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Plasmolysis-like Phenomena in *Paramecium* Cells
Effects of Ca-Precipitant Na-salts and of Non-Electrolytes

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It has already been reported (Wada 1954, 1959) that a phenomenon to be identified with plasmolysis can be induced in *Paramecium* under the conditions in which the protoplast shrinks by exosmosis and, at the same time, the adhesion of the protoplast to pellicle is supposedly weakened by the presence of monovalent cations like Na^+. Furthermore, this action of monovalent cations has been shown to be antagonized by Ca^{2+} ions. In the present study, further kinds of external media were investigated for a possible similar effect; namely, the solutions of Ca-precipitating Na-salts and of non-electrolytes. It was indeed an interesting matter to test these media because of the complete absence either of Ca^{2+} ions or of electrolyte ions at all.

In the following lines, the results obtained will be described, together with discussion concerning the nature and mechanism of the effects observed.

Before going further, the writer wishes to express her sincere thanks to Prof. T. M. Yanagita for his kind guidance throughout this work.

Material and Method

Paramecium caudatum kept in mass culture in hay infusion was used. pH of the culture medium was determined electrometrically as 7.6. The experimental media used were well within the pH range where a difference in pH value had been known to be without noticeable influence on the plasmolytic efficacy of hypertonic salt solutions (Wada, 1959): NaCl (0.2–0.6 M) pH 6.4–6.0, Na-oxalate (0.01–0.28 M) pH 7.3–6.3, sucrose (1.0–2.68 M) pH 7.1–7.1, glycerol (1.0 M–absolute) pH 5.8–5.9.

The method of examining the effect of external media was the same as in the previous studies: the test solution was dropped from a pipette on a population of 30 to 80 *Paramecia* which had been mounted on a glass slide with a minimum amount of culture fluid, and the cells were examined under 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.

Results and Discussion

I. *Effects of the pure solutions of Na-oxalate*

In pure, dilute (0.01–0.06 M) solutions of Na-oxalate, a plasmolysis-like figure consisting of shrinkage of the protoplast and detachment of the protoplasmic surface from pellicle was induced in some percentage of *Paramecium* (Fig. 1). It was similar in general appearance to that obtainable in the hypertonic (0.2–0.6 M) NaCl solutions (Fig. 2), while the area of pellicle separated was generally much larger than in the

