Plasmolysis in *Paramecium*

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Plasmolysis is a phenomenon that can be observed in plant cells when immersed in a hypertonic solution. Is it possible to observe an analogous phenomenon also in the *Paramecium* cells, which are surrounded by a kind of cell wall known as pellicle? Indeed, one can see a fine example of plasmolysed *Paramecium* illustrated in a certain textbook of general biology,[1] though its source is not given. On the other hand, biologists who are handling *Paramecium* every day seem to believe that a phenomenon of that kind will never take place in their material. They would maintain that a *Paramecium* cell, when immersed in a hypertonic medium, will just shrink with its protoplasmic surface remaining in a close contact with the pellicle, instead of shrinking away from the latter.

Many years ago, Massart (1891) observed in some encysted ciliates that detachment of the protoplasmic body from the cyst wall was brought about by exosmosis, and he compared this phenomenon with that of plasmolysis in plant cells. However, as for protozoans in an active phase, there is a likewise early observation of Balbiani (1898) that hypertonicity of the medium merely caused a shrinkage of the cell as a whole. He coind the term "*plasmorrhyse*" for such a phenomenon of exosmotic shrinkage in protozoan cells. In bacterial cell (*Vibrio proteus*), Garbowski (1907) reported that a phenomenon comparable to plasmolysis was observable.[2]

It seems that in *Paramecium* the phenomenon in question has not yet been recorded in the literature. It was a mere shrinkage of the animal body which was meant by Jennings (1898) with the term "plasmolysis", as he made observations on the chemotactic effects of 0.27 M sucrose and 0.17 M NaCl solutions of *Paramecium*. Fortner (1925) described the cases of *Paramecium* whose surface layer came off from the inner protoplasm when placed in hypertonic (0.33 M～0.37 M) solutions of sucrose. The phenomenon was shown in one of his figures (Fig. 3 of Fortner, 1925) and was interpreted by him as due to imbibition of the cell surface.

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1) G. W. Hunter, H. E. Walter and G. W. Hunter, III: Biology: The Story of Living Things. New York, etc.: American Book Co., 1937, page 136.

2) The term "*Plasmoptyse*" used by Garbowski (1907) refers to a phenomenon to be distinguished from either the plasmolysis or exosmotic shrinkage. In this respect, Kalmus made a mistake in the quotation in his valuable monograph (Footnote on page 122, "Paramecium", Jena, 1931.).

In spite of some resemblance in appearance it seems to me different in nature from plasmolysis. It may be said from Fortner's figure, first of all, that there is nothing like a detachment of the pellicle from the protoplasmic surface to be seen, but apparently the pellicle together with some underlying cortical protoplasm has been teared off from the inner protoplasm. Another resembling effect which, too, appears to be of non-osmotic nature has been described in a recent work of Goldacre (1951), when a phenol solution (10～50% saturated) was applied to *Paramecium*.

The present author recently had a chance to discover the fact that *Paramecium*, under a certain condition, may exhibit a phenomenon which is to be identified as that of plasmolysis. Following are the results of some experiments and considerations which have been made on the phenomenon.

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

Material and Method

Paramecium caudatum reared in straw infusion was used.

The test solution was applied to a population of *Paramecia* on a slide glass, and its effect was examined under the microscope. For this, a dense *Paramecium* suspension was obtained by centrifuging the culture medium with a constant and slow rate of rotation (about 1.4 rotations per seconds, for 7 seconds) of a hand centrifuge. A drop from the suspension was placed on the slide and then excess medium was again pipetted off as thoroughly as possible so as to allow an appropriate number of *Paramecia* (plus a minimum amount of the culture medium) to remain on the slide. Immediately, a drop of the experimental medium was applied on them, and the cells were examined 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.

