

# The acclimatization of *Chaos diffluens* to sodium chloride solutions.

By

**Esther Weik Badgley** (University of Washington).

(With 4 figures in the text.)

## Introduction.

In 1869 CZERNY published an article under the title, "Einige Beobachtungen über Amöben", in which he stated that he was able, within a comparatively short time, to acclimate "*Amoeba princeps*" to increasing concentrations of NaCl solutions until the concentration reached 4 ‰. This observation has been cited in a number of general works, as for example, DAVENPORT'S "Experimental Morphology" (p. 86); PARKER'S "Elementary Biology" (p. 22); CALKIN'S "The Protozoa" (p. 297). The experiment seems not to have been repeated, however, and since much recent work on the effects of the common chlorides on protoplasm makes the results of CZERNY'S work appear very exceptional, a series of experiments were designed and carried out as a check on CZERNY'S work.

Although no acclimatization experiments over extended periods of time seem to have been made on the large amebas involving the use of common chlorides, a number of observers have, however, tested the reactions of various species of amebas to various concentrations of NaCl and other chemicals for relatively short periods of time and without specific reference to continued reproduction in these solutions.

Thus, for example, ZUELZER (1907), experimenting with the fresh water ameba, *Thecamoeba verrucosa*, found that the animals lived well in sea water solutions of 0.3 ‰ to 0.5 ‰ salt content, but that in higher concentrations, using 50 ‰ sea water (a salt content

of about 1.5 %) the contractile vacuole was not present. FINLEY (1930) working with the same species of ameba found that it survives 103 % sea water but is different in form and loses its contractile vacuole in 84 % sea water. His counts indicate that at least up to 34 % sea water, *verrucosa* divides. The difference in results may be due to the different culture methods used.

SCHAEFFER (1919, 1926) working with marine amebas at Tortugas, found that different species are variously sensitive to dilute sea water even when belonging to the same genus. *Flabellula citata*, a very hardy type, lives in fresh water, when transferred directly, for several days, while *Trichamoeba sphaerarum* dies quickly in 25 % sea water. This makes it necessary to know exactly what species of ameba one works with, for otherwise the results of various workers cannot be compared.

EDWARDS (1924, 1925) finds that the ectoplasmic surface of "*Amoeba proteus*" ruptures readily in strong solutions of bases and salts, the time required varying with the concentration. The time of inactivation or death in N/300 (0.019 %) NaCl is 266 hours; in N/100 (0.058 %) NaCl is 124 hours.

REZNIKOFF and CHAMBERS (1924, 1925) found that "ameba" lives for less than one hour in M/6.5 (0.9 %) NaCl. If torn even slightly in M/13 (0.447 %) NaCl the ameba did not recover, while with NaCl weaker than M/13 repair took place with increasing ease. The amebas could recover from extensive tears only in M/312 (0.0187 %) NaCl.

PANTIN, investigating the reactions of the marine limax types of amebas (description, PANTIN, 1923) in various salt solutions, found that pure isotonic solutions of NaCl (also KCl, MgCl, and CaCl) will not support ameboid movement. In testing these amebas, PANTIN found that in transferring them too suddenly from very concentrated sea water to 1 % sea water, cytolysis occurred.

CHATTON and TELLIER (1927) experimenting with *Glaucoma pyriformis*, an infusorian, in hypertonic NaCl solutions, were able by using intermediary concentrations, the lowest being 0.085/N (0.49 %) NaCl, to reach a "distal" limit of 0.205/N (1.19 %) NaCl.

WOLF (1927) in attempting to adapt amebas of "genus *Hartmannella*" to NaCl solutions, found the "pure mixed cultures" excellent in "5 to 10 parts in 1000" NaCl solutions. In "30 to 40 parts in 1000" the amebas became more rounded, but he concludes with HOLLAND (1921) that no cysts are formed in the NaCl solutions.

MAST and HULPIEU (1930) in testing the "reaction time" (time in seconds required for streaming to stop when an ameba in the salt solution kept in darkness for two minutes, was subjected to an increase in illumination) of "*Amoeba proteus*" in concentrations of N/10 (0.584 %) NaCl down to N/1500 (0.0038 %) NaCl, found that there was no consistent difference in the increasing concentrations.

MAST (1931) measuring the effect of pure water and different salt solutions upon the length of life of "*A. proteus*" obtained fission in N/10 000 NaCl (0.00058 %). He reports that the maximum concentration lies between N/500 (0.011 %) NaCl and N/1000 (0.0058 %) NaCl. In single salt solutions N/1000 (0.0058 %) the length of life was highest in the sodium salts. Had he used an intermediate concentration of NaCl between N/500 and N/100, it is possible that he would have found a greater maximum range for the amebas than the range which he obtained (according to my results).

This problem was suggested to me by A. A. SCHAEFFER in 1925. It was started under his direction and finished under the supervision of Trevor Kincaid. I am deeply grateful to both for their suggestions and the use of their personal libraries. The gift of a Loretta Denny Fellowship at the University of Washington, made it possible for me to complete this paper.

### Materials and methods.

Pure line amebas only were used in the following experiments. Genetical research has shown beyond question that populations of protozoa of unknown history may contain many races with different characteristics (JENNINGS, HEGNER and others) and that physiological experiments based on animals selected at random out of such populations are therefore likely to give results which are not consistent within a series, and are also open to the further objection that such experiments are not sufficiently well described to allow of checking and repeating by other experimenters. The objections apply, of course, with much greater force if the population should contain two closely related species, which is readily possible, or if the species is unidentified or misidentified. This criticism of prevailing technique is particularly applicable to the present case, for the wide divergence between CZERNY'S results and mine indicate that the ameba he worked with: "*Amoeba princeps*" could not possibly have been *Chaos diffluens*, MUELLER, with which I worked.

All of the amebas with which I worked were descended from a single individual which conformed to the description given for the species (SCHAEFFER, 1916, 1926).

*Chilomonas paramecium* (EHRENBERG) grown in dilute hay infusion was used as food for the amebas. Occasionally a few hypotrichs and *Paramecium caudatum* were present with the *Chilomonas* in the food culture.