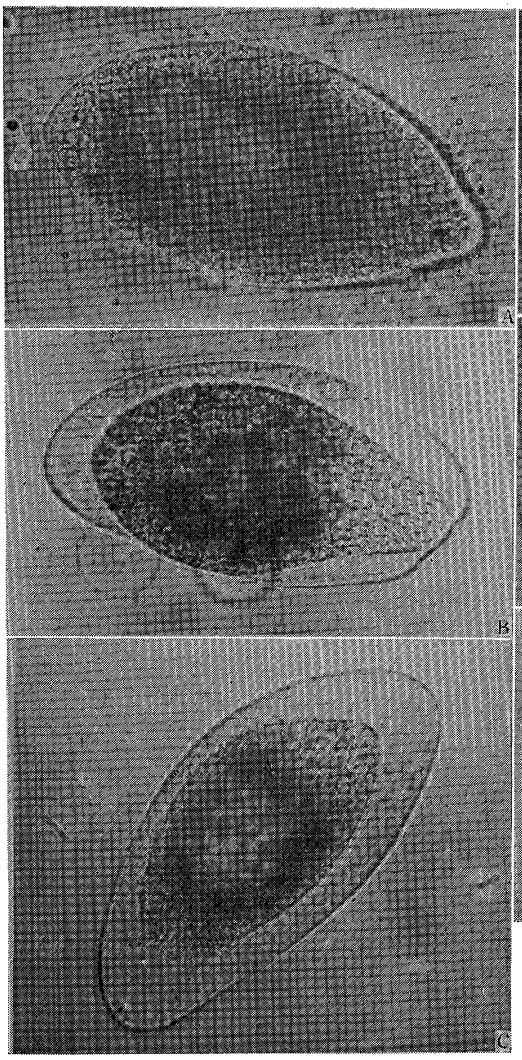

Fig. 1

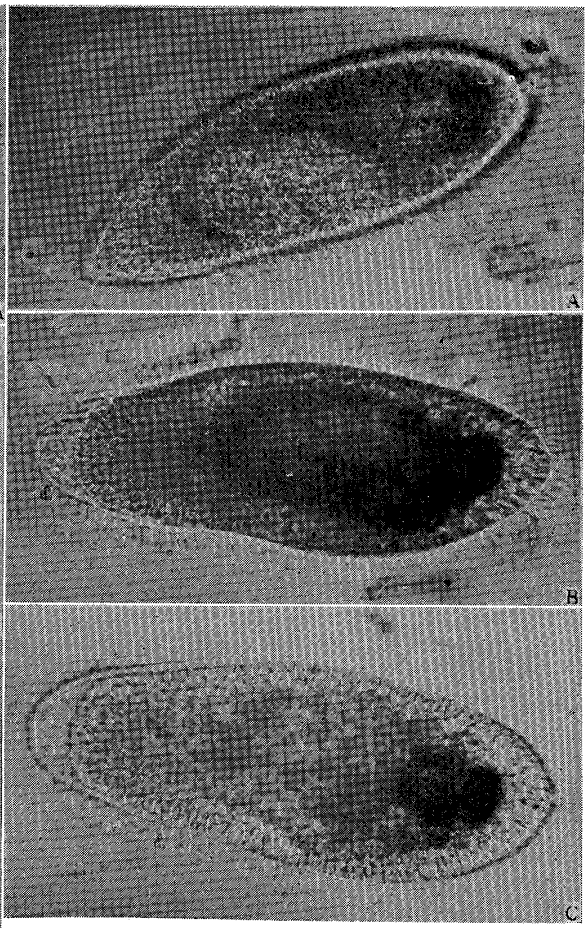

Fig. 2

Fig. 1. A *Paramecium* in the course of "plasmolysis" in 0.02 M Na-oxalate solution. A: 5 minutes of immersion; ciliary movement has stopped and a sign of rounding up becomes apparent. B: 8 minutes; pellicle detachment spreads over the dorsal side. C: 10 minutes; the "plasmolysis" is complete (×320).

Fig. 2. A *Paramecium* in the course of plasmolysis in 0.4 M NaCl solution. A: 3 minutes of immersion; pellicle is separated from the protoplast and ciliary movement is slowing down. B: 4 minutes; the pellicle becomes smooth again. C: 5 minutes; the plasmolysis is complete (×320).

latter. In higher concentrations (0.07–0.28 M) of Na-oxalate, however, no such figure was obtainable. In these solutions, *Paramecia* showed slight flattening of the bodies in 20 to 30 seconds, but after 2 to 3 minutes of immersion the ciliary motion was stopped, whereupon the whole cell bodies began to swell gradually, till they finally were disintegrated in 30 minutes (Fig. 3).

The data obtained are given in Table 1. As can be seen from the table, the optimum concentration of Na-oxalate to induce the "plasmolysis" was 0.02 M. The percentage number of "plasmolysed" cells at that