The experimental media were as follows: sucrose, in solutions of graded concentrations ranging from 0.2 M to 2 M; Na-acetate, NaCl, KCl, KNO_3, $CaCl_2$ and a physiological saline (Herbst's artificial sea water), each in graded concentrations from 0.08 M to 1 M. For the sake of simplicity, all the solutions were used unbuffered, but their pH values were found to be well within the range of neutrality.

Results

Of all the media tested, only NaCl and Na-acetate solutions were

found to cause plasmolysis in *Paramecium*. KCl, KNO_3 and sucrose solutions, which are usually used as the plasmolysing agents for plant cell materials, were without effect on the present material. $CaCl_2$ solutions and the physiological saline were similarly without effect. With KCl and KNO_3 solutions, as well as with the physiological saline, a slight sign of exosmotic flattening was observed in 5 minutes in the solutions of 0.1 M concentration. In the 1 M solutions, an extensive flattening accompanying longitudinal wrinkling of the cell surfaces took place in 20 seconds to 1 minute and the characteristic latticework pattern of pellicular surface was often visible on the surface of the flattened body.

Sucrose solutions also caused the flattening of the *Paramecium* body already in 0.2 M concentration, but a disintegration of the cell body set in after 1 to 3 minutes of immersion in concentrations above 0.4 M. In $CaCl_2$ solutions, the observation was interfered with by the active contraction of the cell induced by the Ca ions, the phenomenen already reported by Kamada and Kinosita (1945).

The lower limit of the concentration inducing plasmolysis was determined to be at 0.2 M for NaCl solutions, and at 0.1 M for Na-acetate solutions (see Tables I and II, and Figs. 1 and 2). In 0.1 M NaCl solution, as well as in 0.08 M Na-acetate solution, *Paramecia* were still

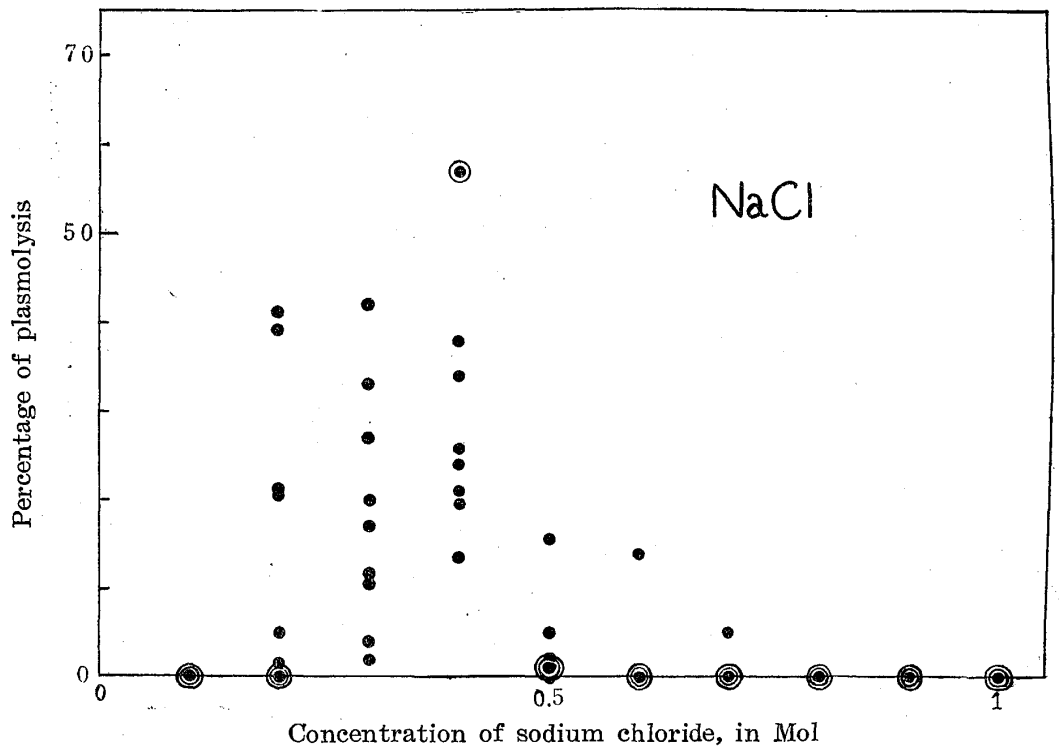

Fig. 1. Percentage numbers of plasmolysed *Paramecia* as obtained with varied concentrations of NaCl solutions. The results of 9 series of tests are plotted in superposition. Circled dot stands for two plots, doubly circled dot for three or more plots.

