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Further Study on the Plasmolysis in *Paramecium*

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It has been reported in the previous note (Wada, 1954) that the *Paramecium* cell can exhibit a phenomenon which is to be identified as that of plasmolysis common in plant cells. In order to obtain this effect two factors were found necessary, i. e., the hypertonicity of the external medium and the presence of sodium ions, and it was suggested that the sodium ions were effective in weakening the adhesion of the pellicle to the protoplasmic surface. It was shown further that calcium ions were likely to antagonize such effect of sodium ions.

In the present study, first, the problem of Na-Ca antagonism was examined quantitatively. Then, the tests for a plasmolysing effect of monovalent cations were taken up again, this time with an extended range of salts, i. e., a further series of sodium salts, on one hand, and the chlorides of potassium and lithium, on the other. Though potassium had already been tested preliminarily in the previous study, leading to the suggestion of its inefficacy, it seemed necessary to reinvestigate its effect with an increased number of *Paramecium* specimens. Lastly, some trials were made to inquire into the problem of reversibility of the process in question in *Paramecium*.

Before going further, I wish to express my sincere thanks to Assistant professor T. M. Yanagita of the Ochanomizu University for his kind guidance throughout this work.

Material and Methods

Paramecium caudatum reared in straw infusion was used as in the previous experiments. The manner of testing a solution for its plasmolysing effect was also the same as before; the test solution was applied to a population (about 50-80 individuals) of *Paramecium* on a glass slide and the cells were examined under the microscope for their appearance changing with time. The number of the plasmolysed cells was counted in each examination and was given in percentage of the total number present.

To study the reversibility of plasmolysis process, a different procedure was adopted, as follows: a single *Paramecium* was taken out of the culture by using a very finely drawn pipette. Then, it was placed on a glass slide with a minimum amount of the culture medium, a drop of 0.4 M NaCl solution (as a plasmolysing agent) was promptly applied to it, and the cell was examined under the microscope. As soon as the plasmolysis was found setting in (in 2 or 3 minutes), the NaCl solution was drained off as thoroughly as possible, and a sufficient amount of the culture medium, which had been filtered through filter paper, was applied. Then, the *Paramecium* was examined microscopically for possible recovery from the plasmolysis.

The pH values of all the salt solutions used were found to fall within the range from 4.8 to 5.6. Since the pH within the range from 4.0 to 9.0 seemed not to affect the plasmolysing effect of a solution, the experimental solutions were used unbuffered.

Results

1. *Sodium-calcium antagonism.* The antagonism between sodium and calcium ions in inducing the plasmolysis in *Paramecium* was studied by making use of mixtures of $\frac{4}{10}$M NaCl and $\frac{4}{15}$M $CaCl_2$ solutions in various volume ratios (9 : 1, 49 : 1, ……, 99 : 1, 999 : 1). The osmotic pressure of all the mixed solutions was thus equivalent to that of the $\frac{4}{10}$M NaCl, the value at which the pure NaCl solution was known to show the maximum effect in inducing plasmolysis.

The results obtained are given in Table 1. They proved to be quite affirmative of the existence of the antagonism, which had been barely suggested in the previous paper. The upper limit of the Na^+/Ca^{++} ratio for a complete inhibition of plasmolysis may be decided as being

Table 1. Frequencies of plasmolysis in the solutions with different Na^+/Ca^{++} ratios. Temp. 17.5°C.

Volume ratio of $\frac{4}{10}$M NaCl to $\frac{4}{15}$M $CaCl_2$	Na^+/Ca^{++} ratio	Percentage number of plasmolysed individuals (average from three tests)
9 : 1	13.5 : 1	0
49 : 1	74 : 1	0
59 : 1	89 : 1	0
69 : 1	104 : 1	0
79 : 1	119 : 1	2.4
89 : 1	135 : 1	3.8
99 : 1	149 : 1	37.7
999 : 1	1500 : 1	37.5
∞ : 1	∞ : 1	38.2

104 : 1. At the ratio 119 : 1, a plasmolysing effect of the solution was already recognizable though slightly. At the ratio 149 : 1, any antagonizing effect of calcium was no longer noticeable, the frequency of plasmolysis (in percentage number) being practically the same as that obtained with the pure hypertonic NaCl solution.

2. *Effects of different sodium salts.* Nitrate, sulphate and thiocyanide of sodium were tested for a plasmolysing effect, in solutions of graded concentrations ranging from 0.08 M to 1 M. This series of tests was further extended to include NaCl, which was studied anew to provide the basis of comparison. First, it must be pointed out that the optimum molarity of the three salts for plasmolysis (0.3～0.4 M) was practically the same as that found for NaCl solution, though the frequency of plasmolysis induced was apparently lower, especially in the case of NaSCN (Table 2). The lower limits of plasmolysing concentration of the three kinds of salts were also found to be the same as that of NaCl, while the upper limits were perhaps somewhat lower. A possible cause of the failure of excessively hypertonic solutions in inducing the plasmolysis has already been discussed in the previous note (Wada, 1954).