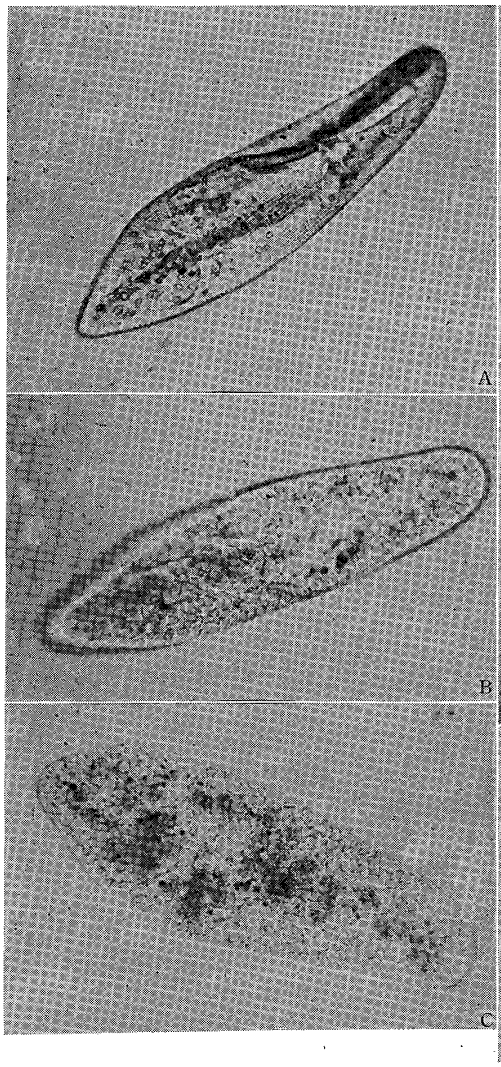

Fig. 3

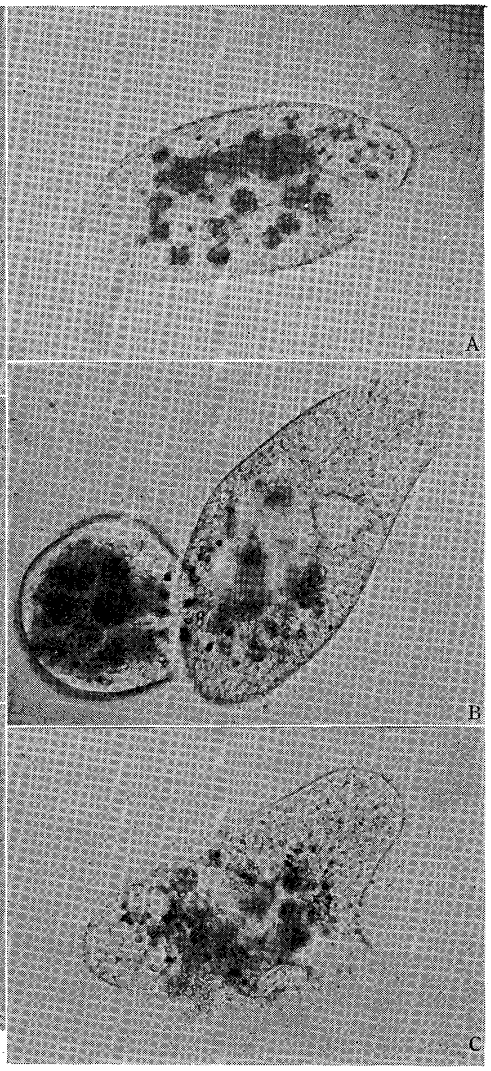

Fig. 4

Fig. 3. A *Paramecium* in 0.2 M Na-oxalate solution. A: 30 seconds of immersion; the cell body flattened. B: 3 minutes; cilia have stopped and the cell body begins to swell. C: 30 minutes; begins to disintegrate (×255).

Fig. 4. A *Paramecium* which failed to "plasmolyse" in 0.02 M Na-oxalate solution. A: 3 minutes of immersion; the cell rounding up, and the cilia stopped. B: 10 minutes; pellicle detachment is not induced, but the cell body swells and a blister forms. C: 30 minutes; the cell body burst up (×250).

Table 1. The effects of pure Na-oxalate solutions on *Paramecium* cells. Results of three repeated tests are presented for each concentration. Temp. 25.5-28.5°C.

Concentration (Mol)	Number of individuals	Percentage number of "plasmolysed" individuals (The averages given in brackets)	Time required for "plasmolysis" (in minutes)
0.008	72 51 43	0.0 0.0 (0.0) 0.0	—
0.01	34 59 79	5.8 0.0 (4.0) 6.3	10
0.02	50 66 48	44.0 34.8 (30.4) 12.5	10
0.03	59 65 86	28.7 21.5 (26.8) 30.2	10
0.04	45 57 52	15.6 17.5 (11.0) 0.0	10
0.05	43 63 48	23.3 9.5 (10.9) 0.0	10
0.06	28 78 62	21.5 12.8 (11.4) 0.0	10
0.07	53 69 55	0.0 0.0 (0.0) 0.0	—
0.08	61 39 56	0.0 0.0 (0.0) 0.0	—
0.09	46 58 53	0.0 0.0 (0.0) 0.0	—
0.1	68 60 52	0.0 0.0 (0.0) 0.0	—
0.2	47 62 44	0.0 0.0 (0.0) 0.0	—
0.28	64 57 54	0.0 0.0 (0.0) 0.0	—

concentration was as high as 30.4, though the time required for the process was considerably extended.

In 0.02 M Na-oxalate solution, though the ciliary movement was first accelerated in all the *Paramecia* within a minute, it soon began to slow down, until all the *Paramecia* stood still after 7 to 8 minutes. A slight sign of the cell rounding up was first noticed in 3 minutes of

immersion, but it became remarkable only about at the time when all the movement had disappeared (Fig. 1A). This was followed rather abruptly by detachment of the shrinking protoplast from pellicle in the above given percentage of *Paramecia*, which set in on the aboral side of the cell bodies within 10 minutes (Fig. 1B). The detachment then spread progressively over the whole surface except for the peristomial region (Fig. 1C). The protoplasmic surface thus detached from pellicle had a quite clearcut contour. This process was found to be irreversble, even when the "plasmolysed" cells were soon brought back to the culture medium. Those individuals which did not show such separation of pellicle in 10 minutes of immersion in the Na-oxalate solution became simply swollen and eventually burst up (Fig. 4).

The data shown in Table 1 were obtained at temperature of 25.5–28.°C. There was a tendency noticed in the course of experiments that the easiness of inducing "plasmolysis" in Na-oxalate solutions varied with temperature, lower temperature decreasing the "plasmolysis" percentage. This is evidenced by the specially provided set of data, which is shown as curves of "plasmolysis" percentage growing with time of immersion at different temperatures in Fig. 5. It is interesting here to note that a rather reverse tendency was observed in the case of NaCl solutions as to the temperature effect on plasmolysis; the plasmolysis percentage in 0.4 M NaCl was found to be considerably lowered (below 10) at 34.0°C.