### Culture methods.

Several preliminary experiments were conducted using old timothy hay infusions without discarding any of the liquid from the boiled hay. In later experiments the food cultures were made up as follows:

One gram of finely cut timothy hay (IDAHO, 1930 crop) was placed in a small beaker. Twenty-five cc. of distilled water were poured over this and it was then quickly brought to boiling over a Bunsen flame. The liquid was then immediately poured off and the hay, with 500 cc. of distilled water was transferred to a sterile glass quart jar and covered with the glass lid (turned upside down). On the second or third day it was inoculated with 25 cc. of a good growing culture of *Chilomonas* taken from a similar culture fluid. This was allowed to stand in diffused daylight at about 21 degrees Centigrade until it reached a  $p_H$  of 6.5 or 6.6 (measured with a Standard Eimer and Amend colorimeter using bromthymol blue as an indicator) and contained a heavy culture of the *Chilomonas* in the clear or slightly cloudy infusion; it was never used if colored, as this shows the presence of excess undesirable bacteria. The  $p_H$  of this infusion (in the stratum where the *Chilomonas* were most numerous) remained in the region of 6.5 to 6.8  $p_H$  for at least two weeks, if the jar was kept carefully covered (lid right side down after the top stratum, where the *Chilomonas* were most abundant at first, reached 6.6  $p_H$ ) and the infusion not allowed to evaporate. The infusion was then used with success, as indicated by the normal division rate of the control amebas kept in this medium. By placing the culture medium in shallow open glass dishes the change toward alkalinity, that is, from  $p_H$  6.5 to  $p_H$  7.0 can be hastened. This is true for the culture medium containing the NaCl as well as for the controls. Over 50  $p_H$  determinations were made (colorimetrically) at various times, all of them showing a change to approximate neutrality, as stated

The culture medium was strained through several folds of white cloth and later portioned to the watch glass cultures. The amount of food present, even in the NaCl solutions far exceeded the needs of the amebas placed in the cultures. The Syracuse type of watch glass was used and the medium was kept from spreading by dropping it at one side of the dish. This gave less opportunity for surface evaporation during examination, for rapid  $p_H$  change, and also made it less difficult to locate the ameba quickly. Upon the ground edge of the watch glass the records of the ameba and the concentration were written in pencil at the time of changing to new medium. Loss by evaporation was negligible, since a few amebas at a time were quickly observed and returned at once to the moist chamber after being transferred to the new medium. Occasionally a single specimen was kept out considerably longer for observation; in this case the transfer to new medium was made just before return to the moist chamber.

The stock solution of NaCl was prepared by weighing out 10 grams of MERK'S Blue Label NaCl and dissolving this in 90 cc. of distilled water. This was kept in a sterile glass-bottle. A second solution (1 % solution) was prepared from this by measuring out 10 cc. of it and adding 90 cc. of distilled water. This 1 % solution was in daily use. Both solutions were kept tightly corked and in diffused daylight.

The increasing NaCl concentrations, addition of a certain amount of the 1 % NaCl solution to the food medium to make up to the desired NaCl concentration, was as follows:

Table I.

Showing the gradual increases of NaCl solutions from 0.05 % to 0.2 % NaCl.

Amount of 0.1 % NaCl solution in drops	Amount of food cul- ture used, in drops	Total amount of solution in drops	Calculated per- centage by weight %
1	19	20	0.05 of 1
2	18	20	0.10 of 1
2	16	18	0.111
2	14	16	0.125
3	18	21	0.143
3	17	20	0.150
4	22	26	0.1665
4	18	22	0.1815
4	16	20	0.20

Special care was observed in the use of pipettes, watch glasses, etc., to prevent contamination and to secure uniformity in technique.

Solutions were usually changed daily although it was found that changes every other day produced the same results. All dishes were examined daily with a binocular microscope and the results recorded. There were two pipettes used for the NaCl solutions, one drawn to a very fine point and used for the transference of an ameba from one watch glass to another and the other ordinary pipette was dipped into the NaCl solution in the bottle. There were other pipettes used for the controls and for the *Chilomonas* cultures fluid. The pipettes were all of the same size in order that all the concentrations should be uniform, since the measurements were all made by holding the pipette vertically and counting the drops. They were cleaned daily and rinsed with distilled water, the rubber bulbs removed and the glass parts placed in a glass dish, covered with a clean white cloth, and allowed to dry thoroughly for use the next day.

It was necessary to keep a supply of dry watch-glasses on hand, since drops of water adhering to recently washed watch-glasses would change the NaCl concentrations.

### Experimental results.

The divergence of view as to just what effect NaCl solutions have on the plasma membrane, is partly due to specific differences in the organisms employed and partly to the different kind of effects which the observers looked for. But even in those experiments in which amebas were employed no direct comparison can be made, owing to inadequate description of species and culture purity. In a general way, however, the results agree in that both the ectoplasm and the endoplasm are affected by immersion in NaCl solutions. In this series of experiments the internal effects are probably of the greater importance seeing that the first visible steps in division are internal; and second, the point in the process of acclimatization where the rate of division is markedly interfered with, corresponds to the point where digestion is markedly interfered with. text, p. 12. Feeding can therefore apparently continue beyond the stage where food can be digested. It may well be the case then that division is inhibited through failure of the digestive and assimilative processes to permit growth. For PHELPS (1926) has shown that increase in volume of *Chaos diffluens* is necessary in order to initiate division. This may mean therefore, that the digestive process cannot acclimate itself to increased amounts of NaCl, and if so, this would in effect prevent any other acclimati-

zation process to proceed beyond the stage where growth is inhibited, although an acclimating mechanism might be present.

