Effects of Various Chemicals on the Behaviour of *Paramecium caudatum*[1)]

By

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(With 7 Text-figures and 2 Tables)

Sears and Gittleson (1964) studied the effects of narcotics on the swimming velocity and characteristics of the spiral path of *Paramecium caudatum*. They found that even narcotics have an excitatory effect on ciliary activity of *Paramecium* if the dosages are lower than the narcotic level. They measured the length, radius, and angle of the spiral path at different pressures of xenon gas, and could not find out any statistically significant change in these parameters. On the other hand, Stephen, Gittleson and Sears (1964) studied the effects of CO_2 on *Paramecium multimicronucleatum* and found that the velocity and length of the spiral increased when CO_2 was applied at pressures from 30 to 150 psi. It is also known that the swimming velocity (Dryl, 1961; Kinosita, Dryl and Naitoh, 1964) and the chemotactic response (Dryl, 1959, a) of *Paramecium caudatum* reached a maximum at a pH value of about 6.

It was suggested in the previous paper (Nakatani, 1968) that there is a correlation between the chemotactic response and the swimming velocity of *Paramecium caudatum*. The present investigation was undertaken in order to examine the effects of various chemicals on the swimming velocity and on the type of movement in *Paramecium caudatum*. The results obtained from these experiments and the relationship between the swimming velocity or the movement type and the chemotactic response are discussed.

Material and Methods

Paramecium caudatum cultured in a vegetable powder infusion was used for experiments. The animals were washed well with adaptation medium having the same composition[2)] of cations as that used in the previous paper (Nakatani, 1968) and kept in this medium for more than 24 hours before an experiment.

1) Contribution No. 874 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) The adaptation medium used was of the following composition; 3.3 mM NaCl, 0.4 mM $CaCl_2$ and 1.0 mM tris (hydroxymethyl) aminomethane per litre (pH=7.2).

Test solutions were prepared as described in the previous paper (Nakatani, 1968). The adapted animals were transfered into each test solution and kept in this solution for ten to twenty minutes which is approximately equal to the time for the chemotactic response of *Paramecium* to reach a maximum. Then several drops of the test solution containing the animals were placed on a slide glass and photographic records of the paths along which the animals moved were taken under a low magnification microscope by stroboscopic illumination (at the frequency of 5 flashes per sec.) and a photographic record was produced on 35 mm film as white streaks on a black background. The negative film was projected on a screen with an enlarger and the following data were recorded: magnification, length of one spiral (z) and radius of the spiral (a). The average length of animal movement paths per second traced by twenty different individuals randamly selected was taken as the velocity under a given experimental condition.

The experiments were carried out at room temperatures ranging from 20° to 22°C.

Results

In a solution of 2.2 mM $CaCl_2$, the swimming velocity was 1625 micra per sec. This value is nearly two times the value measured by Kinosita *et al.* (1964) in a solution of 2 mM $CaCl_2$. In the other solutions also, higher velocity values were obtained those reported by many other authors (Sears and Elveback, 1961; Kinosita, Dryl and Naitoh, 1964; Sears and Gittleson, 1964). This discrepancy may due chiefly to the difference between the experimental methods of the present authors and the others. The other authors covered the chamber containing the *Paramecia* suspension with a coverslip. Prior to the present experiments, the author measured the swimming velocity with the same method and also obtained lower velocities than those obtained with the present method in which the adapted animals were tested in a large quantity of each chemical solution. Therefore, the present experiments were carried out without a coverslip.

The swimming velocity changes with the concentration of each chemical. The mean values of the maximum velocity and the concentrations which produced the maximum value are summarized in Table 1. The standard errors are large due to the variations between individuals rather than to error in measurement, as is clear from the photographic records (Fig. 1).

It is well known that under normal conditions the path of *Paramecium* is a narrow spiral (Jennings, 1899; Sears and Elveback, 1961). The swimming velocity, the length of a spiral and the radius of the spiral in the adaptation medium, culture medium and KCl solutions of different concentrations were measured, the relationship among the measured parameters was examined, and the results are summarized in Table 2. As is clear from this table, an increase in velocity increases the length of the spiral and decreases the radius of the spiral, i.e., the spiral becomes steeper and narrower with increasing velocity. On the other hand, abnormal swimming types occurred when the swimming velocity was very low as in 3.3 mM KCl, 0.66 mM acetic acid and 0.55 mM $BaCl_2$.

