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Comparative Behavioral Studies Between Ciliates. Ong

Comparative Behavioral Studies Between a Parasitic and a Free-living Ciliate

by

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CONTENTS

Page

I. Introduction----------1

II. Materials and methods----------14

III. Some observations on culturing Paramecium in hay infusion----------20

Discussion----------26

IV. Some observations of Opalina's habitat----------29

A survey of the incidence of parasites in the rectum of the frog Rana pipiens from the province of Quebec----------34

Discussion----------41

V. Comparison of behavior in several experimental media

Brief introduction----------44

Precautions and methods----------45

Results and observations----------49

1. Frog-Ringer solution
2. Pütter solution
3. Lactalbumin
4. Gastric mucin
5. Hay infusion
6. Distilled water
7. Tap water

Discussion----------76

Page

VI. Comparison of behavior under different physical conditions

1. Reaction to gravity----------84

Brief introduction

Experiments and observations

2. Reaction to light----------89

Brief introduction

Experiments and observations

Discussion----------93

3. Reaction to heat----------97

Brief introduction

Precautions and methods

Results and observations

4. Reaction to cold----------111

Brief introduction

Precautions and methods

Results and observations

Discussion----------120

VII. Summary----------125

VIII. Bibliography----------131

LIST OF FIGURES

Page

1. Group of Opalina obtrigonoidea showing diversity of form and size in a single infection----------4
2. Descriptive diagram of Opalina obtrigonoidea showing characteristic shape, cilia and nuclei----------6
3. Descriptive diagram of Paramecium caudatum (after Wichterman)----------9
4. Sandon's apparatus for concentrating and cleaning ciliates----------17
5. The growth of Paramecium caudatum as related to the hydrogen-ion concentration in culture medium measured at intervals----------22
6. Different rate in the pH changes of a concentrated and a dilute hay infusion medium----------24

7a,b Cross-sections of the anterior portion of a rectum infected by Opalinas. The relative position of the Opalinas in the rectum is also clearly shown----------30

8. A portion of the middle cross-section of same rectum showing a heavier infection by Opalinas---------31
9. Frequency of occurrence of different parasites in frog Rana pipiens----------38
10. Un-identified parasites found in the rectum of frog Rana pipiens----------40

Page

11. Irregular division of Opalina obtrigonoidea observed in one of the Frog-Ringer solution plus rectal wall-53

12. Some of the visible morphological changes of Paramecium caudatum in Frog-Ringer solution----------54

13. Paramecium caudatum

a. The short and narrow spiral path

b. The long and wide spiral path

c,d. The distorted shape accompanied by considerable narrowing

e. The disintegration into a shapeless mass---------59

14a,b. Opalina obtrigonoidea The swelling of anterior end and subsequent pointing of the posterior end

c-g, Paramecium caudatum The gradual rounding-up accompanied by the formation of large vacuoles in the protoplasm. Finally, the cell membrane bursts.----------62

15. Opalina obtrigonoidea

a-c, Formation of blister in the anterior region

d-g, The rounding-up of the cell accompanied by the formation of large vacuoles in the protoplasm----65

16. The typical course of death and disintegration occurs in Opalina obtrigonoidea----------70

17. The typical course of death and disintegration occurs in Paramecium caudatum----------73

Page

18. a-g, Blister formation in Paramecium caudatum-------75

19. a-e, Reaction to gravity----------86

20. a-c, Reaction to light----------91

21. Wiring diagram of a simple wheatstone bridge-------103

22. Resistance v temperature characteristic Type F1512/
300 thermistor----------105

23. Resistance v temperature characteristic Type F2200
thermistor----------112

LIST OF TABLES

Page

1. Measurement of Opalinas from two different populations----------33
2. Frequency of occurrence of Opalina infection in frog *Rana pipiens* Schreber of Quebec, Canada----------35
3. The number and percentage of the total number of frogs infected by each of the different types of parasites-37
4. A comparison of the longevity of Opalina and Paramecium in several experimental media----------49
5. A comparison of the relative tolerance of Opalina and Paramecium at various temperatures----------107
6. The ability of *Opalina obtrigonoidea* to recover in prolonged cold-exposure after all movements have ceased----------114
7. The ability of *Opalina obtrigonoidea* to recover when exposed to frozen medium----------116
8. The ability of *Paramecium caudatum* to recover when exposed to frozen medium----------117

INTRODUCTION

Nearly three hundred years ago, the protozoa-both free-living and parasitic were first seen by the father of protozoology, Antony van Leeuwenhoek. Ever since various biological studies and experiments have been carried out on this fascinating "little animal." Knowledge about them increases more and more with the advance of microscopy as well as various research techniques and other scientific apparatus. An examination of the previous literature shows that extensive work has been done on the behavior of free-living protozoa, especially Amoeba and Paramecium; the literature on the behavior of parasitic protozoa is comparatively limited. As a matter of fact, the writer has failed to find any other literature that gives a comparison of the general behavioral characteristics of both, with the exception of the one reported by Swezey and Atchley (1935) working on Paramecium (free-living) and Troglotella abrassarti, Balantidium coli (parasitic).

The present investigation involves studies of the comparative behavioral characteristics of a free-living and a parasitic ciliate. The purpose of this study is to find out whether the parasitic ciliate reacts to all stimuli in a manner similar to that of a free-living one. Paramecium, being the elite of protozoan society, is used as the representative of the free-living one; Opalina is

used as representative of the parasitic one because they are easily procurable in large numbers. As is generally known, the work on Paramecium is voluminous. The work on Opalina, was mostly done by European investigators, especially by M. M. Metcalf (1909-1940). The anatomy, life cycle, galvanotaxis, chemotaxis, longevity and continuous culture of Opalinas have been studied to a greater or lesser degree by these earlier investigators. Most of the works have been done on Opalina ranarum and Opalina obtrigona. In the present study, some of the works of the earlier investigators are being repeated at length for the purpose of re-examination and the observations are reviewed in the light of their relationship to earlier workers.

Through out these investigations, Paramecium caudatum and Opalina obtrigonoidea have been used exclusively. Identification to species was based chiefly on body shape and size, and the number and size of the nuclei. The identification of Paramecium caudatum was made by consulting the description given by Wenrich (1928), and of Opalina obtrigonoidea by Metcalf (1923), and of Rana pipiens Schreber by Wright (1949).

Opalina obtrigonoidea is a multinucleate ciliate found in the rectum of frog. It was first seen by Leeu-

wenhoek in 1683 and first described by Metcalf in 1923. The generic name Opalina was first used by Purkinje and Valentin in 1835.

Opalina is almost transparent when studied. Its form shows a great deal of diversity in different infections as well as in a single infection (Fig.1). The Opalinas used in the present experiment are characterized by a flattened triangular shape. It is about 160-325 microns in length. The size varies with the age; it is not uncommon to find one less than 160 microns or much more than 325 microns. The size of the Opalinas used in all experiments is approximately 230-250 microns in length. The entire body is covered by a coat of uniform cilia arranged in oblique lines. Their successive waves are easily observed as the animal swims forward. These cilia are found to be more numerous at the anterior end than in the posterior end. In its active movement, the broader anterior end is always directed forward. It usually swims on a flat side, but may rotate in a spiral course, first in one direction and then, after a hesitation, in the other. The cytoplasm is distinctly divided into a clearer ectoplasm and darker endoplasm. The numerous nuclei are small and mostly spherical. They are scattered throughout the body and are plainly seen as small hyaline spheres which are comparatively more at the

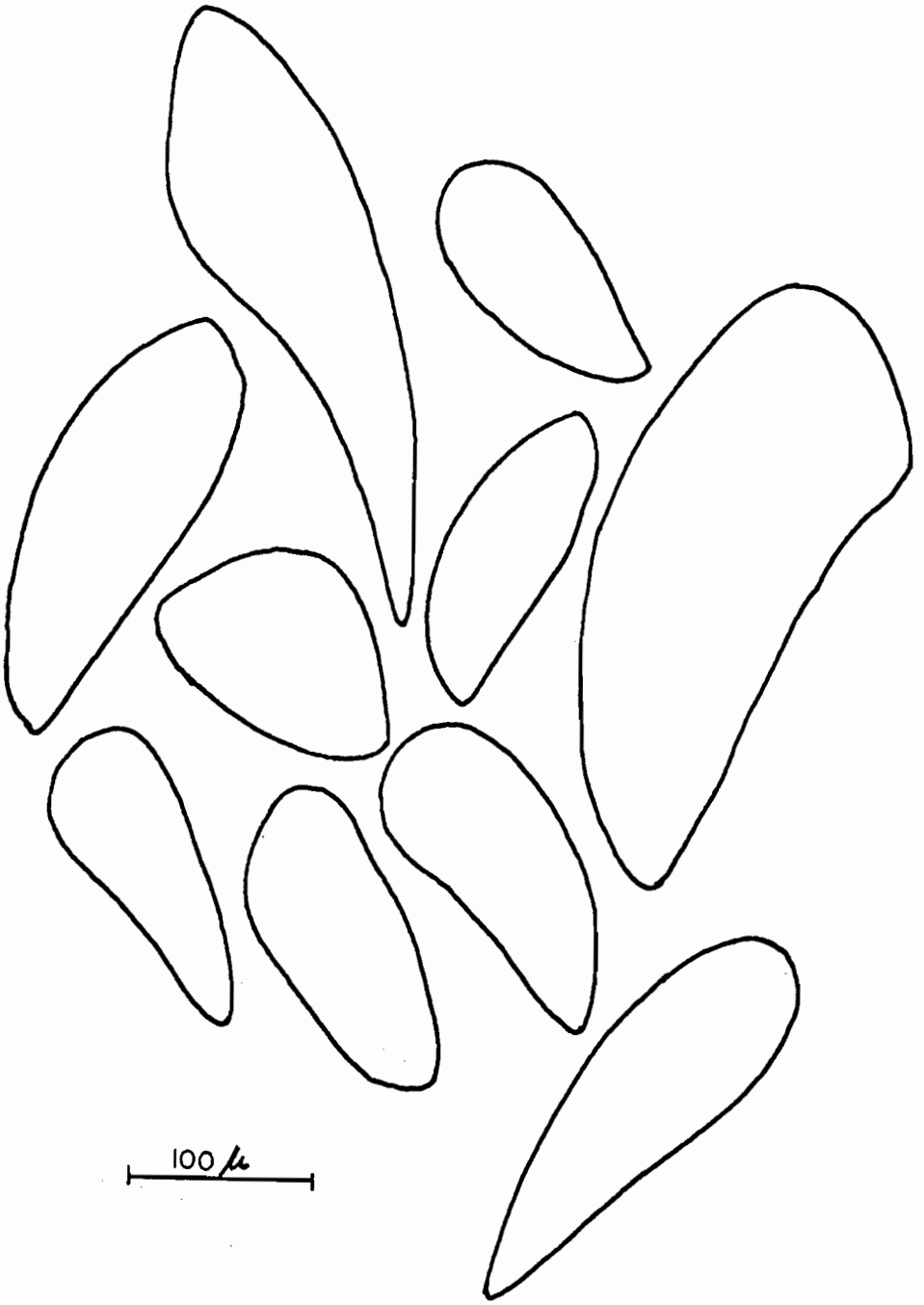


Fig. 1

Group of Opalina obtrigonoidea showing diversity of form and size in a single infection

anterior end than in the posterior end. There is no mouth, since food is obtained by absorption over the entire body surface (Fig.2). The animals reproduce in the intestine of the frog only asexually by binary fission, throughout the year, with the exception of the spring. When the frog begins to reproduce, Opalinas also begin to prepare for sexual reproduction. They encyst and cysts are shed into the water, to be swallowed by small tadpoles. Within the tadpoles, Opalinas divide by multiple fission, forming very small gametes, which then conjugate. Another species, Opalina carolinensis, is also found in Rana pipiens. It is differentiated from Opalina obtrigonoidea by its acute posterior end either tapering to a sharp point or abruptly acuminate.

For the first account of Paramecium, we are also indebted to Leeuwenhoek, the discoverer of protozoa. It is believed that he first saw them as early as 1674 and 1677. Hill (1752) was the first to apply scientific names to microscopic animals and was the first to use the genus name, Paramecium. The recognition of the species caudatum is usually credited to Ehrenberg. In 1833, he not only described the species, but also made the first attempt to distinguish it from Paramecium aurelia on the basis of the shape of the posterior end. Fifty-five years later, Maupas presented

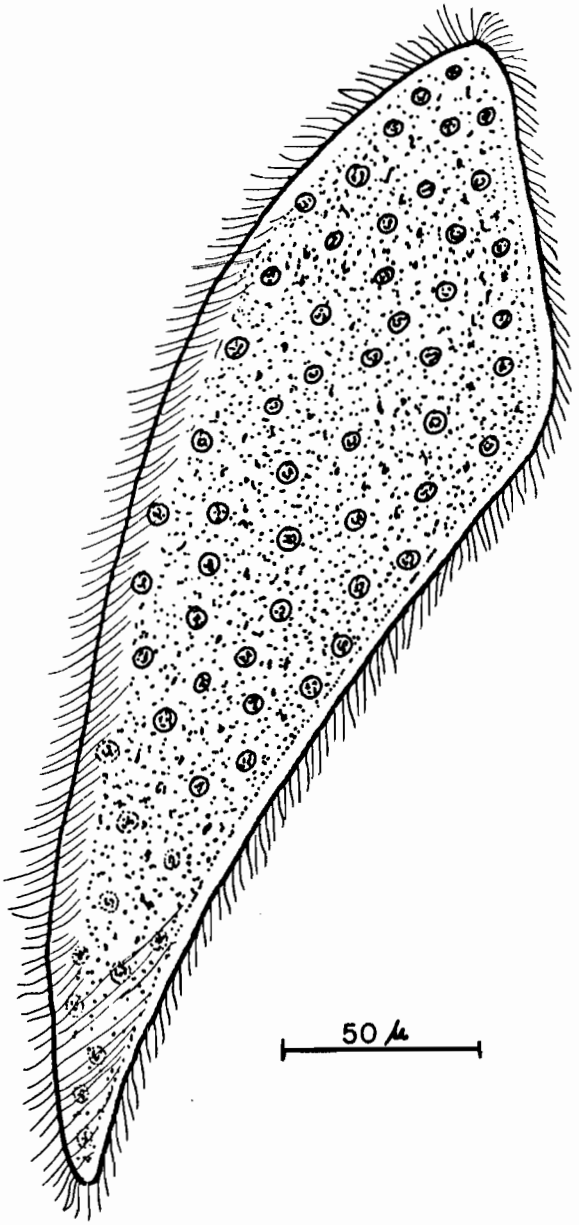


Fig.2

Descriptive diagram of Opalina obtrigonoidea showing characteristic shape, cilia and nuclei

a more accurate description than ever before, with emphasis being placed on the internal structure of Paramecium. He was the first man to notice the different sets of nuclear structures present in these two species.

Paramecium caudatum is a "slipper-shaped" animal with a bluntly rounded anterior end and a somewhat pointed posterior end, appearing a light gray or white minute body when seen by the naked eye. It measures commonly between 160-275 microns; the animals used in the present experiment were approximately 190-220 microns. The entire body is covered by rows of cilia of fairly uniform length except those at the posterior tip of the body which are considerably longer. During its active movement, the bluntly round anterior end is usually directed forward and the animal always swims in spirals when passing freely through the water. This species spirals characteristically to the left. Like Opalina, its cytoplasm is also distinctly divided into a clearer ectoplasm and a darker endoplasm. But unlike Opalina, it has two different types of nuclei; a large compact ellipsoidal-shaped macronucleus and an ovoid micronucleus located close to it. The nuclei are generally found slightly anterior to the middle of the body. It also differs from Opalina by having a broad shallow

obliquely directed oral groove which leads to a cytostome and further to a cytopharynx. The groove is ventrally located and extends slightly more than half the length of the body. In addition to these, it also has two contractile vacuoles with approximately seven radial canals situated near both ends of the aboral side. The two vacuoles contract alternately and empty their contents to the outside of the body by means of a cytopyge which is ventrally located between the posterior end of the oral groove and the posterior end of the body. Paramecium also has numerous special organelles called trichocysts embedded in the ectoplasm. They are generally considered to be organelles of defence, but due to the fact that they are easily devoured by Didinium even when great masses of trichocysts are extruded, their function is open to doubt (Fig.3). The most common type of reproduction is asexually by binary fission. This usually last for a long period of time, but at intervals it may be interrupted by the joining of two animals along their oral surface for the sexual processes of conjugation and cytogamy.

Undoubtedly, Paramecium is taxonomically considered as a ciliate, and for many decades Opalina was also classically considered to be a ciliate through the prodigious works of Metcalf. But in recent years, certain flagellate

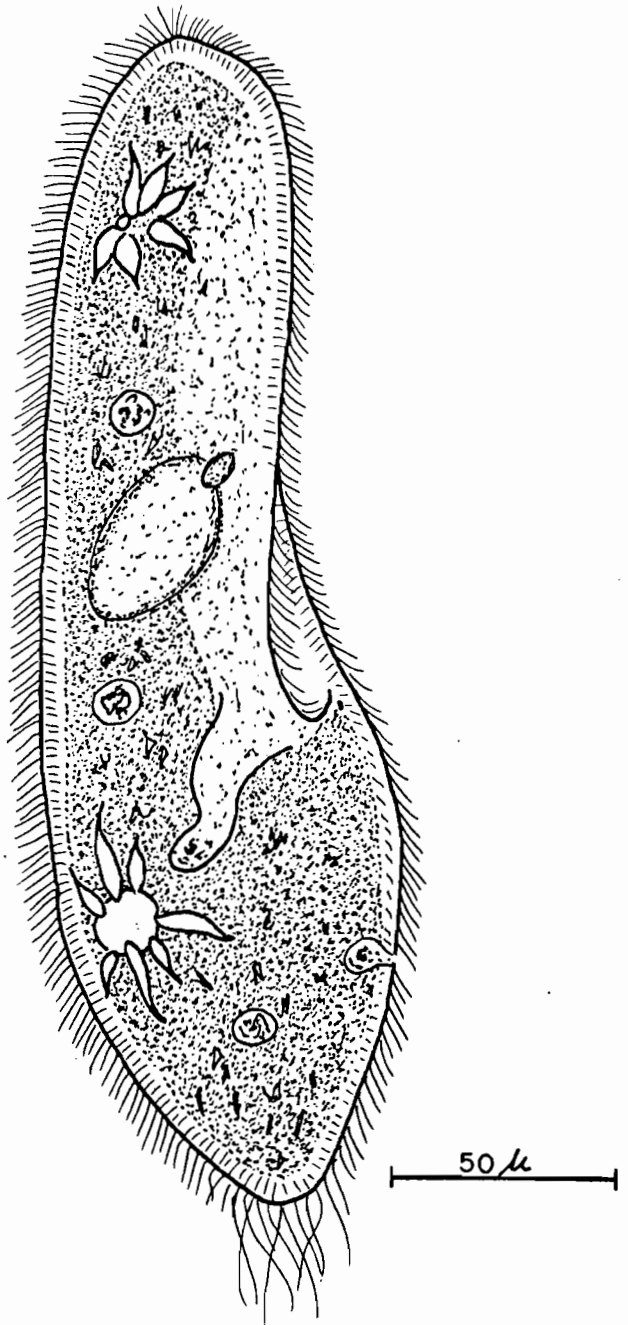


Fig. 3

Descriptive diagram of Paramecium caudatum

(after Wichterman)

affinities have been recognized, so that its proper taxonomic status becomes problematical, particularly when Grassé (1952) placed them among the zooflagellates. Corliss (1955) reviewed and listed the old and modern arguments of the "ciliate" and "flagellate-like" characteristics. Perhaps the most important of all characteristics linking the opalinids with the mastigophora is its mode of fission. Like most flagellates, Opalina divides in such a way that the plane of fission passes through the longitudinal or antero-posterior axis of the body. Not too long ago, Wessenberg (1957) showed that both "longitudinal" and "transverse" fission occur, hence the mode of fission can no longer be a strong ground to differentiate opalinids from the ciliates. Another strong mastigophoran character is the possesion of monomorphic nuclei. This point is again weakened through the work of Devidé (1951). He concluded that there is a "temporary existing of nuclear dimorphism in opalinids." Last year (1964), the committe on taxonomy and taxonomic problem of the society of protozoologists made a revised classification of the Phylum Protozoa. The controversial group of opalinids is considered a separate superclass, Opalinata, within the Subphylum Sarcomastigophora (Corliss and Balamuth, 1963). The revised classification aims at placing them in an intermediate position

between flagellate and ciliate protozoa. Inspite of the many controversies, to many protozoologists, Metcalf's protociliate hypothesis still retains a high popularity. It is thought that further research on the group is essential to establish its systematic position. For the time being, the writer retains Metcalf's protociliate hypothesis.

In the case of any organism, whether high or low, the question of the part played by environmental conditions in their different reactions is one of considerable importance. Therefore, in studying the reaction of any organism, their environmental factors are also being considered. Swezey and Atchley (1935) in their study of the comparative behavioral characteristics of free-living and parasitic ciliates have listed a number of comparative factors in the habitat of both organisms. They are as follows:

Factors	Parasitic	Free-living
1. Gen. Habitat	Intestinal contents of living frog	Fresh-water pools
2. Temperature	Constant at 37.5°C (sic)	Variable
3. Light	Total darkness	Diffuse daylight
4. Food	Abundant, mostly starch	Various materials

Continue on next page

Factors	Parasitic	Free-living
5. Viscosity of Medium	Very high	Comparatively low
6. Pressure	Varied, usually high	Constant, comparatively low
7. Motion in Medium	Peristaltic movement	Gentle currents
8. Organic Materials in Medium	a. Other plants and animals (Protozoa, worms, yeast etc.)	a. Other plants and animals (Algae, Protozoa, Rotifers etc.)
	b. Bacteria abundant (intestinal)	b. Bacteria fewer (fresh-water)
	c. Product of digestion of host	c. Decaying plants and animals
	d. Products of bacterial action (impt.)	d. Products of bacterial action (unimpt.)
	e. Excretory products from neighboring plants and animals (impt.)	e. Excretory products from neighboring plants and animals (unimpt.)
	f. Digestive juices	f. Nothing comparable
	g. Intestinal secretions	g. Nothing comparable

In addition to the above, it is believed that other factors such as hydrogen-ion concentration and oxygen tension of the habitat are also of major importance. In the

parasitic habitat, vertebrate gut, the pH is variable, ranging from 1.5 to 8.4 depending on the amount of CO_2 saturated in the intestinal contents; in the free-living habitat the pH is less variable, ranging approximately from 6-8. It is also evident, that the vertebrate gut is an anaerobic environment; if oxygen is present, the amount is very little. Von Brand (1952) in his study of the oxygen content in some habitats of parasites noted that in the large intestine of some vertebrates, the amount of oxygen present ranged from 5 down to 0 mm. Hg; in the free-living habitat, oxygen may be present in varying quantities, but is always comparatively much higher than in the vertebrate gut.