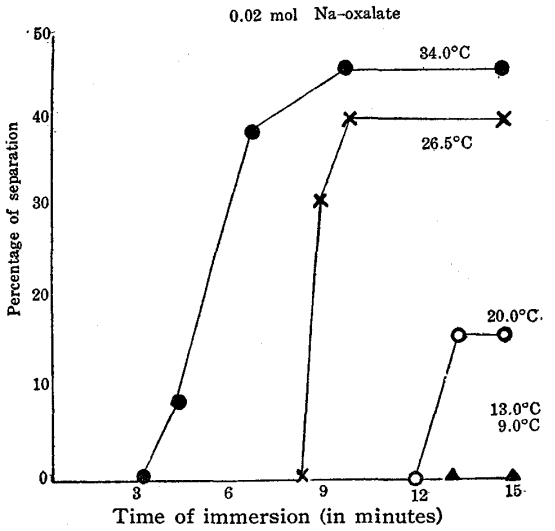

Fig. 5. The effect of temperature upon the "plasmolysis" percentage in 0.02 M Na-oxalate solution. *Paramecia* taken from one and the same culture were used throughout in this set of tests.

The value of the ratio Na^+/Ca^{2+} is supposed to be extremely high in the Na-oxalate media, and from the point of view stated above, this should have been expected to represent a condition quite favourable for producing plasmolysis. However, the actual finding was that the higher concentrations of Na-oxalate failed to induce plasmolysis (except for the initial flattening) but caused swelling of the protoplast. It is possible that an excessivly high Na^+/Ca^{2+} ratio may have an effect on the protoplasm colloid to cause abnormal swelling besides the possible effect of weakening the adhesiveness of surface protoplasm. An effect of high Na^+/Ca^{2+} ratio of causing protoplasmic swelling like this has been reported by Heilbrunn and Daugherty (1932) in the amoeba cells placed in a pure

NaCl solution. At any rate, so far as there was such swelling, it must have necessarily prevented any plasmolytic event from being actually taking place even if there were a tendency to it.

On the other hand, remarkable plasmolysis figures occur in Na-oxalate solutions weaker than 0.06 M. The easy separation of the pellicle from the protoplast in these cases may well be explainable on the basis of the high Na^{+}/Ca^{2+} value which should have weaken the adhesiveness of pellicle. But what is the factor responsible for the marked shrinkage of the protoplast in these solutions? Picken (1936) reported that the osmotic pressure inside the cells of the fresh water ciliate, *Spirostomum ambiguum*, was equivalent to 0.15 per cent. (about 0.026 M) NaCl solution. Further, according to Frisch (1939), exosmotic flattening of *Paramecium caudatum* is barely noticeable in 10% sea water (isosmotic with about 0.05 M NaCl). Since, in the present study, the shrinkage of protoplast was most conspicuous in 0.02 M Na-oxalate solution, and this solution was found by the writer cryoscopically to be isosmotic with 0.028 M NaCl, it would be difficult to consider an ordinary process of water exosmosis as responsible for that shrinkage. In fact, as has been described above, the most of the cells in this same solution, being unplasmolysed, eventually showed simple swelling, as should have been expected in a hypotonic solution. There were no findings that the contractile vacuoles were in activity in the "plasmolysing" individuals, so that the possibility is excluded that the protoplast shrinkage in these cells was due to an abnormally accelerated drainage of water through those organelles. Therefore, it is probable that the protoplasmic shrinkage in question represent a specific event of non-osmotic nature, which is due presumably to some colloidal change under an ionic influence characteristic to the lower concentration range of Na-oxalate. The difference in sign of the temperature effect on the plasmolysis in Na-oxalate and in NaCl solutions, mentioned above, may also suggest that the phenomenon is different in nature in both kinds of media.

II. *Effects of pure solutions of Na-citrate*

Na-citrate solutions were also tested in a similar way and gave results similar to those of Na-oxalate solutions (Table 2). Though their effective range of molarity was situated somewhat lower, the optimum was at 0.02 M similarly to the case with oxalate, and the external appearance of "plasmolysis" was also alike between the two salts. The maximum "plasmolysis" percentage was somewhat lower than in Na-oxalate, while the time required was much longer.

III. *Effects of mixed solutions of Na-oxalate and NaCl*

In this group of observations, Na-oxalate solutions were tested on *Paramecia* as mixtures with a NaCl solution in various mixing ratios,

Table 2. The effects of pure Na-citrate solutions on *Paramecium* cells. Results of three repeated tests are presented for each concentration. Temp. 21.5-24.3°C.

Concentration (Mol)	Number of individuals	Percentage number of "plasmolysed" individuals (The averages given in brackets)	Time required for "plasmolysis" (in minutes)
0.005	53	0.0	—
	60	0.0 (0.0)	
	72	0.0	
0.008	46	30.4	25
	73	0.0 (10.1)	25
	66	0.0	25
0.01	44	18.2	25
	89	16.9 (12.9)	25
	42	3.6	25
0.02	73	28.4	25
	42	15.5 (20.1)	25
	79	16.5	25
0.03	51	0.0	—
	62	11.2 (3.7)	30
	76	0.0	—
0.04	49	0.0	—
	58	0.0 (0.0)	
	56	0.0	
0.06	64	0.0	—
	77	0.0 (0.0)	
	72	0.0	
0.1	52	0.0	—
	61	0.0 (0.0)	
	66	0.0	
0.2	59	0.0	—
	48	0.0 (0.0)	
	63	0.0	
0.3	63	0.0	—
	57	0.0 (0.0)	
	53	0.0	
0.4	55	0.0	—
	69	0.0 (0.0)	
	78	0.0	

