

EXPERIMENTAL CULTURE
AND
REACTIONS OF PARAMECIUM

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EXPERIMENTAL CULTURE AND REACTIONS OF
PARAMECIUM

A Thesis

Submitted to the Faculty of Graduate Studies
and Research of McGill University

By

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the degree of Master of Science.

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ERRATA

in Thesis on Paramecium by Dr.

C. Lhérisson.

-:-

Page 15, Line 9, The first word in this line
should read "disequilibrium".

Page 18, Second line from the bottom of the
page, read
"Relative number of individuals, 1"

Page 37, Third Line, read "Action of acids"

Page 40, Eleventh line, at end of the line, read
"..... it is necessary"

Page 49, Sixth line, first word in the line,
should be Parthenogenesis.

Page 63, Third line from the bottom, for "Aurelis",
shpuld read "Aurelia".

-:-

ACKNOWLEDGMENTS.

The writer is greatly indebted to Professor Arthur Willey, under whose personal supervision this work was done, for suggesting this investigation and for his co-operation in the experiments; to Professor N. J. Berrill for the interest which he has shown and valuable suggestions; to Dr. Georges Préfontaine and Mr. J. Brunel, of the University of Montreal, for facilities in connection with the literature on Paramecium; to Mrs. K. G. Pinhey for the use of her translation of the study of Dembowsky on the vertical movement of Paramecium. He is also very grateful to Mr. J. W. Pollock, of the Department of Zoology, for his constant help and his kind assistance.

"Cautions in viewing objects.

Beware of determining and declaring your opinion suddenly on any object, for imagination often gets the start of judgment and makes people believe they see things which better observations will convince them could not possibly be seen: therefore assert nothing till repeated experiments and examinations in all lights, and in all positions.

When you employ the microscope shake off all prejudice, nor harbour any favourite opinions; for, if you do, 'tis not unlikely fancy will betray you into error, and make you think you see what you would like to see.

Remember that truth alone is the matter you are in search after; and if you have been mistaken, let no vanity seduce you into persisting in your mistake.

Pass no judgment upon things over-extended by force, or contracted by dryness, or in any manner out of their natural state, without making suitable allowances".

Henry Baker, F.R.S. "The Microscope Made Easy";
3rd Ed., pp.62-63,
London, 1744.

INTRODUCTION.

Paramecium, the "slipper animalcule", is a free living ciliate infusorian which can be obtained and cultivated in great abundance, and it has been used extensively during the past thirty years in researches upon the irritability or reactivity of the cell and upon the genetics of unicellular animals. Owing to its accessibility it has been utilized for a great variety of biochemical and bio-physical tests to which it is highly sensitive.

CHRONOLOGY OF PARAMECIUM FROM LEEUWENHOEK TO MAUPAS.

- 1703 - Anonymous description and figure of Paramecium in Philosophical Transactions, suggested by a letter from Leeuwenhoek. In this paper is given the first description of Cilia being referred to as "minute feet", and the first account of habits and transverse fission of Paramecium.
- 1718 - L. Joblot: Contractile vacuole and figure of Paramecium. (Joblot said Dujardin, in 1835, for his picture of Paramecium apparently used his own slipper as a model).
- 1742 - Henry Baker: "The Microscope Made Easy", which received the imprimatur of the Royal Society of

London on October 28th, 1742. Description (p.72) and figure (pl.VII, Fig.1) of Paramecium, the "animalcule of pepper infusion".

- 1752 - Hill, in his Natural History, described various Infusoria under the names of Paramecium, Cyclidium and Enchelys.
- 1769 - H. B. de Saussure carried the first experimental culture of Paramecium.
- 1769 - Eichorn: Observations on Infusoria.
- 1770: J. Ellis: Trichocysts of Paramecium.
- 1774 - Corti described contractile vacuole as a heart.
- 1776 - A. Spallanzani first mentioned the effects of environment on Protozoa and described contractile vacuole of Paramecium as a respiratory organ.
- 1778 - Baron W. F. von Gleichen introduced method of experimental feeding of Paramecium with powdered carmine and indigo. He described the formation of food vacuoles and discharge of undigested residue.
- 1786 - The monograph of Otto Friedrich von Muller on the Infusoria was posthumously published. He described and figured conjugation of Paramecium.

1812 - F. von P. Gruithuisen: Cyclosis, a circulation of the endoplasm in *Paramecium*.

1835 - Felix Dujardin studied the structure of endoplasm and named it sarcode. He described surface precipitation reaction of *Paramecium* under the term diffluence. He made a classification of Infusoria and placed *Paramecium* in the 5th group, 5th order, 17th family of "Parameciens". Later, in 1841, he expressed the view that potential immortality of Protozoa could be controlled by experimentation.

1838 - C. G. Ehrenberg: Food vacuoles of *Paramecium* regarded as many stomachs, hence the class name "Polygastrica". For him all infusoria were hermaphrodite animalcules, capable of self fertilization, and that which was known to be the act of conjugation was one of longitudinal fission. He thought that the macronucleus was the male genital gland and that the contractile vacuoles were the seminal vesicles, their afferent canals being the passages by which the spermatic fluid was conveyed to the ova contained in the body. He considered as ova the granules of chlorophyll or other coloured globules in the body, as in *Paramecium bursaria*, thus opposing spontaneous generation "on the ground

that

that he had discovered genitalia in the Infusoria"(Cole). He made experiments on the influence of heat and cold on Paramecium. Ehrenberg presumed the existence of internal muscles in their bodies to explain the varied and active movements of the Ciliata. Presumption based on his hypothesis of similarity of the organization of higher animals with that of Protozoa, he was the first to consider the immortality of unicellular organisms.

1843 - Martin Barry was the first, according to Butschili, to apply the cell theory to the Protozoa. He expressed the view that cell increase was effected only by division.

1843 - Addison made experiments on the effect of chemical agents on Paramecium and found that acetic acid, alcohol, tincture of iodine, etc., caused "difffluence" of protoplasm.

1845 - Th. von Siebold showed that the central so-called granular body of Ehrenberg is the cell nucleus. He formulated definitely the doctrine of unicellular nature of Protozoa (a name substituted by Godfuss in 1820 for Urken's suggestive "Urthiere" (1805).

1847 - Boeck regarded contractile vacuole as an excretory vesicle.

1852 - Max Perty's classification of Ciliates: Class B

"Monima":- "Animalcules which, although very contractile, neither undergo change of form nor exhibit jerking movements. Family 6, Paramecina: Body covered by longitudinal rows of cilia, Mouth lateral, often situated in a furrow". (after Pritchard).

1853 - Rood published his experiments on reactions of Paramecium. "He states that when a feeble galvanic current is passed through water containing paramecia, the animals are brought to a stand-still, near the negative pole, and after revolving for a time on their own centres cease to move; ciliary action is also arrested and diffluence ensues." On the subject of chemical reagents he makes the following remarks: "Alcohol stopped their motion, coagulated their contents so that they shrunk within the integument, and caused death. Ammonio-chloride of mercury, acetate of lead and perchloride of mercury destroyed life instantly". (after Pritchard).

1854 - Stein put the classification of the Ciliate upon a modern basis.

1856 - N. Lieberkuhn held the view that contractile vacuole was a rudimentary heart.

1858 -

- 1858 - Claparède and Bachmann studied the contractile vacuole and held the view that the apparatus was the homologue of the circulatory system in the more differentiated animals.
- 1861 - H. J. Carter demonstrated the expulsion of fluid from the contractile vacuole by surrounding *Paramecium* with a suspension of Chinese ink.
- 1861 - Andrew Pritchard published in London his valuable history of Infusoria. He mentions "Respecting the effects of cold, it is a general law of the ciliata that their numbers rapidly diminish when winter sets in, and that, on the contrary, they rapidly augment so soon as the warmth of the sun in spring manifests itself, and continues to increase in number and variety until the height of summer is passed".
- 1861 - Balbiani did not accept the unicellular theory of Protozoa and still regarded the Infusoria as being hermaphrodites, but recognized the necessity and the fact of conjugation (after the manner of the earth-worm). According to his opinion the macronucleus was the ovary, and the micronucleus the male gland. Such an erroneous interpretation is not so much to be wondered at, especially in view of the extensive observations which he made and the accuracy of his figures, which by their very wealth of detail could easily lead to a wrong conclusion at that time.

