

THE EFFECTS OF SOME CATIONS UPON THE GROWTH RATE  
AND COLLOIDAL NATURE OF THE PROTOPLASM  
OF PARAMECIUM CAUDATUM

by

RALPH EDWARD PETERSON

B. S., Kansas State College  
of Agriculture and Applied Science, 1940

---

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE  
OF AGRICULTURE AND APPLIED SCIENCE

1941

Docu-  
ment  
LD  
2668  
T4  
1941  
p41  
C.2

TABLE OF CONTENTS

	Page
INTRODUCTION . . . . .	1
REVIEW OF LITERATURE . . . . .	3
MATERIAL AND METHODS . . . . .	23
Procedure for Making Pure Cultures of <u>Paramecium</u> <u>caudatum</u> . . . . .	23
Procedure Used in Measuring the Apparent Viscosity of the Protoplasm of <u>Paramecium caudatum</u> . . . . .	32
RESULTS . . . . .	37
The Normal Growth Curve of <u>Paramecium caudatum</u> . . . . .	37
The Effects of Various Salts upon the Growth Rate of <u>Paramecium caudatum</u> . . . . .	39
The Effects of Various Salts upon the Viscosity of the Protoplasm of <u>Paramecium caudatum</u> . . . . .	60
DISCUSSION . . . . .	69
SUMMARY . . . . .	97
ACKNOWLEDGMENT . . . . .	101
LITERATURE CITED . . . . .	102

## INTRODUCTION

A voluminous amount of literature has been published upon the effects of the alkali and alkaline earth salts, and in particular, the cations of these salts, upon the growth of various kinds of plants and animals. Most investigators who have worked with the effects of different salt solutions on living protoplasm have merely recorded the actual results observed after subjecting the protoplasm to various concentrations of salts. It has only been in recent years that attempts have been made to explain the action of the various salts upon the protoplasm on the basis of experimental evidence.

The purpose of this problem was, first, to measure the effects of different salt solutions (cations) upon the growth rate and longevity of the protoplasm of Paramecium caudatum (Ehren.); second, to compare the normal growth curve with the growth curves of those paramecia grown in different molar concentrations of salt solutions; and third, to determine the reasons why the growth curves for the paramecia grown in the various salt solutions deviated from the curve of normal growth.

Any theory that attempts to explain the changes that occurred in the protoplasm and in the growth rate will undoubtedly lie in an interpretation of the changes that take place in the colloidal properties of protoplasm.

In interpreting the colloidal nature of protoplasm, the biologist is not so fortunate as the chemist, for the former is

limited in the methods available to him for studying the properties of the protoplasmic colloids. The only methods that are of value to him in studying and explaining the colloidal nature of the protoplasm are electrophoresis determinations and determinations of protoplasmic viscosity.

Viscosity studies have, in the past, proved to be the surest guide to an interpretation of the colloidal nature of the protoplasm, for it is known that colloidal systems vary their viscosity rapidly and that these variations may be quite marked. It is for this reason that viscosity changes are perhaps the best indicators of colloidal processes in the living cell. In the present study, this method of analysis was chosen to determine the causes for the effects of various salt solutions (cations) upon the growth rate of Paramecium caudatum.

## REVIEW OF LITERATURE

In making any study of the effects of salt solutions upon the growth rate and physical nature of the protoplasm, it is imperative that a determination be made of the standard or normal growth rate, so that any deviation from the mean may be adequately interpreted.

In the culturing of paramecia, Hargitt and Fray (1917) were among the first experimenters to take cognizance of the necessity of using mono-bacterial strains in making quantitative studies upon the physiology of the protozoa. They found that it was necessary to free the paramecia of all bacteria in order that the paramecia might be grown upon a medium that contained only a single species of bacteria as the source of food.

A number of bacteriologists have pointed to the possible effects that might be produced when two or more strains of bacteria are grown in the same culture medium. These effects are known as synergistic effects. For example, Speakman and Phillips (1924) found that when either Bacillus granulobacter-pectinovorum or Bacillus volutans were cultured separately, no lactic acid was produced in the culture. However, if these two species of bacteria were grown in association with each other, lactic acid was produced. In this instance the production of the lactic acid was found to be due to the partial inhibition of the normal physiological processes of Bacillus granulobacter-pectinovorum by Bacillus volutans.



Other examples of bacterial synergisms are discussed by Sears and Putman (1923), Holman and Meekison (1926), and Castellani (1926).

Several methods have been described for the sterilization of the protozoa. Hargitt and Fray (1917), Phillips (1922), Farpart (1928), and Hetherington (1934) sterilized paramecia by washing them in successive changes of sterile water.

Amster (1922) used the process of cataphoresis to free the bacteria from paramecia.

Cleveland (1928) made use of lateral migration by causing the protozoa to migrate across a 10-inch Petri dish.

Glaser and Coria (1930) took advantage of the phenomenon of negative geotropism exhibited by paramecia to sterilize them by migration through long glass tubes of sterile media.

Kidder and Stuart (1939) used the centrifuge method for sterilizing Colpoda, employing at least 15 washes. This method is, however, limited in its application to situations where the investigator has a high concentration of protozoa with which to start.

Claff (1940) designed an apparatus for sterilizing negatively geotropic protozoa by incorporating both a migration and a dilution factor in a closed system. The organisms were introduced into one end of the system and were not exposed to any chance contamination until they were taken away from the apparatus, after having passed through six flasks of sterile media. This appears to be the most satisfactory method yet devised for the sterilization of paramecia. The method was not employed

with this problem because it was not discovered until the problem was near completion.

There has been an extensive amount of work done on the nutrition and technic of growing paramecia. Most of the investigators (Hargitt and Fray, 1917; Phillips, 1922; Parpart, 1928; Glaser and Coria, 1930; Hetherington, 1934; and Loefer, 1936) have maintained the organisms in a hay infusion culture in the presence of a single species of some secondary microorganisms, such as living bacteria and yeast cells.

Recently Glaser and Coria (1933) claimed to have grown Paramecium caudatum on a nutrient medium in the complete absence of any living microorganisms. Their medium consisted of liver extract, killed yeast cells, and fresh rabbit kidney.

Peters (1920, 1921) grew Colpidium colpoda in the absence of any bacteria in a buffered salt mixture containing glucose, ammonium lactate, and some of the common amino acids.

As the secondary organism for use in growing the paramecia, Hargitt and Fray (1917), Phillips (1922), and Johnson (1936) found that Bacillus subtilis, the "hay bacillus", supported a good growth of paramecia. Phelps (1931) and Hetherington (1934) used Erythrobacillus prodigious to cultivate paramecia.

Glaser and Coria (1930) grew paramecia on the yeast, Saccharomyces cervisial, while Loefer (1936) used Parke Davis and Company yeast, Saccharomyces ellipsoidous with a tryptone medium.

Luck, Sheets, and Thomas (1931) have presented an excellent review on the role of bacteria in the nutrition of protozoa.

It has been noted by Robertson (1923), Yocom (1928), and Peterson (1929) that there is a definite relationship between the volume of culture medium used in growing the ciliates and the rate of growth of such organisms. Since this will be an important variable in making any quantitative studies of paramecia, it seems advisable to review the literature on this subject.

It has been found in the present study that the rate of division of the paramecium is inversely related to the volume of the culture medium used.

Robertson (1923) found that when two ciliates (Enchelya) were isolated into the same drop of culture medium, the division rate was higher than if a single cell was isolated into a drop of the same size. This difference in division rate, according to Robertson, is due to what he calls the "allellocatalytic effect" (autocalalyst), caused by the liberation of a growth catalyzing substance from the cell.

Yocom (1928) found that in Oxytricha cultures that contained only four drops of medium, there was a 14% higher division rate than in the ten drop cultures.

Peterson (1929) found that within certain limits a higher rate of division of paramecium is attained in cultures containing a larger number of ciliates.

Woodruff (1911), Cutlur and Crump (1923), and Greenleaf (1924, 1926), Myers (1927), and Calkins (1933), however, have observed exactly the opposite results in their experiments with various ciliates.

Little work has been done on growth curves for protozoa.



Calkins (1933) gave a good review of the methods used in studying the growth and growth characteristics of the protozoa; but no mention was made of the use of growth curves.

Buchanan and Fulmer (1928) have given a good discussion of the growth rates and of growth curves for bacteria, and this may be applied equally well to the protozoa.

Phelps (1935, 1936) and Kidder (1941a, 1941b) have recently considered the growth characteristics of certain ciliates, and the effects of certain substances upon the growth curves of these organisms.

Jahn (1934) published a review of the problems that have to be taken into consideration in studying the population growth of the protozoa. He maintained that it is not feasible to apply mathematical formulae to studies upon the growth rates of protozoa, because the factors influencing the growth rate can not be well enough controlled. Particularly is this true when one is working with a protozoan form that will not grow in the absence of bacteria.

Jahn (1934) mentioned the following ten factors that might affect the growth rate of protozoa: (1) food supply, (2) waste products, (3) hydrogen ion concentration, which may determine the toxicity of the substance, (4) temperature, (5) light, (6) physiological differences, (7) oxidation-reduction potential, (8) oxygen tension, (9) autocatalysis, and (10) the presence of mixed species.

Fulmer (1928) stated that the rate of logarithmic growth is affected by osmotic pressure, pH, surface tension, interfacial

tension, extent of hydration of cell colloids, and temperature.

In studying Paramecium caudatum, another factor that might be added to the above list is that of endomixis (Erdmann and Woodruff, 1916). This is a type of internal reorganization of the protoplasmic constituents which occurs in certain strains of Paramecium caudatum every 50-60 days.

An enormous amount of literature has been published on the action of the various salts upon plant and animal protoplasm. Since the action of salts upon plant and animal protoplasm seems to show similar effects, a review of the literature from both fields will be presented; however, the review will be limited to the literature that deals with the action of the cations, since this problem has dealt entirely with cation effects.

Loeb (1902, 1924) maintained that the effects of ions upon protoplasm was related to a purely chemical grouping of the ions. He stated that the valence determined the effect of the cations upon the purely physical properties of the colloids, such as, osmotic pressure, swelling, and viscosity. He denied the existence of the Hofmeister or lyotropic salt series, maintaining that such a series becomes a pure valence grouping of ions when acidity is kept constant.

Loeb maintained that the Donnan equilibrium explained fully the action of electrolytes upon the osmotic pressure, and that this was explained solely upon the basis of the valence of the ions concerned, since the equilibrium equation depends only upon the valence of the ions. Thus, he concluded that since the osmotic pressure, viscosity and swelling of proteins are all

influenced in a similar way by electrolytes, all three of these physical properties are, in the last analysis, functions of one and the same property, namely osmotic pressure.

Greely (1904) found that with paramecium the speed of coagulation of the protoplasm is proportional to the valence of the cation in the following order:  $\text{Na} < \text{Ca} < \text{Al}$ . He maintained that the effects of these cations on the protoplasm can be explained purely on the basis of electrostatic action.

Mathews (1904, 1940) attempted to relate the degree of toxicity of the ions to known physical and chemical properties of the ions. He attempted to show the relationship between solution tension or ionic potential, atomic volume, equivalent weight, and the physiological action of the cations.

Mathews referred to the solution tension or ionic potential as the voltage of the ion--that is, the tendency of the ion to give up its charge or the amount of available energy in the ion. The greater the affinity of the element for its ionic charge, the greater the voltage it will take to separate that charge from it. He found that the greater the voltage of an ion, the less toxic it is. He showed that for Fundulus eggs, the toxicity of the cation varied inversely with the solution tension.

Mathews (1904) also attempted to relate the degree of toxicity to the atomic volume or the individual ion. By assuming that the charge is moving about the atom, or the atoms themselves are rotating with the same speed, he came to the conclusion that the atoms of large atomic volume are less active than those of a small volume. He maintained that the toxicity was

inversely proportional to the atomic volume.

In addition, he found that the toxicity of the cations was directly proportional to the equivalent weight of the cation. Thus, those elements with a high equivalent weight and a low solution tension and atomic volume (Hg) are more active than those with a low equivalent weight, and a high solution tension and atomic volume (Na).

Woodruff and Bunzel (1909) ascertained that the specific toxicity of the ions for paramecium was related to the ionic potential or solution tension of the ions. They found that when the ionic potential decreased, the toxicity decreased ( $\text{Ag}^+ \rightarrow 1.165$  and  $\text{K}^+ \rightarrow -2.92$ ).

Estabrook (1910) discovered, in working with the effects of NaCl on paramecia, that a concentration of greater than M/50 inhibited their growth rate, whereas concentrations as high as M/50 were not harmful.

Cramer (1918) has discovered that Ca ions inhibit the growth of mouse carcinoma, whereas Na ions produce no effect. He explained this on the assumption that rapidly growing cells contain more water bound to the protoplasm and that Ca salts inhibit growth because they cause the cell to lose water.

Falk (1918) observed that in addition to the effects that some of the cations might have on the physical structure of the protoplasm, they might also have an effect on the enzyme systems within the cell. He noticed, in vitro experiments, that NaCl stimulated lipase action, and  $\text{CaCl}_2$  definitely inhibited its action.

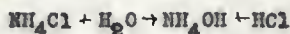


Eisenberg (Hotchkiss, 1923) determined the toxicity series of the cations for bacteria arranged in the following order: Na < K < NH<sub>4</sub> < Li < Mg < Sr < Ca < Ba < Mn < H < Ce < Cr < Fe<sup>+++</sup> < Fe<sup>++</sup> < Zn < Th < Al < Cu < Ti < Ni < Tl < Cd < Pb < Co < Au < Pt < Hg.

Loeb (1920) stated that the ionic or atomic radius is related to the action that an ion might have upon the protoplasm. The radius of a single ion, in this case, being the distance between the positive nucleus and the outermost ring or shell of electrons. A monovalent cation bears one excess positive charge in its nucleus. The cation, according to Loeb, acts through the excessive positive charges in the nucleus and the ionic or atomic radius. The electrostatic action of the nucleus being greater, the smaller the nucleus, i. e., the nearer the positively charged nucleus can get to the body on which it is supposed to act. This explains, according to Loeb, why the accelerating as well as depressing effect of a cation on the rate of diffusion of water through the membrane increases inversely with the radius of the cation in the order Rb > K > Na > Li.

Fulmer, et. al., (1921) found that the optimum concentration of NH<sub>4</sub>Cl for the growth of yeast was the concentration of the salt in which a protein (wheat gluten) swelled the least.

Jacobs (1922) postulated a theory for the action of NH<sub>4</sub>Cl on the isolated cell. He pointed out that an aqueous solution of NH<sub>4</sub>Cl, on account of the weakness of NH<sub>4</sub>OH as a base undergoes partial hydrolysis into



and that because of the greater degree of dissociation of the

acid as compared with  $\text{NH}_4\text{OH}$ , the solution has an acid reaction. It is well known that the  $\text{NH}_4\text{OH}$  molecule (or the  $\text{NH}_4^+$  ion) penetrates the cell much more readily than the  $\text{HCl}$  complex. Therefore, even though the reaction of the solution is acid, the cell contents might be alkaline. Jacobs (1920) has also shown that the reverse may be true in that the cell contents might be acid and the external medium basic, due to the penetration of the  $\text{CO}_2^-$  ions when in high concentration.

Osterhaut (1922) suggested that the toxicity of the monovalent salts is associated with an increase in the permeability of the membranes of living cells (indicated in Laminaria by a fall in electrical resistance), whereby they lose essential constituents, and that divalent salts decrease the permeability of the membrane, thereby preventing necessary exchanges with the medium.

Hotchkiss (1923) discovered that the order of toxicity of the various cations upon the growth of Bacillus coli was  $\text{Na} < \text{K} < \text{NH}_4 < \text{Li} < \text{Sr} < \text{Ca} < \text{Mg} < \text{Ba} < \text{Mn} < \text{Tl} < \text{Tl} < \text{Ni} < \text{Sn} < \text{Cu} < \text{Fe}^{++} < \text{Fe}^{+++} < \text{Al} < \text{Ce} < \text{Pb} < \text{Co} < \text{Cd} < \text{Hg}$ .

Heilbrunn (1923) claimed that  $\text{CaCl}_2$  and  $\text{MgCl}_2$  solutions decreased the viscosity of the protoplasm of Stentor, and that  $\text{NaCl}$ ,  $\text{KCl}$ ,  $\text{LiCl}$ , and  $\text{NH}_4\text{Cl}$  solutions increased it.

Falk (1923) pointed out that the cardinal purpose of the cations in the cell is to act as a regulative mechanism upon the biochemical activities, whereas the anions are more intimately related to nutrition. He maintained that the action exerted by the cation is on the surface interfaces in the cell.

Heilbrunn (1925b) stated that bivalent and trivalent cations are more readily adsorbed than the monovalent cations.

Chambers and Reznikoff (1925) found that the toxicity of the ions depended upon whether the salt acted upon the inner protoplasm or upon the membrane. They discovered that upon immersion of ameba into various salt solutions, the toxicity series was  $K > Na > Ca > Mg$ . With injection experiments, the series was  $Mg > Ca > K > Na$ . In the former, the Na and K ions caused a liquifaction of the membrane, and the Ca and Mg ions caused a solidification. From these facts they assumed that the toxic effects of Na and K were caused by a greater susceptibility of the cell membrane. Upon injection of the salts into ameba, the Ca and Mg ions caused a solidification of the internal protoplasm.

Reznikoff and Chambers (1929) found that with Amoeba dubia the predominant action of the salt is that of the cation, and that the divalent and trivalent cations are more toxic.

Most investigators have expressed the relationships between salt effects upon the basis of molarity or isotonicity. However, Winslow and Dolloff (1928) have used the degree of dissociation of the ions or the ionic activity (strength) as a means of expressing the relationship between different salts. They calculated these from the equation of Lewis and Randall (1923):

$$\mu = \frac{m \cdot v^2}{2} \quad \frac{m \cdot v^2}{2}$$

- $\mu$  = ionic strength or activity of solution
- $m$  = stoichiometric molarity for each ion
- $v$  = valence for each ion

Page (1929) has shown that the toxicity of the cations for

sea urchin eggs (Arbacia) is  $Li > Na > Ca > Mg > K > Rb > Cs$ .

True (1930) postulated a theory to explain toxicity that is in direct opposition to the viewpoint expressed by Loeb (1924). True found no close correlation between a chosen physiological property of the ions and such physical and chemical properties as the atomic number, atomic weight, speed of migration, and solution tension. He suggested that the toxicity of the ions is the result of the adaptation of the living protoplasm through evolutionary history to the soluble constituents of the environment. Alkali metals and alkaline earth metals which are abundant in the soil are relatively non-toxic, compared with heavy metals which are rare or are soluble only in dilute solutions.

Telkes (1931) found that M/20 KCl and M/10 NaCl solutions brought about death in ameba in a few minutes, whereas concentrations of M/5  $CaCl_2$  and  $MgCl_2$  were not lethal to the ameba.

Mast (1931) discovered that in a single dilute salt solution (M/1000) the order of length of life observed, beginning with the highest is Na ( $SO_4$ ,  $NO_3$ , Cl,  $PO_4$ ,  $C_2H_3O_2$ ), Mg, Ca (Cl,  $SO_4$ ,  $PO_4$ ,  $NO_3$ ,  $C_2H_3O_2$ ), K and  $NH_4$ . This series indicates that there was only a very slight difference in the effect of the anion. Mast (1931) also corroborated the evidence deduced by Chambers and Reznikoff (1925) that Na and K are more toxic to the exterior of the cell, and Ca and Mg are more toxic to the interior.

Winslow and Haywood (1931) found that the average specific potency of several cations is Na, 1; K, 1.2; Li, 3.4; Ba, 5.0; Mg, 9.4; Ca, 12; Mn, 400; Zn, 700; and Cd, 3,000; where the



specific potency is expressed as the reciprocal of the ratio of the concentration of a given salt to the potency of Na.

Seifrig and Flowe (1931) found that while all divalent cations increased the extensibility (viscosity) of the protoplasm more than the monovalent ones within each valence group, no two ions had the same effect.

