Environmental Factors Affecting the Life History of Three Soil Species of *Colpoda* (Ciliata).

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[*Read before Wellington Branch, May 26, 1954; received by Editor, May 27, 1954.*]

CONTENTS

Abstract

Colpoda has at least three types of cysts resting cysts, division cysts, and unstable cysts. Unstable cysts are thin-walled, and their formation is due to the inhibition of normal division by environmental factors. Removal of the inhibiting factor results in division and excystment. Resting cyst formation is not affected by the same factors. Temperatures below 5° C. and above 35° C., and salinities over 3% cause the formation of unstable cysts in *C. steinii*. Neither absence of oxygen nor high carbon dioxide concentrations are effective.

Colpoda survives the absence of oxygen and feeds and moves freely. It does not divide. The species differ in their tolerance of high carbon dioxide tensions. *C. steinii* and *C. inflata* are more tolerant than *C. cucullus*.

I. INTRODUCTION

IN its life history *Colpoda* closely resembles other members of the family Colpodidae, particularly in the important role played by encystment and excystment. The two species of related genera which have been most carefully studied are *Tillina magna*, by Beers (1943, 1945, 1946) and his students Bridgeman (1948) and McIntyre (1949), and *Woodruffia metabolica*, which has been studied by Johnson and Larson (1938) and Johnson and Evans (1939, 1940, 1941a, 1941b).

In this family the life history consists of three stages: an active, trophic or feeding stage, a reproductive or division stage, and a resting stage. Both the latter stages involve encystment and excystment (Brown and Taylor, 1938; Kidder and Claff, 1938). Under favourable conditions the ciliate feeds, grows,

* This work was carried out during the tenure of a Victoria University College Research Scholarship.

and then, after a time, encysts to form a reproductive cyst. The encysted ciliate divides once, twice, or three times, and then excysts and begins feeding again. If the food supply is exhausted, resting cysts are formed. These are either temporary cysts or permanent cysts. Temporary cysts may excyst spontaneously, but permanent cysts require a definite stimulus and will not excyst for some time after encystment, during which time there is a period of reorganization.

Although this is the typical life cycle, division may take place without prior encystment (Penn, 1937, 368), and this condition may be experimentally induced (Stuart, Kidder and Griffin, 1939) Reynolds (1936, 49) found that when *C. steinii* is parasitic in snails it divides without forming a cyst. The life cycle of *C. maupasi,* which differs slightly from that of other species, has recently been described by Padnos *et al* (1951, 1953a, b). Conjugation within the family is extremely rare. Both Enriques (1908) and Wenyon (1926) recorded conjugation or syngamy in *C. steinii,* but I have observed conjugation of this species only once.

I have generally endorsed the accepted effects of pH, temperature and salinity on the active and encysted ciliates or have extended these observations. In one important point I differ in conclusion from those of C. V. Taylor *et al.* of Stanford University. Distilled water has proved perfectly effective for excystment without the addition of any excysting factor (cf. Goodey, Bodine and Kidder *et al*). Further, I have shown the particular significance of osmotic diffusion and the importance of osmotic difference between the milieux interieur and exterieur. The survival of *Colpoda* under anaerobic conditions, while agreeing with the ecological data, is in disagreement with Adolph's findings These discrepancies may be attributed to differences in technique and the failure to allow gradual adaptation of the ciliates to changed conditions (Brand, 1946). The results are similar to those with *Tetrahymena* (Thomas, 1942) and *Balantiophorus* (Watson, 1944), in particular in the suppression of division.