in the expectation if there might be any synergy between the effects of both salts. The mixtures were prepared between 0.02 M Na-oxalate (i.e., optimum concentration) and 0.1 M NaCl solutions ; 0.02 M Na-oxalate and 0.4 M NaCl (i.e., optimum concentration) solutions ; 0.04 M Na-oxalate and 0.8 M NaCl solutions, respectively, in different volume ratios (0 : 10, 1 : 9, 5 : 5, 9 : 1, 10 : 0). The solutions tested and the effects observed were given in Tables 3A, B, C.

The overall result was that there was no sign of synergy to be found between the effects of both salt components, and an effect, if any, of a mixed solution was just that which was to be expected from one or the other of the component salts in its actual concentration.

The mixture 0.02 M Na-oxalate+0.1 M NaCl 1 : 9 was quite without effect on *Paramecia*, just as the mixture 0 : 10 (i.e., 0.1 M NaCl) was so (Table 3A). This was rather expectable since both salts were definitely

Table 3A. The effects of mixtures of NaCl and Na-oxalate solutions. Results of three repeated tests are presented for each concentration. Temp. 25.5–27.5°C.

Volume ratio 0.02 M Na-oxalate / 0.1 M NaCl	Number of individuals	Percentage number of "plasmolysed" individuals (The averages in brackets)	Time required for "plasmolysis" (in minutes)	Type of "plasmolysis"
0 : 10	65 48 39	0.0 0.0 (0.0) 0.0	—	—
1 : 9	67 51 48	0.0 0.0 (0.0) 0.0	—	—
5 : 5	19 69 53	16.0 5.8 (9.8) 7.5	10	Aboral
9 : 1	37 69 53	12.0 6.5 (10.2) 12.2	10	Aboral
10 : 0	39 46 104	15.4 26.2 (17.7) 11.5	10	Aboral
(Control) 0.05 M NaCl	42 54 39	0.0 0.0 (0.0) 0.0	—	—
0.01 M Na-oxalate	44 73 68	4.6 5.5 (4.3) 2.9	10	Aboral

subliminal in strength here. In the mixtures 5 : 5 and 9 : 1, on the other hand, there was "plasmolysis" in a certain number of *Paramecia*, and it was definitely of the type which has been described above as characteristic to Na-oxalate effect; it set in first on the aboral side to extend over the whole cell surfaces and took longer time (10 minutes) for its establishment. The percentages of reacting individuals also were such as to be expected from the pure Na-oxalate solution of corresponding molarity (0.01 M and 0.018 M). (Compare the figures in Table 3A for the mixture 10 : 0 (0.02 M oxalate) and for 0.01 M oxalate). These results seem to indicate that the effects observed of the mixtures 5 : 5 ond 9 : 1 were due entirely to the Na-oxalate component, while NaCl could not exert any synergising effect when it is present at concentration of 0.01 to 0.05 M.

Similar relations were found when the mixture 0.02 M Na-oxalate+ 0.4 M NaCl 9 : 1 was tested, in which only the "plasmolysis" of oxalate type was obtained (Table 3B). The percentage of reaction and the time

Table 3B. The effects of mixtures of NaCl and Na-oxalate solutions. Results of three repeated tests are presented for each concentration. Temp. 27.5–28.5°C.

Volume ratio 0.02 M Na-oxalate / 0.4 M NaCl	Number of individuals	Percentage number of "plasmolysed" individuals (The averages in brackets)	Time required for "plasmolysis" (in minutes)	Type of "plasmolysis"
0 : 10	61 55 49	47.5 38.5 (40.9) 36.7	3	Anterior
1 : 9	74 39 26	39.1 20.5 (25.0) 15.4	3	Anterior
5 : 5	50 64 54	12.0 10.9 (12.6) 14.8	3	Anterior
9 : 1	21 17 20	33.0 35.8 (31.3) 25.0	10	Aboral
10 : 0	69 48 45	30.4 22.9 (23.0) 15.6	10	Aboral
(Control) 0.2 M NaCl	62 51 39	16.1 11.8 (13.6) 12.8	3	Anterior
0.01 M Na-oxalate	35 49 51	2.8 8.2 (6.9) 9.8	10	Aboral

Table 3C. The effects of mixtures of NaCl and Na-oxalate solutions. Results of three repeated tests are presented for each concentration. Temp. 20.5–22.0°C.

