EFFECTS OF THE LIGHTEST MONOVALENT CATIONS ON *STENTOR COERULEUS* AND THE ISOLATION OF ITS DNA FROM A MIXED CULTURE

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CHAPTER I

INTRODUCTION

Stentor coeruleus, a ciliate protozoan, responds chemically, morphologically, and physiologically to changes in the chemical composition or radioactivity in its environment. Brief immersion of stentors in solutions of one percent or less of common ions causes a shedding of the pigment granules as a homogeneous blue green halo and the membranellar band (1-6). Sleigh (7) showed that some metallic ions effect the beating and coordination of the peristomial membranelles. A reduced internal viscosity of the protoplasm increases the beating of the cilia.

Tartar (5, 8) found that concentrations of one percent of certain solutions cause stentors to swim about in agitation, contract rapidly, and fimbriate their membranelles. The major portion of the membranellar band and a basement ribbon are usually cast off.

Child (9) demonstrated disintegration of the polar region of stentors in potassium cyanide. Tartar (5) observed similar responses in sodium iodide, potassium nitrate, ammonium chloride, strychnine sulfate, dextrose, urea, and sixteen other salt solutions and chemicals. There was some variation in the order with which the exfoliation of the peristome occurred. When stentors were left in solutions, the disintegration of the ectoplasm started with the frontal field and proceeded posteriorly. Disintegration often began also with the holdfast and moved towards the front in wavelike movement.

According to Heilbrunn (10) when stentors were centrifuged in monovalent ions--lithium, sodium, and potassium,--the endoplasm of Stentor coeruleus increased in viscosity and coagulated. When the ectoplasm of stentor was torn in sodium chloride or potassium chloride solutions, the wound opened and the endoplasm flowed out without any sign of coagulation. The reactions of divalent ions, such as calcium and strontium, were the opposite; that is, when the ectoplasm of stentor was broken, the wound opened and the exposed endoplasm clotted.

Tartar (8) further reports that lithium produces a coarseness of the cytoplasm and causes proteins to become coagulated, fibrillar, and stable. Chambers and Kao (11) microinjected calcium chloride and strontium chloride solutions into the interior of stentor and observed an endoplasmic clotting at the injection site. Both investigators verified that the two solutions had clotting effects on the endoplasm. The ectoplasm of stentor is neither affected by the immersion nor by the injection of the salt solutions.

When stentors were placed in lethal salt solutions for a short time and then returned to normal solutions, they regenerated the missing organelles normally. Lithium chloride had the opposite effect; stentors were unable to regenerate the missing organelles, probably because of the retention of the salt (5).

Lithium chloride also causes the cells to broaden and, in rare instances, forms doublets. This means that one stentor has two sets of feeding organelles and the two sets are separated by a lateral striping. Perhaps the most striking reactions produced by lithium chloride are the shedding of the peristome and differentiating oral primordium (5, 8, 12).

While lithium may influence the formation of doublets in Stentor coeruleus, it inhibits the formation of the oral region in Tetrahymena pyriformis, a protozoan (13). In a similar way as lithium chloride affects tetrahymena, actinomycin D, pruomycin, and cycloheximide prevent the completion of the development of Stentor coeruleus when it is treated in early stages. According to Burchill (14) actinomycin D inhibits the synthesis of ribonucleic acid while puromycin and cycloheximide inhibit protein synthesis.

Merton (15) reported that chloride solutions of monovalent ions--potassium, rubidium, cesium, sodium, and ammonium--induce reversal of the ciliary beatings. After inducing ciliary reversal, sodium chloride and potassium chloride produce partial anesthesia.

Low concentrations of potassium chloride solutions stimulate fission in Stentor. Sodium chloride of comparable strength produces instead an inhibition of division (2). Similar inhibition of fission in stentor is produced by X-rays. Kimball (16) reported that division by radiation is a secondary consequence of inhibiting growth and not a consequence of blocks in the specific morphogenetic processes required for division. Stentors fail to attain fission size or slowly achieve the maximum volume. Burchill and Rustad (17) delayed the regeneration of the missing feeding organelles by ultraviolet-microbeam irradiation of the regenerating stentors. Preece (18) irradiated stentors with gamma rays and reported an inhibition of cell division.

Extremely low concentrations of four cations are essential for stentor's survival. When stentors are not in their natural habitat, pond water, they require sodium, potassium, magnesium, and calcium in the medium. Optimum medium for raising stentors contained 0.15 mM $NaNO_3$,

0.15 mM K_2CO_3, 0.15 mM $MgSO_4$ and 0.55 mM $CaCl_2$ (2). Sleigh (7), de Terra (19), and Randall and Jackson (20) used different amounts of sodium as compared to potassium but in all three cases the amounts of sodium were much greater than potassium.

There are no records of separation and determination of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins in Stentor. Zech (21) studied the distribution of DNA in the macronucleus of Stentor coeruleus. However, methods are recorded for isolation and characterization of DNA and RNA in a number of protists. Marmur (22) and Mandel and Honigberg (23) isolated DNA from trichomonads. A given amount of nucleic acids has been isolated per cell from Tetrahymena. Scherbaum (24) isolated 13.6 picograms of DNA and 0.25 mμg of RNA per cell and Iverson and Giese (25) isolated 1.28 mμg of RNA per cell.

Large amounts of RNA from Amoeba proteus were isolated by James (27), Brachet (28), and Iverson (29) independently, but differed in the amount from 1.0 mμg to 6.8 mμg per cell. Iverson (29) also isolated 1.4 mμg per cell from Amoeba dubia. Maaløe (30) found that the rate of the total RNA synthesis is a function of growth conditions in synchronized bacterial cells. Buetow and Levedahl (31) reported a decline in the RNA content in Euglena gracilis during logrithmic growth. Since the amounts of tRNA, mRNA, and rRNA differ during the various stages of cell division, meaningful comparisons of the total RNA content of cells probably cannot be made except as a consideration of the role of the RNA species in protein synthesis. The general constancy of RNA to protein ratios has been illustrated by Brawerman and Shapiro (32). The total amount of RNA per cell is at least partially a function of the growth condition, state of ploidy, and probably of the genetic make-up.