The inhibition of division, and the consequent formation of what has been called an unstable cyst, under the influence of adverse environmental conditions, is the chief new contribution to our knowledge of the life history of *Colpoda.* In this *Colpoda* resembles *Woodruffia* in which thin walled unstable cysts, larger than the permanent cysts, are formed as the result of extremes of temperature, high concentrations of H-ions, increased salinity, low oxygen tensions, crowding and feeding on starved *Paramecium.* In some respects the unstable cysts of *Colpoda* differ from those of *Woodruffia.* First, although both are large and thin walled and in this resemble the ordinary reproductive cyst, in *Woodruffia* there is no record of the unstable cyst becoming a reproductive cyst upon the removal of the inhibiting factor, as is the general rule with *Colpoda.* Secondly, *Colpoda* is far more acid tolerant than *Woodruffia* and extreme pH does not cause unstable cyst formation. *Colpoda* continues to feed and live under low oxygen tensions, and only very rarely forms unstable cysts. *Woodruffia* ceases feeding and encysts at low oxygen tensions and dies in the absence of oxygen. *Woodruffia* is carnivorous, whereas *Colpoda* is typically bacteriophagous. Crowding of active *Colpoda* is important in determining whether permanent or temporary resting cysts will be formed (Taylor and Strickland, 1938, 1939) but does not cause unstable cyst formation. Generally, then, there are close parallels between the two, but they are not altogether identical. It may be remarked that Johnson and Evans (1939, 95) described a third type of cyst, which they regard as a digestion

and assimilation cyst. In *Colpoda,* temporary cysts which excyst spontaneously are similar to normal resting cysts and are formed in the absence of food and the absence of crowding (Taylor and Strickland, 1939; Garnjobst, 1947). They are therefore different to the *Woodruffia* cyst. In *Woodruffia* the stable resting cyst requires an excystment medium similar to that allegedly required by *Colpoda* resting cysts, according to Taylor *et al.*

Tillina has apparently a simpler life history with only reproductive and resting cysts. The cysts are activated with distilled water. Bridgeman (1948) proposed that encystment was due to an insufficient amount of food, or an insufficient amount of oxygen, or both, and that it was accompanied by water loss. Excystment, she considered, occurred when food and oxygen levels are sufficiently high and there is an entrance of water into the cyst. In dealing with the resting cyst of the Colpodidae she is partially correct. However, she is in error when she considers the two processes—viz., encystment and excystment, as complementary, both under the influence primarily of food and oxygen. Absence of food, or nutritional deficiency, is an absolute condition of resting cyst formation in the Colpodidae, but it is not the only condition. Crowding, for *Colpoda,* is equally important, nor in this case is encystment affected by the oxygen tension. Indeed, encystment, in marked distinction to excystment, is independent of oxygen tension. Moreover, excystment both in *Colpoda* and *Tillina* can be achieved with distilled water. Excystment is then in these cases independent of food. Unlike encystment, however, it is dependent upon respiration, and is accompanied by a marked rise in the respiratory rate (Thimann and Commoner, 1940; von Wagtendonk and Taylor, 1942). The two processes then must be considered quite distinct and the factors affecting them quite distinct also. This distinction is shown in the following table:—

	Encystment	Excystment
Food deficiency	Dependent	Independent
Food	Inhibits	Independent
Respiration	Independent	Dependent
Osmosis	Independent	Dependent

Another problem is the efficacy of potassium ions and alcohol as excysting factors. The protective function of the potassium ion in the alcohol-potassium excystment medium has also not been satisfactorily explained (Thimann and Haagen-Smit, 1937; Haagen-Smit and Thimann, 1938; Strickland and Haagen-Smit, 1947, 1948). This is partly due to the fact that those who worked on these factors were unaware that excystment of *Colpoda* can be achieved simply with distilled water. Such substances as hay extract, organic acids and so on have not, therefore, the role of essential metabolites. In this case Bridgeman is also misleading, for alcohol can hardly be considered as food. She is on safer ground when she stressed the fundamental matter of osmotic differential. It has been shown (Bodine, 1923) that alcohol in low concentrations rapidly penetrates the cyst membrane. It may be presumed that metabolites such as the organic acids readily diffuse through the cyst membrane as shown by Bodine (1923) and Haagen-Smit and Strickland (1938). The particular efficacy of the potassium ions, found by these last authors, may therefore be attributed to their ready diffusion through the membrane and consequently their stimulus to further osmosis. Once osmosis is established, for example with distilled water, the excystment process is largely, though not wholly automatic, provided oxygen is present

(Brown, 1939). No further stimulus is needed, although excystment is more rapid, with a definite excysting medium than with distilled water. The stimulus, must, however, be maintained until the ciliate is activated and differentiation has begun (McIntyre, 1949; Brown and Taylor, 1938). Differentiation of the ciliates without excystment does sometimes take place, which shows that the mechanism is not always perfectly automatic. Nevertheless, basically excystment and differentiation in *Colpoda* and *Tillina* are best understood as a mechanism set off by a trigger reaction, and this trigger is osmosis. In my experiments the mechanism was unexpectedly set off by reduced oxygen tensions and by carbon dioxide, neither of which reactions could be satisfactorily explained.