From the paths of *Paramecium* as recorded on the negative film, a brief classification of these swimming types seems to be useful for further considerations.

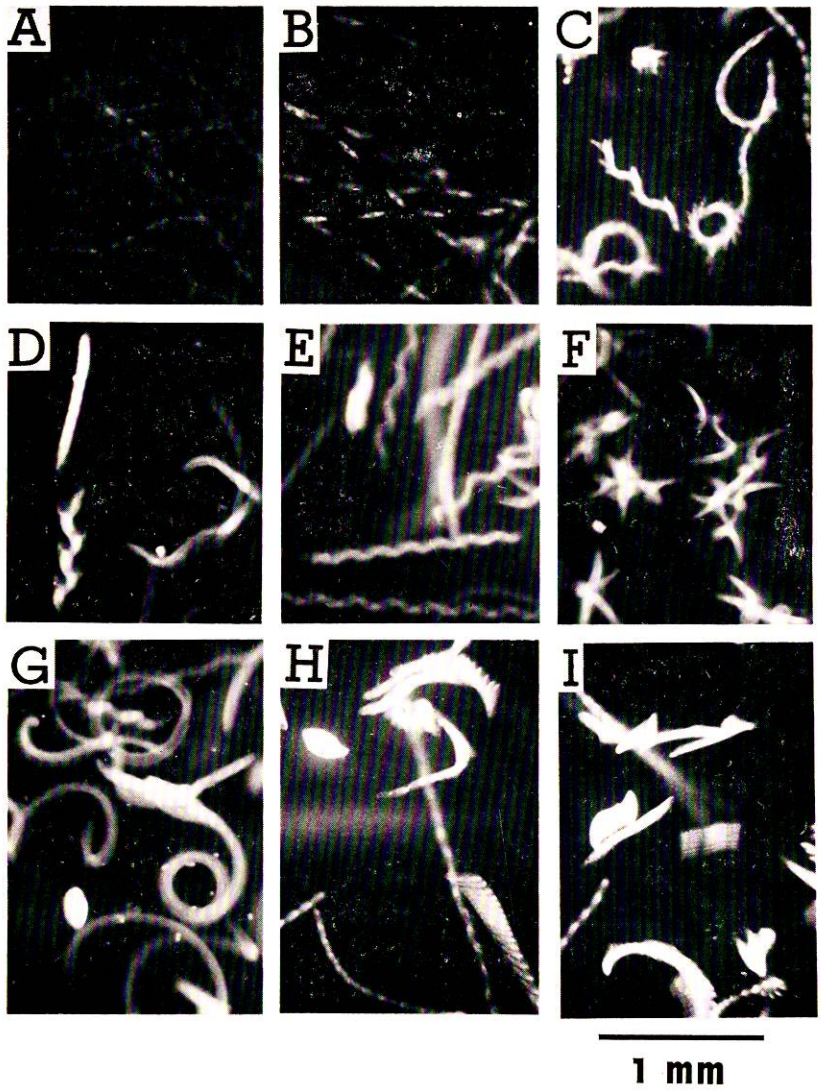

Fig. 1. Photographs showing the paths of swimming *Paramecia* in various test solutions. A,B,C,H and I were obtained with stroboscopic dark field illumination, flash frequency was 5/sec. A: culture medium, B: 2.2 mM $CaCl_2$, C: 3.3 mM KCl, D-G: 0.55 mM $BaCl_2$, H-I: 0.66 mM acetic acid.

Table 1. Swimming velocity of *Paramecium*. The mean maximum values with the standard error in each chemical solution at various concentrations are summarized. %: percentage of velocity calculated with reference to the control value as 100. Each value is the average for 20 animals.

	Concentration	Velocity (μ/sec)	%
Adaptation medium	—	1068± 62	100
$CaCl_2$	2.2 mM	1625±101	152±6
$BaCl_2$	0.017 mM	734± 26	69±4
KCl	0.17 mM	1523± 90	143±6
NH_4Cl	0.83 mM	1175± 56	110±5
Acetic acid	0.17 mM	1835±123	172±7
Milk	0.037 %	2014± 95	189±5
Starch	0.25 %	1589± 73	149±5
Polyvinylpyrrolidone	0.1 %	1523± 99	143±7
Indian ink	0.01 %	1328± 37	125±3
Saccharose	3.14 mM	1123± 53	110±5
Ethyl alcohol	3.3 mM	1499±111	140±7
Culture medium	—	1485± 69	139±5

Table 2. The relationship among the velocity, the radius of the spiral path (a) and the length of the spiral path (z).

