STUDY ON CYCLOSIS OF PARAMECIUM (1)

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Introduction

The cyclosis of Paramecium was discovered in 1836, in *P. bursaria* by FOCKE, and then the existence of cyclosis in *P. caudatum* or *P. aurelia* was brought out clearly. But in spite of the old discovery, considerably few observations on this phenomenon were reported, and at present there are the reports of observations performed by WALLENGREN (1902), NIERENSTEIN (1905), BILLS (1922), and HOSOI (1936). The interesting fact on motive force of the cyclosis of *paramecium* was reported by HOSOI in 1937.

A quite convenient method to stop the *P. rameciium* was discovered by BILLS in 1922. This BILLS' narcotizing method by isopropyl alcohol was very effective to stop *Paramecium* without injuring the endoplasm, and in HOSOI's experiment on cyclosis of *Paramecium*, a method based on the BILLs' narcotizing method was used.

According to the observations carried out before, cyclosis of *P caudatum* is as follows. If one assumes that the side where the oral groove is present is the "ventral" of *Paramecium*, the cyclosis flows counter-clock wise when *Paramecium* is observed from ventral side. It flows to anterior direction in left of the body, and to posterior direction in right of the body. It occupies almost the whole space of endoplasm, and the boundary between the streaming and the rest is obscure. In side view, cyclosis flows to be ∞-shape, because it flows along the inner surface of oral groove at the left anterior part, then flows along the inner surface of oral fold, then streaming removes to the dorsal portion of the body at cytopharynx, at last it removes to the ventral portion of the body at posterior end of the body. Any reversal of the direction of cyclosis has not been observed yet.

This is one of the papers on cyclosis of *Paramecium*, in which the effects of the body-length, temperature and concentration of external medium, and K-, Na-, Ca- and Mg- ions in the medium were descrived and discussed.

Material and Method

Paramecium was the *caudatum* type which was obtained from the hay culture, and their body-length were between 160μ and 210μ. Before

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the observations they were made to adapt to the test solutions for 18 to 20 hours after being washed in 100 volumes of redistilled water to one volume of culture medium. The pH of the test solutions were kept between 7.1 and 7.3 by addition of $NaHCO_3$. At the observation of cyclosis, either of the following two methods was used to stop the *Paramecium*: a narcotizing method by isopropyl alcohol vapour, which was used by HOSOI, and a new method in which viscous medium, 1% tragacantha solution, was used together with the narcotizing by isobropyl alcohol vapour. By using the latter, a good result was obtained.

The velocity of cyclosis was obtained by measuring the time required for the granules in cyclosis to flow for 15μ near of oral groove of *Paramecium.*

Observations and Discussion

1) Effect of the body-length of *Paramecium* on cyclosis.

The result of the measurement of velocity of cyclosis of *Paramecium* which had the body-length of from 160μ to 210μ, was indicated in table 1 and fig. 1. The test solution; pH 7.3 of artificial sea water was used, and the temperature was 26°C. The concentration of the test solution was equivalent to 0.02 M NaCl solution.

Table 1
The body-length on cyclosis of *Paramecium.*

body-length	Velocity of cyclosis
160-170μ	2.9μ/s
170-180	2.9
180-190	3.0
190-200	3.0
200-210	2.9

Remarks: Temperature 26°C
Solution 0.02 Mol artificial sea water pH=7.3.
Adaptation 20 hours.

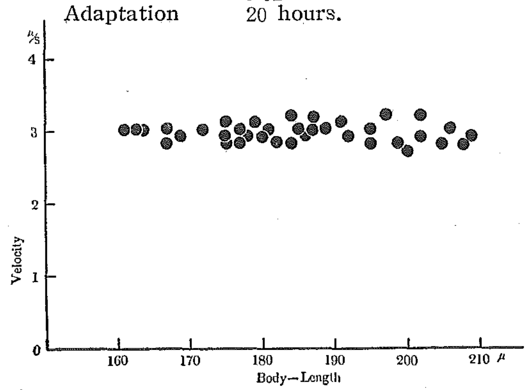

Fig. 1 The body-length on cyclosis of *Paramecium.*
The velocity of cyclosis and the body-length were indicated on the ordinate and the absissa of the graph respectively.
Each circle represents the velocity.