MATERIALS AND METHODS

The Paramecia that I used in this study were secured from Beaver Lake in September of 1964. This was done by the usual method of collecting submersed vegetation and bottom debris from lake. The materials were placed in a wide mouth jar and just enough water was added from the lake to cover the materials. After the water had stood in the laboratory for about three days, many Paramecia appeared in the surface scum, and a few days later, they became abundant. After approximately three weeks or so, Paramecia began to decline in numbers. In order to have a continuous supply of Paramecia at hand for this study, a continuous culture of Paramecia by the hay infusion method was made. Three grams of hay were placed with 500 cc. of distilled water in a wide mouth beaker and boiled for twenty minutes or till the water became brownish in color. The culture was allowed to stand uncovered for two or three days, so that bacteria in the air could fall into it and eventually became the food of Paramecia. This culture was stirred daily to oxygenate. After two or three days, Paramecia from the natural lake culture were added. The culture was kept covered to prevent evaporation of the liquid. Usually, three days after inoculation, Paramecia could be seen in the culture. In a period of approximately 10 to 12 days, Paramecia were abundant in such a culture which lasted for about two and a half weeks or more. New

cultures were set up at intervals of about one month and a few drops of old culture containing abundant Paramecia were inoculated into the fresh culture. Thus, an abundant supply of Paramecia for experimental use was kept continuously and indefinitely.

The parasitic Opalinas were taken directly from the rectum of frogs. These frogs were the common grass frog of Quebec, Rana pipiens. The frogs used in this study were obtained from McGill animal house and probably had been collected in the vicinity of Montreal. These frogs were freshly killed by pithing and were dissected dry. The rectum was immediately removed, slit open and the intestinal contents were washed out with Frog-Ringer solution into a petri dish. Sometimes, if the contents of the gut were too thick or too viscous, Frog-Ringer solution was again added as diluting fluid in order to allow clear observation of the parasites. At times the amount of debris accompanying gut parasites made them difficult to examine. Sandon's (1941) apparatus for concentrating and cleaning rectal ciliates was then used. The method was as follows:

Three 15ml. conical centrifuge tubes were fitted into each other after the ends of the two inner ones had been cut off and replaced by membrane of bolting silk. A mode-

rately wide mesh was used for the top and a finer mesh for the lower tube. Frog-Ringer solution was poured in till the bottom of the inner tube was covered. The medium containing the Opalinas together with their accompanying debris was gently inoculated into the tube with a pipette. The bolting silk retained the dirt but allowed the Opalinas to pass through. After twenty minutes or so, the Opalinas collected as a white mass at the bottom of the outer or lower tube (Fig. 4).

When the fecal mass in the infected rectum was well-formed, a more convenient way of obtaining abundant Opalinas was found through experience during the course of experiment. This omitted the usual opening of rectum, washing out of rectal contents, concentrating and cleaning of ciliates by Sandon's apparatus used by most investigators. The method simply involved putting the unopened rectum in a petri dish with the addition of a small drop of Frog-Ringer solution. Immediately after, the Opalinas were found escaping from the anterior open end of the rectum into the drop of solution surrounding it. Thus, a great concentration of Opalinas devoid of a large amount of debris was obtained easily. This was found to be the most time-saving way of securing considerable amounts of Opalinas for experimental work.

Fig. 4

Sandon's apparatus for concentrating and cleaning ciliates

For all experimental works, Opalinas were examined in Frog-Ringer solution, as they soon die in water. The Paramecia on the other hand, were examined directly in the liquid containing them when taken from the culture medium. Other methods and apparatus used in different behavioral studies will be discussed separately in its particular section.

A series of cross-sections of the rectum was prepared inorder to determine the relative position of Opalinas inside the rectum. The rectum was fixed in Zenker's Fluid with the addition of a small amount of glacial acetic acid and formalin. Washing, dehydration, cleaning and mounting were done in the usual manner. The rectum was embedded in paraffin and sections were cut at 10 microns. Frequently, different sections of Opalinas were obtained side by side. Staining was done by Mallory Triple Stain and Heidenhan's Iron Hematoxylin. Both gave satisfactory results.

The measurements of all organisms were taken during their living state. It is not advisable to measure the organisms after they are dead, because the killing and fixing process tend to produce considerable shrinkage and distortion. Before taking any measurement it is necessary to

slow down their locomotion, for if they are placed on a slide, they move rapidly and cause much difficulty in measuring. The chemical used for slowing them is Methyl Cellulose (trade name, methocel; 4000 centipoises rating). This was first introduced by Marsland in 1943 for quieting Paramecium, and remains the best chemical for slowing both organisms. The solution was prepared by mixing 3 grams of Methocel thoroughly with 45 cc. of boiling water (the writer finds the above proportion for such viscosity rating most satisfactory in her experimental work). Then an equal amount of cold water was added and stirred and cooled immediately in the ice box. A small ring of this solution was made on the slide, after which a drop of culture was placed in the center of the ring and covered with a cover slip to prevent rapid evaporation. The cover slip should be supported by small pieces of glass to prevent crushing the organisms. As the organisms swim outward from the center, they move more and more slowly because of the increasing viscosity and often with only slight distortion of the animal (Stiles & Hawkins, 1947). The actual measurement was made with the aid of a Camera lucida and a stage micrometer.

SOME OBSERVATIONS ON CULTURING PARAMECIUM IN HAY INFUSION

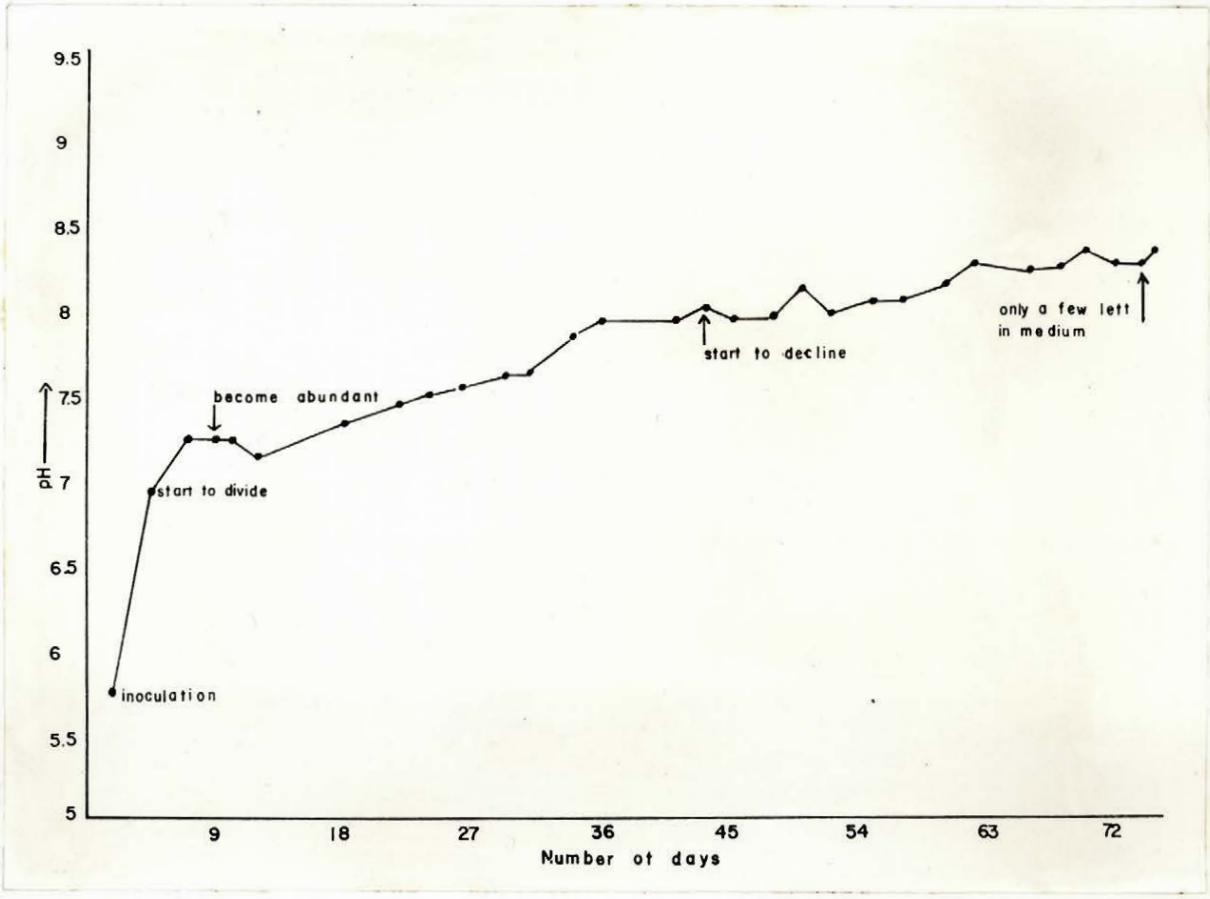
Woodruff observed in 1912 that hay when boiled with distilled water becomes straw color and after standing in the laboratory for three or four days, the color appears darker. There seem to be three distinctive colored-stages in the infusion medium. This enables one to detect the approximate age of the cultures. Usually, a lighter color appears in the first eighteen days. Closely following it a darker color appears and lasts up to thirty-four days; subsequently it turns to dark brownish-green shades. The above results were obtained by averaging the duration of the color changes which took place in five hay infusion media prepared with three grams of hay in five hundreds cc. of distilled water.

Fine (1912) attributed that the lighter color in the infusion to its relative high acidity and the darker color to its relative low acidity. In the course of experiments, the hydrogen-ion concentration of several cultures was measured. Those that have a lighter color usually fall within the pH range 5.5-7.3. They become darker within the range 7.4-7.8 and finally form a brownish-green shade from 7.8-8 and over. Since there is no uniform hydrogen-ion concentration at the top and bottom of culture (Peters, 1907; Fine, 1912; Bodine, 1921), each pH measurement was taken after a thorough, moderate stirring in order to insure uniform concentration. All hydrogen-ion determinations were made elec-

trometrically by the use of Beckman and Canlab pH meters.

The hydrogen-ion concentration of the culture medium is recognized as being an important factor in the origin and sequence of the protozoan fauna of hay infusion (Woodruff, 1912; Fine, 1912; Hyman, 1925). Different kinds of protozoa require a different hydrogen-ion concentration for their best development and will not flourish or will disappear at others; hence it may even become the limiting factor for their growth. In the course of several experiments on culturing Paramecium, it was found that the most favorable pH for growth in the medium is approximately 7 or slightly higher to 7.3. Figure 5 shows the growth of Paramecium as related to the hydrogen-ion concentration in culture medium measured at intervals.

As to the factors concerning the changes of hydrogen-ion concentration in the culture medium, the pH is attributed primarily to the quantity of hay relative to the amount of water, for the amount of water determines the rate at which putrefaction prodeeds which in turn determines the rate of change of hydrogen-ion concentration in the medium. Bacterial fermentation is mainly responsible for the whole process. In the study of the chemical properties of hay infusion by earlier investigators (Peters, 1907; Fine, 1912; Bodine, 1921), it has also been pointed out repeatedly that the acidity of

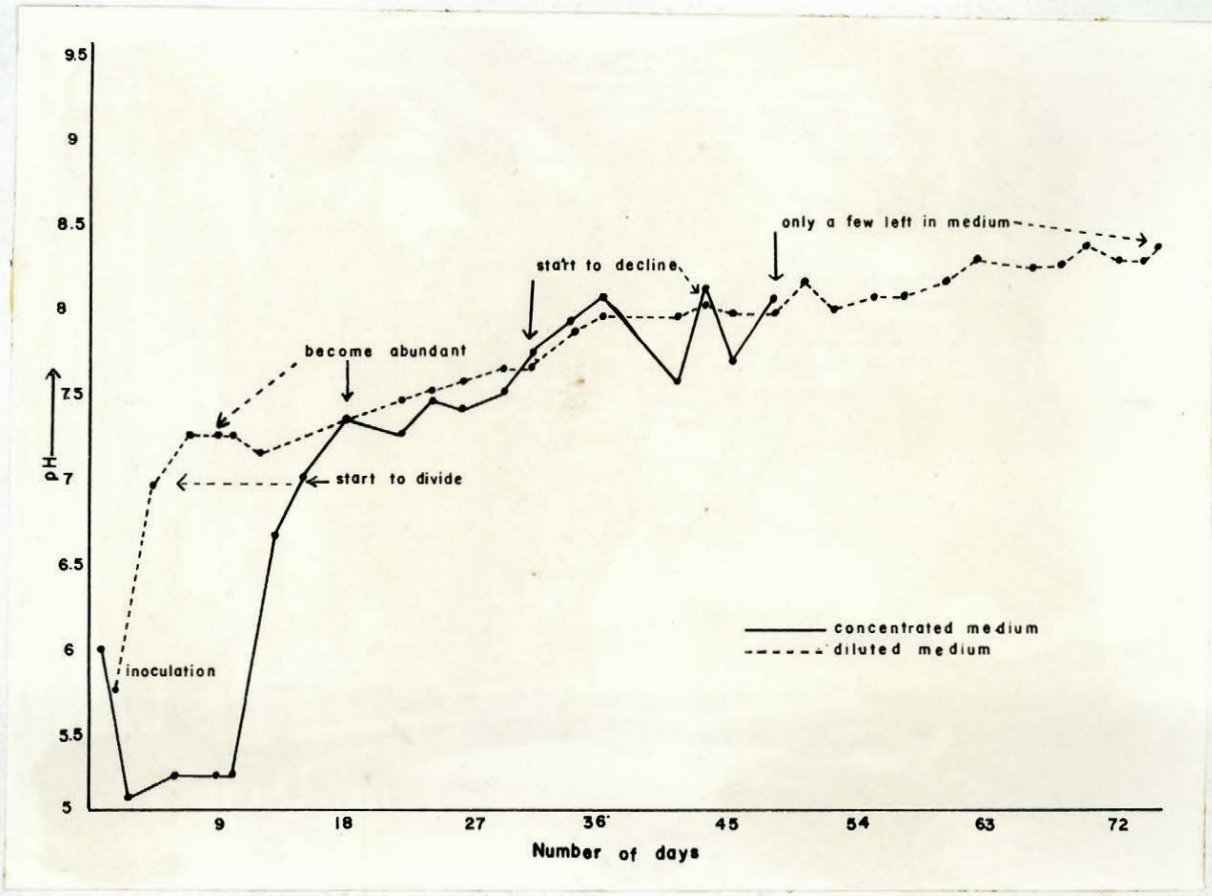


The growth of Paramecium caudatum as related to the hydrogen-ion concentration in culture medium measured at intervals.

Fig. 5

hay infusion is essentially due to bacteria, their efficiency in producing acid being governed by the concentration of the infusion in acid-yielding materials (hay). Figure 6 shows the different rate in the pH changes of a concentrated and a more dilute hay infusion media. The concentrated medium is made up of 6 grams of hay with 500 cc. of distilled water; the dilute one on the other hand is made up of 3 grams of hay with the same amount of distilled water. One can see that the pH changes more rapidly in a more concentrated medium due to the greater amount of hay materials that produce a faster rate of putrefaction. From this experiment, it was observed that the time at which Paramecia dissappear from the culture is different in the two kinds of infusion media. This is again related to the amount of hay materials. The greater the concentration, the faster the rate of putrefaction, the greater the changes in pH value, the shorter the culture lasted. While, the lesser the concentration, the slower the rate of putrefaction, the lesser the changes in pH value, the longer the culture lasted.

The secondary factor, the writer believes, is attributable to the amount of oxygen inoculated during the aeration of the medium by stirring. A medium that has not been aerated by stirring maintains a very low pH measurement; while a medium that is constantly being stirred will increase its



Different rate in the pH changes of a concentrated and a dilute hay infusion medium.

Fig. 6

hydrogen-ion concentration gradually. An attempt not to stir the culture was made in an infusion of 3 grams of hay in 500 cc. of water. In such a medium, the pH value remains very low (5.2-5.6) even after 6 days. In a culture of the same concentration that was constantly stirred, the pH was usually 6.9 or 7 after standing for 3 days. In the former anaerated medium, the inoculated Paramecia never survive; in the latter medium, they multiply immediately and become abundant.

DISCUSSION

Several investigators (Peters, 1907; Fine, 1912; Bodine, 1921) have attributed some factors responsible for the pH changes in an hay infusion medium, but the writer has failed to find in the literature a description of the process in detail. The over all processes concerned with pH changes in an hay infusion medium, the writer believes, occur as follows:

The hay materials which essentially consist of cellulose, by the action of bacteria are broken down into maltose which in turn decomposes into glucose. Glucose under non-aerobic conditions will transform into carbohydrate derivatives, which are mostly in the form of ketone bodies and are acidic in nature. These carbohydrate derivatives will finally be converted into ethanol plus carbon dioxide. The decomposition will continue until all the glucose is converted to ethanol plus CO_2.

The acidity in an hay infusion medium is caused by the abundant presence of acidic carbohydrate derivatives and CO_2. The former comes solely from the cellulose decomposition, the latter may either be derived from air or may also come from the medium through cellulose decomposition. The amount of acid-yielding materials produced is directly proportional to the amount of hay materials available. This is why in a more concentrated medium, because of its greater

quantity of hay materials, a greater amount of acidic bodies is produced, followed by a sudden increase in the acidity of the medium. Because of the abundant supply of bacterial food-glucose-the bacteria that have fallen from the air into the medium will multiply rapidly and act more actively on the cellulose. Thus, the rate of decomposition is accelerated and therefore the culture lasts for a relatively shorter period.

The inoculation of oxygen by stirring the medium will retard the decomposition of glucose, hence the amount of carbohydrate derivatives and CO_2 are diminished and the medium become less acidic. For this reason, an anaerated medium remains highly acidic even after 6 days and a constantly aerated one will gradually decreases its acidity.

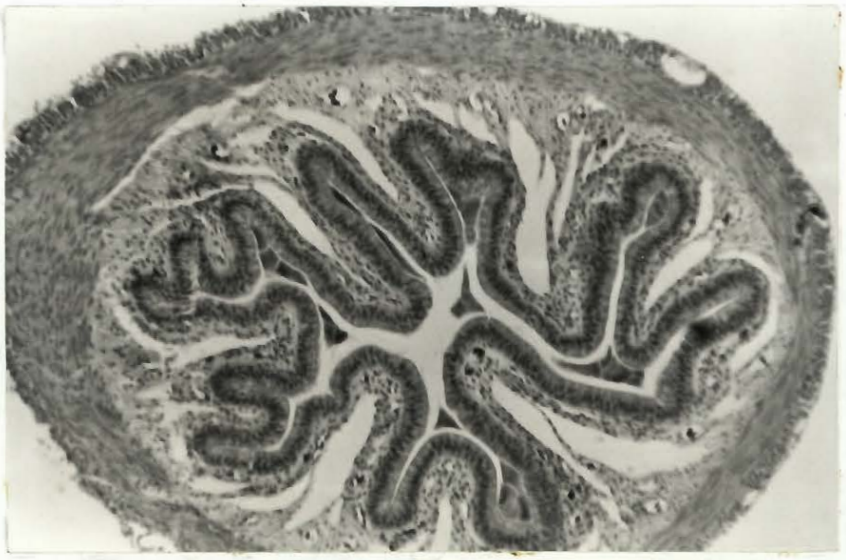
The decline in number of Paramecium after a certain pH has been reached can not be interpreted exactly. It may be due to a direct effect of the hydrogen-ion concentration or may rather be the result of the unavailability of some essential mineral elements or perhaps, as in the view of Woodruff (1912), may be due to the inadequate supply of proper food either in amount or kind or both. As is generally known, the principal food of Paramecium in an hay infusion medium is bacteria. The bacteria in the medium become fewer as the

number of Paramecia increases. Also, due to the covering up of the culture jar to prevent evaporation, fewer bacteria are inoculated into the culture medium. Furthermore, the decrease in number of the bacteria may also be caused by the inadequate supply of their required food-glucose. The deficiency occurs when all the hay materials are being completely decomposed into its constituents (ethanol and CO_2). Therefore, the culture medium no longer has an abundant supply of bacteria, and this results in the lack of food for Paramecia which prevents them from multiplying and causes their decline in numbers.

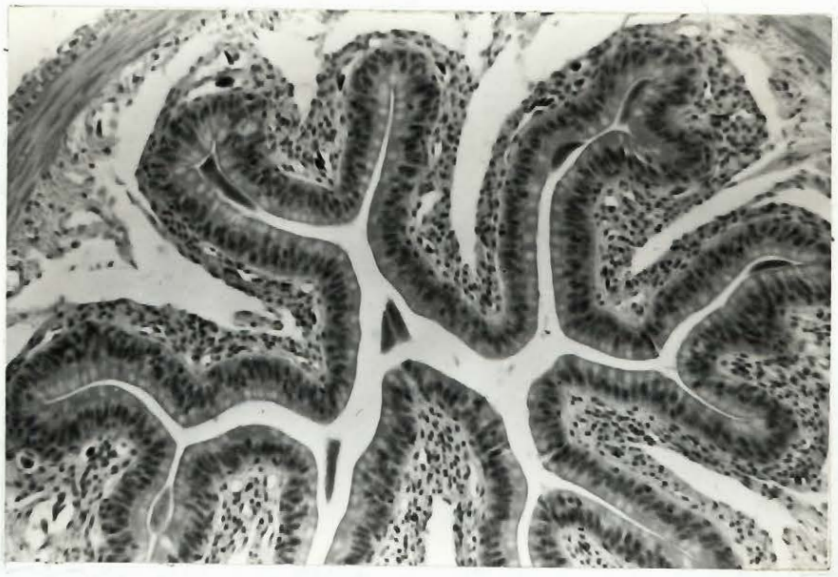
SOME OBSERVATIONS OF OPALINA'S HABITAT

Apparently, the rectum presents two different appearances to the observer. The rectum of some Rana pipiens is filled with fine particles of mud mixed with minute organic debris. They give a grayish color. The rectum of others, contains large pieces of green algae giving the entire rectum a greenish color with an irregular distribution of the contents. These two conditions in the rectum appear to have no relation to infection by Opalinas. Both mud-filled and alga-filled recta may or may not be infected with Opalinas.