Heilbrunn and Daugherty (1931) found that Na and K caused an increase in the viscosity of the protoplasm of Amoeba dubia, and that Ca, Mg, and  $NH_4$  ions caused a decrease.

The following year these workers, Heilbrunn and Daugherty (1932), found that K, Mg, and Na cations produced a decrease in the viscosity of the plasmagel in Amoeba proteus in the order  $K > Mg > Na$ . The Ca ions caused an increase in viscosity. Heilbrunn (1937) maintained that the action of K is inhibited when the surrounding acidity is high. Thus, the viscosity of the plasmagel is decreased in KCl solutions, but no change is produced if the solution be acid enough.

Lindahl (1933) discovered that Li ions inhibit the enzyme activity of cells immersed in a LiCl solution.

Kamada (1934) found that the more dilute the solution ( $> M/40$  NaCl), the more negative was the charge on the cell interior.

Winslow (1934), in a review on the influence of cations on bacterial viability, stressed the importance of the fact that the different effects of various cations upon the cell is purely a matter of degree rather than of kind. That is, each cation exerts a primary effect upon the bacterial cell which is quali-

tatively different for each cation. Every salt has its own stimulative effect on viability, as well as its definite concentration for inhibition. For example, if one compares the effects of one cation with that of another, there is a definite quantitative relationship between all points on the graph of viability at varying salt concentrations. Thus, if the concentration of Salt A, which produces a given degree of stimulation, is called X, and the concentration of Salt B, which causes the same degree of stimulation, is 10X; and if the concentration of Salt A, which produces a given degree of inhibition is 5X, then the concentration of Salt B, which should cause the same degree of inhibition, is 50X.

Barnes (1935) found the toxicity series for the littoral isopod Ligia in an isotonic solution was  $\text{NH}_4 > \text{Li} > \text{K} > \text{Mg} > \text{Ca} > \text{Na}$ .

Thornton (1935) reported that Ca increased the viscosity of the plasmagel of Amoeba proteus, and that K, Na, and Mg decreased the viscosity. He found that Ca speeded up amoeboid movement, and that K, Na, and Mg had the opposite effect.

Heilbrunn (1936) observed that the  $\text{NH}_4$  salts break up the protein-lipin complex in the protoplasm, and this results in fatty or lipin degeneration. This fatty degeneration is believed to occur because the  $\text{NH}_4\text{OH}$  penetrates the cell and the resultant increase in alkalinity liberates the fat. He found that an excess of  $\text{CO}_2$  prevents this action of the  $\text{NH}_4$  ion, probably due to the fact that it tends to keep the intracellular pH low.

Barnes (1937) stressed the necessity of considering the

mobility or speed of migration of the ions in making references to the toxicity of ions.

Oliphant (1938) found that the order in which the monovalent cations produce death in paramecium is  $Li > K > Na$ .

Northen and Northen (1939) discovered that in Spirogyra, the univalent cations of Li and Cs decreased, while Na and K increased the protoplasmic elasticity (viscosity). Of the divalent cations, Ca, Sr, and Ba decreased the elasticity, and Be, Mg, Zn, Cd, and Hg increased it.

McCalla (1940a) has added further support to the theory that cations act upon the cell protoplasm by their degree of adsorption. The order of adsorption for Escherichia coli is  $Na < K < Ca < Ba < Hg$ . McCalla also found that the more highly adsorbable ions tended to replace the less adsorbable ones. By measuring the ability of the cations to replace methylene blue from the bacterial cell, McCalla (1940b) found the following order of adsorption:  $Na < NH_4 < K < Mg < Ca < Ba < Mn < Al < Fe < H$ .

Neilbrunn (1940) has shown that Ca, Ba, Sr, and Mg salts cause a coagulation of muscle protoplasm, but that Mg is only effective in very high concentration.

The results of numerous investigators (Ostwald, 1907; Powers, 1920; White, 1939; and Seifrig and Uraguchi, 1941) seemed to indicate that the toxicity of a cation may be expressed by the fundamental equation  $1,000/t = K \cdot c^b$  in which  $1,000/t$  represents the relative toxicity,  $c$  is the concentration, and  $K$  and  $b$  are constants. Ostwald (1907) and White (1939) have noted the similarity between this equation and the Freundlich adsorp-

tion equation (1926),  $a = ac \ 1/n$ . In this equation  $a$  is the amount of substance adsorbed per gram of adsorbant,  $c$  is the concentration of the solution at equilibrium, and  $a$  and  $n$  are constants. Gause (1933) working with paramecium in  $HgCl_2$ , has also found a similar relationship.

Seifrig and Uraguchi (1941) also explained the order of toxicity of the cations of the heavy metals upon the slime mold, Physarum polycephalum, on the basis of their degree of adsorption. They found that a direct relationship existed between toxicity and the degree of adsorption. The order of toxicity was  $Ag > Hg > Cd > Tl > Cu > Pb > Zn > Y > Sr > La > Rb$ .

Investigators have determined the viscosity of the protoplasm by several different methods, some of which give only arbitrary values.

Neilbrunn (1937) measured the viscosity of the protoplasm of plant cells by determining the rate of fall of starch grains through the cell, while Chambers (1919) determined changes in protoplasmic viscosity by microdissection methods.

Seifrig (1920) introduced mechanically controlled glass needles into both plant and animal cells and into various media of known or surmised viscosity. He then compared the passage of the needles in the living and the non-living materials.

Later Seifrig (1924) used a magnet method for determining protoplasmic viscosity. He introduced small pieces of metal into the protoplasm and then twisted these with an electromagnet to determine the viscosity.

Investigators soon found that the method of determining



protoplasmic viscosity as originally carried out by Heilbrunn, and mentioned previously on plant cells, could not be used on most animal cells because it generally takes a force stronger than that affected by gravity to move granules in the protoplasm. Such a force can only be obtained with the centrifuge, and as a result Heilbrunn (1921, 1926a, 1926b) developed the centrifuge technic for determining protoplasmic viscosity.

With the centrifuge, Heilbrunn was able to arrive at a measurement of the viscosity of the protoplasm by using the formula of Stokes' for the rate of fall of a spherical body through a liquid. Cunningham's (1910) modification of Stokes' formula is

$$v = \frac{2cg(\sigma - \rho)a^2}{9\eta}$$

where  $v$  is the speed of migration of the granules through the protoplasm,  $c$  is the centrifugal force in terms of gravity,  $g$  is the gravity constant,  $\sigma$  is the specific gravity of the granules,  $\rho$  is the specific gravity of the medium through which they travel,  $a$  is the radius of the granules and  $\eta$  is the viscosity of the protoplasm.

Most measurements upon viscosity as determined by the centrifuge give values for the granule-free hyaline protoplasm. However, determinations have been made upon the viscosity of the entire protoplasm--granules plus the surrounding protoplasm. This has been done by employing the formula of Einstein and Hatschek (Heilbrunn, 1925, 1926, 1937) when the viscosity of the hyaline protoplasm and the approximate concentration of granular

material is known.

The formula of Einstein and Hatschek for relative viscosity is

$$\eta_c = \eta_0(1 Kf)$$

in which  $\eta_c$  is the relative viscosity of the protoplasm, where relative viscosity is the ratio of the viscosity of the suspension to that of the solvent at the same temperature,  $\eta_0$  is the viscosity of the dispersion medium (granule-free protoplasm),  $f$  is the ratio of the volume of the suspended particle to the total volume of the suspension, and  $K$  is a constant which Einstein places at 2.5 and Hatschek at 4.5.

Belohradek (1926) has determined the protoplasmic viscosity by the use of a temperature coefficient, where the majority of the biological reactions depend upon the temperature according to

$$y = a/x^b$$

where  $x$  is the temperature in  $C^0$ ,  $y$  is the time, and  $a$  and  $b$  are constants ( $b$  is the real temperature coefficient). Belohradek assumed that since  $b$  increased with age (and with time), under the action of narcotics, and in dry air, that the  $b$  should indicate the degree of protoplasmic viscosity.

Thornton (1932) has determined the viscosity of the plasma-gel of Amoeba proteus at different temperatures by determining the centrifuge time, in seconds, which is necessary to displace all the crystals of the cytoplasm to one end of the cell.

Harris (1935) used the Einstein equation for the rate of Brownian movement to study the protoplasmic viscosity of the

eggs of the worm, Sabellaria alvelata. He centrifuged the eggs and then determined the viscosity by observing the Brownian movement by the use of a microcinematograph. He obtained his values by applying Einstein's formula

$$D \frac{C}{K} = \frac{RTt}{N3\eta a}$$

where D is the displacement of a particle in Brownian movement along one axis, R is the gas constant, N is Avogadro's number, T the absolute temperature, t the time, a the radius of the particle, and  $\eta$  the viscosity.

Northon (1938) measured the elasticity (viscosity) of the protoplasm of Spirogyra by applying the following formula:

$$v = K(C-C_0)$$

where v is the velocity of chloroplastic movement, K is a constant, C is the centrifugal acceleration used, and C<sub>0</sub> is the initial starting centrifugal acceleration at which, or below which, the chloroplasts will not move regardless of how long the acceleration is allowed to act.

Heilbrunn has presented the greatest amount of work on protoplasmic viscosity determinations by means of the centrifuge. In making a study upon viscosity changes introduced during mitosis in the eggs of Cumingia, Heilbrunn (1921) found that, in both maturation divisions and cleavage, appearance of the spindle was always preceded by a sharp increase in viscosity followed by a sharp decrease.

Heilbrunn (1926b) found that the eggs of the clam Cumingia and the sea urchin Arbacia were only slightly more viscous than

water. For the Arbacia he determined the viscosity by the centrifuge method to be 1.8 times as viscous as water. For the eggs of Gummingia he found the viscosity to be 4.3 times that of water.

There has been very little work done upon the relationship between viscosity and growth; however, Weber and Belohradek (Belohradek, 1926) found that protoplasmic viscosity increased with an increase in age.

All types of protoplasm do not have the same viscosity. For example, Fetter (1926) found that the viscosity of the endoplasm of paramecium was more than 8,000 times as viscous as water. Heilbrunn and Daugherty (1932) have demonstrated that the cortex of a cell may have a much higher viscosity than the interior.

On the basis of the Einstein and Hatschek formula, Heilbrunn (1926b) has shown that whereas the viscosity of the granule-free protoplasm of the sea urchin is 1.8 times as viscous as water, the viscosity of the entire protoplasm is approximately six times that of water.

Brown (1940) determined the protoplasmic viscosity of the paramecium to be only 50 times that of water. He used the microscopic centrifuge method and was able to observe the actual movement of the granules through the endoplasm.



## MATERIAL AND METHODS

Procedure for Making Pure Cultures of Paramecium caudatum

Sterilization of the paramecia. The paramecia used in this study were descendants of a single specimen isolated in November, 1940, from a departmental culture.

The paramecia were sterilized to be freed of all bacteria. This was done because the presence of a heterogeneous bacterial flora would furnish the paramecia with a varied nutriment and thus produce changes in the growth rate of the animal.

However, the paramount argument in favor of the use of a mono-bacterial strain for culturing paramecia is that when several different strains of bacteria are present in the same culture, synergistic effects may be produced that could not be produced by a single strain of bacteria.

For this problem, the sterilization procedure of Parpart (1928) was used, with only a slight modification.

All of the glassware used in this experiment was cleaned with a dichromate sulphuric acid cleaning solution. After the glassware was cleaned, it was rinsed in tap water and then in distilled water. After being dried, it was sterilized either by heating in a hot air oven at 160°- 170° C. for 45 minutes, or in an autoclave at 15 pounds pressure for 15-20 minutes. In addition, all of the solutions that were used in this experiment were sterilized before being used.

A series of 10 sterile Petri dishes, with a depression slide in each, were cleaned and sterilized. A few drops of sterile M/100  $\text{CaCl}_2$  solution were placed in each depression. (This is a modification of Parpart's original method, for it was discovered that an excess of Ca ions in the solution greatly speeded up the rate of locomotion of the paramecia, and, as will subsequently be seen, this is a definite aid in the sterilization of the animals). A single paramecium was placed at one edge of the depression in the slide in Petri dish No. 1. When the paramecium swam to the opposite side of the depression, it was drawn out with a micro-pipette, taking care to remove only a small amount of liquid with the paramecium. This paramecium was then placed into the liquid at one edge of the depression in Petri dish No. 2, at the same time observing the rigid use of aseptic technic in making all of the transfers. This procedure was repeated until Petri dish No. 5 was reached. In No. 5, the paramecium was left for seven hours in order that any bacterial spores that were not digested by the animal might be egested. After the paramecium had remained in Petri dish No. 5 for seven hours, it was transferred through the remaining dishes to No. 10 in the series, using the same technic as was used in making the transfers through the first five dishes. Petri dish No. 10 thus contained a single sterile paramecium.

Preparation of the general supply culture (culture solution A). The paramecia used in these investigations were grown in stock cultures (culture solution A) of a hay and wheat infusion

made up with a buffered salt mixture, containing the following constituents:<sup>1</sup>

1 gm. -----	NaCl
0.10 gms. -----	KCl
0.05 gms. -----	CaCl <sub>2</sub>
0.10 gms. -----	MgCl <sub>2</sub>
10.00 cc. M/15	NaH <sub>2</sub> PO <sub>4</sub>
40.00 cc. M/15	Na <sub>2</sub> HPO <sub>4</sub>

Dilute to 1000 cc. with distilled water.

This buffered salt solution represented the stock salt solution and had a pH 7.4. The medium used for culturing the paramoecia (culture solution A) was made up by diluting the buffered salt solution 1:10 with tap water. 2 gms. of Timothy hay and six wheat grains were then added to 250 cc. of this solution in a 500 cc. Florence flask. In this flask, the culture was autoclaved at 15 pounds pressure for 15-20 minutes, cooled sufficiently, and inoculated with 0.1 cc. of a suspension of Bacillus subtilis,<sup>2</sup> which had been grown for 24 hours in 5 cc. of a meat extract medium at a temperature of 37° C. The meat extract medium was prepared as follows:

1.0 gm. -----	peptone (Difco)
0.5 gms. -----	meat extract (Difco)
0.2 gms. -----	NaCl

These ingredients were diluted to 100 cc. with distilled water and the medium adjusted to a pH 7.4 with NaOH. The medium was next sterilized in the autoclave at 15 pounds for 15-20

<sup>1</sup>

This is a modification of the general buffered medium as developed by Brandwein (1935).

<sup>2</sup>

The culture of Bacillus subtilis was kindly furnished by Dr. T. M. McCalla of the Department of Bacteriology at Kansas State College.

minutes. After it had cooled, 10 cc. of a 0.1 per cent sterile aqueous solution of glucose were added to the medium.

The organisms (Bacillus subtilis) were inoculated from an agar slant into 5 cc. of the meat infusion medium in a test tube and then incubated at 37° C. for 24 hours.

Bacillus subtilis was found to be an excellent organism upon which to grow paramecia. The Timothy hay organism, Mycobacterium phlei, also was found to support a good growth of paramecia.

After the hay infusion culture (culture solution A) was inoculated with the suspension of Bacillus subtilis, it was left to incubate at 37° C. for 24 hours, then cooled to 25° C., and inoculated with 100-200 viable paramecia from another culture which contained the single strain of bacteria (Bacillus subtilis) in association with the paramecia. This culture solution A was then left to incubate at 25° C.

Preparation of culture solution B. The culture solution B, which was used to carry out the quantitative studies upon the growth rate and viscosity of the protoplasm of Paramecium caudatum was of an entirely different nature than culture solution A--the general supply culture. This culture solution was an aqueous extract of "Cerophyl".<sup>3</sup>

One tablet of "Cerophyl" was added to 200 cc. of distilled water and ground in a mortar and filtered (2 to 5 l. of culture

---

<sup>3</sup>"Cerophyl" is a form of dehydrated and dried cereal grasses, possessing a high vitamin content. It is manufactured by the American Butter Company, Kansas City, Missouri.



were usually made up). This solution was then sterilized at 15 pounds pressure for 15-20 minutes. Then 0.1 cc. of a 24-hour old suspension of Bacillus subtilis, grown in a meat extract medium at 37° C. was added to each 200 cc. of the "Cerophyl" infusion. This culture, containing the Bacillus subtilis was incubated for 24 hours at 37° C. and then stored in the refrigerator at 0°- 2° C. When a portion of the culture solution was needed, a part of this refrigerated culture was removed and the rest of the culture left to be used at a future date.

This method of refrigerating the culture after incubation was found to insure a more constant supply of living bacteria for use in making comparative growth studies on paramecia. By placing the culture medium in a refrigerator at 0°- 2° C. after incubation, the microorganisms pass into a state of suspended activity and form spores. In this way the bacteria do not continue to reproduce, and therefore, the number of bacteria remains fairly constant. This low temperature also prevents digestion of the bacteria by autolytic enzymes.

This refrigerated culture may be kept in good condition for as long as two weeks, providing it is always maintained at 0°- 2° C. The number of bacteria in a given volume will stay relatively constant for a period of two weeks. This has been verified by making nephelometric determinations upon the suspensions at various times during the two week period. However, it was found that at times the number of bacteria per given volume did vary, and these particular cultures were discarded.

Preparation of salt solutions. These studies were made upon five different molar concentrations of nine different salts--six salts containing monovalent cations and three salts containing divalent cations. The salts used were the alkali salts, NaCl, KCl, NH<sub>4</sub>Cl, LiCl, RbCl, CsCl; and the alkaline earth salts, MgCl<sub>2</sub>, CaCl<sub>2</sub>, and SrCl<sub>2</sub>. With each salt the anion used was common to all, but the cation was varied. Molar concentrations of 1/30, 1/40, 1/50, 1/60, and 1/70 of the monovalent salts (NaCl, KCl, NH<sub>4</sub>Cl, LiCl, RbCl, and CsCl) were used, and 1/42, 1/56, 1/71, 1/85, and 1/99 molar concentrations of the divalent salts (CaCl<sub>2</sub>, MgCl<sub>2</sub>, and SrCl<sub>2</sub>).

The highest concentration used (M/50) was determined by taking as a standard the highest concentration of NaCl that the paramecia would tolerate. A 1/30 molar concentration of NaCl suppressed the growth of the paramecia to a slight degree, but their life span in days was not shortened. Estabrook (1910) found that NaCl did not effect the growth of paramecia in a M/30 concentration, but that a more concentrated solution was harmful. All of the other concentrations of salts were made not to exceed this critical concentration of NaCl.

The monovalent salts were prepared in 1 M. concentrations, sterilized, and stored in prescription bottles. The divalent salts were prepared in 0.5 M. concentrations. The molar concentrations in which the paramecia were grown were then made up by diluting the required amounts of these stock salt solutions

with the proper amounts of culture solution B.<sup>4</sup>

In comparing the effects of these different salt solutions, certain precautions had to be taken. In comparing the effects of the divalent salts and the effects of the monovalent salts upon the paramecia, it was necessary to use solutions of these salts which had nearly the same osmotic pressure. Also, salts had to be selected which did not show any marked changes in the hydrogen ion concentration. The pH of the salt solutions used was approximately pH 7.6, except of  $\text{NH}_4\text{Cl}$ , which was approximately 6.8.<sup>5</sup>

At the present time it is not practicable to use the methods that have been devised for measuring directly the osmotic pressure of electrolyte solutions. It is possible, however, to determine the osmotic pressure of different solutions indirectly

---

<sup>4</sup>It is obvious that in making these molar concentrations by diluting with culture solution B, the final molar concentration is actually greater than any of the above mentioned ones. The osmotic pressure of the "Cerophyl" infusion is around 0.25, whereas that of a M/30 NaCl solution is approximately 1.86. However, since these values are merely for comparison, the important precaution is to keep the molar relationship between the particular salts constant.

<sup>5</sup>Darby stated (Phelps, 1931) that there is a definite pH at which paramecia grow best; however, Phelps disagreed and criticized Darby's work because the latter used a mixed bacterial flora. Phelps found that no differences could be detected in the division rate of Paramecium aurelia through a range of pH 5.9 - 7.7. Mast (1931) found that a range of pH 4.2 - 8.2 would produce an optimum growth of Amoeba proteus.

