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An Analysis of Amoeboid Movement
III. Ionic Effect on the Mechanical Properties of Surface Structure of Amoeba

With 9 Text-figures

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(*Communicated by* H. KINOSITA)

It was found in the previous study that a certain elasticity gradient of surface structure existed along the longitudinal axis of monopodial amoeba which was in active locomotion, the elasticity being the highest at the posterior and the lowest at the anterior region (Kanno, 1964a). Such a gradient of surface structure has also been found to exist by recent workers (Allen and Roslansky, 1959; Allen, 1960; Yagi, 1961). Moreover the effects of chemical agents on the rigidity of surface structure have been quantitatively investigated by Heilbrunn and Daugherty (1932), Brinley (1928) and Mast and Prosser (1932). An extensive study on the effects of various ions on the 'plasma gel' was made by Heilbrunn and Daugherty (op. cit.). They proposed that calcium ions increase the elasticity of surface structure.

In the present study the effects of chemical agents on the elasticity of surface structure were investigated by the suction method (Kanno, op. cit.), using a single organism and the significance of calcium ions on the maintenance of surface elasticity was especially discussed.

MATERIAL AND METHOD

A clone of *Amoeba* (*proteus* type), cultured in rice grain medium (Kanno, 1964b) was used. The surface elasticity was represented by the reciprocal of the length of the surface structure pulled in a glass capillary (diameter: 15 micra) applied locally on the cell (Kanno, 1964a). Measurement was made usually three seconds after application of sucking force of 1.18×10^{-2} dynes, when the length of pulled part became steady. The amoeba, adapted for about four hours in inorganic culture medium* (Kanno 1964b) or control solution, was introduced into a test solution and time change of the surface elasticity was followed. Test solutions used were isotonic solutions of $CaCl_2$ (M/750), KCl (M/500), NaCl (M/500), $MgCl_2$ (M/750), $SrCl_2$ (M/750), $BaCl_2$ (M/750) and of Na-citrate (M/1000). Effect of 10^{-3} and 10^{-4} M EDTA, a strong Ca-chelating agent, was also examined. pH values of test solutions ranged

* The medium is composed of 0.12 g of NaCl, 0.003 g of KCl and 0.005 g of $CaCl_2$ dissolved in one litre of deionized pure water. Osmotic concentration and pH of the medium were M/500 NaCl eq. and 6.0 respectively.

from 5.7 to 7.2, although such a difference in pH was not found to affect the surface elasticity in this work. Before the experiment, the survival times of animals in each solution were measured in order to test the toxicity of the agents on the organism. Since the mechanical property of the anterior region of amoeba has been known to be altered even by a slight mechanical stimulation (Kanno, 1964a), the elasticity of surface structure was measured chiefly at the middle region except where noted. Comparison of the effect of each test solution with that of control solution was carried out on one and the same cell at 20°–24°C.

Results

1. *Survival of organisms in various test solutions.*

Ten organisms, washed repeatedly by deionized water, were immersed in a test solution, and the number of survivals was counted from time to time. Such a measurement was repeated ten times for each kind of test solution. The results are summarized in Table 1 in which the degree of survival is expressed in percentage of the number of survivals. Most of the animals were found to

Table 1
Percentage of survival of amoebae in various test solutions

Test solution	Time of immersion									
	10 min.	20 min.	30 min.	1 hr.	2 hrs.	4 hrs.	8 hrs.	16 hrs.	32 hrs.	64 hrs.
Inorganic culture medium (control solution) pH:6.0	100	100	100	100	100	100	100	100	100	89
10^{-3} M Na-citrate pH:7.2	100	100	100	100	95	89	63	61	47	33
Ca-free inorganic culture medium pH:6.0	100	100	100	100	100	85	85	80	50	30
M/750 $CaCl_2$:Ca-free inorg. culture medium										
1:3 pH:6.3	100	100	100	100	100	100	100	100	92	42
1:1 pH:6.5	100	100	100	100	100	92	92	85	78	70
3:1 pH:6.6	100	100	100	100	100	82	82	77	47	40
M/750 $CaCl_2$ pH:6.7	100	100	100	100	100	91	78	73	70	54
M/500 KCl pH:6.8	100	100	100	100	95	70	62	18	0	0
M/500 NaCl pH:6.6	100	100	100	100	100	95	77	50	37	20
M/750 $MgCl_2$ pH:7.0	100	100	100	100	100	100	80	50	45	35
M/750 $SrCl_2$ pH:5.7	100	100	100	100	100	100	90	76	50	32
M/750 $BaCl_2$ pH:5.7	100	100	100	100	100	66	52	30	0	0
10^{-3} M EDTA pH:6.5	100	100	100	72	62	37	27	0	0	0
10^{-4} M EDTA pH:6.5	100	100	100	100	100	85	50	30	0	0

advance normally at least within two hours in these solutions except in 10^{-3}M EDTA solution in which the organism shrank gradually and became round.