	Velocity (μ/sec)	a in μ	z in μ
Adaptation medium	1068±62	29±3	849±30
Culture medium	1485±69	27±1	964±32
KCl 3.3 mM	418±20	49±2	457±27
1.6 mM	791±23	31±2	867±35
0.83 mM	1455±66	28±2	918±15
0.17 mM	1523±90	20±1	856±32

1. Elongated spiral (ES): the animal swims foward showing an elongated spiral path (Fig. 1, B).

2. Shortened spiral with backward movement (SSBM): the animal swims backward and alternatively changes the rotatory force which causes the animal to rotate about its longitudinal axis (Fig. 1, C and D).

3. Loop (L): the animal moves in a loop or a circle (Fig. 1, G).

4. Sliding (S): the animal rotates rapidly and slides perpendicular to the body axis (Fig. 1, H and I).

5. Periodic ciliary reversal (PCR): the animal swims forward and backward alternatively (Fig. 1, F).

The swimming velocities of the animals in each solution are summarized in Fig. 2 to 6 together with the data on chemotactic response (Nakatani, 1968). In general, as clear from these figures, the swimming velocity increases in the solutions

where a positive chemotactic response was observed (Nakatani, 1968), and it decreases in the solutions where a negative chemotactic response was observed.

In solutions of 3.3 mM saccharose and 3.3 mM NH_4Cl, the velocity was lower than that of the control. The velocity increased and reached a maximum when these solutions were slightly diluted with the adaptation medium, but decreased and approached the control value with further dilution of these solutions. In addition, in 3.3 mM NH_4Cl, an abnormal swimming type occurred which belonged to the type "L". In 0.073% milk (isotonic with 3.3 mM NaCl solution), the swimming velocity increased and reached 167 per cent of the control value, again increased with further slight dilution of the solution, and reached a maximum. These changes in swimming velocity with changes in concentration of the chemical solution are parallel to those changes in the rate of chemotactic response with changes in concentration of the test solution.

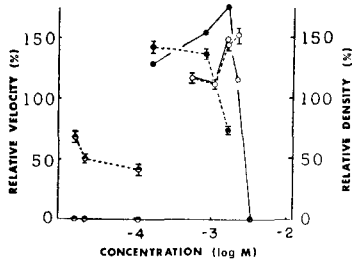

Fig. 2.

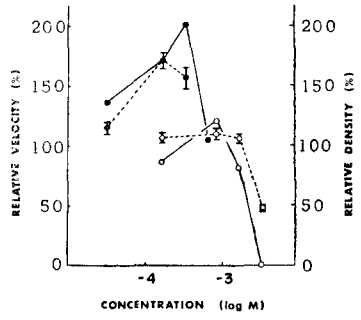

Fig. 3.

Fig. 2. The swimming velocity and the chemotactic response of *Paramecium* expressed in percentage with reference to the control value as 100. Broken lines and solid lines indicate the swimming velocity and the chemotactic response, respectively. Ordinates: the percentage of the swimming velocity and the chemotactic response. Abscissa: molar concentration of test solution in logarithmic scale. ●: KCl, ○: $CaCl_2$, ◓: $BaCl_2$.

Fig. 3. The swimming velocity and the chemotactic response expressed in percentage with reference to the control value as 100. Broken lines: swimming velocity. Solid lines. chemotactic response. ●: acetic acid, ○: NH_4Cl.

Also in solutions of acetic acid and KCl, almost the same relationship was obtained but the concentration at which the maximum velocity was produced was lower than that in the case of experiments on chemotactic response (Nakatani, 1968).