All pH determinations used in this experiment were made by the colorimetric and spot plate methods with the use of a universal indicator (Britton, 1931; and Clark, 1928).

(Heilbrunn, 1937) by calculation from freezing point determinations, and it is in this way that the osmotic relationships between the different salt solutions used in this experiment were determined.

Preparation of isolation cultures. All of the studies upon growth and viscosity have been made with the use of isolation cultures. This method of study is the only method that insures a measurable degree of accuracy in making quantitative measurements upon the vitality of paramecia. Thus, if by isolating a small bit of protoplasm, in the form of a single individual organism such as a paramecium, it is possible, providing the food and other environmental conditions are kept constant, to observe variations in the metabolism of the protoplasm. Such metabolic changes may be recognized by a change in the rate of division, rate of respiration, rate of senescence, physical appearance, and in the viscosity of the protoplasm (Calkins, 1933).

The isolation cultures of paramecia were prepared after first washing the paramecia in the solution in which they were to be grown. In order to make sure that the paramecia used for these experiments were at about the same state of vitality, the paramecia were always taken from a 15-20 day old hay infusion culture (culture solution A), prepared as previously described. At this age the paramecia were at their maximum in numbers (there being practically no increase in the number of organisms present). This period constitutes what is known as the maximum stationary phase of growth (Buchanan and Fulmer, 1928).

1 cc. of a concentrated suspension of paramecia was removed



aseptically from a 15-20 day old stock culture, introduced into a Syracuse dish containing the medium in which the organisms were to be grown, and then washed three times, being left in each washing 10-15 minutes. From the third washing one paramecium, in one drop of culture fluid, was transferred into 0.3 cc. of the same type of culture medium as that contained in the cylindrical depression of culture slide. This slide was placed upon a plain glass slide in the bottom of the Petri dish.

After the paramecium was introduced into the depression, 10-15 cc. of culture solution was added to the bottom of the Petri dish. This furnished a moist chamber for the paramecium. In adding the culture to the bottom of the Petri dish, it was necessary to add a solution which had the same osmotic pressure as that of the solution in the depression. This prevented the loss or gain of water inside the depression due to isothermal distillation.

The Petri dishes were then stored in a constant temperature water bath where the humidity around the dishes was high enough to prevent an excessive loss of water from the dishes. These dishes were left to incubate at a fairly constant temperature of 25° C. Counts of paramecia in each dish were made every 24 hours with a binocular microscope, using the 2X objective. The counts were recorded as numbers of living paramecia present in each depression, and counts were made on each plate until the last paramecium had died.

For each salt solution 20 different isolation cultures were set up for each of the five molarities used. In addition, 100

controls were run.

Procedure Used in Measuring the Apparent Viscosity  
of the Protoplasm of Paramecium caudatum

The method used for determining the viscosity of the protoplasm of paramecium was Heilbrunn's (1921, 1923, 1925a, 1926a, 1926b) centrifuge method, which is based upon a determination of the rate of fall of spherical bodies through a liquid. This method depends upon the application of Cunningham's (1910) modification of Stoke's formula, and is expressed in the following manner:

$$v = \frac{2cg(\sigma - \rho)a^2}{9\eta}$$

- v = velocity of the falling sphere  
 c = the centrifugal force applied  
 g = the gravity constant  
 $\sigma$  = the specific gravity of the sphere  
 $\rho$  = the specific gravity of the medium through which the sphere falls  
 a = the radius of the sphere  
 $\eta$  = the viscosity

In the above formula  $\rho$  as applied to the present problem does not refer to the specific gravity of the fluid or hyaline substance lying between the granules of the endoplasm of the paramecium, but rather to the specific gravity of the entire animal. Since in measuring the viscosity of the internal protoplasm (endoplasm) of the paramecium, it was the viscosity of the entire animal that was being measured, and not just the fluid-like or hyaline substance in which the granules were suspended.

The value for  $\rho$  was determined by placing the paramecia in varying concentrations of sugar solutions and then centrifuging

them. With the more dilute solutions of sugar (sucrose), the paramecia were found at the bottom of the tube, and with heavier concentrations, they were found near the top of the tube. The specific gravity of the sugar solution between the highest concentration at which they were on the bottom of the tube and the lowest concentration at which they were on the top of the tube was taken as the specific gravity of the paramecium. This condition was found to exist when a ten per cent aqueous solution of sugar was used. Consequently, since the specific gravity of a ten per cent aqueous solution of sugar is 1.038, this was taken as the specific gravity of the paramecium. This is the same value as determined by Koebler in 1922, and verified by Fetter (1926), employing the same method.

In order that the viscosity of the protoplasm of paramecium might be compared with the rate of growth, it was necessary to grow the paramecia under the same conditions as those that were used in studying the growth rates. Therefore, the isolation culture method was duplicated in making these determinations of viscosity.

When it was desired to measure the viscosity of a given culture of paramecia after a certain number of days, a heavy suspension (20 per cent) of starch grains was added to the culture of paramecia. The starch suspension was prepared by adding 2 gms. of "cold" starch to 10 cc. of culture medium. This mixture was then shaken and one drop of the suspension was placed in the depression slide. The mixture was allowed to

stand for 10-20 minutes to permit the paramecia to ingest a goodly number of the starch grains. Thereupon, one paramecium was drawn into a capillary tube about 5 cm. in length and 0.1-0.5 mm. in diameter. By using a capillary tube with a small diameter, only slightly greater than the cross-section of the animal, it was possible to keep the animals oriented with their long axis parallel to the line of centrifugal force. This capillary tube was then sealed at one end and placed into a centrifuge tube which contained some cotton to keep the capillary tube from breaking. The centrifuge used was a standard International Centrifuge, with a maximum speed of 2,400 R. P. M.

The paramecium in the capillary was centrifuged until microscopic examination revealed that all of the starch grains were evenly packed in the posterior portion of the cell (usually the posterior one-fifth of the cell).<sup>6</sup> When all of the starch grains were in the posterior one-fifth of the cell, it was assumed that the granules that were originally at the anterior

---

<sup>6</sup>In order that examination of these cells might be made before the granules could have had an opportunity to move away from the posterior region of the cell, the paramecia had to be killed and fixed in a permanent state immediately after the centrifuge stopped. After making an extensive search for a fixative that would not cause an excessive amount of shrinkage or darkening of the cellular protoplasm, it was found that Neguchi's fixative was the most adequate. It contains the following constituents:

100 cc. -----	O.1 M. $K_2HPO_4$
25 cc. -----	O.1 M. $KH_2PO_4$
12.5 cc. -----	formalin

10 cc. of culture solution is added to each 2 cc. of fixative.



portion of the cell had moved four-fifths of the distance down the cell to a point approximately one-fifth of the distance from the posterior end.

Thus, when the paramecium was 300 microns in length, which was the average length, the distance which the starch grains migrated was approximately 260 microns. The time it took the starch grains to migrate this distance was recorded in seconds, and the centrifugal force necessary to move these starch grains 260 microns in a unit of time was calculated from the following formula of Huyghens (Heilbrunn, 1921), when the number of revolutions per second are known for this unit of time:

$$C = 4 \frac{2n^2 r m}{g}$$

C = the centrifugal force

n = the number of revolutions per second

r = the radius of the circle described by the ends of the tubes

m = mass

In order that C may be expressed in terms of gravity:

$$C' = \frac{4 \frac{2n^2 r m}{g}}{g} = \frac{4 \frac{2n^2 r}{g}}{g}$$

C' = the centrifugal force in terms of gravity

g = the gravity constant

In determining the viscosity of the protoplasm of a normal and actively reproducing paramecium, it was found that it required a centrifugal force of 581.95 times gravity, acting for six minutes (360 seconds) to move all of the starch grains into the posterior one-fifth of the cell. If the average length of the paramecium was 300 microns, then the distance that the starch grains migrated was 260 microns, or 0.026 cm. Since the velocity is directly proportional to the distance and inversely

proportional to the time,  $v = d/t$ . Calculating from this, the velocity of movement of the starch grains is 0.0401695 cm. per second.

The specific gravity of the starch is 1.5, and the difference between this and the specific gravity of the paramecium (1.038) is 0.562.

The average radius of the starch grains ingested by the paramecium was found to be 2.5 microns, or 0.00025 cm. When this is squared, the result is 0.000000625 cm.

Using 980.6 as the gravity constant and substituting the above figures into Stokes' formula,  $\eta = 72.58$  c. g. s. units.

The c. g. s. unit of viscosity is the poise, and it may be defined as the tangential force per square centimeter per second in two layers of liquid one centimeter apart. The viscosity of water at room temperature is approximately 0.01 poise, i. e., a centipoise. Thus the value for the viscosity of the protoplasm may be expressed in these terms, and would in this case be 7238 centipoises.

## RESULTS

The Normal Growth Curve of Paramecium caudatum<sup>7</sup>

Figure 1 shows the curve of growth of the paramecia in a "salt-free" control. This normal growth curve corresponds closely with the curve of growth for bacteria as described by Buchanan and Fulmer (1928). However, the curve of growth in Figure 1 does not show the typical initial stationary phase. As described by Buchanan and Fulmer, this is the phase during which the organisms do not divide, or, their numbers remain constant.

The curve in Figure 1 does show the presence of the typical lag or positive growth acceleration phase (a-b). This is the period elapsing between the beginning of multiplication and the beginning of the maximum rate of growth.

This curve of normal growth also shows the logarithmic growth phase (b-c). This is the period during which there is a maximum rate of increase per animal and it is thus represented by a straight line.

The phase of negative growth acceleration is represented

---

<sup>7</sup>It is obvious that the growth of paramecia is not always equivalent to their division rate. This fact will become more apparent when the growth curves for paramecia grown in  $\text{SrCl}_2$  are discussed. However, for simplicity, the terms "growth", "growth rate", and "growth curves" are used frequently in this paper, but it should be borne in mind that only the rate of multiplication or division of the animals has been studied; in other words, the values express the number of paramecia in a culture at a given time.

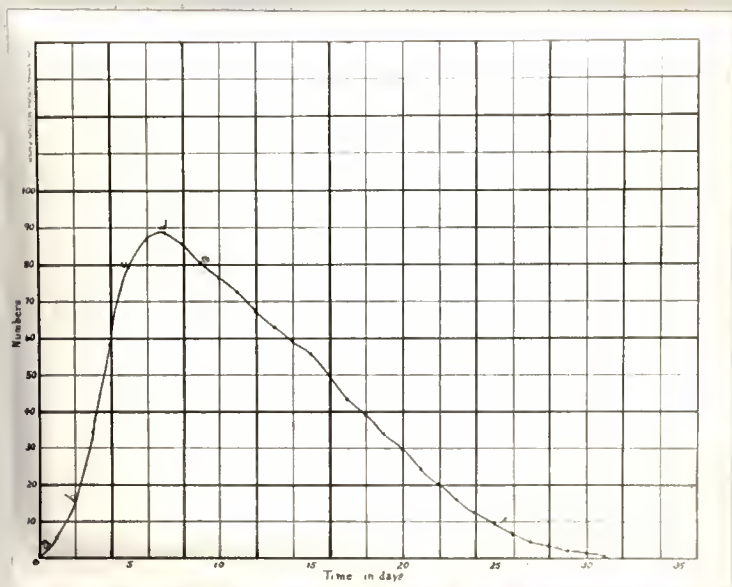


Figure 1. Curve of normal growth for paramecium. a-b is the lag or positive growth acceleration phase, b-c is the logarithmic growth phase, c-d is the negative growth acceleration phase, d-e is the phase of accelerated death, and e-f is the logarithmic death phase.



upon this curve by c-d. This is the period during which the animals continue to increase in numbers, but less rapidly than in the logarithmic period.

The typical maximum stationary phase is absent here, this is the phase in which there is practically no increase in number of animals present.

The phase of accelerated death is represented in the curve d-e, and it is the period during which the rate of death is increasing to a maximum. The logarithmic death is e-f, the phase during which the rate of death is constant and at a maximum. This is represented by the straight line (e-f).

This curve of normal growth shows a maximum at about the seventh day. At this time, the paramecia had attained their maximum individual size, as well as their maximum number. From about the seventh day to the thirtieth day, the paramecia began to decrease in size and became much less active. As the culture approached the thirtieth day, the animals became extremely small (approximately 150 microns in length, as compared with the normal length of 300 microns) and rigid, and in some instances, it was observed that the anterior end was bent slightly towards the oral groove. After 30 days, most of the paramecia were dead.

#### The Effects of Various Salts upon the Growth Rate of Paramecium caudatum

Figures 2 to 10 inclusive show the effects of the various molar concentrations of salts upon the growth rate of the paramecia. Figure 11 contains the curves of growth for paramecia

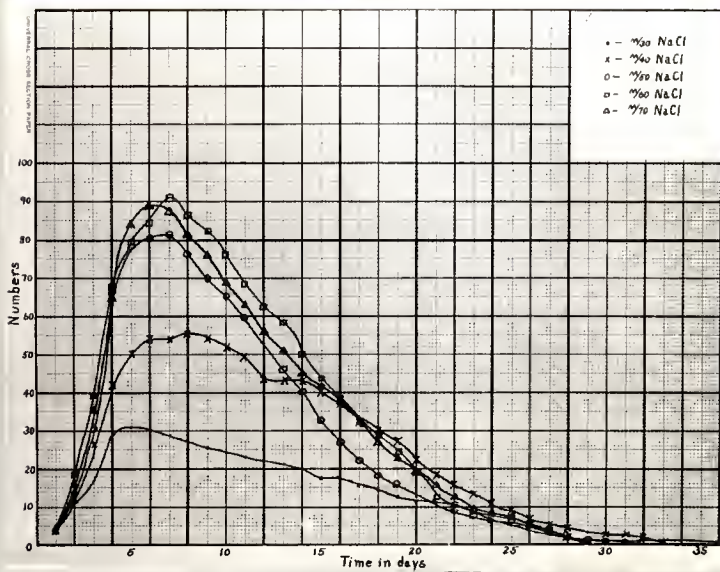


Figure 2. Curves of growth for paramecia grown in various concentrations of NaCl.

grown in M/70 NaCl, KCl,  $\text{NH}_4\text{Cl}$ , LiCl, RbCl, and CsCl, and M/99  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ , and  $\text{SrCl}_2$ .

Sodium chloride (Figure 2). NaCl solutions caused no visible change in the appearance or activity of the organisms in any of the five concentrations used. The maximum number of paramecia were present about the seventh day, which was the same as in the "salt-free" control. In the molar concentrations used, the paramecia normally lived about 30 days.

In the M/50, M/60, and M/70 concentrations, there was no significant difference between those in the NaCl solutions and those in the "salt-free" control. However, in the M/30 and M/40 NaCl solutions, the growth of the organisms was inhibited to a slight extent. Like those animals in the "salt-free" control, the paramecia grown in all five concentrations of NaCl decreased in size and became much less active as the culture aged.

Potassium chloride (Figure 3). KCl in high concentrations was extremely toxic to the paramecia. One of the most characteristic effects produced by the addition of KCl was the almost complete disappearance of any locomotion. There was no evident change in the physical appearance of the animals when placed in any of the five solutions. However, the KCl solutions, especially the higher concentrations, seemed to destroy the cell membrane, for when a paramecium in any of the KCl cultures died, it could not be observed in the culture. Whereas, in "salt-free" cultures, the presence of dead paramecia could be observed several days after death.

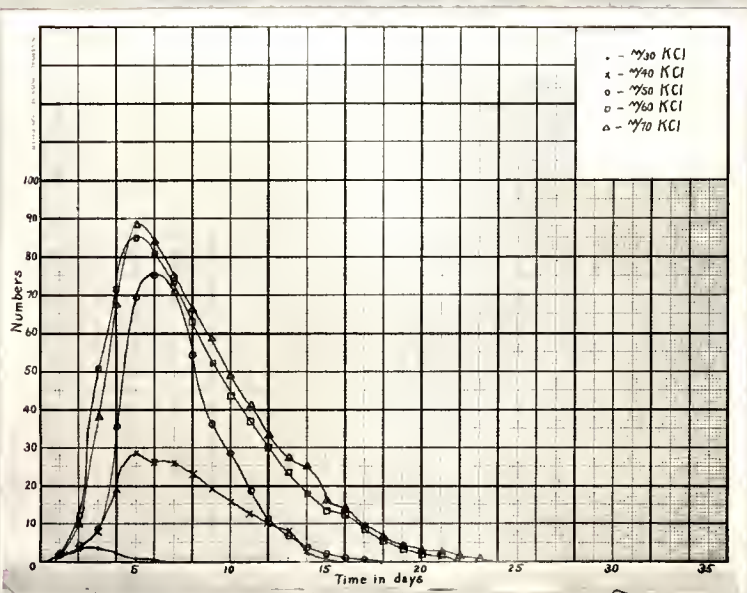


Figure 3. Curves of growth for paramecia grown in various concentrations of KCl.



The maximum number of paramecia were present about the fourth and fifth day, the maximum being reached earlier than in the case of the NaCl solutions. In the M/60 and M/70 KCl solutions there were as many paramecia present at one time as in the "salt-free" control; in all instances those in the KCl solutions died nearly twice as soon as those in the control solution. KCl in certain concentrations stimulated the growth of the paramecia, but in all of the concentrations tried, it brought about an earlier death of the animals. As was the case in the NaCl and "salt-free" control solutions, the organisms began to decrease in size after having attained the maximum number.

Ammonium chloride (Figure 4).  $\text{NH}_4\text{Cl}$  in all concentrations was very toxic.  $\text{NH}_4\text{Cl}$ , generally, like NaCl and KCl, caused no visible changes in the appearance of the paramecia. However, in some cultures containing the  $\text{NH}_4$  ion in high concentration (M/30, M/40, and M/50), various atypical and aberrant "monster-like" paramecia were produced, which had no resemblance to the form or shape of a normal paramecium. The  $\text{NH}_4$  ion did not inhibit the locomotor activities of the animal. The  $\text{NH}_4$  ion acted like the K ion in bringing about a gradual destruction of the cell membrane, although to a minor degree.

All five concentrations of  $\text{NH}_4\text{Cl}$  greatly inhibited the growth of the paramecia. In the most dilute solutions (M/70) the maximum number of paramecia were present from the first to the third days. These paramecia grown in the  $\text{NH}_4\text{Cl}$  solutions reacted peculiarly, for after the first day in the  $\text{NH}_4\text{Cl}$  solution, there were just as many paramecia present (4) as there

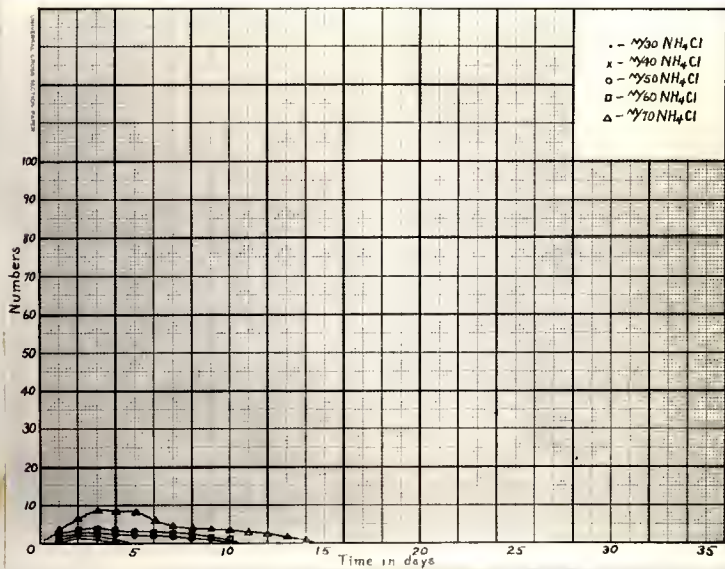


Figure 4. Curves of growth for paramecium grown in various concentrations of  $\text{NH}_4\text{Cl}$ .

were in the "salt-free" controls. After this first day there was hardly any noticeable or significant increase in numbers. In the  $\text{NH}_4\text{Cl}$  solution, the size and activity of the animal decreased with age.