Inspection for parasite was made by dividing the rectum of Rana pipiens into three separate portions: an anterior, a middle and a posterior; and placing them into three separate dishes. These pieces were then examined for parasites by teasing them with a dissecting needle into smaller pieces. It was observed that the Opalinas are found inhabiting the anterior and middle portions of the rectum. But more often the latter has the greatest number of Opalinas (Figures 7,8). If Nyctotherus are also found infecting the rectum, they occupy the same portion of the rectum as the Opalinas. A further study of the exact ecological niche occupied by the different parasites was not possible, but the characteristic distribution of each parasite was determined. Occasionally,

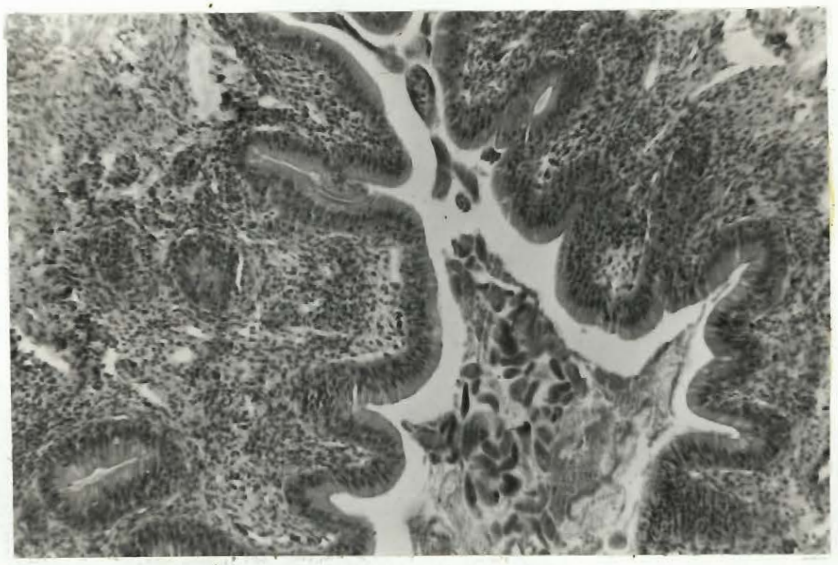


a. Cross-section of the anterior portion of a rectum infected by Opalinas. The Opalinas are found in aggregation between the crypt of the intestinal wall or in close contact with the rectal wall.



b. An enlarged portion of the anterior cross-section of same rectum showing a few Opalinas lie freely in the rectal lumen.

Fig. 7



A portion of the middle cross-section of same rectum showing a heavier infection by Opalinas.

Fig. 8

when the rectal mass contains much fluid, a few Opalinas may be found in the posterior region. Perhaps the more liquid medium facilitates their swimming from one place to another. In a well-formed rectal mass, they are never found in the posterior region. Hitherto, several species of Opalinas have been reported from the small intestine. The most recent observation is that reported by Marx (1963) in Rana pipiens, from one frog with a malformed bowel and two under starvation. None of the small intestines examined by the writer were found infected with Opalinas.

The relative position of the Opalinas in the rectum is clearly shown in a cross-section of a rectum. Most of the Opalinas are usually found gathered in a single group lying in close contact with the rectal wall. Some of them are found in aggregation between the crypt of the intestinal wall, and others may lie freely in the rectal lumen. Cases have been reported in which they may be found scattered through the fecal mass. But in the present observations, none of them were found in the central portion of the rectal mass.

As was mentioned before, besides Opalinas, there are many other parasitic worms and ciliates found in the rectum of Rana pipiens constituting the phenomenon called parasitocoenosis or parasite-mix. No apparent harmful effect was observed from the presence of nematodes, trematodes and other

ciliates on Opalinas. But there is an apparent effect on the number and distribution of Opalinas due to the presence of these other parasites. Usually, if there is a heavy infection with one species it is usually not accompanied by a heavy infection with Opalinas.

Another observation made was that there is a relation between the size and the number of Opalinas in an infection. This relationship is nearly always inversely proportional. That is, the average size of large numbers of Opalinas crowded in a host organ (A) is smaller than the average size of a few Opalinas in the same organ (B). Table 1 shows the various measurements of length of Opalinas. Each measurement in the column is the average length of a group of 10 Opalinas selected at random.

Table 1

	A	B
1.	235	330
2.	230	325
3.	230	335
4.	225	325
5.	230	315
6.	235	320
7.	230	335
8.	225	330
9.	235	315
10.	230	320
Average of the groups :	230	325

Attention has also been paid as to the size of the parasite in relation to the size of its host. It was observed that there exists no intimate relationship between the size of the two. Both large and small Opalinas may be found in any sizes of Rana pipiens, although some parasitologists generally expect to find bigger parasites in bigger hosts.

A SURVEY OF THE INCIDENCE OF PARASITISM IN THE RECTUM OF FROG RANA PIPIENS FROM THE PROVINCE OF QUEBEC

Investigation of the parasites in the frog Rana pipiens of Quebec have been made (Khaner, 1935; Anderson, 1944; Shah, 1959), but a general survey of the incidence of different parasites in these frogs is entirely lacking. The present investigation has the purpose of giving a survey of the incidence of parasites in the rectum only.

FREQUENCY OF THE OCCURRENCE OF OPALINA INFECTION

From October of 1964 up to September of 1965, 332 frogs selected at random from this locality were examined for opalinids, the result was as follows (Table 2):

TABLE 2
FREQUENCY OF OCCURENCE OF OPALINA INFECTION IN FROG
RANA PIPIENS SCHREBER OF QUEBEC, CANADA

Month	No. of frogs	No. of infected frogs	Percentage of infection	No. of female frogs	No. of infected female frogs	Percentage of infection	No. of male frogs	No. of infected male frogs	Percentage of infection
Oct.	15	4	27%	5	1	20%	10	3	30%
Nov.	60	40	67%	13	9	69%	47	31	66%
Dec.	25	13	52%	13	7	54%	12	6	50%
Jan.	25	15	60%	8	4	50%	17	11	65%
Feb.	20	13	65%	2	1	50%	18	12	67%
Mar.	36	11	31%	7	3	43%	29	8	28%
Apr.	26	9	35%	5	1	20%	21	8	38%
May	30	18	60%	13	6	46%	17	12	71%
June	20	12	60%	16	10	63%	4	2	50%
July	36	19	53%	18	10	56%	18	9	50%
Aug.	20	15	75%	9	6	67%	11	9	82%
Sept.	19	11	58%	12	7	58%	7	4	57%
Total	332	180	54%	121	65	54%	211	115	55%

Percentage infection in all frogs: 54%
Range of infection: 27%-75%
Monthly average infection: 54%

Percentage of infected female frogs: 54%
Range of infection: 20%-69%
Monthly average infection: 50%

Percentage of infected male frogs: 55%
Range of infection: 28%-82%
Average infection: 55%

From the data recorded, it was found out that an average of 54% of the Rana pipiens are infected with Opalinas. This was not found to be in entire accord with what the earlier investigators stated, that Opalina occurs in nearly every frog and that all healthy frogs have Opalinas (Thompson, 1929; Neresheimer, 1906).

It has always been observed by many investigators, that in spring, when the reproduction season of the host approaches, the division rate of most of the Opalinas is rapidly increased, so that many small forms are produced. In the frog Rana pipiens from the Province of Quebec, this usually starts to occur at the end of March and gradually ends at the beginning of July.

FREQUENCY OF THE OCCURRENCE OF DIFFERENT PARASITIC INFECTIONS

Besides the ciliate Opalinas, other parasites found during the examination of the rectum were nematodes, trematodes, cestodes and other ciliates. Opalinids and nematodes are very frequent, other ciliates less frequent, while the trematodes and cestodes are the least often found. Following is a table showing the number and percentage of the total number of frogs infected by each of the different types of parasites.

TABLE 3

Parasites	No. of frogs	Percentage
Nematodes	189	40%
Opalinids	180	38%
Other ciliates	30	6%
Trematodes	4	.9%
Cestodes	1	.2%
No parasites	69	15%

From the above table, it clearly shows that most frogs are infected with nematodes. This is especially true upon the advent of warm weather, when they are more heavily infected with larval worms than ever before. This is due to the fact that all worm eggs accumulated during winter are hatched at this time. In the case of nematodes in Rana pipiens from this locality, this usually begins early in July and gradually ends in October. At other times of the year, the nematode infection is very slight, ranging from 1-11 nematodes per frog. Occasionally, numerous small nematodes freshly hatched from mature eggs may also be found.

As mentioned before, the rectum of frogs shows a great deal of parasitocoenosis or parasite mix. Figure 9 in the form of a histogram shows how frequently some of the different types are found in the same host organ.

1. Absent of all kinds of parasites (69)
2. Frogs infected with Opalinas only (63)
3. Frogs infected with nematodes only (71)
4. Frogs infected with nematodes and other ciliates (Nyctotherus) (7)
5. Frogs infected with Opalinas and other ciliates (7)
6. Frogs infected with Opalinas, nematodes and other ciliates (14)
7. Frogs infected with Opalinas, nematodes and trematodes (2)
8. Frogs infected with Opalinas and nematodes (94)
9. Frog infected with trematodes only (1)
10. Frog infected with trematodes and nematodes (1)
11. Frogs infected with Nyctotherus only (2)
12. Frog infected with cestodes only (1)

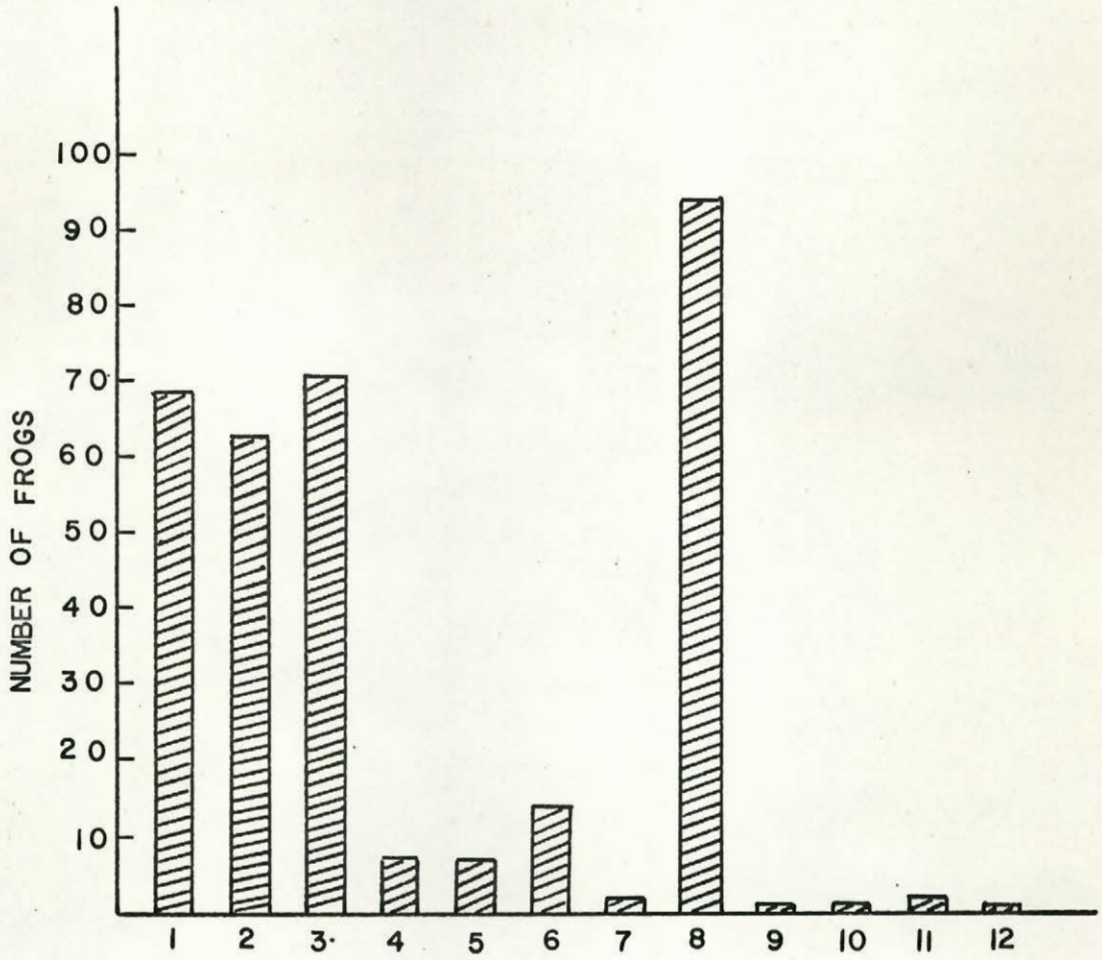


Fig. 9

Frequency of occurrence of different parasites in frog Rana pipiens

As to the identification of the helminthic fauna, a detailed morphological study of the various forms was not made. But in general, the worm which occurred most frequently is species Aplectana americana (Walton, 1929). The numerous worm eggs from which larval worms were hatched identified as those of Rhabdias bufonis (Stiles and Hassal, 1905). The other ciliates found in the rectum were identified as Nyctotherus cordiformis Stein. However, besides these parasitic fauna, there are other unidentified parasites (Figure 10). To my knowledge, these have not been listed as parasites of Rana pipiens of Quebec province, Canada. One of them (Fig. 10d) closely resembles the genus Diplodiscus as found in the rectum of other amphibians (Stafford, 1905; Chandler, 1923; Hunter, 1930).

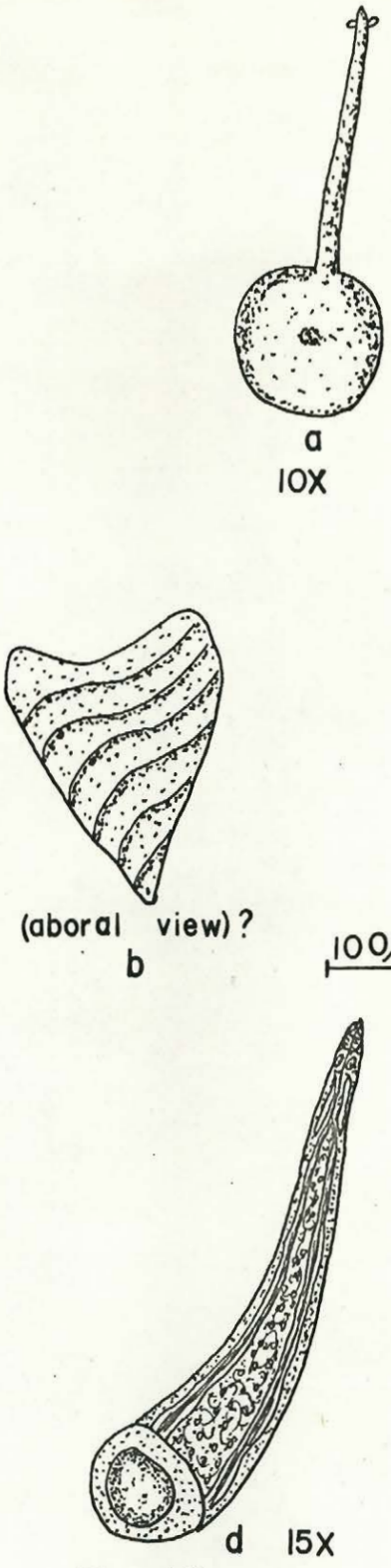


Fig. 10

Un-identified parasites found in the rectum of frog Rana pipiens

DISCUSSION

Bhatia and Gulati (1927) studied Opalinas in Indian frogs and toads and concluded that some of the adult frogs show only a slight amount of infection probably because certain nematodes in the rectum prey upon them. Again, Hazard (1941) found that the absence of opalinids in the adult frog Rana clamitans is due to the fact that Diplodiscus, the trematode parasite of the frog feeds on them as they enter the host. The present writer has observed these parasites for many hours on several occasions and never seen a single nematode or trematode eat Opalinas. It is thought that the inverse ratio between the two populations may be attributable to three possible reasons. First, it may be that the two populations have the same food requirement and that the competition within the confined habitat will eliminate one of them. Or, secondly it may be that the two populations have different specific nutrient or environmental requirements. In other words, each organism requires specifically a particular kind of food, both in quantity and quality, and a particular type of environment for their best survival. When the conditions in the habitat turn out to be favorable to one species, they consequently become unfavorable to the other species, so that their chance of survival becomes smaller. Thirdly, it may be due to the harmful metabolic by-products of the worms that retard the growth of

the Opalinas. The first two reasons, however, are merely theoretical and much experimental research is needed to produce actual evidence for the existence of these phenomena. Perhaps a study of the kind and quality of food found in the cytoplasm of each organism and a study of the environmental conditions such as the chemistry of the surrounding medium, the various hormones of the host in the digestive tract and the possible secretions of the alimentary canal as stimulated by the different parasite will help to solve the problem. The third possibility has some support from the following experimental evidence. Opalinas were placed into two different petri dishes; one contained nematodes and the other was devoid of nematodes. A few hours later, many of those found together with the nematodes became less motile and gradually quieted down while most Opalinas in the other dishes were still actively swimming. The harmful metabolic by-products produced by the accompanying nematodes may be the chief cause of Opalinas quiescent.

The answer to the inverse proportion between the size and number of organisms may possibly be due to inadequate supply of food. A few or a small number of Paramecia or Opalinas in the same habitat have much room in which to grow, but 300 or 500 in the same habitat are crowded for space and have to compete for limited supplies of food. Therefore, a few in the same habitat grow better than a large

number of them growing in the same habitat. Another answer to the retardation of their growth when large number are present may be due to the large amount of harmful metabolic by-products produced by the populations themselves.

In the spring, the average length of a large number of Opalinas crowded in a rectum is smaller. It is also at this time that the Opalinas show very great size differences between individuals. This as a whole is purely a matter of growth, since small Opalinas are young ones which have recently been produced by fission.

COMPARISON OF BEHAVIOR IN SEVERAL EXPERIMENTAL MEDIA

An examination of the past literature shows that many attempts have been made in the culturing of Opalinas in different laboratory media (Engelman, 1876; Pütter, 1905; Metcalf, 1909; Konsuloff, 1922; Tyler, 1926; Larson, Van Epp, Brooks, 1925; Larson, 1928; Larson & Allen, 1928; Yang & Bamberger, 1953). These early investigators achieved varied success in their culturing technique. The greatest achievement was made by Yang and Bamberger in 1953. They were able to culture Opalinas continuously for more than 6 years in modified Boeck Drbohlov diphasic egg slant-saline medium with the addition of inactivated human serum as food for Opalina and the use of two antibiotics to inhibit bacterial growth. Seven years later (1960), Yang was able to develop a more convenient medium by applying aqueous liver concentrate for retarding bacterial growth. Using this medium, the culture needs only to be transferred weekly or biweekly instead of every two or three days as was formerly done. Most of the experimental works were done on species other than Opalina obtrigonoidea. The only papers concerning the behavior of Opalina obtrigonoidea in different laboratory media were those of Larson and her associates (1925-1928). Paramecium caudatum on the other hand, has been studied extensively in respect to its permeability to water and different anisotonic media such as sea water, glycerine, cane sugar, solid

agar medium etc.

The present study has as its purpose, comparing the behavior of Paramecium and Opalina in various experimental media. The purpose is not only to compare their longevity in these different media; but also to observe and describe some of the visible morphological changes occurring when the two species are exposed to these media.

PRECAUTIONS AND METHODS

In the comparative study of the behavior of these two organisms in several experimental media, preliminary experiments show the necessity for a number of precautions. The first and the most important is the size of the drop containing the organisms. The size of the drop has a considerable effect on the results obtained, especially when the drop is very small. Usually, the average lethal time of organisms contained in a small drop is much shorter than those in a large drop. In one experiment, the average lethal time in a small drop was 15 minutes, while the average lethal time in a rather large drop was 32 minutes. This is probably due to the higher concentration of the medium and the greater suddeness with which the organisms are subjected to the various media. While several small drops or one big drop tend to dilute the various media and lessen the

suddeness with which the organisms are subjected to the medium. In order to guard against this source of error, drops were all made as nearly as possible the same size as was the amount of liquid medium; the standard being two drops about 0.2 mm. each in diameter and the amount of liquid medium being 4 ml. The second precaution that can not be neglected is the amount of debris or scum introduced into the various media along with the drop containing the organisms. In the course of the experiments, it was found that if one introduces the organisms into various media along with large bits of debris or scum, the organisms can tolerate a longer exposure to such medium. The same species but without debris or scum, if introduced into a similar medium, reacts in different ways, and survives a shorter time in the medium. This may be due to the fact that the effect of the various liquid media must have been slower due to the hindering action of the debris. Lastly, in the study of the behavior of Paramecium, the pH of the hay infusion medium must be controlled. It has a marked effect on the time in which death occurs. Preliminary experiments show a very wide range in average length of life in these organisms. The difference ranged from 3 minutes to 2 hours or so. By repeating the same experiment many times, the writer finally found out that she had been taking samples from two cultures at different hydrogen-ion concentration; an old one that has a pH of 8 and a freshly made one that has a pH of 7.1. These Parame-

cia taken from a medium of higher hydrogen-ion concentration can withstand the unfavorable condition longer.

