Chemotactic Response of *Paramecium caudatum*[1)]

By

Isamu Nakatani

Zoological Institute, Hokkaido University

(*With 8 Text-figures*)

Jennings (1899) and more recently Dryl (1959, a, b) studied the chemotaxis of *Paramecium* by placing a drop of test solution on a glass plate containing *Paramecium* suspension. In this method the test solution is diluted by surrounding fluid and the reaction to correct concentration of test solution can not be known. In the present paper, chemotactic responses of *Paramecium caudatum* were studied by means of glass capillary filled with test solutions, one end of which was placed in the trough containing *Paramecium* suspension and the other end of which was out of the suspension. By this method the reaction of the animals was clearly demonstrated even to lower test solution, and the density of *Paramecium* in the test solution was measured quantitatively.

Although the balanced experimental solution for *Paramecium* has not been established, the culture medium (Jennings, 1899) or tap water (Dryl, 1959, a, b) has been used as an adaptation medium and the control solution. In the present investigation, a balanced salts solution was used as the adaptation medium and the control solution.

The present investigation was undertaken to examine the reaction of *Paramecium caudatum* to various chemicals and the mechanisms involved in it from point of view of the chemotactic behaviour of this animal, and the biological significance of chemotactic response was discussed.

The terms negative and positive chemotaxis or chemotactic response in the present paper are of the same meanings with those defined by Jennings (1899).

Material and Methods: *Paramecium caudatum*, cultured with the vegetable powder infusion, was used as the experimental material. *Paramecia* were washed well by the adaptation medium (3.3 mM NaCl, 0.4 mM $CaCl_2$ and 1.0 mM tris, or 2.2 mM $CaCl_2$ and 1.0 mM tris, pH=7.2) and kept in the medium for more than 24 hours before experiment. After adaptation about 4 ml of *Paramecium* suspension (the density of the animals is about 5000/ml) were poured in a trough (65 mm × 40 mm × 5 mm).

Five glass capillaries (about 0.6 mm in diameter and about 45 mm in length) were filled with the test solution at various concentrations, and the other one was filled with the adaptation medium as the control. Then one end of capillaries was placed in the above

1) Contribution No. 826 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 16, 1968.

mentioned trough and another end was out of the fluid. The end of capillaries in the trough separated enough from each other, but another end was gathered and approached, and arranged in parallel in order to observe under a trinocular stereoscopic microscope Olympus X-TR) and to take the micro-photograph of capillaries at the same time as is shown in Fig. 1. The micro-photographs were taken under the condition where *Paramecia* in the capillary appeared as bright dots on a black back-ground.

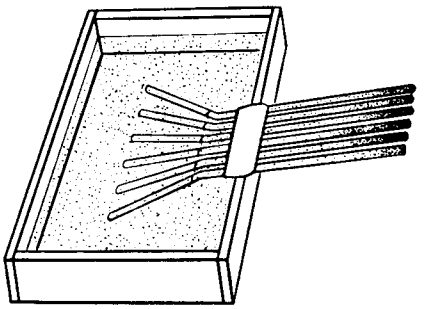

Fig. 1. The trough and the glass capillaries used for the experiments.

As a quantitative method for investigating chemotactic response, the densitometry of the animals gathered in the capillary was carried out. The negative microphotographic film was illuminated by light through a slit, the width of which is approximately same as that of image of the capillary on the film, the light through the film recepted by a photo-transistor and recorded by Kipp & Zonen micrograph (sensitivity: the movement of recording pen 21 cm corresponds to 0.1 microampere, and the pen movement on the recording paper was 0.4 to 1.0 mm by difference of 1% of the *Paramecium* density).

After an interval of five minutes since the end of capillaries was transfered into the trough, the first photograph was taken. Subsequent photographs were taken at 5 or 10 minutes intervals, and the film which exhibited the maximum response was used for the densitometry. The same experiment was repeated five times, and the results were expressed by those mean values.