The great difference between CZERNY's observations and mine is probably due to the difference between the amebas used. CZERNY states that he used "*Amoeba princeps*" which is, historically, the same as "*Amoeba proteus*". Even at the present day "*Amoeba proteus*" is of uncertain specific reference, meaning one species to one writer and another species, or several other species collectively, to other writers. This was probably also the case with the name "*Amoeba princeps*". While it is therefore still possible to suppose that CZERNY had a fresh water species which could be acclimated to NaCl solutions to 4 % concentration, the species, *Chaos diffluens* MUELLER, as described in this paper, does not so acclimate itself. It may be presumed therefore, that CZERNY did not have *Chaos diffluens* among his acclimated amebas, but what species he did have remains unknown.

According to CZERNY, one can begin with a culture solution containing 0.25 % NaCl and increase the concentration of the solution at the rate of 0.16 % NaCl every 24 to 48 hours, so that by the ninth day one would reach a concentration of 1.16 % NaCl. He says that after he had reached a concentration of 1.16 % NaCl there were many amebas present, intimating that there had been divisions during the time he was increasing the NaCl solution in which the amebas were living. It was found in my experiments, however, using division as the measure of growth and thus a positive acclimatization, that 0.25 % NaCl is too high a concentration to use at first.

*Chilomonas paramecium* was tried out in 1 %, 2 %, 3 % and 4 % solutions of NaCl and all these solutions slowed down movement at once. In 30 minutes the *Chilomonas* in 1 % moved still more slowly, those in 2 % were dying and all the *Chilomonas* in 3 % and 4 % were dead. By the next day there were a few living, moving *Chilomonas* only in the 1 % solution. *Chilomonas* were next tried out in 0.1 of 1 %, 0.2 of 1 % and so on up to 0.9 of 1 % NaCl. Some of the *Chilomonas* remained alive in all of the solutions, but as the concentrations increased they tended to form larger and larger clumps somewhat similar in appearance to agglutinating bacteria. In 0.1 % the *Chilomonas* appeared to move much as they did in the control cultures.

Preliminary testing of *Chaos diffluens* in 1 % NaCl solution in which, at the end of 2 hours it had become more or less spherical,

showed that this was too high a concentration to use at first. Even in 0.1 % NaCl the amebas did not stick to the substratum as did the controls in normal medium. CZERNY (1869) and ZUELZER (1907) also found this to be the case as the NaCl concentration rises. MAST (1929) found that sticking to the substratum is best on common glass. The power of sticking to the substratum is also lost when the ameba is enucleated, CALKINS (1910) and PHELPS (1926).

The results of the first series of experiments in acclimating *C. diffluens* are shown in Table II. Some 50 individuals from a pure line culture were used. Each ameba was stepped up, after a division in a culture containing less added NaCl (percentage concentration indicated), to a culture containing a still greater concentration of NaCl, to discover in how high a concentration division could still occur.

Table II.

Showing divisions occurring once in various concentrations of NaCl.

Percent of NaCl solution	No. of individuals used	No. of single divisions	Per cent of divisions
0.05	6	6	100.0
0.10	31	30	96.8
0.125	15	9	60.0
0.15	18	4	22.2
0.20	31	3	9.68

The amebas were placed in the 0.15 % and 0.2 % solutions after they had divided once in a 0.1 % solution of NaCl. Divisions in these concentrations were probably "hang over" or delayed divisions, as evidenced by later experiments. The above results were obtained when the amebas in normal culture medium were dividing at the rate of 12 to 15 times in 30 days. In late December (1930) and early January (1931) when the controls were dividing at a very slow rate, many of the amebas placed directly in 0.1 % NaCl did not divide in that concentration, but among these there was often a preparation for division without its completion. Two daughter amebas having been in 0.1 % for six and seven days respectively, went thru this preparatory phase without results. Twelve days later, however, one of them successfully divided; the other never did, merely shrinking up and finally disintegrating. In Table II, the single ameba which failed to divide when placed directly into the 0.1 % NaCl solution, was returned to 0.05 % NaCl in which it divided. A daughter of this division was



then placed in the 0.1 % NaCl culture where it divided after 2 days. In some cases where an individual ameba was subjected to a 0.1 % NaCl concentration before dividing in 0.05 %, the ameba shrank and division did not occur unless it was put back in 0.05 % NaCl and allowed to divide in that concentration and then again placed in the 0.1 % solution where, under these conditions, it never failed to divide. This is shown in Table III below. Here every ameba was allowed to divide in 0.05 % before being placed in 0.1 % NaCl, with the resulting 100 % divisions in the 0.1 % NaCl. There seems to be evidence that racial acclimatization is possible to some extent as in the case of race "H" which when placed directly in 0.1 %, without previously dividing in 0.05 % NaCl solution, shrank and did not divide. This ameba was then placed in 0.05 % and allowed to divide in that concentration. A daughter of this division placed into 0.1 % divided and continued to divide in that concentration. Only one division of this race occurred in 0.111 % and none in 0.125 %, before shrinkage occurred.

Table III.

Amebas acclimated in new hay medium, starting in a culture medium containing 0.05 % NaCl and after a single division being placed in the next concentration listed.

Percent of NaCl solution	No. of individuals used	No. of single divisions	Percent of divisions
0.05	13	13	100
0.10	13	13	100
0.11	13	12	92.3
0.125	12	6	50.0
0.143	6	0	0

Amebas placed in the NaCl concentrations directly without being first allowed to divide in a solution containing less NaCl show a lower rate of division. These amebas were placed in the concentration indicated just after they had divided in normal medium. See Table IV.

Table IV.

Percent of NaCl solution	No. of individuals used	No. of single division
0.05	16	16
0.10	39	34
0.111	8	3
0.125	8	1
0.143	8	0

The time when most initial divisions occurred is shown in Fig. 1.