Also, it was observed that individual differences in resistance are considerable. For example, the average survival time of organisms from one culture is 5 hours, but some organisms die in 1 hour and others live 10 hours. For this reason, a culture is considered "dead" when more than one-half of the organisms in it are found dead. It was also very difficult to determine the exact moment of death, because vitality seems to remain a long while even after they have became motionless. They are considered dead when they no longer move when touched by a fine dissecting needle and no longer swim when put back into a favorable medium. Moreover, the difference in resistance occurs not only between individuals but may also be found among groups from different cultures or from different hosts. Larson (1928) observed that among those Opalina cultures examined immediately after the removal of the rectum, some are quite active and others are comparatively inactive. Those cultures containing inactive Opalinas usually die first. She also found that there are some cultures, which looking just as good as the others when cultured under same conditions, die off more rapidly

than the others. The writer, also has observed the same phenomenon as that recorded by Larson. Thus in table 4, several experiments were performed for each medium, giving a range in length of life in one column of the table and their average length of life in another.

RESULTS AND OBSERVATIONS

The following table shows a comparison of the longevity of Opalina and Paramecium in several experimental media:

TABLE 4

Medium	Species	No. of expt.	Average longevity	Range
Frog-Ringer solution	O.	7	10 hrs.	4.5-14 hrs.
	P.	12(4)	7'; 1 hr.4'	3'-2 hrs.30'
F.R.S.+Fecal material	O.	7	20 hrs.	10-29 hrs.
	P.	11(3)	22'; 5 hrs.38'	20'-24 hrs.
F.R.S.+Rectal wall	O.	7	48 hrs.	32-60 hrs.
	P.	9(2)	20'; 5 hrs.54'	20'-24 hrs.
Pütter solution	O.	4	16 hrs.	9-24 hrs.
	P.	9(5)	10'; 1 hr.10'	5'-2 hrs.30'
#Boiled Pütter + albumin	O.	3	21 days	16-26 days
	P.	7(4)	52"; 2'7"	46"-2'50"
Lactalbumin	O.	5	62 hrs.	48-96 hrs.
	P.	11(6)	42'; 2 hrs.25'	18'-6 hrs.
Gastric mucin	O.	7	84 hrs.	48 hrs.-7 days
	P.	4(2)	50 hrs.; 72 hrs.	48-84 hrs.
Hay infusion	O.	2	3 hrs.	1-4 hrs.
	P.	1	Mar. 15----?	-------
Hay infusion + debris	O.	4	7 hrs.	5-9 hrs.
	P.	1	Mar. 15----?	-------
Distilled water	O.	4	4 hrs.	3-6 hrs.
	P.	1	Dec. 18----?	-------
Tap water	O.	5	15'; 4 hrs.	10'-7 hrs.
	P.	5	5'; 72 hrs.; months	2'-months

NOTE:

with daily renewal of medium

The numbers in brakects refer to the number of experiment done on Paramecium taken from culture that has a pH of 7.1.

There are two different average lengths of life in Paramecium. The first is obtained from Paramecium in culture having a pH of 7.1; and the other from those whose culture has a pH of 8.

In their behavior in tap water, there are two or more average lengths of life obtained even when organisms are taken from the same culture medium (see discussion for explanation).

1. Frog-Ringer Solution:

Engelman, in 1876, was able, with great difficulty to keep Opalina ranarum alive outside of the host for two or three days in the aqueous humor of the eye. He found that the best results were obtained when there was a complete separation of Opalinas from the rectal contents. Contrary to Engelman's findings, the writer finds that cultures live longer if pieces of the rectal contents and rectal wall are added. Several other early investigators also have observed the same phenomenon, among them were Pütter (1905); Metcalf

(1909); Konsuloff (1922); and Larson (1928). Thus, the fact that the inclusion of rectal contents or rectal wall in the medium will prolong the survival time of Opalinas is once more confirmed. However, this does not only apply to Opalinas but is also true with Paramecia. From the results obtained one can see that Paramecia live longer in Frog-Ringer solution with large bits of debris or scum than in the clear Frog-Ringer solution. The composition of Frog-Ringer solution used is as follows:

NaCl	0.65 gm.
KCl	0.014 gm.
$CaCl_2$	0.012 gm.
$NaHCO_3$	0.02 gm.
NaH_2PO_4	0.001 gm.
Aq.D.	100 ml.

The death of Opalinas in the different preparations of Frog-Ringer solution is characterized by a typical balling-up of the cell. This was also noted by Larson (1928). However, prior to their death, all forward progression of Opalinas slow down and soon after enter into a standstill position. At this moment they are not necessarily dead and the cilia of some still keep on beating for sometime. Usually, after all the ciliary movements have ceased, the orga-

nisms are generally considered dead. They start to round up gradually. Among the several observations, there was no record of bursting of the cell membrane. In one of the experiments where Opalinas were introduced into Frog-Ringer solution with large bits of rectal wall, many individuals were observed fresh from division. Certain parts of their body are drawn out into irregular strands causing some abnormalities in their form and shape (Fig. 11).

The death of all Paramecia are accompanied by a deformation in shape. In most organisms, there is a swelling of the body, an enlargement of the contractile vacuole, retardation of ciliary movements, formation of blisters around the cell surface and finally the bursting of the cell membrane. Only a few discharge their trichocysts (Fig. 12). It is noteworthy that, in those Paramecia taken from a culture of lower pH value, not only the death and disintegration occurs earlier than those taken from a higher pH medium, but the frequency of the bursting of cell membrane and the formation of blisters occurs more frequently.

2. Pütter Solution:

This medium originated from Pütter (1905) and has the following composition:

NaCl .8% ----------100 parts
Rochelle salts 30% ------- 5 parts
Distilled water ----------400 parts

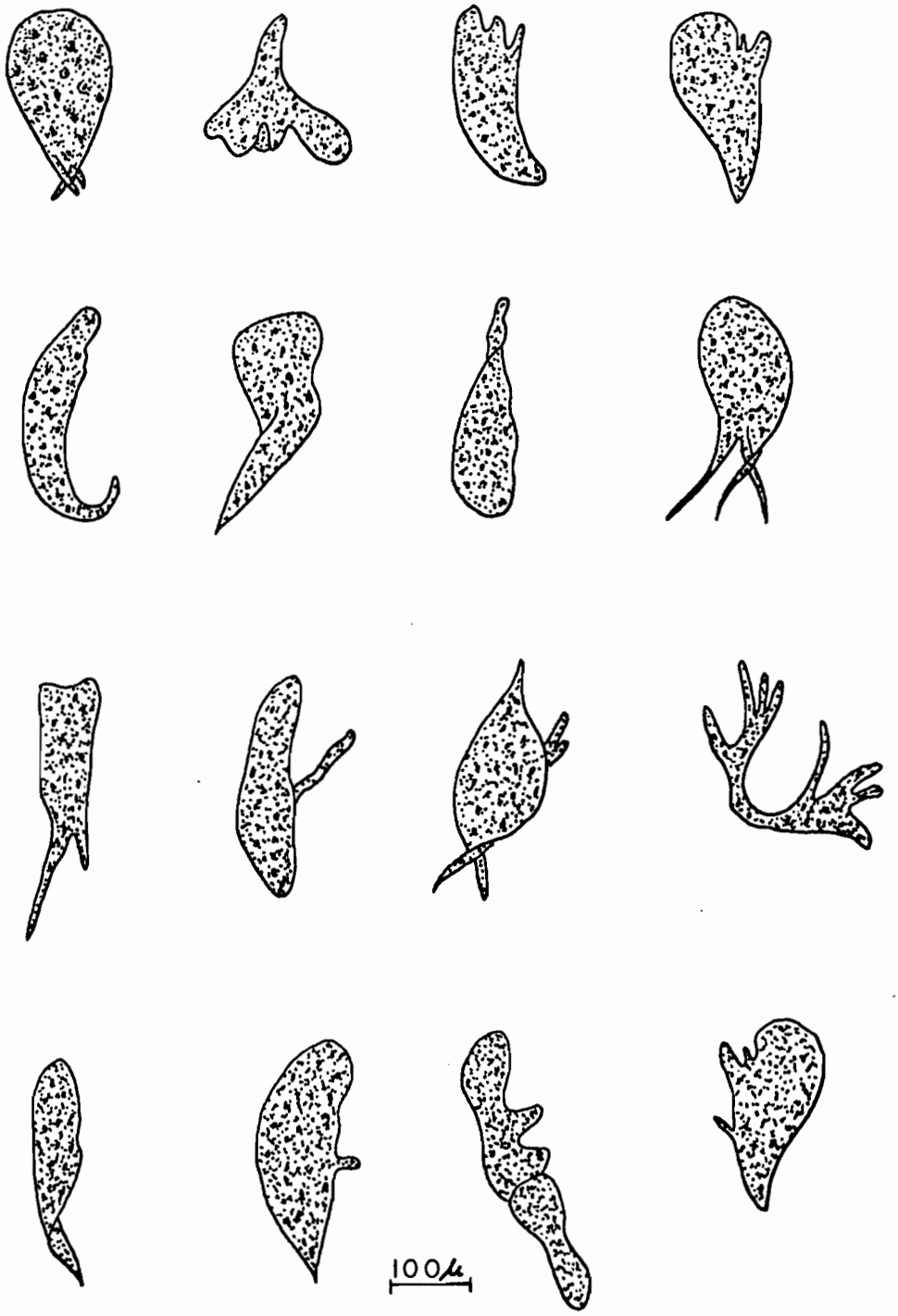


Fig. 11

Irregular division of Opalina obtrigonoidea observed in one of the Frog-Ringer solution plus rectal wall

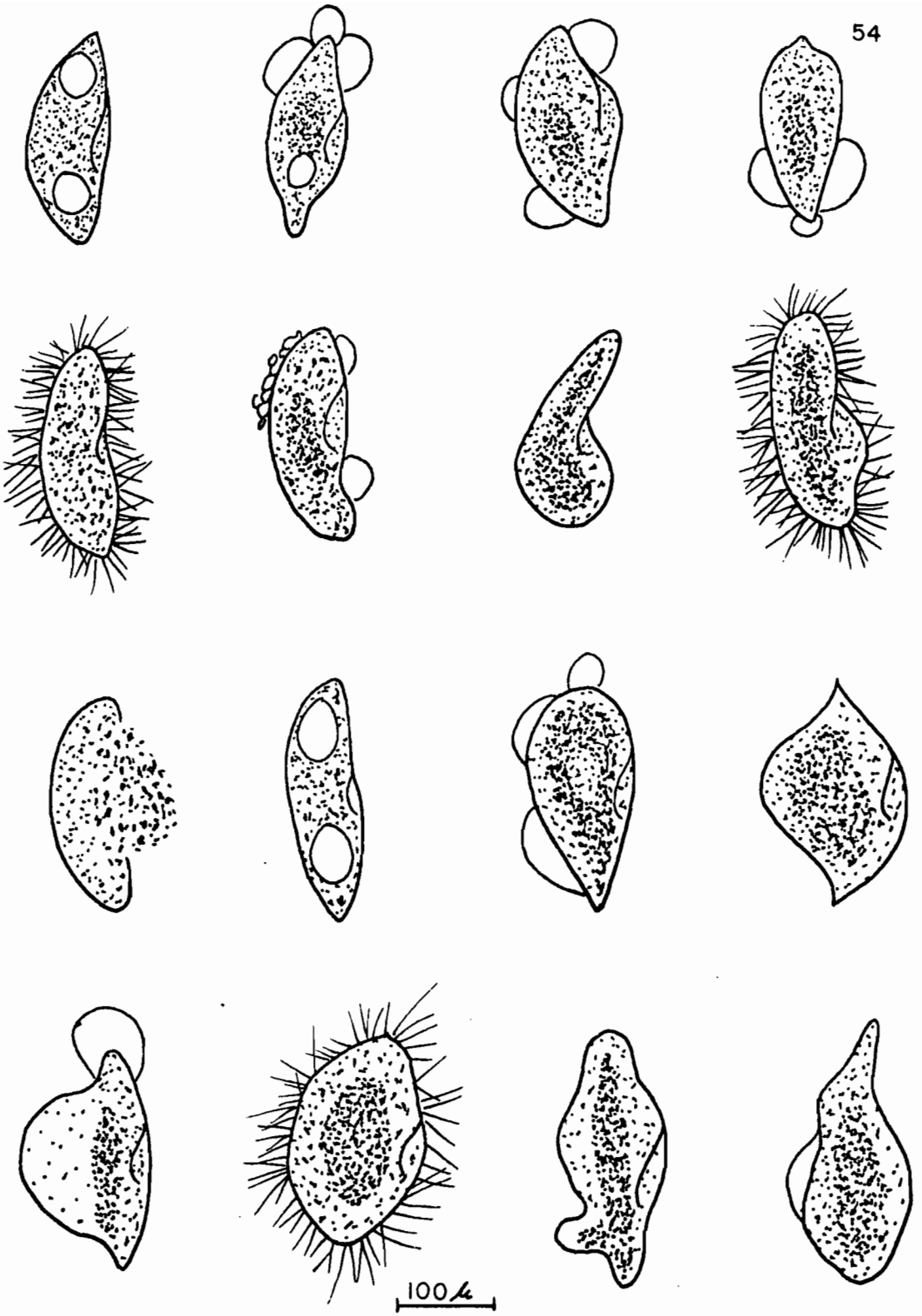


Fig. 12

Some of the visible morphological changes of Paramecium caudatum in Frog-Ringer solution

In this fluid, it was found out that Opalinas can live longer than in Frog-Ringer solution. In one experiment, most of the organisms come to a standstill after 24 hours, and gradually become ovate in shape so that finally the whole animal rounds up at approximately 29 hours. It still proves to be a much better medium for keeping Opalinas especially if the medium is boiled and renewed daily, accompanied by the addition of a drop of albumin. In this preparation, one thing of interest was that many dividing forms are observed. However, as time went by, many of them show signs of abnormal division as also occured in Frog-Ringer solution (Fig. 11). The cultures lasted for different numbers of days, the average being 21 days. All these organisms gradually assume the form of a ball after they die. Prior to their death, locomotion is also retarded, but cilia still keep on beating forcibly for some time. Bursting of the cell membrane does not occur.

As for Paramecia, this medium is not much better than Frog-Ringer solution. As a matter of fact, it was found to be extremely unfavorable especially after the medium has been boiled. The lethal effects occur within a few minutes after the organisms are introduced into the medium, resulting in reduced locomotion, swelling of body, dysfunctioning of

contractile vacuoles, blistering of the body and finally the disintegration of the animals. In most cases, the number of blisters formed in Paramecia left in boiled Pütter solution was comparatively more and may appear simultaneously and instantaneously as small clear droplets at different surfaces of the cell. In an unboiled Pütter solution, the Paramecia, do not die so soon. Usually, only a single large blister starts to form, first at the side becoming larger and larger until the whole animal becomes greatly swollen.

3. Lactalbumin:

5% of aqueous lactalbumin hydrolysate was prepared. Clegg (1965) found that this preparation is isotonic with the mammalian red cells.

Opalinas, when inoculated into this medium show no apparent abnormalities. There is neither a distortion of shape nor swelling or shrinkage of the body. The only thing observed was that the animals become quiescent for some moment after inoculation and then resume their normal locomotion soon after. This medium when left at room temperature, due to its protenoid nature becomes denatured and coagulated. It is at this state that most of the Opalinas are found dead. When the same medium is kept at a low, constant temperature

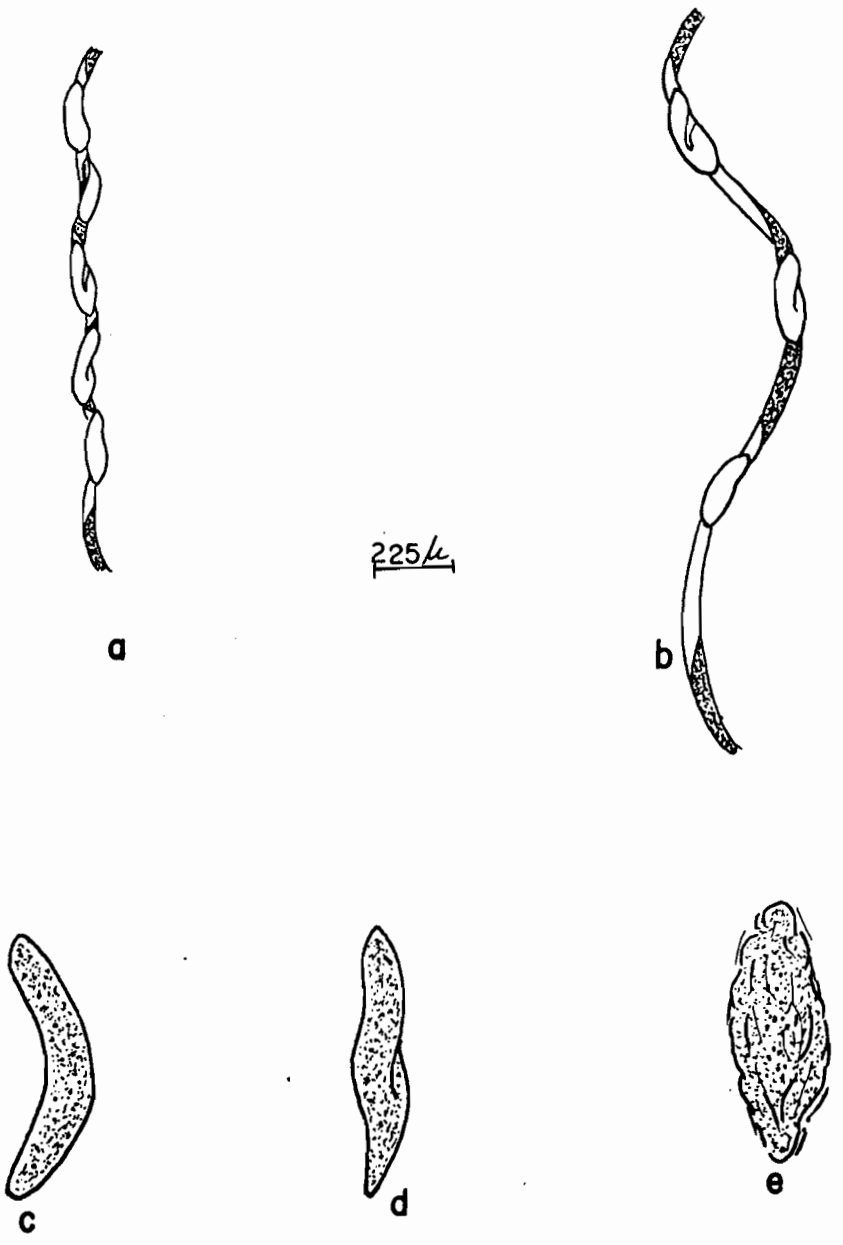
to prevent protein denaturation and also to avoid bacterial growth, Opalinas can live much longer. In one experiment, they survived as long as 4 days. In investigating the longevity of these two organisms in the various experimental media, these have been maintained at low, constant temperatures as well as room temperature. The results obtained at the lower temperature are not much different from those observed at room temperature with the only exception of Opalinas in lactalbumin solution.

This medium, although it does not produce any marked effect on the form, shape and locomotion of Opalinas, has an appreciable effect on Paramecia. At first they swim very fast, first proceeding toward a certain direction then quickly changing to another direction, continuing in this way for about 5 to 10 minutes. After this, they still maintain their rapid swimming but follow a typical spiral path, which due to the great speed, becomes short and narrow (Fig. 13a). As the animals swim in a forward direction, their bodies also swerve from side to side. At times a more striking peculiarity was observed. There is a tendency for the anterior alone to rotate while the animals are making their forward progression. When their locomotion becomes fast and abnormal, their bodies

start to become distorted and are accompanied by a considerable narrowing (Fig. 13c,d). Following this, the animals start to slow down, thus the spiral path increases its length and width (Fig. 13b). This usually occurs at an average of 22 minutes after inoculation. Sometime after they slow down, the animals start to enter a standstill position and finally die. The time for the animals to become quiescent varied in different individuals from about 30 minutes to 6 hours after inoculation. The average length of time is about a little over 2 hours. When the animals die, most of them die as they are, that is, with a narrower bodily form than those freshly taken from hay infusion. When left for a long period of time, they disintegrate into a shapeless mass (Fig. 13e). However, there are still a few that show a slight swelling. For those Paramecia that are taken from hay infusion medium with a pH of 7.1, the interval from the time they slow down to the time they become quiescent remains very short. In most cases, as soon as they slow down they become quiescent immediately and no longer move when touched by a fine dissecting needle.

4. Gastric Mucin:

Originally, this medium was used in culturing Entamoeba terrapinae. It consists of .3% gastric mucin. The solu-



a. The short and narrow spiral path
b. The long and wide spiral path
c, d. The distorted shape accompanied by considerable narrowing
e. The disintegration into a shapeless mass

Fig. 13

tion is warmed to dissolve the salt and mucin, filtered and tubed in 10 cc. amounts. It is sterilized at 15 pounds pressure and stored in the refrigerator until used. Before inoculation, about 2 mg. of sterile rice starch is added to each tube. Cultures are maintained at room temperature and transfer made at weekly intervals. The Entamoeba grows abundantly in this medium.

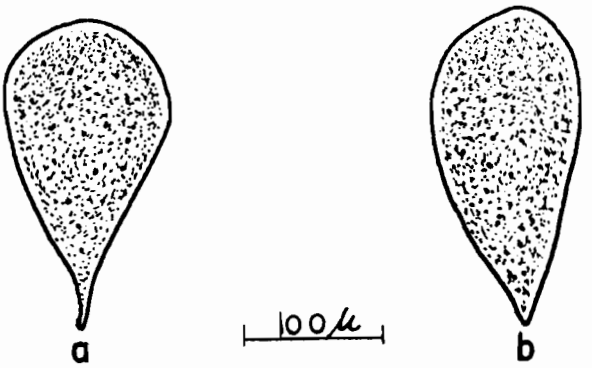
In the present study, no sterile rice starch was added, no transfer was made since the writer simply wanted to compare their longevity in clear medium with that in various other media. Usually, 4 ml. of 0.3% gastric mucin were placed in a petri dish, and two drops of liquid containing Opalinas were also introduced separately into the clear fluid of the various media. It was found that the Opalinas in this medium nearly always live much longer than any of those in the other media, with the exception of one experiment in which those in the lactalbumin outlived them. From table 4, one can see that the Opalinas can live from two days to a week, although very rarely the latter. Prior to their death, the anterior end swells in size so that the posterior end becomes greatly pointed (Fig. 14a,b). The animals will remain in this shape for many hours before they round up.