In 0.66 mM acetic acid, the swimming velocity was greatly decreased and many *Paramecia* could not swim forward in a spiral path but moved perpendicular to the body axis, i.e., type "S" movement. Even in such a concentration of acetic acid, *Paramecia* responded with a positive chemotactic response (Nakatani, 1968). This may be due to following facts; in the experiments with the chemotactic

response, the test solution in the capillary was diluted with the adaptation fluid in the trough, while in the case of the measurement of swimming velocity, the animals were transfered directly into the test solution, and the solution was only slightly diluted. The abnormal swimming types "SSBM", "PCR" and "L" occurred in 3.3 mM KCl as shown in Figure 1. These types resemble the types observed by Grebecki (1965) in 1 mM acetylcholine (periodic ciliary reversal) and in a mixture of 1 mM $CaCl_2$ and 32 mM KCl solution (partial ciliary reversal).

In ethyl alcohol, $CaCl_2$ and starch, the maximum velocity was obtained at the concentration which was isotonic with the adaptation medium. On the other hand, in the chemotactic response to these chemicals, the maximum response was obtained at slightly lower concentrations. Thus, there is a difference between the concentrations at which the maximum velocity and the maximum chemotactic response were produced. This may be explained as follows: if the velocity increases, the density of the animals in the solution decreases.

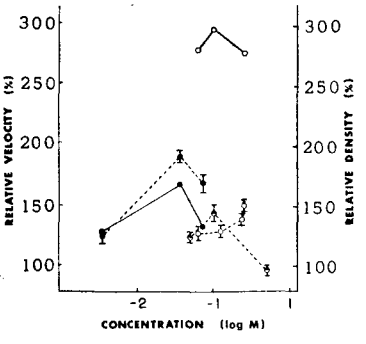

Fig. 4.

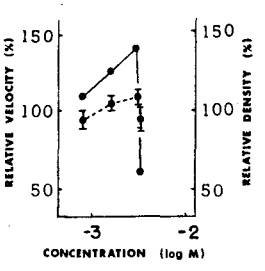

Fig. 5.

Fig. 4. The swimming velocity and the chemotactic response expressed in percentage with reference to the control value as 100. The abscissa is the same as that of Fig. 2 and 3. Broken lines: swimming velocity. Solid lines: chemotactic response. ●: milk, ○: starch, ◓: polyvinylpyrrolidone.

Fig. 5. The swimming velocity and the chemotactic response in saccharose solution. The ordinates and abscissa are the same as before. Broken line swimming velocity. Solid line: chemotactic response.

In the solution of $BaCl_2$, the velocity decreased even in the dilute solutions and the velocity recovered and approached the control value gradually with greater dilution of the solution. In the culture medium, the swimming velocity was 139 per cent of the control value. Also in the solutions which contain small particles, such as polyvinylpyrrolidone (PVP) and Indian ink, the velocity was increased. Particularly in the former the velocity increased more than that in the culture medium and the value of the velocity reached 142 per cent of the control value.

Swimming velocity and chemotactic response in each chemical solution was plotted on a graph to show the relationship between velocity and the chemotactic response. The result is shown in Figure 7. It is clear that there is a correlation between the swimming velocity and the chemotactic response. When the swimming velocity is higher than the control value, the chemotactic response is higher than the control value. There is a tendency that the chemotactic response reaches its maximum when the velocity in the test solution is roughly equal to the velocity in the culture medium but when the velocity increases further, the chemotactic response decreases.

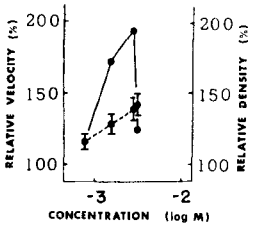

Fig. 6.

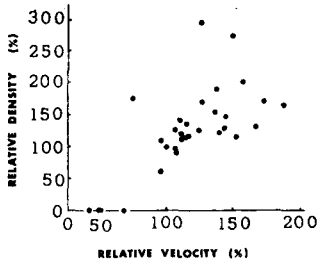

Fig. 7.

Fig. 6. The swimming velocity and the chemotactic response in ethyl alcohol solution. The ordinates and abscissa are the same as before. Broken line swimming velocity. Solid line: chemotactic response.

Fig. 7. Relation between the swimming velocity and the chemotactic response measured in various chemical solutions. Ordinate: chemotactic response. Abscissa: swimming velocity. %: percentage with reference to the control value as 100.