1873 - Bütschli, after observing that a continued asexual reproduction in *Paramecium* resulted in decreased size and a general lowering of life of the individuals, concluded that the function of conjugation is to bring about a renewal of youth of the participants. "Verjungjung".

1881 - 82 - W. Saville Kent published his valuable manual on the Infusoria.

1888 - Griffiths mentioned that he was able to detect uric acid in the contractile vacuoles of *Paramecium* by means of murexide and adopted the view that the vacuole is a kidney.

1888-89 - Emile François Maupas published his researches on karyogamic rejuvenation (conjugation). It is not excessive to consider him as the first authority on *Paramecium* and Infusoria. His valuable work will remain one of the most interesting achievements in the field of Zoology.

Maupas observed that the causes which determined conjugation in cultures of Infusoria vary according to whether they are wild or pedigree cultures. In wild cultures it sets in generally at the transition from plethora to hunger. In pedigree cultures he says it occurs after a long series of fission, variable

with

with the species, and only when they begin to show signs of senescence. According to Maupas the impulse to conjugation is inherent in the organism and arises from internal factors which operate independently of external conditions.

Maupas' work was the beginning of a series of interesting investigations on *Paramecium*.

MATERIAL AND CULTURE.

A preliminary study was made to find satisfactory methods to carry on the main experiments. The material was derived from a wild mass culture, started in a fish infusion, developed at room temperature from a small dead catfish in September 1929. To this was added later some pieces of hay, and, from time to time, small amounts of standard beef extract (oxo), and a sprinkling of bread crumbs (dried wheat bread). Sub-cultures were made in hay infusion prepared by boiling. This infusion was found highly toxic at first, with a pH of 5.2, but after it had been exposed for a few days and altered by bacterial contamination, it became gradually neutral and was found to be a very good medium for *Paramecium*, especially when diluted. It was also found that the pH of hay infusion kept in a closed bottle remained high for a long period.

It was

It was possible to make a comparison between the fresh and the old hay infusion and determine the effects of the fluid on the rate of reproduction and also on the reactivity of the infusoria.

The rate of fission was higher (average of three a day) in a fluid of three days old, with a pH of 6.8, in which chemical conditions were favourable and the bacterial content quite high. The infusion would be more or less alkaline at the end. In the early stages of the cultures, *Paramecia* are mostly clumping close to the surface, on the wall of the container, later, further from the edges of the jar, and finally distributed through the fluid and at the bottom. Periods of crisis were noted during which a decrease in the fission rate would be marked and most of the animalcules found at the bottom of the jar. Effects of the accumulation of CO₂ with the lowering of the pH was found to stimulate agglomeration of the population of the Infusoria at the bottom of the watch glasses. Under this condition, at room temperature, the pulsations of the contractile vacuoles of *Paramecium* were found to be 20 to 25 per minute. It is wondered also if the lack of oxygen in the culture fluid would not bring the formation of toxic substances which otherwise would be oxidized. The waste products of the *Paramecia* are also responsible for the constant chemical changes in the culture fluid.

Various

Various kinds of water have been used such as tap water, distilled water and Laurentian water.

Experiments were made to test the various nourishing fluids and the general reactions of *Paramecium* to different media. Filtered and unfiltered fluids were used; no apparent difference was found between them.

A solution of one per cent Oxo in five per cent alcohol was used with success. Junker's solution was found of no important value. Egg emulsion proved to be an ideal culture fluid, the best emulsion being made of one yolk in 2,000 c.c. water.

Various tests were made with dilute salt solutions and each of them was found to be toxic when used alone. Zaugg, cited by Strohl*, has pointed out that almost all soluble substances are poisonous when used alone. The infusions in which *Paramecium* grows contain many inorganic salts; when any one of them is used alone in distilled water, it is poisonous to the Infusoria. When dissolved together they make a balanced solution in which the toxic effects of the individual salts are neutralized. The action of solutes, when not too quickly fatal, is reflected in the behaviour of the contractile vacuoles, a matter which will be discussed in another chapter.

Paramecium placed in a 0.5 per cent solution

of

*J. Strohl (1925)

of CaCl_2 showed a rate of movement of 3 mm. in six seconds, being very active - but this same solution has no effect upon the division rate of the Infusoria.

Observations and records were made daily at different intervals so that the course of reproduction and decline was closely studied. An average of one fission a day was observed at room temperature, and none found at 5°C . to 7°C , the Infusoria being very sluggish in that case. In time the population of Paramecium was too large to be successfully counted.

The stock cultures were kept throughout the session in battery jars and evaporating dishes, the pedigree cultures being kept in moist chambers.

Stock and renewed cultures were regularly under control in order to keep them as uniform as possible. During the winter the cultures were all in flourishing condition. Every two days the cultures were stirred and aerated to eliminate CO_2 formation. As it was desirable to obtain evidence from miscellaneous populations, under different conditions, cultures from various sources were later employed. New groups of Paramecium were collected during the fall, and at the beginning of the winter, from Crawford Pond (near Verdun) and from the St. Lawrence River. The changes in these different series were characteristic of variations due to environmental conditions. The fall specimens showed a

rate

rate of over one hundred conjugations a day.

Pure cultures of *Paramecium* were used during the various experiments by the writer for different means, but principally for the experimental ingestion of *Paramecium*.

The method used to obtain the bacterial sterilization of *Paramecium* is as follows: A series of the *Infusoria* taken from one of the stock cultures was washed in distilled water contained in a centrifuging tube for two hours. (They were taken from a medium rich in bacteria to another free from bacteria). According to their negative geotaxis, the *Paramecium* would be collected at the top of the tube, and then removed after the period of two hours, centrifuged ten times in 5 c.c. of distilled water at intervals of five minutes up to the fifth wash; in the fifth they would be left for three hours before passing on to the tenth. This long interval was found necessary to purge the *Paramecium* from their bacterial content. A microscopic examination was made after the second wash to be sure that no other infusoria was present and that there was no debris of any sort in the fluid, and also in an attempt to make a count of the individuals.

To study the effect of low temperatures on *Paramecium*, the animalcules were contained in petrie dishes placed in a square jar having a current of running water

with

with a constant temperature easily moved from 3°C. to 10°C. They were also placed outside, or between the windows of the laboratory, at temperatures varying from 0°C. to 7°C.

The study of higher temperature was made in an incubator.

Repeated observations showed for the different lines of *Paramecium* that where conditions were the same results were uniform, and for the same conditions in different culture fluids results were closely parallel. So the essential conditions have been adequately controlled and the results quite dependable to permit conclusions. All observations were checked by different people to be sure that no mistake of interpretation was taking place. Some experiments made by others were repeated and sometimes showed different results. No experimental means for producing the encystment of *Paramecium*, as mentioned by Linder*, could be found, till it was known that Linder had mistaken a *Colpoda* for *Paramecium*, according to his own figure. Specimens were stained in mass. The fixative used was a sublimate-acetic solution (i.e., 3 drops of glacial acetic acid added to 10 c.c. of corrosive sublimate). After the fixation period of five minutes, the solution was removed and replaced by 70 per cent alcohol for another period of five minutes. The alcohol was replaced by iodized alcohol for five minutes. Fresh 70 per cent alcohol was used again for five minutes

*G. Linder: Die Protozoenkeime in Regenwasser. Biol. Zentralbl., XIX, pp.423, 426 and 427. 1899. and

and was replaced by a picrocarmin solution of 1 per cent in 30 per cent alcohol. If the preparation was stain to excess, acidulated 70 per cent alcohol was used for five minutes, after which the preparation was cleared with an equal part of glycerine and pure acetic acid.

For the cutting experiments, a very fine cataract knife was found to be very practical. India ink and neutral red were used in the study of food vacuoles in Paramecia.

Tannic and picric acids were employed to study the discharge of Trichocysts and the formation of Tectine. The "double drop" method was used in studying the reactions of Paramecium to different salts. This method, devised by W. H. Johnson, consists of placing a drop of medium containing Paramecia side by side with a drop of the salt solution and mixing both, using for that purpose the point of a needle. The colorimetric method of determining hydrogen ion concentration was used for the different tests, the standard tube covering a range from pH 3.0 to pH 8.4.