It is preferable to isolate RNA and DNA from axenic cultures because there is no additional amount of DNA and RNA resulting from the mixed protozoa which serve as food. Cummins and Plaut (33) and later Mandel (26) showed that complications are occasioned by the holophagous ingestion of live protozoa. They found considerable difference between the average base composition of the RNA from mixed cultures and from cultures where the organisms were starved for several days. Thus, RNA and DNA are preferably isolated from axenic cultures but measurements of both nucleic acids can be obtained from individual cells even in mixed populations by quantitative cytochemical techniques (24-26, 28).

CHAPTER II

EXPERIMENTAL PROCEDURE

A culture of Stentor coeruleus inoculated with rotifers, paramecia, Vorticella, and several other protozoa was obtained from the General Biological Supply House, Chicago, Illinois. For additional foor, Colpidium were added as recommended by Heterington (34). Since the maintenance of abundant growth was vital, several methods were tried (8, 9, 35).

The growth rate, morphology, and movement of stentors in three different ionic solutions were studied in the first part of the experiment. Harden and Holland's (35) method was used. Stentors were maintained in gallon jars containing natural spring water from Eureka Springs, Arkansas, buffered to pH 7.4 with 5 mM Tris buffer. Besides the protozoa in the culture, skim milk was added to provide bacteria and more nutrients. About ten cooked wheat grains were added per three liters of culture. For the fission rate studies in the control, potassium, sodium, and lithium solutions, stentors were placed in Petri dishes which were coated with 3% agar. The agar environment served as a place of attachment for the organisms and also made it easier to count the stentors with naked eyes.

The second part of the studies is concerned with the isolation of DNA from stentor. Since spring water was not readily available, de Terra's (19) technique was used. The inorganic basal medium was

modified to be 0.55 m$\underline{M}$ $CaCl_2$, 0.15 m$\underline{M}$ $MgSO_4$, 0.75 m$\underline{M}$ Na_2CO_3, and 0.30 m$\underline{M}$ $KHCO_3$. The medium was made up in deionized water and adjusted to a pH 7.5 with 2 N HCl. Colpidium were grown in semi-stagnant conditions in 250 ml beakers on other smaller protozoa and added to fresh cultures of stentor. Cooked wheat grains were used for additional food. Cultures of stentor were maintained in large flat glass dishes with 800 ml of medium per dish. Containers were covered with Saran Wrap to prevent evaporation and provide means for exchange of gasses. All cultures were kept in the dark at 20° to 22°.

In both culture methods, subculture was accomplished by repeating this process as opposed to either the addition of nutrient material or to pouring off most of the medium and adding fresh water and wheat seeds. Dishes were subcultured whenever the organisms appeared small, unpigmented, or whenever vigorous growth had ceased, approximately every three weeks. Lack of pigmentation is an indication of sickly stentors. Usually additional colpidium and other protozoa were added weekly.

Nine millimolar medium was prepared by adding 25 ml of 18 m$\underline{M}$ solution to 25 ml of the conditioned medium in Petri dishes coated with 3% agar. Five organisms were placed in each dish containing 9 m$\underline{M}$ LiCl, 9 m$\underline{M}$ NaCl, or 9 m$\underline{M}$ KCl. The control medium contained one-half spring water and one-half conditioned medium.

Counts of the organisms were taken daily usually for two weeks either manually or on the New Brunswick Colony Counter. To study the acclimation of stentors to a medium, five stentors were taken from the two-week old medium and transferred to a fresh medium of the same

ionic strength. Counts of stentors were again taken daily from one to two weeks.

A comparative study of several ionic strengths of lithium, sodium, and potassium--0.1 M, 0.05 M, 0.018 M, and 0.009 M--was made on the movement and morphology of stentors. Observations were made using an Olympus Zoom Dissecting Microscope and the photomicrographs were taken with a Nikon M-35 Camera and Leitz Wetzler Phase Microscope or with a Brownie Star Camera and an Olympus Zoom Dissecting Microscope. Kodak Tri-X Pan Film was used in the Nikon M-35 Camera and Kodak Verichrome Pan Film in the Brownie Star Camera.

Sequential quantitative separation of RNA, DNA, and proteins by Shibko, Koivistoinen, Tratnyek, Newhall, and Friedman (36) was attempted. Samples containing from 1200 to 2400 organisms were counted manually and placed in 20 ml of either 9 mM NaCl, 9 mM LiCl, or 9 mM KCl. After 48 hours stentors were centrifuged in Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge for 20 min at 2000 rpm at 0°. After washing with fresh media, equal numbers of stentors were placed in 5 ml of 0.25 M sucrose. Lysis of cells and precipitation of proteins was accomplished with 70% perchloric acid.

DNA was determined by Burton's (37) diphenylamine method. Herring sperm DNA was used as a reference standard. The absorbance was measured at 600 nm on a Gilford-Beckman Spectrophotometer.

The orcinol method was used to characterize RNA (38) with yeast sRNA used as a standard. Absorbance was read at 660 nm.

Protein content was measured by the method of Lowry, Rosebrough, Farr, and Randall (19). Bovine serum albumin was used as a reference standard. Absorbance was measured at 700 nm.

Marmur's (22), Mandel and Honigberg's (23), Kirby's (40), and Mandel's (41) modified procedure was used to isolate and characterize the DNA from 20 to 25 mg (approximately 2500 to 3000 organisms) of Stentor grown with the variable conditions: starvation by transfer to filtered conditioned medium at 24, 48, and 72 hours; addition of 20 µg per ml of streptomycin sulfate; complete mixture from the culture; and making the culture 9 mM with lithium chloride for several hours prior to the isolation of the DNA. Concentrated complete mixture from the culture without the stentors was used as a control. After preparation, the DNA was refrigerated at 0° for several hours before the CsCl gradient analysis was employed.