The chemicals were dissolved in redistilled water and bring its osmotic pressure to the same one of the adaptation medium, and further dilution of the chemicals was made by the adaptation medium.

During the experiment, the trough and capillaries were kept in the dark box, excepting light illumination for the microphotographic records.

All experiments were carried out at room temperature (21–23°C).

Results

At first in the present experiments, the most suitable adaptation medium for *Paramecium caudatum* was searched. Yamaguchi (1963) proposed a balanced salt solution having the following composition as the basic experimental solution for *Paramecium caudatum*; M/2000 $NaHCO_3$, M/300 KCl and M/2500 $CaCl_2$ in one

litre of H_2O, since the hay infusion used for culture of the animals contains those salts of the same quantity.

The test of the effect of adaptation medium was made on the survival of *Paramecium*. The animals were kept in following three kinds of isotonic salts solutions without any nutritive substance: (1) 3.3 mM KCl and 0.4 mM $CaCl_2$ in one litre of redistilled water, pH was adjusted to 7.2 by 1.0 mM tris buffer, (2) 3.3 mM NaCl, 0.4 mM $CaCl_2$ and 1.0 mM tris, (3) 2.2 mM $CaCl_2$ and 1.0 mM tris.

The all animals in the solution of (1) died within a half of month, and the animals in the solutions of (2) and (3) survived longer than one month.

Kinosita *et al.* (1964) found that the membrane potential of *Paramecium* was more increased in the mixture of 2 mM Na and 2 mM Ca solution than in other solutions. From these facts, the solutions of (2) was adopted as the adaptation medium for *Paramecium*. As above mentioned, the medium satisfies the physiological condition for the animals. But the chemotactic response of the animals to the medium is less than to the culture medium, since the medium does not contain organic substances which were contained in the culture medium. And this medium was convenient for ranking the sensitivities of chemotactic responses of the animals to many chemicals. Also the solution of (3) was used as the adaptation medium in comparison of the chemotactic responses of the animals.

In the case of positive chemotaxis, the animals near the lower end of the

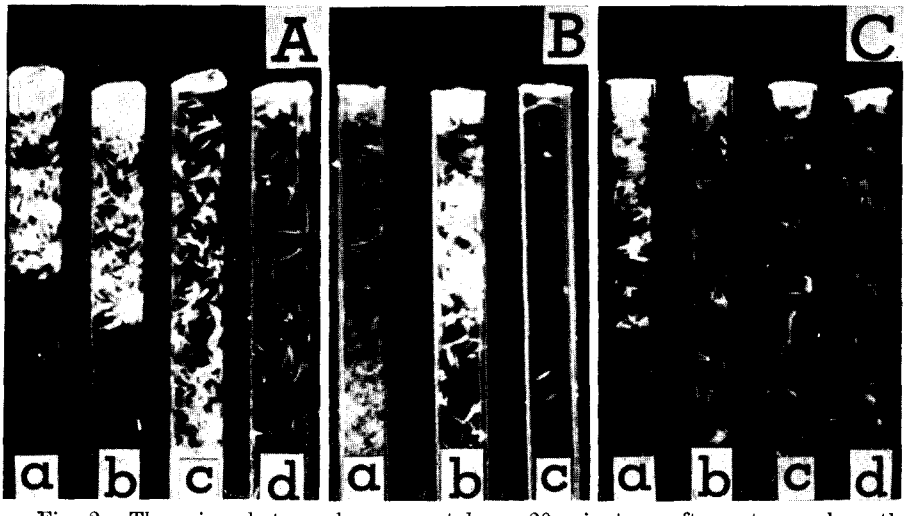

Fig. 2. The microphotographs were taken 20 minutes after set-up, show the chemotactic responses of *Paramecium caudatum* to some solutions. A. a: 0.28% starch, b: 0.067% milk, c: 0.16 mM acetic acid, d: the control. B. a, b: diluted India ink, c: the control. C. a: 0.5% polyvinylpyrrolidone, b: 0.1% polyvinylpyrrolidone, c: 0.05% polyvinylpyrrolidone, d: the control.

glass capillary inserted in *Paramecium* suspension, entered into the capillary without avoiding reaction, swam to its upper end, many of them gathered there and the other swam around in the capillary. In the case of negative chemotaxis the animals avoided the test solution at the lower end of capillary, even though they entered into the capillary, they changed the swimming direction in it and went away from there.

