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The relation of inorganic salts to growth and reproduction in *Amoeba proteus*[1].

By

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(With 8 figures in the text.)

Introduction.

In recent years *Amoeba proteus* has been used extensively for the study of the elementary properties of protoplasm. CHAMBERS and REZNIKOFF (1926), REZNIKOFF (1926), REZNIKOFF and CHAMBERS (1927) and CHAMBERS and HOWLAND (1930) from results obtained by microinjection studies on *Amoeba* claim that monovalent salts tend to solate while divalent salts tend to gelate the protoplasm. BRINLEY (1928) using Brownian movement as a means for determining solation and gelation in *Amoeba*, agrees with CHAMBERS, etc. However, contrasting results have been obtained by HEILBRUNN (1923), and HEILBRUNN and DAUGHERTY (1931) who claim that the salts have just the opposite effect on arbacia and amoeba protoplasm to that described by CHAMBERS, etc. EDWARDS (1923), PANTIN (1928), and HOPKINS (1929) studied the effects of various salts on locomotion in *Amoeba* but like the work of CHAMBERS and HEILBRUNN, deal with immediate effects.

In their immersion experiments, CHAMBERS and REZNIKOFF have shown that amoebae may live for over five days in salts of certain concentrations but they did not follow out the length of life for

[1]) The writer wishes to acknowledge his indebtedness to Prof. D. L. HOPKINS who suggested the problem and under whose supervision the work was done.

more than five days. Mast (1931) has shown what effects various concentrations of certain salts have on length of life in *Amoeba proteus*. The work was carried out without the presence of food in the solutions.

The purpose of the writer's work was to ascertain the effects of certain salts in various concentrations, on growth and reproduction in *Amoeba proteus*. In order to obtain these effects, it was necessary that food be present in the media.

Material and methods.

Amoeba proteus was used throughout the investigation described in this paper. At first, it was necessary to obtain a medium in which the amoebae grew well. Various spring waters were used as culture media and the one which supported growth best was taken for analysis. General quantitative methods were used in this procedure. After several changes in the salts found by the analysis the medium shown in Table 1 was devised and used in all the experiments, varying only in the salt that happened to be under investigation.

Table 1.

The inorganic composition of the culture medium used as control throughout the experiments.

Na_2SiO_3	16.4 mg
NaCl	12 „
Na_2SO_4	6 „
$CaCl_2$	6.5 „
$MgCl_2$	3.5 „
$FeCl_3$	8 „
Dist. H_2O to	1000 cc.

All the salts used were C. P. Baker's analysed. The water which was used was distilled three times as described by Hopkin's (1928). In the distillation and in mixing the solutions only pyrex glass was used. The hydrogen ion concentrations of the cultures were taken colorimetrically by a La Motte apparatus, model 5 B, and electrometrically by a Leeds and Northrup quinhydrone outfit.

All the experiments were performed in the following manner: Using 125 cc. pyrex-glass flasks, ten cultures of each of the different concentrations of the salt under investigation were prepared, 50 cc. of the medium being put into each flask. Each culture contained the salt under question in the specified concentration plus all the other salts in the concentrations given in Table 1. The number of

cultures, the results from which the curves to follow represent, is equal to the number of points represented on each curve multiplied by ten. Thus the curves of Fig. 1 represent the results from eighty cultures. To each culture were added fifty amoebae taken from a clone culture. Practically equal numbers of chilomonas were put into the cultures as food for the amoebae. One grain of wheat was added to each culture, care being taken to get grains of equal size, at least so far as the eye could detect. Naturally it would be impossible to get exactly the same amount of organic material in each culture by this method, and the variation in each wheat grain would be a probable cause for error in the experiments. After the cultures were inoculated they were plugged with cotton and placed in a dark constant temperature box. The temperature was held between 20° C and 23° C by circulating water around the outside of the compartment and by means of a thermo-regulator within. The amoebae were allowed to grow and multiply for four weeks, then the cultures were taken out and the numbers of amoebae per cubic centimeter of culture media ascertained.