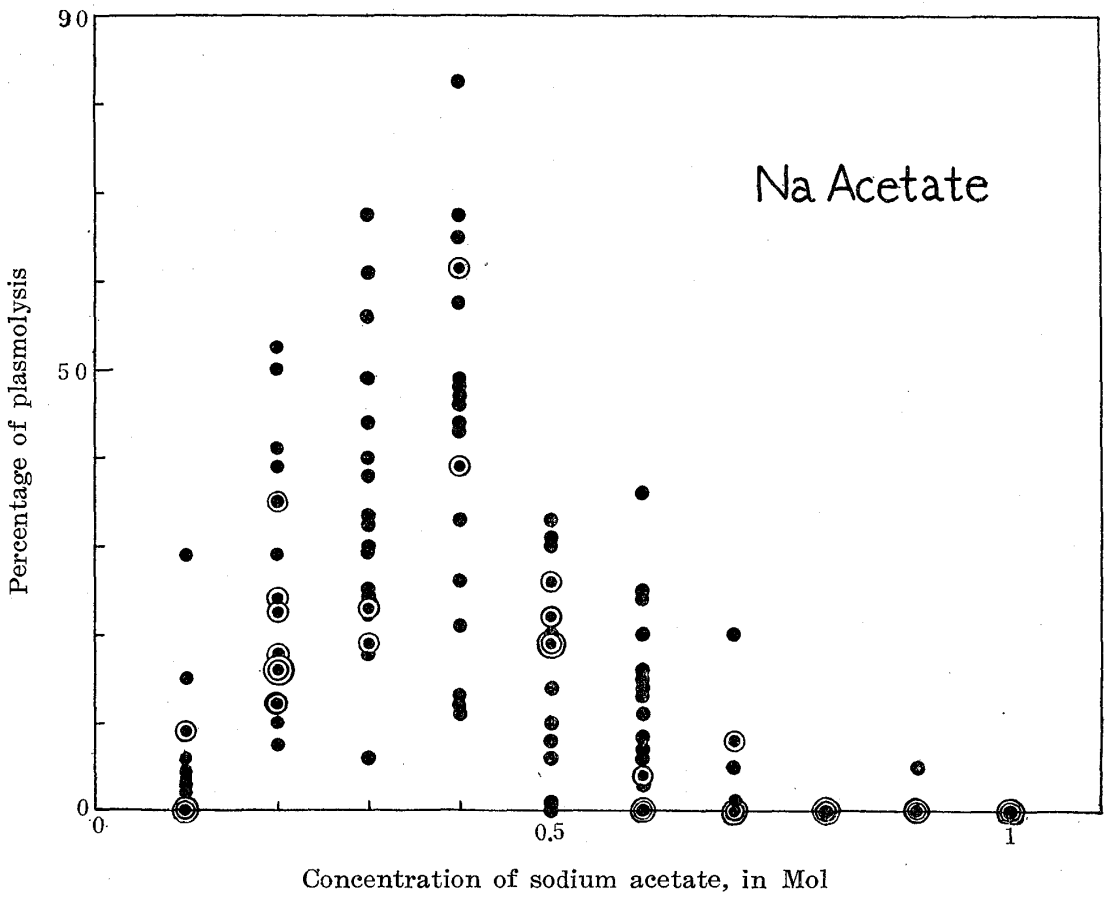

Fig. 2. Percentage numbers of plasmolysed *Palamecia* as obtained with varied concentrations of Na-acetate solutions. The data from 20 series of tests. The notation same as in Fig. 1.

moving about after 20 minutes, without showing any sign of plasmolysis, although there is slight flattening to be noticed. Kamada and Kinosita's (1945) observation of an exosmotic flattening of *Paramecium* in M/8 (=0.125 M) NaCl solution is in accord with the present finding.