2. *Effects of calcium ions on the elasticity of surface structure.*

At first the elasticity of surface structure of amoeba was measured in the inorganic culture medium. The medium was completely replaced with a test solution, taking care not to remove the cell from the bottom of the trough. The elasticity was measured in each of the mixtures of M/750 $CaCl_2$ and Ca-free inorganic culture medium, the mixture ratio being 0 to 100, 0.2 to 99.8, 0.4 to 99.6, 0.8 to 99.2, 1.6 to 98.4, 6.6 to 93.4, 13.2 to 86.8, 19.8 to 80.2, 26.4 to 73.6, 50 to 50, 75 to 25 and 100 to 0*.

Effect of Ca-depletion, given by Ca-free inorganic culture solution is shown in Figure 1. The figure clearly demonstrates that the elasticity of surface structure is lowered reversibly in Ca-free solution. Such an effect was found to be less

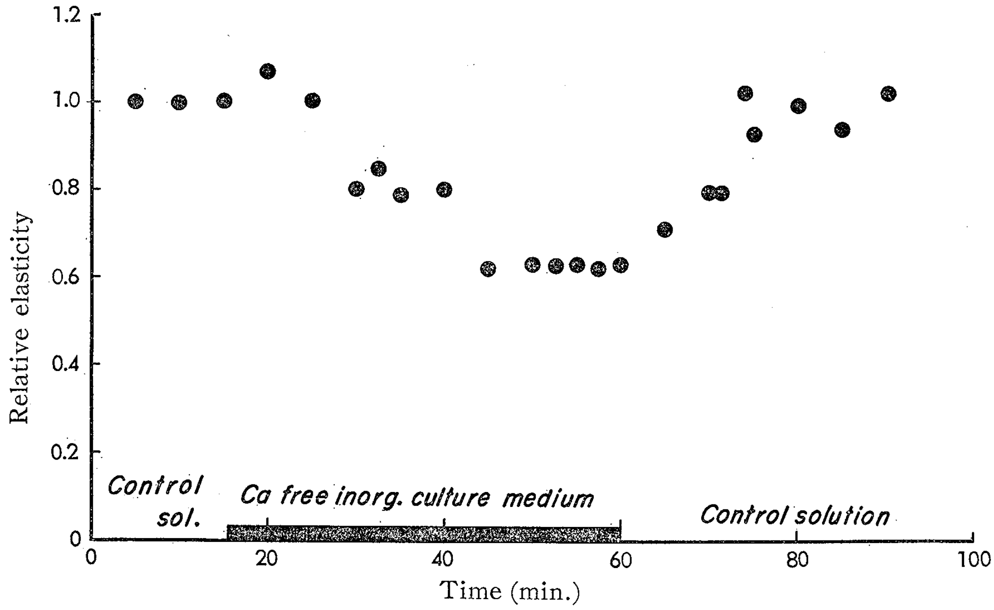


Fig. 1. One of the typical results showing the reversible change in the surface elasticity at the middle region of amoeba immersed in Ca-free inorganic culture medium. Thick solid line on the abscissa indicates time of application.

marked in the posterior region than in the middle part. Yet noteworthy effects of Ca-deficiency were found in the cell immersed in 10^{-4}M EDTA solution, as is shown in Figure 2. Elasticity decreased rather rapidly, though the organism continued normal locomotion. When immersed in 10^{-3}M EDTA, the shrinkage of surface occurred and the animal soon assumed a spherical form with a number of small pseudopodia on it. Figure 3 shows the reversible change in elasticity of surface structure of an animal immersed successively in 10^{-3}M and in 10^{-4}M EDTA. It will be seen that the surface elasticity of the rounded cell is almost the same as that of the posterior region measured in the control solution. It is also shown in the figure that the surface elasticity of the posterior region is less

* In the inorganic culture medium the ratio was 3.3 to 96.7.