GENERAL REACTIONS.

GENERAL REACTIONS.

Paramecium is physiologically a well organized infusoria, morphologically a single cell, and genetically a germ cell. As well as all living organisms, it reacts to changes in its relations to its environment by exhibiting characteristic alterations in its activity and finally adapting itself to certain environmental conditions.

One of the main reactions which produces a remarkable effect on the animalcule as a whole is the energetic equilibrium which is the result of various metabolic changes. The increase and loss of energy of Paramecium affects the reproductive synthesis and growth of the individual, and is the cause of whythmical variation in the rate of fission. Both internal and external factors are included in the production of a period of crisis, that is, the time when trophic disequilibrium sets in. The same factors are responsible for the stimulation of conjugation. Each Paramecium continually or intermittently receives energy from the surroundings; later it returns this energy to the environment. The interesting fact is that the rate and character of this interchange are controlled or regulated by the activity of the infusoria itself. It must be stated that the vital activities cannot be considered apart from the external conditions. The animalcule reacts positively to

food

food and temperature and other conditions necessary to life, and negatively in avoiding injurious conditions, the result of these activities being of a chemical nature with even variations of pH in the culture fluid. But the chemical reaction of the Paramecium, with oxidation of the protoplasmic constituents, are largely determined by the integrity of the protoplasm and the catalytic factor appears to be operative only when the protoplasmic layers are intact. The structural stability of the protoplasm and some features of its chemical organization and behaviour depend on the presence of the external network fibres by which the structural elements are enclosed, and which can be demonstrated by the action of tannic acid on the paramecium. Dead Paramecia show an increased opacity of the protoplasm indicating coagulation of its constituents. It shows also changes of physical consistency, death rigor, and increase of viscosity.

The different stimuli employed in the experiments were mechanical, electrical, thermal, chemical and osmotic. A uniform physical condition is not sufficient for stimulation; it must change, and the variations must be of a certain degree and rate. Where Paramecium is not highly sensitive, it can be rendered so by adding some absorbing compounds in the medium. Then specific artificial anaphylaxis will be shown. Temperature has polymorphous effects which are direct or indirect; directly on the vital processes and indirectly on

the

the food and conditions in the environment.

Thigmotaxis and stereotropism of *Paramecium* are very high. With most agents used the constant effect noticed is a change of shape and the appearance of blister-like elevations, shortening and rounding of the individual with more or less complete disappearance of the oral groove. A slight pressure on the blisters causes them at once to collapse, thus liberating all endoplasmic content, and generally vacuoles may be observed wandering about in the surrounding fluid after the *Paramecium* has burst. In high concentrations of various salts such as potassium chloride, sodium chloride, potassium iodate, barium chloride, sodium carbonate, these changes are instantaneous, and bursting occurs in a few minutes. With decrease in concentration, the changes occur more slowly and complete cytolysis after a longer period of time. The same effect has been found with new hay infusion, even when diluted, where probably concentration of tannic acid is quite high. In strong acids cytolysis is not extended, and appears with little change in the shape of the *Paramecium* and immediately followed by complete fixation of the infusoria.

When the action of the agent is slow a reversal change in ciliary movement is seen. The cortical pellicle coagulates, becomes thick and hard, and is the seat of the secretion of protective substances. Discharge of antitoxic

matter

matter which Bresslau* called Tectine, forming a marked film around the infusoria. This tectine was demonstrated in our experiments especially by diluted tannic acid and cresyl blue (1:10,000). The experiments of Bresslau consist in testing different numbers of Paramecium in the same volume of culture fluid, to which is added an equal volume of the toxic solution of a basic stain.

The three principal basic stains employed are methylene blue, Victoria blue, and neutral red. Divers numbers of individuals are introduced into a certain amount of a standard culture fluid. The addition of the staining reagent causes the Paramecia to secrete the tectine, and if the concentration of the toxic colouring matter is not too high the Paramecia will soon slip out of their tectine coats again. The animals do not stain at first, but the tectine capsules are stained and collect in coloured clumps corresponding to the mass of infusoria. If the solution of methylene blue is a 1:10,000, the following results are obtained:

Methylene Blue	1 c.c.
Volume of Culture Fluid,	1 c.c.
Relative Number of Individuals,	
Viability of the individuals,	25 minutes.

* E. Bresslau (1923).

Methylene Blue	Vol. of Culture Fluid	No. of Individuals	Viability
c.c.	c.c.		
1	1	20	68'5"
1	2	1	138'
1	2	20	1200'

The tectine coat adsorbs collargol and many other poisonous products.

The toxic substances are more effective when the Infusoria are confined in limited volumes of culture fluid.

Relation of density of population to survival is there evident.

CONTRACTILE VACUOLES.

It was noticed that conditions which are injurious to *Paramecium* usually increase or decrease the rate of pulsation of the contractile vacuole, and often bring about a complete paralysis of the apparatus, blocking the radiating canals, forming a permanent rosette around the vacuoles. Depression in the rate of contraction is also synchronous with the occurrence of the process of division, when two vacuoles are formed. With this total of four vacuoles the number of pulsations of each vacuole varies at room temperature between three and four times a minute, each small newly-formed vacuole being synchronous with the old in each segment of the dividing individual. In the normal state the anterior contractile vacuole pulsates on the average faster than the posterior.

After division, in the new individuals the rhythm of the pulsation is equal for both vacuoles during the first twelve hours. Temperature has a marked effect on the behaviour of the vacuoles. At 3° to 5°C, there is a complete paralysis of the vacuole, with increase of osmotic pressure within the *Paramecium*. In the metabolic forms obtained at low temperature, the rounded animalcules show no function of the contractile vacuoles. From 5° to 10°C. there is a slow rhythm of two to three contractions per minute, and, in the

same

same individual, it is striking to see the change with variation of the temperature. The history of the normal pulsation of the contractile vacuoles in Paramecium has recently been outlined by Professor Francis E. Lloyd (1928). Jean Port (1928) found that the pre-oral vacuole pulsates more rapidly than the caudal. This is shown in the sub-joined table compiled from his data and showing variations of rate with different temperatures.

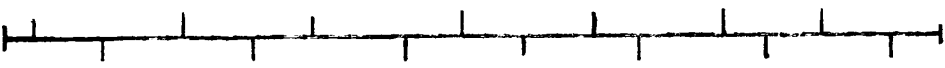
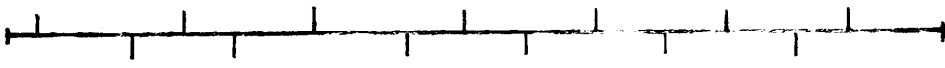
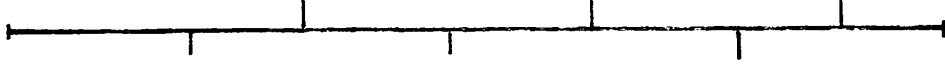
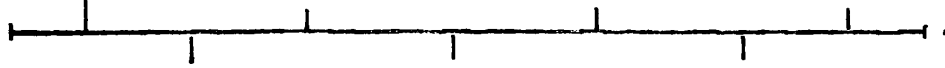
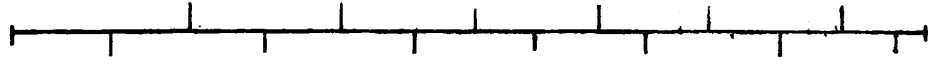
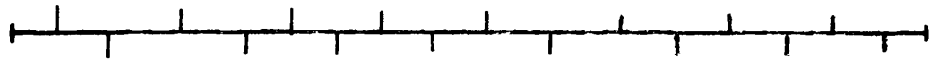
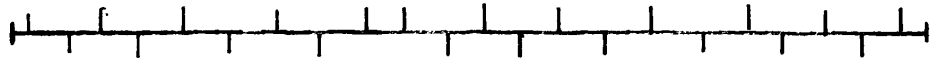
TABLE I.