Volume ratio 0.04 M Na-oxalate / 0.8 M NaCl	Number of individuals	Percentage number of "plasmolysed" individuals (The averages in brackets)	Time required for "plasmolysis" (in minutes)	Type of "plasmolysis"
0 : 10	66 69 57	0.0 0.0 (0.0) 0.0	—	—
5 : 5	52 68 74	0.0 0.0 (0.0) 0.0	—	—
10 : 0	72 48 56	0.0 4.2 (3.2) 5.3	12	Aboral
(Control) 0.4 M NaCl	59 44 63	27.1 29.5 (29.4) 31.7	3	Anterior
0.02 M Na-oxalate	57 66 70	10.5 21.2 (16.8) 18.6	10	Aboral

required were also similar to those to be found in pure Na-oxalate solution of corresponding concentration. On the other hand, in the mixtures 0.02 M Na-oxalate+0.4 M NaCl 1 : 9 and 5 : 5, the plasmolysis observed was exclusively of the type characteristic to NaCl effect; i.e., quick (establishing in 3 minutes) but only partial one commencing from the anterior end. The percentages of the effect, too, were largely comparable to those to be expected from the corresponding concentration (0.36 and 0.2 M) of pure NaCl solution, so that any synergising effect of Na-oxalate present at concentrations of 0.002 to 0.01 M was not noticeable. It may be pointed out that in the mixture 5 : 5 both Na-oxalate and NaCl were just at such a concentration as would have been effective if alone, and yet there was only the NaCl effect apparent. An assumption which may serve as an explanation for this will be that Na-oxalate can no longer be effective when NaCl has once attained its own effect on the cells. Indeed, in 0.2 M pure NaCl solution (tested as a control for the present set of observations), the plasmolysis set up in 3 minutes, and all *Paramecia* had lost every sign of life within 5 minutes, whereas the effect of pure Na-oxalate at concentration of 0.01 M (control) appeared only in 10 minutes.

Lastly, it is interesting to note that the mixture 0.04 M Na-oxalate +0.8 M NaCl 5 : 5, in which both components were at the optimum concentration for inducing plasmolysis, was without any effect on *Paramecia* (Table 3C). In this solution, the ciliary motion was stopped in 2 to 3 minutes, and a slight flattening of the cell bodies took place, being followed soon by swelling, which lead to cell destruction in 5 minutes. It may be suggested that the present mixture was equivalent in its effect to the concentrated (above 0.07 M) pure Na-oxalate solution, since the change in external appearance of the cells as well as the time required for the stoppage of ciliary motion was alike in the two media. If this is the case, Na ions from NaCl component will have to be looked upon as having a capacity of substituting excess Na-oxalate in abolishing the " plasmolysis "-inducing effect due to dilute Naoxalate.

It may be concluded from all these results that the effects of NaCl and Na-oxalate do not only fail to synergize in producing plasmolysis-like figures in *Paramecium* but sometimes interfere with each other. This seem to strengthen further the suggestion already stated that there is a difference in nature between the plasmolysis-like phenomena induced by NaCl solutions and by solutions of Ca-precipitant Na-salts.

IV. *Effects of hypertonic solutions of non-electrolytes*

As has been reported formerly (Wada, 1954), hypertonic solutions (up to 2 M) of sucrose do not produce plasmolysis in *Paramecium* cells but cause quick disintegration of cell bodies. However, since there was afterwards a personal communication from Mr. Iwamatsu[1] of the Tokyo

College of Agriculture that extremely concentrated glycerol sometimes caused a plasmolysis-like change in *Paramecia*, concentration range beyond 2 M of sucrose as well as of glycerol was taken up as test solutions to be included in the present survey.

The results obtained were as follows. Concentrated sucrose solutions were found to have no plasmolysing effect on *Paramecia*, even at 2.68 M, which represented the limit of water solubility of sucrose at 25°C. In sucrose solutions at concentration up to this, there was only flattening of the cell bodies (accompanying longitudinal wrinkling of the surfaces) followed by rapid disintegration (without swelling) in a few minutes of immersion (Fig. 6), like the one which was to be found in the less concentrated (1–2 M) solutions. The findings were much the same when