Lithium chloride (Figure 5).  $\text{LiCl}$  inhibited the growth of the paramecia in all concentrations, and in the highest concentrations (M/30, M/40, and M/50), it was the most toxic of all the nine salts used.  $\text{LiCl}$  in these three molarities produced some visible change in the appearance of the paramecia; they often developed small raised areas upon their surfaces. The Li ion, like the K ion, inhibited the locomotion of the paramecia in all the molar dilutions used.

The maximum number of paramecia in the M/60 and M/70  $\text{LiCl}$  solutions were present around the twelfth to fifteenth day. This delay in the increase in number was due to the presence of an initial stationary phase of growth, which was characterized by the absence of any increase in number of organisms present. The lag phase in these cultures was also long. These paramecia in the M/60  $\text{LiCl}$  solution also showed the presence of the maximum stationary phase, so typical of growth curves for bacteria.

The paramecia grown in the M/60 and M/70 solutions of  $\text{LiCl}$  reacted atypically in that they did not decrease in size with age, but rather, they stayed large and healthy looking until they died. In the M/60 and M/70 solutions, they lived as long as these paramecia grown in the "salt-free" controls.

Rubidium chloride (Figure 6).  $\text{RbCl}$  produced no evidence

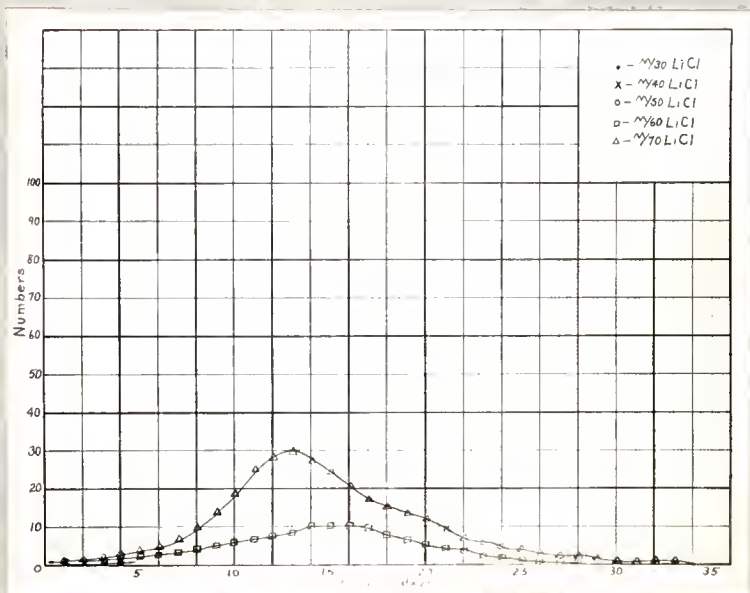


Figure 5. Curves of growth for paramecia grown in various concentrations of LiCl.



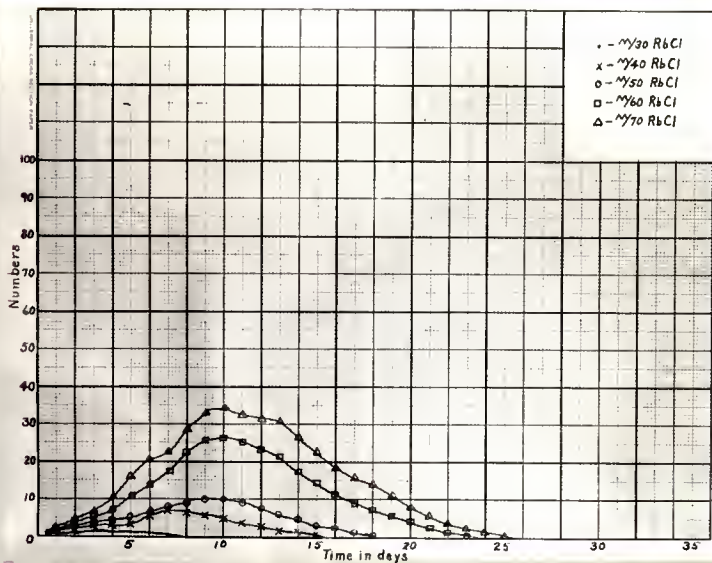


Figure 6. Curves of growth for paramecia grown in various concentrations of RbCl.

of change in the form of the paramecia, and in addition, it did not inhibit the normal locomotor activities of the animal.

RbCl in all molar dilutions that were used caused a marked inhibition of growth. In the M/70 solutions, the maximum number of animals were usually present around the tenth day. This lengthened time was due to the longer lag period. After the maximum in numbers was reached the paramecia became smaller and less mobile. The length of life of these paramecia grown in the RbCl solutions corresponded closely in time to that for the paramecia that were grown in the KCl solutions.

Caesium chloride (Figure 7). CsCl salt solutions produced no change in the physical appearance of the paramecium; although it slowed the locomotion of the animal.

CsCl in all the molarities used caused an even more marked inhibition in the growth than did the RbCl solutions. The maximum number of animals were present in the most dilute solutions (M/70) about the tenth day. This lengthened time may again be attributed to the long lag period. After the maximum in numbers was reached, the organisms became increasingly smaller in size. In the M/70 CsCl solution, all of the paramecia were usually dead by the twentieth day.

Magnesium chloride (Figure 8). MgCl<sub>2</sub> solutions in the various molarities used produced no visible change in the physical appearance of the cell, but the Mg divalent cation caused a slight decrease in the speed of movement.

The paramecia in the M/71, M/85, and M/99 solutions of

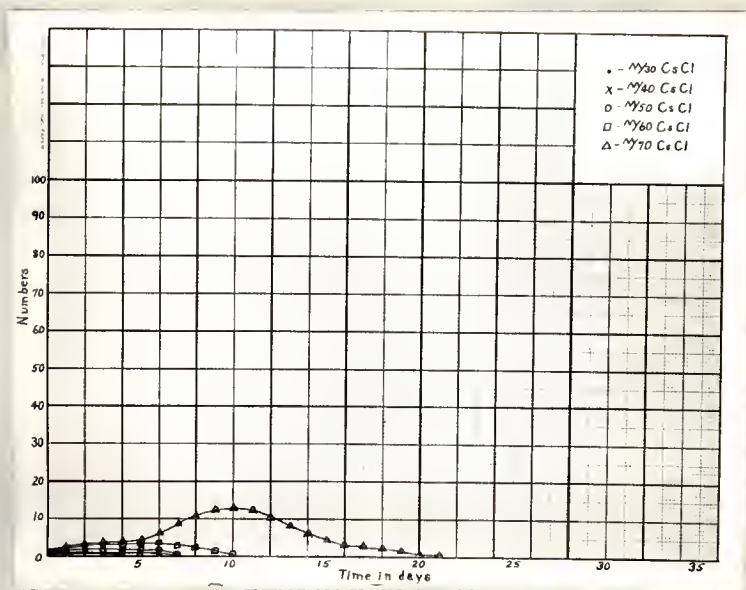


Figure 7. Curves of growth for paramecia grown in various concentrations of CsCl.

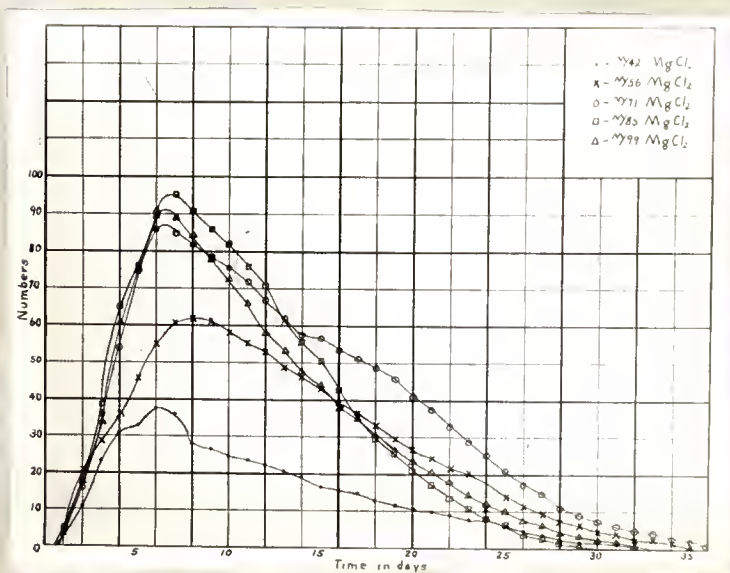


Figure 8. Curves of growth for paramecia grown in various concentrations of  $MgCl_2$ .



$MgCl_2$  produced no significant deviation in the growth curve from that of the organisms grown in the "salt-free" cultures.  $MgCl_2$  in M/42 and M/56 concentrations caused a slight inhibition of the growth. The maximum number of paramecia were present at about the seventh day. From the seventh to the thirtieth day, the animals began to decrease in size, as well as in numbers.

Calcium chloride (Figure 9).  $CaCl_2$  solutions in the concentrations used produced no visible changes in the physical appearance of the paramecia. The most marked effect was an increase in the rate of locomotion.

$CaCl_2$  in all concentrations used caused a pronounced decrease in the growth of the paramecia. In the M/85 and M/99 solutions of  $CaCl_2$ , the maximum number of paramecia were present at about the seventh day. From the seventh day until about the fifteenth day, when most of them had died, the paramecia became very small and bent at the anterior region of the body. They also moved about with jerky movements.

Strontium chloride (Figure 10).  $SrCl_2$  solutions showed a wide range of activity. In the M/99 solutions, the Sr ions caused a definite stimulation in the growth rate of the paramecium. In the M/85 solution, there was a slight inhibition of growth and in the more concentrated solutions, a very marked inhibition in growth.

The maximum number of paramecia were observed to be present on about the twelfth day. This longer period was due to the lengthened lag phase.

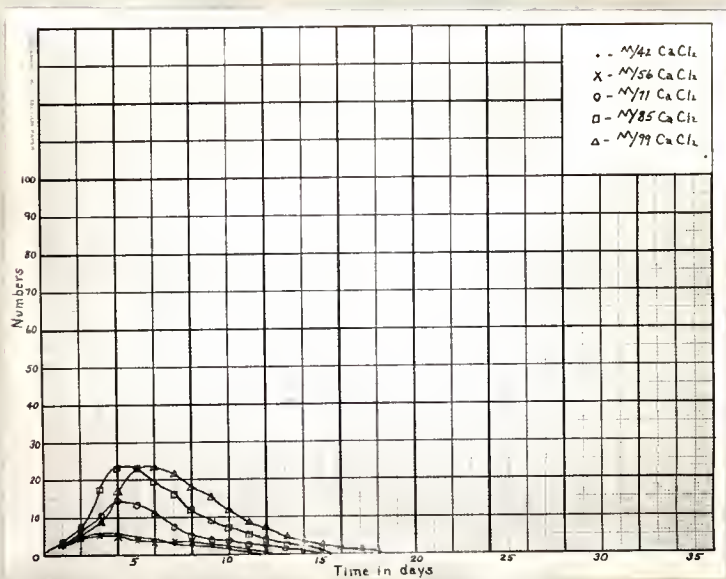


Figure 9. Curves of growth for paramecia grown in various concentrations of  $\text{CaCl}_2$ .

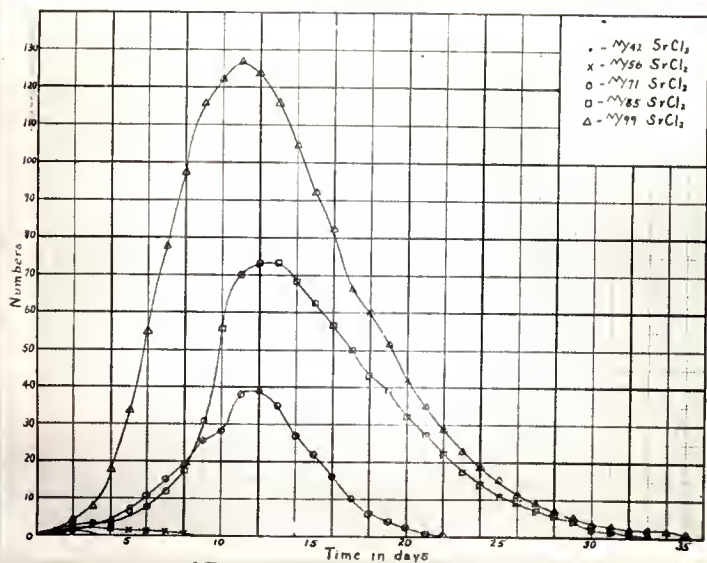


Figure 10. Curves of growth for paramecia grown in various concentrations of  $\text{SrCl}_2$ .

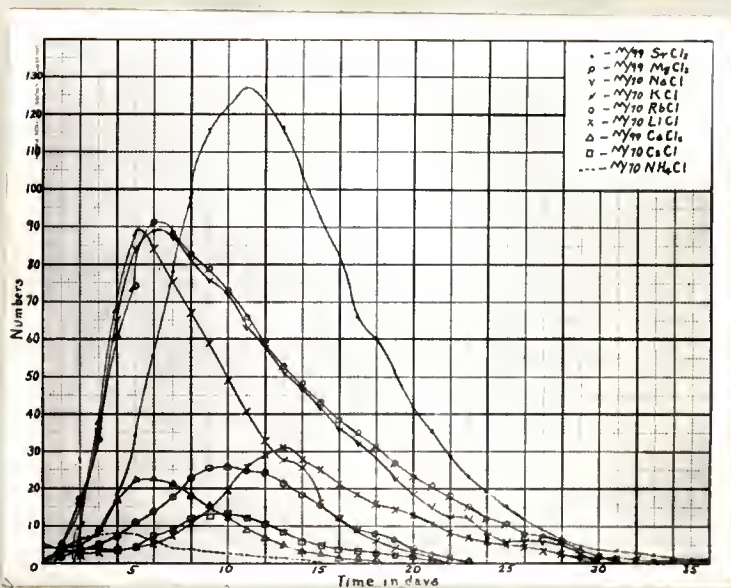


Figure 11. Curves of growth for paramecia grown in M/70 molar solutions of the monovalent salts and M/99 molar solutions of the divalent salts.



The  $\text{SrCl}_2$  solutions did not produce any change in the rate of locomotion of the paramecia during the first few days. The size of the individual paramecium shortened to 150 microns from the average initial length of 300 microns, and remained this size until death intervened at approximately the thirtieth day. After they shortened to 150 microns, their locomotion became very "jerky".

Attempts were made to grow the paramecia in M/100 solutions of  $\text{AlCl}_3$ ,  $\text{FeCl}_3$ ,  $\text{FeCl}_2$ ,  $\text{ZnCl}_2$ ,  $\text{MnCl}_2$ , and  $\text{BaCl}_2$ , but without success. When the paramecia were placed in M/100 solutions of the  $\text{Al}^{+++}$  and  $\text{Fe}^{+++}$  chlorides, they were killed instantly. In the  $\text{Fe}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Mn}^{++}$ , and  $\text{Ba}^{++}$  chloride solutions, the paramecia lived from 10-30 minutes. The length of time in which they were able to survive the toxic effects of these solutions was in the following order:  $\text{Ba}^{++} > \text{Mn}^{++} > \text{Zn}^{++} > \text{Fe}^{++} > \text{Fe}^{+++} > \text{Al}^{+++}$ .

Figure 12 presents the relationship between the per cent of the maximum growth of these animals in the alkali and alkaline earth salt solutions as compared with the "salt-free" control. Table 1 is a single complete protocol that indicates the type of results obtained.

The number listed under molar concentration represents the maximum number of paramecia present in that particular salt solution at any one time. The average results, expressed for each concentration of each salt as a percentile value of the number of paramecia present in the "salt-free" control (viz. 88) are presented in the column to the right (marked X) of the

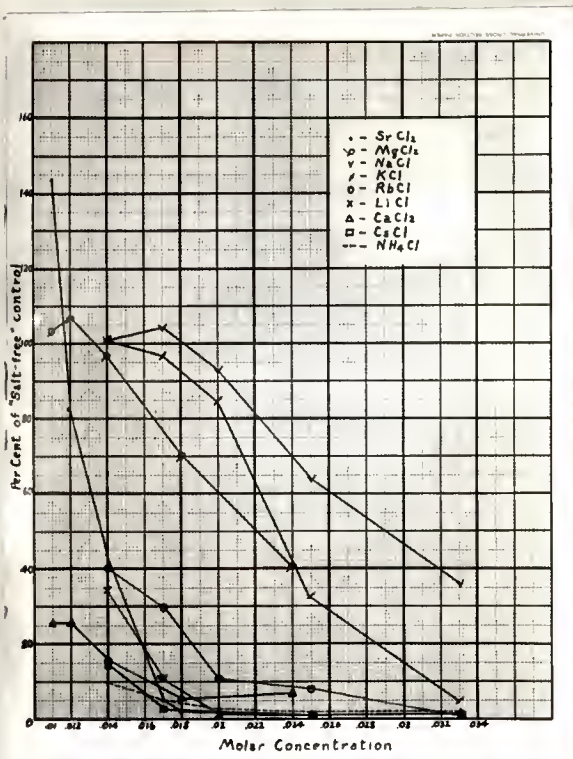


Figure 12. Curves showing the maximum number of paramecia present in the various salt solutions as compared with the "salt-free" control. See text for explanation.

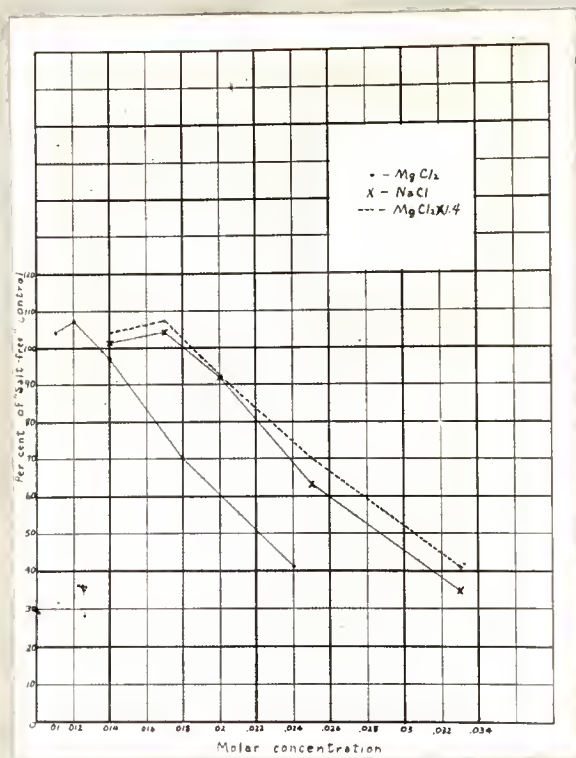


Figure 13. Curve showing the relationship between the potency of NaCl and MgCl<sub>2</sub> to paramecia. See text for explanation.

molarity in Table 1, and by means of curves in Figure 12.

The curves presented in Figure 12 show graphically the extent of inhibition and stimulation produced by these nine different cations upon the growth of the paramecium. Thus it may be seen, that with the exception of the Sr cation, none of the cations used caused any significant increase in development of the paramecia.

Table 1. The maximum number of paramecia produced in salt solutions of various molarities, as compared with a "salt-free" control (per cent).

Salt	M/30	X	M/40	X	M/50	X	M/60	X	M/70	X
NaCl	31	35	55	65	81	92	91	104	89	101
KCl	4	5	29	33	75	85	85	97	89	101
NH <sub>4</sub> Cl	2	2	2	2	3	3	4	5	9	10
LiCl	1	1	1	1	1	1	10	11	31	35
RbCl	1	1	7	8	10	11	26	30	35	40
CsCl	1	1	1	1	2	2	3	3	13	15

Salt	M/42	X	M/56	X	M/71	X	M/85	X	M/99	X
CaCl <sub>2</sub>	6	7	5	6	14	16	23	26	23	26
MgCl <sub>2</sub>	36	41	62	70	85	97	94	107	91	104
SrCl <sub>2</sub>	1	1	2	2	39	44	73	83	127	144

Number present in "salt-free" control = 80.