Temperature	Pre-oral Vacuole	Caudal Vacuole	Mean num- ber of Pulsations per minute
°C.	Mean rate of Pulsation. Seconds	Mean rate of Pulsation. Seconds	
5	151.	161.8	0.38
10	44.4	48.1	1.3
15	26.4	28.	2.2
20	11.9	13.25	4.75
25	8.2	9.4	6.8
30	6.2	7.3	8.8
35	5.8	6.5	9.8
38	5.7	6.1	10.1
40	5.1	5.7	11.

TABLE II.

PULSATIONS OF CONTRACTILE VACUOLES.

Number of contractions per minute in culture fluid at room temperature (22° to 25°C.). Rate determined by means of a stop-watch.

1.	$\frac{A}{P}$		$\frac{7}{6}$	
2.	$\frac{A}{P}$		$\frac{7}{7}$	
3.	$\frac{A}{P}$		$\frac{9}{8}$	
4.	$\frac{A}{P}$		$\frac{7}{6}$	
5.	$\frac{A}{P}$		$\frac{3}{3}$	Contractions at long intervals, then quickly one after the other
6.	$\frac{A}{P}$		$\frac{4}{3}$	Paramecium escaped immediately after the minute.
7.	$\frac{A}{P}$		$\frac{8}{5}$	
8.	$\frac{A}{P}$		$\frac{6}{7}$	
9.	$\frac{A}{P}$		$\frac{8}{8}$	
10.	$\frac{A}{P}$		$\frac{12}{10}$	Very small Paramecium $\frac{1}{4}$ the size of the others (dwarf)

I found that in moving *Paramecium* the rate was slower than in resting individuals. It is more active in the dwarf forms than in normal sized individuals, as can be shown in the preceding table (Table II, Observation 10).

By testing various salts in concentrations of 0.5 to one per cent, I have found that the vacuoles enlarge to a great extent, cease to pulsate owing to the increased concentration of the fluid in which the *Paramecium* is living. By centrifuging *Paramecia* in water several times, the vacuoles again become dilated and cease to pulsate; this is due to increased viscosity of the protoplasm (See Heilbrunn, 1926, and 1928; also Dorothy Fetter, 1926). In general the anterior vacuole is more susceptible to changed conditions, and it is retarded more than the posterior until brought again to normal conditions. Variations also occur in the pulsatory rhythm, between diastole and systole of the vacuole.

Regarding the causes of the changes of frequency of pulsation of the contractile vacuoles, Kanitz (1907, *Biol. Zentralbl.*, 27) was of the opinion that the activity of the vacuoles depends upon the rate of metabolism in the protoplasm. With increase of temperature the metabolic processes are accelerated and waste products are released, drawing more water into the vacuoles. But the correlation between the effects of temperature and metabolism is not exact, since the viscosity of the protoplasm is a disturbing

factor

factor. The observations of C. M. Child and Ezda Deviney (1926) on the rate of pulsation at room temperature of four different cultures, the records being given separately because the rates often differ widely, indicate generally a much slower rate than that observed in my table. This discrepancy will be due to some imponderable factors such as might result from variations of room temperature and constitution of local races. It would seem that Paramecium has a tendency to form local races, but there are no precise data available on this point. Some of Child's figures agree fairly closely with those tabulated by Jaan Port at 10° to 150°C.

The following figures, compiled from Child and Deviney (1926), gives the results of counts I to III of ten individuals each, and of five individuals for IV.

"In the case of culture I. the counts are given as the number of beats occurring while one vacuole gains one entire beat on the other. Here in all cases the anterior vacuole is the faster. The records for cultures II, III and IV. are given as number of seconds between the successive contractions, the figures being the averages of from 5 to 20 beats." He noted that the posterior vacuole was faster in individuals which had been two to four hours under slight pressure, and where ciliary activity had almost ceased. The vacuolar rate was decreasing and the animals evidently approaching death. In his culture II the rate per minute

varies

varies from 1.44 to 3.64 for the posterior vacuole.

TABLE III.

I		II		III		IV	
An- terior	Pos- terior	An- terior	Pos- terior	An- terior	Pos- terior	An- terior	Pos- terior.
8	7	32	37.5	6.9	8.7	26.4	40.6
5	4	42.5	42.5	10.2	10.7	34	52
5	4	36	41	9.4	11.8	44	53
7	6	36.7	46.3	10.8	11.6	31.5	45.4
5	4	38	40	11	13.7	34.5	54
8	7	30	33	9.8	11.2		
4	3	31	37	15	17		
5	4	21.7	22.7	12.7	11.7		
6	4	21.7	24	33.6	32.4		
7	6	16.7	16.5	42	28		

Child also made experiments on the susceptibility of Paramecium to KCN and found that the anterior end was more susceptible than the posterior.

W. B. Unger (1926) found that if the data for the contractile vacuole activity are considered from the angle of the actual number of individuals in which the anterior or
posterior

posterior vacuole pulsated more rapidly, it is evident that in 81 per cent of Paramecia, the anterior vacuoles beat more rapidly. His figures for the average number of contractions per minute at room temperature are as follows:

	<u>Anterior</u>	<u>Posterior</u>
Paramecium aurelia	7.69	8.26
Paramecium caudatum	8.27	7.44

With the various toxic agents I used I found that the anterior contractile vacuole was slower than the posterior, or became paralyzed, while the posterior was still beating and the rate of pulsation of the posterior vacuole under these conditions was found higher at room temperature than usual. From a series of beats I found the average to be fifteen pulsations per minute before any blister formation upon the protoplasm. After the surface precipitation reaction the number of pulsations would decrease down to zero.

Conjugation also brings variation in the rhythm of vacuolar contraction, which is more rapid at that period. In Paramecium from new wild culture I found, after several counts, the following average results per minute, at room temperature:

11 for the anterior vacuole; 8 for the posterior.

SITOTROPISM.

The study of digestion in Paramecium started with the experiments of Von Gleichen (1778) introducing with food particles, coloured matter, which delimits the digestive activities of the "gastric vacuoles". Since these early experiments, much work has been done on the feeding reactions of Paramecium. Dogiel (1929) uses a combination method, while, together with the action of a salt, he uses various vital stains at the same time, as well as feeding with India ink or carmine suspension. Experiments with magnesium salts $M/64 \text{ MgCl}_2$, $M/178 \text{ MgSO}_4$, or $M/50 \text{ MgSO}_4$, gave the following results:

Instead of forming separate food vacuoles, full of India ink, there is an uninterrupted stream of ink particles into the endoplasm. So that after from two to five minutes long looping black bands arise. After three to five minutes this band elongates by addition of ink, so that it becomes coiled spirally up to four turns. This Dogiel calls the ink-gut. This arises at the bottom of the cell gullet, in the depths of the intestinal tract, occupying half of the posterior end, resembling the digestive canal of many metazoa. In this way Paramecia behave from ten to twenty minutes, after which time a gradual regulation of the endoplasmic condition begins. The regulation starts by the inner extremity of the ink coil becoming concentrated into a

thick

thick knot, finally rounding up completely to a large ink vacuole. From this moment the ingested ink takes the form of the usual food vacuole, and the conclusion is that the animal has acclimatized itself to the action of $MgCl_2$. Referring to Nierenstein (1905), he describes an abbreviated cyclosis. The ink rope remains as a sausage-like body not breaking out in the water at once. He observed this three times. On such occasions for a certain length of time one can see the emptied place in the endoplasm occupied formerly by the ink rope.

Similar results were obtained with Cobalt sulphate, nickel sulphate, iron sulphate and manganese chloride. The similarity of effects in these salts is due to the cations. G. Hober (1925) used $MgSO_4$ without India ink and found no food rope built, but found large food balls in the endoplasm, which indicates a specially active ingestion of food. Under the action of these various salts, these changes are caused by an alteration in the consistency of the endoplasmic contents. These experiments point to a far-reaching plasticity of the paramecium organization. It is remarkable how, under chemical influence, the digestive "organelles" acquire a certain structural and functional resemblance to the digestive system of metazoa.