In this figure it is shown that while nearly all the amebas in a culture medium containing 0.05 % NaCl had divided by the second day, those in 0.10 % had not divided until the third day in that concentration. The wide range in the rate of division among the various individuals in 0.1 % NaCl is very noticeable, although most of them had divided for the first time by the end of the fifth day. The short range of those in 0.125 % may be accounted for by the fact that all of them had divided in 0.1 % and some in 0.11 %

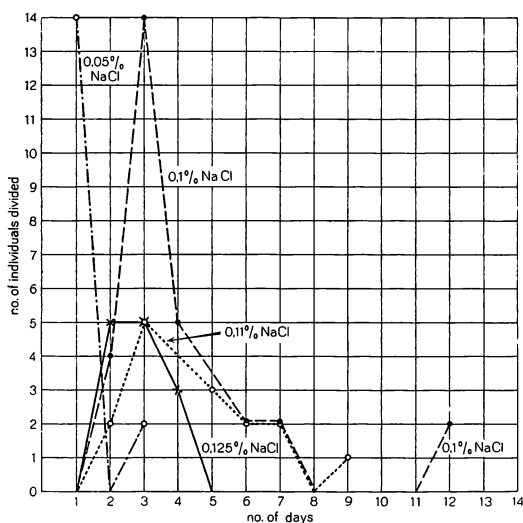


Fig. 1. Showing the time when most initial divisions occurred.

once before they were placed in 0.125 %, although the rate of division in 0.125 % was not more rapid when the individual had divided twice instead of only once in 0.1 %. One individual which had divided twice in 0.1 % divided in 0.125 % on the third day it was in that solution, while another individual which had divided only once in 0.1 % divided on the first day it was placed in 0.125 %. In both cases the individuals were put into the

0.125 % on the day that they had divided in 0.1 % NaCl. The divisions in 0.125 % may also be partially explained on the basis of "hang over" or delayed divisions since the ameba in 0.10 % often grew to a large size and then showed a tendency to divide twice in fairly rapid succession; the first division of an ameba kept in 0.1 % NaCl solution, being followed by a second division one or three days later. Ameba "R", for example, which was kept in a 0.1 % culture medium, kept on dividing at intervals from Mar. 7, until May 13, 1931, the last division having occurred May 12. This ameba divided in this concentration (0.1 %) on the 18<sup>th</sup> and again on the 21<sup>st</sup> and 25<sup>th</sup> of March and then grew in size until the 4<sup>th</sup> of April

when again it divided 3 times in succession: April 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup>. Other individuals in 0.1 ‰ have shown a similar tendency to divide in this irregular manner, particularly if there is a food change to become adjusted to.

Another series of experiments was run using 10 individuals and keeping them in the various NaCl solutions in which they were acclimated for a period of at least 30 days, to see how long they would continue to di-

vide and at what rate division occurred when a daughter ameba (result of the initial division in the specified concentration of NaCl culture medium) was retained in that particular concentration, while the other daughter was placed in the next higher concentration. In these experiments it was found that when the controls were dividing at the rate of 10 to 15 times a month in new hay infusion *Chilomonas* culture, as before explained, the amebas retained in the same medium but containing 0.1 ‰ NaCl,

continued to divide slowly (average 5 times a month) for at least two months; the amebas kept under the same conditions in 0.05 ‰ NaCl divided at practically the same rate as the amebas in the culture medium where no NaCl was added. Control amebas were also used for each different concentration (see Fig. 2); for example, as one of the daughters (Fig. 2 "B3") from the first division in 0.1 ‰ NaCl medium, was moved into the next NaCl medium (0.11 ‰), an ameba (Fig. 2 "BBB"), also a recently divided daughter taken from one of the control cultures containing no added NaCl (example,

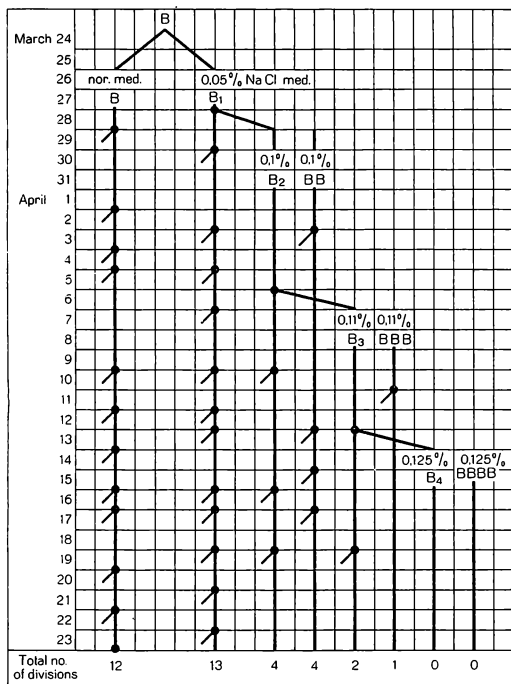


Fig. 2. Showing acclimatization of ameba "B" and its descendants to NaCl solutions during a period of 30 days.

"B"), was placed directly into the 0.11 % NaCl culture medium. Thus, we have in Figure 2 a direct comparison of the rate of division between two newly divided amebas: one (Fig. 2 "B3"), which had been first acclimated to 0.05 % and 0.1 % NaCl before being placed in the higher concentration of 0.11 % NaCl, and one (Fig. 2 "BBB"), which had not been acclimated to NaCl solutions of less concentration, but placed directly from normal medium containing no added NaCl, into the 0.11 % NaCl culture medium. Thus, for instance Fig. 2, shows that the acclimated ameba, "B3", divided twice in 0.11 %, while "BBB" placed directly in the same NaCl medium failed to divide more than once: in fact, in most cases of the controls placed directly in 0.11 % there was no division in this concentration (see Table IV, above). The daughter of the second division and all successive divisions of an ameba kept in the same concentration of NaCl medium, were generally discarded. Fig. 2 shows the acclimatization of ameba "B" and its descendents to increasing NaCl solutions over a period of 30 days. Each division is indicated by a heavy dot and short line. The NaCl concentration of the culture medium and the name of the ameba kept in that concentration, is indicated at the head of each line; where the line passes to the right there is the indicated increase in NaCl concentration. The date on which each division occurred, of each individual ameba kept in the same concentration, is also specified.

Table V.

Summary of the results obtained in acclimating 10 amebas in the same manner as "B" in Fig. 2, was acclimated.

