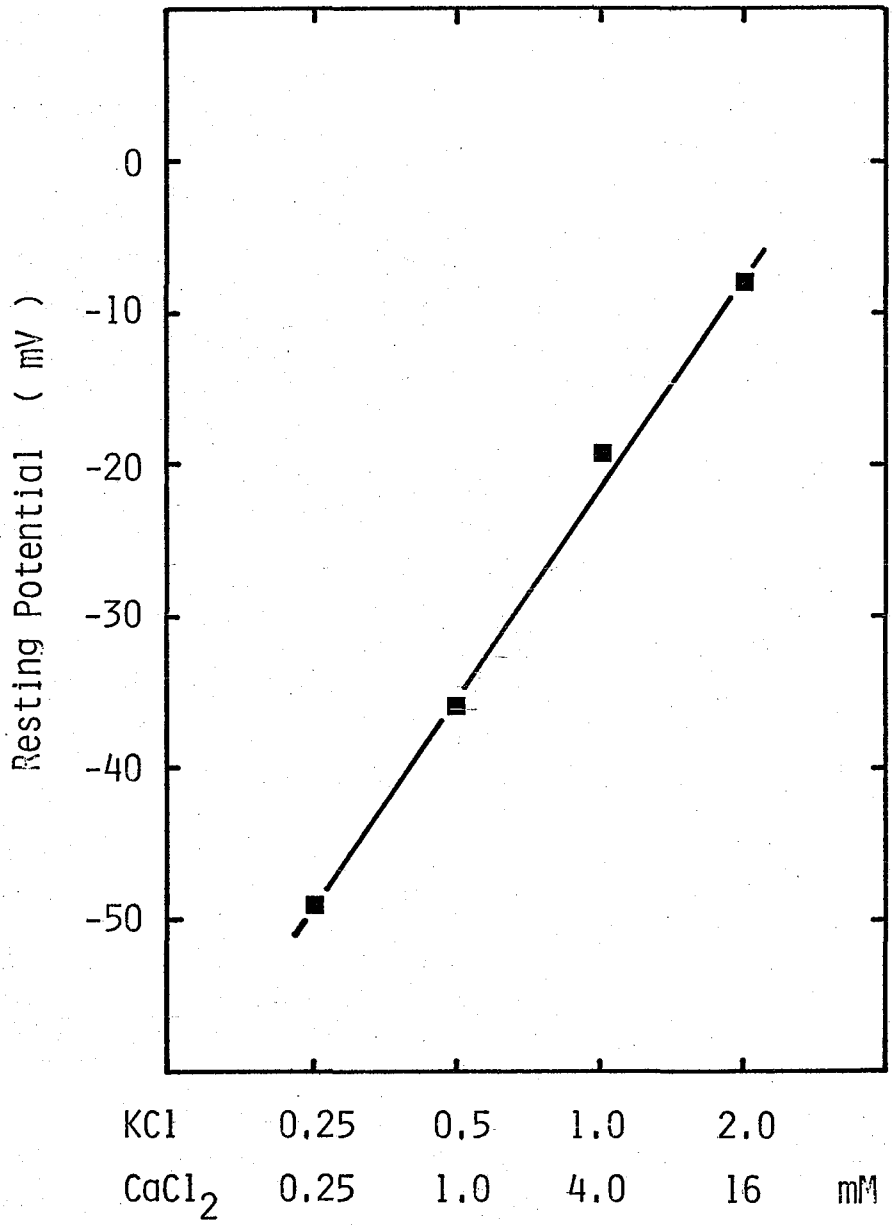


Fig. 20. Effect of the ion concentration at a constant ratio $[K]/[Ca]^{1/2}$ on the resting potential. In this case the ratio was kept at 0.5.

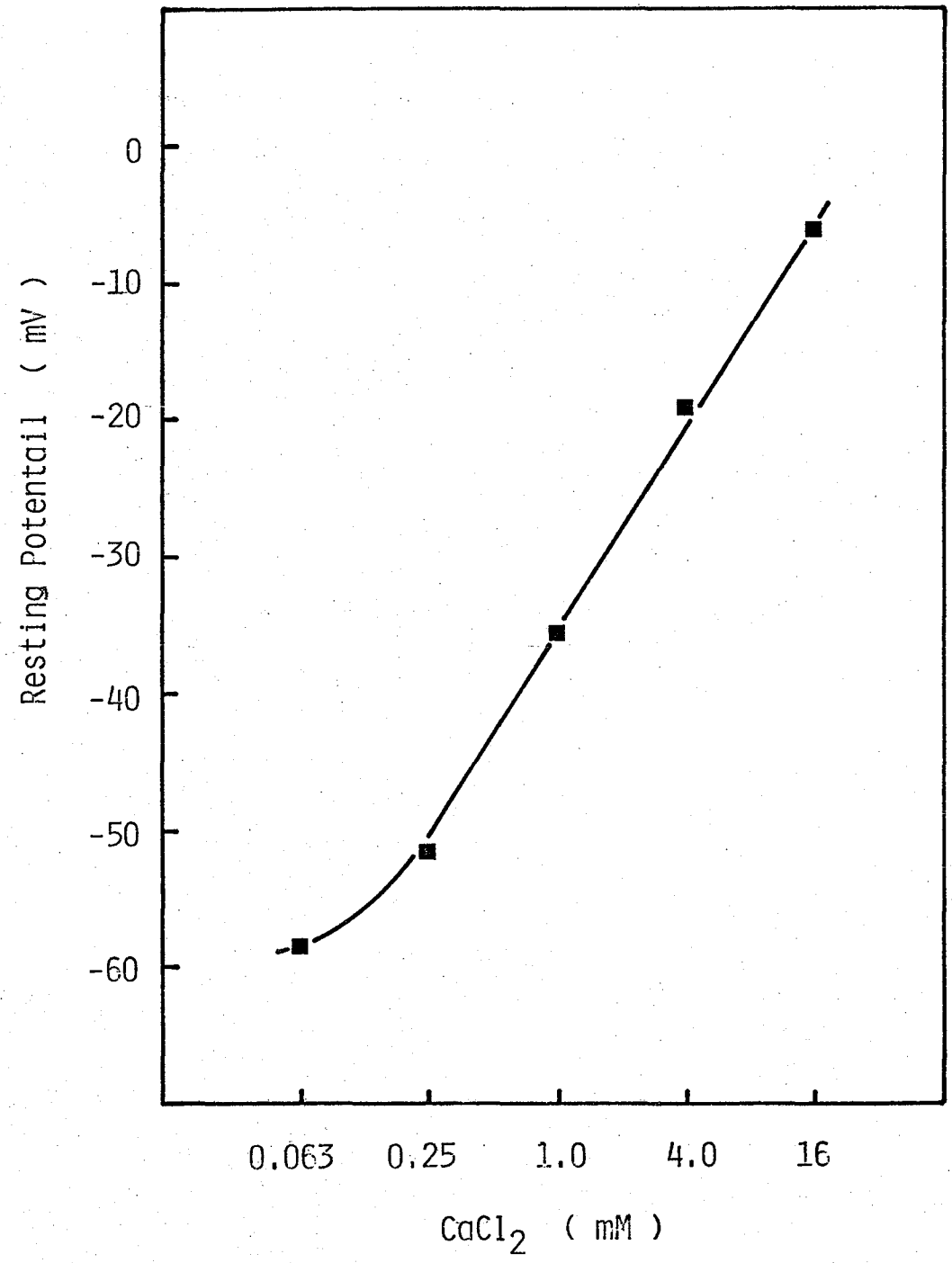


Fig. 21. Effect of the $CaCl_2$ concentration on the resting potential in the absence of KCl.

of the power of fluctuation, while in the Ca series the depolarization of resting potential was associated with the increase of the power. When the ratio $[K]/[Ca]^{1/2}$ was kept constant, the power of fluctuation was maintained at a constant level, while the resting potential showed a wide change with the change of the total ion concentration.

IV. DISCUSSION

A. Resting Potential, Local Current and Ratio $[K]/[Ca]^{1/2}$

The power of fluctuation changed with the change of the ratio $[K]/[Ca]^{1/2}$, regardless of the intracellular membrane potential. In the K series the resting potential depolarized with the increase of the ratio, whereas it resulted in the decrease of the power of fluctuation. In the Ca series the resting potential hyperpolarized with the increase of the ratio, whereas it resulted in the decrease of the power. In two cases the depolarization of the resting potential was associated with the opposite effects on the power of the potentail fluctuation. On the other hand, when the ratio $[K]/[Ca]^{1/2}$ was kept constant, the power was kept in the same order of magnitude, while the resting potential changed

in proportion to the logarithm of the total ion concentration.

Potassium ions and calcium ions are considered to be main contributors to the membrane potential of Paramecium. The potassium current I_K and the calcium current I_{Ca} across the membrane, which are local circuit currents, are given by

$$I_K = g_K(E_m - E_K) \tag{3}$$

$$I_{Ca} = g_{Ca}(E_m - E_{Ca}) \tag{4}$$

and satisfy a relation

$$I_K + I_{Ca} = 0 \tag{5}$$

where, E_m is the membrane potential and g_K and g_{Ca} are the conductances of potassium ion and calcium ion, respectively; E_K and E_{Ca} denote the equilibrium potential of potassium ions and calcium ions. E_K and E_{Ca} are defined by the following equations;

$$E_K = (RT/F)\ \ln([K_i]/[K_o]) \tag{6a}$$

$$E_{Ca} = (RT/2F)\ \ln([Ca_i]/[Ca_o]) \tag{6b}$$

where R is the gas constant, T is the absolute temperature and F is the Faraday constant. The factor RT/F is equal to 25.69 mV at 25 °C. The suffix i or o means the concentration inside or outside the cell.

Under the assumption (5) that the total current across the membrane is zero, E_m is given by

$$E_m = [g_K/(g_K + g_{Ca})]\ E_K + [g_{Ca}/(g_K + g_{Ca})]\ E_{Ca}; \tag{7}$$

using Eq. (6a) and (6b), Eq. (7) is rewritten as follows

$$E_m = (RT/F)[g_{Ca}/(g_K + g_{Ca})] \ln([K_o]/[Ca_o]^{1/2}) + (RT/F) \ln([K_i]/[K_o]) - (RT/F)[g_{Ca}/(g_K + g_{Ca})] \ln([K_i]/[Ca_i]^{1/2}) \quad (8)$$

The third term in the right hand of Eq. (8) becomes constant if we assume that the internal concentrations of potassium ions and calcium ions were constant. This equation explains the result of Fig. 20.

The potential difference between the resting potential E_m and the equilibrium potential of each ion, which is the motive force for each ion, is given as follows

$$E_m - E_K = [g_{Ca}/(g_K + g_{Ca})] (E_{Ca} - E_K) \quad (9a)$$

$$E_m - E_{Ca} = -[g_K /(g_K + g_{Ca})] (E_{Ca} - E_K) \quad (9b)$$

The difference between the equilibrium potential E_{Ca} and E_k which appeared in Eqs. (9a) and (9b) is given as follows from Eqs. (6a) and (6b)

$$E_{Ca} - E_K = (RT/F) \ln([K_o]/[Ca_o]^{1/2}) + \text{constant} \quad (10)$$

We used the assumption that the internal concentrations of potassium ions and calcium ions were constant. The Eq. (10) means that the difference $E_{Ca} - E_K$ changes in proportion to the logarithm of the ratio $[K]/[Ca]^{1/2}$ of the medium. Therefore, the motive force for each ion given in Eqs. (9a) and (9b) is also a function of the logarithm of the ratio $[K]/[Ca]^{1/2}$ of the medium.

Oertel, Scein and Kung reported that the internal potassium

ion concentration of Paramecium is between 17 and 18 mM [21]. The equilibrium potential of potassium ion E_K can be calculated according to the Eq. (6a). The calculated values of E_K are shown in Table V. Naitoh and Kaneko reported that the ciliary reversal was induced by an increase in cytoplasmic calcium concentration using a chemically skinned model of Paramecium [22]. They reported that a critical concentration of calcium ions to induce the ciliary reversal was 10^{-6} M. This suggests that the internal calcium concentration in Paramecium must be below 10^{-6} M under the physiological condition. Considering this result we assume that the calcium concentration is 10^{-7} M. The equilibrium potential of calcium ions was calculated according to the Eq. (6b). The calculated values of E_{Ca} are shown in Table VI.

From Eqs. (9a) and (9b), numerical ratios of g_{Ca}/g_K were obtained using the calculated values of equilibrium potential, E_K and E_{Ca}, and the measured values of resting potential E_m. The values of g_K and g_{Ca} were determined using the ratio g_{Ca}/g_K and the reported values of the total resting resistance [23]. Then I_K and I_{Ca} were calculated according to Eqs. (3) and (4). The results and the data used for calculation were shown in Table V and VI, which represented the results in the K series and in the Ca series. The equilibrium potential of potassium ions, E_K, changed from -92 mV to -3 mV in the K series and the equilibrium potential of calcium ions, E_{Ca}, was fixed at 207 mV in the same series.