Concerning the intimate process of digestion, it

is presumed that enzymes are secreted into the vacuoles and that products of digestion are assimilated by the surrounding cytoplasm. According to Nierenstein (1905) a food vacuole in normal conditions requires an interval of forty to forty-five minutes to conclude the cyclosis from mouth to end. During that length of time food vacuoles exhibit changes which can be divided into two periods: in the first, the vacuole shows an acid reaction and the ingested organisms are killed; in the second, the vacuole has an alkaline reaction, when the solution of the food substance is at an end. In the first period, the bacteria, after being rendered immobile, are clumped together, enveloped in a turbid substance which makes the outlines indistinct. The reaction of the vacuole is strongly acid, due to the presence of mineral acid in the vacuole. During this period of about ten minutes in normal conditions, the vacuole diminishes in size, till it is not more than one third of its original size. Metalnukoff found that some vacuoles never give an acid reaction, some show the acid reaction throughout, while still others in the same organism are first acid and then alkaline as usual. Different food substances incite these different responses (Minchin, 1912). When the vacuole was first formed, its wall was surrounded by a number of granules which stain distinctly with neutral red; these granules pass suddenly into the interior of the vacuole after it becomes diminished considerably in size. Nierenstein

regards

regards the red staining granules as bearers of a tryptic ferment. Von Uexkull (1921) divides the tryptic digestion in nine phases:

1. Formation of the food vacuole.
2. Food vacuole passes into circulation.
3. Contents of food vacuole become acid.
4. The acid reaction disappears.
5. Contents of food vacuole become alkaline.
6. Granules of enzyme enter the vacuole.
7. Tryptic digestion begins.
8. Vacuole reaches hinder region of endoplasm.
9. Vacuole discharges contents (defecation).

The continual intake of surplus water through the mouth in connection with the formation of food vacuoles is compensated by the activity of the contractile vacuole. Zwoff and Ronkellmann (1922) observed in pure cultures of Infusoria that nitrogen compounds were dominant on account of a trypsin diffused from the animals. According to recent work of Nierenstein, Paramecia under natural conditions contains a great quantity of fat. But the quantity of fat in the endoplasm can be increased greatly. The fat granules serve as reserve nutriment and disappear under starvation. It is curious how the form of Paramecium changes under starvation, showing a marked emaciation and a flat shape. In that case the endoplasm is very transparent. I noticed

on starved individuals a decrease in the pulsatory rhythm of the contractile vacuole, and also a slow ciliary activity. Temperature and other environmental conditions react on the circulation of the food vacuoles and upon the effects of starvation. In cultures kept at room temperature (about 22°C) death from starvation occurs around the seventh day. Where starved specimens were kept a month at 5°C, with the progressive increase of temperature up to 36°-38° death occurred after the third day.

The function of digestion is activated or retarded when the animal is exposed to high or low temperature. Parallel with the variations in metabolic activity food vacuoles present show great variations in size and number.

CENTRIFUGING.

Centrifugation of Paramecium in distilled water has three effects on the Infusoria - one, packing them together at the bottom of the tube, causing them to clump in a mass adhering to zooglea; another in inducing conjugation, and, finally, death, if centrifuged for one hour at high speed, with swelling of the protoplasm.

The centrifuging of the Paramecium transferred directly from stock culture to distilled water must have the definite effect of accelerating endosmosis and causing a trophic disequilibrium. Lyon (1905) has shown that the body of Paramecium, in normal conditions, contains substances of varying specific gravity, some of which collect under strong centrifugation at one end of the animal which is at the outer end of the tube.

Centrifugation was used by Dorothy Fetter (1926) to determine the viscosity of Paramecium protoplasm. The Paramecia for that purpose were fed on powdered iron particles and on starch grains, and the viscosity determined by the rate of movement of these inclusions in the protoplasm. The specific gravity of Paramecium was found to be 1.038, and the average length 0.026 cm. The results obtained were for the endoplasm only, for no granules ever penetrated the ectoplasm. The measurements were made at room

temperature

temperature between 20° to 25°C . Considering the viscosity of water as 0.01, the viscosity of paramecium protoplasm was found about 80.27, Of course there is some variation in the viscosity of paramecium protoplasm, but the presence of strands in the animalcule is the logical explanation of the high viscosity values obtained by Fetter. If Paramecium is centrifuged for a long time at moderate, or even at high speed, the granules which are scattered throughout the protoplasm do not move at all. The intergranular medium is probably too viscous to permit the free movement of the granules, or the granules do not differ very markedly in specific gravity from this medium, or perhaps the cell is so full of granules that there is no possibility of their moving into a limited portion of the cell. With all these reasons and other imponderable factors, we cannot consider this value of 80.27 as absolute.

Centrifugation alone will increase the viscosity of the protoplasm, and this effect is reflected upon the behaviour of the contractile vacuole which is affected by a complete paralysis of the apparatus or a wide dilatation of one of the vesicles with slow beat of the other. Paralyzed vacuoles sometimes become very large and burst through the ectoplasm or give rise to endoplasmic extrusions. Much of that, of course, is due to increase of internal pressure by endosmosis.

In the

In the reaction to gravity the animals place themselves with anterior end directed upward, and as a result swim to the top of the vessel containing them forming a thick collection there. During our experiments it was possible to show living paramecium in a tube through a low micro-projector successfully, to demonstrate the negative geotropism of the animalcule. If the tube is inverted after the collection is formed, they again come to the top. But if the upper end of the tube is closed, accumulation of CO₂ will force them to go to the bottom. But before clumping at the bottom, a constant current of Paramecium will be seen moving upward on one side and downward on the other, while at the top and at the bottom a mass of individuals will be collected. If the upper end is open again they will come back to the top and only dead individuals will be found at the bottom among debris and zoogloea.

HALITROPISM AND ACID REACTIONS.

Paramecium is stenohaline, i.e., intolerant of salts alone and dilute in sea-water, but tolerant of the salts when mixed in very low concentration of fresh water. The action of salts on Paramecium is often complicated by participation of other factors. The concentration of the salt must not exert any osmotic effect and the solution used must neither be hypertonic nor hypotonic. We have found that it is not a simple matter to find the exact strength of solution to use without producing immediate death of the animalcule. The pH of the solution is also another important factor in the experiment.

Solutions of potassium chloride or sodium chloride alone are toxic, but the addition of a small amount of magnesium or calcium chloride is sufficient to offset the toxicity. The effects vary with the type of water used - although all the salts used were fairly toxic at concentration of 0.2 and above. Very few of them remain so at lower concentration. The action of the salts are also variable at the same concentration with increasing proportion of individuals, up to the point where they will remain unaffected. Differences in the physiological conditions of the protoplasm may exist in various individuals from different populations of Paramecium, collected in ponds or rivers. Differences in acidity or

alkalinity

alkalinity of the culture medium, even though very slight, types of bacteria present, exposure to light, CO₂ concentration, - all these factors play a rôle in determining the conditions of the individual itself and its avoiding reaction. Some salt solution may affect the animal chemically on account of its chemical properties only, another may have physical effects. Of course, since both sets of properties co-exist in the same solution, it is possible that the Infusoria may be affected in both ways at the same time. The solute may produce a response in the protoplasm by diffusing into it and reacting with it in some way as yet not understood, producing marked changes in the morphology of the animal. It may affect the cell by plasmolyzing it or reducing its turgor pressure. The effects of the salts solutions sometimes vary according to whether the solutions are fresh or old. The salts solutions react on the division rate of the paramecium in increasing or decreasing it and often in preventing fission entirely. They induce conjugation, as stated by Chatton (1923) and Hopkins (1921). They reverse the irritability of the Infusoria by provoking sudden change in the osmotic surroundings. A high osmotic pressure is manifested upon the behaviour of the contractile vacuole, which at first show a very active pulsation, and progressively the beats decrease and then the apparatus is completely paralyzed. At that period a swelling of the protoplasm is seen, which gives to the Paramecium a butterfly shape, as can be seen in

Figure

Figure 4, before the rupture of the ectoplast.