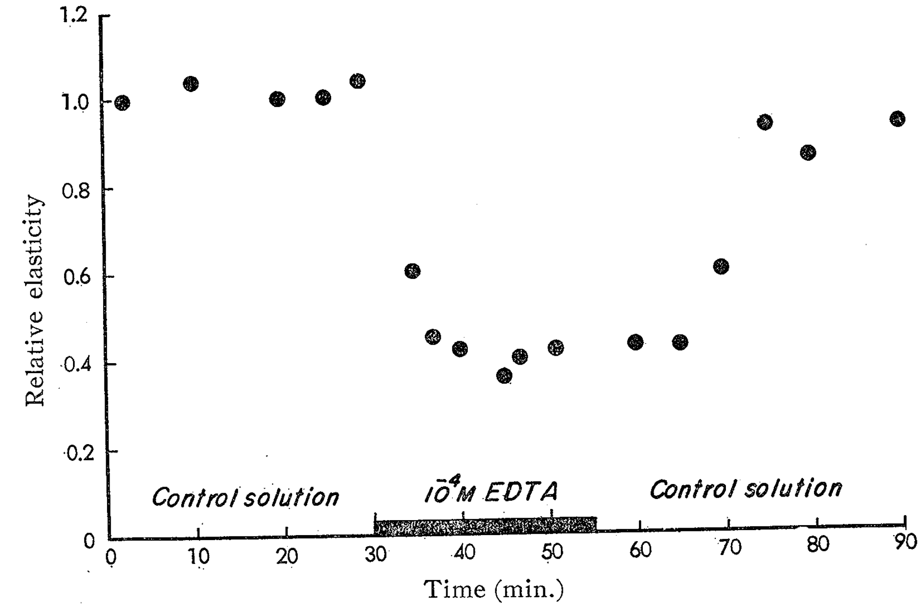


Fig. 2. Change in elasticity of surface structure of amoeba immersed in 10^{-4} M EDTA solution. Thick solid line on the abscissa indicates time of application.

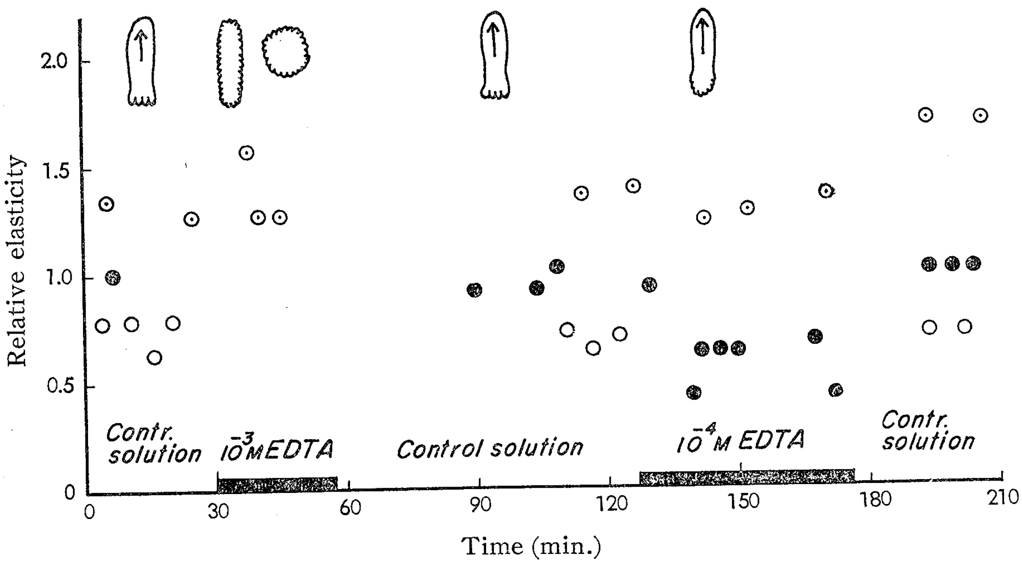


Fig. 3. Change in elasticity of surface structure of amoeba in 10^{-3} M and 10^{-4} M EDTA solution. Hollow circles; anterior region, solid circles; middle region, double circles; posterior region. Thick solid lines on the abscissa indicate times of immersion. The diagrams at the top indicate the change in shape and movement (arrow) of amoeba.

affected by EDTA than that of the middle region. In 10^{-4} M EDTA the anterior region of amoeba came to be cut off by sucking force more readily than in the control solution. Another effect of Ca-depletion was found when the organism was immersed in M/1000 Na-citrate solution. The animal became round as in the case of 10^{-3} M EDTA, but after a short period it began to move normally. The surface elasticity of the middle part decreased reversibly as in the case of

Ca-free inorganic culture medium or of 10^{-4} M EDTA, while that of the posterior region did not change in this medium as is shown in Figure 4.