Figure 13 brings out more clearly the relative effects of the cations of Na and Mg as computed from their specific potencies. The specific potency is calculated by determining the reciprocal of the ratio of the concentration of a given salt to the amount of NaCl necessary to produce the same effect. Thus,



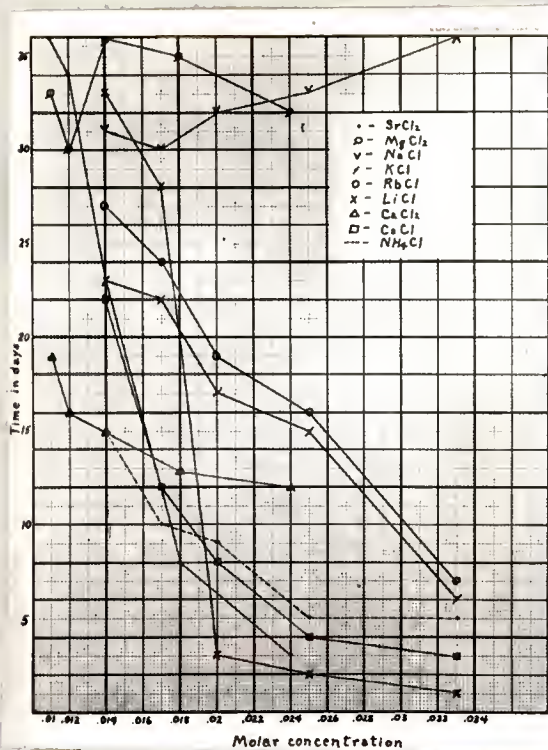


Figure 14. Curves showing the effect of various concentrations of the salts on longevity of paramecia.

the specific potency of Na is 1 and of Mg 1.4. The broken-line curve of Mg in Figure 13 is plotted by multiplying the molarity of each point on the continuous-line curve for Mg by this value of 1.4, i. e., the specific potency of Mg. This curve should then follow closely that for Na.

Effect of salt concentration on longevity. Figure 14 is a curve designed to point out the relationship between the longevity of the paramecia in the different salt solutions and the respective molarities of these salt solutions. This curve also shows the effect of different cations upon aging in paramecia.

The points on this curve, as related to the molarity are obtained by determining the time, expressed in days, when less than an average of one paramecium was still contained in the entire group of 20 different isolation cultures that were used for each molar concentration of each salt.

With the exception of the Mg and Na salts, as the molar concentration of the particular salt solutions is decreased, the length of life or the age of the animals is increased. With the Na and Mg salts, particularly the former, the life span of the animals was slightly decreased with a decrease in concentration.

#### The Effects of Various Salts upon the Viscosity of the Protoplasm of Paramecium caudatum

The normal curve of viscosity. Figure 15 shows the curve of viscosity and the curve of growth for the paramecia grown in the "salt-free" control. As the number of animals in the culture began to increase, the viscosity of the protoplasm decreased

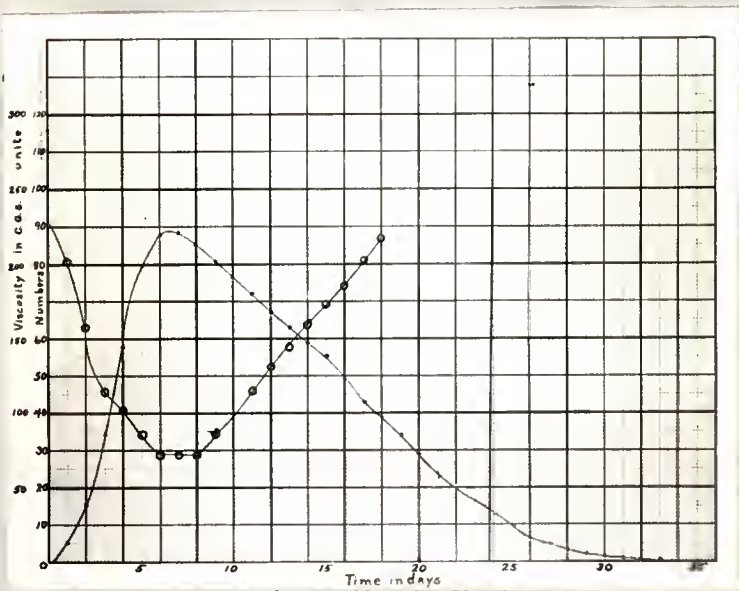


Figure 15. Curves showing the relationship between the rate of growth of the paramecium and the viscosity of the protoplasm. The continuous line represents the growth curve, and the broken line represents the curve of viscosity expressed in c. g. s. units (poise).

to a low point of 72 on the sixth, seventh, and eighth days. As the number of paramecia in the culture began to decline in numbers and those still present became less active, there was a coincident increase in viscosity. It was not practical to record viscosity readings after 18 to 20 days, because the results were too irregular and the paramecia frequently failed to ingest the starch grains.

Thus, Figure 15 indicates that the rate of growth of the paramecium was inversely proportional to the viscosity of the protoplasm (endoplasm). In addition, it indicates that the viscosity of the protoplasm increased with an increase in the age of the paramecium.

Figure 16 shows the trend of viscosity changes in cultures of paramecia grown in M/40 concentrations of NaCl, KCl,  $\text{NH}_4\text{Cl}$  and RbCl, and M/56 concentrations of  $\text{SrCl}_2$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$ . Those paramecia grown in M/40, LiCl and CaCl did not ingest the starch granules and consequently no determinations of viscosity could be made. In these molarities the Na and Mg cations decreased the viscosity of the paramecium protoplasm to nearly the same extent as those in the "salt-free" controls, whereas the K, Rb, NH, Sr, and Ca cations all produced a great increase in the viscosity over that in the "salt-free" controls.

Figure 17 shows the changes in viscosity of paramecia grown in M/70 concentration of NaCl, KCl,  $\text{NH}_4\text{Cl}$ , LiCl, RbCl, and CsCl, and M/99 concentration of  $\text{SrCl}_2$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$ . The K, Na, and Mg ions produced a slight decrease in the viscosity of



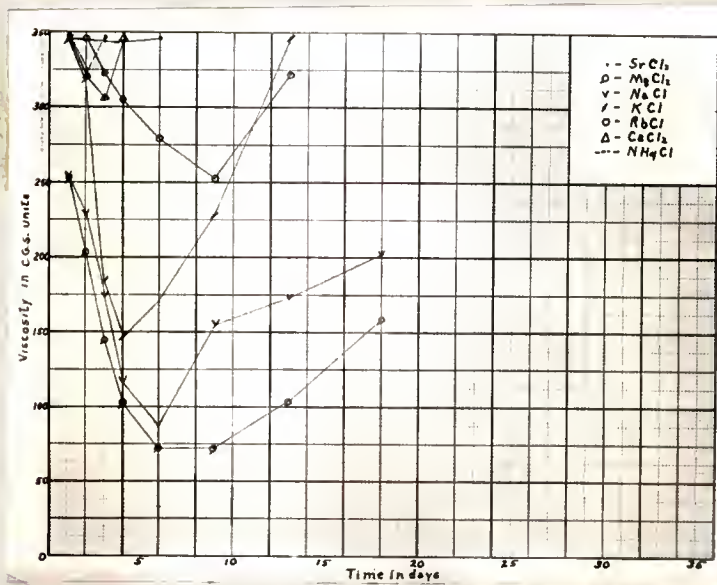


Figure 16. Curves of viscosity for the protoplasm of parametia grown in  $M/40$  molar concentrations of the mono-valent salts and  $M/86$  molar concentrations of the divalent salts.

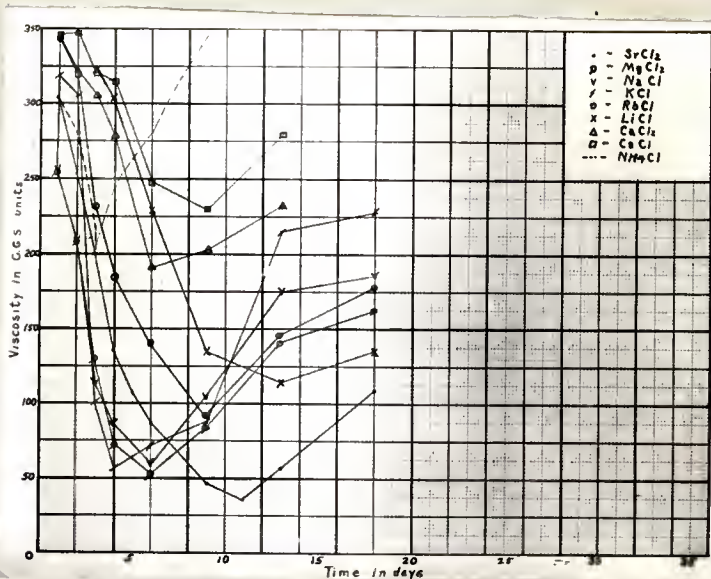


Figure 17. Curves of viscosity for the protoplasm of paramecia grown in M/70 molar concentrations of the mono-valent salts and M/99 molar concentrations of di-valent salts.

the protoplasm of the paramecia over those in the "salt-free" control. Sr cations produced a marked decrease in the viscosity as compared with that of the control. The viscosity in the case of Sr reached a low value of 31 c. g. s. units. The remainder of the group of cations used caused a progressive increase in the viscosity of the protoplasm.

Figures 18, 19, and 20 bring out the relationship between the curves of growth and the curves of viscosity of the paramecia grown in M/40 and M/70 solutions of NaCl (Figure 18), M/70 solution of LiCl (Figure 19), and M/56 and M/99 SrCl<sub>2</sub> solutions (Figure 20). These curves are representative of the relationship that exists between the growth rate and the viscosity of the protoplasm of paramecium.

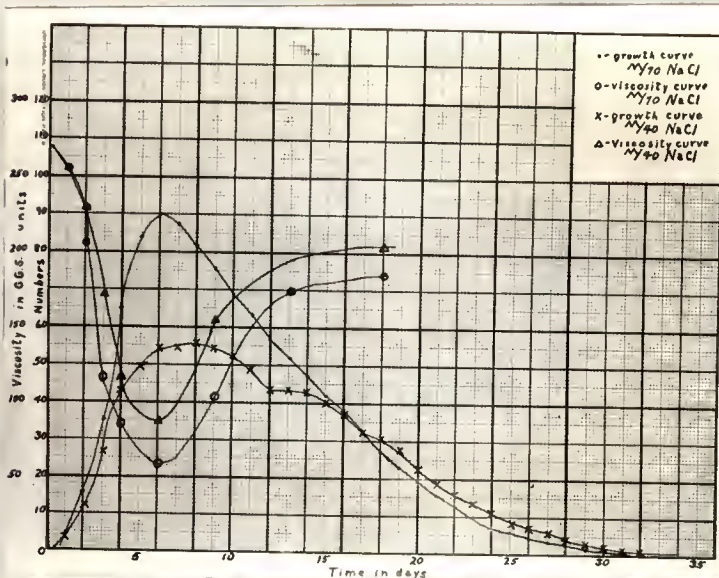


Figure 18. Curves showing the relationship between growth rate and viscosity of the protoplasm of paramecia grown in M/40 and M/70 concentrations of NaCl.



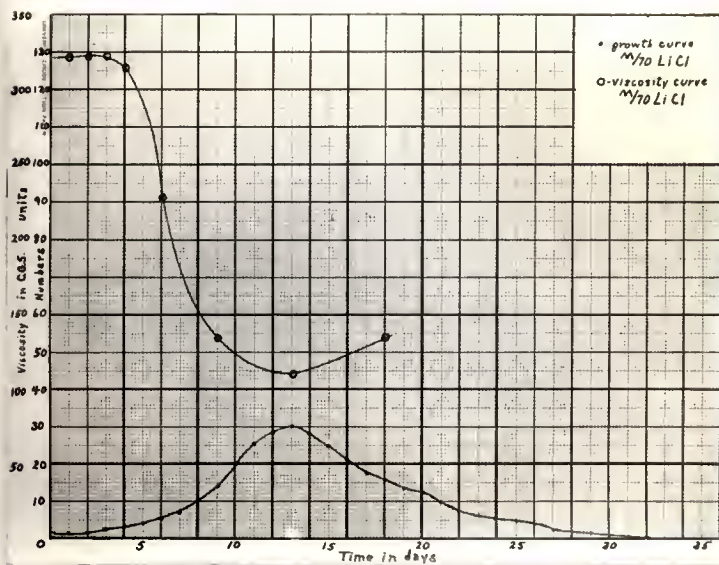


Figure 19. Curves showing the relationship between growth rate and viscosity of the protoplasm of paramecia grown in M/70 LiCl.

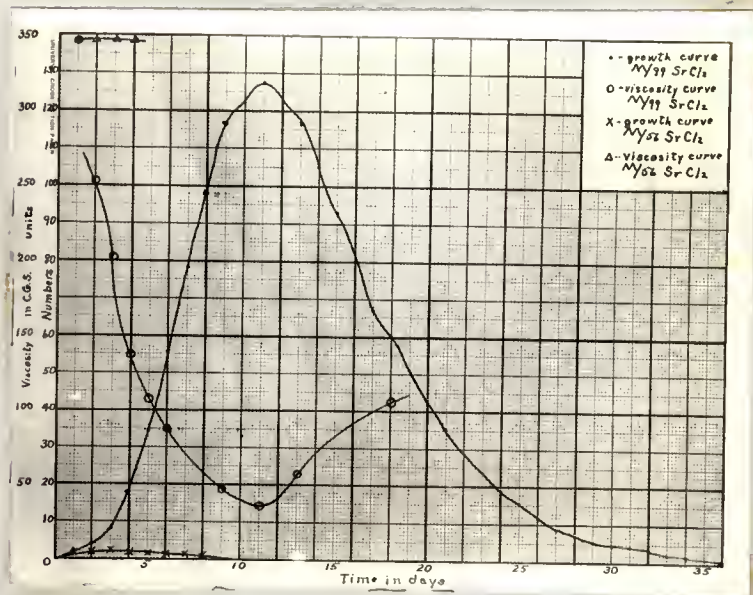


Figure 20. Curves showing the relationship between growth rate and viscosity of the protoplasm of paramoecia grown in M/40 and M/70 concentrations of SrCl.

## DISCUSSION

The fact that a paramecium is not an extremely good osmometer makes it difficult to determine whether a given salt solution is hypertonic or hypotonic for the animal cell. In addition, the cell membrane of the paramecium is not a perfect semi-permeable membrane which makes it difficult to determine if a given salt solution is isotonic with the cell. (The osmotic pressure values, as calculated, refer to perfect semi-permeable membranes). Too, the effect that the salt has upon the permeability of the cell membrane may effect the permeability of it and thus change its osmotic balance. One cannot be sure that a given concentration of a salt like NaCl is isotonic with some other concentration of  $\text{CaCl}_2$  that is calculated to be isotonic with the NaCl.

These difficulties may be obviated to a great extent, if dilute enough solutions are used, which, if not equal in their osmotic effects, are unequal in such a way that any osmotic differences would favor division rate and viscosity changes of an opposite nature from those obtained in the experiment.

In the dilute solutions which were used, it may be assumed that the monovalent salts were completely ionized and the divalent salts nearly completely ionized. If it is assumed that the cell membrane surrounding the paramecium is a perfect semi-permeable membrane, a solution of a divalent salt, since it

dissociates into three ions, would have an osmotic pressure one and a half times that of a solution of a monovalent salt of the same molar concentration. This is true, because the osmotic pressure, being a "colligative" property, is not dependent upon the kind, shape, or size of the particles, but rather upon the number of particles or ions present. Thus, for example, M/45 solution of  $\text{CaCl}_2$  would be isosmotic to a M/30 NaCl solution. By calculating the osmotic pressure from the depression of the freezing point, it is found that this relationship does not hold exactly, since the NaCl solutions are slightly more highly ionized than the  $\text{CaCl}_2$  solutions. Thus, instead of using a M/45 solution of  $\text{CaCl}_2$ , a M/42 solution was used.

In the past, there has been some criticism of the use of the centrifuge in making viscosity determinations upon living cells. Chambers (1917) insisted that the drastic action of centrifugal force invalidates its use, as such forces completely alter the delicate state of viscosity in the cell.

However, Chambers' basis for criticism is entirely unfounded. The centrifugal forces applied in this experiment had no visible effect upon the paramecia, and after treatment for as long as 30 minutes at more than 1200 times gravity, the paramecia, when returned to a suitable culture medium, continued to grow and reproduce in a normal fashion. However, in some of the animals that contained the starch grains, the centrifugal force applied caused the paramecia to separate into two parts, due to the added force supplied from the inside by the starch grains.

That the paramecia can withstand high centrifugal forces is



demonstrated by the work of King and Beans (1934), who centrifuged Paramecium caudatum and Paramecium multimicronucleata with an ultracentrifuge at 150,000 times gravity. Paramecia that were centrifuged at this speed for ten minutes recovered their normal vigor and soon began to reproduce again. If they were centrifuged for longer than ten minutes, they began to disintegrate.

Guyer and Claus (1936) have presented even more convincing evidence, which vividly demonstrates the high gravitational forces that living cells are able to tolerate with impunity. These investigators studied the redistribution of the components of the anterior pituitary cells, after ultracentrifuging at 400,000 times gravity for 20-60 minutes. They then transferred these cells back into the animal, and observed that the animal continued to live normally.

The value obtained for the apparent viscosity of the normal paramecium (approximately 7000 times the viscosity of water) is much higher than the values obtained for other types of protoplasm. It is difficult to explain the reason for this. Heilbrunn (1940) maintains that the high viscosity of the protoplasm of paramecium is due to the very dense packing of the granules. Brown (1940), who was actually able to observe the movement of the starch grains through the protoplasm as the paramecia were being centrifuged, noticed that the rate of fall of the starch grain was not the same throughout all parts of the protoplasm. He observed that the starch grains were often

completely stopped in their movement, and then after a short interval of time, proceeded to move downwards again. This seemed to suggest that there was some sort of an elastic mesh-work structure within the endoplasm of the paramecium.

It is possible that the complex structure of the paramecium may invalidate the use of a moving body in making accurate viscosity measurements. Fetter (1926) suggested that the high viscosity which she found might be due to the presence of a neuro-motor system, as described by Rees (1922). However, in opposition to this view, Lund (1933) has pointed out that the neuro-motor system in paramecium is located almost entirely at the periphery. If this is so, the structure of it could not have any effect on the viscosity measurement.

It thus becomes increasingly obvious that these measurements upon the viscosity of the protoplasm are not implicitly accurate, and do not represent numerical values for the actual absolute or even relative viscosity of the protoplasm. It is for this reason that the term apparent viscosity has been used.

In applying any of the methods of the physical chemist to biological systems, there is always a certain amount of accuracy sacrificed, but due to the complexity of biological systems, this is only natural. However, these values, as obtained, even though they may not be accurate measurements of viscosity, are quite suitable for comparison, and to the biologist, comparative values are as sufficient as accurate quantitative data.

Even with these types of protoplasm, such as the sea urchin

eggs and ameba, where the values obtained for viscosity are fairly low, the term absolute or true viscosity should not be applied. Heilbrunn (1926, 1937) misrepresented the term viscosity by referring to the absolute viscosity of the protoplasm of these animals.

Schmidt (1938) insists that even in homogeneous systems, it is so difficult to measure absolute viscosity that only units for comparison are of any value. These units are the relative viscosity and the specific viscosity. He asserts that viscosity values can only be accurate when the system contains uncharged particles, particles in suspension which are rigid spheres, that have a diameter which is small in comparison with the distance between the spheres.

McBain (1926) maintains that it is best to use the term, apparent viscosity, when referring to the viscosity of colloidal systems, rather than absolute or true viscosity, because viscosity is found to depend upon the rate of shear and the interference caused by ramifying particles present within the system.

The results of the experiments presented here seem to show a difference in the effects of the various cations, when the cations are present in varying concentrations. The nine cations that were used showed a difference in the toxic effect upon the longevity of the paramecium, and upon the growth rate.