There are two phenomena which are particularly cha-

racteristics of Paramecium when introduced into 0.3% gastric mucin. First, there is a marked change in form; secondly, there is a tendency towards the formation of large vacuoles in the protoplasm. The body, originally slipper-shaped, after one or two days often becomes broadly ovate or sometimes almost circular in outline. At this state, the animals are still seen swimming either actively or sluggishly. Towards the end, the animal becomes rounded and finally the cell membrane bursts (Fig. 14 c-g).

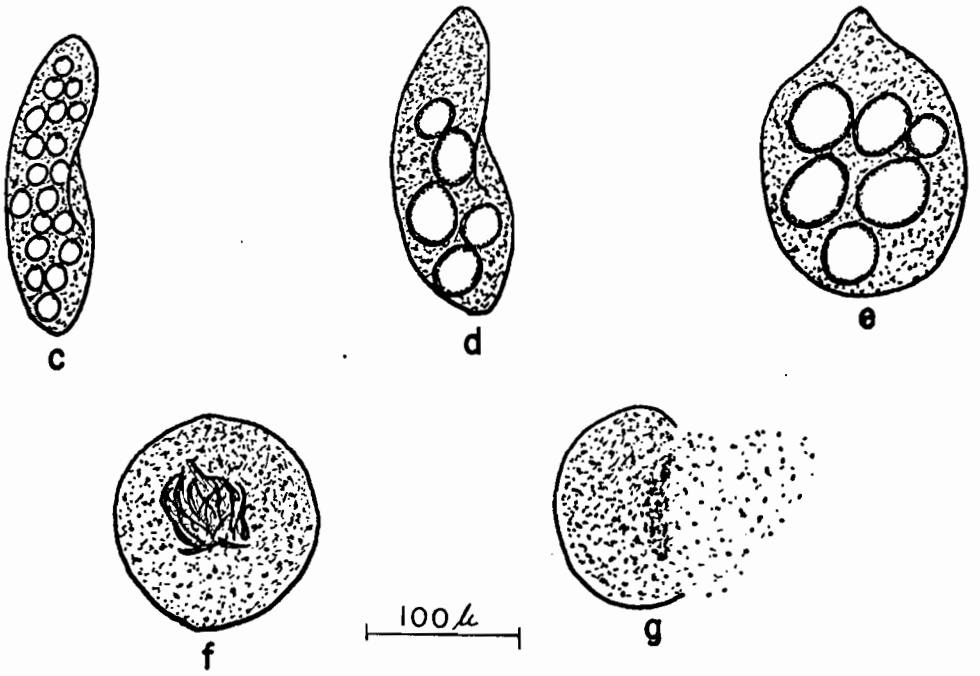
5. Hay Infusion Medium:

It is really beyond the writer's expectation that Opalinas accompanied with large bits of rectal debris could live for long in hay infusion medium, for if they are placed in distilled water or tap water they soon die. In clear hay infusion media, those that are devoid of any sort of debris, either rectal wall and its contents or decayed organic materials, Opalinas can not tolerate that long. Usually, they live from one hour to three hours. In most cases, all forward progression ceases after an hour, with the exception of 1 or 2 individuals that posses extremely high resistance. Some of them start to round up an hour later. Most of their bodies enlarge and become ovoid in shape. It is in this state that the cilia still keep on beating and may continue



Opalina obtrigonoidea

The swelling of anterior end and subsequent pointing of the posterior end.



Paramecium caudatum

The gradual rounding-up accompanied by the formation of large vacuoles in the protoplasm. Finally, the cell membrane bursts.

Fig. 14

even after 2 hours. The nuclei become less distinct and can not be clearly seen under the binocular microscope.

Undoubtedly, of all the media, this is the best medium for the growth of Paramecia. The different conditions in the medium that affect the growth, population and size of these organisms together with the factors that will determine the life of a certain culture medium are discussed in the first part of the thesis. As a matter of fact, when 2 drops of liquid containing Paramecia are inoculated into 4 ml. hay infusion medium that has a pH of 7, they can live *indefinitely and move about in a fairly normal manner. One thing of interest was that, there is not only an appreciable gradual narrowing of the body, but the body also becomes remarkably more and more transparent. The Paramecia in this medium do not show any active division and hence never become abundant. On the other hand, those that are inoculated into the same hay infusion medium as above but accompanied with some bits of debris, do not show any considerable narrowing and transparency. It should also be noted that in both media, the same kind of hay infusion medium is added whenever it is necessary to compensate the liquids that have evaporated when left over for a long period of time.

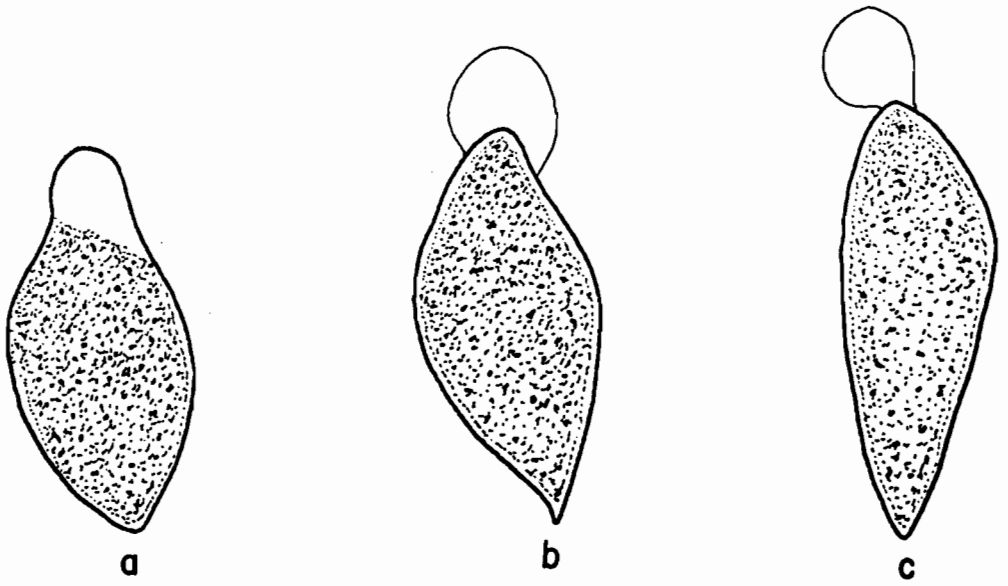
* still alive at the time of writing

6. Distilled Water:

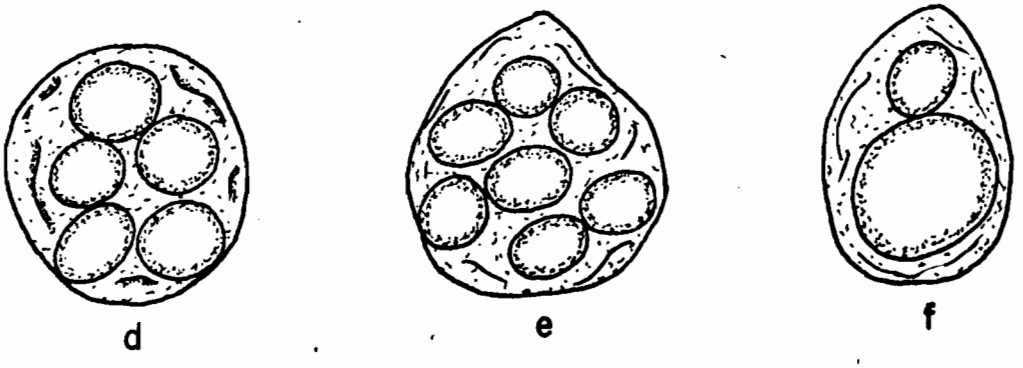
The distilled water used in this experiment is the common laboratory distilled water. No attempt was further made to re-distill it.

Opalinas, when being introduced into distilled water can survive from 3 to 6 hours depending upon individual resistance. Usually, after an hour or so, their body seems to be swollen and nuclei remarkably clear. At the moment when the body seems to be swollen, blisters start to form at one end of the body. This occurs mostly in the anterior region (Fig. 15a-c). Even in this state, the Opalinas still keep on swimming slowly. Approximately two hours later, some of them start to round up and become more and more ovate. As they assume the spherical form, there is a tendency towards the formation of large vacuoles in the protoplasm (Fig. 15d-f), but this phenomenon does not commonly occur in all Opalinas.

As for the Paramecia, approximately 3 days after inoculated into the petri dish containing distilled water, they start to show change in their form by a decrease in width just as those that have been left in clear hay infusion medium. The transparency and narrowing of the body also becomes more and more marked as days go by. All the animals



Formation of blister in the anterior region



The rounding up of the cell accompanied by the formation of large vacuoles in the protoplasm

100 μ Opalina obtrigonoidea

Fig. 15

swim fairly normally at a slow speed, but if mechanically stimulated, such as with a fine dissecting needle, they will increase their speed of locomotion immediately. They resemble those in clear hay infusion, in that they do not show any sign of active division and thus never become abundant inspite of the fact that they can live in this medium *indefinitely.

7. Tap Water:

As is generally known, the amount of minerals present in the tap water differs not only from time to time but also from place to place. This has a marked influence in the results obtained. For example, in a series of experiments on Opalinas, one have different average lengths of life from 15 minutes to 4 hours. Especially in the case of Paramecia, the average length of life ranges from 2 minutes to 72 hours and even up to months. In other words, the higher the concentration of minerals in the tap water, the higher and the faster the death rate of the animals.

The morphological and locomotional changes as shown by the lethal effects of tap water upon Opalinas are more or less similar to those brought about by the effect of distilled water. For the Paramecia, those that are in tap water of lower mineral concentration can live very long and at the

*still alive at the time of writing

same time show the same narrowing and transparency of the body as those in distilled water. But for those that are introduced into a higher concentration of minerals, death may occur within a few minutes. As soon as they are introduced into the medium, they tend to show different pronounced abnormalities in their locomotion. Some Paramecia stop making any forward progression at once, but keep on rotating the body rapidly; others swim forward at a surprisingly rapid speed with the body continuously swerving from side to side; and in still others, there is a tendency for the anterior end alone to swing about a large circle. Prior to their death, there is also swelling of the body so that they become oval in shape. The enlargement of the contractile vacuoles is very prominent here and the macronucleus is remarkably distinct. There are also more blisters formed in tap water of higher mineral concentration wherein Paramecia die very soon. Usually, a single blister starts forming at the side first, then a smaller one follows in the anterior region and finally in the posterior region. Quite often too, many small clear droplets appear simultaneously and instantaneously on different surfaces of the cell. In all cases, the length of life, the time in which disintegration begins and the rate of advance of disintegration over the body are faster in tap water of higher mineral concentration.

In general, the typical course of death and disintegration occurring in each organism is the same in all cases. But the length of life, the time in which disintegration begins and the rate of advance of disintegration over the body vary in different media as well as in different individuals and groups within wide limits.

The usual course of death and disintegration taken by both organisms is described separately as follows:

Opalinas, when placed in the various media, at once begin to swim faster than before, either in a forward progression with occassional spiralling or rotating in a circular direction. This peculiarity in locomotion is comparatively less remarkable than in Paramecia. The movement may continue for a period ranging from a few minutes to half an hour. Gradually, it will become less and less marked until the animal slowly quiets down. Nevertheless, its cilia still keep on beating until the advancing wave of disintegration reaches it. Before disintegration begins, some of them lose their flattened triangular form and become slightly swollen (Fig. 16a); others may have a slight swelling or none at all. The degree of swelling is different in different media and with differing susceptibilities of the individuals.

At this stage, their movement still continues and the anterior end is still distinguishable by its broader shape. Most disintegrations start to occur while the animal is slowly quieting or when it is completely motionless. The disintegration when it finally occurs, often starts in a region near the anterior end by the formation of a blister. It very rarely occurs at the sides or in the posterior region and the number of blisters formed is seldom more than one. This blister is mostly crystal-clear and devoid of any cytoplasm, so that the boundary between it and the body proper is always sharp (Fig. 16b). It may also occur in the other way, instead of forming a blister, a region in the anterior end just swells and bulges giving a clear swollen area devoid of any amount of granular cytoplasm (Fig. 16c). After all the ciliary movements have ceased and the animal no longer moves when touched by a fine dissecting needle, it is generally considered dead. Sometime after death, the cell body characteristically rounds up (Fig. 16d), the time ranging from approximately 10 minutes to several hours after all movements have come to a stop. All the cellular structures are dissolved and the nuclei are no longer distinguishable. The cytoplasm merely assumes a granular appearance (Fig. 16e). The cilia covering the entire body may be completely shed or may still be seen irregularly attached to the surface of the body (Fig. 16f). This

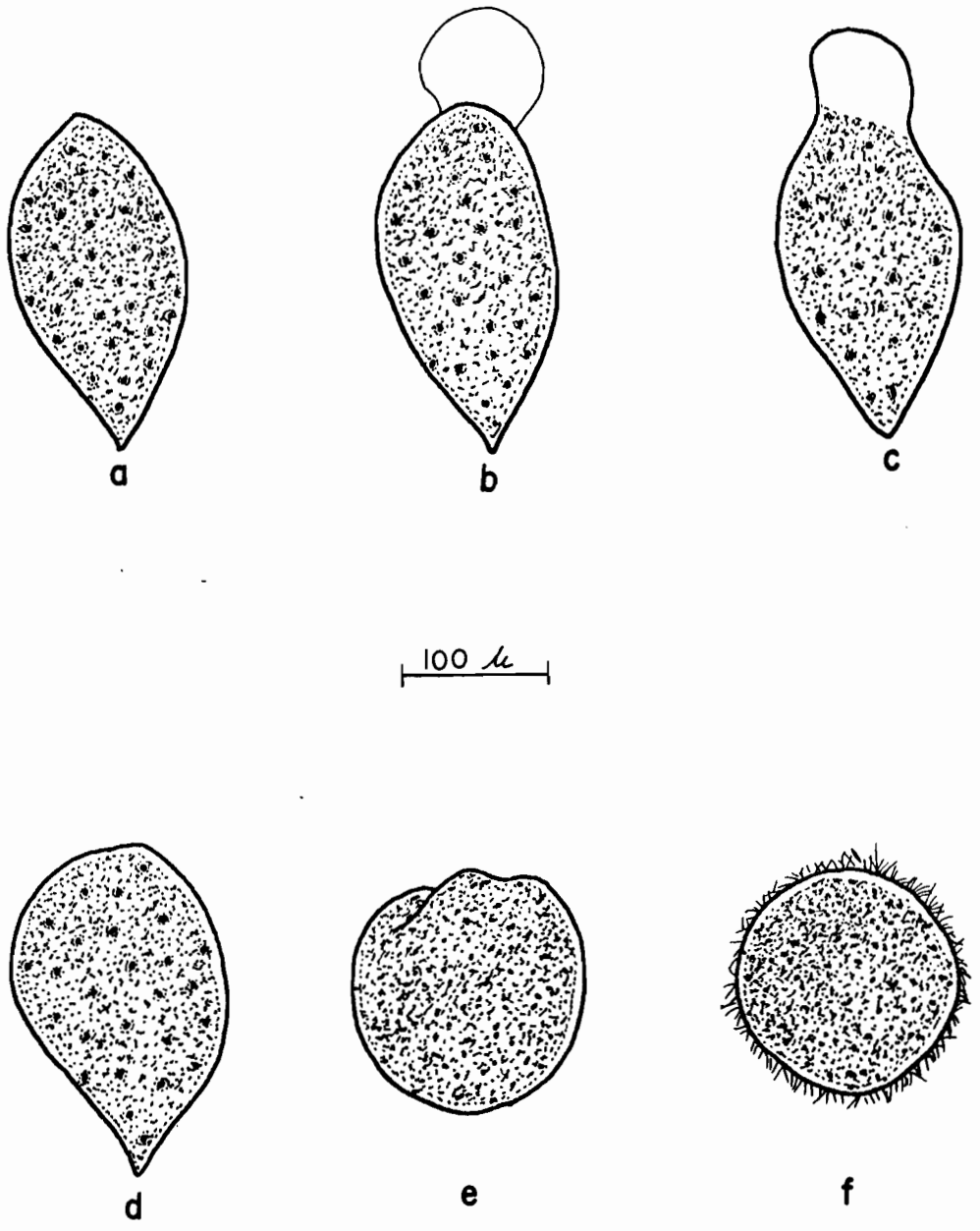


Fig. 16

The typical course of death and disintegration occurs in Opalina obtrigonoidea

rounded form, when left over-night will gradually decrease in diameter until it completely dissappears.

The Paramecia on the other hand, when transferred to the various media, at once show surprisingly rapid speed in swimming. Several striking peculiarities in their swimming may be frequently observed. Such as, the tendency of the anterior end alone to rotate while making its forward progression; the short and narrow spiral path; the continuous swerving of body from side to side; the anterior end alone swings about a large circle. This movement may continue for a period ranging from a few minutes to half an hour, or in some cases longer. Like the Opalinas, the peculiar movement will gradually becomes less and less marked until the entire animal comes to a standstill position. Their cilia seldom at once cease beating while the animal becomes motionless and will only come to a stop when disintegration reaches it. Also, before disintegration begins most of them swell and become ovate in shape (Fig. 17a). The degree of swelling also differs very considerably with the resistance of each individual to different media. The swelling may occur simultaneously with the enlargement of the contractile vacuoles (Fig. 17b) or may sometime occur after the enlargement. The contractile vacuoles, while undergoing enlargement, gradually lose their rhythmic pulsation and subsequently completely

dissappear. One of the most striking peculiarities that distinguish Paramecia from Opalinas in the course of disintegration is that the blistering of the body, when it occurs, in a majority of cases begins on the mid-ventral side instead of the anterior region. Usually, while the animal is still slowly swimming, a region near the oral groove first begins to form a blister (Fig. 17c). This blister is also characterized by the complete absence of the ectoplasmic structure and cilia. It merely appears as a clear hyaline structure, so that the boundary between it and the body proper is also sharp. From the mid-ventral region, the blister enlarges; as a result the animal decreases in size (Fig. 17d,e), and finally the cell membrane bursts. Droplets of cytoplasm can be seen flowing out from the burst membrane into the water (Fig. 17f). Finally the animal disintegrates and assumes a shapeless mass (Fig. 17g). However, in many cases, there is more than one blister formation. If it does occur, the first blister also starts forming at the mid-ventral side, then proceeds to the anterior region and lastly to the posterior region (Fig. 18a,b,c). In a few cases, blisters may also start at the anterior region near the anterior contractile vacuole, or they may start at the posterior region near the posterior contractile vacuole (Fig. 18d,e). In such cases, the blister occurs more frequently in the anterior

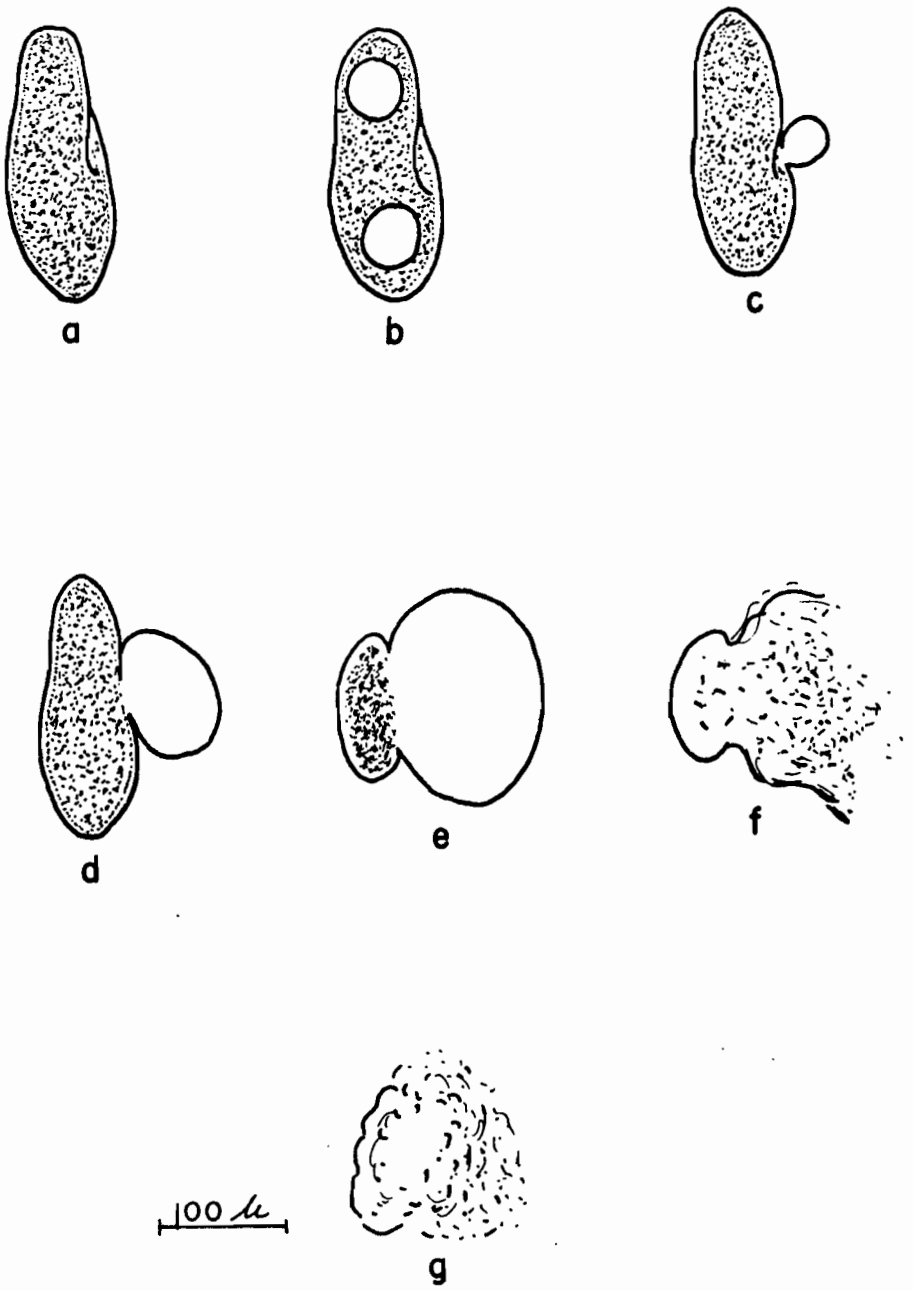
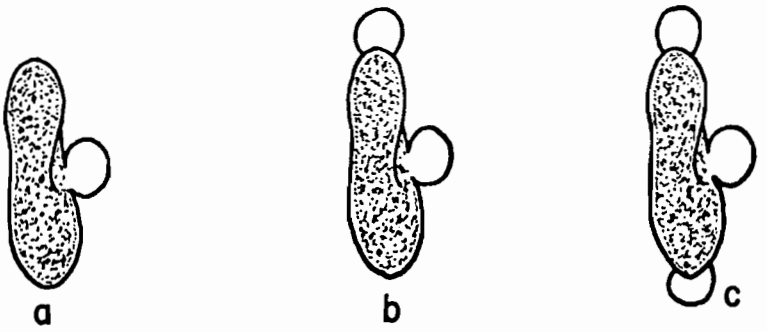


Fig. 17

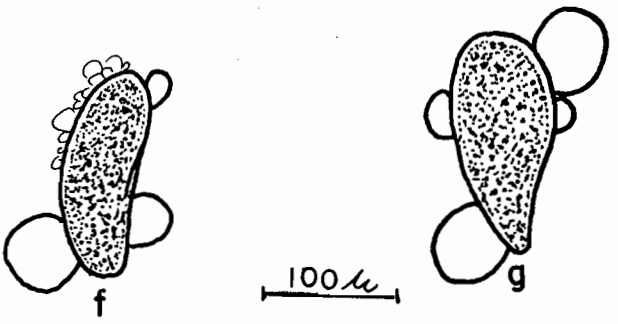
The typical course of death and disintegration occurs in Paramecium caudatum

region than in the posterior region. Where Paramecia die very quickly in those media, blisters of different sizes form simultaneously and instantaneously at different surfaces of the body in a few seconds (Fig. 18f,g). It is in this state, or at this stage that the usual course of death and disintegration can not be followed completely. At this point, they show very great differences from the Opalinas, in which very rarely more than one blister is formed under any circumstances. Moreover, they also greatly differ from Opalinas by their higher incidence of membrane rupture.