Contrary to the above-mentioned results on the effect of Ca-deficiency, the

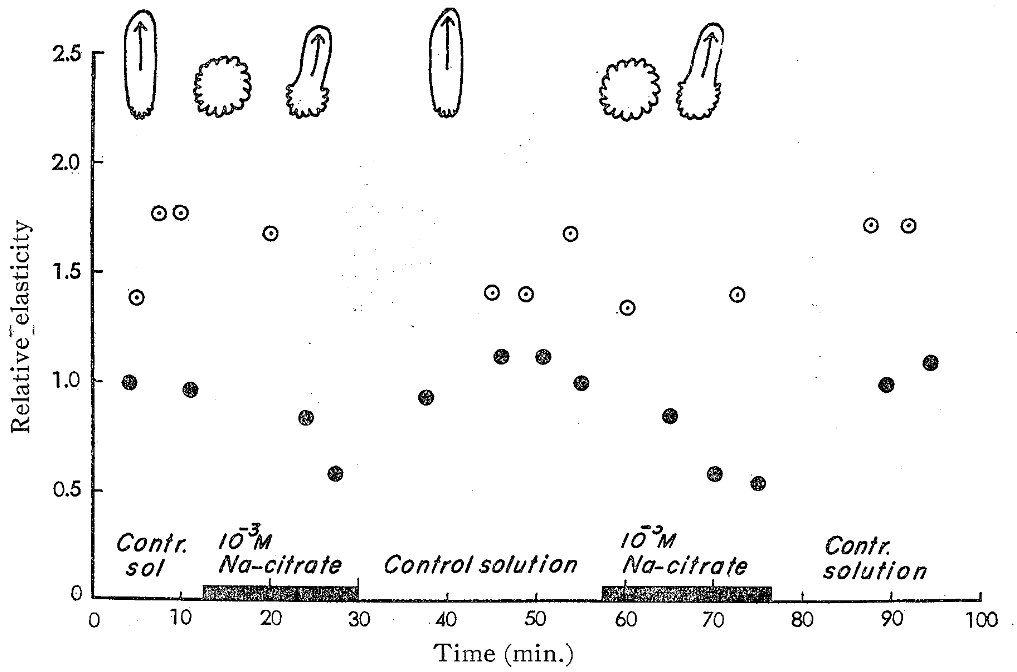


Fig. 4. Repeated change in the surface elasticity of the organism immersed in M/1000 Na-citrate solution. Solid circles; middle region, double circles; posterior region. The diagrams at the top indicate the change in shape and movement (arrow) of amoeba.

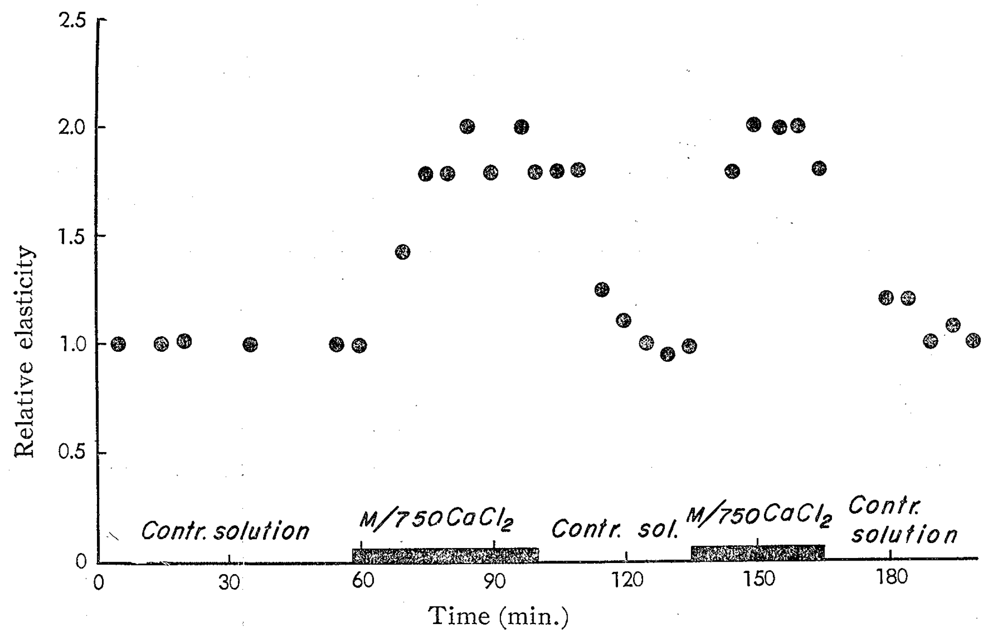


Fig. 5. Repeated change in the surface elasticity of amoeba immersed in M/750 $CaCl_2$ solution. Thick solid lines on the abscissa indicate the time of application.

surface elasticity of amoeba immersed in M/750 $CaCl_2$ solution was found to increase reversibly (Fig. 5), while the shape of the moving organism in this solution remained normal.