The density of animals in the test solution expressed in percentage with reference to the control (the adaptation medium), especially in the case of positive chemotaxis, reached a maximum value and then lowered. The decay after the maximum was due to the following facts; when the animals too crowded in the capillary, the physiological condition became to be unfavourable for them, the crowded animals dispersed away from the capillary (see Fig. 2, A, c); the density of animals in the control became to be higher slowly with time, reached the maximum, and again lowered. The time course was different between the responses to the test solution and the control solution.

A. Paramecia adapted to the mixture of 3.3 mM NaCl, 0.4 mM $CaCl_2$ and 1.0 mM tris solution.

(1) Inorganic ions. According to Kinosita *et al.* (1964), the swimming velocity of *Paramecium* increases in the solution of 2 mM $CaCl_2$ and decreases in one of 2 mM KCl, further Ba-ions inhibits strongly the ciliary activity of these animals. And it is known that NH_4-ions induce the ciliary reversal (Mast and Nadler, 1952; Oliphant, 1938).

The solutions of KCl, NH_4Cl, $CaCl_2$ and $BaCl_2$ which related closely with ciliary activity as above mentioned, were used as the test solution. *Paramecia* gathered in the solution of 2.2 mM $CaCl_2$. When solution was diluted with the adaptation medium to 1.65 mM *Paramecia* more gathered in it. The animals avoided the solution of 3.3 mM KCl, but they gathered in KCl solution when it was diluted with the adaptation medium to 1.65 mM. These facts mean that response was positively increased by co-existence of Ca- or Na-ions. This tendency was observed in many other solutions. The animals avoided the solution of 3.3 mM NH_4Cl, but they entered in the solution of lower concentration. However, the response to diluted NH_4Cl solution could not be positive taxis. To $BaCl_2$ solution, the animals responded strongly with negative chemotactic reaction and they did not entered at all even in the solution of lower concentration (0.016 mM) which contains more amounts of Na and Ca than that of Ba. These results are shown in Fig. 3.

(2) Organic ions. It has been well known that *Paramecium* exhibit positively chemotactic response to acid solution (Jennings, 1906; Dryl, 1959, a). In the present experiments, *Paramecia* responded with positive taxis to the solutions of acetic acid at lower concentration than 0.33 mM (0.002%), and this concentration is lower than that (0.02%) reported by Jennings (1906). The

animals avoided surrounding medium of the capillary end in the *Paramecia* suspension, when the capillary filled with 3.3 mM acetic acid solution. This fact due to the animals avoided the solution dispersing from the capillary.

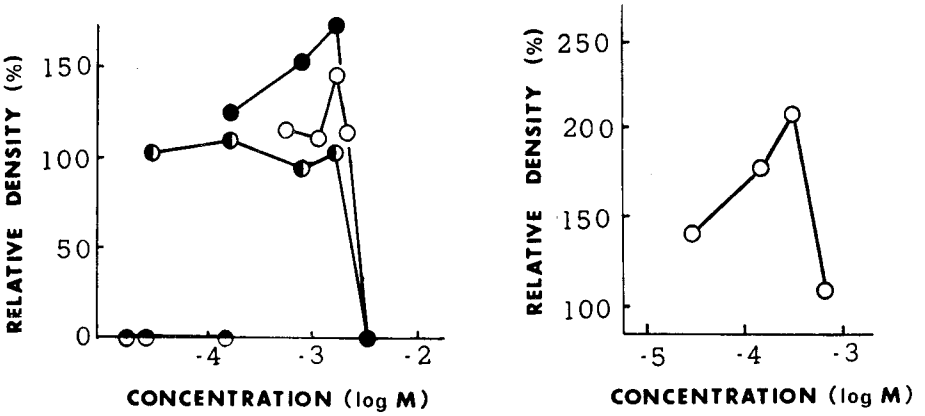

Fig. 3. The density of *Paramecia* in the test solution expressed in percentage of the control value. Ordinate: percent of *Paramecia* density. Abscissa: molar concentration of the test solution in logarithmic scale. ●: KCl, ◐: NH_4Cl, ◓: $BaCl_2$, ○: $CaCl_2$.