Base compositions of the samples have been determined by the measurement of the bouyant density in CsCl (43). Beckman model E analytical centrifuge was used with 12 mm Kel F cells. Centrifugation in 5.7 molal cesium chloride established equilibrium in 24 hours at 42,040 rpm or in 22 hours at 44,770 rpm at 25°. The ultraviolet absorption photographs on Kodak professional film were traced with a Joyce-Loebl double-beam recording densitometer. Each cell included 1 to 2 µg of the DNA to be determined and .5 µg of standard DNA (bacteriophage 2C; density 1.742 g/cm^3) as a reference.

The buoyant density was calculated by the method described by Schildkraut, Marmur, and Doty (44) and by the DuPont 310 curve resolver. In the latter technique, the bands of the DNA were plotted on graph paper and the areas under the curves were determined by the DuPont 310 curve resolver. With the known percent of the areas under the curve, the distance from the centripetal end of the cell, and the

known amount of the standard DNA, the amount of the unknown DNA was calculated.

CHAPTER III

RESULTS

Effect of Li^+ on *Stentor* Growth

Low molar concentrations (0.1 - 0.009 M) of lithium chloride caused *Stentor coeruleus* to slough a light halo of blue green pigment granules. The organisms swam vigorously backwards with reversed ciliary movement. Next the cell contracted into a sphere and gyrated in all directions. As the motion decreased gradually, the cells elongated occasionally and broadened. The responses were similar in all the tested concentrations; however, the time and rate of reaction were proportional to the concentration. Since the 9 mM solution was the highest concentration conducive to the growth of the organisms without morphological effects, except for slight shedding of the pigment granules, it was used throughout the experimentation.

Figure 1 shows no difference in growth rate of stentors in 0.009 M lithium chloride and control cultures.

Effect of Various Cations

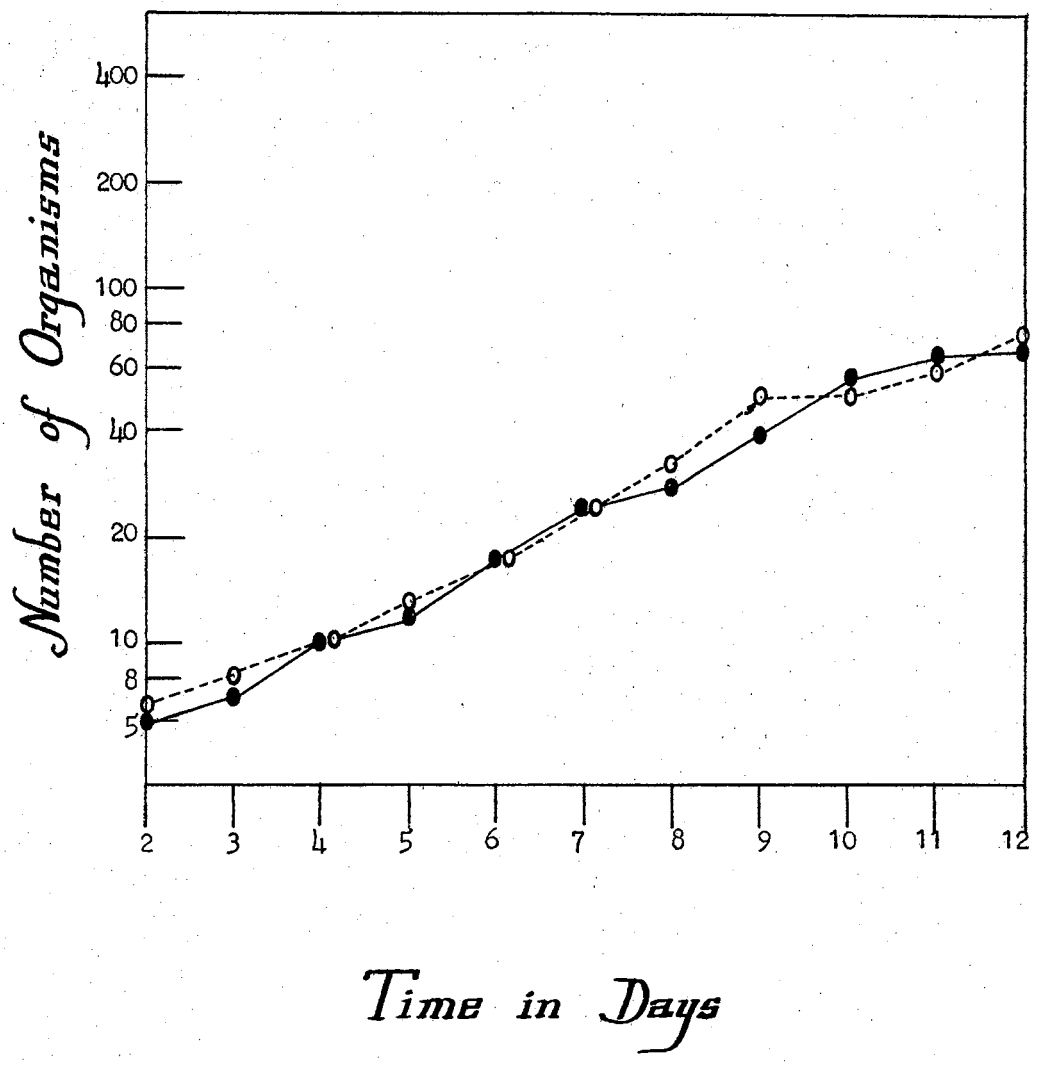
A question arose--are the lithium ions or chloride ions responsible for the shedding of pigment granules? Two other monovalent cations, sodium and potassium, with common chloride anions were tested and compared with the control and lithium solutions.

Figure 1

Average Division Rate of Stentor Coeruleus in Control and Lithium Chloride Solutions

Five stentors were inoculated in Petri dishes containing 50 ml of 9 mM LiCl solution and 50 ml of control medium. Counts of stentors were taken manually for twelve days. The average fission results of three experiments show no significant difference.

Li^+, O---- ; Control, ●—— .


Number of Organisms
400
200
100
80
60
40
20
10
8
5
2
3
4
5
6
7
8
9
10
11
12
Time in Days

Upon immersion in sodium solution, stentors sloughed large amounts of pigment granules and continued shedding the pigment granules in a thread-like form from the anterior region. Figure 2a illustrates this phenomenon. The cells usually rolled about slowly in a clockwise direction.