The changes in surface elasticity of amoeba immersed in solutions containing

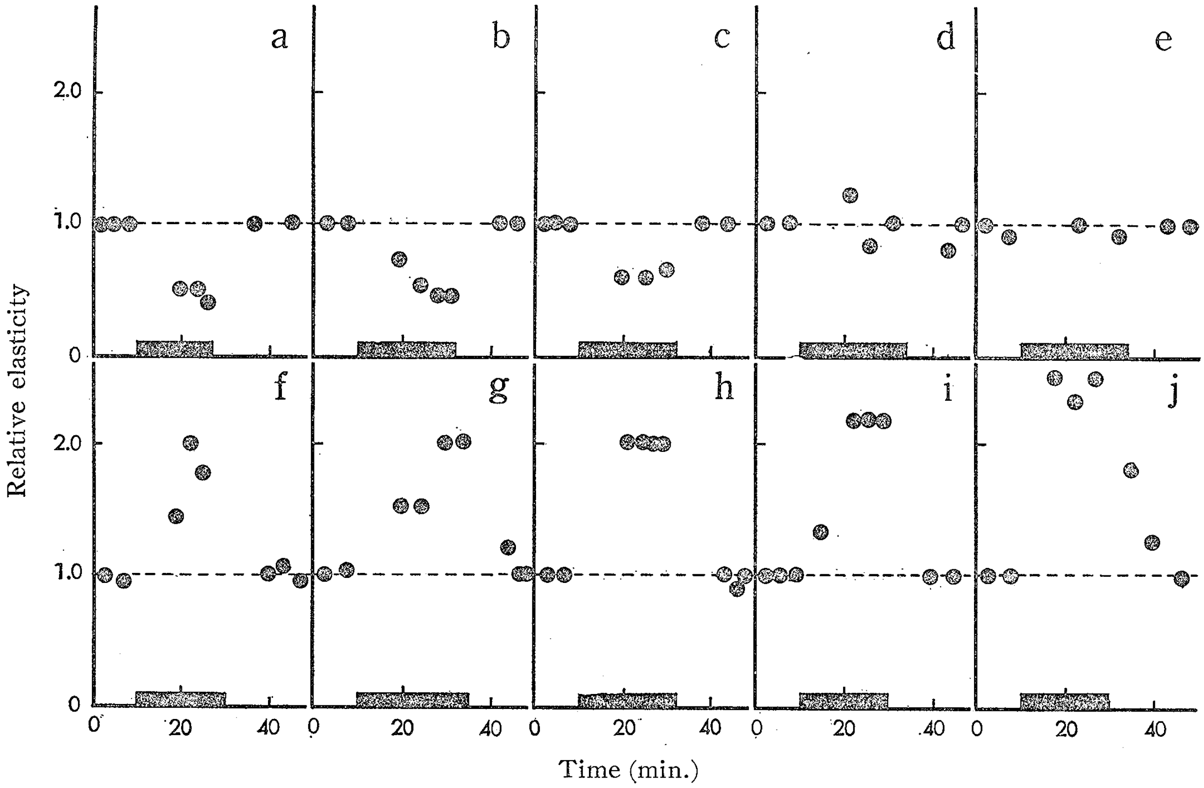


Fig. 6. Change in elasticity of surface structure of amoeba in the solutions containing $CaCl_2$ of various concentration. Solutions a, b, c, d, e, f, g, h, i and j were prepared by mixing M/750 $CaCl_2$ solution with inorganic Ca-free culture medium in the following ratios.

Experimental solution	Mixture ratio		Concentration of $CaCl_2$ in 10^{-6} M
	M/750 $CaCl_2$ solution	Inorganic Ca-free solution	
a	0.2	99.8	2.8
b	0.4	99.6	5.5
c	0.8	99.2	11.0
d	1.6	98.4	22.0
e	6.6	93.4	89.0
f	13.2	86.8	177.0
g	19.8	80.2	266.0
h	26.4	73.6	354.0
i	50.0	50.0	666.0
j	75.0	25.0	999.0

pH of these experimental solutions was 6.0. Thick solid lines on the abscissae indicate times of immersions.

$CaCl_2$ of various concentrations are shown in Figure 6a-j.

The results show that the mixture containing $CaCl_2$ in concentration four times higher than that in the control solution* was enough to induce a remarkable increase in the surface elasticity, the degree of the elasticity increase being nearly unaffected by further increase in calcium concentration (Fig. 6f-j). On the other hand, the elasticity decreased markedly when the calcium concentration of the medium is less than one-fourth of that of the control solution (Fig. 6a-c).

3. *Effects of other divalent cations on the elasticity of surface structure.*

Isotonic solutions of some of the divalent salts, namely, M/750 $SrCl_2$, M/750 $BaCl_2$ and M/750 $MgCl_2$ solutions, were used for tests. The animal kept normal shape and locomotion in these media, however, contrary to the case of M/750 $CaCl_2$ solution, the surface elasticity underwent clear and reversible decreased as is shown in Figure 7.