Table I. A representative set of data with NaCl solutions (temp. 15°C).

NaCl concentration (Mol)	Number of individuals	Percentage of plasmolysed individuals	Time required for plasmolysis to be complete (minutes)
0.1	62	0	—
0.2	40	5.0	5
0.3	18	16.7	3~5
0.4	58	34.4	3~5
0.5	57	5.2	3
0.6	41	0	—
0.7	57	0	—
0.8	59	0	—
0.9	46	0	—
1.0	38	0	—

Table II. A representative set of data with Na-acetate solutions (temp. 15°C).

Na-acetate concentration (Mol)	Number of individuals	Percentage of plasmolysed individuals	Time required for plasmolysis to be complete (minutes)
0.08	32	0	—
0.1	64	4.6	3~5
0.2	44	15.9	3~5
0.3	37	40.6	3~5
0.4	44	47.7	2~3
0.5	83	25.3	2
0.6	73	20.5	1~2
0.7	100	1.0	0.5~1
0.8	72	0	—
0.9	40	0	—
1.0	24	0	—

In 0.2 M to 0.6 M NaCl solutions there were some percentage of *Paramecia* in which an unmistakable figure of plasmolysis gradually appeared in the anterior end of the body in the course of a few minutes. The rest of the individuals only showed flattening of the body, to a more or less extent according to the concentration of the media. The same was true of the cases with 0.1 M to 0.7 M Na-acetate solutions.

The process of plasmolysis always began with some decrease in the body length about 2 minutes after the application of the NaCl solution, the movements of the animals having been much slowed down at that time. This was followed by detachment of the protoplasmic surface from the pellicle at the anterior end of the body. The protoplasmic surface thus detached had a quite clearcut contour. The pellicle in that region was showing a longitudinal wrinkling on it, and was found to remain as a pointed cap upon the receding head end of the body (see Fig. 3, A and B). After more than 5 minutes of immersion the wrinkles were already disappeared, and the pellicle cap was rounded in outline, thus completing a typical figure of plasmolysis (Fig. 3, C). Similar sequence of events was observed in *Paramecia* immersed in Na-acetate solutions from 0.1 M to 0.7 M. The plasmolysing process went on in these cases apparently rather more rapidly than in the case of NaCl solution. It appeared that the plasmolytic process was not appreciably affected in velocity by a temperature change within the range of 10°~20°C. Concerning reversibility of the process, no inquiry has thus far been made.

It is particularly important here to note that, both with NaCl and with Na-acetate, there was an upper limit to the concentration exhibiting a plasmolysing effect on *Paramecium*. Namely, in NaCl solutions above 0.7 M, and in Na-acetate solutions above 0.8 M, there was no longer plasmolysis taking place. Maximum rate of plasmolysed individuals

was thus obtained at 0.3~0.4 M, both with NaCl and with Na-acetate.