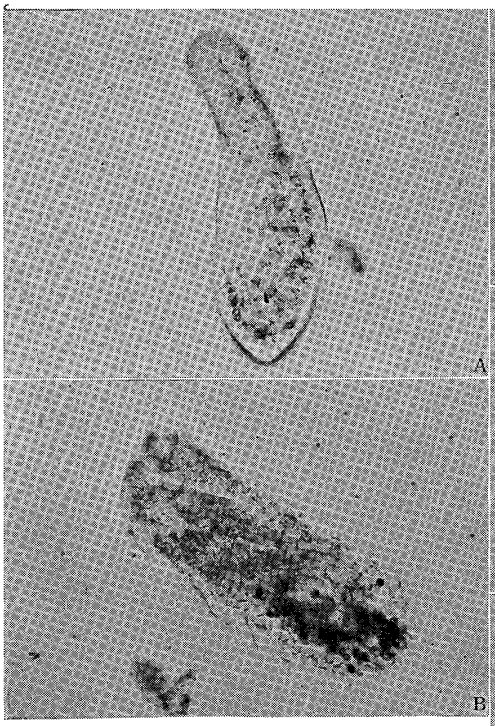

Fig. 6

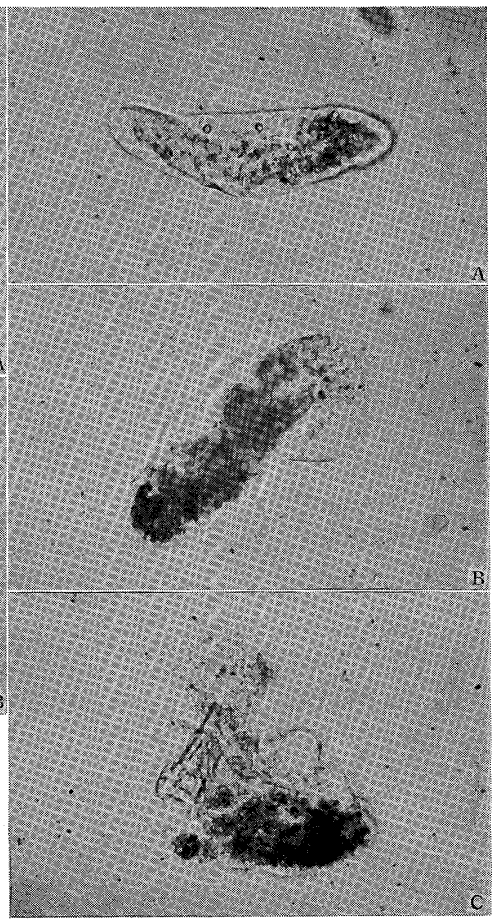

Fig. 7

Fig. 6. A *Paramecium* in 2.5 M sucrose. A: 1 minute of immersion; slight flattening of the cell body. B: 3 minutes; disintegration of the cell body beginning (×250).

Fig. 7. A *Paramecium* in 2.5 M glycerol solution. A: 1 minute of immersion; the cell body flattened. B: 2 minutes; disintegration of the cell body beginning. C: 3 minutes; the cell body burst up (×255).

1) See the postscript to this paper.

glycerol solutions at concentration up to 5 M were tested on *Paramecia* (Fig. 7).

However, the effect of glycerol solutions higher in concentration than 6 M (6 M, 10 M, and 13.7 M=absolute) was quite different. There was immediately a marked change in the appearance of all the *Paramecium* cells in these solutions, which consisted of protoplasmic coagulation and extensive shrinkage (not simple flattening) of cell bodies, while there was no sign of cell disintegration (Fig. 8A). After 20 minutes to 1 hour, the protoplasmic surface began to detach from the pellicle in one region or another of the cell surfaces in nearly 40 per cent. of individuals present there. The detachment then spread progressively over the whole surface, so that a typical figure of the "plasmolysis" became complete (Fig. 9A). The polygonal lattice pattern appeared clearly on the pellicle which had thus been detached and the cilia, though already stopped, became visible with unusual clearness. When *Paramecia* "plasmolysed" in this way in the glycerol solution were pressed gently