Fig. 4. Chemotactic response of *Paramecium* to acetic acid solution. Ordinate: *Paramecia* density per cent of the control value. Abscissa: molar concentration of the test solution in logarithmic scale.

The solution of 0.33 mM acetic acid had the value of pH=5.7, which agrees with the optimum chemotactic pH range for *Paramecium caudatum* reported by Dryl (1959, a). The result is shown in Fig. 4.

(3) Solutions containing colloidal particles. According to Mueller *et al* (1965) *Paramecium multimicronucleatum* ingest non-nutritive materials as polystyrene latex particles, as same as ingest bacteria which are usual food for the animals. It is quite possible that *Paramecium* gather to small particles as bacteria. From these reasons, the taxic responses to the solutions of milk, starch, polyvinylpyrrolidone and India ink which contain colloidal particles, were examined.

The concentration of isotonic solutions of starch and milk with 3.3 mM NaCl solution were 0.275 % and 0.073% respectively, which were determined by Barger's method (1904). To the solutions of 0.275% starch and 0.073% milk, *Paramecia* responded with positive taxis. The response to starch solution was particularly strong, and in the case of 0.14% solution the response of high percentage as nearly 300 was obtained. Many *Paramecia* gathered to diluted India ink and 0.05 to 0.5% polyvinylpyrrolidone solution before the animals gather to the control as is shown in Fig. 2.

The response to milk is not only due to suspending particles, because it containing many kinds of ions such as Ca, K, etc. From the present results,

it can be thought that *Paramecia* gather to colloidal particles. This nature of *Paramecia* may have an important significance for uptake of their food, because their usual food is small particles such as bacteria or other organic particles.

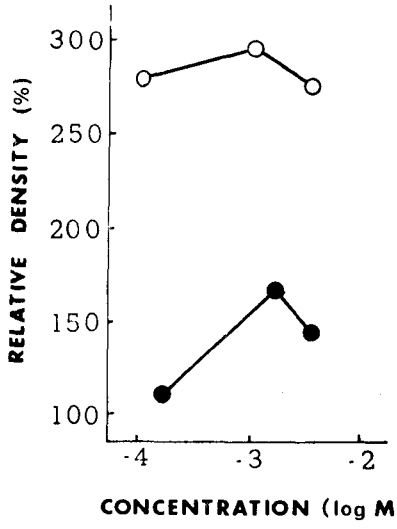

Fig. 5. Chemotactic response of *Paramecium* to the solutions of milk and starch. Ordinate: *Paramecia* density per cent of the control. Abscissa: molar concentration of the test solution in logarithmic scale. ○: starch, ●: milk.

(4) Non-ionic substance not containing colloidal particles. It has been known that *Paramecia* enter into sugar solutions having the osmotic pressure many times higher than that of the normal solution, and they swim into the 20 percent sugar solution without avoiding reaction (Jennings, 1906). Goldschmied-Hermann (1935) reported that *Paramecium caudatum* introduced into a drop of 1 or 3 percent solution of ethyl alcohol gathered after a few minutes on the periphery of the drop. The fact that *Paramecium* avoids the central area of the drop, suggests rather than ethyl alcohol itself. On the other hand, Dryl (1959, b) reported that *Paramecium caudatum* exhibited a negative chemotactic reaction with regard to all kinds of lower alcohols. In his experiments too hypertonic solutions for *Paramecium*, 400 to 700 mM solutions of ethyl alcohol were used.