In NaCl solutions of concentration above 0.7 M up to 1 M, *Paramecia* came to show a simple exosmotic flattening within 25 seconds to 1 minute. These flattened *Paramecia* at once ceased all their movements, while the pellicular pattern, which was never observed in the solutions below 0.6 M, made its appearance on their body surface. Exactly the same was true with the case of the *Paramecia* which were

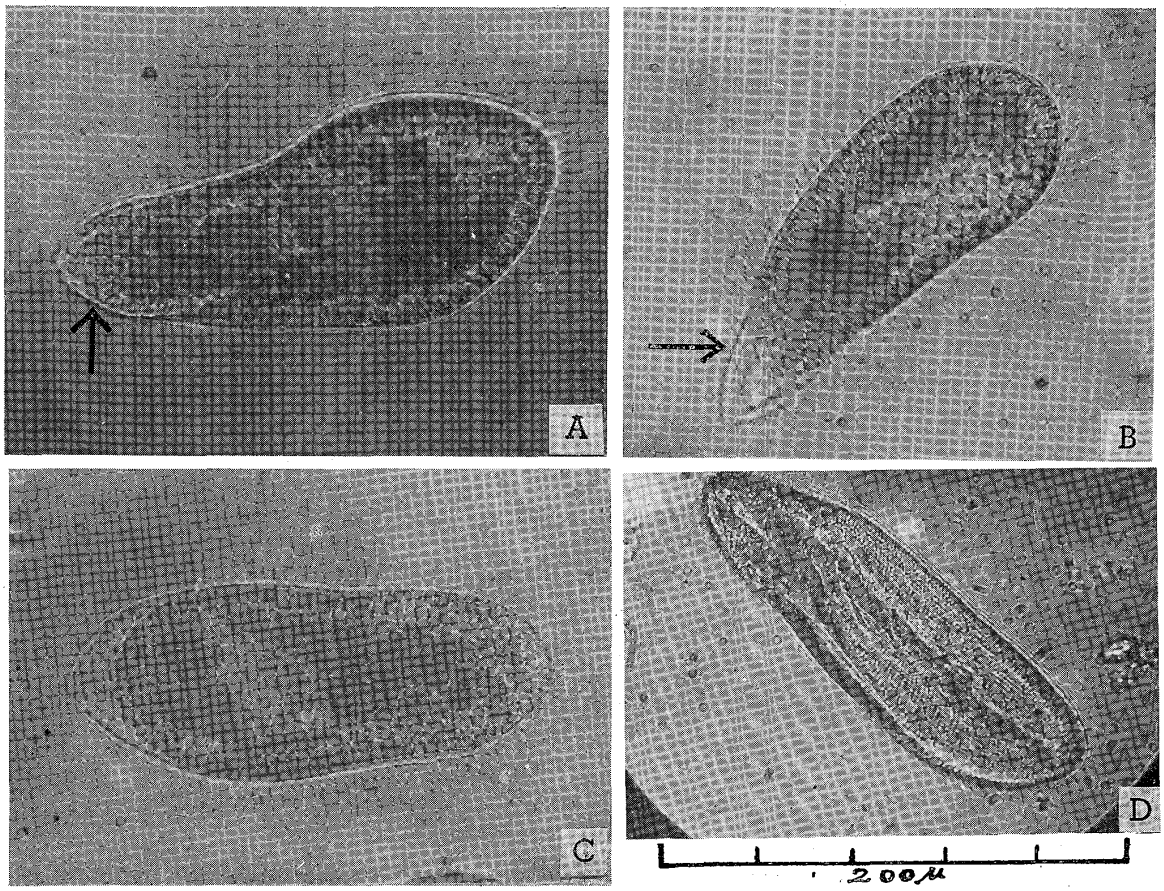

Fig. 3. *Paramecium* in the course of plasmolysing in 0.25 M NaCl solution (A~C), and one exhibiting exosmotic flattening in 1 M Na-acetate solution (D). A: after 3 minutes of immersion, the pellicle wrinkled and pointed, B: after 3~4 minutes, the pellicle being smoothed, C: after more than 5 minutes, the plasmolysis complete, D: after 1 minute in the Na-acetate solution, the pellicular pattern showing.

exposed to the Na-acetate solutions of concentration above 0.8 M (see Fig. 3, D). In the *Paramecia* placed in such concentrated Na-acetate solutions, the cilia were rendered invisible under a magnification of one hundred times, for some unknown reason. This made the pellicle's surface pattern appear even more clearly than in the NaCl solutions. In the latter, the cilia were remaining so well visible that they tended to keep the pellicular pattern from a clearer appearance.