In fig. 1, the velocity of cyclosis and the body-length were indicated on the ordinate and on the absissa of the graph respectively. Each circle on this graph represents the velocity of cyclosis. In these table and fig., it is apparent that the velocity of cyclosis is not concerned with the body-length of *Paramecium.*

2) Effect of the medium temperature on cyclosis.

The velocity of cyclosis was investigated by changing the temperature. The medium concentration of artificial sea water was equivalent to 0.01 M NaCl and its pH was 7.3. The time of adaptation was 20 hours. The observation was started 5 minutes after the temperature was changed. The *Paramecium* was observed at higher temperature firstly, and, after the observation, at lower temperature, was again observed at the same temperature with the first observation. And the data were employed only when the velocity of cyclosis at last observation was equal as the first value. The result is indicated in fig. 2. In this figure, the velocity of cyclosis is indicated on the ordinate and the temperature on the absissa of the graph respectively, and each circle represents the mean value.

As is apparent in fig. 2, the velocity of cyclosis changes linearly with the change of the medium temperature, and if the velocity is indicated by Vμ/s, and the temperature is by t° C, the relation between the velocity and the temperature is represented by the following formula, *i. e.* V=at+b, where a=0.1, b=0.7, between 5° C and 30° C. The velocity-change by temperature-change in the medium was reversible in most *Paramecium.* The relation of the velocity with the temperature below 5° C was expected very interesting, but it could not investigated from lack of a suitable apparatus.

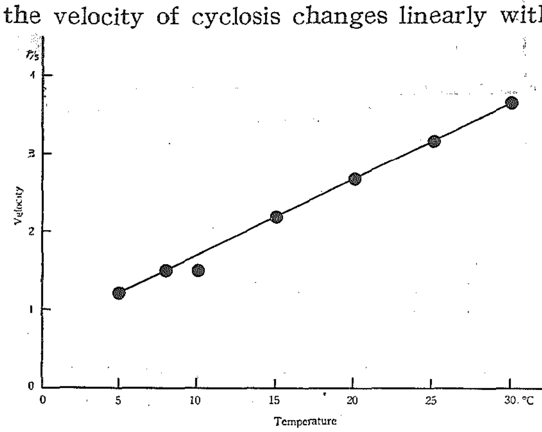

Fig. 2 Effect of the medium temperature on cyclosis.
The velocity of cyclosis is indicated on ordinate and the medium temperature on absissa. Each circle represents the mean value.

3) Effect of the concentration of medium on cyclosis.

It was expected that the velocity of cyclosis must be affected greatly by the concentration of the external medium, for the velocity of cyclosis is affected by both of the change of motive force and the change of the viscosity of protoplasm, and then the viscosity of protoplasm changes with the change of the colloidal state of protoplasm, and the colloidal state of protoplasm is affected by the osmotic pressure of the medium. Hence, an investigation of the relation between the velocity and the concentration of the medium was carried out.

Paramecia were adapted for 20 hours in the test solutions. Various concentrations of artificial sea water were used, their pH being set at 7.3 by adding $NaHCO_3$, and their temperature at 26° C. The result is indicated in fig. 3. The ordinate indicates the velocity and the absissa the concentration of medium. The concentration of the medium is shown as the mol-concentration equivalent with NaCl solution. And each circle in this figure represents the mean value of the velocity of cyclosis.

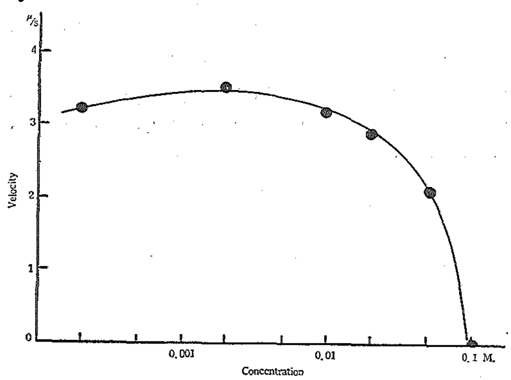

Fig. 3 Effect of medium concentration on cyclosis.
The ordinate indicates the velocity and the absissa the medium concentration. The medium concentration is shown as the mol-concentration equivalent with NaCl. And each circle represents the mean value of cyclosis.