In the present experiments, the chemotactic responses of the animal to saccharose and ethyl alcohol were examined in an isotonic solution with the adaptation medium. Only few animals entered into 3.3 mM saccharose solution. But when the solution was diluted by the adaptation medium to 3.0 mM, the animals responded remarkably with the positive taxis to it. This suggests the solution of saccharose must contain a small amounts of Ca or Na for the animal

to respond positively. The solution was more diluted, the value in percentage of *Paramecia* density in the diluted solution decreased and approached to 100.

On the other hand, to 3.3 mM ethyl alcohol solution which does not contain Na- and Ca-ions a distinct positive chemotactic reaction was found. And in 3.0 mM solution of ethyl alcohol, the response was more increased. It is clear that the gathered animals in the solution was never anaesthetized by alcohol, since the active swimming of the animals was observed in the capillary filled with the solution. These results are shown in Fig. 6.

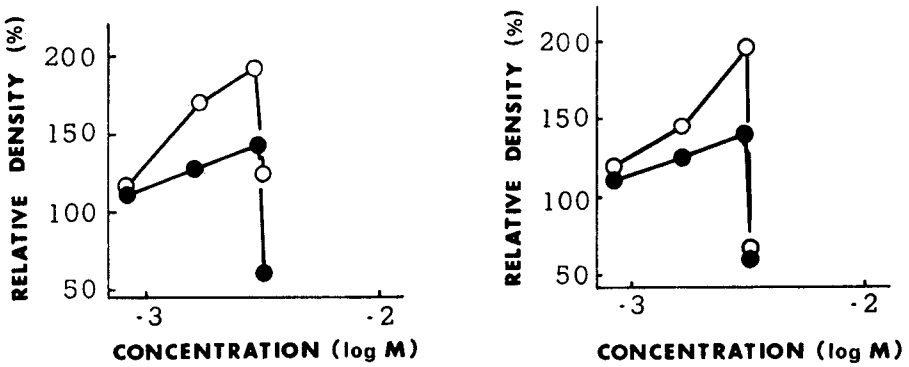

Fig. 6. Chemotactic response of *Paramecium* to the solutions of saccharose and ethyl alcohol. ○: ethyl alcohol, ●: saccharose. Ordinate: *Paramecia* density per cent of the control value. Abscissa: molar concentration of the test solution in logarithmic scale.

Fig. 7. Effect of the adaptation medium on the chemotactic response of *Paramecium* to saccharose solution. ●: the result of the adaptation to mixture of 3.0 mM NaCl, 0.4 mM $CaCl_2$ and 1.0 mM tris solution. ○: the result of the adaptation to mixture of 2.2 mM Ca Cl_2 and 1.0 mM tris solution. Ordinate: *Paramecia* density per cent of the control value. Abscissa: molar concentration of the test solution in logarithmic scale.

B. Paramecia adapted to the mixture of 2.2 mM $CaCl_2$ and 1.0 mM tris solution.

From the above experiments, it is suggested that the chemotactic response of *Paramecium* relates closely to its ciliary activity. It was known that the swimming velocity of *Paramecium* increase in the solution containing 2 mM Ca (Kinosita *et al*, 1964).

The case which 2.2 mM $CaCl_2$ solution was used as an adaptation medium and as a control, was examined and it is confirmed that *Paramecium* adapted even to such a solution shows clearly a chemotactic response.

The chemotactic response to ethyl alcohol solution is essentially the same as that in the case which *Paramecia* were adapted to the solution of NaCl-$CaCl_2$. On the other hand, the cases of solutions of saccharose and acetic acid show more positive response than the case adapted to the NaCl-$CaCl_2$ solution (see Fig. 7 and

8). It is due to the following reason; when *Paramecia* were adapted to $CaCl_2$ solution for long time, the animals swarmed each other with a thigmotactic response in the adaptation medium and comparatively small number of animals entered