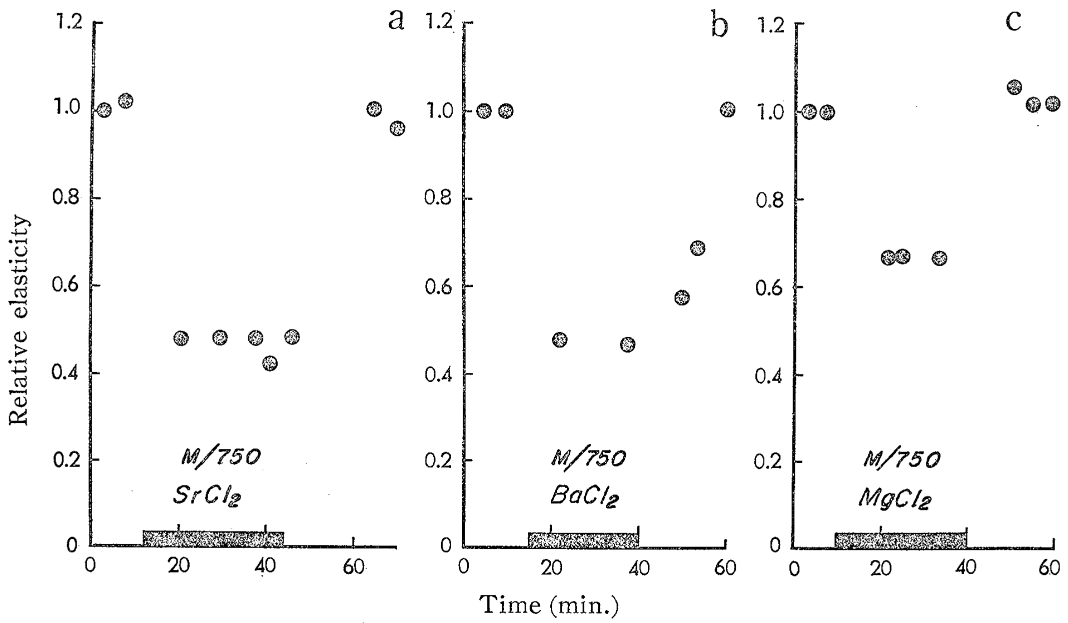


Fig. 7. Reversible changes in elasticity of surface structure of the amoeba immersed in various divalent salt solution. a, M/750 $SrCl_2$; b, M/750 $BaCl_2$ and c, M/750 $MgCl_2$. Thick solid lines on the abscissae indicate times of immersions in the test solutions.

4 *Effects of sodium and potassium ions on the elasticity of surface structure.*

The surface elasticity of amoeba decreased markedly and reversibly in solutions of isotonic KCl (M/500) and NaCl (M/500) as is shown in Figures 8a and 8b. The organism became gradually inactive in isotonic solution of KCl, while the animal advanced normally in isotonic solution of NaCl, which was the chief component of the inorganic culture medium. The surface elasticity of the posterior region was found to be less affected by M/500 KCl than that of the middle region as was the case with EDTA and Na-citrate solution.

* Concentration of $CaCl_2$ in control solution was 44×10^{-6} M.