II. Experimental: Material

The chief species studied was *C. steinii*. *C. cucullus* and *C. inflata* were also studied for the effect of some of the factors. All these species occur in the soil, and it was from soil that the strains employed were isolated. Generally they were maintained free of other protozoa on yeast extract, except *C cucullus*, which grew better on lettuce infusion. For some purposes *C. steinii* was washed and suspended in a hanging drop with a loop of bacteria as food The sterile medium devised by Garnjobst (1942) was not used.

In comparing these experiments with published work it is necessary to remember the confusion in nomenclature which obtained up till the publication of Burt's (1941) paper. C. V. Taylor *et al.* worked with *C. steinii*, Goodey's experiments were probably done with *C. inflata*, while the identity of Adolph's and Bodine's ciliates is uncertain.

i Oxygen and Carbon Dioxide Tensions: Methods

A modification of Kitching's (1939) apparatus was used. A small chamber with inlet and outlet tubes was placed on the stage of the microscope. A hanging drop preparation was sealed to the chamber and the gas phase replaced with either nitrogen, carbon dioxide or an oxygen-carbon dioxide mixture Before passing through the chamber the nitrogen was washed in dilute sulphuric acid alkaline pyrogallol and distilled water, and on escaping from the chamber through distilled water and alkaline pyrogallol The carbon dioxide and oxygen-carbon dioxide mixture were passed through distilled water. The gases used were from commercially prepared cylinders. Rubber leads were used to connect the apparatus.

Effect of Anoxia

The present experiments give clear evidence of anaerobic survival by *Colpoda*, and this is in accord with the ecological evidence (Noland, 1925) Adolph (1929), however, found that *Colpoda* was killed when oxygen was suddenly taken away and, further, that when partly grown individuals were subjected to low oxygen tensions they finished growing and formed their division cysts several hours sooner than the controls under the oxygen tension of room air. Their sizes were much smaller than would be accounted for by the shortened period of growth, indicating a lessened rate of assimilation. The smaller individuals each gave only two progeny in place of the usual four In some experiments the individuals under low oxygen tension formed permanent cysts, whereas no such cysts were found in the controls. Adolph cites Rhumbler (1888) who also stated that decrease of oxygen tension causes the formation of protective cysts in *Colpoda*.

Adolph did not find any variation in oxygen consumption of *Colpoda* with oxygen tensions of 157 to 750 mm. Hg. but with a tension of about 5mm Hg. the oxygen consumption fell to 31% of its normal value.

In my experiments with *Colpoda,* unstable cysts were formed on two occasions Once, a single ciliate (*C steinii*) encysted when there was adequate food in the drop and a trace of oxygen, and then excysted, without dividing, when the oxygen was removed. Secondly, three ciliates (*C. inflata*) formed unstable cysts which excysted when the drop was placed in air, but did not encyst again when they were returned to the nitrogen chamber. In this case also there was ample food, and the ciliates continued to feed in the nitrogen atmosphere. At no time, however, were resting cysts formed as described by Adolph and Johnson and Evans (1941a). Such cysts are formed only on the exhaustion of food and the rate of encystment is dependent solely on the concentration of the ciliates and is independent of the oxygen tension. The formation of unstable cysts is very unusual, but it may be due to a sudden, as contrasted with a gradual, change of oxygen tension. The inhibition of division is similar to the results with *Woodruffia,* and the inhibition of excystment. Brown (1939) demonstrated that excystment was independent of oxygen tension above 15mm. Hg., and was completely inhibited below.