If there is more than one blister formation, the first blister usually starts forming at the mid-ventral side, then proceeds to anterior region and lastly to the posterior region.

In some cases, blister may also starts at the anterior region near the anterior contractile vacuole, or at the posterior region near the posterior contractile vacuole.



Where in Paramecia die very soon in those media, blisters of different sizes form simultaneously and instantaneously at different surfaces of the cell.

Fig. 18

DISCUSSION

The exact cause of the variation in individual and group resistance can not be clearly interpreted. This question is likely to prove to be a complex one dependent on many interrelated factors. It includes not only the complex environmental factors such as food, temperature, hydrogen-ion concentration, viscosity, osmotic pressure, water, oxygen tension etc, but it also depends on the physiological state of the animals. For example, in the case of Opalinas, whose habitat is the rectum, the rectum is always undergoing regular physiological changes relative to the feeding habits of the frogs. There may be times when the specific environmental conditions necessary for the growth and development are absent, so that they are weaker and become less resistant when subjected to unfavorable conditions. The physiological state of an animal may also influence the degree of resistance. This was frequently observed in a medium where both large and small Opalinas are present. The large Opalinas usually swim slower than the small Opalinas and they also die more readily than the small ones when subjected to unfavorable environmental conditions. For this reason, in every experiment, the writer tried to get the animals as nearly the same size as possible. The writer believes, each of these factors, to a greater or lesser degree influence the resistance of the organisms whether as an individual or as a group, and suggests that much more

experimental work will be required before any specific answer to the cause can definitely be determined.

The writer agrees with the view of Larson (1928), that the reason Opalinas live longer in a culture containing fecal material and pieces of rectal wall is probably that these may have furnished food in some form and brought about more favorable food conditions. In addition to this, it is believed that the presence of the debris or scum may also hinder the effect of the various liquid media so that their longevity are prolonged.

The abnormal division that was observed in one experiment cannot be due to the direct effect of the unfavorable environmental conditions, for it did not occur in the many other experiments that were repeated under identical conditions. It cannot also be the attempted copulation of the microgametes and macrogametes, although they resemble each other to a certain extent. One reason for doubting this is the fact that the Opalinas have never been seen coming together as a result of fusion. Rather, it is thought that the division is one of the three kinds of division observed by Metcalf (1909) in Opalina obtrigona and Opalina ranarum. The abnormal division described above is probably an irregular division of this type.

Daniel (1908), Estabrook (1910) attributed the inability of the Paramecia to reproduce abundantly in distilled water as mainly due to the deficiency of food in the environment. It is believed that this lack of food also brought about the narrowing and transparency of the body, for the Paramecia have to utilize the reserve food materials contained in their vacuoles. Thus the food vacuoles are gradually lost and the animals become narrow and transparent. There is reason to believe so, because when they are supplied with food in the form of bread crumbs, the Paramecia returned to normal again. In addition to this, it was observed that in clear hay infusion medium devoid of any organic materials, this same phenomenon of narrowing and paling also occurred.

The Pütter solution proved to be a better medium for keeping Opalinas especially if boiled and renewed daily, accompanied by the addition of a drop of albumin. But for Paramecia, it produces an immediate fatal effect. This suggests strongly that one of the important factors related to the growth and survival of both organisms is oxygen. By boiling, oxygen is liberated and the medium which is devoid of oxygen creates a more favorable environment for Opalinas and a most unfavorable environment for Paramecia. In other words, oxygen is a vital factor that determines the survival of these orga-

nisms. Opalinas favor an anaerobic environment and Paramecia favor an aerobic environment. To avoid free oxygen in the Opalina culture, according to Metcalf (1930) is considered as one of the major desiderata in culturing Opalinas. The earlier investigators such as Konsuloff (1922), Tyler (1926), Larson and her associates (1925-28) also made the same observation. Therefore, great care to exclude oxygen as much as possible must always be taken in the inoculation. Kitching (1939) in a paper on the effects of a lack of oxygen and of low oxygen tension on Paramecium, observed that Paramecium has a limited period of anaerobic survival, during which period it undergoes visible abnormal cytological changes. The cytological changes observed by the writer accompanying exposure of Paramecium to anaerobic environment is in entire accord as that description.

The death of all Paramecia are characteristically accompanied by a swelling in size, bursting of the cell membrane and finally the disintegration of the animals. According to Bovie (1916) the swelling and disintegration may possibly be due to hydrolytic cleavage of molecules of the protoplasmic constituents and an accompanying increase of internal osmotic pressure. It is believed that the result is not due merely to an increase of osmotic pressure but is at least

partly due to actual injury of the cell membrane and of the contractile vacuole, whereby the former loses its protective impermeability and the latter loses its water regulatory power. Frequently one finds that the bursting of cell membranes occur even if the animal has not become markedly swollen and may also occur long after the animal has swollen. Furthermore, after one blister has formed, thus supposedly relieving the internal pressure, others may form in quick succession at other parts of the cell surface. There is also reason to believe that the swelling in size is partly due to the actual injury of the contractile vacuole, since it is often observed that swelling takes place at the same time while the contractile vacuoles are undergoing enlargement or after they have enlarged and are no longer seen performing their rhythmic pulsation.

As to the frequency of the bursting of cell membrane and the formation of blisters which occur more often in Paramecia than in Opalinas, this may be associated with the differing delicacy of their cell membranes. The Opalinas inhabiting one of the most hazardous habitats-vertebrate gut, must have possessed a cell membrane of higher powers of resistance than those of Paramecia. In other words, the delicacy of the cell membrane may be correlated with the low pow-

er of resistance. It is thought that this different delicacy does not exist only in the cell membrane of different species but might also exist in different parts of the cell membrane itself. This may be supported by the fact that the course of disintegration, when it begins, usually starts uniformly among Paramecia and Opalinas in a certain region of the body surface, and is found to be specific in different species. For example, in Paramecia, disintegration is most likely to begin at the mid-ventral side, in Opalinas, at the anterior end. The writer believes that the weakness of the cell membrane in these regions plays a part in the localization of the swelling.

In the investigation of the reaction of Opalinas in various experimental media, it was found out that the results obtained are somewhat different from those of earlier investigators (Larson, Van Epp, Brooks, 1925; Larson, 1928). In most cases, the longevity of Opalinas in the same media under the conditions of the present investigation are shorter. The possible reasons are believed to be due to the differences in the size of drops containing Opalinas, in the amount of liquid media used, and in the size of Opalinas inoculated. The earlier investigations did not mention the standard size of drops, the exact amount of liquid used and the approximate

size of Opalinas. It is thought that the present experiments may have inoculated a larger drop, or used a larger amount of liquid media as well as larger Opalinas.

There also exists a great difference in the longevity of both organisms in tap water. In working with Opalinas, the earlier investigators obtained a range in length of life from 5 to 13 hours; while in the present observation there is a much wider range as from 10 minutes to 7 hours. In Paramecia, this appears to be much the same. At one time they can survive for months, at another for approximately 72 hours and still at another for only 5 minutes. The difference is thought to be related to the different concentrations of minerals present in the tap water. Those that survive a longer time were inoculated into tap water obtained from the old biology building which may have contained a lower concentration of minerals and those that survive only for a very short time were inoculated into tap water obtained from the new Stewart biology building which possibly contains higher concentration of minerals. Moreover, not only tap water from different places gives different results but that taken from the same place at different times also gives great differences in average length of life. In all cases, the different results obtained in the longevity of both organisms in tap

water is believed to be due to a difference in the concentration of minerals. The higher the concentration the shorter their survival time, the lower the concentration the longer their survival time.

COMPARISON OF BEHAVIOR UNDER DIFFERENT PHYSICAL CONDITIONS

1. REACTION TO GRAVITY:

Ever since the last few decades of the 18th century, many papers have been published dealing in whole or in part with the reaction of Paramecium to the pull of gravity. As a matter of fact, the negative reaction of Paramecium to the pull of gravity is an old well-known fact. Glaser and Coria (1933) have used this reaction in freeing Paramecium caudatum from living micro-organism in their culture. It might well be pointed out that there always has been a wide divergence of opinions concerning the mechanism involved, so that a satisfactory explanation has never been clear. Here and there, the subject is still open to investigation. To my knowledge, the reaction of Opalina obtrigonoidea to the pull of gravity has not yet been investigated. In the present study, the writer wished to find whether or not the parasitic Opalina reacts to gravity in a manner similar to that of Paramecium.

EXPERIMENTS AND OBSERVATIONS

The apparatus and method employed by Jennings (1904) in investigating the reaction of Paramecium to gravity was used. This was done by inoculating abundant Paramecia and Opalinas separately into the base of two U-tubes, then distilled water and Frog-Ringer solution were added respectively to fill up the two arms of both tubes. The U-tubes

used in this experiment are 4½ inches in length and 0.6 mm. in diameter.

In the tube where Paramecia are found, after a period of 5 minutes or a little longer, one can see the Paramecia move as two separate groups toward the two ends of the base, forming two well-defined rings about the wall of the tube which gradually travel toward the end of the arms after a period of 35 to 45 minutes (Fig. 19a). Whereas in the tube where Opalinas are found, if they are inoculated into the base, they remain at the bottom; but if they are inoculated into the end of both arms, most of them can settle down rapidly within a period of approximately 15-20 minutes (Fig. 19b), thus indicating that Opalinas react differently from Paramecia to the pull of gravity. That is, instead of reacting negatively to the pull of gravity, they reacted positively. These observations are strongly confirmed when the U-tube is inverted. Within 20 minutes or less, Opalinas at the base are seen collected at the end of both arms (Fig. 19c). Their reaction to gravity can also be easily observed under a microscope by placing both organisms separately in a depression slide with a concave center, the Opalinas tend to gather around the center of concavity (Fig. 19d), while the Paramecia can be found swimming all around (Fig. 19e).

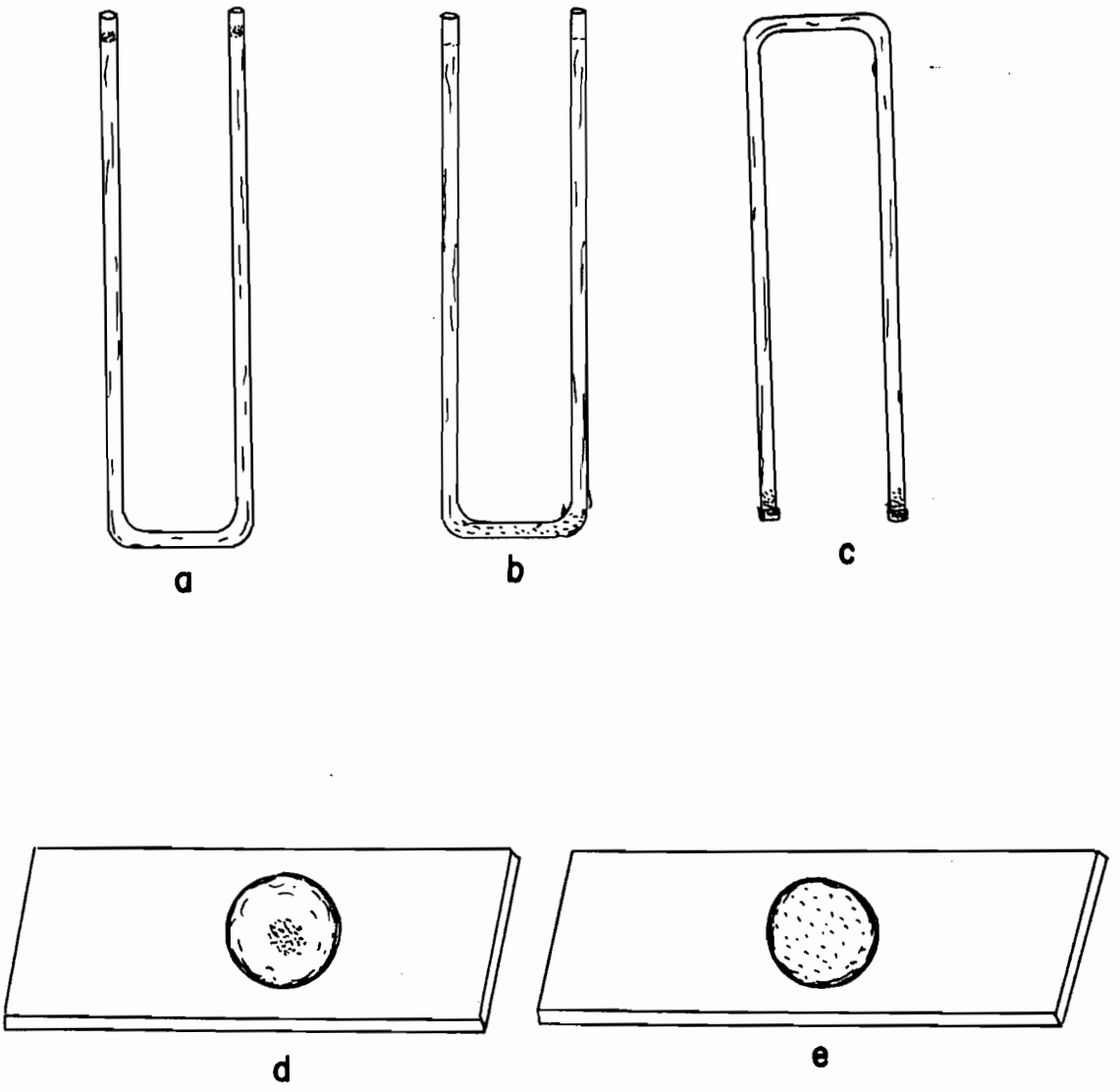


Fig. 19

Reaction to gravity

In behavioral studies, great care must be taken to always use a flat-bottomed depression slide or a flat-bottomed petri dish, so that the distribution of the Opalinas will not be affected by the concave center.

Why do the organisms swim toward or against the pull of gravity? The writer considers this reaction as due to some external conditions. Apparently, there are two factors which might determine the direction of their movement. These factors may be correlated with their habitat. As was stated before, Paramecia favor an aerobic environment and Opalinas an anaerobic one, so that the former tend to swim up to the surface where there is much oxygen and the latter tend to settle down at the bottom of the tube where there is less oxygen. This oxygen effect was tested by two simple ways: first, by placing a drop of paraffin oil on top of the liquid; second, by sealing the open end of the arms with aluminum foil. This was done inorder to prevent the diffusion of oxygen from the air into the surface of the water. Subjected to these conditions for many days, Paramecia and Opalinas show the same reaction. Thus, oxygen can not be the all important cause of their specific reaction to the pull of gravity. Another factor in their habitat which may be correlated with their reaction to gravity is light. Para-

mecia live in diffuse daylight and Opalina in total darkness; this may cause the former to swim toward the source of light and the latter to swim away from the source of light. This factor was again found not to be the prime important cause of their reaction to gravity when the same experiment was performed at night in a darkroom in which the U-tube is illuminated from below. Under such conditions, the specific reaction of both organisms to the pull of gravity remains unchanged. Moreover, even if the upper-half of the tube is shaded with photographic paper, both organisms again show the same specific reaction to the pull of gravity. The assumption that their specific reaction to gravity is mainly due to attraction to such external factors as oxygen and light is not tenable.

2. REACTION TO LIGHT:

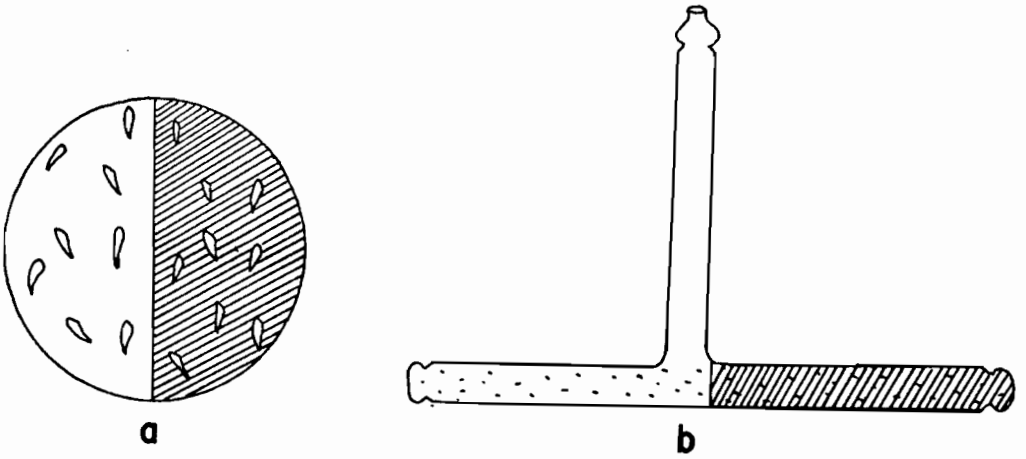
The reaction of the different species of Paramecium to light has been reported as being indifferent to ordinary light, with the exception of the green Paramecium, P. bursaria, which reacts positively to light (cited by Wichterman, 1953, p.243; Paramecium bursaria is the only one of the eight well-defined species that exhibits a symbiotic relationship because of the presence of green unicellular algae, Chlorella in the cytoplasm). Moreover, in a number of well-controlled experiments performed by earlier investigators, it has been found that there is no difference in the growth of ciliates in light and in darkness. Both maximum growth and highest fission rates can be obtained from infusion that have been kept in light and in darkness (Maupas 1888; Woodruff, 1905; Eddy, 1928). On the other hand, as far as the writer knows, no work has yet been reported concerning the reaction of Opalina obtrigonoidea to light.

EXPERIMENTS AND OBSERVATIONS

When Paramecia and Opalinas were inoculated separately into a flat-bottomed petri dish, one-half of which was shaded by photographic paper, it was seen that both of them swam freely from the light region into the dark region or vice versa (Fig. 20a). This indicating that not only Paramecia

react indifferently to ordinary light but Opalinas also react similarly. When they were inoculated separately into an inverted T-tube (Swezey & Atchley, 1935), one arm of which was darkened, both organisms also showed no preference to light or darkness. They were equally distributed in both regions of different light intensity. Their reaction to light was again studied at night in a darkroom. A flat-bottomed, half-shaded depression slide was used to contain the organisms. The slide was placed on the microscopic stage enclosed in a box lined with photographic paper. A circular opening was made on the box to permit the objective to pass through with a close fit. In this way, it will exclude the passage of any other light and allows only light thrown from the lantern on the substage mirror to be directed upward to the slide containing the organisms. To prevent the effect of heat upon these organisms, a thick glass container filled with alum solution was placed between the light source and the mirror. Under these conditions, the reaction of both organisms to light remained unchanged. Both Paramecia and Opalinas were found swimming freely from the light area into the dark area or vice versa. In all investigations, one thing worthy of note was that, after some moments, the majority of the organisms were found swimming at the darker edge of the slide (Fig. 20c).

Their reaction to light of different colors was



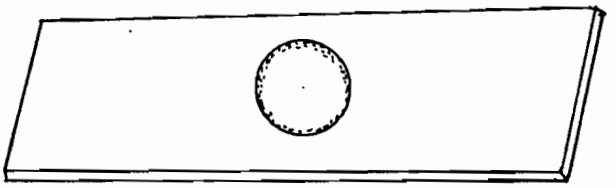


Fig. 20

Reaction to light

also investigated in the same way as mentioned above, with the only difference that the black screen was replaced by a series of color filters. Nine different color filters were used in this study. They were as follows:

1. Dark blue
2. Light blue
3. Pink
4. Light yellow
5. Dark yellow
6. Orange
7. Red
8. Light green
9. Violet

The experiment was performed in two ways, both during daylight and at night. First, by subjecting the organisms to the slide that was half-replaced by any of the color filters, until every color has been used. Second, by subjecting them to the slide both halves of which were replaced by different colored filters until all of them paired off. By manipulating the mirror, the organisms on the dish can be suddenly subjected to these different colors of light. In both ways, it was found that both organisms show no specific preference toward any particular color of light. All the organisms swim freely and are evenly distributed among microscopic fields of different colors of light.