The degree of toxicity of the cations upon the longevity of the protoplasm of paramecium, when the salt solutions which were isotonic with M/30 NaCl were compared, was found to be  $Li > Cs >$

$\text{NH}_4 > \text{Sr} > \text{Rb} > \text{K} > \text{Ca} > \text{Na} > \text{Mg}$ . The toxicity, when solutions which were isotonic with M/70 NaCl were compared, was  $\text{NH}_4 > \text{Ca} > \text{Ca} > \text{Rb} > \text{K} > \text{Na} > \text{Mg} > \text{Li} > \text{Sr}$ .

When the toxicity of the cations was based upon their relative degree of inhibition of growth, it was found that the order for those solutions which were isotonic with M/30 NaCl was  $\text{Li} > \text{Cs} > \text{Sr} > \text{NH}_4 > \text{Rb} > \text{Ca} > \text{K} > \text{Na} > \text{Mg}$ . The order of toxicity on division rate when the solutions were all isotonic with M/70 NaCl was  $\text{NH}_4 > \text{Cs} > \text{Ca} > \text{Li} > \text{Rb} > \text{K} > \text{Na} > \text{Mg} > \text{Sr}$ .

Thus, it may be seen that it would be unwarranted to speak of one cation as being more toxic to the protoplasm than another, unless the statement was qualified in some way. For example, in M/30 concentration the Li ion is the most toxic, whereas in M/70 concentration it is nearly the least toxic of all. In addition, while in M/42 concentration the Sr ions barely support any division of paramecium, a molarity of 1/99  $\text{SrCl}_2$  caused the greatest degree of multiplication.

These results substantiate those obtained by Hetchkiss (1925), Winslow and Dolloff (1928), and Winslow and Haywood (1931) for bacteria. These workers also found that the effects of cations upon the protoplasm is purely a matter of degree and not one of kind. Every cation has its own point for inhibition and point for stimulation, however dilute the solution may be.

The toxicity series for the effect of these nine different cations on the growth rate is in the same order as the series for the effect of the nine different cations upon the viscosity of



the protoplasm, for the data presented in this paper show that the rate of growth is related, in a definite manner, to the viscosity of the protoplasm.

Since the degree of toxicity exerted by these cations on the protoplasm of the paramecium was found to be inversely proportional to the growth rate and directly proportional to the viscosity, it is reasonable to assume that the growth and the speed at which the organism reproduces is dependent upon the state of viscosity of the protoplasm existant at a given time. Thus, the faster the division rate, the lower must the viscosity be, and conversely, the lower the rate of division or the fewer the number of generations, the greater would the viscosity be. With these points in mind, it is possible to conclude that any change in the biological activity of the protoplasm, such as vitality and growth, is associated with changes in viscosity. These changes in viscosity are commensurate in degree with the changes in the rate of growth.

Thus, it would appear that the cations of Na, K,  $\text{NH}_4$ , Li, Rb, Cs, Mg, Ca, and Sr only indirectly effected the rate of growth and longevity of the paramecia. The direct effect, in this case, being due to a change in the colloidal nature or physical structure of the protoplasm, brought about by the cations.

In order to explain the mechanism or the causes that might be responsible for such changes in viscosity, it is first necessary to arrive at some explanation of the manner in which changes in the physicochemical make-up of the protoplasm might cause its

viscosity to increase or decrease.

The explanation for changes in viscosity in colloidal solutions are based upon the changes that take place in the physical properties of the dispersed phase of the colloidal system.

Loeb (1924) asserted that an increase in viscosity is a result of an increase in the degree of hydration of the suspended particles, and that such an increase in the case of protein solutions is purely a Donnan equilibrium effect.

Rice (1926) believed that the increase in viscosity is brought about by a coagulation of the smaller into larger particles. However, most investigators (Alexander, 1926) believe that an increase in viscosity consists of something more than a simple aggregation of several small particles to form one large particle. They believe that the suspended particles coalesce in some manner to form a meshwork system out of the dispersed phase.

The explanations as postulated by Loeb and Rice for viscosity changes in colloidal systems are not confirmed by experimental or empirical evidence. McBain (1926) maintains that according to the Einstein and Hatschek formula for viscosity, the enhanced viscosity is related to the total bulk of the particles independent by their degree of subdivision. Thus, increased viscosity could not be due to increased solvation of the particle or agglomeration of the smaller particles into larger ones. He attributes high viscosity values to the formation of ramifying aggregates of colloidal particles which embrace and immobilize parts of the solvent.

Bancroft (1926b) states that the viscosity of a system may be changed by one of the following means. If the suspended particles aggregate into chains, the viscosity is increased. If the particles form larger spherical particles which are homogeneous, there will be a decrease in viscosity, because of the decrease in surface and consequently in the amount of bound water. If the particles simply agglomerate loosely into spherical masses, the viscosity will increase because the water in the voids inside the spherical agglomeration no longer may be counted as free water.

Dhar and Chakravorti (1928), Abramson (1934), and Schmidt (1938) also believe that an increase in viscosity is due to an increase in agglomeration that involves a decrease in the available free water.

It is difficult to find an explanation for the decrease in viscosity that occurs in colloidal systems. If it is assumed that all of the particles of the dispersed phase are free and that no aggregates of particles are formed, then the only tenable theory or hypothesis that could explain a decrease in the viscosity of such a system is that the suspended particles become more finely subdivided, or they become peptized. This explanation is, however, contrary to the theory upon which the Einstein and Hatschek formula is based. Another explanation that might explain the decrease in viscosity is that a certain amount of aggregation is normally present in the colloidal system, and that the viscosity of such a system is decreased when

some of the aggregates are broken up into individual particles.

Thus, it is manifest that an increase in viscosity may be caused by a coalescing of the dispersed particles, either by a grouping into chains or into loose clumps. Also, a decrease in viscosity may be the result of a more complete subdivision of the particles, or of a dispersion of the aggregated particles in the system.

There now remains to be explained the mechanism of the action of the various cations upon the protoplasmic colloids, and the method by which these cations act to aggregate the suspended particles when the viscosity is increased or to disperse them in case the viscosity is decreased.

Kruyt (1926) gives the following two conditions as necessary for coagulation to occur: (1) the probability of collision, and (2) the probability of adhesion. According to this view, the mechanism of coagulation in a colloidal system in which the dispersed particles are deprived of any stabilizing factor would be governed merely by the probability of collision of the particles. However, with a dynamic colloidal system, such as that present in the living cell, there are always some stabilizing factors present, and consequently the probability of adhesion would be the main factor to be considered.

The probability of adhesion is determined by such effects as the viscosity of the suspension, temperature, rate of Brownian movement, degree of hydration of the suspended particles, and the electrical charge on the particle, or more accurately,



the electrokinetic or  $\zeta$  (zeta) potential. It is because of the existence of these last three mentioned effects that lyophilic colloidal suspensions are kept from settling out. It is patent that protoplasm is a lyophilic colloid, for if it were a lyophobic colloid, even the small concentration of salt normally present within the cell would cause a precipitation.

By adding various agents to the suspension of colloids, it is possible to remove the stabilizing effect offered by the hydration of the particles, or to remove the stabilizing influence exerted by the electrical charges, or to remove both. This latter effect of electrical charge is the most important one to consider when adding dilute salt solutions to protoplasm. Thus, if the charge on the particle is completely removed, or only partially altered, the probability of adhesion is greatly increased.

The cations may alter the charge on the surfaces of the suspended particle to such an extent so as to (a) completely remove it and produce a neutral particle, (b) reverse the sign of the charge, or (c) only partially disturb it by lowering the electrokinetic potential ( $\zeta$ ). The change in the electrokinetic potential is the most important one to consider.

The electrokinetic potential is the potential produced at the surface of the colloidal particle or micelle as a result of the adsorption of a double layer of ions about the particle. All particles in a given colloidal system tend to adsorb an excess of either positive or negative ions on the surface. This

charged surface tends to attract ions of opposite sign to it by the electrical forces of attraction, forming two layers of oppositely charged electricity, as in a condenser.<sup>8</sup> These electrical fields due to ions constitute what is known as a double layer, and the outer movable layer is known as the diffuse layer.

Against the electrical forces set up by this "condenser" and striving for osmotic equilibrium is the movement of all ions tending to diffuse away from the surface. Since the kinetic energy of the suspended particles gives rise to diffusion gradients, the presence of electrostatic forces leads at equilibrium to a uniform distribution of ions so that the number of ions opposite in sign of charge in the immediate neighborhood of the particle will be, over a period of time, greater than that of the ions of the same sign of charge. In this way, an "atmosphere" is built up, extending from the surface of the particle to the bulk of the solution.

Where  $\underline{\epsilon}$  is the potential difference between the colloidal micelle and the solution, the  $\zeta$  (zeta) potential difference is only a part of the total drop,  $\epsilon$ .  $\xi$  or the electrokinetic potential is that portion of the potential drop between the liquid adhering to the wall of the particle and the movable liquid.

Bull and Gertner (1931) explained the importance of the electrokinetic potential in maintaining the stability of the

---

<sup>8</sup>Michaelis (1926), Höber (1928), and Hauser and Hinson (1939) discuss the various theories that attempt to explain the manner in which these charges are produced upon the surface of the colloidal micelle.

colloidal suspension. They found that the zeta potential decreased with an increase in salt concentration in the aqueous phase; however, the decrease could be accomplished by an actual increase in the charge on the particle, the decrease in potential being more nearly related to a decrease in the thickness of the double layer. Thus, according to Bull and Gortner, salts do not reduce the electrical charge on the micelle to zero or even to a "critical threshold", (except in the case of polyvalent ions which may even reverse the sign of charge on the particle) but instead, they cause a decrease in the thickness of the diffuse layer which allows the particles to approach each other closely enough so that they adhere to each other by electrostatic forces of attraction. This is in agreement with the statement of Abramson (1934), who claimed that the electrokinetic potential does not have to be zero for aggregation of the colloid particles to occur. If zeta is greater than zero, coagulation may occur, although more slowly. This slow coagulation is probably the type which occurs in living systems when dilute salt solutions are added to protoplasmic material.

Thus, the coagulation of the colloidal particles and the subsequent increase in viscosity resulting from the addition of dilute salt solutions to the protoplasm may be assumed to occur in the following manner:

As the salt solution is added to the protoplasmic colloids, the electrokinetic potential decreases. However, Brownian movement persists and the colloidal particles still contain most of their bound water. As the zeta potential is decreased and the

suspended particles still exhibit Brownian movement, the probability of adhesion of these particles is increased. The probability of adhesion, in this case, results from the decrease in electrokinetic potential. Therefore, the degree of adhesion or aggregation is enhanced and the microscopic colloidal micelle forms larger ramifying aggregates. (Abramson, 1934, has actually observed this phenomenon of aggregation through the microscope) As the particles form into larger aggregates, the viscosity is increased, and this initial increase in viscosity then causes a greater rate of aggregation or coagulation. (Kruyt, 1926).

Before it is possible to arrive at any conclusion as to the effects of the various ions on the protoplasm, the charge on the protoplasmic micelle must first be ascertained.

This is difficult to determine by objective methods, and thus, empirical methods must be resorted to. Notwithstanding a few attempts have been made to determine the charge upon the protoplasmic micelle by objective methods. Kühne, in 1860, (Abramson, 1934) determined the electrical charge on muscle tissue to be negative by the process of electroosmosis. He observed that if a thin strip of muscle with parallel fibers was placed between two electrodes, after the first contraction identical to the completion of the circuit, swelling of the muscle took place at the negative pole with wave-like movements occurring in the same direction. With reversal of the direction of the current, reversal of the direction of flow occurred. The increase in volume which occurred at the cathode was



accompanied by a corresponding decrease at the anode.

Taylor (1925) introduced electrodes into the protoplasm of the slime mold, Stemonitis elegans and observed the electrophoretic migration of the particles. He observed that the microscopic granules migrated towards the anode and an increase in viscosity reduced their speed of migration. He noticed that the ultramicroscopic particles moved to both the anode and the cathode and that some even failed to migrate.

Seifrig (1928) maintained that most types of protoplasm are negatively charged. Nöber (1928) also stated that in all types of protoplasm investigated he found the electrical charge of the protoplasm to be negative.

Greeley (1904) observed that under normal conditions, paramedia migrated towards the cathode when placed in an electric field. This indicates, according to Greeley, that the charge on the cell surface is positive, and that the charge on the individual protoplasmic micelle is of the opposite sign, or in this case, negative.

This evidence seems to indicate that the protoplasmic micelles are charged negatively; however, additional evidence for assuming that the particles are negatively charged is offered by the results of this experiment. Since the paramedia used in this experiment were grown in an alkaline medium, pH 7.6, which is definitely on the alkaline side of the isoelectric point for protoplasm), the excess OH or negatively charged ions would tend to give the micelle a negative charge. Also the fact that the cations used produced such a definite increase in protoplasmic

viscosity in dilute salt solutions would further indicate that the protoplasmic particles are negative charged, since colloidal suspensions are most effectively precipitated by ions possessing the opposite sign to that of the colloid particle (Hardy, 1900).

This latter statement of cation action is in line with the findings of other investigators (Falk, 1923), who have shown that with the living cell, it is the cation which has the most drastic effect upon the physicochemical make-up of the colloids, and that the effects of the anions are more often of only minor importance. Thus, it is concluded that the cations have the most drastic action because the protoplasmic micelles are negatively charged.

Several questions at once present themselves: (1) What caused the cations used in this experiment to effect the viscosity of the protoplasm in a definite manner, and (2) Why didn't all the cations effect the protoplasm in the same manner, since they all carried positive charges and the protoplasm is assumed to have carried a negative charge? Nearly all of the investigators who have worked with the action of cations on animate and inanimate colloids have attempted to relate the effect produced by the ions to some physical or chemical property of the ions. Table 2 is a protocol, showing at the top of the table the order of toxicity for the various cations for paramecium at M/30 and M/42 concentrations, and below this, a list of values for various physical and chemical properties of the ions. A great number of investigators have, in the past,

Table 2. Protocol showing the relationship between the toxicity of certain cations on Paramecium caudatum and various physical and chemical properties of the cations.

Cation	M/30	Al	Fe <sup>++</sup>	Zn	Mn	Ba	Li	Cs	NH <sub>4</sub>	Sr	Rb	K	Ca	Na	Mg
Atomic Number	13	26	30	25	56	3	55	-	38	57	19	20	11	12	
Atomic Weight	26.97	55.84	65.38	54.93	137.36	6.94	132.91	15.01	87.63	85.48	39.09	40.08	22.99	24.32	
Speed of ions <sup>2*</sup>	-	6.1	4.5	6.5	-	5.7	3.4	7	6.7	5.6	6.8	6.7	5.3	4.6	4.5
Degree of Hydration <sup>2Δ</sup>	-	-	-	-	4	124	13	17	8	14	16	10	60	14	
Ionic Radii <sup>Δ</sup>	0.50	0.67	0.85	0.74	0.91	1.55	0.60	1.69	-	1.13	1.48	1.33	0.99	0.95	0.65
Atomic Radii <sup>Δ</sup>	1.48	1.26	1.24	1.33	1.24	2.17	1.50	2.62	1.99	2.14	2.45	2.27	1.97	1.86	1.56
Atomic Volume <sup>3o</sup>	-	7.2	7.2	9.2	7.3	3.6	-	71	-	35	57	45	25	23	-
Solution Tension	0.99	0.05	0.44	0.50	0.80	2.54	2.37	-	-	2.49	2.54	2.92	2.28	2.54	2.26
Valence	III	III	II	II	II	II	I	I	I	II	I	I	I	I	II

1. From Lang's "Handbook of Chemistry (1939).  
 2. From Creighton's "Principles of Electrochemistry, Vol. I (1935).  
 3. From Mathews (1904). Amer. Jour. Physiol.  
 4. Microns per second.  
 Δ. Listed as number of layers of water molecules surrounding the ion.  
 o. Angstrom units.  
 o. Relative figures.  
 +. Volts.

related the degree of toxicity to one or more of these properties.

By examining Table 2, it will be seen that there is no relationship between the degree of toxicity and any of the nine different physical and chemical properties of the cations listed. There appears to be no evidence for believing Loeb's (1920) hypothesis that the toxicity is related to the ionic or atomic radius of the ions. Neither does there seem to be any vindication of Barnes' (1937) statement that the toxicity is related to the speed of movement and the degree of hydration of the ions. Nor does Mathews' (1904) statement that the toxicity is related to the atomic volume seem to have any bearing upon the order of toxicity of the various cations, when applied to paramecia.

However, there does appear to be a certain degree of relationship between the solution tension of the ions and their toxicity, as pointed out by Mathews (1904, 1940) and Woodruff and Bunzel (1909). Though this relationship is not quantitative and is only an approximation, it may have some bearing upon the extent of toxicity that a particular cation has upon the protoplasm.

Loeb (1902, 1924) was one of the first investigators to attribute the toxic effects of the ions to their valence. He maintained that all of the ions that had the same valence produced the same degree of effect upon the physical properties of proteins, such as, their osmotic pressure, viscosity, and swelling. Loeb asserted that the effectiveness of the ions followed the Schulze-Hardy Rule. Schulze (Hardy, 1900) was the first



experimenter to accurately describe the power of salts to destroy colloidal solutions. He showed that the power which various salt solutions possessed to precipitate a hydrosol of sulfide of arsenic is related to the valence of the metal in the order  $R'$ ,  $R''$ ,  $R'''$ ; 1:50:1650. Linder and Pieton (1895) obtained similar results with inorganic colloids. Hardy (1900), working with inorganic colloids, found that the coagulative power of a salt is determined, not only by its valence, but also by the sign of charge of the colloid upon which it is acting. The precipitating ion is the positive ion when the colloid is negatively charged. Thus, the Schulze-Hardy Rule states that the power of an ion to coagulate a colloid is increased with an increase in valence, and that the coagulating ion is always of opposite electrical charge to that of the particle it is coagulating.

Burton (1926) concurred with these views concerning the power of ions to coagulate the inanimate colloids. Heilbrunn (1923, 1940) and other biologists still maintain, as does Burton, for inanimate colloids, that the coagulating power of an ion for protoplasm is related in a definite manner to its valence.

Such a viewpoint no longer seems to be tenable. Bancroft (1915) stated that the Schulze Rule is only an approximation, and that while there is unquestionably a propensity for ions of a higher valence to be adsorbed more strongly onto the colloid particles than those of a lower valence, there are many exceptions.

Abramson (1934), like Bancroft, expressed the opinion that the Schulze Rule works well in some cases, but that it is complicated by the effects produced by the nature of the dispersed system and the specific action of individual ions.

If it were only the valence of an ion that determined the effect it might have on the colloidal system, then it should be possible to replace one monovalent cation by another in the living cell. Boissevain (1928) found that although some bacteria were capable of substituting Rb for K in their growth, Cs, Na, and Li ions were not able to replace K. Seifrig (1928) also found that the reaction of protoplasm to all divalent ions is not the same. For example, he found that Ca decreased the permeability of plant cell membranes and increased the viscosity of the protoplasm, and that Sr increased the permeability of the membrane and decreased the viscosity of the protoplasm.

If none of these nine different properties of the ions enumerated in Table 2 are responsible for the toxicity of the salts and their effects on viscosity, then what factor is most important in regulating the degree of toxicity and viscosity?

At the present time, one of the most widely accepted theories is the adsorption theory. That is, the more highly adsorbed the cation is, the more toxic it is.

Abramson (1934) contended that when coagulation occurs, the ions concerned were adsorbed on the colloid. Docking and Heymann (1939) have recently shown that proteins possess a high adsorption capacity for cations. Jenny (1932) has shown that the same condition exists with the inorganic colloids.

Bancroft (1915) maintains that the Schulze Rule of valency is not applicable because all univalent ions are not adsorbed alike, and neither are bivalent and trivalent ones. The adsorption order is specific for a given colloid and therefore, depends upon the nature of the cation, the anion associated with the cation, and the colloid. More recently, Bancroft (1926a) has advanced a new theory to explain the effect of the different ions on the viscosity of a colloidal suspension. His theory is based upon the effects of the ions upon the water equilibrium. Thus, if water is considered an equilibrium between hydrol ( $H_2O$ ) and other forms  $(H_2O)_2$ ,  $(H_2O)_3$ , and  $(H_2O)_n$ , this equilibrium, according to Bancroft, may be disrupted by various agents. He explained how a change of the equilibrium might effect either the peptizing or pectizing (coagulating) properties of the water.