Percent of added NaCl	Maximum no. of divisions during a 30 day period	Average no. of divisions during a 30 day period
0.0 (normal med.)	15	13.33 (ave. of 3 amebas)
0.05	15	13.33 ( " " 6 " )
0.10	6	4
0.11	3	2
0.125	1	0.6
0.143	0	0

According to these experiments, it is evident that *diffluens* grows normally in 0.05 % NaCl concentrations with the same reactions and rate of division as do the amebas in normal media. In 0.1 % NaCl division is delayed with less adherence to the substratum. From this concentration onward, it was necessary to increase the

concentrations by smaller amounts, namely, by 0.01 of 0.1 % NaCl. Individuals started in 0.05 % NaCl, divided once successively, in each concentration up to 0.125 % in a period of 13 days. A division in 0.125 % was not always accomplished; as soon as a division had occurred in this concentration, however, shrinking slowly proceeded and although the ameba was able to form food cups for a few days after division in this concentration, the food remained undigested in the body of the ameba. Undigested food is also present to some extent in amebas which finally shrink in a 0.11 % NaCl medium. In this concentration, after the second or the third division, the ameba lives for some 20 days before disintegrating, while in 0.125 %

there is marked shrinkage after the first 4 or 5 days (from the time the ameba divided in that concentration) with final disintegration in about two weeks. Controls placed directly in the 0.11 % NaCl medium increased in size for a time, and some divided once in this concentration (Table IV; Fig. 2). After

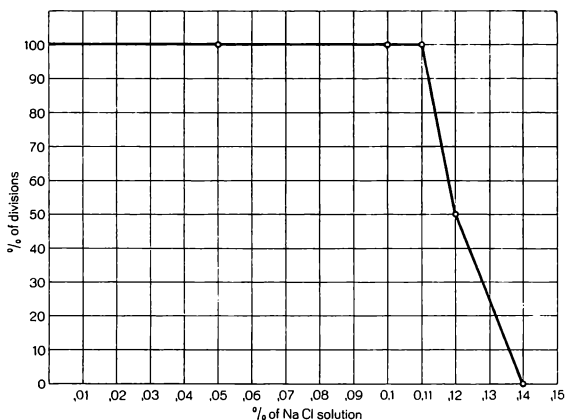


Fig. 3. Curve showing the percent of the NaCl solution and the percent of divisions in the various solutions, of four individuals acclimated ad the same time.

this time they began to shrink and in about 15 days disintegrated. Amebas placed directly in 0.125 % (Table IV), did not divide in that concentration, although one individual was seen forming a food cup on the second day in this medium. Shrinkage was apparent by the 4<sup>th</sup> day, sometimes sooner, and the ameba disintegrated in about 10 days, kept in this concentration. Amebas placed directly in 0.143 % never divided in that concentration of NaCl, and no food cups were formed, tho food cups are formed in amebas placed directly in still higher concentrations. However, no special effort was made to record the occasions when food cup formation was taking place in the various solutions. Shrinkage of amebas placed directly in 0.143 % NaCl medium was gene-

rally marked on the second or third day in that concentration and final disintegration occurred in about a week: on the other hand, acclimated amebas, placed in 0.143 % on the day of their division in 0.125 % NaCl, while they did not divide in this concentration, did not disintegrate for 9 or 10 days in most instances.

Thus it was found in this series of experiments that (1) the rate of division and the total number of divisions in 0.05 % NaCl parallels that of the controls; (2) that divisions in 0.1 % are slowed up; (3) that in 0.11 % the rate of division is still slower than in 0.1 % although the time of initial division is similar to that in 0.1 %. Also divisions do not continue over long periods of time if the individual ameba is kept in this concentration; (4) the initial and only division in 0.125 % NaCl occurs within the first few days or not at all, and is probably a "hang over" division.

Figure 4 shows the effects of the NaCl concentrations upon the amebas after they have passed some time in those concentrations. An ameba subjected to NaCl media in acclimating to the point of disintegration, passed through the stages of normality, 1; branchlike and rayed stages 2, 3, 4 and 5, and finally to knobby and contracted stages, in 6 and 7. From this point the ameba grows roughly spherical and disintegrates in a short time thereafter.

No. 1. A control ameba in normal culture fluid. No. 2. Rayed type which had been in 0.1 % NaCl for 4 days; four days later it divided. No. 3. Rayed type also somewhat branched which divided in 0.1 % after 2 days. It was kept in 0.1 % for 5 days and then returned to normal medium in which it divided after one day. It was then placed in 0.15 %. The drawing was made at this time. No. 4 divided in 0.1 % twice and has been in 0.125 % for 2 days without dividing. No. 5 a "knobby" or "warty" type divided twice in 0.1 %; it had been in 0.125 % for 5 days without dividing. No. 6, also "knobby", divided in 0.1 % after 12 days and was in 0.143 % for 2 days. No. 7 divided once in 0.1 % and was in 0.2 % for 2 days. No. 8, in three positions; it divided in 0.1 % twice and was in 0.2 % for 3 days. No. 9 divided in 0.15 % ("hang over" division from 0.1 % where it had increased greatly in size and divided once before being placed in the 0.15 % NaCl medium) and was in 0.2 % for 4 days.

CZERNY (1869), ZUELZER (1907), SCHAEFFER (1926), PANTIN (1926), MAST (1927), WOLF (1927) and others tell of various changes in shape when amebas are immersed in salt solutions. Very similiar changes occur when there is insufficient oxygen or too much

hydrogen, HULPIEU (1930). Amebas in 0.05 % NaCl medium showed no difference in shape or degree of adhesion to the substratum as compared with the controls. Those living in 0.1 % were often loose and branched, sometimes with pseudopods extending outward from the body like so many fine rays. L. LOEB (1921) believes that this type of pseudopod is formed because of liquifaction at the advancing tip of the pseudopodium, followed by gelation at the sides, since in these solutions the ameba is gelated because the loss of water has