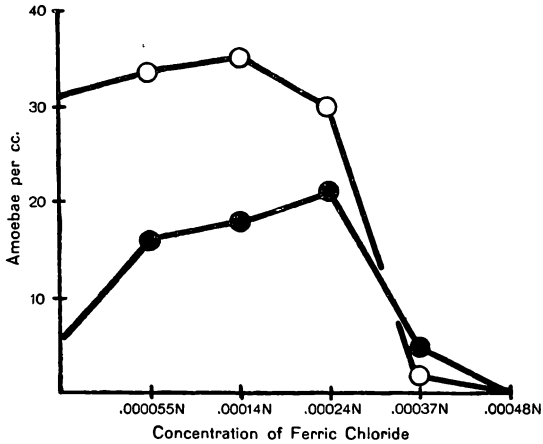

Fig. 1. Graph showing the relation between the concentration of ferric chloride and growth and reproduction in *Amoeba proteus*. ● first experiment; ○ second experiment.

In counting, the contents of the flasks were stirred well and 1 cc. of the culture was quickly transferred to a syracuse watch dish by means of a graduated pipette. The amoebae were then counted under a dissecting microscope. Three to five counts were made for each culture. The hydrogen ion concentration was taken for all solutions at the beginning and at the end of each experiment.

Results.

Ferric chloride: Cultures were set up containing different concentrations of ferric chloride ranging from 0 to .00048 N. There was some iron present in the wheat which was added to the cul-

tures so that it was impossible to obtain an absolute iron deficiency. Later (about ten months) this experiment was repeated. The results obtained from both are given in Fig. 1.

It will be noted that the concentrations of ferric chloride given in the figure are the concentrations that exsisted immediately after mixing the solutions but because of the hydrolysis of the ferric chloride these would not be the correct concentrations. An analysis was made to determine the amount of ferric chloride that remained in solution, the results of which are given in Table 2.

Table 2.

Showing the relation of the concentration of iron remaining in solution after hydrolysis to the concentration actually added.

Concentration of $FeCl_3$ put into solutions	Concentration of $FeCl_3$ after hydrolysis
.000055 N	.0000031 N
.00014 N	.0000065 N
.00024 N	.000001 N
.00037 N	.0000013 N
.00048 N	.000019 N
.00053 N	.000066 N
.0007 N	.00013 N

According to the analysis and the results of the experiment, ferric chloride in concentrations above .00001 N is decidedly toxic to growth and reproduction.

No attempt was made to buffer the solutions so that due to the hydrolysis of the ferric chloride in the higher concentrations, the hydrogen ion concentration was high. This was noted in the first experiment so that when it was repeated, several control series were set up with hydrogen ion concentrations varying about the same as the cultures containing ferric chloride, but no ferric chloride was added to these controls. The cultures were brought to the desired hydrogen ion concentration by addition of hydrochloric acid. A comparison of the results obtained from the controls and from the ferric chloride experiment is given in Table 3.

It will be seen that while the hydrogen ion does have a retarding effect, the retardation is not as great as in the cultures containing $FeCl_3$ and having the same H-ion concentration.

Magnesium chloride: Ten series of cultures were set up, the concentration of magnesium chloride ranging from 0 to .021 N. This salt as well as all the others used in this investigation occurs

Table 3.

Showing the relation between solutions of high H-ion concentrations containing iron to solutions of the same H-ion concentrations containing no iron.

	Results of $FeCl_3$ experiment			Control (no iron present)		
Conc. of $FeCl_3$	H-ion conc. when cultures were set up p_H	H-ion conc. at end of experiment p_H	Number of amoebae per cc.	H-ion conc. when cultures were set up p_H	H-ion conc. at end of experiment p_H	Number of amoebae per cc.
.000013 N	5.4	6.4	2	5.2	6.3	21
.000019 N	4.2	6.1	0	4.4	6.0	11
.000066 N	3.8	5.0	0	3.9	5.6	14
.00013 N	3.2	4.6	0	3.2	5.9	7

to some extent in wheat so that small quantities were probably present in the cultures to which magnesium chloride had not been added. The hydrogen ion concentrations of the solutions when the cultures were set up, were between p_H 6.6 and 6.8. At the end of the experiment the hydrogen ion concentrations were still between p_H 6.6 and 6.8. The results are given in Fig. 2. It will be noted that two optima occur in the growth curve. At first it was thought that this was due to an experimental error but in the other salt experiments and in the second magnesium chloride experiment (Fig. 2) two optima also occur. This second magnesium chloride experiment was carried out exactly as the first. The hydrogen ion concentrations were the same as in the previous experiment.