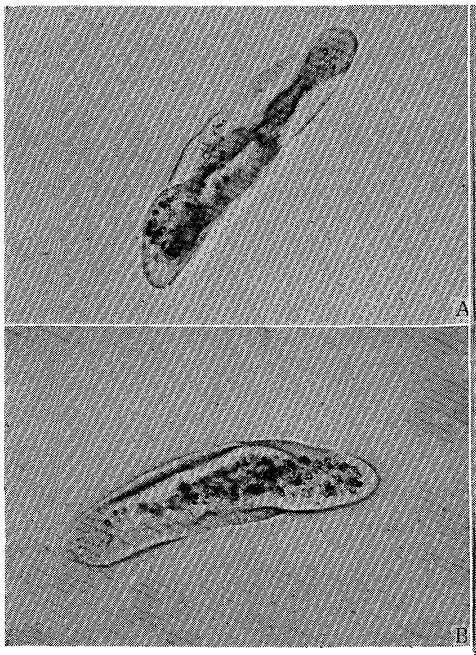

Fig. 8

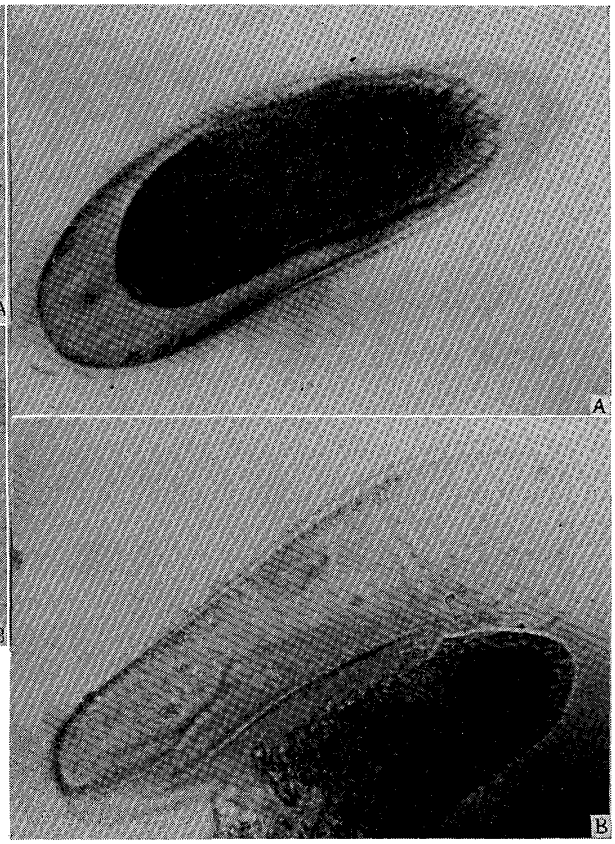

Fig. 9

Fig. 8. A *Paramecium* which failed to plasmolyse in 6 M glycerol solution. A: 30 minutes of immersion; the cell body shrinked. B: 1 hour; no change (×220).

Fig. 9. A *Paramecium* plasmolysed in 6 M glycerol solution. A: 40 minutes of immersion; the plasmolysis is complete. B: the protoplast squeezed out of the plasmolysed cell. The polygonal lattice pattern is apparent on the pellicle (×345).

against the glass slide, the pellicle was ruptured at some spot and the protoplast became extruded as a whole through this opening (Fig. 9B). The unplasmolysed remainder of the cells remained in the state of shrinkage, with their protoplasm coagulated, for as long as 24 hours (Fig. 8B).

It is very probable that the flattening as well as shrinkage (which may lead to the "plasmolysis") of the cell bodies observed in the higher concentrations of the non-electrolytes was due, at least primarily, to exosmotic loss of water. On the other hand, it was successful only with a low rate of frequency (percentage) to actually obtain plasmolysis, even with very hypertonic solution of glycerol, and this may be ascribed to the absence of such ionic conditions (high Na^+/Ca^{2+} ratio) in these media as should facilitate the detachment of the protoplasmic surface from the pellicle. It may be inferred, however, from the above results that an extremely intense shrinkage of protoplast may be able by itself to cause somehow the detachment from pellicle, so as to induce a "plasmolysis", in an ion-free condition. The considerable slowness with which the pellicle detachment becomes apparent in concentrated glycerol may indicate that it takes so much time to overcome completely the pellicle adhesion in an ion-free medium, rather than that the pellicle permeability to glycerol is relatively low as compared with that to inorganic salts.

As to the reason why the cell surface has so marked a tendency to disintegrate in sucrose as well as in glycerol solution, hardly anything may be stated at present. So far, it is possible only to refer to in this connexion some known instances of the effects of non-electrolyte media which render cell surfaces either abnormally leaky or mechanically unstable, such as reported in muscle fibres (Embden and Lange, 1923), erythrocytes (Davson, 1939), sea urchin eggs (Lucké and McCutcheon, 1928), some plant cells (Küster, 1909) and acontial epithelium of sea-anemone (Yanagita, 1959). Whatever the cause of disintegration of *Paramecium* cells in the non-electrolyte solutions may be, it seems that the tendency to disintegration can be counteracted by the dehydration due to an extremely hypertonic external medium.

Summary

1. A very marked phenomenon of "plasmolysis" (protoplast shrinking away from the pellicle) was shown to take place in *Paramecium* cells, when they were immersed in Na-oxalate (and also Na-citrate) solutions at lower concentrations (0.01 to 0.06 M). It seemed to be different in nature from the true plasmolysis which had already been reported to be obtainable in hypertonic solutions of NaCl, etc., in that it was apparently of a non-osmotic origin.

2. In higher concentrations of Na-oxalate (and Na-citrate) there

was only flattening of the cell bodies, which was followed by abnormal swelling of protoplasm.

3. Sucrose and glycerol caused only flattening of *Paramecia* even in very hypertonic solutions, and this was soon followed by cell disintegration. Only extremely concentrated (above 6 M) glycerol solution was effective in setting up quite remarkable and stable form of plasmolysis in a fairly high percentage of the cells. These findings in non-electrolytic media were discussed as to their nature and mechanism.

Postscript added in proof: Iwamatsu's published report (Iwamatsu and Ohfuchi, 1959) was received by the present writer after the manuscript of this paper had gone to the editors. The threshold concentration of glycerol for plasmolysis turned out somewhat lower by these authors. Their results were otherwise largely confirmative of the previous and present results reported by the writer, inclusive of the electrolyte effects, except for the effective concentration range of Na_2SO_4 falling very high (about twice as high as the writer's). This was possibly due to their failure to take into account the crystallization water in preparing the molar solution.

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