Table V K series ($CaCl_2$ 1 mM)

$[K]/[Ca]^{1/2}$	0.5	1	2	4	8	16
E_m	-36	-33	-28	-23	-14	-1
E_K	-92	-74	-57	-39	-21	-3
$E_m - E_K$	56	41	29	16	7	2
E_{Ca}	207	207	207	207	207	207
$E_{Ca} - E_m$	243	240	235	230	221	208
R (MΩ)	37	42	47	45	32	24
g_t ($\times 10^{-8}$ ℧)	2.70	2.38	2.13	2.22	3.13	4.17
g_K (")	2.19	2.03	1.90	2.08	3.03	4.13
g_{Ca} (")	o,51	o.35	0.23	0.14	0.10	0.04
I_K (nA)	1.23	0.83	0.55	0.33	0.21	0.08
I_{Ca} (nA)	1.23	0.83	0.55	0.33	0.21	0.08
(current)^2	1*	0.46	0.20	0.072	0.029	0.004
(potential)^2	1*	0.27	0.17	0.017	0.013	0.004
$\left(\frac{g_K}{g_t}\right)^2 (E_m - E_K)^2$	1*	0.59	0.32	0.11	0.022	0.0019
$\frac{\delta g_K^2}{g_K^2} + \frac{\delta g_{Ca}^2}{g_{Ca}^2}$	1*	0.46	0.53	0.15	0.59	1.89
$1/g_K^2$	0.209	0.243	0.277	0.231	0.109	0.059
$1/g_{Ca}^2$	3.85	8.16	18.90	51.02	100	625

Table VI Ca series (KCl 1 mM)

$[K]/[Ca]^{1/2}$	0.5	1	2	4	8	16
E_m	-18	-32	-45	-51	-56	-
E_{Ca}	225	207	189	171	154	-
$E_{Ca} - E_m$	243	239	234	222	210	-
E_K	-74	-74	-74	-74	-74	-74
$E_m - E_K$	56	42	29	23	18	-
R (MΩ)	19	27	47	46	30	25
g_t ($\times 10^{-8}$ ℧)	5.26	3.70	2.13	2.17	3.33	-
g_K (")	4.27	3.15	1.90	2.08	3.03	-
g_{Ca} (")	0.99	0.55	0.23	0.09	0.27	-
I_K (nA)	2.39	1.32	0.55	0.45	(0.55)	-
I_{Ca} (nA)	2.39	1.32	0.55	0.45	(0.55)	-
(current)^2	1*	0.31	0.053	0.035	(0.053)	-
(potential)^2	1*	0.38	0.30	0.042	0.011	-
$\left(\frac{g_K}{g_t}\right)^2 (E_m - E_K)^2$	1*	0.62	0.32	0.24	0.13	-
$\frac{\delta g_K^2}{g_K^2} + \frac{\delta g_{Ca}^2}{g_{Ca}^2}$	1*	0.61	0.94	0.18	0.085	-
$1/g_K^2$	0.055	0.101	0.277	0.231	0.109	-
$1/g_{Ca}^2$	1.02	3.31	18.90	123.5	13.72	-

Note on Table V and Table VI

E_m is the resting potential. E_K and E_{Ca} are the equilibrium potential of potassium ions and calcium ions calculated by Eqs. (6a) and (6b). †R is the membrane resistance measured by Naitoh and Eckert [23]. g_t is the total conductance derived from the membrane resistance R. g_K and g_{Ca} are the potassium and calcium conductance derived from Eqs. (3), (4) and (5) (see text). I_K and I_{Ca} are the local circuit current defined by Eqs. (3) and (4). $(\text{ current })^2$ is the squared current normalized to be 1* at 0.5 mM-KCl and 1.0 mM-$CaCl_2$. ††$(\text{ potential })^2$ is the normalized value of potential fluctuaiton in each series. $(g_K/g_t)^2(E_m - E_K)^2$ is calculated using the data shown above and is normalized to be 1* at 0.5 mM-KCl and 1.0 mM-$CaCl_2$. $(\delta g_K^2/g_K^2 + \delta g_{Ca}^2/g_{Ca}^2)$ is derived from $(\text{ potential })^2$ by $(g_K/g_t)^2(E_m - E_K)^2$. $1/g_K^2$ and $1/g_{Ca}^2$ are calculated using the data shown above.

† In the K series, the membrane resistance R was measured in the presence of 1.0 mM $CaCl_2$ and in the Ca series it was measured in the presence of 2.0 mM KCl.

†† Normalized power of potential fluctuation in the K series at $[K]/[Ca]^{1/2} = 16$ was obtained using the empirical relation between the MRR and the power of potential fluctuation given in Eq. (17) and Fig. 32.

On the other hand, the equilibrium potential of potassium ions, E_K, was fixed at -74 mV in the Ca series and the equilibrium potential of calcium ions, E_{Ca}, changed from 225 mV to 154 mV in the same series. Therefore, the motive force of potassium ions $(E_m - E_K)$ given by Eq. (9a) changed widely in both series. The motive force of calcium ions $(E_m - E_{Ca})$ given by Eq. (9b), however, changed only a little, in these series. The relative change of g_{Ca} is larger than that of g_K. The calcium conductance g_{Ca} showed five times increase with decreasing ratio $[K]/[Ca]^{1/2}$ while the potassium conductance g_K showed two times increase with the increasing ratio $[K]/[Ca]^{1/2}$. These results suggested that the change of I_K was due mainly to the change of motive force of potassium ions $(E_m - E_K)$ and the change of I_{Ca} was due mainly to the change of conductance g_{Ca} : The origin of the change of local circuit current was different between I_K and I_{Ca}.

B. Power of Fluctuation

Fig. 22 shows the relation between the logarithm of the power of potential fluctuation and the logarithm of the motive force of potassium ion current $(E_m - E_K)$. A linear relation was found between these two quantities. When the motive force of potassium current $(E_m - E_K)$ is greater than 15 mV, the proportional coefficient of the relation is 3.15. When the $(E_m - E_K)$ was less than 15 mV, the coefficient became

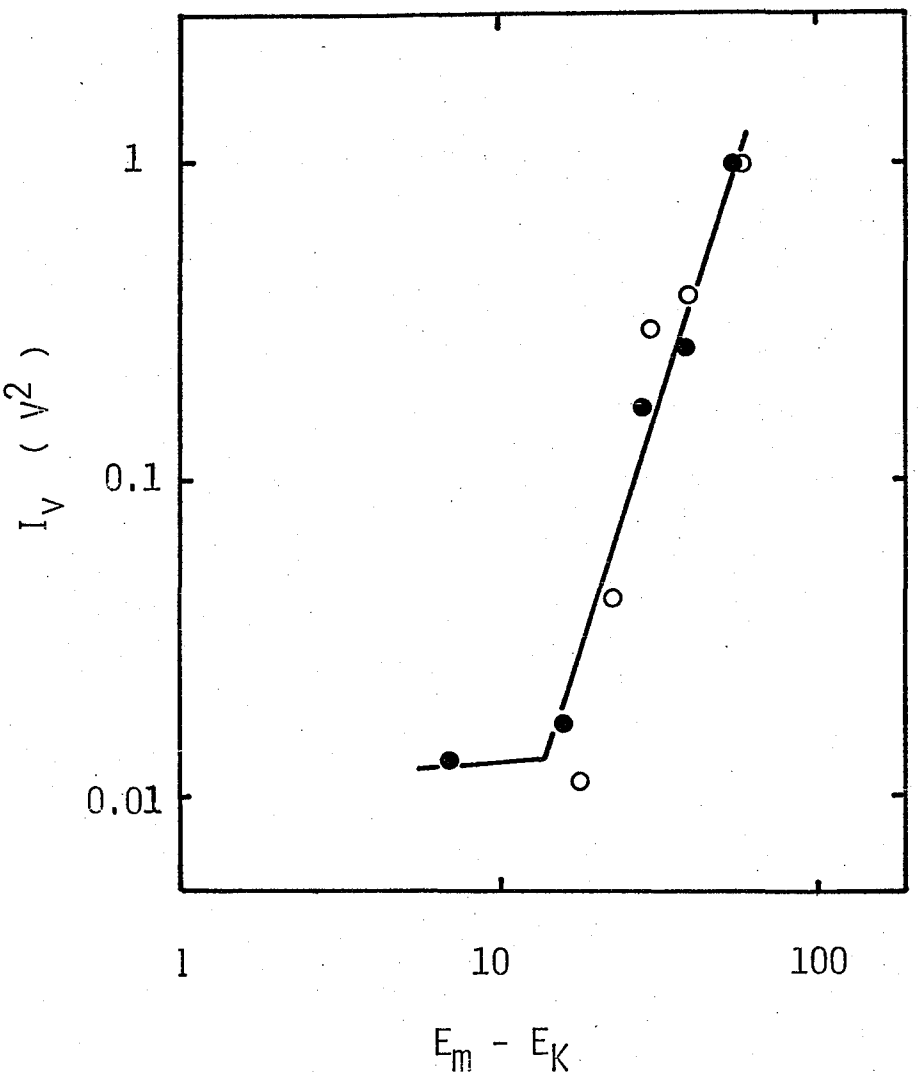


Fig. 22. Relation between the normalized power of potential fluctuation (I_v) and the motive force of potassium ion ($E_m - E_K$). K series (●) and Ca series (O).

smaller , less than 0.3. Moolenaar et al. reported the result of noise analysis in Paramecium under the voltage clamp [13]. Their experiment was performed at 4.0 mM KCl and 1.0 mM $CaCl_2$. Fig. 23 shows the relation between the membrane potential and the intensity of current fluctuation under the voltage clamp reported by Moolenaar et al. The replotted figure of the same data is shown in Fig. 24. The power of current fluctuation and the potential difference $E_m - E_K$ were both plotted in the logarithmic scale. In this case, a linear relation similar to that previously obtained in Fig. 22 also holds. The proportional coefficient is 3.2 when $|E_m - E_K|$ is greater than 13 mV and is 0.08 when $|E_m - E_K|$ is less than 13 mV. The value of 13 mV is a critical value at which the dependence of fluctuation on the motive force changed. The results of these two experiments showed good agreement. The magnitude of the power of potential fluctuation or the magnitude of the power of current fluctuation depended on the motive force for potassium current. The dependence of the power of fluctuation on the motive force of potassium current, however changed drastically. In the usual case, the power of fluctuation is expected to be proportional to the second power of the motive force. However, above the critical value of the motive force, 13 mV, the power of fluctuation increased much more with the increase of the motive force, and below the critical value it changed much less than that expected. The results are summarised as follows

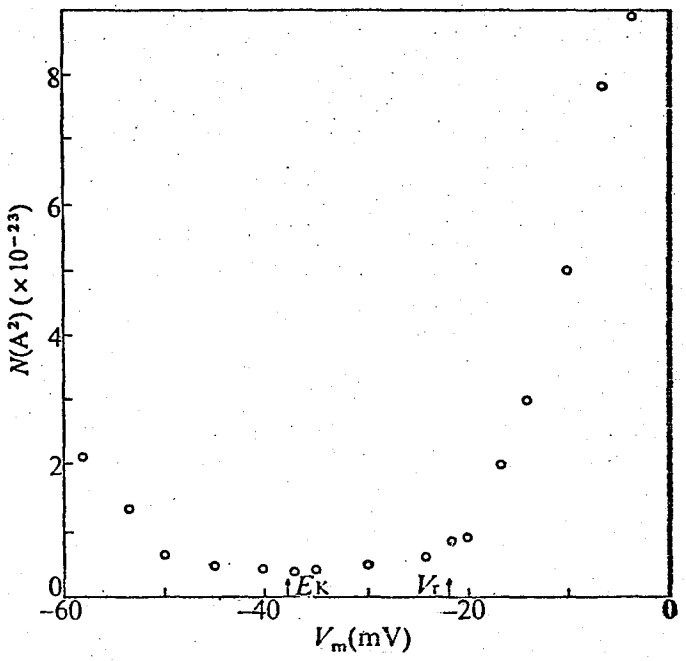


Fig. 3 Intensity N of $1/f$ noise plotted against membrane potential V_m. N was determined as the value of $S_I(f)$ at 1 Hz. Resting potentjal $V_r = -22$ mV, K^+-equilibrium potential $E_K = -38$ mV in the standard solution.

Fig. 23. Relation between the intensity of current noise and the membrane potential at which the potential was clamped in Paramecium. From Moolenaar, W. H., de Goede, J. and Verveen, A. A. Nature, 260;344-346 (1976) [13].

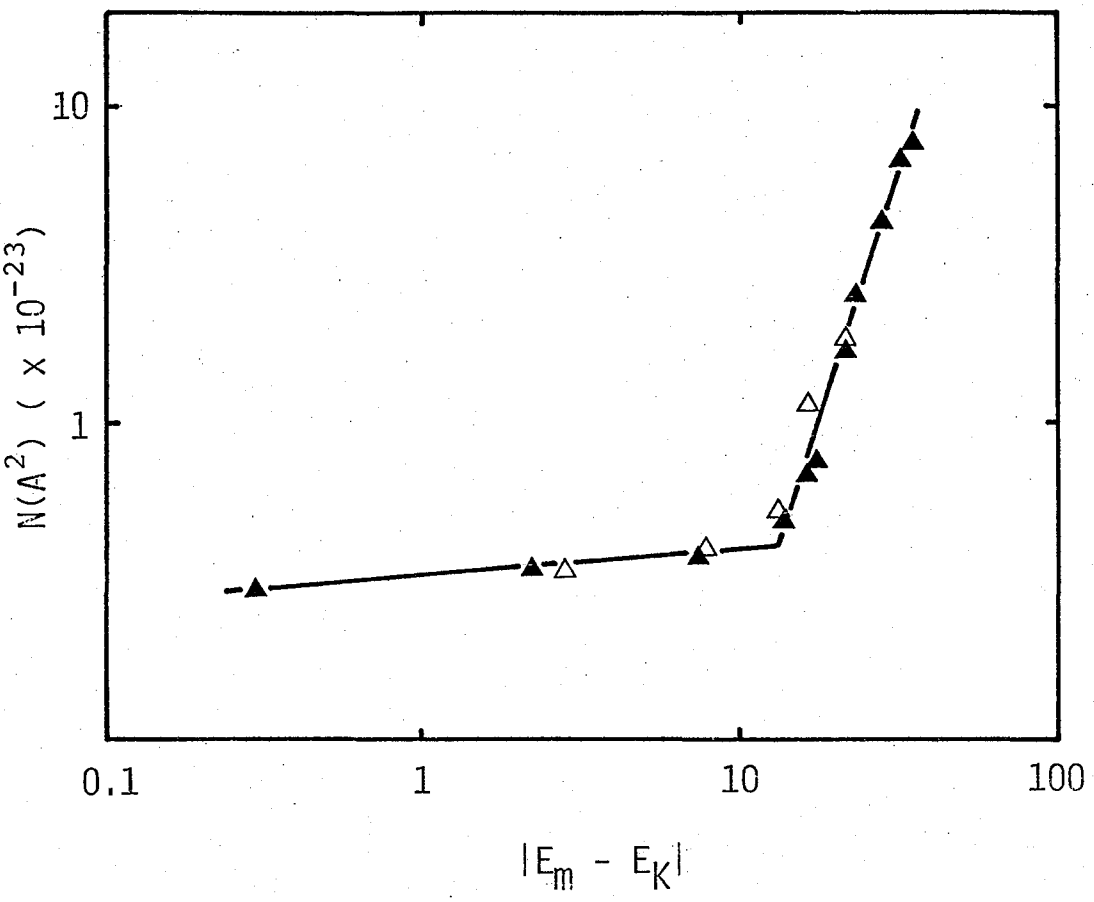


Fig. 24. Relation between the intensity of current noise (N) and the motive force ($E_m - E_K$). Positive value of $E_m - E_K$, (▲) and negative value of $E_m - E_K$, (△).

$$I = \beta(|E_m - E_K|)^{3.2} + \text{constant} \qquad (11a)$$

for $|E_m - E_K| > 13$ mV

$$I = \beta'(|E_m - E_K|)^{0.08} + \text{constant} \qquad (11b)$$

for $|E_m - E_K| < 13$ mV

where I is the power of fluctuation, β is proportional coefficient.