In this figure, the velocity decreases slowly in both sides of 0.002 Mol, and in the medium concentrated more than 0.02 Mol, the curve drops in considerable rapidity, and at last in the medium concentrated more than 0.1 Mol the cyclosis disappears completely.

It is said that the osmotic pressure of protoplasm of *Paramecium* is almost equal to that of 0.05 Mol or 0.1 Mol Nacl solution. If this is true, it can be said that the cyclosis of *Paramecium* disappears in the hypertonic external solution, in other word, cyclosis appears only in the hypotonic enternal solutions. This fact suggests that cyclosis of *Paramecium* requires the entrance of water into protoplasm from external medium. The requirement of entrance of water into protoplasm from the vacuole or external medium in the protoplasmic streaming of *Nitella* has been discussed by OSTERHOUT in 1952. It is very interesing with the case of the protoplasmic streaming connected that the cyclosis of *Paramecium* appears in the hypotonic solutions in which the entrance of water into protoplasm may take place.

4) Effect of Na-, K-, Mg- and Ca-ions on cyclosis.

It has been well known that various ions in which K-, Na-, Mg- and Ca-ions are contained, affect protoplasm. The effect that these ions given to the cyclosis when *Paramecium* is kept in the medium containing K, Na, Mg or Ca ion in various concentrations was investigated. The effect of each ion was observed by making the solution in which two ions were contained in various ratios. Every test solution had a constant pH, 7.1 or 7.2, and a constant concentration, equivalent to 0.01 M NaCl-solution. The temperature at observation was 20° C, and the time of adaptation of *Paramecium* was between 18 and 20 hours. *Paramecium* was stopped by a narcotizing method where the viscous medium was used with isopropyl alcohol vapour. Every observation was started after 30 minutes from narcotizing treatment.

The results are demonstrated in the following tables. Table 2, table 3, table 4, table 5 and table 6 indicate the results in the series of NaCl-KCI mixtures, NaCl-$CaCl_2$ mixtures, NaCl-$MgCl_2$ mixtures, KCl-$CaCl_2$ mixtures and KCl-$MgCl_2$mixtures respectively.

Table 2
Velocity of cyclosis in NaCl-KClmixtures.

log [Na˙]/[K˙]	velocity	variance	number of observations
−∞	0 μ/s		15
−4	0		15
−3.65	1.5	0.030	10
−3.4	1.5	0.012	10
−3	1.8	0.0925	10
−2	2.5	0.075	10
−1	2.9	0.055	10
0	2.7	0.104	9
1	3.0	0.045	10
2	2.9	0.023	10
3	3.0	0.033	10
4	2.9	0.045	10
5	3.1	0.025	10
∞	3.2	0.082	9

Table 3
Velocity of cyclosis in NaCl-$CaCl_2$mixtures.

log [Na˙]/[Ca˙˙]	velocity	variance	number of observations
−∞	2.7μ/s	0.024	10
−3	2.5	0.033	10
−2	2.3	0.030	6
−1	2.3	0.0478	13
0	2.3	0.0480	9
1	2.4	0.0290	10
2	2.7	0.0720	10
3	2.5	0.034	10
∞	3.3	0.062	10

Table 4

Velocity of cyclosis in NaCl-$MgCl_2$mixtures.

log [$Na^{\cdot}$]/[$Mg^{\cdot\cdot}$]	velocity	variance	number of observations
$-\infty$	2.8 μ/s	0.0625	9
−5	2.7	0.046	10
−4	2.2	0.033	10
−3	2.3	0.0223	9
−2	2.4	0.013	10
−1	2.7	0.079	10
0	2.8	0.043	10
1	2.9	0.071	10
2	2.8	0.073	10
3	2.8	0.027	10
4	3.2	0.020	11
5	3.2	0.019	6
∞	2.8	0.0976	8

Table 5

Velocity of cyclosis in KCl-$CaCl_2$mixtures.