Stentors placed in a potassium environment shed a delicate halo of pigment granules and usually floated or moved slightly counter-clockwise. They gyrated in a vertical position and then with the holdfast attached strongly to the base of the container, twisted and tumbled gently from side to side. Finally they relaxed quietly as if anesthetized. Figure 2b shows a relaxed stentor about ten minutes after immersion in 0.1 M KCl. Again, in both sodium and potassium solutions, the time and rate of reaction of the organisms were dependent upon the concentration of the solution.

Effect of Ions on Growth Rate

Several experiments studied the growth rate of stentor in the control and three different ionic solutions. Figure 3 shows the average results of the five experiments of three ionic and control solutions. Sodium stimulated the growth rate while lithium and potassium were inhibitory.

After two weeks of daily observation, the cultures were left standing for ten days. The stentors in the sodium cultures continued to divide; those in the control cultures decreased to approximately one-half; less than one-fourth survived in the lithium cultures; no life was found in the potassium cultures. These results initiated the study of acclimation of stentors to ionic solutions. Stentors were grown for two

Figure 2

Changes in Stentor Coeruleus Caused by 0.1 M NaCl and 0.1 M KCl

Figure 2a shows stentor sloughing additional pigment granules in thread-like form from the anterior region in two drops of 0.1 M sodium chloride on a concave glass slide. Picture was taken 5 min after immersion in the sodium chloride solution. X 800.

Anesthetized stentor 10 min after immersion in 2 drops of 0.1 M potassium chloride on a concave slide (Figure 2b). X 800.