Clowes (1916) has produced some evidence which seems to indicate that the anion may play a role in adsorption, even when the micelles are charged negatively. He attempted to explain the effects of Na and Ca ions on the basis of the antagonistic effects they produced upon an oil-water emulsion. He found that in the case of  $CaCl_2$ , the cation Ca is far more readily adsorbed than the anion Cl, while with NaCl, the anion Cl is somewhat more readily adsorbed than the cation Na. Weiser (1926) also pointed out that the precipitating value of an electrolyte is dependent upon the adsorption capacity of the anion as well as that of the cation. The precipitating value of an electrolyte with a strongly adsorbed stabilizing ion is higher of necessity

than that of an electrolyte with a weakly adsorbed stabilizing ion. Thus the order of precipitation for K salts on ferrous hydrosol is  $\text{Ca} > \text{SO}_4 > \text{FeCN} > \text{citrate}$ , and according to Weiser, this is probably the order of increasing adsorbability of the stabilizing anions.

McCalla (1940a, 1940b) was one of the first workers to actually measure the degree of adsorption of the cations upon living protoplasm. His findings agree with those of numerous experimenters who have worked with inorganic and organic colloids. He found that the more toxic the cation was for bacteria (or the more readily it coagulated the protoplasm), the more highly adsorbed it was. Seifrig and Uraguchi (1941), who worked with the cations of the heavy metals upon the protoplasm of the slime mold, Physarum patycephalus, obtained results which also seem to show that the degree of toxicity of a particular cation is directly proportional to its adsorption capacity. These workers measured the degree of adsorption of the cations upon blood charcoal and then likened this to the adsorption on the protoplasm of the slime mold.

McCalla (1940b), working with bacteria, and Jenny (1932, 1936), working with inorganic colloids (aluminum silicates), have revealed additional facts which further substantiate the adsorption theory. They measured the degree of adsorption of the various ions by determining the extent to which a particular ion would replace another ion from the surface of the colloidal particles, and then itself be adsorbed. They found that



in general the order of adsorption or ionic exchange ability was  $\text{Li} < \text{Na} < \text{NH}_4 < \text{K} < \text{Mg} < \text{Ca} < \text{Ba} < \text{Mn} < \text{Al} < \text{Fe} < \text{H}$ . This order of adsorption corresponds closely to the well-known Hofmeister or lyotropic series.

Michaelis (1924) mentioned the importance of considering the indirect or hydrophilic effect of the cations upon colloids when the salt concentration is high. This hydrophilic effect of the cations gives rise to the famous lyotropic series, and results from different degrees of hydration, possessed by the different ions. This effect is not considered to be of any great import in interpreting the results of this experiment, except possibly in the cultures, where high concentrations of  $\text{LiCl}$  was used, for the  $\text{Li}$  ion is very highly hydrated as compared to the other ions (o. g. Table 2, p. 85). This effect may be interpreted as a competition for the water between the  $\text{Li}$  ions and the micelles which are also highly hydrated in lyophilic colloids. Thus, if there is an excess of  $\text{Li}$  ions in the suspension, they will tend to remove water from the colloidal micelle and weaken one of the stabilizing influences which tend to keep the particles suspended. This loss of bound water on the colloidal particle will then increase the probability of adhesion which will in turn cause an increase in viscosity.

It does not appear therefore, that any of the physical and chemical properties of the  $\text{Na}$ ,  $\text{K}$ ,  $\text{NH}_4$ ,  $\text{Li}$ ,  $\text{Rb}$ ,  $\text{Cs}$ ,  $\text{Mg}$ ,  $\text{Ca}$ , or  $\text{Sr}$  ions (Table 2, p. 85) played a major role in bringing about the increase in viscosity of the protoplasm by a coagulation of the colloidal micelle. However, the weight of evidences seems to

point to a relationship between the rate of adsorption and the degree of toxicity of the various ions. If it is apparent that such a relationship holds true for paramecium protoplasm, then the rate of adsorption for a particular cation must of necessity vary with the concentration, since it was shown that the degree of toxicity varies with the concentration used.

Although this theory of adsorption may be accepted as the principal factor which controls the state of aggregation, and hence the viscosity of the protoplasmic material, it is evident that conditions other than the physical state of the protoplasm may effect the rate of growth. Even in so "simple" an organism as the paramecium, it is only reasonable to conclude that these various cations may have had an effect upon some other mechanism of the cell, such as, the respiratory system, which in turn, would have affected the growth rate and probably the viscosity. Lindahl (1933) found that the  $Mg$  ion inhibited the action of the enzymes of living cells.

In addition to the effect upon the respiratory system, the cations may have had an effect upon the cell membrane, which may have been entirely different from their effect upon the inner protoplasm. The experimental results seem to suggest that the  $K$  ion has a very drastic effect upon the cell membrane when present in high concentration.

Also, the chemical nature of the ion itself may have caused it to produce a condition within the cell which would complicate any valid interpretation upon the basis of purely physical effects. Such a situation is believed to be the case as regards

the rather anomalous effect of the  $\text{NH}_4$  ion upon the vitality of the paramecium.

It was noticed that after centrifuging the paramecia and then staining them with Sudan III (a lipin specific stain), the anterior portion of the animal showed a more intense stain in those paramecia that had been grown in the  $\text{NH}_4\text{Cl}$  solutions prior to centrifuging, than those that had been grown in the control solutions or the solutions of the other salts.

The fact that the anterior region of the paramecia stained more intensely in those animals that had been grown in  $\text{NH}_4\text{Cl}$  solutions must indicate that there was more free lipin present in this region. After centrifuging the paramecia, the lipin material, being lighter than the granular substance, came to lie at the anterior portion of the cell.

Most of the lipin present in protoplasm is bound up with the protein, and if this protein-lipin combination is broken up, there will be an increase in the amount of free lipin present in the cell.

These results seem to indicate that the  $\text{NH}_4\text{Cl}$  ionizes into  $\text{NH}_4\text{OH}$ , and then penetrates the cell easily and produces a stronger alkaline condition within the cell, even though the water surrounding the cell may be acid (Jacobs, 1922).

This strong alkaline reaction within the cell causes the lipin fraction of the protein-lipin combination to split off, giving rise to more free lipin. This is the same type of condition which results when fatty degeneration occurs in higher animals, except that it is usually not due to the presence of

an alkali. Heilbrunn (1936, 1940) has described similar effects for  $\text{NH}_4\text{Cl}$  solutions with the sea urchin egg and the amoeba. This condition of fatty degeneration, as induced by the  $\text{NH}_4\text{Cl}$ , is undoubtedly harmful and will result in the death of the paramecium if it is carried far enough.

This destructive action of the  $\text{NH}_4$  ion may be removed by growing the paramecia in a nearly anaerobic environment. It has been observed that when paramecia grown in such concentrated solutions of  $\text{NH}_4\text{Cl}$  as  $M/30$ , will reproduce at a normal rate providing they are kept in a tightly stoppered flask. The explanation for this probably lies in the fact that with the accumulation of large amounts of  $\text{CO}_2$  in the water, the  $\text{CO}_2$  being readily permeable to the cell membrane, passes into the cell and tends to keep the pH down to normal.

Another question requiring solution confronts us. Why is a system which has a high viscosity less conducive to rapid growth than a system which has a low viscosity?

Belehrádek (1926, 1929) and Bayliss (1915) maintained that the viscosity of the protoplasm, by determining the rate of diffusion into the protoplasm, determines the rate of biological reactions. Since the rate of any chemical reaction depends solely on the rate of diffusion of the reacting substances, an increase in viscosity of the protoplasm, by decreasing the rate of diffusion, would, according to Behlradek, and Bayliss, likewise decrease the rate of biological reactions. Stiles (1930), however, found that in solutions of substances of non-electrolytes and in heterogeneous systems, the coefficient of diffusion



of substances is not inversely proportional to the viscosity of the medium, and consequently, according to Stiles, the assumptions of Belehrádek and Bayliss are not valid.

Since colloidal systems contain such a tremendous amount of surface area for chemical reactions to take place, the most plausible explanation for the decrease in growth rate concurrent with an increase in viscosity may be explained on the basis of a decrease in surface area due to a coagulation of the suspended particles. Since the amount of chemical change in a unit of time is proportional to the absolute surface area (Bartell, 1931; Gortner, 1933), any agent which decreases the surface area will also decrease the growth rate, and any agent which increases the surface area will tend to increase the free surface energy and thus increase the rate of growth.

In summarizing the discussion, the following general statements may be made: (1) The rate of growth is dependent upon the viscosity of the protoplasm. As the viscosity increases, the growth rate decreases.<sup>10</sup> (2) An increase in viscosity is due to an agglomeration of the suspended particles in the colloidal system into larger aggregates with a loss of available free water. (3) This agglomeration of the particles, in the case of an increase in viscosity, is brought by a decrease in the electrokinetic potential on the particle surface. The

---

<sup>10</sup>Essentially, it is correct to say that as the viscosity of the protoplasm increases, the growth rate decreases; however, in some cases the viscosity may actually decrease with age, due to the fact that the proteins of the colloids begin to hydrolyze. (Schmidt, 1938). This latter effect need not be considered except in pathological cases.

increased dispersion of the particles, in the case of a decrease in viscosity, is due to an increase in the electrokinetic potential. (4) Cations have definite effects upon the electrokinetic potential and consequently the viscosity of the protoplasm, because the protoplasm is negatively charged. (5) It appears that the extent of adsorption of the cations upon the protoplasmic colloids is the most satisfactory explanation for the action of cations on the protoplasm. That is, the greater the degree of adsorption of an ion, providing that it has a charge opposite in sign to the protoplasmic colloids, the more effective it will be in lowering the electrokinetic potential and in this manner cause an increase in viscosity and a decrease in the growth rate. And (6) the decrease in growth rate with an increase in viscosity is the result of a decrease in surface area from which chemical reactions may occur.

## SUMMARY

1. An improvement in the Parpart method for the sterilization of paramecia is described. The improvement in technic calls for the addition of an excess of  $\text{CaCl}_2$  to the washing and dilution medium.

2. An improvement in the Brandwein general culture medium for protozoa has been developed. This improvement in the medium was developed specifically for culturing Paramecium caudatum.

3. The use of "Cerophyl" as the organic "base" for the culturing of paramecia in making quantitative determinations upon the physiology of Paramecium caudatum is described.

4. The development of a new technic for controlling the number of bacteria present in a given volume of culture medium over a period of time is outlined.

5. It was found that the use of either Bacillus subtilis or Mycobacterium phlei in the culture medium supported a good growth of paramecia.

6. The curve of normal growth of paramecium follows closely the curve of bacterial growth as described by Buchanan and Fulmer (1928).

7.  $\text{CaCl}_2$  solutions, in the dilutions used, speeded up the rate of locomotion of the paramecium, whereas K, Li, and Cs retarded the rate of movement.

8.  $\text{ErCl}_2$ , in the more dilute solutions (M/71, M/85, and M/99), caused the paramecia to become decreased to one-half their

normal size.

9. Of all the cations used, the Sr ion (M/99) was the only one which caused a significant increase in the rate of growth as compared with the "salt-free" control.

10.  $\text{AlCl}_3$ ,  $\text{FeCl}_3$ ,  $\text{FeCl}_2$ ,  $\text{ZnCl}_2$ ,  $\text{MnCl}_2$ , and  $\text{BaCl}_2$  solutions as dilute as M/100 failed to support the growth of the paramecia. The organisms were all dead after a few minutes immersion in these solutions.

11. The apparent viscosity of a normal paramecium was determined to be approximately 7238 times that of water.

12. As the rate of growth of the paramecia culture was increased, the viscosity of the individual paramecium was decreased, and conversely, as the rate of growth was decreased and the organisms began to age, the viscosity was increased.

13. The viscosity of the protoplasm of the paramecium is capable of wide variation. By the centrifuge technic employed, it was possible to measure a viscosity range of from 30-350 c. g. s. units.

14. The toxicity of the cations upon the longevity of the protoplasm of paramecium, when the salt solutions that were isotonic with M/30 NaCl were compared, was found to be  $\text{Li} > \text{Cs} > \text{NH}_4 > \text{Sr} > \text{Rb} > \text{K} > \text{Ca} > \text{Na} > \text{Mg}$ . The toxicity, when solutions which were isotonic with M/70 NaCl were compared, was  $\text{NH}_4 > \text{Cs} > \text{Ca} > \text{Rb} > \text{K} > \text{Na} > \text{Mg} > \text{Li} > \text{Sr}$ .

15. The toxicity of the cations, when based upon their relative degree of inhibition of growth when compared with M/30



NaCl was found to be  $\text{Li} > \text{Cs} > \text{Sr} > \text{NH}_4 > \text{Rb} > \text{Ca} > \text{K} > \text{Na} > \text{Mg}$ . The order of toxicity, when compared with M/70 NaCl, was  $\text{NH}_4 > \text{Cs} > \text{Ca} > \text{Li} > \text{Rb} > \text{K} > \text{Na} > \text{Mg} > \text{Sr}$ .

16. The effects produced by the different cations varied with each cation. In every case the effect produced by a single salt varied with the concentration.

17. The cations affected the growth rate of the paramecia only indirectly. The rate of growth was affected directly by the viscosity of the protoplasm, and the viscosity of the protoplasm was in turn affected in a definite manner by the particular cation added and its concentration.

18. It is suggested that the  $\text{NH}_4$  ion decreased the growth rate of the paramecia by causing fatty degeneration to occur within the cell. The free  $\text{NH}_4\text{OH}$  within the cell acted to break-up the protein-lipin bond.

19. The protoplasmic micelles of the paramecium were negatively charged.

20. There did not appear to be any direct relationship between the degree of toxicity and any of the physical and chemical properties of the ions; such as, valence, atomic number, atomic weight, atomic volume, atomic radii, ionic radii, solution tension, mobility of ions, or extent of hydration of the ions.

21. It is suggested that the toxicity of the various cations for the paramecium is proportional to its degree of adsorption. However, it is not wise to be too dogmatic about such theories of behavior in colloidal systems. Kopaczewski (1926)

also cautions against making such statements, as may be noted in the following:

The colloidal state is intermediate between suspensions of solids dominated by physical laws, and true solutions governed by chemical reactions; the same substance may, according to conditions, be in the colloidal state (soap in water, NaCl in benzene), or in the soluble state (soap in alcohol, NaCl in water).

The laws governing colloidal phenomena cannot, therefore, be exclusively physical laws of classical chemistry. To the intermediate state there must correspond intermediate properties--and intermediate laws.

The degree of dispersion controls these properties and these laws; the more dispersed a substance is, the more it approaches the state of true solutions, and the more closely its properties approximate chemical properties; with decrease in dispersion there reappears the preponderance of physical laws. That is why with variations in the degree of dispersion of the substances experimented with, we find ourselves confronted with so many chemical principles, and so many physical laws. And having in mind this importance of the degree of dispersion, we must be cautious against generalizing and building chemical or physical theories, which are always one-sided, and against being satisfied with one part of the truth, which is itself scattered among'st all.

## ACKNOWLEDGMENT

Indebtedness is acknowledged to Dr. E. J. Wimmer, Associate Professor of Zoology, for directing this study; to Dr. B. L. Smits and Dr. H. W. Marlow of the Department of Chemistry for valuable suggestions and loans of essential equipment; and to members of the Department of Bacteriology for advice and equipment which greatly facilitated the work of the problem.

## LITERATURE CITED

- Abramson, Harold A.  
Electrokinetic phenomena. Amer. Chem. Soc. Mono.  
New York. Chemical Catalog Co. 351 p. 1934.
- Alexander, Jerome.  
Colloid chemistry. Vol. 1. New York. Chemical Catalog  
Co. 974 p. 1926.
- Amster, V.  
Ein neues zuchtungsverfahren für protozoen. Centralb.  
f. Bakt., Orig. 89:166-168. 1925.
- Bancroft, F. W.  
The control of galvanotropism in paramecium by chemical  
substances. Univ. Calif. Publ. Physiol. 3:31-51. 1906.
- Bancroft, W. D.  
Neutralization of adsorbed ions. Jour. Phys. Chem.  
19:363-376. 1915.
- Bancroft, W. D.  
The water equilibrium. Colloid Symposium Monograph,  
4:29-37. 1926a.
- Bancroft, W. D.  
Applied colloid chemistry. New York. McGraw-Hill.  
489 p. 1926b.
- Barnes, T. C.  
Salt requirements and orientation of Ligia in Bermuda.  
Biol. Bul. 69:259-268. 1935.
- Barnes, T. Cunliffe.  
Textbook of general physiology. Philadelphia.  
P. Blakiston's. 554 p. 1937.
- Bartell, E. S.  
Wetting of solid by liquid. In Colloid chemistry. Vol.  
III. Edited by Jerome Alexander. New York. Chemical  
Catalog Co. 655 p. 1931.
- Bayliss, William Maddock.  
Principles of general physiology. London Longmans, Green.  
650 p. 1915.



✓  
 Belehradek, J.

Protoplasmic viscosity as determined by a temperature coefficient of biological reactions. *Nature*, 118:478-480. 1926.

✓  
 Belehradek, J.

Sur la signification des coefficient de temperature. *Protoplasma*, 7:252-255. 1929.

Boiszevain, C. H.

Replacement of potassium by other elements in culture mediums. *Jour. Infect. Dis.* 43:194-199. 1928.

Brandwein, P. T.

The culturing of fresh water protozoa and other small invertebrates. *Amer. Nat.* 69:628-632. 1935.

Britten, Hubert Thomas Stanly.

Hydrogen ions. New York. Van Nostrand. 589 p. 1931.

Brown, R. H. J.

The protoplasmic viscosity of paramecium. *Jour. Expt. Biol.* 17:317-324. 1940.

Buchanan, R. E. and Fulmer, Ellis I.

Physiology and biochemistry of bacteria. Vol. I. Baltimore. Williams and Wilkins. 516 p. 1928.

Buchanan, R. E. and Fulmer, Ellis I.

Physiology and biochemistry of bacteria. Vol. II. Baltimore. Williams and Wilkins. 709 p. 1930.

Bull, H. B. and Gortnor, R. D.

Studies on electrokinetic potentials VI. Electrical phenomena at interfaces. *Colloid Symposium Monograph*, 8:309-330. 1931.

Burton, E. F.

Helmholtz double layer related to ions and charged particles. *Colloid Symposium Monograph*, 4:132-144. 1926.

Calkins, Gray N.

Biology of the Protozoa. Philadelphia. Lee and Febiger. 588 p. 1933.

Castellani, A.

Importance of symbiosis or close association of different species of organisms. *Amer. Med. Assoc. Jour.* 87:15-22. 1926.

Chambers, R.

Microdissection studies. I. The visible structure of cell protoplasm and death changes. *Amer. Jour. Physiol.* 43:1-12. 1917.

- Chambers, R. J.  
Changes in protoplasmic consistency and their relation to cell division. *Gen. Physiol.* 2:49-68. 1919.
- Chambers, R. and Reznikoff, P. J.  
Microsurgical studies in cell physiology. I. The action of the chlorides of Na, K, Ca, and Mg on the protoplasm of Amoeba proteus. *Jour. Gen. Physiol.* 8:369-401. 1925.
- Claff, C. L.  
A migration-dilution apparatus for the sterilization of Protozoa. *Physiol. Zool.* 13:334-344. 1940.
- Clark, W. Mansfield.  
The determination of hydrogen ions. Baltimore. Williams and Wilkins. 717 p. 1928.
- Cleveland, L. R.  
The separation of Trichomonas of man from bacteria. *Amer. Jour. Hyg.* 5:8-12. 1928.
- Clowes, G. H. A.  
Protoplasmic equilibrium. *Jour. Phys. Chem.* 20:407-451. 1916.
- Cramer, W.  
XVIII. On the biochemical mechanism of growth. The effect of sodium and calcium ions on the growth of a transplantable mouse carcinoma. *Biochem. Jour.* 12:210-220. 1918.
- Creighton, H. Jermain.  
Principles and applications of electro chemistry. Vol. I. London. John Wiley and Sons. Chapman and Hall. 502 p. 1935.
- Cunningham, E.  
On the fall of spherical particles through fluid medium. *Proc. Roy Soc. (A)* 83:357-365. 1910.
- Cutler, D. W. and Crump, L. M.  
The rate of reproduction in artificial culture of Colpidium colpoda. *Biochem. Jour.* 18:905-912.
- Dhar, N. R. and Chakravorti, D. N.  
Hydration and viscosity of sols in the presence of electrolytes. In *Colloid chemistry*. Vol. II. Edited by Jerome Alexander. New York. Chemical Catalog Co. 1029 p. 1928.
- Docking, A. R. and Heymann, E.  
Studies on the lyotropic series. II. The adsorption of salts on gelatin. *Jour. Phys. Chem.* 43:513-530. 1939.