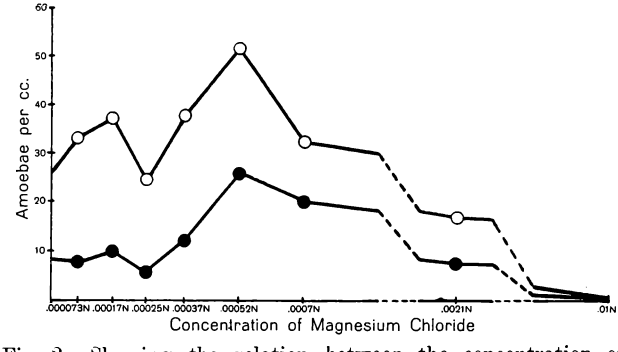

Fig. 2. Showing the relation between the concentration of magnesium chloride and growth and reproduction in *Amoeba proteus*. ● first experiment; ○ second experiment.

In all the later experiments a larger number of amoebae were obtained than in the earlier experiments. This was probably due to the higher rate of reproduction and growth in the stock cultures which were used in the later experiments.

Potassium chloride: Two experiments of ten series each were carried through, with each series ranging in concentration from 0 to .013 N potassium chloride. The hydrogen ion concentrations of the solutions of both experiments were all between p_H 6.6 and 6.8 when the cultures were set up and remained so throughout the experiments. The results of both are given in Fig. 3.

Again, as in the case of magnesium chloride there is first an increase in numbers of amoebae, then a decrease followed by another increase to an optimum. The numbers of amoebae drop off to zero

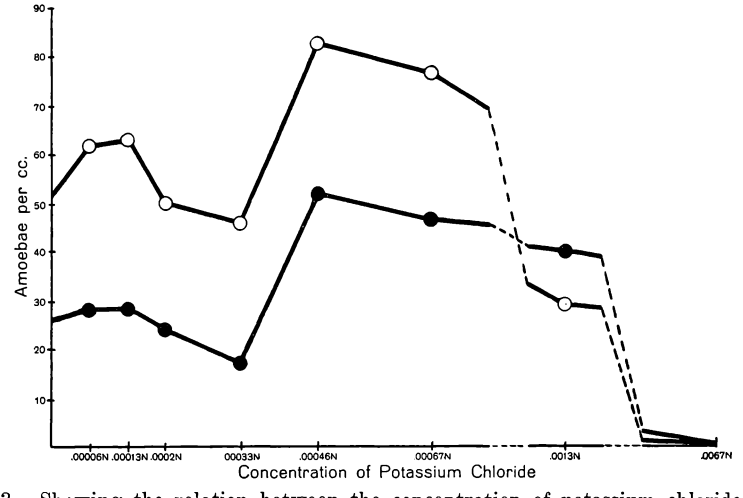

Fig. 3. Showing the relation between the concentration of potassium chloride and growth and reproduction in *Amoeba proteus*. ● first experiment; ○ second experiment.

in the cultures containing .0067 N potassium chloride. However, in these cultures of higher salt concentration the numbers of chilomonas present were about the same as in the other cultures so that the amoebae did not lack food.

Calcium chloride: This experiment was carried out in the usual manner and a repetition made about ten months after the first experiment was set up. The concentrations ranged from 0 to .018 N calcium chloride. The results are shown in Fig. 4. A depression point between two optima occurs at .0045 N $CaCl_2$. As in the previous experiments, chilomonas were numerous in the cultures of higher salt concentration although no amoebae were present. The hydrogen ion concentrations of all the solutions at the beginning of

the experiments were at p_H 6.6 and at the end of the experiments, between p_H 6.7 and 6.8.