Anaerobic survival of ciliates is summarised by Thomas (1942) and Brand (1946). The only other evidence of prolonged anaerobic survival of facultative anaerobes is that of Watson (1944, 46) for *Balantiophorus minutus* and Thomas (1942) for *Tetrahymena pyriformis* (syn. *T. geleii*). Watson's experiments and results are very similar to the present work. Thomas recorded survival of *T pyriformis* for 52 hours with no deaths. Both authors found that division was inhibited. The ecological evidence (Noland, 1925) supports Thomas's results It seems evident then from the experimental and ecological evidence that these two ciliates—viz., *Balantiophorus minutus* and *Tetrahymena pyriformis,* and the three species of *Colpoda* tested—viz., *C. cucullus, C. inflata* and *C steinii,* are all characterised by the ability to survive anaerobic conditions for some time and. in the case of *Colpoda,* ultimately to encyst None of the ciliates can divide in the absence of oxygen, and this is a general rule, illustrated by mammalian epithelial tissue also (Medawar, 1947), which will be discussed later. The results of Adolph with *Colpoda* may have been due to a sudden rather than a gradual removal of oxygen, but they were not reproduced in my experiments.

Experimental Results

Without oxygen the ciliates continued to move and feed Without food they encysted to form resting cysts. On two occasions unstable cysts were formed With food the ciliates survived several days in the absence of oxygen, often increasing in size, but in no case did the ciliates divide. The most important effect of lack of oxygen was the complete inhibition of division. This is illustrated by the following experiments.

EXPERIMENTS WITH *C. steinii.* Ciliates in hanging drops with food.

A. Time (Hours)	0	21	25
Nitrogen	1 active	1 active	1 active
Control	1 active	1 active, 1 div. cyst	5 active

B. Time (Hours).	0	5.30	29.30	98
Nitrogen	3 active	3 sluggish	3 very sluggish and large	3 still active
Control 1	1 active	1 active	5 active	
Control 2	2 active	2 active	c. 10 active	

C. Time (Hours).	0	30	53.30	77.30
Nitrogen	1 active	1 cyst	1 active	1 active
Control	2 active	3 active	5 active	c. 40 active
			2 div. cysts	2 resting cysts

In Experiment C., the current of nitrogen slowed down and stopped within the first 30 hours, permitting oxygen to diffuse back into the chamber. The ciliate encysted to form an unstable cyst which subsequently excysted when the current of nitrogen was renewed. It then continued to move and feed in the drop. After 80 hours the current of nitrogen stopped and was not renewed. With the return of oxygen to the chamber, the ciliate then began to divide. Similarly, in Experiment B, the three ciliates kept in an atmosphere of nitrogen for 98 hours were then returned to air and within two and a-half hours had formed division cysts Within 46 hours there were about 100 ciliates in the drop. These ciliates had continued to feed and increase in size in the nitrogen atmosphere.

Resting cysts are formed upon the depletion of food and by concentration of the ciliates. The following experiments show that resting cyst formation is independent of the oxygen tension.

Ciliates in nitrogen and ciliates in oxygen showed no significant difference of encystment rate. Rate of resting cyst formation, however, depended upon the initial concentration of the ciliates.

EXPERIMENTS WITH *C. steinii.* Ciliates in hanging drops without food.

D. Time (Hours)	0		5.30		% Encystment
Control Active	94		21		70
Cysts			73		
Control Active	125		15		88
Cysts			110		

E. Time (Hours)	0		6		% Encystment
Control Active	101		6		95
Cysts			95		
Control Active	29		6		80
Cysts	2		26		
Oxygen Active	50		8		87
Cysts	11		65		

F. Time (Hours)	0	3.45	6.25	23 30	% Encystment
Control Active	14	13	12	8	43
Cysts		1	2	6	
Oxygen Active	25	23	19	8	68
Cysts		2	6	17	

G. Time (Hours)	0	1.45	2 35	23.45	% Encystment
Control Active	4			4	0
Cysts	6			6	
Nitrogen Active	12	7	7	5	42
Cysts		3	5	5	
		2 encysting		2 dead	