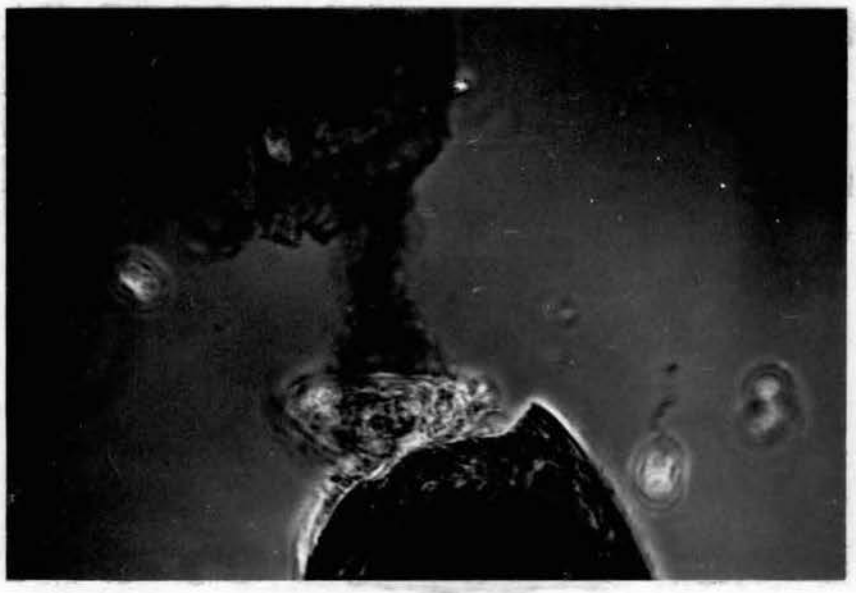


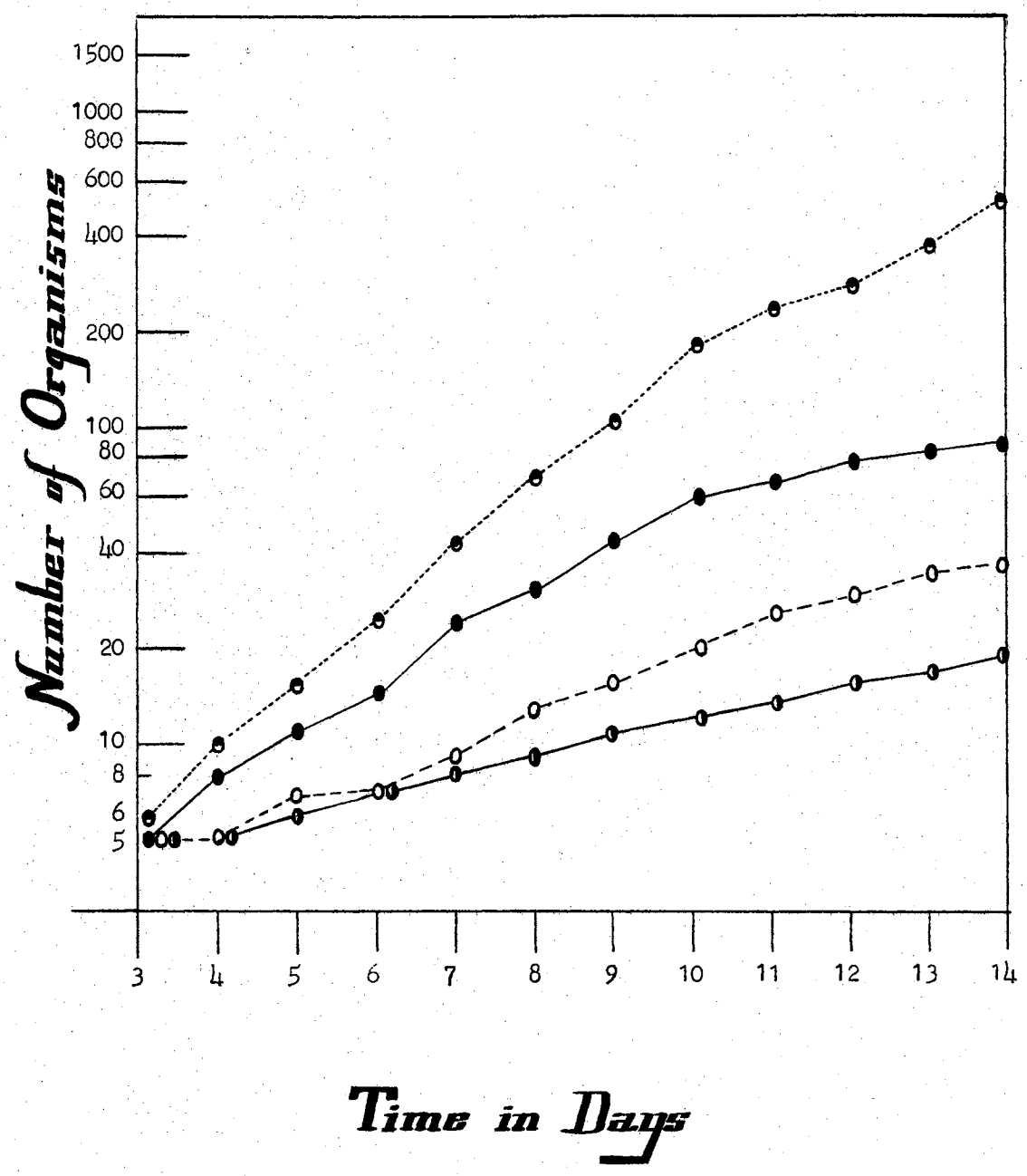
Figure 2a

Figure 2b

Figure 3

Results of the Rate of Division of Stentor Coeruleus in Control, Lithium, Sodium, and Potassium Solutions

Five stentors were inoculated in Petri dishes containing 50 ml of the respective media --- 9 mM LiCl, O----O; 9 mM NaCl, ●----●; 9 mM KCl, ●——●; and control, ●——●. The organisms were counted daily either manually or on the New Brunswick Colony Counter for two weeks. The average fission results of five experiments were recorded.


Number of Organisms
1500
1000
800
600
400
200
100
80
60
40
20
10
8
6
5
3
4
5
6
7
8
9
10
11
12
13
14
Time in Days

weeks in the respective four media and five stentors were inoculated into the corresponding fresh solutions. The average of the three experiments is given in Figure 4.

Morphological Observations

During the study on the acclimation of stentors to the ionic environments, there were pronounced changes in the morphology of most stentors in the lithium and potassium cultures. Stentors in the potassium cultures were deformed, inactive, large, and attached to the base of the container upside down. Several doublets were observed in the lithium cultures. The organisms in the sodium cultures were most active.

In the 30 individual cases studied in each of the three ionic solutions, the stentors in lithium chloride shed large amounts of pigment granules and began to swell vertically in about one-half minute after immersion. Stentors in potassium chloride environment shed a delicate halo of pigment granules and swelled after two minutes. Figure 5 shows the size of swelled stentors in a sodium chloride environment. In the process of swelling, over 25% of the potassium treated cells burst. This disintegration occurred instantaneously and uniformly in the entire cell, leaving an outline of the frontal field with the membranellar band floating for a few seconds intact. Before disintegration, the cells released tiny crescent particles and more pigment granules from the cytopyge area. These crescent particles were also resistant to the 0.1 M EDTA which was used in the isolation of DNA. Figure 6 illustrates the crescent bodies released by stentors in the potassium chloride environment. The extrusion of the crescent bodies has been observed occasionally in sodium chloride (Figure 6c) and rarely in lithium chloride.

Figure 4

Average Results of the Number of Organisms in Control, 9 mM Lithium, Sodium, and Potassium Solutions in the Three Experiments on Acclimation of Stentor

Stentors were grown for two weeks in 9 mM LiCl, O----O; 9 mM NaCl, ●·····●; 9 mM KCl, ●——●; and control, ●——●; and five stentors were inoculated into the corresponding fresh solutions. Organisms were counted daily for two weeks, either manually or on the New Brunswick Colony Counter. The average fission results of three experiments were recorded.