These results seem to state that the fluctuation depends on the motive force of the passive potassium current. However, the calcium current is not excluded from possible contributors of the fluctuation. From simple calculation an expression of the total power of fluctuation in terms of the motive force of potassium ions is obtained. Under the assumption that the conductance g_K and/or g_{Ca} were fluctuating, we consider the current fluctuation given by

$$\delta I_K = \delta g_K (E_m - E_K) \qquad (12a)$$

$$\delta I_{Ca} = \delta g_{Ca} (E_m - E_{Ca}) \qquad (12b)$$

Since the time constant of the membrane impedance is much shorter than that of the fluctuation, the current fluctuation is related to the potential fluctuation with a relation given by

$$\delta E = \delta I_t / g_t \qquad (13)$$

where g_t is the total conductance and I_t is the total current defined by Eq. (5). If the fluctuations of conductances g_K and g_{ca} were independent, then, the relation

$$\overline{\delta E^2} = \overline{\delta I_t^2} / g_t^2 = (\overline{\delta I_K^2 + \delta I_{Ca}^2})/(g_K + g_{Ca})^2 \qquad (14)$$

holds. Substituting Eqs. (12a) and (12b) into Eq. (14) and considering Eqs. (9a) and (9b), we obtain

$$\overline{\delta E^2} = (\frac{\overline{\delta g_K^2}}{g_K^2} + \frac{\overline{\delta g_{Ca}^2}}{g_{Ca}^2})[\frac{g_K^2}{(g_K + g_{Ca})^2}](E_m - E_K)^2 \qquad (15)$$

As we examined above (Table V and VI), g_{Ca} was much smaller than g_K and the relative change of g_{Ca}, however, was larger than that of g_K when the salt condition was changed. The calculated values of the factor $[g_K^2/(g_K + g_{Ca})^2](E_m - E_K)^2$, which were normalized at the ratio $[K]/[Ca]^{1/2}$ of 0.5, are shown in Table V and VI. The factor $[g_K^2/(g_K + g_{Ca})^2](E_m - E_K)$ is approximately proportional to the second power of the motive force $(E_m - E_K)$, while, as stated above, the observed power of fluctuation was not exactly proportional to the second power. By dividing the observed values of the power of fluctuation by the calculated values of the above factor, the values of the factor $(\overline{\delta g_K^2}/g_K^2 + \overline{\delta g_{Ca}^2}/g_{Ca}^2)$ were obtained, which are shown in Table V and VI. The value of this factor $(\overline{\delta g_K^2}/g_K^2 + \overline{\delta g_{Ca}^2}/g_{Ca}^2)$ decreased with increase of the ratio $[K]/[Ca]^{1/2}$ and then increased with ratio exceeding 4. This change of the factor depending on the ratio $[K]/[Ca]^{1/2}$ corresponds to the previous result of analysis that the power of fluctuation increased with the motive force $(E_m - E_K)$, with a power smaller than 2 at small values of $(E_m - E_K)$ and with a power larger than 2 at large values of $(E_m - E_K)$.

Assuming the open-close kinetics of the channels, let us denote the average number of opened channels for K^+ and Ca^{++} in the membrane as n_K and n_{Ca} and the unit conductance as γ_K and γ_{Ca}, respectively. Then the conductance g_K and g_{Ca} are given by $\gamma_K n_K$ and $\gamma_{Ca} n_{Ca}$, and the fluctuation of the conductance due to the fluctuation of the number of opened channels is given by $\gamma_K \delta n_K$ and $\gamma_{Ca} \delta n_{Ca}$, respectively. Usually, the mean square of fluctuation of the number, δn_K^2 and δn_{Ca}^2, is of the order of n_K and n_{Ca}, respectively. Thus we have approximately

$$\frac{\delta g_K^2}{g_K^2} + \frac{\delta g_{Ca}^2}{g_{Ca}^2} \simeq \frac{1}{n_K} + \frac{1}{n_{Ca}} \qquad (16)$$

and the above mentioned dependence of the factor $(\overline{\delta g_K^2}/g_K^2 + \overline{\delta g_{Ca}^2}/g_{Ca}^2)$ on the motive force $(E_m - E_K)$ may be translated into the dependence of the numbers of n_K and n_{Ca} on the motive force. The decrease of n_K or n_{Ca} results in the increase of the factor. n_{Ca} might decrease with increasing $[K]/[Ca]^{1/2}$ or decreasing $E_m - E_K$. However, it is not sure if the above change of the value of the factor would be explained on the basis of the possible change of the number n_K or n_{Ca} with $(E_m - E_K)$.

The interpretation that the observed fluctuation was due to the fluctuation of the number of opened channels must be discussed, taking the observed PDS into consideration. The profile of the PDS was changed from the $1/(1 + (f/f_c)^2)$

type to the 1/f type and the total power was decreased from unity to 0.16, by the application of 1.0 mM TEA in the presence of 0.5 mM KCl and 1.0 mM $CaCl_2$. The effect of TEA on Paramecium cells is known to block outward potassium current. Therefore, the above result suggests that most of the $1/(1 + (f/f_c)^2)$ type component came from the potassium current through the channels which can be blocked by TEA . A similar observation was made on the giant axon membrane [10]. It is likely that the large increase of the potential fluctuation at large values of $(E_m - E_K)$ was mainly due to the increase of the fluctuation of potassium current and to this fluctuation, the open-close kinetics of the channels may be applicable. The remaining component of fluctuation might come from other origins including the contribution of the calcium current. Even so, however, a simple open-close kinetics of channels is not directly applicable to this component, because it was not of the $1/(1 + (f/f_c)^2)$ type but of the 1/f type.

The two components, the $1/(1 + (f/f_c)^2)$ type and the 1/f type, both decreased with decreasing $(E_m - E_K)$ or increasing ratio $[K]/[Ca]^{1/2}$. At small values of $(E_m - E_K)$ the PDS totally became of the 1/f type. Therefore, it is probable that the fluctuation at this condition was due to other origins than the potassium channels.

C. Comparison of the Total Intensity with that of other cells

The magnitude of potential fluctuation in Paramecium is relatively larger as compared with other biological membranes. Fishman [24] and Fishman, Moore and Poussart [10] applied a technique of patch voltage clamp by the sucrose gap method and analysed the fluctuation in a small area of a squid axon. They estimated the size of the area to be 10^{-4} to 10^{-5} cm^2. The membrane resistance is 10 to 100 MΩ and the capacitance is 10 to 100 pF. These values are almost equivalent to that of Paramecium. The surface area of Paramecium which includes the surface membrane of cilia is 5×10^{-4} cm^2 taking the averaged cell dimension of 160 × 40 μm [25]. The membrane resistance is 35 MΩ and the capacitance is 900 pF. Thus, we can directly compare the magnitude of fluctuation in these materials. Fig. 25 shows the potential fluctuation in the squid axon reported by Fishman et al. [10]. Notice the fluctuation observed at 24 °C. The amplitude of fluctuation is about 50 μV. The resting potential of the squid axon is about -60 mV or so. This magnitude of fluctuation is much smaller than 3.2 mV in Paramecium in the presence of 0.5 mM KCl and 1.0 mM $CaCl_2$ at 25 °C. The resting potential of Paramecium at this condition is -36 mV. The magnitude

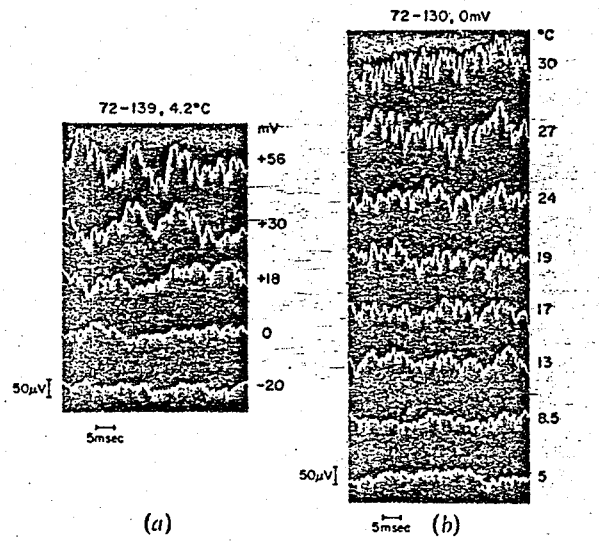


Fig. 4. Voltage fluctuation waveforms, reproduced from tape (effective noise bandwidth 1.25 kHz) of noise from excitable patches (spike amplitude >90 mV). (*a*) Changes of potential from patch "rest" (0 mV) at fixed temperature. (*b*) Another axon patch at "rest" at eight different temperatures. These very short time-segments were taken randomly from 1-min lengths of recorded data

Fig. 25. Potential fluctuation waveforms of isolated patch in squid axon membrane. From Fishman, H. M., Moore, L. E. and Poessart, D. J. M. J. Memb. Biol. 24,305-328 (1975) [10].

of fluctuation in Paramecium is about sixty times larger than that of squid axon. This anomalous fluctuation resulted in the difficulty of number estimation of channels on the Paramecium membrane. Assuming the open-close kinetics of the channel, we can roughly estimate the number of channels by the comparison of the fluctuating potential with the motive force of ions. Comparing the amplitude of 1.6 mV with the motive force of 56 mV for potassium ions, we obtain the number of channels as 1200. The channel density of Paramecium is $0.024/\mu m^2$. The number of cilia in Paramecium is calculated to be 5600 [25]. This means that the number of channels on a cilium is of the order of unity. This value is very small as compared with the reported values in the other species for delayed K channels or for Na channels. For the delayed K channel in squid axon the density is $40\text{-}70/\mu m^2$ [26]. For the Na channel in squid axon the density is $483\text{-}553/\mu m^2$ [27 - 29] and about $2000/\mu m^2$ for the Na channel in frog myelinated nerve fibers [30]. For the Na channel in lobster giant axon the density is $13\text{-}35/\mu m^2$. A small value of channel density was observed in artificially hyperpolarized tunicate egg cells [31]. The mean number of 6400/cell was obtained for the anomalous rectifier channel. This gives the channel density of $0.028/\mu m^2$.

Thus, the number of channels effective to fluctuation

was very small in Paramecium as compared with, for example, the giant axon. On the other hand, the static conductance of the membrane was not very different in two cells. Therefore, the unit conductance of the channel in Paramecium must be very large. In other words, a certain number of single channels may work cooperatively as a unit for fluctuation.

Chapter II

POTENTIAL FLUCTUATION AND MEMBRANE EXCITAION

I. INTRODUCTION

In the previous chapter we studied the effect of the environmental condition on the membrane potential fluctuation. In this chapter we study the effect of the environmental condition on the degree of membrane excitation or the excitability of the membrane. The excitability must be one of the important factors which determine the sensitivity of the organism to the environmental change. To see the excitability, a current stimulation is applied to the organism, and an action potential is evoked. The maximum rate of rise of an action potential and the potential where the maximum rate is observed are chosen to represent the excitability. Similarly to the analysis in the previous chapter, special attention is paid to the effect of potassium ions and calcium ions in the medium.

II. METHODS

A depolarizing current pulse of 10^{-9} amp × 1 ms was given to Paramecium cells through intracellular microelectrodes to induce an action potential. The potential change was recorded and the maximum rate of rise of the potential, MRR, a maximum value of the time derivative of evoked action potential dV(t)/dt, was measured under the same condition as the fluctuation was analysed. The value of the potential where the rate of rise was maximum E_{MRR} was also measured.