- Erdmann, R. and Woodruff, L. L.  
The periodic reorganization process in Paramecium caudatum.  
Jour. Expt. Zool. 20:59-84. 1916.
- Estabrook, A. H.  
Effect of chemicals on the growth in paramecium. Jour.  
Expt. Zool. 8:489-534. 1910.
- Falk, I. S.  
The influence of certain salts on enzyme action. Jour.  
Biol. Chem. 36:229-247. 1918.
- Falk, I. S.  
The role of certain ions in bacterial physiology. Abs.  
Bact. 7:35-50, 87-103, 135-147. 1923.
- Fetter, D. J.  
Determination of the protoplasmic viscosity of paramecium  
by the centrifuge method. Jour. Expt. Zool. 44:279-283.  
1926.
- Freundlich, Herbert.  
Colloids and capillary chemistry. New York. E. P. Dutton.  
883 p. 1922.
- Fulmer, E. I.  
Colloids and the growth of microorganisms. In Colloid  
chemistry. Vol. II. Edited by Jerome Alexander. New  
York. Chemical Catalog Co. 1029 p. 1928.
- Fulmer, E. I., Nelson, V. E., and Sherwood, F. F.  
Jour. Amer. Chem. Soc. 43:191. 1921.
- Gause, G. F.  
Certain properties of the curves of toxicity. Protoplasma,  
17:548-553. 1933.
- Gelfan, S.  
The electrical conductivity of protoplasm and a new method  
of its determination. Univ. Calif. Pub. in Zool. 29:  
453-465. 1927.
- Glaser, R. W. and Coria, N. A.  
Methods for the pure culture of certain protozoa. Jour.  
Expt. Med. 81:787-806. 1930.
- Glaser, R. W. and Coria, N. A.  
The culture of Paramecium caudatum free from living micro-  
organisms. Jour. Parasitol. 20:33-37. 1933.
- Gortner, Ross Aiken.  
Outlines in biochemistry. London. Chapman and Hall.  
793 p. 1933.

- Greeley, A. W.  
Experiments on the physical structure of the protoplasm of paramecium and its relation to the reactions of the organism to thermal, chemical, and electrical stimuli. Biol. Bul. 7:3-32. 1904.
- Greenleaf, W. E.  
The influence of culture medium and cell proximity on the rate of reproduction in protozoa. Soc. Expt. Bio. and Med. Proc. 21:405-406. 1924.
- Guyer, M. P. and Claus, P. E.  
Recovery changes in transplanted anterior pituitary cells stratified in the ultra-centrifuge. Biol. Bul. 71:462-469. 1936.
- Hardy, W. D.  
A preliminary investigation of the condition which determines the stability of irreversible hydrosols. Proc. Roy Soc. Lond. 66:110-129. 1900.
- Hargitt, C. T. and Fray, W. W.  
The growth of paramecium in pure cultures of bacteria. Jour. Expt. Zool. 22:421-456. 1917.
- Harris, J. E.  
Studies on living protoplasm. I. Streaming movements in the protoplasm of the egg, Sabellaria alveolata. Jour. Expt. Biol. 12:65-79. 1935.
- Hauzer, E. A. and Hirson, S.  
The behavior of colloidal suspension with electrolytes. Jour. Phys. Chem. 43:1015-1036. 1939.
- Heilbrunn, L. V.  
Protoplasmic viscosity changes during mitosis. Jour. Expt. Zool. 34:417-446. 1921.
- Heilbrunn, L. V.  
The colloid chemistry of protoplasm. Amer. Jour. Physiol. 64:481-498. 1923.
- Heilbrunn, L. V.  
The colloid chemistry of the protoplasm. Colloid Symposium Monograph, 3:135-151. 1925a.
- Heilbrunn, L. V.  
The electrical changes of living cells. Science, 51: 236-237. 1925b.
- Heilbrunn, L. V.  
The centrifuge method for determining protoplasmic viscosity. Jour. Expt. Zool. 43:313-320. 1926a.



- Heilbrunn, L. V.  
The absolute viscosity of protoplasm. Jour. Expt. Zool.  
44:255-278. 1926b.
- Heilbrunn, L. V.  
Protoplasm in colloid chemistry. In Colloid chemistry.  
Vol. II. Edited by Jerome Alexander. New York. Chemical  
Catalogue Co. 1029 p. 1928.
- Heilbrunn, L. V.  
Protein lipid binding in protoplasm. Biol. Bul. 71:299-305.  
1936.
- Heilbrunn, L. V.  
An outline of general physiology. Philadelphia.  
W. B. Saunders. 550 p. 1937.
- Heilbrunn, L. V.  
Protoplasm and colloids. In the cell and protoplasm.  
Edited by Forest Ray Moulton. Washington, D. C. Science  
Press. 210 p. 1940.
- Heilbrunn, L. V. and Daugherty, K.  
The action of chlorides of sodium, potassium, calcium, and  
magnesium on the protoplasm of Amoeba dubia. Physiol.  
Zool. 4:635-651. 1931.
- Heilbrunn, L. V. and Daugherty, K.  
The action of chlorides of sodium, potassium, calcium,  
and magnesium ions on the plasmagel of Amoeba proteus.  
Physiol. Zool. 5:254-274. 1932.
- Hetherington, A.  
The sterilization of protozoa. Biol. Bul. 67:315-321.  
1934.
- Höber, R.  
Colloidal state and physiological function. In Colloid  
chemistry. Vol. II. Edited by Jerome Alexander. New  
York. Chemical Catalog Co. 1029 p. 1928.
- Holman, W. L. and Meekison, D. M.  
Gas production by bacterial synergism. Jour. Infect.  
Dis. 39:145-172. 1926.
- Hotchkiss, Margaret.  
Studies on salt action. VI. The stimulating and inhibi-  
tive effect of certain cations upon bacterial growth.  
Jour. Bact. 8:141-162. 1925.
- Jacobs, M. H.  
The production of intracellular acidity by neutral and  
alkaline solutions containing carbon dioxide. Amer. Jour.  
Physiol. 53:457-463. 1920.

- Jacobs, M. H.  
The influence of ammonium salts on cell reaction. Jour. Gen. Physiol. 5:181-188. 1922.
- Jahn, T. L.  
Studies on the physiology of the Euglenoid flagellates.  
I. The relation of the density of population to the growth rate of Euglena. Biol. Bul. 57:81-106. 1929.
- Jahn, T. L.  
Problems of population growth in the protozoa. Symposia on quantitative biology. 2:167-180. 1934.
- Jenny, H.  
Studies on the mechanism of ionic exchange in colloidal aluminum silicates. Jour. Phy. Chem. 36:2217-2258. 1932.
- Jenny, H.  
Simple kinetic theory of ionic exchange. Ions of equal valency. Jour. Phys. Chem. 40:501-517. 1936.
- Johnson, W. H.  
Studies on the nutrition and reproduction of paramecium. Physiol. Zool. 9:1-14. 1936.
- Kamada, T.  
Some observations on potential differences across the ectoplasm membrane of paramecium. Jour. Expt. Biol. 11:94-102. 1934.
- Kidder, G. W. and Stuart, R. T.  
Growth studies on ciliates. I. The role of bacteria in the growth and reproduction of Colpoda. Physiol. Zool. 12:329-336. 1939.
- Kidder, G. W.  
Growth studies on ciliates. V. The acceleration and inhibition of ciliate growth in biologically conditioned medium. Physiol. Zool. 14:209-226. 1941a.
- Kidder, G. W.  
Growth studies on ciliates. VII. Comparative growth characteristics of four species of sterile ciliates. Biol. Bul. 80:50-68. 1941b.
- King, R. I. and Beams, H. W.  
Effect of ultracentrifuging on paramecium. Soc. Expt. Biol. and Med. Proc. 31:707-708. 1934.
- Kopcegowski, Dr. W.  
Physics, chemistry and the colloidal state in colloid chemistry. Vol. I. p. 547-574. Edited by Jerome Alexander. New York. Chemical Catalog Co. p. 1926.

- Kruyt, H. R.  
The mechanism of coagulation. In Colloid chemistry. Vol. I. Edited by Jerome Alexander. New York. Chemical Catalog Co. 974 p. 1926.
- Lange, Norbert Adolph.  
Handbook of chemistry. Sandusky, Ohio. Handbook Publishers. 1543 p. 1939.
- Lewis, G. V. and Randall, M.  
Thermodynamics and the free energy of chemical substances. New York. McGraw-Hill. 653 p. 1923.
- Lindahl, P. E.  
Über animalisierte und vegetativsurte seogellaruem. Arch. entw-weck. Org. 128:661-664. 1933.
- Linder, S. E. and Ploton, H.  
IX. Solution and pseudo-solution. Part II, Some physical properties of arsenious sulfide and other solution. Jour. Chem. Soc. 67:63-74. 1895.
- Lipman, C. B.  
Toxic and antagonistic effects of salts as related to ammonification of Bacillus subtilis. 48:105-125. 1909.
- Loeb, J.  
Studies on the physiological effects of the valency and possibly the electrical charges of ions. Amer. Jour. Physiol. 6:411-433. 1902.
- Loeb, J.  
Ionic radius and ionic efficiency. Jour. Gen. Physiol. 2:673-687. 1920.
- Loeb, Jacques.  
Proteins and the theory of colloidal behavior. New York. McGraw-Hill. 380 p. 1924.
- Loefer, J. B.  
A simple method for maintaining pure line mass cultures of Paramecium caudatum on a single species of yeast. Trans. Amer. Micros. Soc. 55:255-256.
- Luck, J. M., Shoets, G. and Thomas, J. O.  
The role of bacteria in the nutrition of protozoa. Quart. Rev. Biol. 6:46-53. 1931
- Lund, E. E.  
A correlation of the silverline and neuromotor system of paramecium. Univ. Calif. Pub. in Zool. 39:35-77. 1933.

- McBain, J. W.  
The apparent viscosity of colloidal solutions and a theory of neutral colloids as solvated micelles capable of aggregation. *Jour. Phys. Chem.* 30:239-247. 1926.
- McCalla, T. M.  
Cation adsorption by bacteria. *Jour. Bact.* 40:23-32. 1940a.
- McCalla, T. M.  
Physico-chemical behavior of soil bacteria in relation to the soil colloid. *Jour. Bact.* 40:33-43. 1940b.
- Mast, S. O.  
Effect of salts, hydrogen ion concentration and pure water on length of life in Amoeba proteus. *Physiol. Zool.* 4:58-71. 1931.
- Mathews, A. P.  
The relation between solution tension, atomic volume, and the physiological action of the elements. *Amer. Jour. Physiol.* 10:290-323. 1904.
- Mathews, Albert P.  
The physical chemistry of protoplasm. In *Physiological Chemistry*. New York. William Wood. 1223 p. 1940.
- Michaelis, L.  
General principles of the effects of ions on colloids. *Colloid Symposium Monograph*, 2:1-15. 1924.
- Michaelis, L.  
Electrical phenomena in colloid chemistry. In *Colloid chemistry*. Vol. I. Edited by Jerome Alexander. New York. Chemical Catalog. 974 p. 1926.
- Myers, E. C.  
Relation of density of population and certain other factors to survival and reproduction in different biotypes of Paramecium caudatum. *Jour. Expt. Zool.* 49:1-43. 1927.
- Northen, H. T.  
Studies of protoplasmic structure in Spirogyra. I. Elasticity. *Protoplasma*. 31:1-8. 1938.
- Northen, H. T. and Northen, P. T.  
Effects of cations and anions on protoplasmic elasticity. *Plant Physiol.* 14:539-547. 1939.
- Oliphant, J. F.  
The effect of chemicals and temperature on reversal of ciliary action in paramecium. *Physiol. Zool.* 11:19-30. 1938.



- Osterheut, W. L.  
On the decrease in permeability due to certain bivalent kations. Bot. Gaz. 59:317-330. 1915.
- Osterhaut, W. L.  
Some aspects of selective adsorption. Gen. Physiol. 5:225-230. 1922.
- Osterhaut, W. L.  
Is living protoplasm permeable to ions? Gen. Physiol. 8:146. 1925.
- Ostwald, W.  
Über die Beziehungen zwischen adsorption und giftigkeit von salzlösungen für Süßwassertiere (Gammarus), 120:19-30. 1907.
- Page, I. N.  
The toxicity of monovalent and divalent cations for sea urchin eggs. Biol. Bul. 57:449-457. 1929.
- Parpart, A. K.  
The bacteriological sterilization of paramecium. Biol. Bul. 55:113-120. 1928.
- Peters, R. A.  
Nutrition of the protozoa. The growth of paramecium in sterile culture media. Jour. Physiol. 53: Proceedings. 1920.
- Peters, R. A.  
Substances needed for the growth of a pure culture of Colpidium colpoda. Jour. Physiol. 55:1-32. 1921.
- Peterson, W. A.  
The relation of density of population to rate of reproduction in Paramecium caudatum. Physiol. Zool. 2:221-254. 1929.
- Phelps, Austin.  
Effect of hydrogen ion concentration on the division rate of Paramecium aurelia. Science, 74:595-596. 1931.
- Phelps, Austin.  
Growth of protozoa in pure cultures. I. Jour. Expt. Zool. 70:109-130. 1935.
- Phelps, Austin.  
Growth of protozoa in pure cultures. II. Jour. Expt. Zool. 72:479-496. 1936.
- Phillips, R. L.  
The growth of paramecium in infusions of known bacterial content. Jour. Expt. Zool. 36:135-183. 1922.

- Powers, E. B.  
Influence of temperature and concentration on the toxicity of salts to fishes. *Ecology*, 1:95-112. 1920.
- Rees, C. W.  
The neuromotor apparatus of paramecium. *Univ. Calif. Pub. in Zool.* 20:353-364. 1922.
- Reznikoff, P.  
Microsurgical studies in cell physiology. II. The action of chlorides of Pb, Cu, Fe, and Al on the protoplasm of amoeba. *Jour. Gen. Physiol.* 10:9-21. 1921.
- Reznikoff, P.  
Microsurgical studies in cell physiology. The antagonism of cations in their cation on the protoplasm of Amoeba dubia. *Jour. Gen. Physiol.* 11:221-232. 1927.
- Reznikoff, P. and Chambers, R.  
Microsurgical studies in cell physiology. III. The action of CO<sub>2</sub> and some salts of Na, Ca, and K on the protoplasm of Amoeba dubia. *Jour. Gen. Physiol.* 10:731-738. 1929.
- Rice, O. K.  
Equilibrium in colloid systems. *Jour. Phys. Chem.* 30: 189-204. 1926.
- Robertson, T. B.  
The chemical basis of growth and senescence. Philadelphia. J. B. Lippincott. 389 p. 1923.
- Schmidt, C. L. A.  
The chemistry of the amino acids and proteins. Springfield. C. C. Thomas. 1031 p. 1938.
- Sears, H. J. and Putman, J. J.  
Gas production by bacteria in symbiosis. *Jour. Infect. Dis.* 32:270-280. 1923.
- Seifrig, W.  
Viscosity values of protoplasm as determined by microdissection. *Bot. Gaz.* 70:360-386. 1920.
- Seifrig, W.  
An elastic value of protoplasm with further observations on the viscosity of protoplasm. *Jour. Expt. Biol.* 2:1-11. 1924.
- Seifrig, W.  
The physical properties of protoplasm. In *Colloid chemistry*. Vol. II. Edited by Jerome Alexander. New York. Chemical Catalog Co. 1029 p. 1923.

- Seifrig, W. and Plowe, J.  
The effect of salts on the extensibility of protoplasm.  
Jour. Rheology 2:263-271. 1931.
- Seifrig, W. and Uraguchi, M.  
The toxic effects of heavy metals on protoplasm. Amer.  
Jour. Bot. 28:191-197. 1941.
- Speakman, H. B. and Phillips, J. F.  
A study of a bacterial association. The biochemistry of  
the production of lactic acid. Jour. Bact. 9:183-197.  
1924.
- Stiles, W.  
Viscosity of protoplasm as determining the rate of bio-  
logical reactions. Biol. Rev. 5:171-176. 1930.
- Taylor, C. V.  
Cataphoresis of ultramicroscopic particles in protoplasm.  
Sec. Expt. Biol. and Med. Proc. 22:533-556. 1925.
- Telkes, Maria.  
Bioelectrical measurements on amoebae. Amer. Jour. Physiol.  
93:475-483. 1931.
- Thornton, F. E.  
The viscosity of the plasmagel of Amoeba proteus at dif-  
ferent temperatures. Physiol. Zool. 5:246-253. 1932.
- Thornton, F. E.  
The action of sodium, potassium, calcium, and magnesium  
ions on the plasmagel of Amoeba proteus at different  
temperatures. Physiol. Zool. 8:246-254. 1935.
- True, R. H.  
The toxicity of molecules and ions. Proc. Amer. Phil.  
Sec. 69:231-245. 1930.
- Weiser, H. B.  
Ionic antagonism in colloid systems. Colloid Symposium  
Monograph, 4:354-373. 1926.
- White, T.  
The toxicity of copper nitrate solutions to Polycelis  
nigra. Protoplasma, 32:289-297. 1939.
- Winslow, C. E. A.  
The influence of cations on bacterial viability. Quart.  
Rev. Biol. 9:259-274. 1934.
- Winslow, and Dolloff, A. F.  
Relative importance of additive and antagonistic effects  
of cations upon bacterial viability. Jour. Bact. 15:67-92.  
1928.

- Winslow, C. E. A. and Fleeson, E. H.  
The influence of electrolytes upon the electrophoretic migration of bacteria and of yeast cells. *Jour. Gen. Physiol.* 8:195-214. 1925.
- Winslow, C. E. A. and Haywood, E. T.  
The specific potency of certain cations with reference to their effect on bacterial viability. *Jour. Bact.* 22:49-69. 1931.
- Woodruff, L. L.  
An experimental study on the life history of hypotrichous Infusoria. *Jour. Expt. Zool.* 2:585-632. 1905.
- Woodruff, L. L.  
The effect of excretion products of paramecium on its rate of reproduction. *Jour. Expt. Zool.* 10:857-881. 1911.
- Woodruff, L. L. and Bunzel, H. H.  
The relative toxicity of various salts and acids towards paramecium. *Amer. Jour. Physiol.* 25:190-194. 1909.
- Yocom, H. B.  
The effect of the quantity of culture medium on the division rate of Oxytricha. *Biol. Bul.* 54:410-416. 1928.



EXPLANATION OF PLATE I

Normal paramecium showing distribution of starch grains before centrifuging.

## Plate I



EXPLANATION OF PLATE II

Normal, six day old paramecium showing displacement of starch grains after centrifuging for six minutes at 1600 R. P. M.

## Plate II





EXPLANATION OF PLATE III

Paramecium showing displacement of starch grains after having been grown in M/70 solution of NaCl for six days and centrifuged for six minutes at 1600 R. P. M.

## Plate III



#### EXPLANATION OF PLATE IV

Paramecium showing displacement of starch grains after having been grown in M/70 solution of KCl for six days and centrifuged for ten minutes at 1600 R. P. M. In this paramecium the centrifugal force applied has caused the animal to begin to separate into two parts.

## Plate IV