Consideration

Metz *et al.* (1953) recently published an electron-micrographic study on the fine structure of the body surface of *Paramecium*. He confirmed that the pellicle is a structure distinctly separate from the cortical layer of the protoplasm, and that the cilia, as projections from the protoplasmic surface, rise through the minute pores which are arranged over the pellicle, to protrude over the body surface. It is important in considering the pellicle permeability as well as the pellicle detachment to remember that the pores are likely to fit only loosely around the basal parts of the cilia.

The results which have been given in the foregoing pages indicate that, in order to cause a plasmolysis in *Paramecium*, a certain factor is needed besides that of hypertonicity of the external medium. As such factor, one may now reasonably name the Na ions. And, as to the role of the Na ions in causing the plasmolysis, one can suggest their action of decreasing the adhesive power between the pellicle and the protoplasmic surface.

The fact that, in lower concentrations of NaCl and Na-acetate, only exosmotic flattening and not a plasmolysis takes place may either be due to the lower intensity of exosmosis or due to the still insufficient Na ion concentration. A determination of the lowest effective concentration of Na ions with use of mixed solutions of a Na salt and some indifferent substance (such as sucrose), for various fixed values of the external osmotic pressure, may be expected in future.

The existence of an upper concentration limit to the plasmolysing effect of the Na salts may provisionally be interpreted by an assumption that, in the media of higher osmotic pressure, the exosmotic process is already far gone before the pellicle-releasing action of the Na ions becomes effective. One has thereby only to assume in addition that, once the pellicle has reached a statically stable position by the flattening, there is no longer the force which would pull off the pellicle from the protoplasmic surface even when the pellicle comes to be released by the Na ion action. It may be pointed out in this connection that, whereas the plasmolysis in the 0.2 to 0.6 M NaCl solutions required from 3 to 5 minutes for its establishment, the flattening in the 0.2 to 1 M NaCl solutions took place in a distinctly shorter interval of time (25 seconds to 2 minutes). This may be understood in the way that it took more than 3 minutes for the Na ions to permeate the pellicle and to act on the subpellicular layer. A further series of experiments are again to be expected to determine the time factor involved in the Na ion action, controlling by addition of some non-electrolyte the total osmotic concentration, and consequently the velocity of exosmosis, with

the Na ion concentration being kept constant. The maxima found in the plasmolysis percentage-concentration curves are to be explained as due to a resultant of the effects of both the increasing Na ion concentration and of the increasing osmotic value of the medium. Further, in view of the negative result obtained with the use of the balanced saline, it must here be added that the above described action of Na ions seems antagonized by other cations.

There is hardly any doubt that the protoplasmic shrinkage concerned in the present phenomenon does not represent an active contraction as was described by Kamada and Kinosita (1945). According to these authors, the active contraction in *Paramecium* takes place only in media rich in Ca or Sr ions. Moreover, either in their observation or in the present study, the contraction in $CaCl_2$ solution never led to plasmolysis.

The very reason why the plasmolysis in *Paramecium* has not been taken notice by observers in the past is likely to be in the fact that it is an event which takes place under so narrowly limited conditions.

Summary

1. A phenomenon identified as plasmolysis was found to be obtainable in *Paramecium*, when immersed in NaCl solutions from 0.2 M to 0.6 M or in Na-acetate solutions from 0.1 M to 0.7 M.

2. In solutions of the same salts either above or below the said concentration ranges, there may be exosmotic flattening of the cell body induced, but no plasmolysis at all.

3. Hypertonic solutions of KCl, KNO_3, $CaCl_2$, artificial saline and sucrose were never found to have such a plasmolysing effect.

4. Possible mode of action of the Na ions in causing plasmolysis in *Paramecium* was discussed.

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