III. RESULTS

A. Effect of the Ratio $[K]/[Ca]^{1/2}$ on the Membrane Excitation

A current pulse was given to the organism at various concentrations of KCl and $CaCl_2$. Records of the membrane potential depolarization and its time derivative are shown in Fig. 26. Time courses of potential depolarization in different cells are similar when the ratios $[K]/[Ca]^{1/2}$ of the medium are the same. Table VII shows the effect of the ratio $[K]/[Ca]^{1/2}$ on the MRR. In each experimental series, K series and Ca series, the MRR decreased with the increase

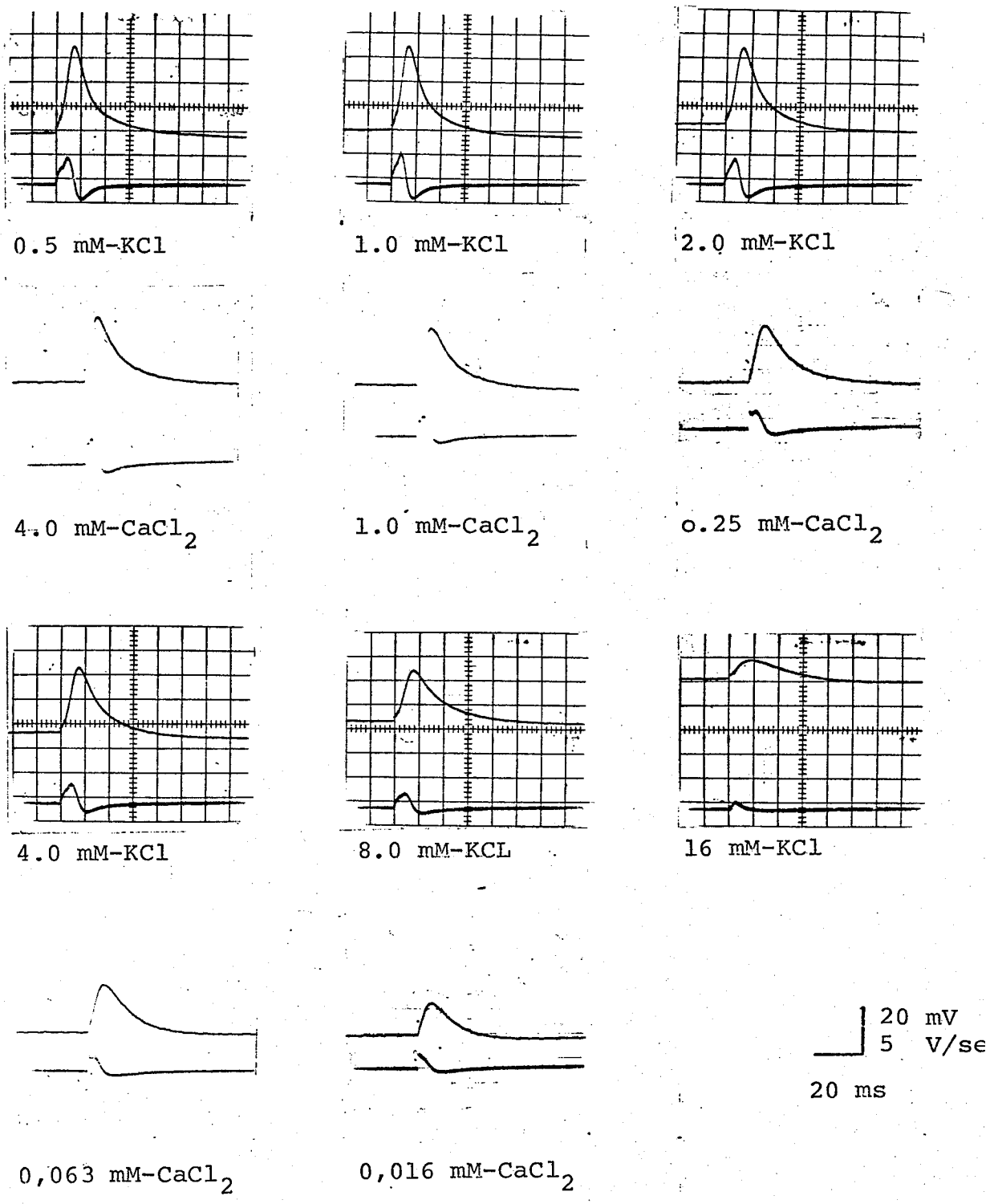


Fig. 26. Records of action potential (upper trace) and its time derivative (lower trace) at various concentrations of KCl and $CaCl_2$. Solutions containe 1.0 mM-$CaCl_2$ (K series) or 1.0 mM-KCl (Ca series).

Table VII Effect of the Ratio $[K]/[Ca]^{1/2}$ on the MRR

Ratio $[K]/[Ca]^{1/2}$		0.5	1	2	4	8	16
K Series	(KCl mM $CaCl_2$ = 1 mM)	0.5	1	2	4	8	16
MRR	(V/sec)	7.0	6.5	5.4	4.2	3	2
Ca Series	($CaCl_2$ mM KCl = 1 mM)	4	1	0.25	0.063	0.016	*
MRR	(V/sec)	6.5	5.9	5.8	4.5	3	2*

of the value of the ratio. The same value of the ratio $[K]/[Ca]^{1/2}$ in different series gave the same value of the MRR. This result denotes that the degree of membrane excitation estimated by the MRR also depends on the ratio $[K]/[Ca]^{1/2}$, as the power of potential fluctuation did.

To analyse the relation between the MRR and the ratio $[K]/[Ca]^{1/2}$, MRR^{-1} were plotted against $[K]/[Ca]^{1/2}$. Fig. 27 shows the result. MRR^{-1} increased in proportion to the ratio $[K]/[Ca]^{1/2}$, in both experimental series, in K series and Ca series. The relation between the MRR and the calcium concentration of the medium is shown in Fig. 28. The MRR was saturated at high concentrations of $CaCl_2$.

Fig. 29 shows the effect of KCl on the peak of action potential, E_{peak}, and the value of potential, E_{MRR}, where the rate of rise was maximum. E_{MRR}(or E_{peak})- E_m would be another quantity to be useful to measure the excitability. The resting potential is also shown. The E_{peak} slightly depolarized with increasing KCl; however, it was maintained at a constant level below 4.0 mM KCl. The E_{MRR} changed with increasing KCl. It depolarized 3.5 mV per ten times change of KCl below 2.0 mM KCl and 12 mV per ten times change of KCl above 4.0 mM KCl. The effect of $CaCl_2$ on the peak of action potential and the E_{MRR} is shown in Fig. 30. In this case, the E_{peak} depolarized with increasing $CaCl_2$. It changed 32 mV per ten times change of $CaCl_2$ above 0.25 mM $CaCl_2$ and 18 mV below 0.063 mM $CaCl_2$. The E_{MRR} also changed

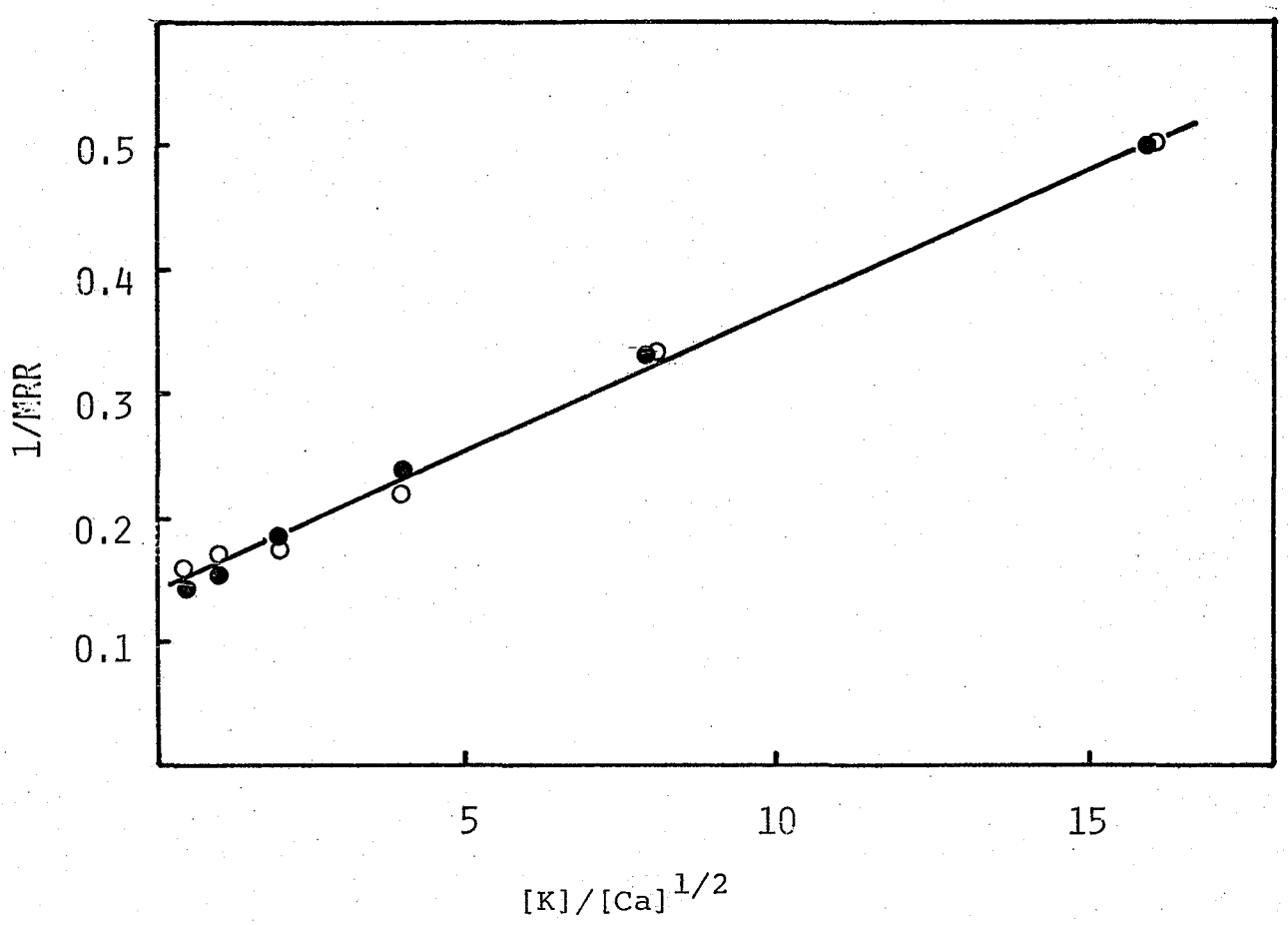


Fig. 27. Relationship between MRR^{-1} and $[K]/[Ca]^{1/2}$. MRR^{-1} increases in proportion to the ratio.
K series (●), Ca series (○).

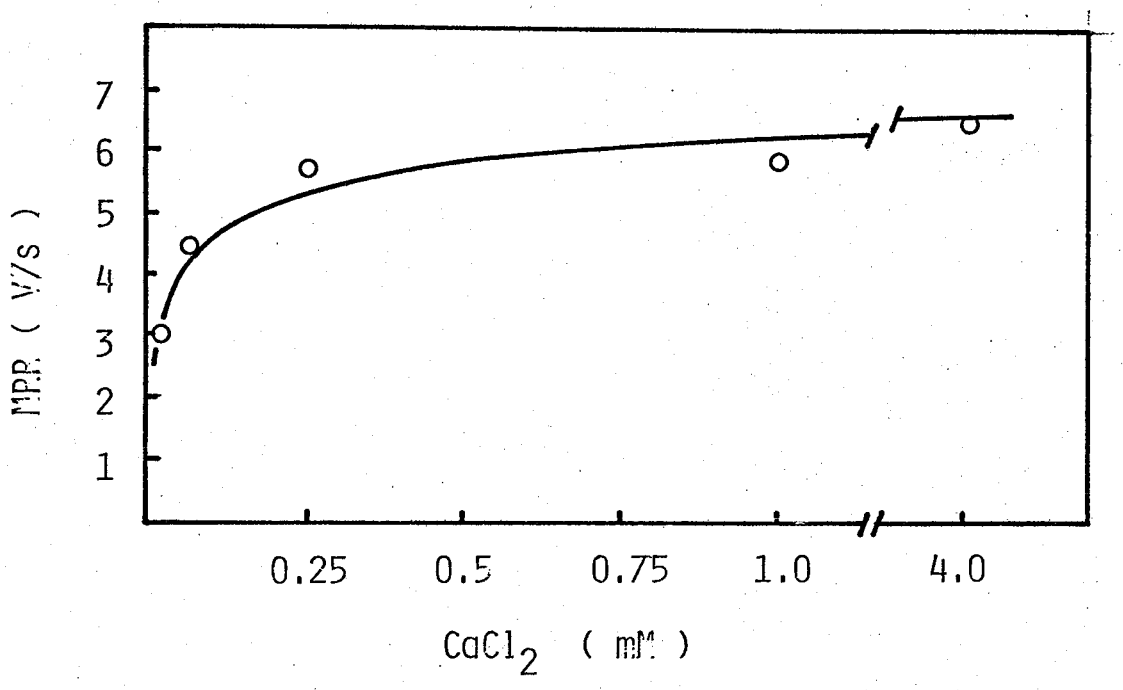


Fig. 28. Relationship between the Ca concentration and the maximum rate of rise of the spike potential in Paramecium.