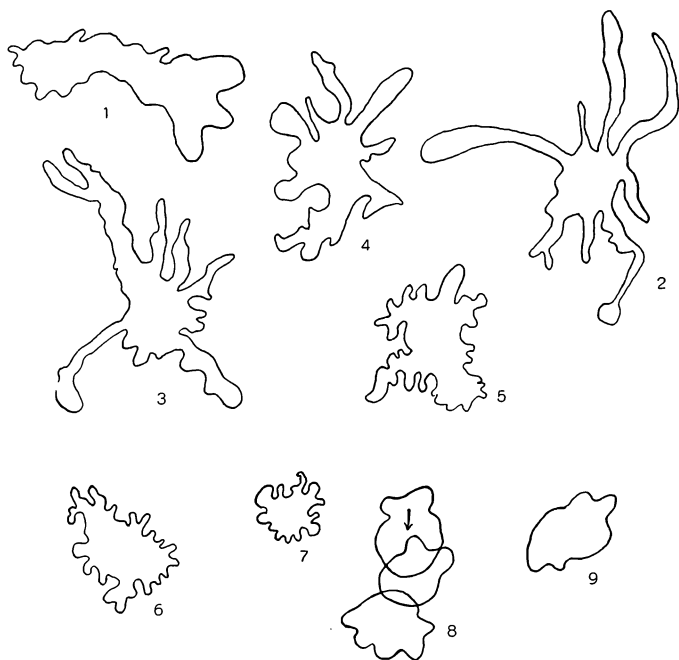


Fig. 4. Camera lucida sketches showing typical shapes of amebas in the various NaCl concentrations.

caused an increase in consistency. The raylike pseudopods were sometimes thin and almost hyaline. As the concentration became increasingly effective, the pseudopods were withdrawn more and more, extending shorter and shorter distances from the body, and often being branched. Finally the ameba assumed a knobby appearance and took on a more and more spherical form, which at last disintegrated. At the knobby stage it never appeared to have moved any distance from its position of the day preceeding. It changed shape slightly and almost imperceptibly. CALKINS (1910) says that in all cases of harmful action upon the Rhizopoda the

reaction is expressed by withdrawal of the pseudopods, rounding out of the body and final disintegration.

Even in the higher concentrations of NaCl my amebas frequently formed food cups. A *Chilomonas* was found struggling in the food cup of an ameba which had been placed directly into 0.2 % NaCl the day before. This ameba did not divide in this concentration but became spherical 4 days later.

There was considerable variability among the daughter amebas, two of the same division showing different susceptibility to the same NaCl solution. SCHAEFFER (1926) and HOGUE (1923) found this to be true in the case of amebas they worked with. This susceptibility to NaCl solutions is correlated with the rate of division of the daughter amebas, at least in such cases as were noted. For example, two daughter amebas, "R1" and "R12", the results of a second division of ameba "R" in 0.1 % NaCl medium, were both kept in a 0.1 % NaCl medium at the same time and under the same conditions. "R1" divided 3 times in this concentration, while "R12" divided only 2 times and later, when the food was changed rather abruptly, "R12" did not recover from the bad effects of the change while "R1", although slowed up in the rate of division, continued to divide again, nearly three weeks later. This same susceptibility to changes in environment between daughters is seen in normal conditions SCHAEFFER (1916).

Examination of the amebas under a high power lens in NaCl solutions as high as 0.15 %, showed the presence of the contractile vacuole when the ameba had not taken on the spherical form but was merely shrunken somewhat with unequal distribution of the cytoplasmic granules. Although it seemed to remain for a considerable time, the rate of pulsation was not obtained, since in the cases investigated the crystals of the ameba were so numerous that the contractile vacuole was often lost sight of. It was seen sometimes at the end of a pseudopod as well as in the body of the ameba. Decrease in the rate of pulsation of the contractile vacuole in salt solutions are reported by ZUELZER (1907), ADOLPH (1926), and DEGEN (1905). FINLEY (1929) experimenting with *Thecamoeba verrucosa* found that in 44 % sea water the contractile vacuole pulsed only once in 40 minutes, with an interval of 10 to 15 minutes before its reappearance; in 84 % sea water the contractile vacuole was absent. HOGUE (1923) working with *Flabellula (Vahlkampfia) calkinsi*, a parasitic species, obtained a gain of 1 to 4 vacuoles when subjecting this ameba to a decreased salt medium.



BOTSFORD (1926) believes that the mechanism of systole is related to surface tension and DEGEN (1905) suggests that if the NaCl is washed out in time the contractile vacuole returns to normal. It seems that a contractile vacuole is necessary for the existence of *Chaos diffuens* for it soon forms another if it loses the one it has (METCALF (1910) and BOTSFORD (1926).

The granular clumping so evident in amebas exposed to NaCl solutions where there is maladjustment in concentrations above 0.05 %, has been observed in many solutions (MAST and HULPIEU, 1930, for example, consider it due to "poor physical condition"). Division in 0.1 % could take place as long as the lack of uniformity of the granules in the endoplasm of the ameba was only slightly noticeable. REZNIKOFF and CHAMBERS (1926) found sinking and clumping of the granules in ameba in 0.89 % NaCl solutions, within one hour after immersion. They maintain, also SEIFRIZ (1929), PANTIN (1926) and BRINKLEY (1928), that NaCl causes liquifaction of the cytoplasm of the ameba, while MAST and HULPIEU (1930) agree with HEILBRUNN (1928) that the effect of NaCl is a gelating one and in the series:  $\text{Na} > \text{K} > \text{Mg} > \text{Ca}$ .

Individuals recover in a few hours when returned to normal or 0.05 % NaCl medium shortly after division in 0.1 %. If the ameba has remained in 0.1 % for several days without dividing and the granules are unevenly distributed, the pseudopods are drawn in until a knoblike appearance is presented (No. 5 and 6 of Fig. 4) with the ameba rolling about loosely in the dish. There will be recovery when the ameba is returned to normal media, but the time will be longer, about 6 days, before the ameba sticks consistently to the substratum of common glass. If the ameba has reached the above condition in any of the higher concentrations of NaCl (which takes a shorter time with the average ameba) the time of recovery to normal is about the same. ZUELZER (1907) obtained the recovery of amebas that had been in a high concentration of salt for 8 or 10 weeks by adding fresh water, a drop at a time.