Sodium chloride: It was desirable to study the relation between sodium chloride and growth and reproduction without the presence of other sodium salts. This would have necessitated the

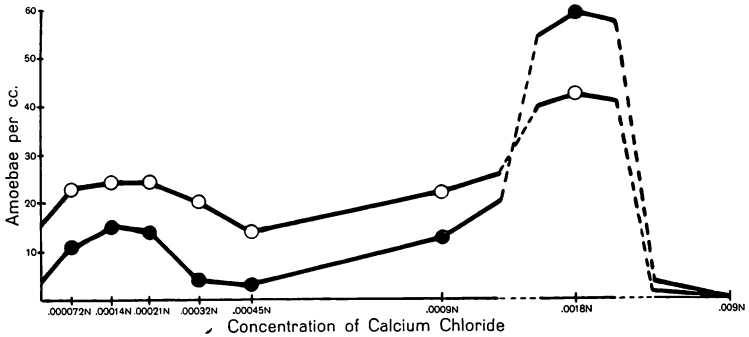

Fig. 4. The relation between the concentration of calcium chloride and growth and reproduction in *Amoeba proteus*. ● first experiment; ○ second experiment.

addition of buffers in order to keep the hydrogen ion concentration constant. The presence of ferric chloride and the absence of sodium silicate would leave the solution with a high hydrogen ion concen-

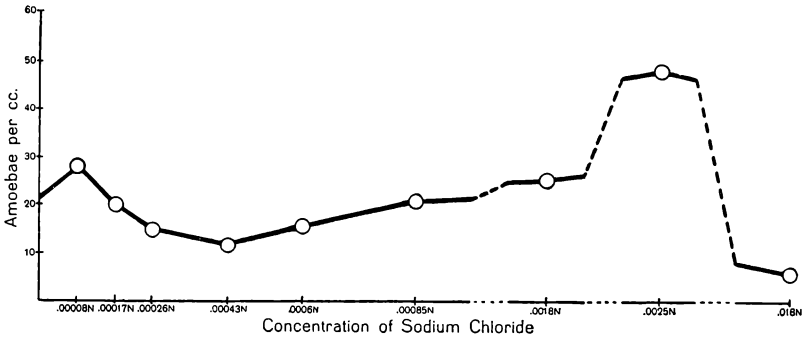

Fig. 5. The relation between the concentration of sodium chloride and growth and reproduction in *Amoeba proteus*.

tration, so sodium silicate and sodium sulphate were added in their original concentrations. The concentration of sodium chloride ranged from 0 to .018 N. The experiment was not repeated. However the same general form of growth curve is obtained as for the previous salts (Fig. 5).

Sodium silicate: Before arriving at a medium that was suitable for culturing *Amoeba proteus*, it was noticed that whenever

sodium silicate was left out, the amoebae did not occur in such large numbers as they did when this salt was present. In order to test these results further, an experiment was carried out using different concentrations of sodium silicate (0 to .016 N). Sodium silicate on hydrolysis gives an alkaline reaction so that in the higher concentrations a very small amount of hydrochloric acid was added in order to bring the hydrogen ion concentration up to normal.

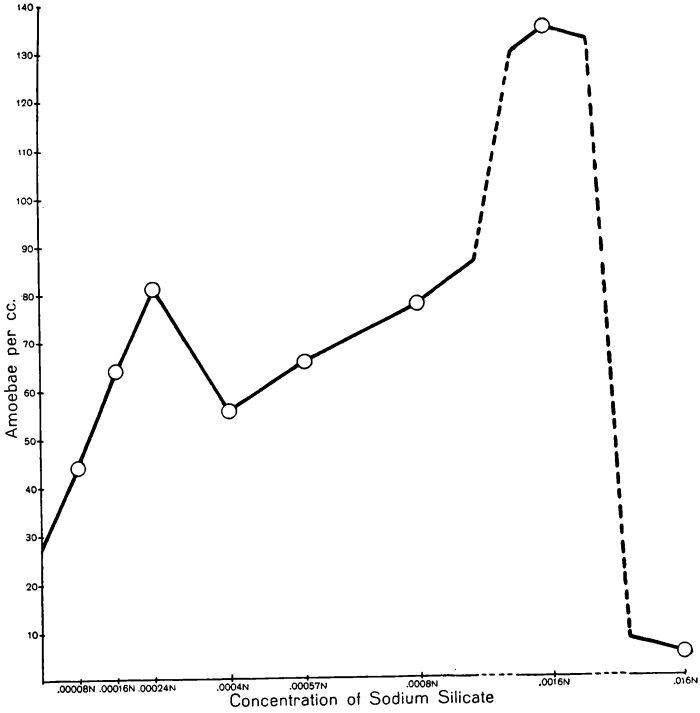

Fig. 6. Showing the relation between the concentration of sodium silicate and growth and reproduction in *Amoeba proteus*.