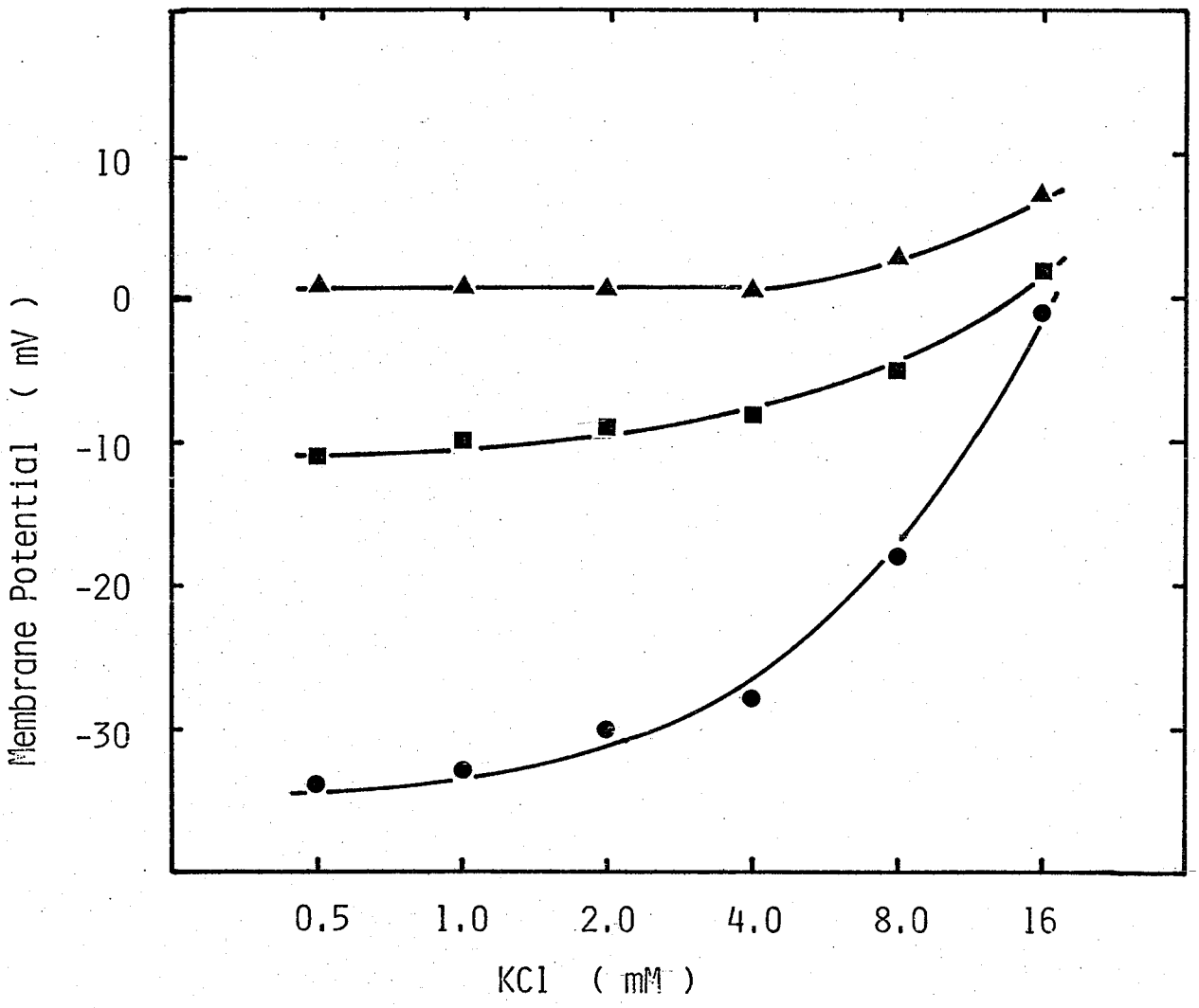


Fig. 29. Effect of the KCl concentration on the peak of action potential (▲) and the value of potential at which the rate of rise was maximum (■). The resting potential (●) is shown.

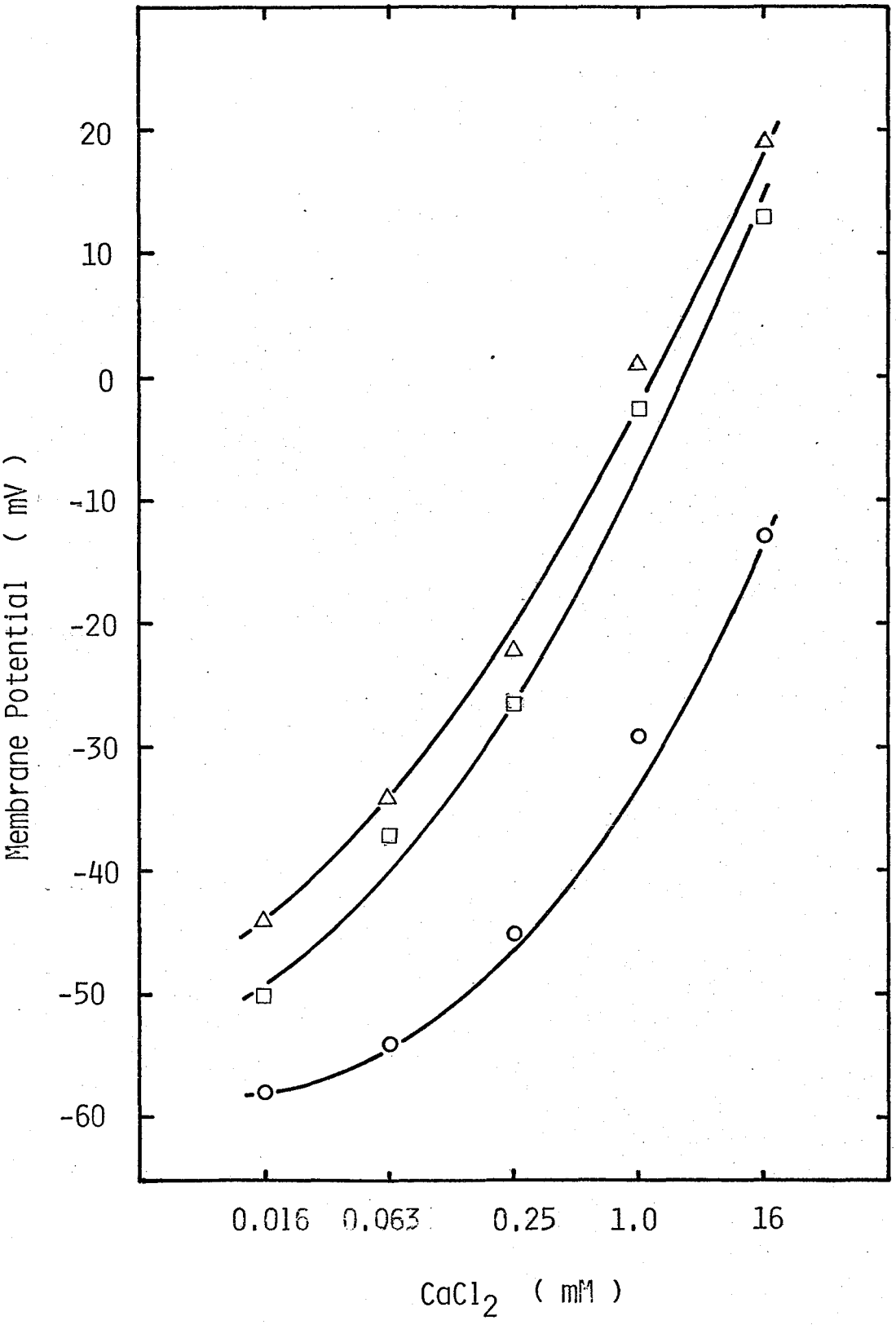


Fig. 30. Effect of the $CaCl_2$ concentration on the peak of action potential (△) and the value of potential at which the rate of rise was maximum (□). The resting potential (o) is shown.

with increasing $CaCl_2$. The dependence of the E_{MRR} on $CaCl_2$ was parallel to that of the peak potential.

The relation between the height of action potential ($E_{peak} - E_m$) and the motive force of calcium ions ($E_{Ca} - E_m$) in K series is shown in Fig. 31. The height of action potential ($E_{peak} - E_m$) changed in proportion to the motive force of calcium ions ($E_{Ca} - E_m$). However, the dependence of ($E_{peak} - E_m$) on the motive force of calcium ions changed at $E_{Ca} - E_m$ = 228 mV. For larger values of the motive force than 228 mV, the height of action potential showed a smaller change than that showed for smaller values of the motive force than 228 mV. The difference between the E_{MRR} and the resting potential, ($E_{MRR} - E_m$), is also shown in Fig. 31. The dependence of ($E_{MRR} - E_m$) on the motive force ($E_{Ca} - E_m$) shows the same tendency as the height of action potential.

B. Relationship between the Potential Fluctuation and the Degree of Membrane Excitaion

As we studied in chapter I, the power of potential fluctuation depended on the environmental salt condition represented in terms of the ratio $[K]/[Ca]^{1/2}$. The degree of membrane excitation estimated with MRR was also dependent on the ratio $[K]/[Ca]^{1/2}$ of the medium. This means that the power of fluctuation and the degree of membrane

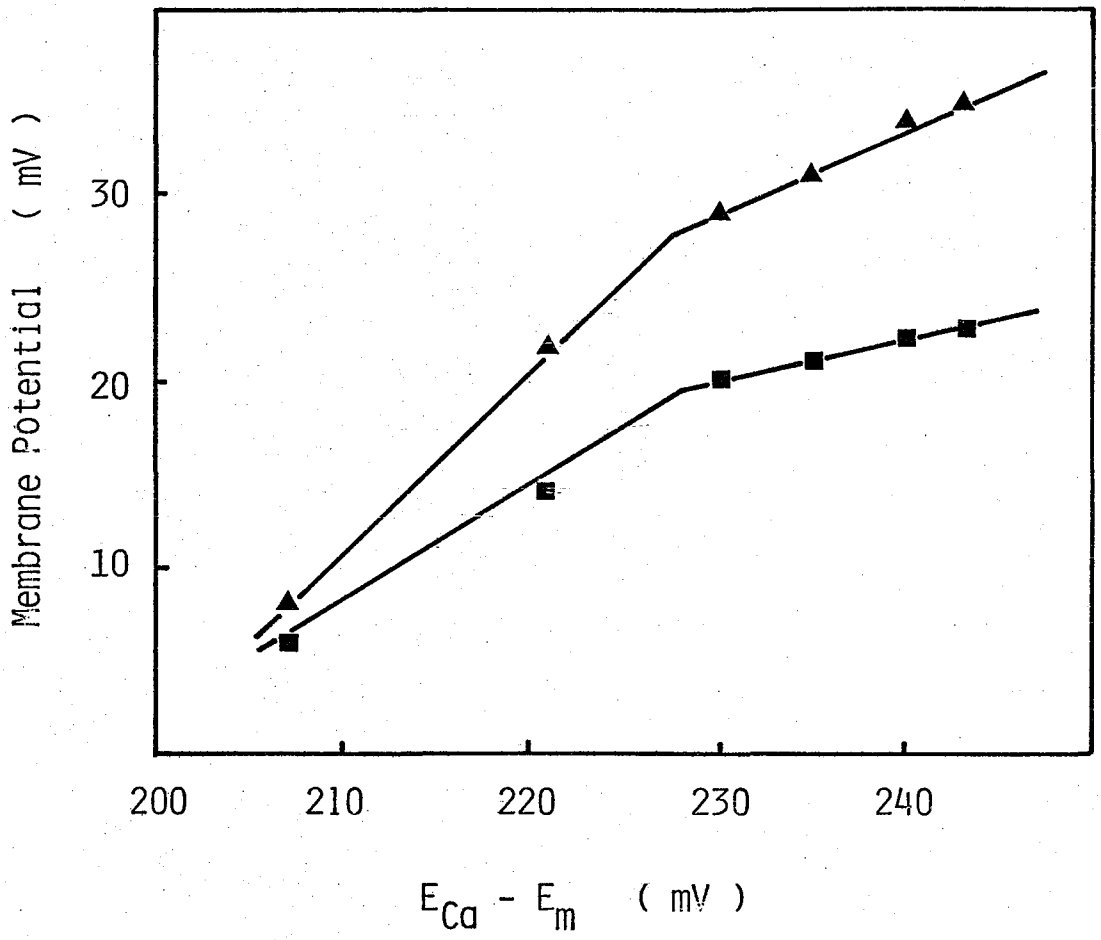


Fig. 31. Relationship between the height of spike ($E_{peak} - E_m$) (▲), the potential difference ($E_{MRR} - E_m$) (■) and the motive force of calcium ions ($E_{Ca} - E_m$)

excitation is related with each other by the parameter $[K]/[Ca]^{1/2}$. From Table I in chapter I and Table VII in this chapter, an empirical relation between the power of potential fluctuation and the degree of membrane excitation by the applied stimulus was derived. Fig. 32 shows the relation. The MRR increased approximately in proportion to the logarithm of the power of fluctuation. In the other words, the relation may be written as

$$\Delta MRR = \kappa \Delta I/I \qquad (17)$$

where I is the normalized power of potential fluctuation and κ is coefficient. Eq. (17) means that a change of MRR regarded as a change of the degree of membrane excitation is proportional to a relative change of the power of potential fluctuation. At large values of I, MRR is also large, but its change may be small. In addition, it should be remarked that Eq. (17) represents a relation between a response of the excitable membrane to an external disturbance and an internal fluctuation of the membrane in the absence of the disturbance. The above equation is only an approximate one and implicitly contains a parameter $[K]/[Ca]^{1/2}$. It is not certain if a similar relation is satisfied when the environmental condition is changed in another way.