DISCUSSION

So far as the writer is aware, among the various opinions concerning the mechanism involved in the negative geotropism of Paramecium, there are four theories which appear to be more popular. They are the mechanical, pressure, resistance and statocyst theories proposed by Verworn, Jensen, Davenport and Lyon respectively (see works and reviews as those by Davenport, 1897; Jennings, 1904a; Lyon, 1905; 1918; Kanda, 1914; 1918; Wichterman, 1953, pp.239-240). Like the writer, the first three attempted to explain the protozoan's reaction as due to some external conditions, but these interpretations have been shown to be inadequate (Lyon, 1905; Kanda, 1914). There is more literature supporting Lyon's idea that the geotropism of Paramecia is due chiefly, if not entirely, to the internal conditions of the organisms rather than external ones. He was of the opinion that "gravity acts directly upon the inner constitution of the cell. This must involve pressures or stresses within, which could only come about in a system of substances of different densities." In addition to this, the writer while investigating the comparative reaction of Opalinas and Paramecia to the pull of gravity noticed that there is an appreciable difference between the morphology of both organisms as related to the number, distribution, and length of cilia as well as a difference in their body shape. It is thought that these differences may

in one way or another be responsible for their different reaction to the pull of gravity. The cilia in the posterior tip of the Paramecia are longer than in the peripheral region; while in Opalinas, the number of cilia in the anterior region is comparatively more than those in the posterior region. Those longer cilia at the posterior tip of the Paramecia are most likely to beat more forcibly than the others, consequently propel the animal to move with its anterior end directed upward. The more numerous cilia at the anterior region of the Opalinas may also work likewise, thus inducing the animal to swim with its anterior end directed downward. The shape of the body of Paramecium would indicate that the posterior region is heavier, this heavier posterior region with its stronger ciliary activity may reenforce the upward movement of its anterior end. On account of the broader anterior region in Opalinas, the anterior region must be heavier than the posterior region. This heavier anterior region may also work together with the more forceful anterior ciliary beating in determining their downward direction of movement.

The observation of Swezey and Atchley (1935) that Paramecia prefer the darkened portion of a slide has not been corroborated; rather, the Paramecia have been observed to distribute themselves evenly in dark and light areas of a

half-shaded slide. Opalinas have been observed to react in the same manner. It is true that both organisms tend to gather around the edge of a slide, which is apparently darker than any other areas. But it is thought that this is not a matter of their preference to darkness. For if they definitely react negatively to light, the gathering of more organisms in dark areas should also occur in a half-shaded slide. The writer believes that the tendency of gathering around the darker edge of a slide may be due to the attraction of a solid contact-glass surface. There are reasons to believe so, for they are always found adhering to the side of a pipette and also at the wall of a wide mouth beaker in an hay infusion medium. Moreover, when teased pieces of paper are placed into the dish containing the organisms, the organisms tend to rest at the sides of these papers after some time. They also react similarly to the food particles present in the medium. The fact that both organisms produce no pronounced response to any particular color of light may be related to their habitats. Paramecia live in diffuse daylight, wherein they are always exposed to different colors of light, hence produce no marked response when re-subjected under same conditions. Opalinas on the other hand, live in total darkness, which may render them unable to produce any marked response toward the different colors of light.

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3. REACTION TO HEAT:

In the present investigation, the writer has the purpose to determine the end points of temperature tolerance for Opalina and Paramecium and to compare their relative tolerance at various intermediate temperatures. The end point is defined as that temperature at which all the organisms were dead after a certain number of hours.

It has been shown by many early investigators that the problem of the resistance of protozoa to temperature is not a simple one. The temperature characteristics vary in same species under different conditions. For example, Kahler, Chalkley and Voegtlein (1929) noted that the same species of Paramecium from different cultures differ slightly in their resistance. A year later (1930), Chalkley further reported that the pH of the medium containing Paramecium has a close correlation with the time required to kill the organisms. He obtained a curve of resistance that is bimodal with the different pH values, and observed a maximum of resistance in the alkaline and acid ranges and a minimum at neutrality. Garner (1934), in his study of the relation of numbers of Paramecium caudatum to their ability to withstand high temperature verified Robertson's (1924b) observation that a culture initially containing a small number of individual shows less re-

sistance to heat than a densely populated one. Doudoroff (1936) reported that in tests with heat and cold, Paramecium multinucleatum was found to be more resistant when starving than when well-fed, and the same phenomenon was found to occur in Paramecium caudatum by Giese and Reed (1940).

It is a well known fact that organisms can stand a higher temperature, if the latter is raised gradually than if it is raised suddenly. The phenomenon is referred to as adaptation. Dallinger (1887; cited by Giese, 1963, p.193) stated that he was able to adapt certain protozoa to a temperature of 70°C by raising their temperature very gradually over a period of seven years. Therefore, in considering the upper and lower lethal temperature limits that required to kill the organisms, it is extremely important to mention the temperature range to which the organisms have been previously adapted. In the matter of adaptation, Sukhanova (1959) reported that Opalina ranarum shows quite definite changes in their temperature tolerance with the seasons of the year. In 1963, she indicated again that the resistance of Opalina ranarum depends on the temperature of the surrounding medium; they have high heat resistance at high temperature and low heat resistance at low temperature, the same general effect

was found to be the case in Paramecium caudatum and Opalina studied by Poljansky (1963). In the same year (1963), Poljansky studied the capacity of Paramecium caudatum to stand sub-zero temperature reported that clones of Paramecium caudatum which have been previously cultured at relatively high temperature appear to have a very low cold-resistance; while same clone of Paramecia cultivated at a relatively low temperature are observed to possess a much higher cold-resistance.

As shown by the earlier investigations, the effect of temperature upon the protozoan is accompanied by many variable factors. In studying the reaction of any organism to temperature, it is very important to remenber that all the other environmental factors helping to determine such a relation must be taken into consideration and exact statements of these conditions must be made. In the present investigation, the writer aimed at accomplishing the purpose of this study by taking into consideration as much as possible the known factors that will affect the longevity of these organisms.

PRECAUTIONS AND METHODS

It should be mentioned that all the precautions observed in the previous experiments were all taken into consideration at the present investigation. In addition to

those, it is necessary to note that preliminary experiments had shown that Paramecia taken from the same culture medium on different days show greater difference in their resistance than those taken from different cultures of the same age. Therefore, no attempt was made to take them out from the same culture; rather many infusion media were set up at various intervals, so that during the course of the experiment, a continuous supply of Paramecia from hay infusion media having approximately the same age and pH value could be obtained. The age of the cultures was usually 9-12 days old, with a pH of approximately 7.2. These media were well provided with food as well as densely populated. The temperature of the medium was approximately 22°C-24°C. The Opalinas on the other hand, were removed from the frog's rectum well provided with food particles. These frogs were kept at room temperature. All the time, only large number of Opalinas crowded in the same host organ were used in the investigation of their longevity and behavior at a particular temperature. The standard drops containing Opalinas and Paramecia were two, about 0.1 mm. in diameter each and the amount of liquid medium being 0.2 ml. The approximate number of organisms inoculated is about 30-40. The investigation of their comparative behavior to high and low temperatures was carried out during the month of June and August respectively.

The organisms were placed in a small glass dish mounted on a piece of wood. This small glass dish has a diameter and depth similar as that of an ordinary flat-bottomed depression slide. Its base was made of a thin cover slip so as to facilitate the penetration of heat into the medium containing the organisms when the glass dish was directly in contact with the heated water. A can, on which two pairs of parallel incisions were made from the open ends down to the middle of the wall was used as a water bath. The portions between the two incisions on each side of the wall were folded down to form two flaps. One of which was folded in such a way that it becomes parallel with the base of the can; it was on this flap that the wood-mounted glass dish was rested. The other flap was folded down against the wall and eventually allowed a place for the inlet and outlet of the water. This can was filled with water preheated to the experimental temperature. The water was allowed to cover over the flap that holds the wood-mounted glass dish, so that when the dish was placed, its base was in direct contact with the heated water. In this way, the diffusion of heat into the glass dish took place faster. The temperature of the water was kept constant at the experimental temperature by withdrawing or adding water through the outlet and inlet. A glass rod was used to stir the water whenever necessary to aid the diffusion of

the hot water, so that thermal equilibrium could quickly be established.

When a sudden exposure was desired, the water in the water bath and in the glass dish was first allowed to assume the proper temperature. Temperature was measured by the insertion of a thermistor in the small glass dish and a thermometer in the water bath. Thermistor has various types depending on their different application. The thermistor used is type 1512/300 manufactured by Standard Telephones and Cables Limited of England. This thermistor gives a big change in resistance over the temperature range $0^{\circ}C$-$50^{\circ}C$. Thermistor is considered to be more advantageous over a thermocouple commonly used by most investigators in measuring temperature (see Platt & Wolf, 1950). It has long been widely used in industry and in the physical sciences. But the use in academic biological science has expanded only in recent years. To measure the temperature in the small glass dish with the use of a thermistor, the writer employed the simple portable wheatstone bridge as circuit design for direct temperature indication. Figure 21 is a wiring diagram of the wheatstone bridge used. The general characteristic of a thermistor is that its resistance decreases rapidly with the rising temperature and increases rapidly with lowering temperature. By knowing the re-

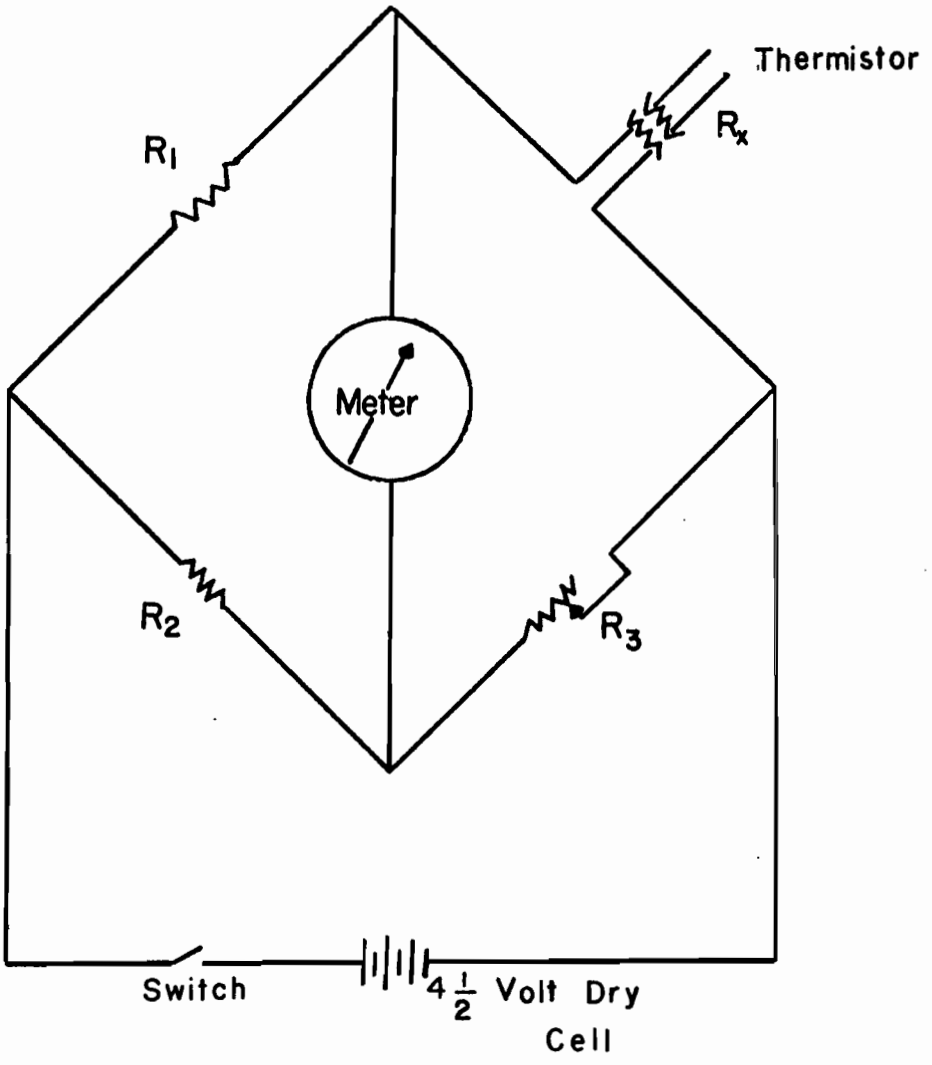

Fig. 21

Wiring diagram of a simple wheatstone bridge

sistance, one will be able to find a quite good approximation of the temperature at that particular resistance by using the graph provided by Standard Telephones and Cables Limited of England (Fig. 22). There is one point that the writer found by experience that one should be aware of before using any thermistor. Each thermistor must be independently calibrated against an accurate thermometer so that the resistance-temperature curve looks like the one in the graph.

When the water in the small dish has reached approximately the temperature of that water bath, a considerable number of the organisms in the smallest possible quantity of water were inoculated into the small glass dish in a capillary pipette. This method of subjecting the organisms to a given temperature immediately will eliminate the period of adaptation. It should be noted of course, that the inoculation of organisms causes a slight drop from the experimental temperature as indicated by the deflection of the galvanometer. But the difference rapidly decreased and the temperature in the glass dish assumes the same temperature as that of the water bath in a few seconds.

The time element recorded for various reactions at any desired temperature was made with the use of a stop watch. A binocular dissecting microscope giving magnifications from

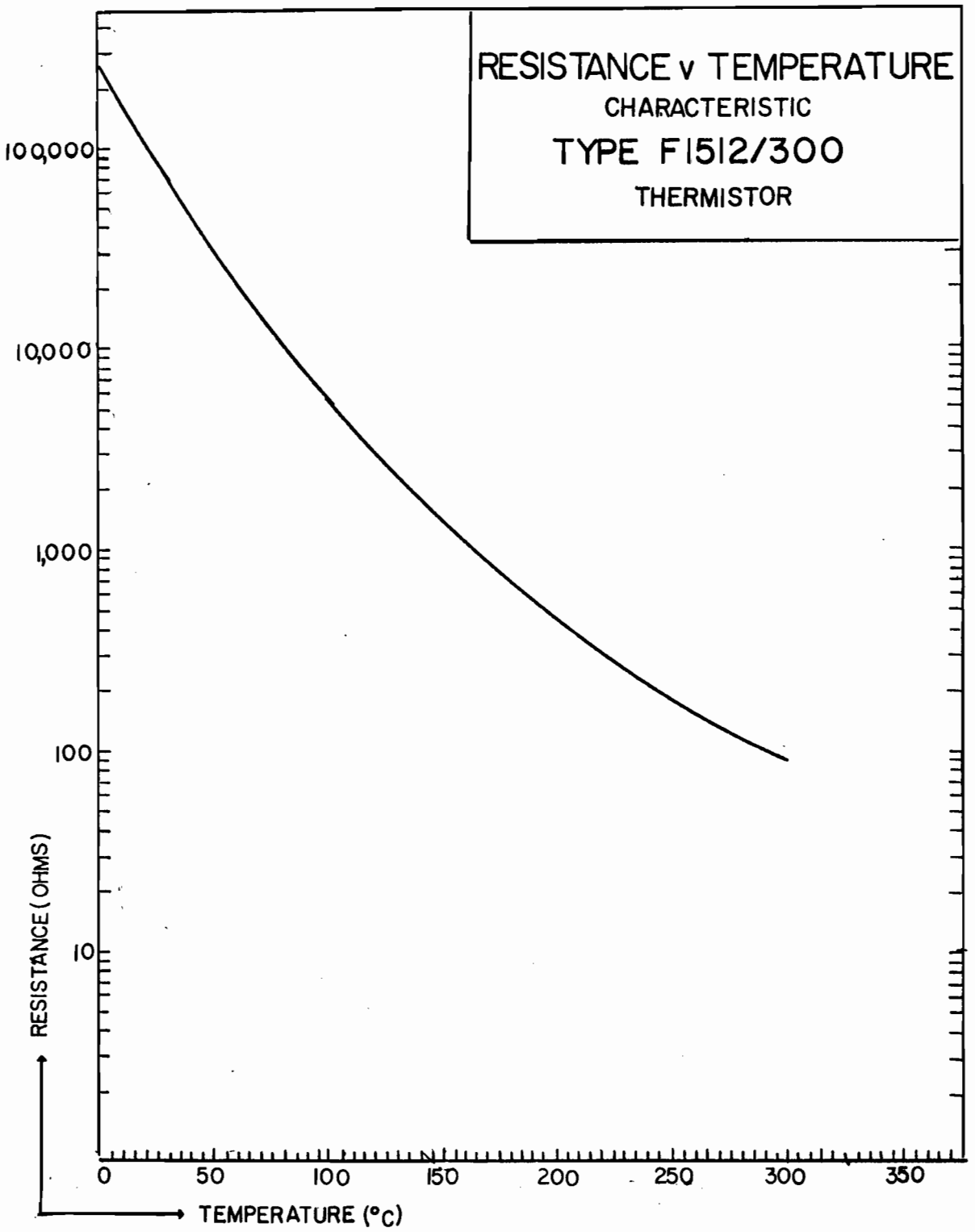
RESISTANCE v TEMPERATURE
CHARACTERISTIC
TYPE F1512/300
THERMISTOR
100,000
10,000
1,000
100
10
RESISTANCE (OHMS)
0
50
100
150
200
250
300
350
TEMPERATURE (°C)

Fig. 22

6X-40X was used for observation. The ciliary activity however, was measured without any mechanical aid but simply by direct observation in their movements, and were described in such terms as "fast", "slow" or "stopped."

As was mentioned before, the individual differences in resistance are considerable. Frequently, a few individuals possesing high resistance are still found swimming slowly long after all the others had succumbed. For this reason, the sample being considered dead if 98% of the organisms in it were dead. Also, the sample rather than the organisms was taken as a unit; this will eliminate the loss of time accompanying individual counts (Doudoroff, 1936).

RESULTS AND OBSERVATIONS

The following table shows a comparison of the relative tolerance of the organisms under study at various temperatures:

TABLE 5

Temp.	No. of expt.	Species	Average Length of Life	Range in Length of Life
30°C	3	O.	All survived	
	3	P.	All survived	
32°C	3	O.	All survived	
	3	P.	All survived	
34°C	3	O.	All survived	
	3	P.	All survived	
36°C	3	O.	All survived	
	3	P.	All survived	
38°C	3	O.	All survived	
	3	P.	All survived	
39°C	5	O.	11'57"	11'45"---12'08"
	5	P.	25'21" (50%)	24'22"---28'24"
40°C	5	O.	6'54"	5'59"---7'56"
	5	P.	11'34"	11'12"---11'59"
42°C	5	O.	2'10"	2'08"----2'14"
	5	P.	2'42"	2'10"----2'59"
44°C	5	O.	1'32"	1'-------2'05"
	5	P.	1'40"	1'12"----1'58"
46°C	5	O.	38"	33"------40"
	5	P.	43"	38"------48"
48°C	5	O.	30"	25"------38"
	5	P.	40"	38"------45"

Temp.	No. of Expt.	Species	Average Length of Life	Range in Length of Life
50°C	5	O.	22"	19"-----28"
	5	P.	36"	30"-----40"
52°C	5	O.	19"	16"-----32"
	5	P.	30"	26"-----32"

The writer has observed Opalinas and Paramecia inoculated into media preheated to the experimental temperatures as 30°C, 32°C, 34°C, 36°C, 38°C for 3 hours each. It was found that at the end of the 3 hours, all of them are still found alive. Many of them may appear to the observer as being dead, for after a period of general restlessness, they become quiescent. But when they are mechanically stimulated by being touched by a fine dissecting needle, they start to move again. It was at 39°C, that the difference in their thermal resistance was clearly shown. At this temperature, all Opalinas are killed at an exposure averaging 11'57"; while, only half of the Paramecia are killed at a comparatively longer exposure of 25'21". The remaining half of the Paramecia are still found alive at the end of 3 hours. It was at 40°C, that all Paramecia are killed. Thus, it clearly shows, that both organisms differ in tolerance to extremes of temperature. The thermal end point of Opalinas and Paramecia is 39°C and 40°C respectively. Furthermore, it also shows that when

they are subjected to temperatures above their thermal end points, Paramecia nearly always tolerate them longer than Opalinas, indicating that Paramecia can resist heat better than the Opalinas. This phenomenon can easily be demonstrated by placing a heated penny on one side of a depression slide containing the organisms. Paramecia can be seen swimming with a surprisingly rapid speed to the unheated side without being injured. Those Opalinas near the heated side of the slide also try to swim away from the unfavorable condition but they never suceeded. A few may be able to escape to the center, but due to their lower resistance to heat die after a few seconds. It was only those few Opalinas that stayed close to the unheated portion which were able to reach the unheated side un-injured. It will be seen from the results, that a lesser rate of temperature increase above the end point is more favorable to both, for the time in which all the organisms will be killed is shorter.

Some incidental observations were made on the general behavior of both organisms and the visible structural changes produced upon the organisms by heat. The most striking reaction of both organisms when exposed to high temperature is the exhibition of a general restlessness. The cilia at once begin to beat faster than ever before, resulting in an amaz-

ingly rapid movement. The organisms, due to the sudden change of environmental temperature, trying to swim away in every direction, move in a typical circular pattern. This peculiar movement is more remarkable in Paramecia than in Opalinas. The restlessness may last violently till death occurs or may slow down some time before death. In the meantime, certain other changes have also been found commonly occurring in both organisms. That is, a change in the shape of both bodies which usually become swollen into a more spherical shape. During the course of the experiment, although Paramecia are always found to tolerate heat longer than Opalinas, their death is often accompanied by the bursting of the cell membrane and subsequently the flowing out of droplets of clear protoplasm. Another further effect of heat which was only observed in Paramecia is the acceleration in the rate of vacuolar pulsation. The contractile vacuoles are observed to contract more often than before. In these cases, the anterior contractile vacuole contracts comparatively faster than the posterior contractile vacuole.

4. REACTION TO COLD:

The environmental factors and the various other conditions that will generally affect the reaction of any organism to temperature have been mentioned in the introductory part in connection with the investigation of the reaction of Opalina and Paramecium to heat. Although most of the works are concerned with higher temperature, it is believed that in the study of the effects of lower temperatures, they should not be neglected. In the present study, the points especially noted by the writer are the time required to stop normal locomotion, the time required completely to stop the beat of the cilia, and the longest possible exposure which permit recovery after normal conditions are restored.