Again (Fig. 6) the same general form of the growth curve is obtained. It appears that sodium silicate has a very stimulative action on the reproduction rate of *Amoeba*, especially at .0016 N. At this point as many as 210 amoebae per cc. were found in some of the cultures.

Sodium sulphate: This experiment was carried out in the same manner as the others with concentrations of sodium sulphate ranging from 0 to .007 N. As is shown in Fig. 7, sodium sulphate

in concentrations above .00007 N inhibits reproduction. Although this inhibition takes place, the second optimum is present.

Total salt concentration: Since, so far, the investigation had dealt only with single salt effects, it was desirable to see what effects might be obtained when the concentrations of all the salts were changed in the same way, that is, concentrations of KCl, NaCl, $MgCl_2$, and $CaCl_2$. The medium used as control was that given in Table 1. To this was added in equal concentrations, the various salts mentioned.

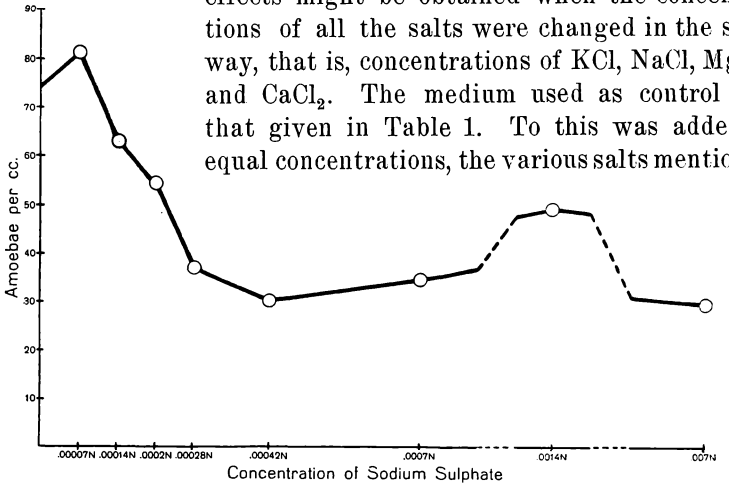

Fig. 7. Showing the relation between the concentration of sodium sulphate and growth and reproduction in *Amoeba proteus.*

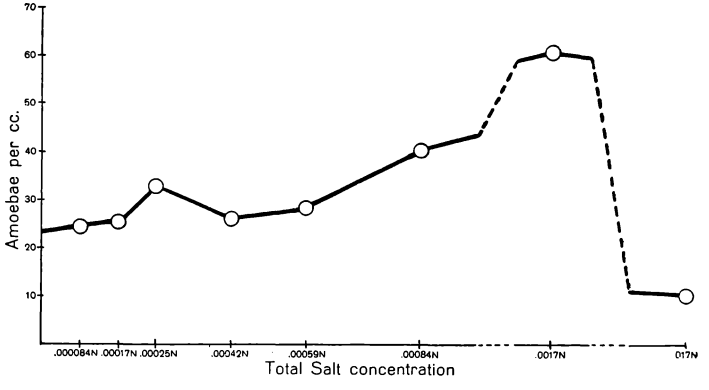

Fig. 8. Showing the relation between the total salt concentration (i. e., concentrations of KCl, NaCl, $CaCl_2$ and $MgCl_2$) and growth and reproduction in *Amoeba proteus.*

For example, when the total concentration was equal to .0017 N, the concentration of each of the above salts was one fourth of this or .00042 N. The results which when plotted form a curve very similar to those obtained with single salts are given in Fig. 8.

Discussion.

Food: The amount of food put into the cultures in the form of wheat was of course slightly variable. However, it is not probable that such a slight variation would effect the cultures to any great extent as it is certain that the food present in the wheat would be sufficient in all cases. It might be argued that the presence of certain salts in particular concentrations might have such an effect on the protoplasm of chilomonas that they could not be used as food by the amoebae. This might be possible, but not probable, for if the salts cause changes extensive enough in Chilomonas as to render them unfit for food they would not have lived and reproduced so well in the cultures. There was no correlation between numbers of chilomonas and numbers of amoebae.