log [$K^{\cdot}$]/[$Ca^{\cdot\cdot}$]	velocity	variance	number of observations
$-\infty$	2.2 μ/s	0.0225	10
−4	2.3	0.0155	10
−3	2.2	0.0133	10
−2	2.3	0.0175	10
−1	2.2	0.0235	10
0	2.0	0.0175	10
1	2.6	0.0100	10
2	2.4	0.0133	10
3	1.3	0.0135	10
3.4	1.4	0.0100	10
3.7	1.3	0.0260	10
4	0		10
∞	0		10

Table 6

Velocity of cyclosis in KCl-$MgCl_2$mixtures.

log [$K^{\cdot}$]/[$Mg^{\cdot\cdot}$]	velocity	variance	number of observations
$-\infty$	2.7 μ/s	0.073	9
−5	2.3	0.060	6
−4	2.3	0.032	10
−3	2.0	0.049	9
−2	2.7	0.138	10
−1	2.8	0.123	10
0	2.9	0.050	10
1	2.4	0.050	9
2	2.5	0.068	10
3	1.7	0.065	10
3.4	1.5	0.013	10
3.7	1.3	0.0367	10
4	0		10
∞	0		10

In figure 4, the results obtained in the series of NaCl-KCl, KCl-$CaCl_2$ and KCl-$MgCl_2$ mixtures were drawn in one graph. The velocity of cyclosis is indicated on ordinate, and the values of logalithm of the ratio of K-ion concentration to the ionic concentrations of the others are indicated on absissa of the graph. Each of the symbols represents each of the results obtained in these observations, *i. e.* circle represents the velocity of cyclosis in NaCl-KCl mixtures, cross the velocity in KCl-$CaCl_2$ mixtures and triangle the velocity in KCl-$MgCl_2$ mixtures. Each value of the figure represents the mean value of the velocity.

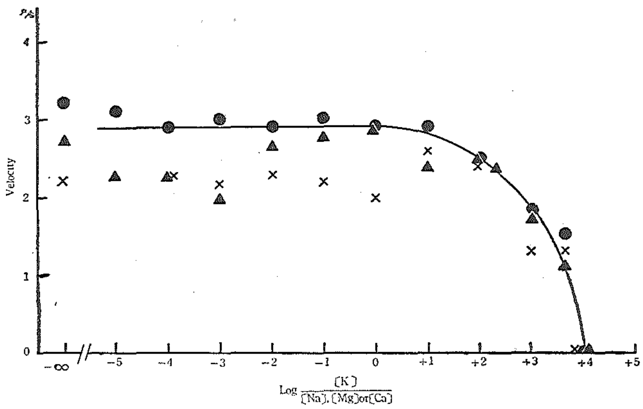

Fig. 4 Effect of Na-, K-, Mg- and Ca-ions on cyclosis.
The velocity is indicated on ordinate, and the values of logalithm of the retio of K-ion concentration to the ionic concentrations of the others are indicated on absissa. Each of the symbols represents each of the results obtained in these observations; circle represents the velocity in NaCl-KCl mixtures, cross the velocity in KCl-$CaCl_2$ and triangle the velocity in KCl-$MgCl_2$ mixtures. Each value represents the mean value of the velocity.

If the facts in these tables and figure were noted, it is obvious that in the mixtures containing much volume of K-ion the cyclosis disappears. Moreover, it is clear that three curves in figure 4 show the same tendency in higher K-ion concentration. In the other ions, however, no remarkable effect given to cyclosis is to be seen.

When the cyclosis disappeared in the K-ion-rich solutions, it appears normally again when Na ion is added to the solutions, except the time after the disappearance is so long. The same effect is also obtained by the addition of the rest of ions. Thus, it is clear that the disappearance of cyclosis by K-ion is reversible. The cause of this disappearance of cyclosis cannot be attributed to such a fact that the coagulation of protoplasm occurs by K-ion, for the fluidity of protoplasm can be recongnized in such a condition.