Discussion

As was discussed in the previous paper (Nakatani, 1968), the cations of the adaptation medium which was used in the present experiments are Na- and Ca-ions. There are no other cation nor any organic substance in the medium. Therefore, in those cases where the swimming velocity of the animal in the test solution is higher than the control value but lower than that in the culture medium it might be thought that the ionic condition of the test solution approaches that of the culture medium. It may also be possible, as Sears and Gittleson (1964) pointed out, that even the poisonous chemicals have excitatory effects on ciliary activity when their dosages are lower than the narcotic level. In fact, Dryl (1959, b) did the experiment on chemotaxis of *Paramecium caudatum* with regard to various kinds of alcohols and he concluded that all kinds of lower alcohols had toxic properties for this animal. On the other hand, in the present experiments, the swimming velocity was higher in the 3.3 mM ethyl alcohol solution than that in the culture medium. These facts may support the statement by Sears and Gittleson (1964) rather than that by Dryl (1959, b).

The suggestion in the previous paper (Nakatani, 1968) that the mechanical action of small particles accelerates the ciliary activity was confirmed by the present experiment. According to Mueller *et al.* (1965), the mechanical action of small particles accelerates the food vacuole formation in *Paramecium multimicronucleatum*. If the particles accelerate the ciliary activity, the action of peristomal cilia is also accelerated, and it is natural that the acceleration results in an increase in the chance of taking particles into the cell. Further, reception of mechanical stimulation by *Paramecium* was found by Naitoh and Eckert (1969), i.e., ciliary reversal occurs if a mechanical stimulus is applied to the anterior part of the animal. The frequency of ciliary beat is increased if the mechanical stimulus is applied to the posterior part of the animal. As the result the velocity of forward swimming is increased.

The mechanism of abnormal swimming types such as "SSBM", "L", "S" and "PCR" must be discussed. The swimming path of *Paramecium* has been described as spiral (Ludwig, 1929; Sears and Elveback, 1961). Further, it is well known that the path is a narrow spiral and the aboral side is always directed toward the outside of the spiral (Jennings, 1899). There are two components of force which are supplied by the effective stroke of cilia, i.e., one is a propelling force which propels the animal forward or backward and the other is a rotating force which causes the animal to rotate about its longitudinal axis. The rotating force was explained by Grebecki *et al.* (1967) as a hydrodynamical force.

If both the propelling force and the rotating force decrease, the radius of the spiral increases but the length of the spiral decreases. When the rotating force ceases but the propelling force remains, the animal may move along in a loop because there is a difference in the forces of cilia on the oral and aboral surfaces. On the other hand, when the propelling force ceases but the rotating force remains, the animal may move in a direction perpendicular to its longitudinal axis which is classified as "S" type movement. In this case the movement can not be explained by the hydrodynamical force but it must be explained by the direction of the effective stroke of the ciliary beat. In the case of the "SSBM" type, the direction of animal movement is reversed continuously, and the direction of rotating force alternatively changes (Fig. 1. C and D).

The relationship between the swimming type and the chemotactic response may be summarized as follows: if the velocity of forward swimming in the test solution is lower than that in the control, negative chemotaxis occurs. If the velocity is higher than that in the control, positive chemotaxis occurs. The experiment on chemotaxis was carried out with small glass capillaries filled with test solution (Nakatani, 1968). Under such a condition, *Paramecium* can hardly enter into the capillary, when the swimming types are "SSBM", "PCR", "S" or "L".

Summary

1) Effects of various chemical solutions on the swimming velocity and the swimming type of *Paramecium caudatum* were investigated.

2) *Paramecium* swims along a spiral path under normal conditions. If the swimming velocity increases, the length of the spiral increases but the radius of the spiral decreases. If the swimming velocity decreases greatly, the swimming type and the locus of animal movement is a shortened spiral, loop, or sliding in the direction perpendicular to its logitudinal axis.

3) It seems that the swimming type changes with differences between the propelling force and rotating force. If the rotating force ceases and the propelling force remains or the propelling force ceases and the rotating force remains, the swimming type becomes "L" or "S" respectively. And if, during the reversal of the propelling direction, the periodic reversal of rotation occurs, the swimming type becomes to "SSBM".

4) Even a poisonous substance has an excitatory effect on the ciliary activity if the concentration is low enough. The mechanical stimulation of small particles has an excitatory effect on the ciliary activity.

5) If the swimming velocity is lower than that in the control or the swimming type belongs to "SSBM", "PCR", "S" or "L", the negative chemotactic response occurs. If the swimming velocity is higher than that in the control, the chemotactic response is positive but if the velocity is too high, the density of animals in such a solution decreases.

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