Salts: No attempt was made to obtain an absolute deficiency of the various salts used in these experiments. It would have been useless to do so unless a synthetic medium had been used since all the salts investigated occur to some extent in wheat.

Ferric chloride as was expected had a toxic action in very low concentrations. Reznikoff (1926) working on the effects of salts of heavy metals on *Amoeba proteus* found that in .000024 N ferric chloride amoebae die in less than one day whereas in concentrations of .000006 N or less they may live for over five days. In the writer's experiments no reproduction or growth occurred in concentrations above .000013 N, while very good cultures were obtained when using concentrations of .00001 N and below. The latter concentration is close to that in which Reznikoff was able to keep the amoebae living for over five days. In all the other salt experiments curves were obtained which had the same general form although variations occurred in some cases. It appears that this form is due to some general electrolytic effect and not to any specific ion effect. This seems highly probable when the results of the last experiment are taken into consideration and when the total salt concentration is calculated for each experiment. Table 4 gives the results of such a calculation showing the total concentration at the first optimum, depression, and second optimum of each of the curves. The concentrations are quite similar, especially at the first optimum and depression. Due to the wide variations in concentrations taken at the second optimum it would not be expected that they would be very similar as it is probable that

Table 4.

The results obtained by calculating the total salt concentration that existed in the cultures which were responsible for the 2 optima and the depression in the curves.

Experiment	1st optimum	depression	2nd optimum
2. $MgCl_2$	.00083 N	.00093 N	.0011 N
3. KCl	.00086 N	.0010 N	.0012 N
4. $CaCl_2$	.00082 N	.0010 N	.0024 N
5. NaCl	.00064 N	.00096 N	.003 N
6. Na_2SiO_3	.00073 N	.00088 N	.0020 N
7. Na_2SO_4	.00074 N	.0010 N	.0020 N

they are not the true optima. The height or extent of the curve may vary on account of some specific ion effect. This can be seen on studying the curves of sodium silicate and sodium sulphate and comparing them with that of sodium chloride. The silicate ion and the sulphate ion each have their own specific effect. The similarity of the curves for sodium silicate and sodium sulphate to the curves for the chlorides would rule out the possibility that it might be the chloride ion which is the active agent in causing the general form of the curves.

Hopkins (1929) working on the effects of divalent cations found that a depression occurred in the locomotion curve of *Amoeba proteus* at certain concentrations of calcium chloride and strontium chloride and that this is followed by an increase in rate of locomotion with further increase in salt concentration. He does not give any explanation for its occurrence. It would be hard to say just what causes these results which give curves having two optima. It is possible that it might be due to a neutralization of the charges on the membrane which would bring the reactions taking place to a minimum, while with further increase of electrolytes the opposite charge is increased, making the potential difference greater. It would be interesting to see if such a change really occurs.

The hydrogen ion concentration of the cultures in each experiment, with the exception of the ferric chloride experiment, was fairly constant so the variations could not have been due to differences in H-ion concentration. D. Hopkins (1928) and Mast (1931) have shown that two optima exist in the hydrogen ion curves for locomotion and length of life in *Amoeba proteus*. It is possible that the effects of the hydrogen ions and other ions are the same.

Summary.

1. The rate of growth and reproduction in *Amoeba proteus* depends in part on the inorganic salts present in the medium.

2. Ferric chloride in low concentrations has a stimulative action on reproduction but becomes quite toxic in concentrations above .00001 N.

3. With all the other salts (KCl, NaCl, Na_2SiO_3, Na_2SO_4, $CaCl_2$, and $MgCl_2$) which were investigated, there is at first an increase in numbers of amoebae with an increase in salt concentration. This is followed by a decrease in numbers. However, with further increase in concentration the amoebae increase in numbers until a second optimum is reached which is followed by a decrease in numbers of amoebae with increasing salt concentration.

4. By increasing the total salt concentration in the same way as that of a single salt, the same type of growth curve is obtained.

5. The effects of the salts studied seem to a large extent to be due to electrolytic properties rather than to any specific ionic property, although the general form of the growth curve may be modified as a result of specific ionic effects.

6. The silicate ion is highly stimulating to growth and reproduction in *Amoeba.*

7. The sulphate ion has a stimulating action in low concentrations but in solutions above .00007 N has an inhibiting action.

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