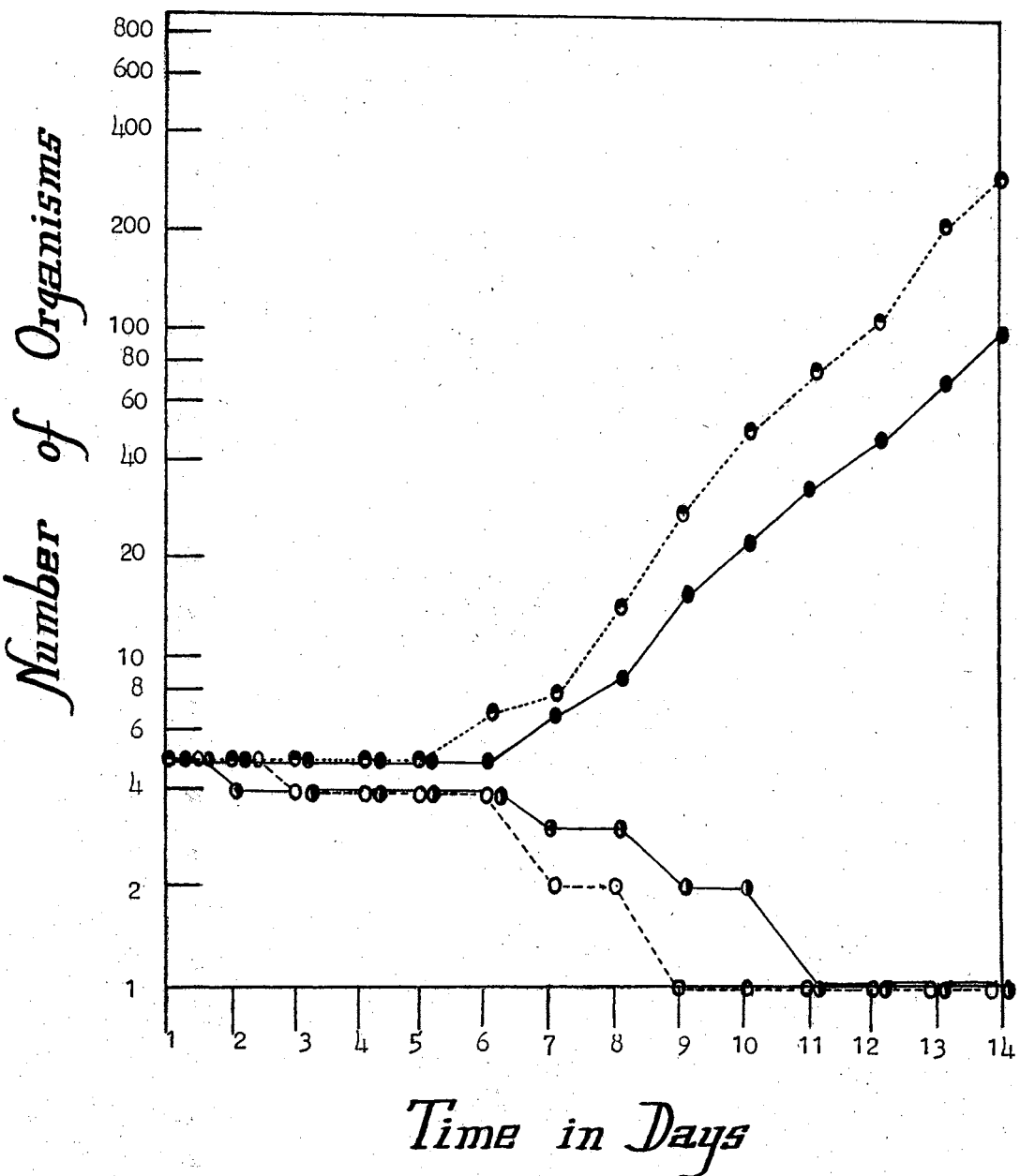

Number of Organisms
800
600
400
200
100
80
60
40
20
10
8
6
4
2
1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Time in Days

Figure 5

Comparison of Stentor's Sizes in 0.1 M Solutions of Lithium, Sodium, and Potassium

Figure 5a and 5b show the broadening of the cells nine minutes after immersion into 2 drops of 0.1 M LiCl and 0.1 M KCl. X 800. Figure 5c shows the size of stentor under similar conditions in 0.1 M NaCl four minutes after immersion and shortly before death. X 800.

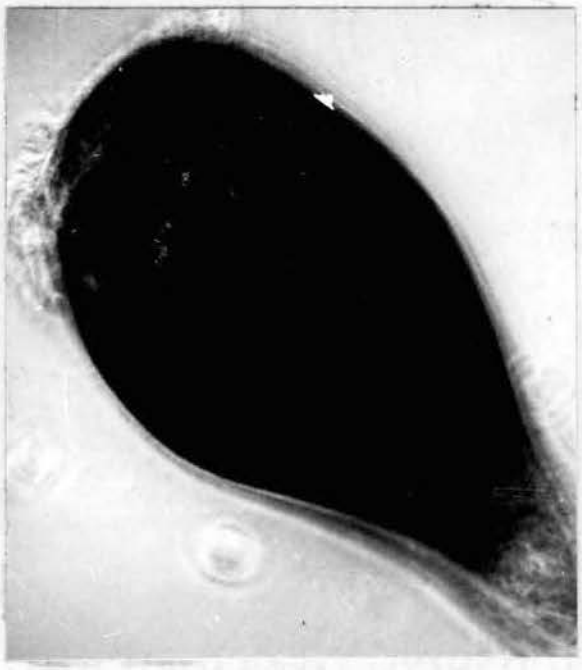


Figure 5a

Figure 5b

Figure 5c

Stentors in sodium chloride solution shed a light halo of pigment granules and continued sloughing the pigment granules in threadlike form (refer to Figure 2a); there was no broadening of cells in sodium chloride. Only one cell burst in its middle area in the lithium chloride and two cells disintegrated in the sodium chloride.

Disorientation of the pigment rows began in the anterior region and in many cases the frontal disorientation was followed by a corresponding reaction from the holdfast. When stentors were treated with potassium chloride, the disorientation of the pigment lines began by a break in the membranellar band.

Some stentors survived as long as 20 min in a 0.1 M KCl; 12 min in 0.1 M LiCl and 10 min in 0.1 M NaCl. The average time of the 30 stentors' death, the region of disorientation of the pigment rows and disintegration are given in TABLE I.

Base Composition of Stentor DNA

DNA was isolated by disrupting the cells and eliminating proteins and other components. After centrifugation the DNA was removed from the top layer and an equal amount of 95% alcohol was added. A small spool of delicate strands of DNA was obtained and suspended in 70% alcohol. Before refrigeration, the DNA was suspended in 0.1 N sodium citrate and a drop of chloroform.

The absorbency was measured on the Zeiss spectrophotometer to determine the amount of DNA and then the buoyant density of this unknown DNA and the known bacteriophage 2C DNA was measured in 5.7 molal CsCl. After 20 hrs of centrifugation at 44,770 rpm or 22 hrs at 42,040 rpm in the Model E analytical centrifuge, photographs were taken and the films

Figure 6

Crescent Bodies Released from Stentor's Anterior Area

Figure 6a (X 800) shows the crescent bodies which were released by stentor 2 min after immersion in 2 drops of 0.1 M KCl. This reaction of extruding large amounts decreased with time. Figure 6b (X 800) shows stentor releasing a few crescent bodies after 12 min.

This phenomenon has been observed occasionally in sodium chloride solution of the same concentration and rarely in lithium chloride. Figure 6c (X 840) shows stentor 2.5 min after immersion in 4 drops of 0.1 M NaCl solution.