Table 2. The frequencies of plasmolysis (in percentage numbers of plasmolysed cells) in the solutions of various salts. Results from three repeated tests, with the average value in the parentheses, are given for each concentration of a given salt. Temp. 15.6～19.0°C.

Salts	Concentration (Mol)						Time required for plasmolysis to be complete (in mins.)
	0.1	0.2	0.3	0.4	0.5	0.6	
NaCl	0.0 0.0(0.0) 0.0	1.6 10.2(9.6) 17.0	11.8 10.8(16.5) 26.8	44.6 38.0(38.0) 31.4	6.2 5.0(6.9) 9.6	0.0 0.0(0.0) 0.0	2～3
NaSCN	0.0 0.0(0.0) 0.0	5.6 0.0(1.9) 0.0	2.1 0.0(1.7) 2.9	16.6 1.5(6.0) 0.0	0.0 0.0(0.0) 0.0	0.0 0.0(0.0) 0.0	3～4
$NaNO_3$	0.0 0.0(0.0) 0.0	11.1 0.0(4.3) 1.8	15.3 6.6(8.8) 4.4	6.2 22.4(13.6) 12.2	0.0 0.0(0.0) 0.0	0.0 0.0(0.0) 0.0	3～4
Na_2SO_4	0.0 0.0(0.0) 0.0	29.0 7.1(13.6) 4.8	42.0 8.1(25.1) 25.3	15.8 20.9(25.6) 40.0	4.8 0.0(1.6) 0.0	0.0 0.0(0.0) 0.0	2～3
KCl	0.0 0.0(0.0) 0.0	1.6 0.0(0.5) 0.0	0.0 2.8(2.5) 4.6	2.5 4.0(3.2) 3.2	0.0 3.3(3.1) 5.9	0.0 0.0(0.0) 0.0	4～5
LiCl	0.0 0.0(0.0) 0.0	2.9 6.4(5.3) 6.7	8.0 12.1(11.0) 12.9	27.0 17.0(25.5) 32.5	0.0 5.5(1.8) 0.0	0.0 0.0(0.0) 0.0	3～4

For all the three salts studied, the appearance as well as the time factor of plasmolysis was similar to that found for the NaCl solutions. Further, those *Paramecia* which failed to be plasmolysed in a hypertonic medium showed also in these cases a tendency to carry through the volume reduction by a flattening. Thus, in the concentrations 0.08 M and 0.1 M, which were quite subliminal as to the plasmolysis, a slight sign of exosmotic flattening was found in nearly all the individuals in five minutes. In the concentration range from 0.2 M to 0.5 M, similar exosmotic flattening was observable among that fraction of population which failed to show plasmolysis, while some showed rather a sign of rounding up. In the solutions from 0.6 M to 1 M, there being again no plasmolysis at all, an intense flattening accompanied by a longitudinal wrinkling of the cell surfaces and by stoppage of ciliary motion took place in twenty seconds to one minute in all the *Paramecia*.

3. *Effect of potassium ions.* Though in the preliminary examination reported in the previous note a plasmolysing effect had seemed to be absent to the KCl solutions, the present re-investigation with an increased number of *Paramecium* specimens has shown that they were certainly effective, though with a low frequency (Table 2). The optimum as well as the lower and upper limits of plasmolysing concentration was found to be equal to those obtained with NaCl solutions, respectively. The plasmolysis process was also similar in appearance as well as in time course.

4. *Effect of lithium ions.* As shown in Table 2, the LiCl solutions were nearly as much effective in causing the plasmolysis as the NaCl solutions. At the optimum concentration, which was 0.4 M also in this case, the plasmolysis percentage was about 26 in an average. The lower and upper limits of plasmolysing concentration also were found to be similar to those obtained with NaCl.

5. *Reversibility of the process.* It was confirmed that the plasmolysis caused by the NaCl solution was reversible, though with a very low rate of succesful cases (see Table 3). When *Paramecium* plasmolysed

Table 3. Reversibility of plasmolysis. Temp. 16.0~18.5°C.

Duration of immersion in the plasmolysing solution (in mins.)	Number of plasmolysed individuals observed	Number of individuals successfully deplasmolysed	Rate of reversibility (in %)	Time required for the reversal
1~2	36	12	33	40 mins. ~2 hrs.
2~3	21	3	14	33 mins. ~3 hrs.

was brought back to the hypotonic culture medium after two minutes of immersion in 0.4 M NaCl solution, the cell could survive at the rate

of 33 per cent. When the immersion in 0.4 M NaCl lasted for three minutes, the rate of survival of the plasmolysed *Paramecium* in the hypotonic solution was reduced to 14 per cent.