Collett (1921) has shown the antagonism of the toxic action by inorganic chlorides. Toxicity of acetic acid, for example, is considerably lessened by the addition of NaCl, CaCl₂, BaCl₂, MnCl₂, MgCl₂, but barium chloride alone at a concentration of 0.2 kills *Paramecium* in less than a minute, and produces a vacuolization of the protoplasm and dilatation of the contractile vacuole, and Collett found that the power of BaCl₂, 0.005 M, to antagonize the toxic action of acetic acid is increased 100 per cent by addition of NaCl, which is lethal to *Paramecium* at any concentration (but addition of NaCl does not, at other concentrations, improve the antitoxic power of BaCl₂). In well balanced mixtures of salt and acid the protoplasm remains for a long time apparently normal, as if the acid were unable to penetrate the surface. The swelling which eventually appears in most of the mixtures is due to the small amount of acid which is able to penetrate the surface in spite of the salt protection.

Calcium is a more active antagonist than sodium, and at a much lower concentration.

Acids used as antagonists of salts are very efficient. The efficiency of a salt or an acid when used as antagonist depends upon the balance existing between its power to protect the membrane against the entrance of the

acid

acid molecule or anion and its power to antagonize the pH.

Nierenstein, from his work in vital staining, concluded that protoplasm of *Paramecium* not only at the surface, but also within the cell body, behaves like a lipoid containing traces of an organic acid and an organic base. Collett concluded from his experiments that the phenomena of acid-induced hydration and coagulation suggest the presence of some protein at the surface of the cell. Bresslau, as we have seen previously, concluded that antitoxic power of the tectine existed.

In the experiments of Dogiel, already mentioned, with $M/64 \text{ MgCl}_2$ and $M/50$ or $M/128 \text{ MgSO}_4$ the food vacuoles of *Paramecium*, as shown by ingested ink, do not break off from the inner end of the gullet, but from a long band with three to four loops which may be called an "ink intestine". This forms an extraordinarily large globular food vacuole after about twenty minutes. Thereafter ingestion is normal. In $M/100 \text{ FeSO}_4$ a similar "ink intestine" is formed, but the ink is usually extended through the wall of the gullet instead of forming a large food vacuole. In $M/100 \text{ BaCl}_2$ the food vacuoles became spindle-shaped, and were only one-tenth the usual size.

Neutral red and methylene blue were found to be very toxic. In concentrations from $1/1000$ to $1/10,000$ the

Paramecium stain rapidly with lethal effect after a few minutes to a few hours, retarding locomotion almost instantly.

Neutral red evidently penetrates the ectoplasm with extreme rapidity, for entoplasmic staining begins almost at once, the ectoplasm apparently not being able to hold the dye against the adsorptive capacity of the endoplasmic constituents. The posterior end is generally the first stained. Discharge of trichocysts may occur at the same time. In the high concentrations the deep staining is accompanied by death changes in the protoplasm, and these lead to fixation rather than cytolysis of the individuals (see plates). I have kept in very weak solution, added to about 0.025 per cent solution of Oxo, Paramecium alive for a week. They were slightly stained at the posterior end; with more penetration of the dye, they progressively died. The cytolytic changes, formation of vesicles, occur generally in lateral regions.

Several acids were used in testing irritability of Paramecium. Tannic acid was found toxic at the weakest concentration, provoking immediate formation of tectine, dilatation of contractile vacuoles and bursting of the protoplasm. Picric acid was used at different concentrations with various effects. Osmic acid produced immediate fixation of the infusoria. Paramecium reacted positively to all pH concentrations higher than that of their culture fluid up to pH 5.2 inclusive, when the pH is produced either by organic

or

or inorganic acids. Among the avoiding reactions of the *Paramecium* to acids and salts is one worth mentioning. It is a torpedo-like movement (see Figure 13). As a result the posterior end of the animal swings in a large circle, the animal advancing slowly, revolving on its axis, while the spiral course becomes wider. It is not a long reaction, but it is present in most of the experiments where strong solutions are used and when all parts of the body are equally affected by the stimulant.

Paramecia show other avoiding reactions already mentioned by Jennings (1905), and I do not think it necessary to repeat them here. *Paramecia* react to all stimuli and certain disturbances induced by gravity and centrifugal force, so that they escape from the condition or area that acts as a stimulus. A constant reversal of movement is produced by a weak solution of cresyl blue. The animals move towards the posterior end, then turn around after a few minutes. But as a rule the animal reacts when the change occurs in its environment, but ceases to react after the change is completed.

Reactions in the movements are also produced by simple addition of distilled water in the culture fluid containing *Paramecium*. They collect in the drop of water for a moment as they collect in acid solutions. Distilled water as is mentioned in another chapter, induces conjugation in *Paramecium*.

GALVANOTROPISM.

The first experiments made upon the reaction of Paramecium to electricity were made by Rood in 1853. My experiments were made with a very simple apparatus composed of a slide bearing a small trough with a platinum electrode at each end, a $1\frac{1}{2}$ volt dry cell with resistance in series and a reversing switch. As a current is passed, the Paramecia which were scattered all over the slide will move rapidly toward the cathode, and if the current is reversed they will immediately turn and swim to the new cathode. With progressive increase in the current they will remain at the negative pole till death. When induction shock occurs the animals react especially at the end facing the anode. In that case the ciliary activity is reversed and trichocysts discharged. With greater shock all the protoplasm react and the animal burst. Under a constant current vacuolization occurs in the protoplasm. The reversal of ciliary movement under electric current is probably due to disturbance in the equilibrium of the chemical process of the protoplasm. Addition of salts produce antagonistic effect of the current upon the infusoria. Under the action of potassium iodate, potassium chloride, sodium chloride, sodium carbonate and barium chloride, Paramecia are brought to the anode. But if they are accustomed to a solution of one of the salts cited they will swim to the cathode as usual if

that

that salt is added to the water containing them. If the solution of potassium chloride is more concentrated they will stand still, avoiding both poles.

Coehn and Barratt (1905) hold that *Paramecia* in ordinary water are positively charged, through the escape into the water of the negative ions of the electrolytes which the body hold while the positive ions are retained. In a solution containing more electrolytes positive ions escaped from the protoplasm and the animals became negatively charged. Statkewitsch (1903), mentioned by Jennings, subjected *Paramecia* that had been stained in the living condition with certain chemical indicators - neutral red and phenolphthalein - to the influence of the electric current. He found that the current caused chemical changes within the protoplasm, the endoplasmic granules and vacuoles becoming more alkaline in reaction. Unfortunately, I did not study the behaviour of the contractile vacuole under the influence of electricity. Increase of viscosity of the protoplasm, as well as the current itself, may produce interesting changes on the pulsatory rhythm.

CUTTING EXPERIMENTS

CUTTING EXPERIMENTS.

Cutting experiments were made on Paramecia after the method of Calkins, and truncated individuals were kept for some time. The monster forms derived from that merotomy were observed. Immediately after the cutting the two separate parts of the individual swim about. At the cut end an apparent evidence of the ectoplasmic pellicle is seen. In some of the Paramecia surface precipitation occurred as the protoplasm began to emerge from the bounding membrane, and in these cases only the experiment was successful. Otherwise the endoplasmic content would flow in the surrounding fluid. A very fine and sharp knife with uniform blade is necessary, for a rough knife would tear the striated net-like protoplasm and produce bursting of the infusoria, thus demonstrating the physical structure of protoplasm.

EXPERIMENTAL INJECTION.

L. R. Cleveland (1927) by injecting Paramecium into frog rectum claimed to have obtained encystment of the Infusoria. These experimental injections were made and rounded individuals, like those obtained at low temperature, were obtained from two to twenty-four hours after the experiment had started. Some individuals in a complete state of turgidity, and others in a state of turgescence, were found even later, but generally the Infusoria would continue to live in the frog rectum, without encysting.

EXPERIMENTAL INGESTION.

No data existed regarding pathogenic effects of Paramecium and it was never noted among the intestinal protozoa of man. I started some experimental ingestion of three hundred Paramecia a day in distilled water (sterilized,

as

as mentioned in a preceding chapter). The ingestion was made over a period of ten days at different times in the day.

Repeated examinations of my stools were made and no *Paramecia* were found at any time. No symptoms of infection were noted either, no diarrhoea, and not the least discomfort felt. I considered this experiment as negative. *Paramecium* could easily resist the temperature of the body, but probably not the gastric juice. During my experiments on thermotropism of *Paramecia*, individuals were kept at 39°C for fifteen minutes.