H. Time (Hours)	0	4.15	52.15	72.15	77.15	% Encystment	
Control Active	36				8	80	
Cysts	24		54		56		
Nitrogen Active	40	33	14	4	3	93	
Cysts	21	28	47	57	58		
I. Time (Hours)	0	3.45	22.45			% Encystment	
Control Active	101	1	0			100	
Cysts		100	101				
Nitrogen Active	43	24	3				
Cysts	3	22	43			93	
J. Time (Hours)	0		24			% Encystment	D.R
Control Active	15		25			30	2.4
Cysts			11				
Oxygen Active	20		25			49	2.55
Cysts			26				

Although these experiments show that encystment is dependent upon the initial concentration of the ciliates and not upon the gas phase in which they are suspended, Experiment J. shows that the division rate of the ciliates is slightly higher in pure oxygen than in atmospheric air. This is similar to the findings of Pace and Ireland (1945, 349) with *Tetrahymena pyriformis.*

Excystment of *Colpoda* is strictly dependent upon the presence of oxygen Experiments with cysts washed and suspended in hanging drops with bacteria showed complete excystment of controls in air and excystment of only three or four ciliates in the nitrogen chamber. Using the alcohol-phosphate excystment medium (Strickland and Haagen-Smit, 1948), about 500 to 600 cysts were suspended for 20 hours in nitrogen. At the end of this period there was one active ciliate. Returned to the air there were about 300 active ciliates, or about 50% excystment, within four hours.

When a trace of oxygen was permitted in the nitrogen chamber, the excystment of *Colpoda* appeared to be stimulated. A hanging drop with 13 active ciliates and about 120 cysts was placed in the chamber, and the current of nitrogen, with a trace of oxygen, turned on. After 3 hours there were 15 active and 20 cysts activated. After 5 hours there were 50 active, 5 activated cysts and 77 unactivated cysts. After 23 hours there were about 41 active ciliates and 89 cysts. The unpurified nitrogen was turned off after 24 hours and oxygen turned on. Later there were 14 active ciliates and about 100 cysts.

The foregoing experiments were conducted with *Colpoda steinii.* The reactions of *C. cucullus* and *C. inflata* are similar With *C. inflata,* three ciliates were suspended in a hanging drop preparation in the nitrogen chamber, together with phosphate buffer and live yeast. After 22 hours one of the ciliates had encysted (to form an unstable cyst) and the other two encysted within 66 hours. The hanging drop was then placed in air, and within 24 hours the ciliates had excysted. They were then returned to the nitrogen chamber, where they did not again encyst, but, after 70 hours, died By this time the ciliates had greatly increased in size.

Effect of Carbon Dioxide

No previous experimental work appears to have been done on the carbon dioxide sensitivity of *Colpoda,* although the work of Jennings and Moore (1901)

Jennings (1915), Jacobs (1912, 1922), Root (1930), Watson (1944) and Pace and Ireland (1945) have shown the general reactions of ciliates to carbon dioxide and the susceptibility of different species. The ecological significance of the present findings will be discussed in another paper. Here it is sufficient to note that the reactions of *Colpoda* are similar to those of other ciliates, although *C. inflata* and *C. steinii* are generally much more resistant than *C. cucullus*. The inhibition of division by high carbon dioxide tensions is recorded for *C. steinii*, and the apparent stimulation of excystment for *C. cucullus*.

Experimental Results

The effect of high carbon dioxide tension is, first, to slow the movements of the ciliate, then to cause the cessation of movement, and, finally, the death of the ciliate. Recovery could be obtained by returning the ciliate to air, provided it had not blistered. In no case did high carbon dioxide tensions cause encystment, although they did appear to stimulate excystment.

Survival of the ciliates depended largely upon their nutritive state, well fed ciliates surviving better than starved ones. The following experiments are with *C. steinii* in an atmosphere of pure carbon dioxide.

Material.	Exposure Time.	Final Condition.	Recovery
Old culture	1 hr.	dedifferentiation	majority
	6 hrs.	cytolysis	none
	1 hr.	dedifferentiation	50%
	1 hr. 25 min.	dedifferentiation	50%
	40 min.	dedifferentiation	50%
	2 hrs 50 mins.	dedifferentiation	50%
	2 hrs 25 mins	cytolysis	none
Fresh culture	6 hrs. 40 mins	some still active	25%

Cysts were kept in the carbon dioxide chamber for 24 hours and 30 minutes and then taken from the chamber and fresh yeast extract added. Excystment took place within four hours.