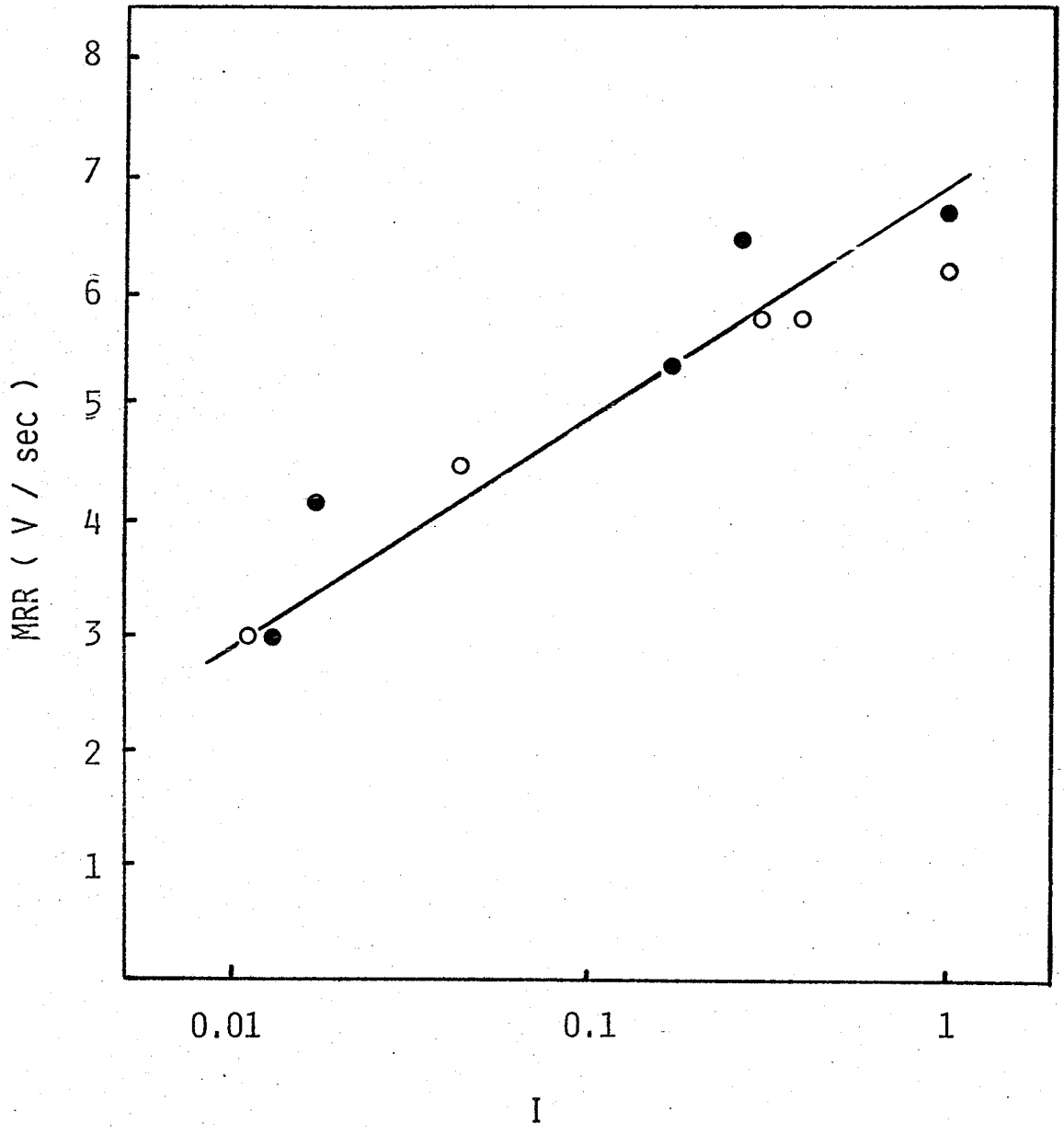


Fig. 32. Relationship between the logarithm of the power of potential fluctuation and the maximum rate of rise of the action potential.

IV. DISCUSSION

A. Degree of Membrane Excitation and Ca Binding

Table VII and Fig. 27 show the dependence of MRR on the ratio $[K]/[Ca]^{1/2}$. MRR^{-1} changed in proportion to the ratio This result is well explained by following three assumptions: (1) There are a finite number of cation binding sites on the membrane; (2) Potassium ions and calcium ions bind competitively to the cation binding sites; (3) MRR is proportional to the amount of Ca occupying sites. Under these assumptions we consider a competitive reaction as follows

$$PCa_{1/2} + K \rightleftharpoons PK + 1/2\ Ca \tag{18}$$

$$k = [PK][Ca]^{1/2}/[PCa_{1/2}][K] \tag{19}$$

$$[P_t] = [PK] + [PCa_{1/2}] \tag{20}$$

where $[PK]$ and $[PCa_{1/2}]$ are the amount of the sites occupied by mono- and divalent cation and $[P_t]$ is the total amount of sites. From Eqs. (18),(19) and (20), we obtain

$$1/[PCa_{1/2}] = (1 + k[K]/[Ca]^{1/2})/P_t \tag{21}$$

Assuming that MRR is proportional to the amount of Ca occupying sites, $PCa_{1/2}$, Eq. (21) is rewritten as follows

$$C/MRR = k[K]/[Ca]^{1/2} + 1 \tag{22}$$

Using Eq.(22) and Fig. 27, k and C were calculated. The calculated equilibrium constant k was 0.17 $mM^{-1/2}$ and proportional constant C was 7.4 sec/V.

In the case of barnacle muscle fiber studied by Hagiwara [19], the number of Ca binding sites at the surface membrane rather than the calcium concentration of the medium determines the flux of Ca. The potential level at which the maximum Ca influx was observed under the voltage clamp experiment in barnacle muscle fiber [32] was nearly equal to the potential level at which the MRR was observed. From these results it was concluded that the MRR should be proportional to the Ca influx. Our results in Paramecium suggest that the MRR is proportional to the number of Ca occupying sites and is also regarded to be proportional to the Ca influx. Thus, the degree of membrane excitation estimated by the MRR roughly corresponds to the maximum Ca influx.

B. Excitability and the Motive Force

The potential difference $(E_{peak} - E_m)$ and $(E_{MRR} - E_m)$ both depended on the motive force of calcium ions $(E_{Ca} - E_m)$ and potassium ions $(E_m - E_K)$. As shown in Fig. 31, the change of $(E_{peak} - E_m)$ with the change of $(E_{Ca} - E_m)$ was large for small values of $(E_{Ca} - E_m)$ and small for its

large values. The boundary was found around $(E_{Ca} - E_m)$ = 228 mV. This value of $(E_{Ca} - E_m)$ corresponded to the value of E_m where the motive force for potassium ions $(E_m - E_K)$ was about 13 mV. This was the potential where the dependence of the power of potential fluctuation on the motive force showed a drastic change, as shown in Fig. 22, 24 and Eq. (11).

It should be remarked also that the value of the ratio $[K]/[Ca]^{1/2}$ which gave the motive force $(E_m - E_K)$ of 13 mV corresponded approximately to that which gave about half saturation of the Ca binding sites, as found in Fig. 28. Therefore, summarizing the results in Chapter I and II, the power of potential fluctuation and the excitability both showed different behaviors in two different environmental conditions, where more than half of the calcium binding sites have actually bound calcium ions or where less than half of the sites have bound calcium ions.

It is useful to introduce the following relation between the peak potential and the conductance which is satisfied under the condition that the total current is zero.

$$E_{peak} = (g_{Ca}^* E_{Ca} + g_K^* E_K)/(g_{Ca}^* + g_K^*) \qquad (23)$$

where g_{Ca}^* and g_K^* are conductances of calcium ions and potassium ions at the peak of the action potential. Then, the difference $E_{peak} - E_m$ is written as

$$E_{peak} - E_m = (R_{peak} - R_m)(E_{Ca} - E_K) \qquad (24)$$

where R means the relative contribution of the calcium

conductance to the total conductance, that is,

$$R_m = g_{Ca}/(g_{Ca} + g_K) \text{ and } R_{peak} = g_{Ca}^*/(g_{Ca}^* + g_K^*).$$

Therefore, the ratio of $E_{peak} - E_m$ to $E_{Ca} - E_K$ is equal to the increase of the relative contribution of the calcium conductance at the peak of the action potential.

At the condition where only a small fraction of the calcium binding sites were occupied by calcium ions, $E_m - E_K$ was smaller than 13 mV and $E_{Ca} - E_K$ was smaller than 236 mV. There, as shown in Fig. 33, $E_{peak} - E_m$ increased very much with increasing Ca concentration or decreasing K concentration, or increasing $E_{Ca} - E_K$. Taking into consideration the above Eq. (24), $R_{peak} - R_m$ increased very much. The relative increase of the calcium conductance at the peak was very large. At the same condition, the power of potential fluctuation was not very much dependent on $E_m - E_K$ and the spectrum was of the 1/f type. This type of spectrum was not sensitive to TEA and the contribution of the fluctuation of the calcium current seemed to be larger than that of the potassium current.

On the other hand, at the condition where most of the binding sites were occupied by calcium ions, $E_m - E_K$ was larger than 13 mV and $E_{Ca} - E_K$ was larger than 236 mV. There, $E_{peak} - E_m$ increased only a little with increasing Ca concentration. $E_{peak} - E_m$ was almost constant. Not only the increase of the calcium conductance but also the increase of the potassium conductance at the peak would

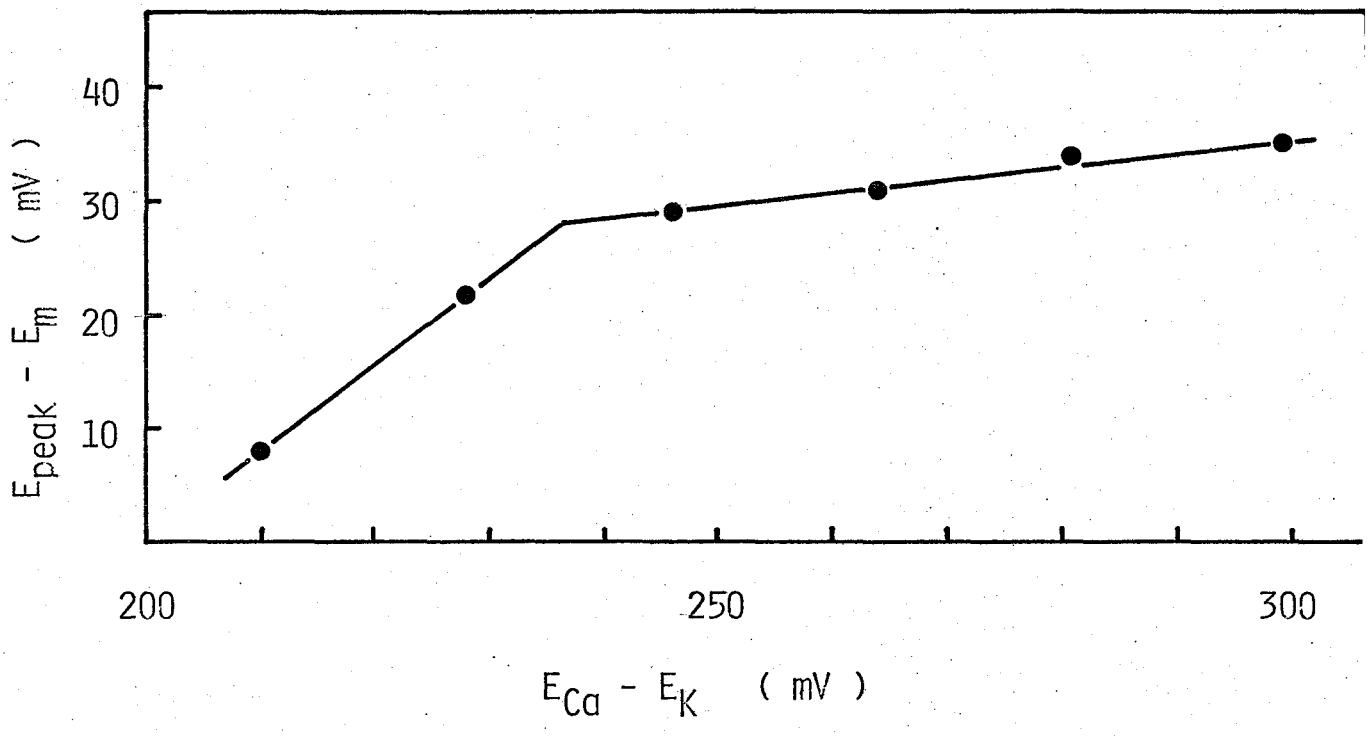


Fig. 33. Relationship between the height of spike ($E_{peak} - E_m$) and the difference of equilibrium potential ($E_{Ca} - E_K$).

be remarkable. At the same condition, the power of potential fluctuation very much increased with $(E_m - E_K)$. There, the fluctuation was sensitive to TEA and the contribution of the potassium current to the fluctuation was predominant over the contribution of the calcium current.

If the analysis developed in chapter I were applied, the minimum value of the factor $(\overline{\delta g_K^2}/g_K^2 + \overline{\delta g_{Ca}^2}/g_{Ca}^2)$ would be found at the condition where half of the binding sites were occupied by calcium ions. The value of this factor would increase with any change which could break the condition of equal partition of potassium and calcium ions to binding sites.

C. Potential Fluctuation and Membrane Excitation

The empirical and approximate relation derived between the degree of membrane excitation and the power of potential fluctuation stated that a change of the degree of membrane excitation is proportional to a relative change of the power of potential fluctuation. In the condition where the power of potential fluctuation is small, a small change of the power of fluctuation corresponded to a large change of the degree of membrane excitation and in the condition where the power is large a larger change of the power of fluctuation corresponded to the same change of the degree of membrane

excitation. Such a property as mentioned above would be important for a sensory reception of the organism. Organisms swimming under the environmental condition where the power of fluctuation is low would be more sensitive than those swimming under the condition where the power is large, if any disturbance were applied.

Chapter III

POTENTIAL FLUCTUATION AND BEHAVIORAL RESPONSE

I. INTRODUCTION

Living organisms show responses to various kinds of stimuli applied by the environmental condition. These responses caused the organism some kind of behaviour. For example, tactic behaviours of microorganisms are a result of the response to a gradient of the environmental condition. The magnitude of the response changes with the change of the sensitivity of the organism affected by the environmental condition. In this chapter we examine the tactic behaviours of Paramecium to chemical substances under various environmental conditions and compare the result with that previously obtained in chapter I and II. The purpose of this study is to examine the relationship among the membrane potential fluctuation, the degree of membrane excitation and the sensitivity of organisms to the environmental disturbance and to discuss the possible role of fluctuation in the sensory reception.