W. H. Johnson (1929) concluded from his experiments on the reaction of *Paramecia* to solutions of known hydrogen ion concentration that "Paramecia are better able to live in solutions of high pH concentration than many have supposed".

But considering the effect of temperature allied to the pH concentration of the gastric contents, and duration of the exposure to that pH concentration, there is all probability that *Paramecia* cannot live in the stomach.

Parasitic ciliates always encyst and the cysts are responsible for infections when ingested. *Paramecia* never encyst. The parasitic forms are adapted to the body reactions. *Paramecium* is highly sensitive to any change in the environment, and would never be able to adapt itself to the body conditions.

THERMOTROPISM.

THERMOTROPISM.

One of the most interesting reactions of Paramecium is that produced by change in temperature. The action of heat and cold on the protoplasm finds expression in colloidal changes. Between the extreme limits of heat and cold in which Paramecium can remain alive, there is a range of temperatures which produce marked effects on the activity of the Infusoria.

The viscosity of the protoplasm is increased as the temperature decreases, and goes to the maximum at 3°C. Coagulation of the protoplasm is produced by heat. After twelve hours' exposure to cold, Paramecium progressively changes its original slipper shape to take, by distension of the posterior end, a flask shape. As distension progresses, a complete spherical form is obtained (see figures). Vacuoles of watery fluid appear in the protoplasm. Continued concentration of the solution within the protoplasm cannot go on without affecting the energetic transformation of vital activity. An alteration in the activities of the protoplasm thus produced may result in changes in its permeability, and changes of this sort, accompanied by changes in the chemical activity within the protoplast, may account for the formation of these numerous vacuoles in it. The turgidity of Paramecium may be said to be due to osmotic pressure of the cell and to the semi-permeability of the surrounding protoplasmic layer. Intra-vacuolar pressure arises from the permeability of the protoplasm as a whole.

Osmotic pressure became so great as to burst the animalcule. When the spherical form is obtained in *Paramecium* ciliary action continues for a while, but movement is very sluggish, and finally the Infusoria cease to be motile and die. Some individuals show metabolic movements (See Figure 11), but die just the same after a moment.

The period of conjugation is longer at low temperature and shorter at high temperature. The process of fission shows the same variation. New separated individuals from fission taking place at 5° to 10°C do not grow, and remain one-quarter the size of normal individuals (dwarf forms), but resist the temperature better than mature individuals. A definite resting stage was obtained by submitting the animalcule to progressive decrease of temperature down to 5°C. Two single individuals were placed in two extreme temperatures - one at 5°C., the other at 36°. The individual in 5°C. did not divide, while the other one did divide. Division rate at 36°C. was even one a day. No cysts were obtained and probably the cyst-like individuals have been mistaken for real cysts at a period of metabolic movement.

Jaen Port (1928), considering protoplasm as a system of colloidal phases, studied the coagulation of *Paramecium* protoplasm by different means, and at different temperatures. He found a mean of 229 seconds at 40°C. in culture fluid to determine coagulation. Below 38° coagulation is very slow. For example, at 36° the coagulation time is 60 to 70 minutes.

Above 44° coagulation is too rapid to be followed. Since at 40° and 38°, and at lower temperatures, Paramecium has maximum resistance to heat at pH 7.7, this value, 7.7, has great significance in regard to the life of the animal cell. In the following table from Jaan Port's experiments are given data on the coagulation of the protoplasm of Paramecium at different temperatures and under different conditions.

TABLE IV.

HEAT COAGULATION IN SECONDS.

Temp.	Pure Water	Culture Fluid	Neutral With HCl			Neutral with H2SO4		Neutral with citric acid			+ KOH		+ NH4OH
°C	pH 7.2	pH 7.7	pH 7.4	pH 7.0	pH 6.8	pH 7.4	pH 7.0	pH 7.4	pH 7.0	pH 6.8	pH 8.0	pH 8.4	pH 8.0
44	9	8.5	8.8	9	9.3	10	11.5	11	12	10	8.	7.5	9
42	50	33	105	120	126	40	53	58	62	38	26	22	37
40	200	253	330	310	220	200	192	168	140	137	165	143	185
38	660	1240	1050	920	900	428	380	625	325	280	900	880	910

VITALITY OF PARAMECIUM.

Fluctuations are noticed in the reproduction rate of Paramecium. Woodruff (1905) used the term "Rhythm" to describe this phenomenon, which I believe is due to extrinsic and intrinsic causes combined together. Woodruff and Erdmann (1914) discovered an endomixis, equivalent to pathogenesis, which realizes under a new form the cyclic evolution of Maupas. According to Child (1915) "it is evident that the rhythm and the process of endomixis represent a senescence-rejuvenescence period". Conjugation is the beginning and the end of a series of reproductive activity. Protoplasm gradually tends toward a state of stable equilibrium by release of potential energy, resulting ultimately in decreased activity restored by conjugation. This power of paramecium protoplasm is shown by protective reaction under unfavourable conditions and by regeneration of the individual during cutting experiments. Conjugation develops a stabilizing effect, not only against environmental conditions, but also against a greater loss of energy, by inducing a resting phase in the life of the infusoria which replaces encystment. Chatton (1923) pointed out a fact which was verified during our experiments, that zygogenic influence of trophic disequilibrium is manifest and that fission rate is feebler in zygogenic cultures than in azygogenic. The incidence of conjugation reduces the fission rate. Binary fission occurs

during

during the vegetative phase, when the animal is actively feeding, growing, and reproducing itself.

The increase of metabolism is the result of a period of crisis manifested about every eight days, and manifested by the fluctuations in the fission rate. Decrease of metabolism is manifested and restored by conjugation or by an automatic process of endomixis. In the laboratory cultures, the chemical changes of the medium by bacterial effects, or by waste products of the Infusoria themselves, produce a marked effect on the morphology of the animal as well as on the fission rate. Under a probable increase in osmotic pressure with decompensation in the function of the contractile vacuole, somewhat of an edema occurs in the individual, the slipper shape is lost and the posterior end of the animal is rounding up. The protoplasm is homogeneous and opaque, with the appearance of a cloudy swelling. Movement is sluggish and rhythm of the contractile vacuole very slow. The animal can recover from that state if placed in a more favorable medium.

As a conclusion we can say that the vitality of Paramecium is the combined result of chemical and physical reactions of the individual upon the medium and of the medium upon the individual. It is manifested by the adaptation of the Infusoria and its metabolic activity.

PROBABILITY OF

PROBABILITY OF CONDITIONS IN NATURE.

AN INTERPRETATION OF MASS CONJUGATION IN PARAMECIUM.

"To study life we must consider three things: first, the orderly sequence of external nature; second, living organisms and the changes which take place in them; and third, the continuing adjustment between the two sets of phenomena which constitute life". - - W. K. Brooks.

The natural environment of Paramecium is determined by different factors which can be divided into two main groups: 1. The physical factors, including the material in suspension or in solution in the water, the variability of the water temperature, depth of the water, speed of water current, winds, light, etc. 2. The chemical factors, including acidity or alkalinity of the water, gases, salts present, living or dead organisms, which make the biological medium.

When freshly gathered material for the study of Paramecium is assembled at the spring of the year shortly after the great thaw in this latitude, it may be observed after a few days that a strong tendency to conjugation manifests itself in the wild culture. Under these conditions the presence of one hundred to upwards of three hundred couples in a single slide culture may be regarded as an indication of maximum conjugation. This has been

observed

observed in the spring of 1926, in the autumn of 1929, and again this spring, the greater number being recorded in the spring of 1926. The occurrence of so many conjugants precisely at the turning points of the year suggested a reference to climatic conditions. The spring and autumn maxima occur at what Chatton (1923) has called periods of crisis characterized by trophic disequilibrium. It is indeed enough to witness the decline of aquatic vegetation in late autumn in order to convince one of the fact that the nutritive conditions in the water are undergoing rapid change at that season, and the well-known sensitiveness of *Paramecium* to change of temperature points in the same direction. If a slide culture is placed for a few minutes between double windows in freezing weather, all movement ceases until they are restored to the warm room; if exposed too long to the cold, they die without encysting. No experimental means have yet been discovered to make *Paramecium* encyst, and it is sometimes stated positively that *Paramecium* never becomes encysted (Perrier, 1897). Métalnikov (1924) established the fact, from laboratory cultures in Russia, that the mean daily fission rate was greater in summer than in winter; and in 1915, when, owing to defective heating, the room temperature fell below normal, the number of annual generations dropped from about four hundred to two hundred and thirty-eight.