Starved ciliates were placed in the chamber and a mixture of carbon dioxide and oxygen passed through There was no encystment after several hours.

Three ciliates with a loop of bacteria were placed in the chamber and carbon dioxide and oxygen passed through. The ciliates were still active after 24 hours, though they were moving very slowly and they had not divided nor were they feeding. They recovered fully when returned to air.

The following experiments are with *C. inflata* in an atmosphere of pure carbon dioxide.

Material.	Exposure Time	Final Condition	Recovery
Fresh culture	2 hrs 15 mins	cytolysis	none
	1 hr. 10 mins	dedifferentiation	50%
Old culture	50 mins.	cytolysis	1 recovered (2%)
	1 hr 35 mins	cytolysis	none
Fresh culture	2 hrs.	cytolysis	none
	2 hrs 30 mins	some still active	100%
	1 hr 30 mins.	some still active	50%
	3 hrs	some still active	25%
Cysts (10)	22 hrs. 25 mins.	1 excystment after 48 hrs.	
Cysts (100)	18 hrs. 45 mins.	25% excystment after 3 days.	

Whereas *C. steinii* and *C inflata* were cultured on yeast extract for these experiments, *C. cucullus* was cultured on lettuce infusion Tests were made both with unbuffered and buffered medium. In the latter case *C. steinii* was used as a control.

The following experiments are with unbuffered lettuce infusion and pure carbon dioxide.

Material.	Exposure Time	Final Condition.	Recovery.
Fresh culture	10 mins.	cytolysis	none
	13 mins.	cytolysis	none
10 active and cysts	7½ mins.	cytolysis	none
			1 excystment after 2 hrs.
50 active	15 mins.	cytolysis	none of active ciliates
7 active and cysts	11 mins	cytolysis	none of active ciliates
10 active and 1 cyst	11 mins	cytolysis	none of active ciliates

Buffered lettuce infusion with *C cucullus* and *C. steinii*, using pure carbon dioxide.

Material		Exposure Time	Recovery.
C. cucullus	10 active and cysts	11 mins	3 excysted
C steinii	3 active		2 still viable
C cucullus	6 active	12 mins	none
C. steinii	20 to 30 active		still viable
C cucullus	3 active	18 mins.	none
C. steinii	20 to 30 active		still viable
C. cucullus	5 active	25 mins	none
C. steinii	20 active		still viable

In these experiments there was cytolysis of all the trophic *C. cucullus* after 9 minutes, *C steinii* alone showed recovery. The only exception were the cysts of *C. cucullus*, in the first experiment, which recovered and were apparently stimulated to excyst.

ii. Hydrogen Ion Concentration

It is now agreed that *Colpoda* will grow over a wide range of hydrogen-ion concentration, and it is apparent from my experiments that growth is inhibited only at extreme pH values The experiments suggest that even where growth is inhibited at extreme pH values this may be due to the toxicity of high concentrations of buffer—viz., tartrate (3·5) and borate (9 2), rather than to the pH itself.

Koffman (1924), however, in a paper now generally discredited (cf. Barker and Taylor, 1933), attributed the excystment, multiplication and encystment of *Colpoda* to changes in pH. Using *C steinii* and *C. cucullus* he followed the pH of hay infusion cultures and correlated rising pH with encystment. The pH of his cultures rose steadily in 72 hours from 6·05 to 8·80. His cultures were started with cysts, and after 72 hours there were only cysts left. The ciliates had excysted, multiplied, and encysted Koffman recorded that the ciliates decreased in size, and there can be no doubt that encystment in this case was simply due to the exhaustion of food. My experiments with *C. steinii* show quite clearly that

it is this factor, and not pH, which is significant. Excystment and encystment are not affected by pH.