If the effect of an external medium on protoplasm is discussed, the permeability of protoplasm must be considered in each case. According to AKITA's data, when *Paramecium* was immersed to the medium in which KCl and NaCl were contained in various ratio of concentrations, K-ion content of the cell was always kept over the definite value, and if the ratio of K-ion to Na-ion became larger than the definite value K-ion content of the cell increased repidly, while Na-ion content of the cell decreased reciprocally, because an ionic exchange between Na-ion in the cell and K-ion in the medium occurred. The fact that K-ion in external medium was more permeable than Na-ion, was also indicated in his data. Thus, it is easy to consider that a large amount of K-ion exchanges to Na-ion in the cell, so that the nature of protoplasm changes greatly, to affect the cyclosis greatly, when the ratio of K-ion concentration to Na-ion concentration of the external solution is large. But in the reciprocal condition, it is considered that the cyclosis is not affected by the external medium, because the nature of protoplasm does not change so greatly, for the ionic ratio of protoplasm does not change greatly. According to HEILBRUNN, the permeability of protoplasm to K-ion or Na-ion is considerably larger than the permeability to Mg or Ca-ion. Hence, it can be expected that Ca-ion or Mg-ion in the external solution is less ready to enter into the protoplasm than Na-ion, so that the ionic change in external medium by the former ion is less ready to introduce the change of protoplasm.

Another fact in the figure and the tables is that in the solution containing $CaCl_2$, the cyclosis shows the lower velocity than in the other solutions. This phenomenon seems to occur by a decrease of permeability of protoplasm, because the decrease of permeability of ectoplasm occurs by Ca-ion in the solution. In the result of the observation of the velocity of cyclosis in a series of NaCl-$CaCl_2$ mixtures, an unexpected result that the cyclosis gets more rapidity in Ca-free solution than the others, was obtained. But this result may be explained by the effect of Ca-ion on the permeability, that Ca-ion sets the ectoplasm in a less permeable condition. The lack of Ca-ion in external medium increases the permeability of ectoplasm of *Paramecium*, but Na-ion in external medium is kept still almost impermeable, hence the nature of protoplasm does not change except the entrance of water increases, and here the increase of the velocity of cyclosis occurs. If a considerable amount of K-ion is contained in the external solution, the lack of Ca-ion in the solution brings a change of the nature of protoplasm, for the K-ion of the solution exchanges with the ions in the protoplasm. Thus, the cyclosis disappears in the Ca-lack solution in which a considerable amount of K-ion is contained.

In closing this paper, I wish to thank Prof. YUITIRO IKEDA, and Prof. HARUO KINOSITA of Tokyo University, for their guidances and encouragements throughout this work.

Summary

The effects of some factors on cyclosis of *Paramecium* was observed. Those factors were the body-length of *Paramecium*, the temperature of

the test solution, the concentration of the external medium and the Na-, K-, Ca-, and Mg-ion in the external medium.

In *Paramecium,* of which body-length is between 160μ and 210μ, the velocity of cyclosis was not concerned with the body-length.

The velocity change of cyclosis occurred in proportion to that of the temperature of test solution as far as the temperature was between 5°C and 30°C. These changes of the velocity by temperature-changes were reversible in most cases.

The cyclosis disappeared in the hypertonic medium, and it appeared in the hypotonic medium. This fact suggests that the cyclosis requires the entrance of water into protoplasm.

The increase of K-ion and the simultaneous decrease of the other cations in the external medium made the cyclosis disappear. This disappearance of cyclosis was generally reversible, and it recovered by the increase of Na-, Ca- or Mg-ion. The effect of the change of the rest of the cations was almost negligible. In the explanation of the actions of these cations in the external medium, it will be considered how the permeability of protoplasm plays a rôle to the ions and how the permeablility of protoplasm is changed by the ions.

Literature

AKITA, Y. Mem. of the Fac. of Sci. and Agri. Taihoku Imp. Univ. Formosa, Japan, Vol. 23: p.99, 1941.
BILLS, C. E. Biol. Bull. Vol. 42: p.7, 1922.
HEILBRUNN, L. V. An Outline of General Physiology. 3rd ed: p.150, 1952.
HOSOI, T. Zool Mag. Vol. 48: p.469. 1936.
HOSOI, T. J. Fac. Sci. Tokyo Imp. Univ. Sec. 4. Zool. Vol. 4: p. 299 1937.
NIRENTEIN, E. Zeits. allg. Physiol. Bd. 5: s. 435, 1905.
OSTERHOUT, W. J. V. J. Gen. Physiol. Vol. 35: p.519, 1952.
WALLENGREN, H. Zeits. allg. Physiol. Bd. I, s. 67, 1902.