II. METHODS

A. Behavioral Analysis

To examine the sensitivity of organisms to the environmental condition, the taxis of Paramecium toward a supernatant of the bacterial suspension was studied by means of the capillary method. Cells were filtrated and washed with the test solution and incubated for 30 min at 25 °C before the experiment and suspended into a small vessel (36 mm long, 22 mm width and 3 mm depth) containing the same solution. After incubation of 10 min in the vessel at 25 °C, a capillary (1.5 mm of diameter) filled with the supernatant of bacterial suspension was put into the center of the vessel. The vessel was photographed by dark field illumination at intervals of 30 sec. The magnitude of the tactic response was measured using the equation

$$T = [\, n(t) - n(0) \,] \,/\, n(0) \qquad (25)$$

where n(t) denotes the number of the organisms in a particular circle around the opening of the capillary at time t after the insertion of the capillary and n(0) denotes the average number of organinsms in the same circle before the insertion.

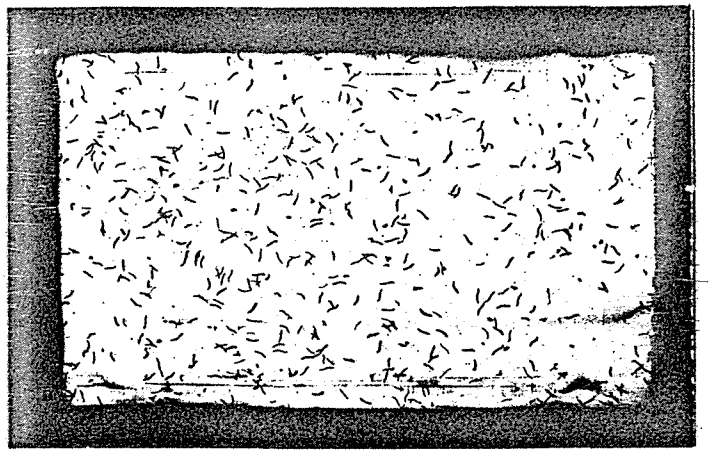
B. Preparation of Attractant

Aerobacter aerogenes were cultured on a 1 % agar plate containing 5 gr yeast extract, 0.1 gr dextrose and 1000 ml of distilled water at room temperature. Bacteria were suspended in 200 ml of distilled water and separated by centrifugation at 9000×g for 10 min. Supernatant was used as the attractant.

III. RESULTS

A. Effect of the Ratio $[K]/[Ca]^{1/2}$ on the Behavioral Response of Paramecium

Taxis of Paramecium toward the supernatant of bacterial suspension was studied as an example of behavioral response. A capillary filled with the supernatant was put into a vessel which contains Paramecium cells. After a while the organisms began to accumulate around an opening of the capillary. Fig. 34 shows an example of accumulation. A dependence of the magnitude of tactic response on the environmental salt condition was examined. It was very remarkable that when the concentration of potassium ions and/or calcium ions were changed, the extent of accumulation depended on the ratio



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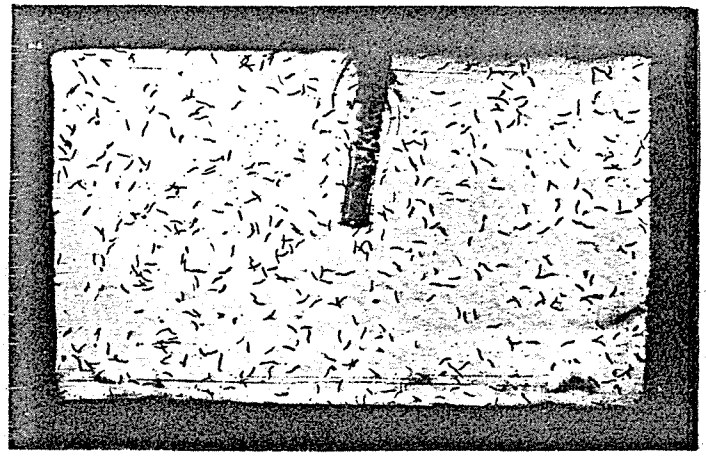

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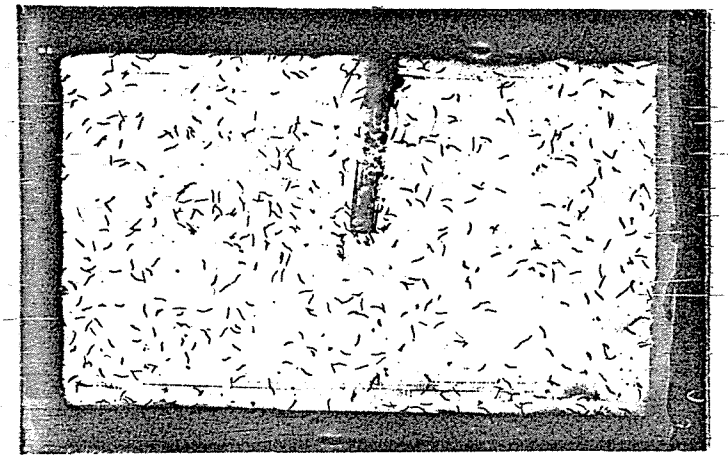

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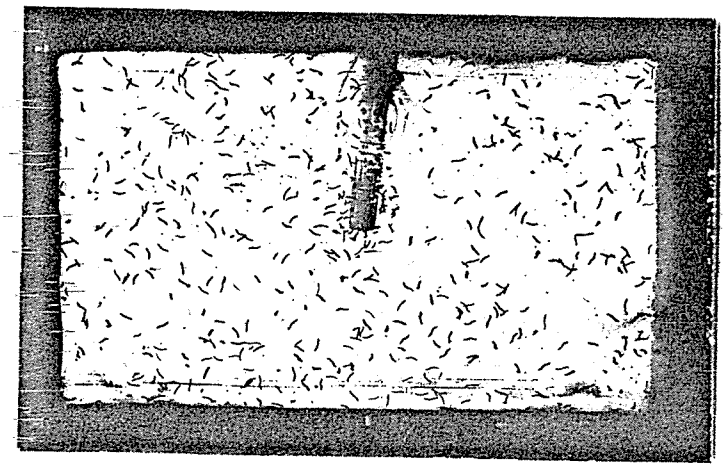

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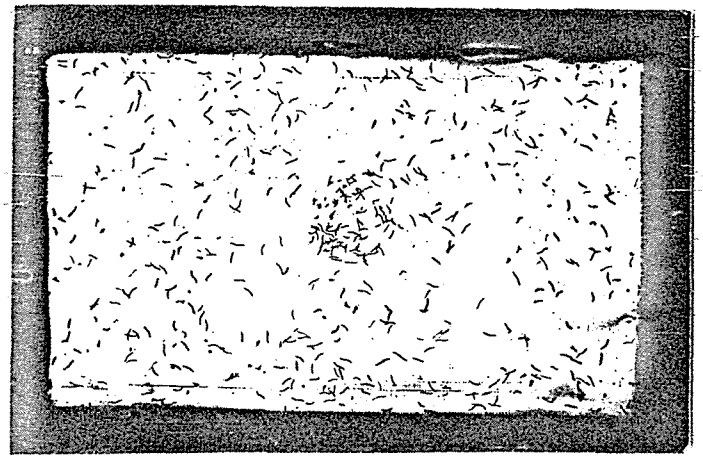

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Fig. 34. Accumulation of Paramecium cells at 8.0 mM-KCl and 1.0 mM-$CaCl_2$.

$[K]/[Ca]^{1/2}$. Table VIII shows the effect of the ratio $[K]/[Ca]^{1/2}$ on the extent of accumulation, or the magnitude of taxis, under various concentrations of the attractant. The magnitude increased with increasing ratio and reached a maximum around $[K]/[Ca]^{1/2} = 8$. It decreased with further increase of the ratio. The magnitude became smaller with dilution of the attractant. However, a similar dependence on the ratio $[K]/[Ca]^{1/2}$ was observed even after the dilution. The magnitude of response increased with time and reached a final level about 10 min after the affording. The final level was almost the same in each experimental condition. At low concentrations of the attractant , the final level was lower than at high concentrations. The 2 min was the time for Paramecia to begin the accumulation at the experimental condition of $[K]/[Ca]^{1/2} = 0.5$. Thus the magnitude of response at this time represents the initial rate of accumulation.

In this experiment, the attractant was the supernatant of a bacterial suspension. Further analyses on the supernatant have not yet been performed to identify the effective component as the attractant.

Table VIII Effect of the ratio $[K]/[Ca]^{1/2}$ on the Behavioral Response

Ratio $[K]/[Ca]^{1/2}$		0.5	1	2	4	8	16
K Series	Attractant × 1	0.4	0.6	0.8	1.1	1.8	1.5
	Attractant × 0.25	0.3	0.2	0.5	0.4	0.8	0.6
Ca Series	Attractant × 1	0.3	0.3	0.5	1.0	1.6	1.2
	Attractant × 0.25	0.1	0.1	0.2	0.6	0.9	0.3

B. Relationship between the Potential Fluctuation and the Behavioral Response

As we investigated in chapter I, the power of potential fluctuation depended on the ratio $[K]/[Ca]^{1/2}$ of the medium and the magnitude of behavioral response also depended on the ratio. Thus, we are able to obtain a relationship between the power of fluctuation and the magnitude of behavioral response using the ratio $[K]/[ca]^{1/2}$ as a parameter. Fig. 35 shows the relationship between the logarithm of the power of potential fluctuation and the behavioral response. The magnitude of behavioral response increased with the decrease of the power of fluctuation and reached a maximum at $[K]/[Ca]^{1/2} = 8$, or some times at $[K]/[Ca]^{1/2} = 4$. In any case, it decreased with further decrease of the power of fluctuation. The magnitude of behavioral response at $[K]/[Ca]^{1/2} = 16$ was about three quarters of the maximum. The characteristic profile of the relationship is a rapid increase and then a gradual decrease of the response with the increase of the power of fluctuation. It is interesting that the condition where the fluctuation is very large is not convenient for the tactic response. Organisms swimming in the environmental condition where the power of fluctuation is low are more sensitive to the change of the environmental condition than those swimming in the condition where the power is high.

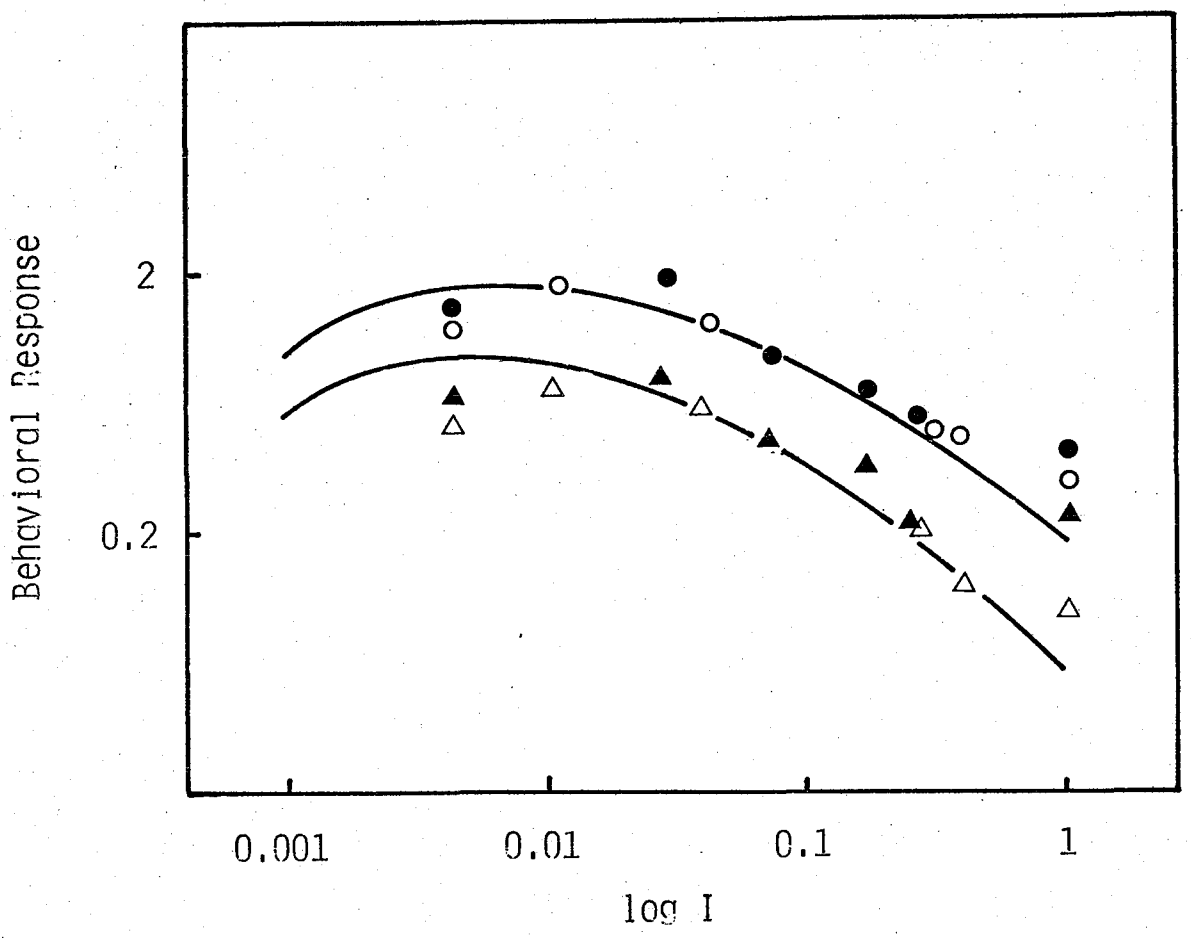

Fig. 35. Relationship between the logarithm of the power of potential fluctuation and the behavioral response.
K series at high concentration (×1) of attractant (●).
Ca series at high concentration of attracrant (○)
K series at low concentration (×1/4) of attractant (▲).
Ca series at low concentration of attractant (△)

IV. DISCUSSION

A. Behavioral Response and Fluctuation

The potential fluctuation may work as a kind of trigger for spontaneous membrane excitation in Paramecium, which, as mentioned previously, would play an important role in their swimming behaviors. If the degree of spontaneous membrane excitation were equal everywhere in the environment, the organism would accept the environment as a uniform one. A change in the degree of spontaneous excitation with a change in the environmental condition is indispensable to the tactic behavior. The sensitivity for the taxis is regulated by the sensitivity of spontaneous excitation to the environmental change. The membrane excitability would be estimated by the MRR and/or the potential difference $E_{MRR} - E_m$. A change in the degree of spontaneous excitation would be caused by a change in the power of potential fluctuation and/or a change in the membrane excitability. Here we consider that a change of the environmental condition, for example, addition of an attractant would give a perturbation to the potential fluctuation. The attractant would induce an increase of the power of fluctuation.