The change in shape and shrinkage of the ameba is probably due, ultimately, to the permeability of the cell membrane to NaCl. LILLIE (1909), OSTERHOUT (1912) and others. BAYLISS (1929) says it is the ions of NaCl that cause the reactions in very dilute solutions. PANTIN (1926) thinks that the NaCl solutions increase the permeability of the plasma membrane so that the result is either a penetration of the medium into the cell or a loss of a substance including Ca, from the cell. He found that Ca alone is

necessary for movement. REZNIKOFF and CHAMBERS (1924, 1925) believe that NaCl has a disintegrating effect upon the surface of amebas, and is more toxic in contact with the external surface than when injected into the ameba. DEGEN (1905) says the most marked effects upon protoplasm and the plasma membrane are caused by Na and Cl salts. CLOWES (1916) and LILLIE (1909) suggest that death may be the result of this increase in permeability, since an organism cannot live with more than a temporary loss of permeability. CLOWES (1916) and DEGEN (1905) agree that it is the anion of NaCl which permeates the membrane and FLEISCHMANN (1929) says that the cell membrane is not permeable to cations but is permeable to anions.

### Acclimatization of *Polychaos dubia* to NaCl solutions.

*Polychaos dubia*, SCHAEFFER (1916, 1926) brought in from the Freshman Pool on the University of Washington campus, was used in these experiments. All individuals used were from a pure line culture of this ameba. This ameba has often been confused with *Chaos diffluens*, hence it was interesting to discover that its reactions to NaCl solutions were not like the reactions of *diffluens*. Seven pedigreed individuals were used, three being kept in normal media and four being acclimated in the same method used with *diffluens*, (Fig. 2). At the same time, and under the same conditions, a single individual, *Chaos diffluens* with controls was acclimated. Results over a 15 day period were as follows:

Table VI.

Showing number of divisions in each concentration for the 15 day period.

Individuals	No. of divisions of amebas kept in Nor. cul. medium	No. of divisions of amebas kept in 0.05% NaCl medium	No. of divisions of amebas kept in 0.1% NaCl medium	No. of divisions of amebas kept in 0.11% NaCl medium
<i>C. diffluens</i> ave. of 3 controls	6			
<i>diffluens</i> "Z" acclimated		7	2	1
<i>P. dubia</i>				
"A" control	12			
"B" "	11			
"C" "	13			
"D" acclim.		5	0	
"E" "		3	0	
"F" "		3	0	
"G" "		5	0	

Table VI shows that the average for the control amebas of *dubia* is 11 divisions during the period of 15 days while the average of the four different individuals, "D", "E", "F" and "G" kept in 0.05 % NaCl medium is 4 divisions. There were no divisions in the 0.1 % NaCl medium, of the species *dubia*.

Compared with the division of *diffluens* in NaCl concentrations, it appears thus, that *dubia* is much less resistant to those NaCl solutions in which the former divides and continues to divide in at a slow rate, namely, 0.1 % NaCl medium. At the end of the 15 day period, none of *dubia* had divided in the 0.1 % NaCl solution and camera lucida drawings showed that the individuals remaining in this concentration had shrunk to at least one-half their original size.

The reactions of *dubia* to 0.05 % NaCl is like that of *diffluens* to 0.1 %: it grows large and then divides at a slow rate. When one compares the rate of division of these two species of amebas in the NaCl concentration in which each continues to divide, to the rate of their division in a normal medium, there seems to be a correlation: each divides in the NaCl concentration at about one-third the rate in normal medium.

### Summary of Results.

1. In this series of experiments no such marked acclimatization of an ameba to NaCl solutions could be demonstrated as that reported by CZERNY.

2. *Chaos diffluens* cannot be acclimated to NaCl solutions by starting directly with 0.25 %.

3. NaCl concentrations cannot be stepped up by such large amounts as 0.16 %, in acclimating amebas of this species.

4. A successful starting point for acclimating *diffluens* seems to be 0.05 of 1 % NaCl, with the next increase to 0.1 of 1 % NaCl followed by increases, in steps, no greater than 0.01 of 1 %.

5. Lack of food was not a factor in preventing growth and division of the amebas as the *Chilomonas* always existed in excess.

6. This ameba may remain alive (show some protoplasmic streaming) for at least three days in 0.2 % NaCl solution (in which it does not divide).

7. This ameba retains its contractile vacuole, without increase in number, in concentrations as high as 0.15 % NaCl.

8. "About 60 % of these amebas divide in 0.125 % NaCl. These divided daughters recover when placed in normal medium on the day of their division. Those which do not markedly shrink after having been left in 0.125 % NaCl for several days, will recover when returned to normal medium — the time depending on the condition of the ameba".

9. Different individuals of the same pedigreed line vary somewhat in their susceptibility to NaCl solutions. There is evidence that this is correlated with the rate of division in the NaCl solutions.

10. There is some evidence that racial acclimatization is possible when judged by reproduction, for some races of amebas will not divide in 0.1 % NaCl solutions unless they have first divided in 0.05 % NaCl solution (Table II, III, and text).

11. These amebas do not adhere to the substratum in concentrations greater than 0.125 % NaCl.

12. *Polychaos dubia* does not continue to divide in concentrations higher than 0.05 % NaCl, and is less resistant to NaCl than *Chaos diffuens*.