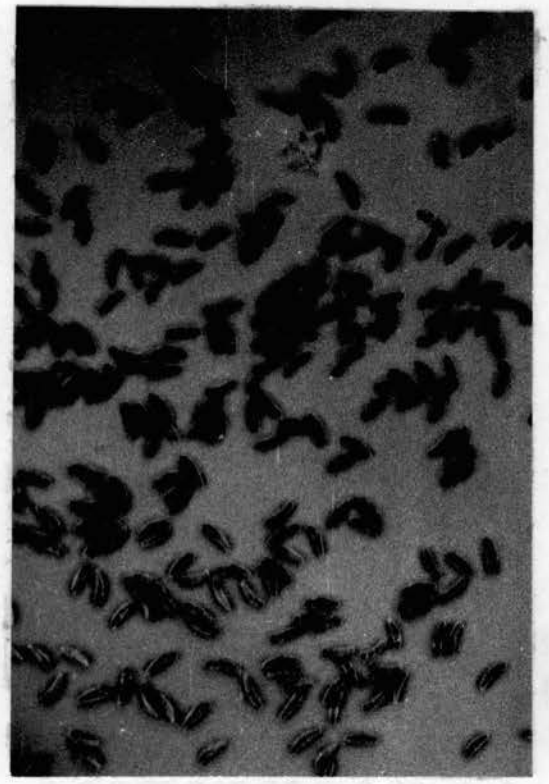


Figure 6a

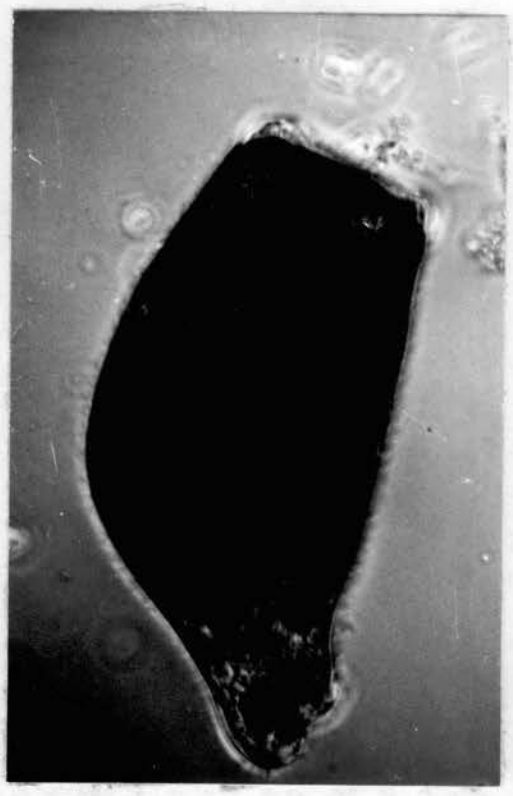


Figure 6b

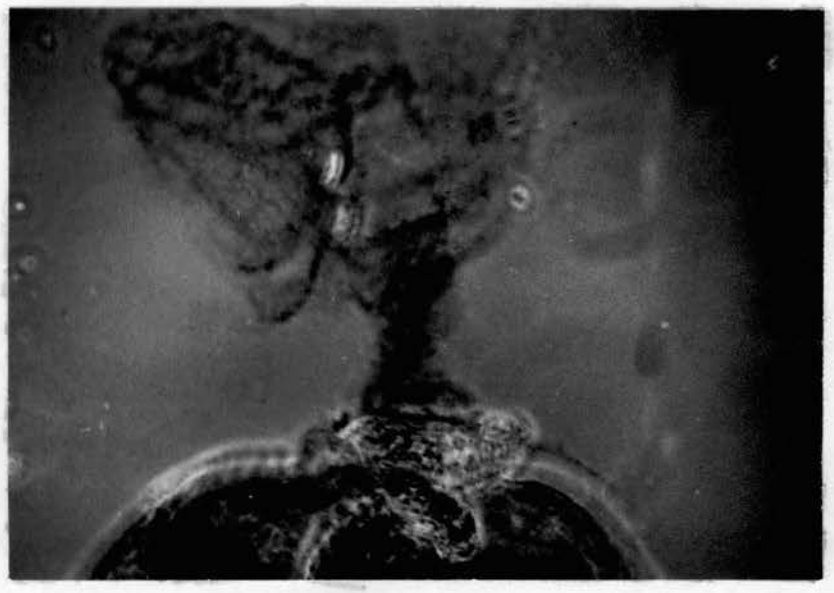


Figure 6c

TABLE I

SOME EFFECTS ON STENTORS OF 0.1 M SOLUTIONS OF LITHIUM CHLORIDE, SODIUM CHLORIDE, AND POTASSIUM CHLORIDE

	REGION OF DISORIENTATION OF THE PIGMENT ROWS		COMPLETE DISINTEGRATION	AVERAGE TIME OF DEATH
	Anterior	Posterior and Anterior		
LiCl	6	23	1	6.2 min
NaCl	25	3	2	4.5 min
KCl	13	9	8	12.5 min

Each time an experiment was conducted, all stentors were taken from the same culture. Deeply pigmented stentors were taken at random; no attention was given to the stage of fission.

The time of death of each stentor was taken with a stopwatch and the average was calculated for the thirty stentors in the respective 0.1 M solution.

were developed. The films of seven samples indicated two DNA bands. The darker band was the bacteriophage 2C DNA band of known density, 1.742 g/cm^3; the lighter band which occurred consistently was assumed to be the DNA of stentor. A sample of concentrated complete mixed culture from which stentors were removed was used as a control. In this complete mixed culture four bands appeared.

Tracings were made by the Joyce-Loebl double-beam recording microdensitometer. The densities were calculated using the position of the bacteriophage 2C DNA (1.742 g/cm^3). The density of the unknown samples was 1.692 g/cm^3 (TABLE II). The densities of the four bands in the concentrated complete mixed culture sample without stentors were calculated to be 1.724, 1.716, 1.703, and 1.695 grams per cubic centimeter.

TABLE II

THE BUOYANT DENSITY OF STENTOR DNA AND THE VARIABLES USED IN THE PREPARATION OF THE CELLS

SAMPLE NO.	VARIABLE	BUOYANT DENSITY in g/cm^3	
1	Complete Mixture from the Culture	1.715	1.701
2	Washed and Starved for 24 hrs	1.692	
3	Washed and Starved for 44 hrs	1.692	
4	Washed and Starved for 48 hrs	1.692	
5	Washed and Starved for 72 hrs	1.693	
6	Added Streptomycin Sulfate 20 µg/ml for 72 hrs	1.692	
7	Prepared in 9 mM LiCl	1.692	

The base composition, moles % guanine plus cytosine, as calculated from the buoyant density is about 33%.

In sample 8 concentrated mixture from the culture without stentors was used as a control. The four densities were calculated to be 1.724, 1.716, 1.703, and 1.695 grams per cubic centimeter.