At large values of the ratio $[K]/[Ca]^{1/2}$ where the power of fluctuation was small, the MRR was so small that, even if the perturbation was given by the environmental change,

the level of spontaneous excitation was too small to show the tactic response. With decreasing ratio $[K]/[Ca]^{1/2}$ or with increasing power of fluctuation, the MRR increased and the tactic response increased, reaching a maximum. With further increase of the power of fluctuation, the tactic response decreased. If the environmental change or the attractant addition were assumed to cause a constant increase of the power of fluctuation, the relative increase of the power of fluctuation, that is, $\Delta I/I$ became smaller with the increase of the total power. Therefore, if not a change of the total power but a relative change of the power were effective to change spontaneous excitation, the decrease of tactic response at large values of the total power would be understandable. In the previous chapter, we derived an empirical relation between the MRR or the membrane excitability and the power of fluctuation by using the ratio $[K]/[Ca]^{1/2}$ as an environmental parameter. Here we assume a similar relation between them when an attractant was added at a constant value of the ratio $[K]/[Ca]^{1/2}$.

According to the above idea, one of the factors which represents a change of the membrane excitability due to an environmental change is given by the following function

$$S(I, \delta I) = \int_{I}^{I+\delta I} dMRR \Big| \\ = \log[(I + \delta I)/I] \qquad (26)$$

where δI is a displacement of the power I assumed to be

induced by addition of attractant.

The integration in the above equation was performed at two different values of δI, corresponding to different concentrations of the attractant. The calculated values of $S(I, \delta I)$ are shown in Fig. 36. It increased monotonously with decreasing power I. However, as shown previously, the sensitivity of the organism estimated by the behavioral response had a maximum and decreased with further decrease of the power. As discussed already, this may be owing to the limitation in the excitability itself, at large values of $[K]/[Ca]^{1/2}$. The increase of $[K]/[Ca]^{1/2}$ resulted in the decrease of the power and also in the decrease of the amount of the Ca binding sites, which was proportional to the MRR. Therefore, we introduce another factor, MRR or the number of Ca binding sites, given by

$$MRR = \beta \log I + \text{constant} = A(I) \qquad (27)$$

Then, the magnitude of the behavioral response is assumed to be proportional to the product of the function $S(I, \delta I)$ and $A(I)$.:

$$R(I, \delta I) \propto S(I, \delta I) \times A(I) \qquad (28)$$

where $R(I, I)$ is the magnitude of behavioral response. The solid line in Fig. 35 and 36 shows the calculated values of this product. The line in Fig. 35 shows good agreement with experimental data.

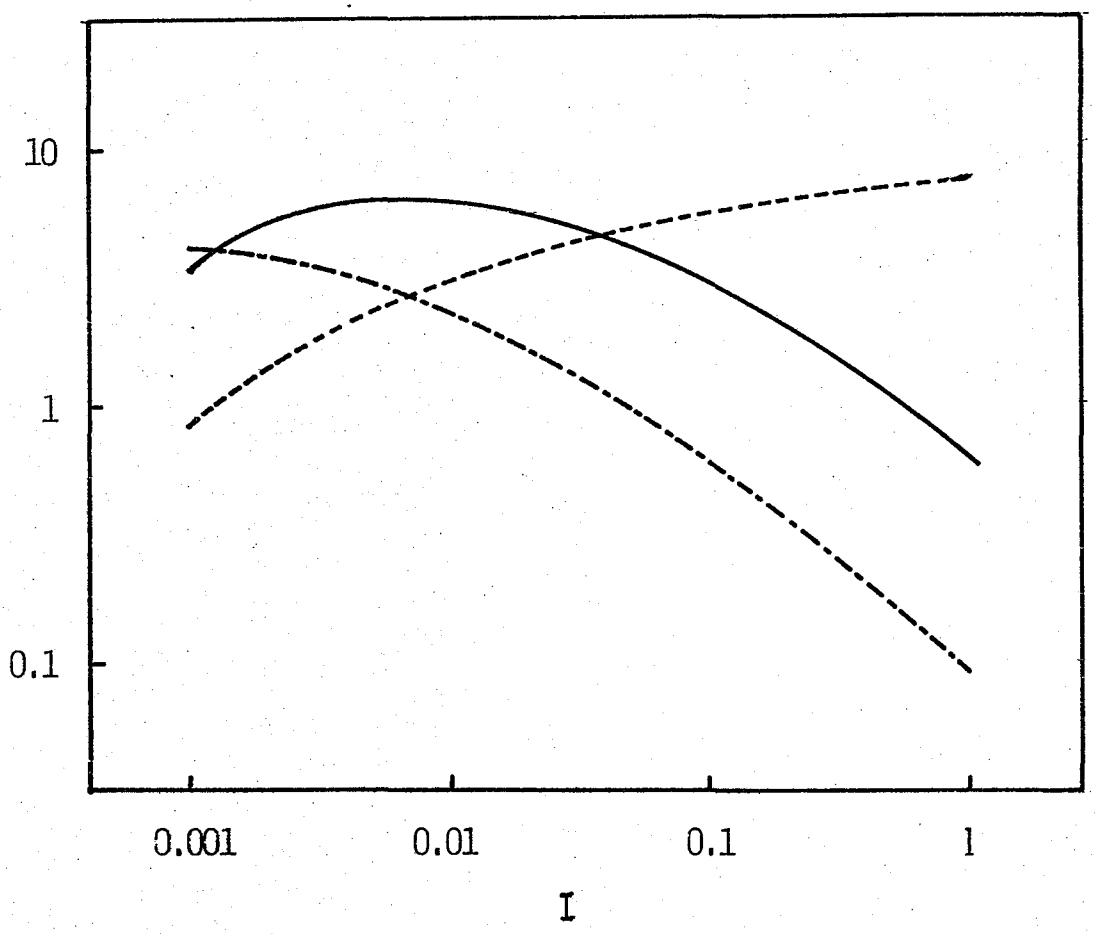

Fig. 36. The calculated values of S(I, δI) (—·—), A(I) (----) and R(I, δI) (——).

B. Behavioral Response and Motive Force

In the above discussion, we treated the potential fluctuation as a kind of input to the organism. A change in the environment was assumed to be accepted by the organism as a perturbation in the potential fluctuation. Another possibility is that the change in the environment would induce a perturbation in the potential E_m or more probably in the motive force $(E_m - E_K)$ or $(E_{Ca} - E_m)$. If the perturbation were constant, it would be possible to derive a relation between the magnitude of the response and the environmental condition represented by the ratio $[K]/[Ca]^{1/2}$, because, as shown previously, the MRR can be related to the motive force by the following approximate equation;

$$MRR = \zeta \log (E_m - E_K) + \text{constant} \tag{29}$$

where ζ is $3.2 \times \beta$ for $(E_m - E_K) > 13$ mV and $0.08 \times \beta$ for $(E_m - E_K) < 13$ mV. Therefore, a change in the motive force results in a change in the excitability;

$$\Delta MRR = \zeta \, \Delta \log (E_m - E_K) = \zeta \, \Delta E_m / (E_m - E_K) \tag{30}$$

The relation between MRR and $\log (E_m - E_K)$ is shown in Fig. 37. The slope shows a discontinuous increase at $(E_m - E_K) = 13$ mV. Below this value of $(E_m - E_K)$ the MRR is small. Above this value, the change of MRR becomes large, but at very large values of $(E_m - E_K)$ the MRR becomes insensitive to the change of $(E_m - E_K)$ again.

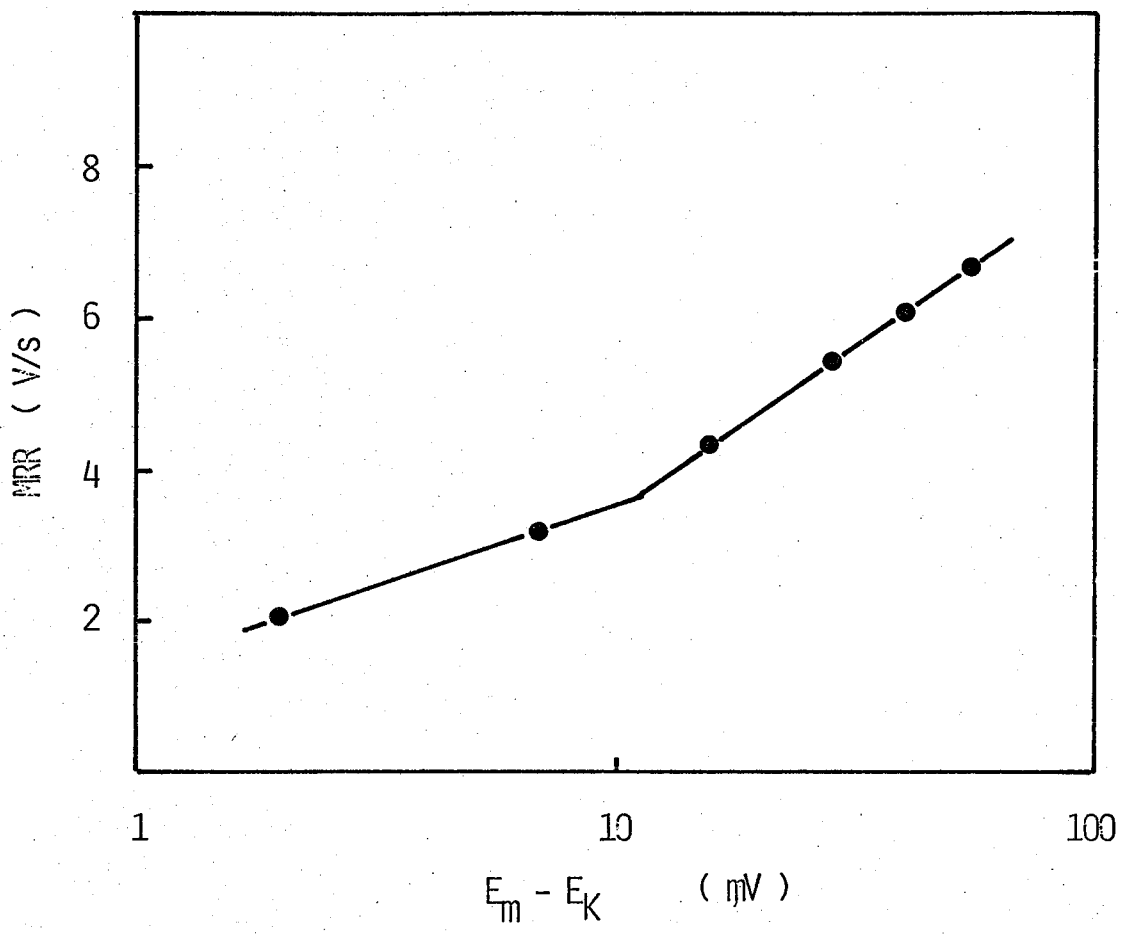

Fig. 37. Relation between the MRR and the motive force of potassium ions ($E_m - E_K$).

Concluding Remarks

Finally it must be emphasized that the value of the ratio $[K]/[Ca]^{1/2}$ where the tactic response was maximum nearly corresponded to the condition where the dependence of the potential fluctuation on the motive force showed a remarkable change. This condition was also nearby the condition where Paramecium cells were cultured. Therefore, Paramecium cells showed a maximum tactic response at the condition where they were cultured.

The fluctuation is a common phenomenon in physical systems. Various quantities are fluctuating. It is impossible for the organisms living in a physical world to be free from the fluctuation. Two ways are possible for them to deal with the fluctuation. One is to suppress the effect of fluctuation by reducing the sensitivity of the systems and the other is to use the fluctuation positively. A high threshold system corresponds to the former case. On the other hand, if the threshold of the system is settled comparable to the amplitude of fluctuation, the system is driven by fluctuation. The swimming of Paramecium under a uniform environmental condition would be the case.

Oosawa discussed the possibility of thermal fluctuation as an underlying mechanism of spontaneous fluctuation of the membrane potential and treated the subthreshold fluctuation on the basis of calculations of the effect of fluctuating

field on the state of macromolecules in the membrane [33, 34].

The excitable membrane is the system which is in non-equilibrium steady state, where the electrochemical potential difference is prevalent. It is likely that in such a biological system, the potential fluctuation is enough effective on the state of macromolecules in the membrane and amplification of fluctuation occurs.

Such spontaneous membrane depolarization induces spontaneous direction changes of swimming organisms. A relationship is found between the fluctuation and the response of the organism to the applied disturbance. In physical systems we know such a relation as the "fluctuation-dissipation theorem" which states a general relationship between the response of the given system to the external disturbance and the internal fluctuation of the system in the absence of the disturbance [35]. In biological systems the fluctuation plays a more important role than in physical (non-living) systems. Living organisms would keep the potential fluctuation at an appropriate level and accept an environmental change as a disturbance on the fluctuation. The response seems to be determined by the fluctuation and its change.

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