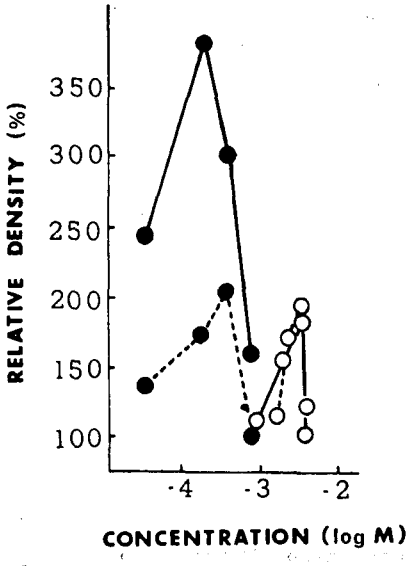

Fig. 8. Effect of the adaptation medium on the chemotactic response of *Paramecium* to the solutions of acetic acid and ethyl alcohol. Broken lines: mixture of 3.3 mM NaCl, 0.4 mM $CaCl_2$ and 1.0 mM tris solution. Solid lines: mixture of 2.2 mM $CaCl_2$ and 1.0 mM tris solution. ●: acetic acid, ○: ethyl alcohol. Ordinate: *Paramecia* density per cent of the control value, Abscissa: molar concentration of the test solution in logarithmic scale.

into the control solution, so if the density of animals is compared between the control and the test solution, the latter tends to be higher than that in the case that the animals were adapted to the mixture of NaCl and $CaCl_2$ solution.

Discussion

According to Jennings (1906) *Paramecia* gather to acid solutions, all reactions to chemicals take place through the avoiding reaction, there is an optimum concentration of each chemical at which *Paramecia* do not show the avoiding reaction, and the chemicals at the regions of either higher or lower concentration than the optimum cause the avoiding reaction, so that the animals tend to remain in the region of the optimum concentration. If this region is small, a dense collection is formed there. Goldschmied-Hermann (1935) reported that *Paramecium caudatum* introduced into a drop of 1 or 3 percent ethyl alcohol solution gather in the periphery of the drop. According to Dryl (1959, b) *Paramecium*

caudatum respond with negative reaction with regard to all lower alcohols. The correct concentration of the test solution which produced the reaction, could not be known by their method, since the test solution was directly introduced into *Paramecia* suspension on the glass plate and the solution was diluted by the surrounding fluid. In general, by their method only comparatively strong solution produced the reaction of the animals, and it is not clear whether the reaction dues to osmotic pressure or to chemical stimuli. Even though the reaction due to the latter, it could be thought that the avoiding reaction was produced by too strong chemicals. In fact, Dryl (1959, b) used the ethyl alcohol solutions, the concentrations of which were hundreds times higher than those used in the present experiment.

On the other hand, by the present method, dilution of the test solution is in a comparatively small degree and the solution was not diluted by *Paramecium* suspension in at least the two thrids of the length of capillary. The chemotactic response of the animal was quite clearly demonstrated even to very low solutions of chemicals. There was no effect of osmotic pressure on the chemotactic responses in the present experiments, since the test solution were isotonic with the adaptation medium.

The results of present experiments can not be directly compared with those by other investigators, since the method, the adaptation medium and the control of the present experiments are different from those by other investigators. Hitherto, the culture medium (Jennings, 1906) or tap water (Dryl, 1959, a, b) was used as the adaptation medium and the control solution. The culture medium is the most reasonable medium for the adaptation and control solution. But, in the present experiment they were not used, because other investigators can hardly repeated the experiments with the exactly same culture medium and tap water.

In the present experiments, the solution which containing the definite ions was used for the control. The solution may be less suitable than culture medium in chemotactic response since it does not contain any organic substance. But the solution was convenient for studies of chemotactic response, because the observation of response could be made clearly and a kind of graded response to chemicals could also be observed.