### Bibliography.

- ADOLPH, E. G. (1926): The metabolism of water in ameba as measured in the contractile vacuole. Journ. Exp. Zool. Vol. 44 p. 365—379.
- BAYLISS, W. M. (1927): Principles of General Physiology, 4th. ed. London, New York (etc).
- BOTSFORD, E. F. (1926): Studies on the contractile vacuole of *Amoeba proteus*. Journ. Exp. Zool. Phila. Vol. 45 p. 95—139.
- BRINKLEY, F. S. (1928): Effect of Chemicals on viscosity of protoplasm of ameba as indicated by Brownian movement. Protoplasma Vol. 4 (2) p. 177—182.
- CALKINS, G. N. (1910): The Biology of the Protozoa.
- CHATTON, E. and TELLIER, L. (1927): Sur les limites et les vitesses de retour a l'équilibre physiologique du *Glaucoma pyriformis* porté dans les solutions hypertoniques des NaCl. Compt. Rend. Vol. 97 (25) p. 780—784.
- CLOWES, G. H. A. (1916): Protoplasmic Equilibrium. Action of antagonistic electrolytes on emulsions and living cells. Journ. Physic. Chem. Vol. 20 p. 407—451.
- CZERNY, V. (1869): Einige Beobachtungen über amoeben. Arch. f. mik. Anat. Vol. 5 p. 158—163.
- DAVENPORT, C. B. (1897—99): Experimental Morphology Part I. New York.
- DEGEN, A. (1905): Untersuchungen über die kontraktile Vakuole und die Wabenstruktur des Protoplasmas. Bot. Zeitung Bd. 63 p. 160—226.

- EDWARDS, J. G. and FORGRAVE, H. S. (1923): The rate of locomotion in ameba in alkali chlorides. *Bull. Johns Hopkins Hosp. Balt.* Vol. 34 p. 387—389.
- EDWARDS, J. G. (1924): Action of certain reagents on amoeboid movement. *Brit. Journ. Exp. Biol.* Vol. 1 p. 571—595 1 pl.
- (1925): Formation of food cups in ameba induced by chemicals. *Biol. Bull.* Vol. 48 p. 236—239.
- FINLEY, H. E. (1930): Toleration of fresh water Protozoa to increasing salinity. *Ecology* Vol. 11 No. 2 p. 337—347.
- FLEISCHMANN, W. (1929): Permeabilität der Leukocyten. *Klin. Wochenschr.* Bd. 8 (26) p. 1223.
- HEILBRUNN, L. V. (1928): The Colloid Chemistry of Protoplasm. Berlin, 365 pages.
- HOGUE, M. J. (1923): Contractile vacuoles in amebas-factors influencing their formation and rate of contraction. *Journ. Elisha Mitchell Sc. Society* Vol. 39 No's 1 and 2.
- HULPIEU, H. R. (1930): The effect of oxygen on *Amoeba proteus*. *Journ. Exp. Zool.* Vol. 56 No. 3 p. 321—359.
- LILLIE, R. S. (1909): The general biological significance of changes in the permeability of the surface layer of living cells. *Biol. Bull.* Vol. 17 p. 188—208.
- LOEB, L. (1921): The consistancy of protoplasm and the character of amoeboid movement. *Am. Journ. Physiology* Vol. 55 p. 280.
- MAST, S. O. (1926): Structure, movement, locomotion and stimulation in amoeba. *Journ. Morph. and Physiol.* Vol. 41 p. 347—425.
- (1928): Form not specifically and directly correlated with any environmental factor. *Journ. Exp. Zool.* p. 51.
- (1929): Mechanics of locomotion in *Amoeba proteus* with special reference to factors involved in attachment to substratum. *Protoplasma* Vol. 8 p. 344—377.
- (1931): Effects of salts, hydrogen ion concentration and pure water on length of life in *Amoeba proteus*. *Physiol. Zool.* Vol. 4 No. 1 p. 58—71.
- and HULPIEU, H. R. (1930): Variation in the response to light in *Amoeba proteus* with special reference to the effects of salts and hydrogen ion concentration. *Protoplasma* Vol. 9 No. 3 p. 412—431.
- OSTERHOUT, W. J. V. (1912): The permeability of protoplasm to ions and the theory of antagonism. *Science* Vol. 35 p. 112—115.
- PARKER, T. J. (1914): *Elementary Biology*.
- PANTIN, C. F. A. (1923): The physiology of amoeboid movement. *Journ. Mar. Biol. Assn.* Vol. 13 p. 24—69.
- (1924): The physiology of ameboid movement. II. Effects of temperature. *Brit. Journ. Exp. Biol.* Vol. 1 p. 519—537.
- (1926): Physiology of ameboid movement, III. Action of Calcium. *Brit. Journ. Exp. Biol.* Vol. 3 p. 275—312.
- PHELPS, L. A. (1926): Experimental analysis of factors concerned in division in ameba. *Anat. Rec.* Vol. 31 p. 312.
- REZNIKOFF, P. and CHAMBERS, R. (1924—25): The reaction of protoplasm of the living ameba to injected salts. *Proc. Soc. Exp. Biol. and Med. New York* Vol. 22 p. 320—322.
- (1924—25): Immersing and tearing ameba in NaCl solutions. *Ibid.* Vol. 22.

- SCHAEFFER, A. A. (1916): Notes on the specific and other characters of *Amoeba proteus* PALLAS (LEIDY), *A. discoides* spec. nov. and *A. dubia* spec. nov. Arch. f. Protistenk. Bd. 37 p. 204—228.
- (1920): Ameboid Movement. Princeton Univ. Press.
- (1926): Recent discoveries in the biology of the ameba. Quart. Rev. Biol. Vol. 1 p. 95—118.
- (1926): Taxonomy of the Amebas. Papers from the Dept. of Marine Biology, Carnegie Inst. of Wash. Vol. 24 p. 1—116.
- SEIFRIZ, W. (1929): The viscosity of protoplasm. Bull. Nat'l Research Council No. 69 p. 229—261.
- WOLF, E. (1927): Sur quelques particularites de la division et de l'enkystement d'une Amibe d'eau douce. Compt. Rend. Acad. Sc. Vol. 184 p. 283—285.
- ZUELZER, M. (1907): Über den Einfluß des Meerwassers auf die pulsierende Vakuole. Arch. f. Entw. d. Org. Bd. 29 p. 632—640.

# ZOBODAT - [www.zobodat.at](http://www.zobodat.at)

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Archiv für Protistenkunde](#)

Jahr/Year: 1932

Band/Volume: [76\\_1932](#)

Autor(en)/Author(s): Badgley E.W.

Artikel/Article: [The acclimatization of Chaos diffluens to sodium chloride solutions. 235-256](#)