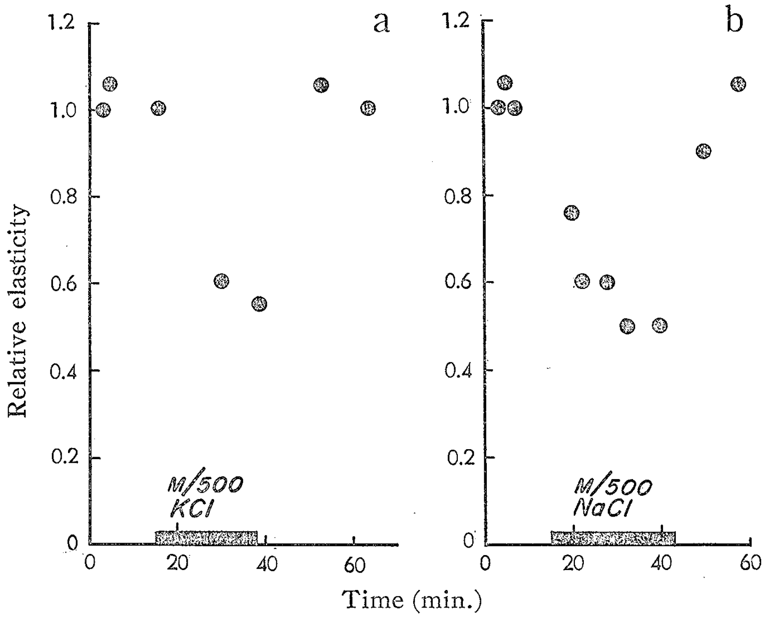
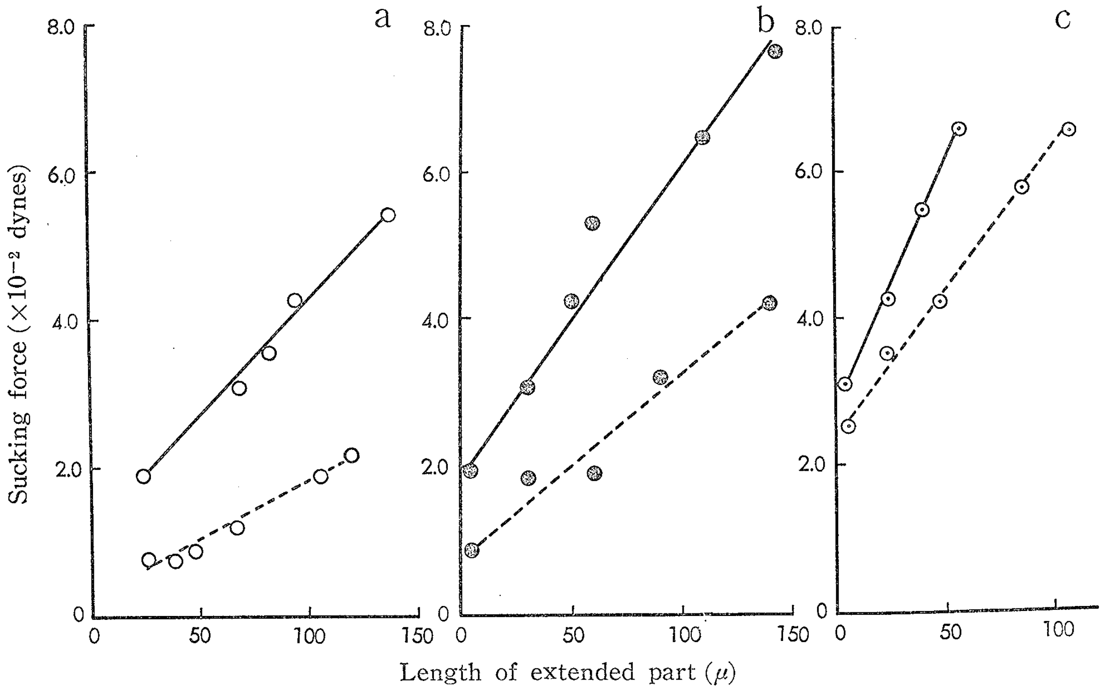


Fig. 8. Reversible changes in elasticity of surface structure of amoeba immersed in; a, M/500 KCl solution and in; b, M/500 NaCl solution. Thick solid lines on the abscissae indicate times of applications.

5. Relationship between applied negative pressure and length of extended surface structure.

Relationship between the value of applied negative pressure and the length



Eig. 9. Relation between applied negative pressure and the length of extended surface structures measured on a single specimen. Broken lines indicate the results on inorganic culture media, while solid lines represent those on M/750 $CaCl_2$. a (hollow circles), anterior region; b (solid circles), middle region; c (double circles), posterior region.

of extended surface structure was examined in order to obtain more quantitative information on the mechanical property of surface structure in various conditions. Suction was applied at various regions of one and the same organism immersed both in the inorganic culture medium and in M/750 $CaCl_2$ solution for more than one hour (Fig. 9a, b and c). The figure indicates that the length of extended surface structure was directly proportional to the applied pressure and that the slope of the length-force lines, which may well represent the elastic property of the surface structure, in the case of M/750 $CaCl_2$ was about twice as steep as in the control solution, irrespective of the region selected for measurement. Therefore, it may be said that calcium ions affected evenly whole surface of the cell, resulting in increase of its elasticity.

Discussion

Suction method used in this experiment was considered to be a favourable one for measuring the elasticity of surface structure of advancing amoeba as was already refered to in the previous paper (Kanno, 1964a). The method was especially suitable for the present study because it enabled the measurement of the time-change of the local surface elasticity of a single animal immersed in various kinds of salt solutions. The surface structure of a certain region, relatively fixed to the cell, was always found to be pulled to the same extent by repeated application of a constant negative pressure, although the structure itself was always circulating (Kanno, 1964a).