Treatment of laboratory cultures at room temperature with distilled water will also induce mass conjugation. We

have

have observed this effect in the month of February, this year, both in subcultures and in renewed cultures. In watchglasses containing a small quantity of freshly matured and diluted hay infusion, seeded with a few individuals from the main culture, after two days of preliminary multiplication the addition of a few drops of distilled water brought about a large proportion of conjugants within the next twenty-four hours. H. S. Hopkins (1921) found that the addition of certain salts to his renewed cultures made them more susceptible to conjugation after a preliminary period of multiplication lasting from three to five days.

From the various observations which we have made from first to last and in general accordance with the known reactions of *Paramecium*, we feel that there is sufficient foundation for plotting a tentative growth curve representing the annual cycle of *Paramecium* under natural conditions in the climate of Montreal.

Experiments on *Paramecium* have yielded results of the highest importance since the classical researches of Maupas, but it seemed worth while to make the attempt to frame a picture of the probable course of events in the open waters, especially under a climate where the seasonal changes are sharply accentuated and consequently where such questions can best be put to the test. The point marked "A" in Figure 15 stands for the turn of spring; the point "B" marks the

autumn

autumn transition. At both these seasons, as mentioned, mass conjugation has been found to take place, suggesting a two-fold significance for it: first, that of activating fission for the summer; secondly, that of stiffening resistance for the winter. The apparent simplicity of the annual cycle (Figure 16), commencing with the vernal conjugation, passing through the summer fission to the autumn climax and ending in the winter rest, is perhaps an argument for its probable approximation to the truth. The spring maximum may be compared with the formation of reproductive cysts in other Infusoria, and the autumn maximum is paralleled by the resting cysts.

A prominent feature of mass conjugation is its simultaneity, comparable in this respect with the simultaneous cleavage of embryonic cells. At the annual changes from winter to spring and from summer to autumn, the Paramecium population responds en masse to the exigencies of the times. The normal summer fission rate is likewise fairly uniform in its incidence, so that the multiplication of an actively feeding community is essentially a synchronous reaction of large numbers of individuals to alterations in the internal medium or endoplasm, while the so-called epidemics of conjugation are mass demonstrations following upon alterations in the external medium. Conjugation, which can be induced experimentally in a mixed culture without the addition of salts, thus appears to result from the interaction or

concurrency

concurrence of two sets of factors, external and internal, not one to the exclusion of the other; and under natural conditions, the seasonal changes are enough to provoke similar responses.

DESCRIPTION OF FIGURES.

Figure 1. Plasmolysis of Paramecium, under the action of strong solution of neutral red. The endoplast has rounded up and has shruken away from the ectoplast.

Figure 2. Effect of weak solution of neutral red after ten minutes.

Figure 3. Same effect on same individual two hours later.

Figure 4. Swelling effect of neutral red solution. Butterfly-like shape, before the rupture of the ectoplast.

Figure 4a. Blister formation on lateral sides of Paramecium. Dilatation of posterior contractile vacuole - under action of neutral red solution.

Figure 5. Karyolysis: Effect of continuous action of neutral red over a period of three days.

Figure 6. Lateral view of Paramecium. Note extrusion of the posterior contractile vacuole due to action of potassium iodate, 1 per cent solution.

Figures 7 and 8: Cyst-like forms obtained by low temperature at 3°C.

Figure 9: Complete rounded Paramecium in a state of turgidity. Note vacuolization of protoplasm and beginning cytolysis. From frog rectum.

Figure 10.

Figure 10. Progressive rounding of Paramecium exposed to 3°C. The flask-shaped individual may be said to be in a state of turgescence. Note vacuolization of the protoplasm.

Figure 11. Metabolic movements of Paramecium exposed to low temperature.

Figure 12. Cytolysis of Paramecium under the action of weak solution of tannic acid, about 1 per mille. Note dilatation of both contractile vacuoles. Paramecium is surrounded by trichocysts.

Figure 13. Diagram to illustrate one of the avoiding reactions of Paramecium - the torpedo movement.

Figure 14. One of the first pictures of conjugation in Paramecium after Balbiani.

Figure 15. Chart of the probable growth-curve of Paramecium under natural conditions as deduced from open cultures in the laboratory.

A. Vernal conjugation equivalent of reproductive encystment (activating fission).

B. Autumnal conjugation equivalent of resting encystment (inducing resistance).

Figure 16. Annual cycle of Paramecium.

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Fig. 1

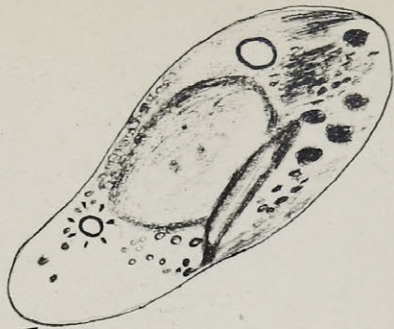


Fig 2.

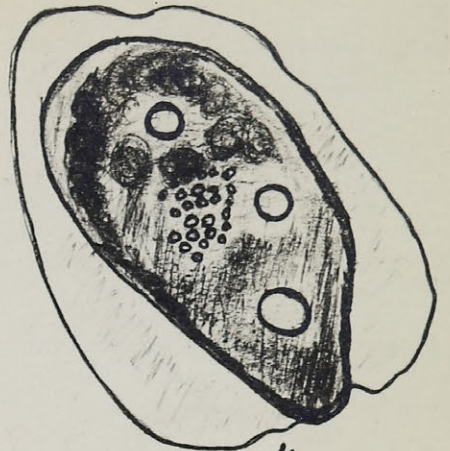


Fig 4



Fig 3.

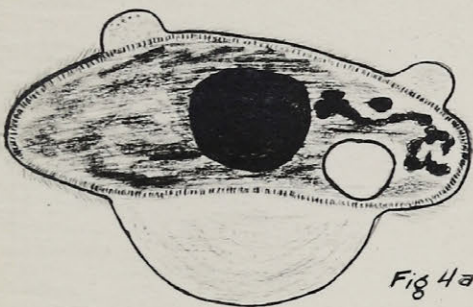


Fig 4a.

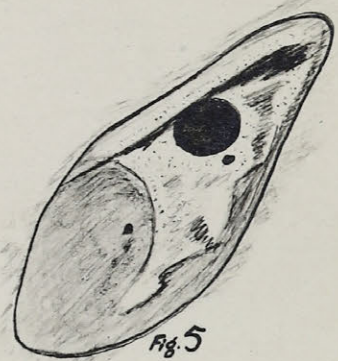


Fig. 5



Fig 6.

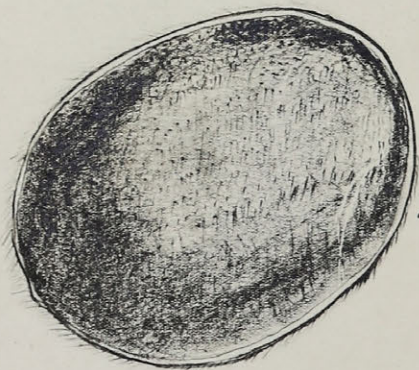


Fig 7.

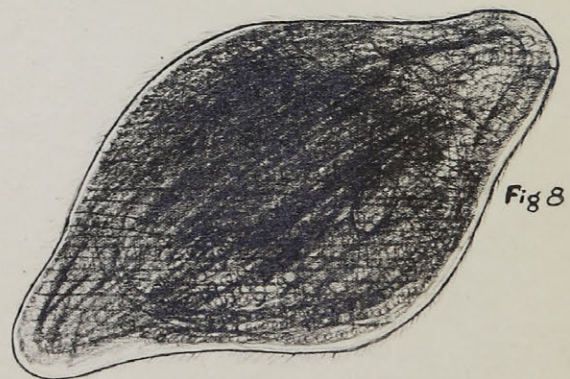


Fig 8