PRECAUTIONS AND METHODS

All the precautions and methods used in the present investigation are similar to those employed in the previous experiment on high temperature. The desired low experimental temperatures were brought about by the dissolution of dry ice in alcohol. In measuring the low temperature, type F2200 thermistor was used. Figure 23 shows its resistance-temperature characteristics.

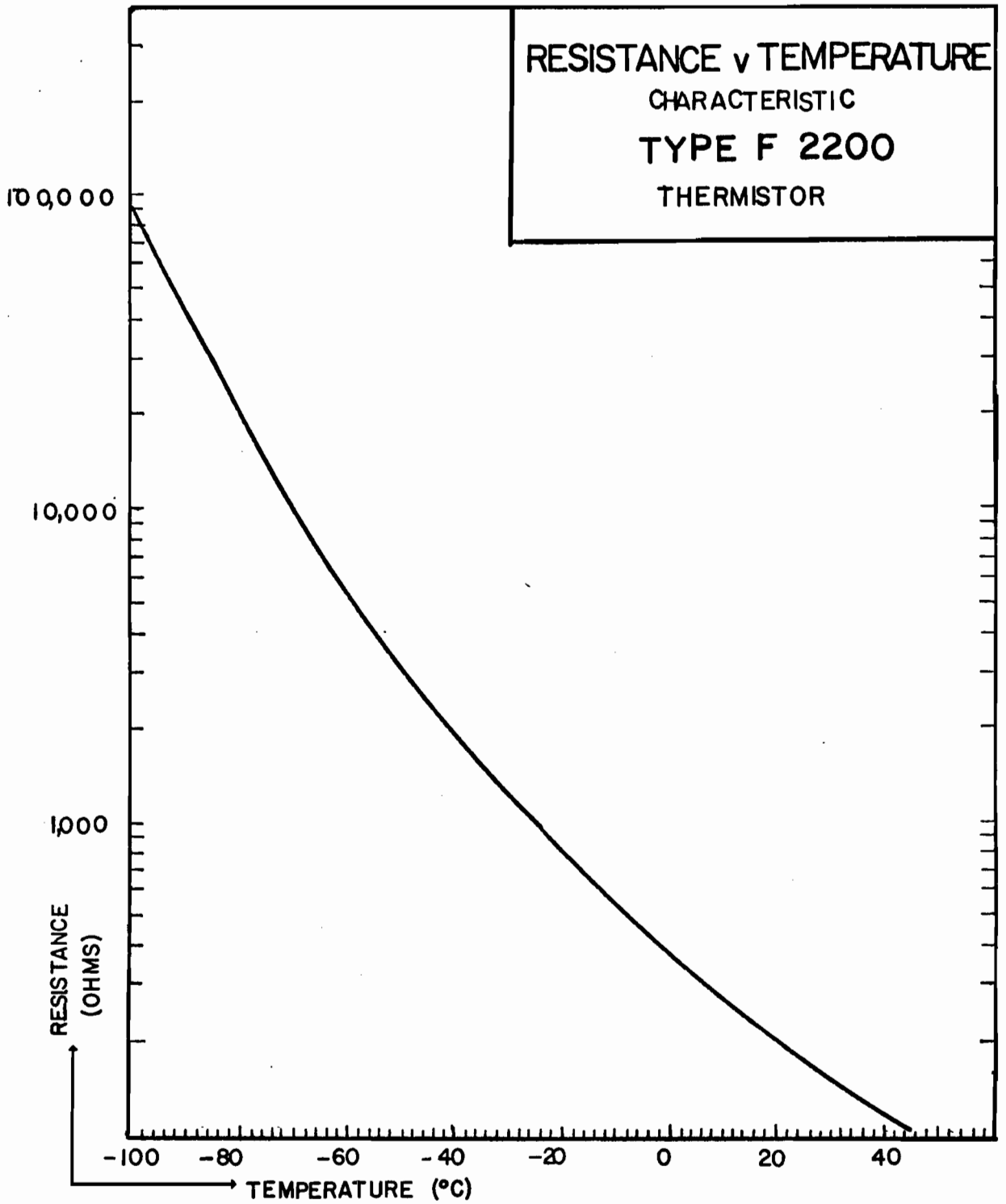
RESISTANCE v TEMPERATURE
CHARACTERISTIC
TYPE F 2200
THERMISTOR
100,000
10,000
1,000
RESISTANCE (OHMS)
-100
-80
-60
-40
-20
0
20
40
TEMPERATURE (°C)

Fig. 23

RESULTS AND OBSERVATIONS

As is generally known, the rate of movement of the organism is proportional within a limited range to the temperature. As heat increases, the rate of locomotion in an organism becomes accelerated; as it decreases, the rate of locomotion in the organism becomes retarded. This was found to be true in the case of Opalina and Paramecium. It can well be said that as soon as they are exposed to a low temperature ($5^{\circ}C$), the normal locomotion stops immediately and both organisms swim in every direction. A typical circular pattern results which Jennings referred to as "avoiding reaction." In Opalinas, this negative response is not pronounced to an observer, for at this time, their speed of locomotion are already greatly reduced and some Opalinas have even quieted down. At approximately 40 minutes after and for a long period, there is either no locomotion or this is very slow. In Paramecia, this typical circular pattern of reaction lasted at an average of 3 minutes and 8 seconds. Among the observations made, the range of time is from 2'15"-3'23". After this typical reaction, the Paramecia swim more and more slowly until they come to a standstill in a time that may vary from 25 minutes to several hours. It is worthy of note that in Paramecia, the rate of pulsation of the contractile vacuoles gradually declined and failed to reveal any pulsation during long exposure to low temperature.

Even after locomotion has ceased, the cilia of both organisms, however, keep on beating and beat more and more slowly until they eventually stop. This continuation of ciliary movement after locomotion has ceased may last for an hour or more. In low temperature, after all ciliary movements have ceased, the organisms are not necessarily dead. The cilia would quickly recover when returned to the temperature of the room. Experiments were tried to determine the longest possible exposure after which recovery is possible when normal conditions are restored. Both organisms were further left at experimental temperatures for various lengths of time after all visible movements ceased. It was observed that when the small glass dish containing Opalinas is removed from the cold bath and placed in room temperature, a considerable percentage of the individuals may recover. Table 6 shows the results of the various experiments with Opalinas.

TABLE 6

Prolonged cold-exposure time after all movements have ceased	Average exposure time in room temperature where recovery occurred	Percentage of recovery
1. 10'	12'	100%
2. 20'	18'	100%
3. 30'	24'	100%
4. 45'	46'	100%
5. 1 hr.	50'	100%
6. 1 hr. 20'	1 hr.	85%
7. 1 hr. 40'	1 hr. 20'	70%
8. 2 hrs.	1 hr. 47'	50%
9. 2 hrs. 20'	2 hrs. 5'	25%
10. 2 hrs. 40'	2 hrs. 30'	5%

In the first three experiments, the locomotion of recovered Opalinas is normal. While in experiments 4 and 5, they are comparatively less active with the locomotion gradually slowing down. The locomotion is much reduced in experiments 6, 7 and 8. Finally, in the last two experiments, those that are able to recover may swim slowly for some time and ultimately come to rest again, soon after which they round up and disintegrate. There is no record of the rupture of cell membrane in Opalinas. The results recorded in table 6 doubtless indicate that shorter exposure of the Opalinas to low temperature ($5^{o}C$) gives a higher percentage of recoveries. As for Paramecia, after the cilia have gradually slowed down and stopped, and they are immediately restored to the normal temperature, they become motile again after a period of approximately an hour. They may swim around very slowly for a while with the contractile vacuole slowly pulsating again. But soon after, all movements cease again and blisters start to form at the sides of the organisms. Similar to Opalinas, their cell membrane does not rupture, but their ability to recover when restored to the room temperature falls far behind Opalinas. It is only those Paramecia that have ceased locomotion but with cilia still continuously beating which are able to restore their normal motile condition for a longer period of time.

The ability of the Opalinas and Paramecia to recover was also tested when exposed to a frozen medium. When the temperature was lowered to the sub-zero degree and ice was allowed to form in the dish containing these organisms, all locomotion immediately quieted down. Due to the solidification and opaqueness of the surrounding medium at the sub-zero temperature, it was not possible to observe any further structural changes that occurred during this period. The following tables give the experimental results:

TABLE 7
The ability of Opalina obtrigonoidea to recover when exposed to frozen medium

Temp. of ext. cooling medium	Duration of exposure	Time required for the medium to freeze	Time exposed in frozen medium	Recovery in percentage
-20°C	1'20"	1'07"	13"	100%
	1'50"	1'08"	42"	100%
	2'10"	1'	1'10"	100%
	2'30"	1'07"	1'23"	65%
	2'50"	1'09"	1'41"	20%
	3'	1'08"	1'52"	0%
-25°C	1'05"	47"	18"	100%
	1'10"	47"	23"	100%
	1'20"	40"	40"	100%
	1'30"	1'06"	24"	95%
	1'50"	60"	50"	90%
	2'	50"	1'10"	20%
	2'10"	50"	1'20"	0%
-30°C	40"	32"	8"	100%
	54"	28"	26"	100%
	60"	30"	30"	100%
	1'10"	34"	36"	100%
	1'15"	30"	45"	50%
	1'20"	30"	50"	20%
	1'40"	28"	1'12"	0%

continuation of TABLE 7

-35°C	54"	26"	28"	100%
	1'	25"	35"	50%
	1'10"	20"	50"	5%
	1'15"	23"	52"	0%

TABLE 8

The ability of Paramecium caudatum to recover when exposed to frozen medium

Temp. of ext. cooling medium	Duration of exposure	Time required for the medium to freeze	Time exposed in frozen medium	Recovery in percentage
-20°C	55"	48"	7"	80%
	1'	47"	13"	60%
	1'10"	44"	26"	5%
	1'15"	42"	33"	0%
	1'25"	40"	45"	5%
	1'50"	44"	1'06"	5%
	2'05"	45"	1'20"	0%
-25°C	45"	38"	7"	80%
	50"	37"	13"	50%
	60"	35"	25"	0%
	1'15"	38"	37"	30%
	1'25"	38"	47"	5%
	1'50"	37"	1'13"	0%
-30°C	30"	23"	7"	80%
	35"	23"	12"	50%
	45"	23"	22"	25%
	55"	22"	33"	10%
	1'10"	25"	45"	10%
	1'25"	21"	1'04"	0%
-35°C	25"	18"	7"	80%
	34"	19"	15"	60%
	43"	18"	25"	5%
	60"	20"	40"	0%

It will be noticed in both tables, that the percentage of recovery of both organisms at any temperature bears

a fairly definite relation to the time exposed in the frozen medium. The shorter the exposure time, the higher the percentage of recoveries; the longer the exposure time, the lower the percentage of recoveries. It also shows that the lower the temperature, the shorter is the exposure time in a frozen medium required to kill all the organisms. For example, at -20°C, and at -30°C, the exposure time required to kill all Opalinas in the frozen medium is 1'52" and 52" respectively. Comparison shows that Paramecia are less resistant to the frozen medium than Opalinas. Even when the Paramecia were immediately restored to room temperature after the medium had frozen, there were always some Paramecia that are unable to recover. Opalinas in every case proved to be much more resistant. All of them live long after the Paramecia had succumbed. The experimental results with the Paramecia are rather complicated. One will find that many of the data recorded overlap. Thus at -20°C, an exposure of 33" in the frozen medium will be sufficient to kill all Paramecia, but in another experiment when exposed for 1'06", 5% of the Paramecia are still able to recover. This phenomenon happens quite frequently in many experiments with Paramecia, which, however, shows the great difference in resistance present among the individual Paramecium. On thawing, all the Opalinas and Paramecia that were unable to recover assumed an extremely

irregular outline, often greatly shrunken. They remained intact for a short time, but soon gradually rounded up.

DISCUSSION

The statement made by Hutchison (1913) that in his cultures of Paramecium caudatum thermal resistance does not change with age in a culture, has not been corroborated. On the contrary, the writer finds that the thermal resistance of Paramecium caudatum did greatly differ with age in a culture. This was also observed to be the case in cultures of Paramecium multinucleatum studied by Doudoroff (1936). It was known that the age in a culture bears an intimate relationship with the pH of that culture, for cultures of different ages have different pH measurements (Fig. 5). It is thought that this change in the pH value may result in the change of some essential mineral elements which in turn have a direct effect on the thermal resistance of the Paramecium. This may possibly be so, because Chalkley (1930) found that there is an increase in thermal resistance of Paramecium on the addition of Ca and a decrease on addition of K.

It is clearly evident that the rate of biological process is altered by a change in the temperature. Ciliary activity is in no exception. High temperature tends to increase ciliary activity; while, low temperature tends to decrease ciliary activity. Thus, the rate of locomotion in an organism is also being affected. Locomotion increases with the increased ciliary activity and decreases with the decreased ciliary ac-

tivity. All these changes are attributable to the generally known fact that the raising of the temperature results in a decrease in the viscosity of the protoplasm through absorption of water, and the lowering of temperature results in an increase in the viscosity of the protoplasm through withdrawal of water. The former increases the motility of an organism and the latter decreases its motility. The phenomenon is strongly supported by the fact that contractile vacuoles of Paramecium were observed to pulsate faster with the rising temperature and slower with the lowering temperature, suggesting that there is an excess of water in the protoplasm so that the vacuoles have to pulsate more often in order to maintain the water content of the protoplasm constant by eliminating them. For this same reason, their pulsation becomes slower when the water in the protoplasm has became minimized, so as to retain as much water as possible. These facts also implicate the osmotic regulatory mechanism of the contractile vacuoles as studied by the earlier investigators. Moreover, when Opalinas and Paramecia are exposed to high temperature, their bodies become swollen; and when exposed to low temperature their bodies become shrunken. These facts make it more probable that absorption of water and a loss of water occurred with respect to the rising and lowering of the temperature.

The fundamental factors that determine the effective-

ness of the ciliary activity in the movement of any ciliated organism are the form and rate of beat of cilia and the mode of their co-ordination. The disruption of any of these factors results in the failure of complete metachronal wave movements responsible for the locomotion of the organism through the water. It is thought that the inability of the organism to propel through water despite the continuous beating of the cilia is due to the fact that the form and rate of beat of some individual cilia are seriously affected by such unfavorable conditions as extreme heat and cold. Thus, their movements are poorly co-ordinated and their metachronal waves are unable to complete. The degree of injury to the individual cilia will determine their power of recovery when returned to favorable conditions. When the exposure time of Opalinas and Paramecia under unfavorable conditions is not prolonged, the cilia will be able to recover their normal activity and the organism moves about again in a fairly normal manner. This ciliary recovery is not a typical characteristic confined to ciliate protozoa. The cilia of Mytilus gills are found to react in the same way. In the absence of oxygen, the cilia of Mytilus gills gradually slow down and stop, but they will quickly recover in aerated water if the oxygen lack is not prolonged (Aiello, 1960).

It has been known for many decades, that protoplasm

resembles that class of chemical solution known as a colloidal solution. This fact was first pointed out by Hardy in 1899. Also, it is generally known that the physical state of organic colloid varies directly with certain external conditions. A variation in the temperature, action of electric current, and certain chemical changes will undoubtedly change the physical state of the colloidal solution. One finds exactly the same in protoplasm as in organic colloids, that the viscosity varies directly with the temperature within certain limits. When the temperature is raised above the normal, the viscosity of protoplasm is decreased. This is due to the liquefaction of the protoplasm through absorption of water and an increase in the kinetic energy acquired by the protoplasmic particles that brings about a finer suspension (Greeley, 1904). With further increase in temperature, the protoplasm suddenly coagulates irreversibly and death occurs. Greeley (1901-1904), one of the pioneer workers in the colloid chemistry of the protoplasm suggested that the coagulation is due to some chemical change in the protoplasm itself. The writer agrees with the view of Greeley, for it is commonly known that protoplasm contains a clloidal complex of proteins which are readily coagulable and denatured by heat. When the temperature is lowered below the optimal, the protoplasmic viscosity increases probably as a result of decreased kinetic energy of the protoplasmic particles. This

change is accompanied by loss of water and a gradual cessation of the vital activities of the cell (Greeley, 1904). The coagulation is nearly always reversible as long as the exposure is not prolonged. When returned to higher temperature, the organism is able to absorb more water and its activities are re-accelerated. With prolonged exposure to low temperature in a frozen medium, the organism loses so much of its water content and consequently an increase in the concentration of the solute within the cell contents takes place. Such high concentration of solute produces a fatal effect upon the organism (Scholander, 1950; 1953), so that when restored to room temperature, their ability to recover to normal motile condition is generally lost.

SUMMARY

1. The purpose of this study is to find out whether the parasitic Opalina obtrigonoidea reacts to all stimuli in a manner similarly to that of free-living Paramecium caudatum. It was found that the two forms reacted similarly to certain stimuli, and had distinct, characteristic reactions to other stimuli.

2. In the comparative study of the behavior of Opalina and Paramecium in eleven experimental media, the length of life, the time in which disintegration begins and the rate of advance of disintegration over the body vary in different media as well as in different individuals and groups within wide limits. The possible reasons that bring about such differences are discussed. However, the typical course of death and disintegration occurring in each organism is the same in all cases. In Opalina, disintegration often starts near the anterior region by the formation of a blister, while in Paramecium, the blistering of the body, when it occurs, in a majority of cases begins on the mid-ventral side. The writer believes, that the weakness of the cell membrane in these regions plays a part in the localization of the blister formation. Also, it is thought that Paramecium has a much more delicate membrane than the Opalina, since Paramecium was observed to have a higher frequency of cell membrane ruptures as well as a larger number of

blister formations.

3. A very conspicuous difference in the behavior of both organisms is the positive reaction of Opalina and the negative reaction of Paramecium to the pull of gravity. The writer attempts to explain such difference as due to difference in the number, distribution and length of cilia as well as a difference in the body shape. The broader heavier anterior region of Opalina together with its more forceful ciliary beating may be responsible for their downward direction of movement, while, the heavier posterior region of Paramecium together with its stronger ciliary activity may play an important role in determining their upward movement.

4. As to their reaction to light, both organisms are indifferent to ordinary light as well as light of different colors despite certain obvious differences in their habitats. It is thought that their indifferent reaction to light and light of different colors may be related to their habitat of life. Opalina lives in total darkness, which may render it incapable of producing any marked response; while Paramecium lives in diffuse daylight , which makes it unable to produce marked responses when subjected to the same conditions.

5. In studying the reaction of Opalina and Paramecium to temperature, the writer took into consideration as much as possible, the known factors that will affect the longevity of

these organisms. Under such conditions, both organisms are found to differ in tolerance to heat. The thermal end point of Opalina and Paramecium is 39°C and 40°C respectively. Furthermore, it also shows that Paramecium can resist heat better than Opalina, for they nearly always subsist longer than Opalina when exposed to temperatures above their thermal end points. The general behavior and the visible structural changes produced upon both organisms by heat are similar with respect to the acceleration of locomotion and swelling into a more or less spherical shape. In Paramecium there is a much greater tendency for the membrane to rupture and there is also an acceleration of the pulsation of contractile vacuoles. The heat death is explained as due to the irreversible coagulation of the colloidal complex of proteins at high temperature.

6. The effects of cold differ greatly from heat, since heat increases the rate of locomotion, while cold reduces it. Moreover, both organisms are observed to shrink in size instead of swelling, thus there is also a lesser tendency for the cell membrane to rupture. Further, both organisms stop their normal locomotion at once when exposed to low temperatures, immediately followed by the "avoiding reaction." In heated water, the "avoiding reaction" is continued violently till the organisms are dead; in cold water, the organism after a time becomes motionless while the cilia

continue to keep on beating for an hour or in some cases longer, until they eventually stop. These cilia will quickly recover when returned to the temperature of the room. Comparison shows that Opalina can resist cold better than Paramecium, for at a prolonged cold-exposure of approximately an hour after all ciliary movements have ceased, all Opalinas are able to recover when returned to room temperature after 50 minutes. While, Paramecium, after the cilia have gradually slowed down to a stop, when immediately returned to room temperature, becomes motile again after a period of approximately an hour, but soon after all movements come to a stop again. Also, during cold-exposure, the pulsation of the contractile vacuoles gradually declined. The ability of Opalina and Paramecium to recover was also tested when exposed to frozen medium. Again, it was found that Opalina can resist frozen medium better than Paramecium. The cold death is explained as possibly due to coagulation of protoplasm at low temperatures accompanied by an accumulation of solute within the cell which produces a fatal effect upon the organisms.

7. In the course of culturing Paramecium caudatum in hay infusion medium, some observations have been made:
   a) Detection of the approximate age of culture through the different intensities of color produced by the infusion medium.
   b) The relativity of the different intensities of the color to the acidity of the culture medium.

c) The growth of Paramecium caudatum as related to the hydrogen-ion concentration of the culture medium.

d) A pH value approximately 7 or slightly higher up to 7.3 yields the best growth for Paramecium caudatum in the hay infusion medium.

e) The possible factors that may affect the changes of hydrogen-ion concentration and the over all processes involved in the pH changes in an hay infusion medium are discussed.

8. Notable observations that have been made on Opalina habitat are as follows:

a) Opalina obtrigonoidea are found inhabiting the anterior and middle portions of the rectum of the frog Rana pipiens. But more often the middle portion has the greatest number.

b) Some Opalinas are found as a single group lying in close contact with the rectal wall. Some are found in aggregation between the crypts of the intestinal wall, and others may lie freely in the rectal lumen.

c) There is a relation between the size and the number of Opalinas in an infection which is nearly always inversely proportional. As to the size of the Opalinas in relation to the size of its host, there exists no intimate relationship.

9. A survey of the incidence of different parasites in the

rectum of Rana pipiens from the province of Quebec shows 54% of the frogs are infected with Opalinas. Besides Opalinas, nematodes are very frequent, other ciliates less frequent, while trematodes and cestodes are least often found.

10. In the present investigation, some of the experiments of the earlier investigators were repeated at length for the purpose of re-examination and the observations were reviewed in the light of their relationship to those of earlier workers.

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