The ciliary movement which had been weakened by the immersion in the NaCl solution became again active in the culture medium, but in a little while, many individuals quickly swelled, and ciliary movement became slow till it stopped altogether in 30 mins. to 1 hr. After a while such cells were broken down. Only in the smaller number of individuals that kept on living successfully after having been brought back to the hypotonic medium, the plasmolysis was found going reversed. The time required by each individuals for the reversal (deplasmolysis) was from about 40 mins. to 3 hrs. First, the space between the pellicle and protoplast became narrowed and both came in contact with each other eventually. After some two days it was observed in two cases of *Paramecia* thus treated that cilia had been newly regenerated at the anterior part of the body and were in motion quite normally. Even a cell division was found occurring in these specimens in the course of three days.

Consideration

The results described above indicate that not only a number of sodium salts but chlorides of other monovalent cations (K and Li) were effective in causing the plasmolysis in *Paramecium* in hypertonic solution and that calcium ions antagonized such effects of monovalent ions. This may lend some further support to the explanation formerly proposed that a certain external factor which weakens the adhesion of the pellicle to the protoplasmic surface is necessary beside that of hypertonicity of the medium.

There are plenty of published informations as to the antagonism between Na (or K) and Ca ions relative to the physical property of protoplasm. Heilbrunn and Daugherty (1932) reported that in the amoeba, calcium caused a marked stiffening of the cortical sol, and the effect tended to be antagonized by sodium, potassium or magnesium. Chambers and Howland (1930) found that in a $CaCl_2$ solution the protoplasm of *Actinosphaerium eichhornii* was converted into a coagulum, while in NaCl and KCl solutions the protoplasmic membrane underwent a dissolution. Further, the cortex of the egg of *Chaetopterus* was shown by Wilson and Heilbrunn (1952) to be liquified by potassium ions.

As for the plant cells, since Cholodny and Sankewitsch (1934), working with *Spirogyra*, have found that a concave type of plasmolysis was caused by hypertonic solutions of $CaCl_2$, whereas a convex type of plasmolysis was found in sodium or potassium salt solutions, such has

been taken for a rule of general applicability. Seifriz (1936) interpreted this by assuming that establishment of the concave type is due to a high tensile strength and adhesive quality of the protoplasm, while the convex type occurs when the protoplasm is low in viscosity.

In the present case of *Paramecium* cell, therefore, it may be fair to suppose that the monovalent cations act to decrease the viscosity of the sub-pellicular substance (if such exists) or of the cortical protoplasm, in a way similar to the cases cited above. In the present type of cell, which possesses an easily yielding cell-wall (i. e., the pellicle) on one hand and no large-sized vacuoles (cell-sap spaces) on the other, it might well be possible that a plasmolysis was so difficult a matter to be brought about that it was impossible at all if the adhesion of protoplasmic surface to the pellicle had not first been weakened by such agents. Indeed, Engmann (1934) reported that the amount of difficulty in inducing the plasmolysis in water-storage cell of *Mesembryanthemum* was dependent upon the strength of adhesion of the protoplasm to the cell wall. It may be interesting here to note, further, that according to Sakamura (1940), the stamen hair cells of *Tradescantia* in younger stages, where the cell wall is not yet much stiffened, only show wrinkling or folding of the cell wall on the exosmotic shrinkage of the protoplasm, and there is no separation of the cell wall from the protoplasm.

The fact that the plasmolysed *Paramecia* tend to die sooner or later in the normal hypotonic medium suggests some injury which may have been exerted by the pure sodium or potassium salts on the protoplasm. In fact, analogous results are known to be obtainable in plant cells. Thus, Sakamura (1933) found that the protoplasm of *Spirogyra* showed an abnormal swelling in NaCl solutions, which was followed by an irreversible coagulation. Further, according to Höfler (1928), the plasmolysis induced in solutions of sodium or potassium salts in *Allium cepa* cells takes the form of "Kappenplasmolyse", which indicates an abnormal change produced on the part of protoplasm.

Summary

Further features of the plasmolytic phenomenon as was observable in *Paramecium* cells placed in a hypertonic solution of Na salts were studied. The phenomenon proved to be reversible, though in a small percentage of plasmolysed cells, when the cells were brought back to the normal, hypotonic medium. It was shown that K^+ as well as Li^+ had an effect similar to that of Na^+ in enabling the plasmolysis to take place. Ca^{++} ion, on the other hand, was found to antagonize the said action of Na^+ ion at Na^+/Ca^{++} values below 135, the plasmolysis being

inhibited completely by the presence of Ca^{++} in a molar concentration higher than 1/100 of that of Na^{+}. The possible modes of action of external cations in facilitating and preventing the plasmolytic event were discussed.

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