What is the direct stimulus which elicits the chemotactic response of *Paramecium*? It is known that Ca-ions activate the ciliary movement, Ba- and K-ions inhibit it, and the addition of Ca to the solution of Ba or K restores the ciliary activity (Kinosita *et al*, 1964). On the other hand, according to Chase and Glaser (1930), Dryl (1961) and Kinosita *et al.* (1964), the swimming velocity of *Paramecium* showed a maximum at pH value of about 6.

pH value of the acetic acid solution which showed the maximum chemotactic response was 5.7. From these facts, it may be possible that the responses at least to $CaCl_2$, $BaCl_2$, KCl and acetic acid are related to the ciliary activity, *i.e.* the chemotactic response is positive to the solution which enhances the ciliary activity and *vice versa*. But in the enhancement of ciliary activity over the natural animal movement in the culture medium, the relation may not be held,

since in such a case the density of animals may be decreased by rapid animal movement than that in the control.

Jennings (1906) reported that *Paramecium* responded with positive taxis to acid solutions and with negative taxis to other chemicals. On the other hand, in the present experiments, many chemicals exhibited positive response. This discrepancy may be due to chiefly to the difference between the control solutions. Also by the present method, as far concerning to merely positive or negative taxis, almost the same results with those by Jennings (1906) could be obtained, if the culture medium was used as the control.

It has been well known that the earth alkali ions (Ca, Sr, Ba) has a repellent power for *Paramecium* (Jennings, 1899). But the mechanism of repellent power of Ca and Ba is quite different from each other, *i.e.* the former accelerates the ciliary activity and the latter have noxious properties.

It is quite possible that the physiological condition relates with the responses to chemicals in *Paramecium*. In general, all animals respond positively to a physiologically good condition and *vice versa*. The culture medium contains various ions and organic substances. The adaptation medium contains only Na- and Ca-ions but did not contain any other ions nor organic substances, in the present experiments. It may be thought that the physiological condition of the adaptation medium approaches to that of culture medium by addition of other chemicals. The following facts may support it; in many chemicals as far as used in the present experiments, *Paramecia* exhibited the maximum positive response to the diluted solution of them with the adaptation medium to 3.0 or 1.6 mM.

It can be thought that mechanical stimuli relate also to the reaction of *Paramecium*. *Paramecia* ingest bacteria or small particles of organic substance as their usual food. Mueller *et al.* (1965) found *Paramecium multimicronucleatum* ingest non-nutritive materials as polystyrene latex particles, as well as ingest bacteria. This fact suggests, in uptake of food, *Paramecium* does not distinguish between nutritive and non-nutritive material. Thus, the probably mechanical action of particles stimulate the cilia, and may elicit the positive taxis to small particle suspension. This was supported by the results of reaction to the diluted solution of India ink and the solution of polyvinylpyrrolidone.

Summary

1) Chemotactic responses of *Paramecium* were investigated by means of the glass capillary filled with test solution, one end of which was placed in the trough containing *Paramecium* suspension.

2) According to the test of culture of *Paramecia*, a mixture of 3.3 mM NaCl, 0.4 mM $CaCl_2$ and 1.0 mM tris solution was used as the adaptation medium.

3) The test solutions of the same osmotic pressure with the adaptation medium were used. Solutions of saccharose, acetic acid, milk, starch, ethyl alcohol, KCl, NH_4Cl, $CaCl_2$ and $BaCl_2$ were used as the test solutions.

4) Distinct positive chemotactic responses were observed in the solutions of above mentioned chemicals except those of $BaCl_2$ and NH_4Cl.

5) *Paramecia* avoided strongly the solution of $BaCl_2$, even comparatively diluted solution of it.

6) These results can not be compared directly with results by other investigators, since the control condition and the method adopted here differed from those adopted by them.

7) When *Paramecia* were adapted to a mixture of 2.2 mM $CaCl_2$ and 1.0 mM tris solution for a long time, they swarmed each other and only few of them entered into the control solution.

8) Ciliary activity, physiological condition and small particles in the test solution may be closely related to the chemotactic responses of *Paramecium.*

The author wishes to express his hearty thanks to Professor Mituo Tamasige for his suggestion of this subject and for improvement of the manuscript.

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