In this experiment, measurements were made mainly on the middle region of amoeba because of the following reasons. The anterior region was ready to respond to the weak contact stimulation given by a glass capillary, resulting in the momentary cessation of locomotion (Kanno, 1964a), while wrinkles of the surface usually prevented the capillary tip from tightly attaching to the cell surface, if weak negative pressure, such as applied in most of the present experiment, is applied through the glass capillary attached to the posterior surface.

The result obtained on the increase in surface elasticity as an effect of excess Ca ions coincided well with those reported by former workers (Heilbrunn and Daugherty, 1932, 1934, Brinley 1928), although their methods were quite different from the present one.

The elasticity of surface structure decreased markedly in Ca-free medium. The surface rigidity remained normal, if the concentration of calcium ions in the medium was between 2.2×10^{-5} M and 8.9×10^{-5} M.

Contrary to the general effect of Ca-deficiency, an increase in surface elasticity was found in a stronger (10^{-3} M) solution of EDTA, a Ca-chelating agent. In this solution the animal gradually took a rounded shape, similar to one induced by repeated mechanical stimulation. According to Yagi (1961), amoebae, rounded by repeated mechanical agitation, lose their intracellular water, resulting in gelation and marked decrease in cell volume. The increase in surface elasticity in 10^{-3} M EDTA solution observed in the present study may be due to the gelation occurring similarly in such spherical cells.

Though magnesium, barium and strontium ions are divalent and alkaline as are calcium ions, these ions reduced the elasticity of surface structure. Similar effects were also found in the case of potassium and sodium ions. However, it is still unknown whether these divalent and monovalent ions act on the cell by themselves or whether the effect is due to Ca-deficiency.

It has been reported in the previous paper (Kanno, 1964a) that the relation between negative pressure applied and extended length of cell surface structure is linear. This relation was also confirmed in the present experiment in which amoeba was immersed in M/750 $CaCl_2$ solution. It may be considered that the inclination of the lines is one of the exact expressions of surface elasticity of amoeba. Similar extent of increase in the surface elasticity of three different regions of the animal transferred to M/750 $CaCl_2$ solution from control solution might clearly indicate that calcium ions act on the whole surface structure.

In the study of the effects of ions on the surface elasticity of amoeba, all the former workers (Chambers and Reznikoff, 1926; Heilbrunn and Daugherty, 1932) attempted to use solutions which are considerably hypertonic, namely, M/60 $CaCl_2$, M/60 $MgCl_2$ and M/40 KCl etc. In such hypertonic solutions animals used in the present study became wrinkled and rounded until they cytolysed within an hour. However, the order of the degree of toxicity of test solutions determined in this work, namely 10^{-3} M EDTA, M/500 KCl, 10^{-4} M EDTA, M/750 $BaCl_2$, M/500 NaCl, M/750 $SrCl_2$ and M/750 $CaCl_2$, was found to be not much different from those obtained by these earlier works.

It has been frequently found that amoeba advanced quite normally in a certain test solution, in which the surface elasticity of cell underwent a definite change. Therefore it may be said that the elasticity of surface structure, at least of the middle region does not play an important roll in amoeboid movement, contrary to the view supported by many workers (e. g. Mast, 1926), that the contraction of the ectoplasmic gel at the middle or posterior region drives the cell forward.

Although the mechanism of hardening or softening the surface structure has not yet been known, it may be considered that the effect is superficial, because the change is reversible and occurs within a considerably short period.

Summary

1. Elasticity of local surface structure of amoeba immersed in various solutions was measured, using suction method.

2. Calcium ions in higher concentrations caused a reversible increase in elasticity of surface structure, while calcium deficiency induced a reversible decrease.

3. It was considered that Ca-ions acted evenly on the whole cell surface.

4. Potassium, sodium, magnesium, strontium and barium ions caused decrease in surface elasticity.

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References

Allen, R. D. 1960 J. Biophys. and Biochem. Cytol., **8**, 379.

Allen, R. D. and J. Roslansky 1959 ibid., **6**, 437.
Brinley, F. J. 1928 Protoplasma, **4**, 177.
Chambers, R. and P. Reznikoff 1926 J. Gen. Physiol., **8**, 369.
Heilbrunn, L. V. and K. Daugherty 1932 Physiol. Zool., **5**, 254.
——— and ——— 1934 J. Cell. Comp. Physiol., **5**, 207.
Kanno, F. 1964a Annot. Zool. Japon., **37**, 12.
——— 1964b ibid., **37**, 1.
Mast, S. O. 1926 J. Morphol. and Physiol., **41**, 347.
——— and C. L. Prosser 1932 J. Cell. Comp. Physiol., **1**, 333.
Yagi, K. 1961 Comp. Biochem. Physiol., **3**, 73.