CHAPTER IV

DISCUSSION

Fission curves of the control and the ionic solutions reveal that a 9 mM sodium chloride solution accelerates fission in Stentor coeruleus. Lithium chloride and potassium chloride of comparable strengths suppressed division (Figure 3). Although there is experimental variation, a comparison of the average of the five experiments indicates that:

1. Stentors divide more rapidly in the 9 mM NaCl, followed by the control, 9 mM LiCl and 9 mM KCl;
2. After a lag, the number of stentors usually doubled after the third day;
3. The division after the fourth day usually increased consistently until the fourteenth day in the respective solutions.

Peters (2) found that within the range of 0.01 M potassium chloride solution, fission in Stentor coeruleus was stimulated and sodium chloride of similar concentrations suppressed division. He conducted his experiments between 6 and 48 hours and considered the process of division to be the maximum during this initial time. Tartar (8) comments that Peters' experiments merely left the suggestion that possibly potassium chloride may supply an impulse to division. Experiments carried out for one week at a time in 1.2 mM lithium chloride solutions indicated slight acceleration in the division of

Stentor coeruleus (45). The first part of the present study with the 9 mM LiCl and control solutions showed no significant difference in the division rate (Figure 1).

As in other protozoa, division in Stentor is usually preceded by growth to a definite size. But there is no comprehensive understanding of growth in the individual stentor and no reports occur in literature. Tartar (8, 46) observed that the number of granular and clear stripes increases with the size of the organism. He also observed the visible changes during cell division and grouped them into eight stages. He reported that the maximum size is about 2 mm in an extended form and 0.7 mm in a swimming or contracted form.

The growth and fission rates are dependent on several factors; namely, other smaller protozoa in the culture, additional food nutrients, a definite ration of certain minerals, pH, temperature, amount of light, length of time in a medium, and other factors. There must be an ecological balance attained between these factors to provide a habitat conducive to the growth and fission of stentors. This point can be illustrated in all the experiments because it took an average of four days for all five stentors to divide. Even when stentors became acclimated to the 9 mM concentrations for two weeks and were transferred to fresh media of the same concentration, it took five days before fission began in the sodium chloride and the control solutions. Stentors in the potassium chloride and lithium chloride did not divide (Figure 4).

According to Peters (2), Daniel (3), and Tartar (8), stentors can acquire appreciable tolerance to 0.001% $HgCl_2$, acids and bases, glycerine, and alcohols of low concentrations. Stentors became acclimated

to dilute solutions and were able to withstand lethal concentrations for several hours longer than stentors grown under normal conditions. All experiments on acclimation reported thus far lasted only for a few hours; the results in this experiment show that stentors can become acclimated to a 0.01 and 0.009 M sodium chloride and remain in this environment successfully for 28 days.

The three different monovalent cations have both similar and different effects on the movement and morphology of stentor. Stentors in lithium and potassium ions broaden. The broadening of cells in lithium chloride is accompanied by an increase of kinety and, therefore, a considerable multiplication of cilia and related structures. Tartar (5) observed not only the broadening of the cells in the sub-lethal concentrations of lithium chloride, but also a partial or complete inhibition of the development of the oral primordium. This effect can be compared to the results of lithium ions on higher forms of animal embryos living in water. Reports reveal that the effects of lithium ions on a developing embryo supress the development of the animal pole and increase the development of the vegetal pole (47-52).

The broadening of stentor in 0.1 M KCl is slow; as the cell elongates (Figure 2b), it appears to be anesthetized. Merton (15) reported that potassium chloride and sodium chloride produce partial anesthesia of the cilia. In the present studies, the entire cell relaxed and became inactive in the potassium chloride; 25% of the cells broadened and then burst. Stentors in the 0.1 M NaCl retained the general morphological structure as they shed a light halo of pigment granules from the cytopharynx. The differences in the resistance of the ectoplasm to the three different cations and the disorien-

tation of the pigment rows are recorded in TABLE I.

DNA was isolated from Stentor coeruleus and the buoyant density and the guanine (G) -cytosine (C) contents were measured. In spite of the varied physiological differences, the buoyant density of five samples remained constant. TABLE II shows the variables which were used and the buoyant density of Stentor coeruleus to be 1.692 g/cm^3. The concentrated mixture from the culture which was used as a control, indicated four densities: 1.724, 1.716, 1.703, and 1.695 g/cm^3. Since none of the control DNA buoyant densities compared to the constant buoyant density, 1.692 g/cm^3, of the stentor samples, it was concluded that the buoyant density of Stentor coeruleus' DNA is 1.692 g/cm^3.

Densities were calculated using the position of a standard bacteriophage 2C DNA as a reference. Its density is taken to be 1.742 g/cm^3. This known DNA banded in a position outside the region of the density gradient in which most DNA samples band, and can be used for most purposes without fear of masking the presence of unexpected satellite bands. The GC content was obtained from the linear relation of Schildkraut, Marmur and Doty (44):

$$(GC) = \frac{\rho - 1.660 \text{ g/ml}}{0.098},$$

where (GC) = mole fraction of guanine plus cytosine in native DNA and ρ = buoyant density in g/cm^3. Stentor has 33% guanine plus cytosine.

Stentor coeruleus has a unimodal distribution of DNA molecules. There is no evidence of satellite components such as reported in protozoa by Schildkraut, Mandel, Levisohn, Sonneborn-Smith and Marmur (53). This technique shows that DNA can be isolated from organisms grown in cultures with mixed populations.

CHAPTER V

SUMMARY

The division of Stentor coeruleus is accelerated in a 9 mM NaCl and suppressed in LiCl and KCl of the same concentration. The organisms became acclimated and continued to divide in the sodium chloride solution but were unable to acclimate and divide in potassium chloride and lithium chloride.

All these monovalent cations have pronounced effects on the movement and morphology of stentor. The most conspicuous is the broadening of the cells in 0.1 M LiCl and 0.1 M KCl; the anesthetizing effect of KCl; and the abundant shedding of the pigment granules in two forms in 0.1 M NaCl.

The DNA of Stentor coeruleus was isolated from a mixed population culture. The CsCl density gradient analysis showed the density of Stentor coeruleus' DNA to be 1.692 g/cm^3 and the GC content, 33%.

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