Experiment 1. Methods:

The pH of unbuffered yeast autolysate was adjusted with HCl and KOH. Cultures were inoculated with *Colpoda steinii* and both the growth of the ciliate and the change of pH recorded. When the ciliates had encysted, the pH of the cultures was again changed, using KOH and HCl, and the cultures examined for any signs of excystment.

Results

Initial pH.	After 7 days.	Changed to.	After 2 days.
3.5	4.5	9.4	7.1
5.25	5.6	10.7	7.4
6.5	6.1	7.7	6.5
9.2	6.1	10.1	6 7
10 2	6.5	3.0	2 0
10	8 0	4.8	5 0

After 7 days the initial pH of the cultures had changed and they were all within the acid range, except for one culture which had been buffered with phosphate. The ciliates had grown in all the cultures, but growth had been slower in the very acid (pH 3·5) culture wherein after two days there were only about ten large and slow-moving ciliates. These were, however, dividing. After 7 days all the cultures had formed many cysts, and these were tested for viability with yeast extract. The effect of changing the pH at this stage was to cause the death of most of the remaining active ciliates. There was no excystment.

Experiment 2. Methods:

Ciliates (*C. steinii*) were suspended in buffer solutions with a loop of bacteria and the division rate observed. The pH of the buffers used were 3 57, 4 0, 7 0, and 9·2.

FIGURE 1

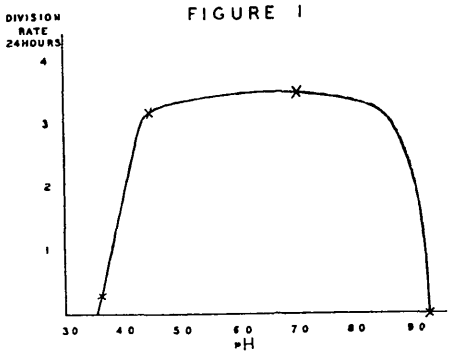

Fig. 1.—Division Rate of *Colpoda steinii* as a function of pH.

Results

The results are shown diagrammatically in Figure 1. In the buffer pH 9 2 the ciliate lived for 24 hours, moved slowly but did not divide. It later died Ciliates at pH 3·5 showed inhibition of division over the first 24 hours and then divided rapidly. There was no apparent difference between the division rate at pH 4·0 and pH 7·0.

Experiment 3 Methods:

Cysts were taken from completion cultures on strands of cotton wool and placed in Syracuse watch glasses. Alkali, acid and distilled water were added and the excystment observed.

Results

	Initial pH	Final pH	% Excystment
KOH	8.5	7 8	95
HCl	4.7	5 5	75
Double Glass Dist. H_2O	7.0	6.8	67

Excystment took place in all three cases without any special excysting factor.

Experiment 4 Methods:

To cysts of *C. steinii* formed in buffer pH 3 57, loops of bacteria were added

Results

Excystment was obtained although the percentage was not high.

iii. Osmotic Tension

Greig-Smith (1912, 655) reported that *Colpoda* encysts when washed in 0 2% saline. Yakimoff, Wassilewski, and Zweitoff (1925) found that *C steinii* lived only 15 days in 0 07% NaCl. Thimann and Barker (1934, 37) alleged that *C steinii* is sensitive to saline Finley (1930) recorded the tolerance of a number of fresh water ciliates to direct and indirect transfer to higher salinities. He employed artificial sea water, total salinity 3 51% (sodium 2 73%, magnesium 0 38%) *C cucullus* tolerated 5% (direct transfer) and 10% (indirect transfer), *C. steinii* 25% and 40% and *C. aspera* 100% in both cases. Doflein (1916) apparently transferred *C. cucullus* to almost pure sea water. There is obviously, therefore, considerable disagreement over the salinity tolerance of *Colpoda.* It would seem from Finley's evidence that Doflein was really dealing with *C. aspera* Finley made some interesting observations on encystment and excystment

"Experiments indicated that those protozoa which encysted readily could be recovered from their cysts by placing them in a saline medium; but the concentration of that medium was in no case greater than the maximum concentration tolerated in direct transfer. This conclusion is supported by attempts to recover *Colpoda, Stylonychia Euplotes, Halteria* and *Chilodon.* In no case were they recovered from cysts placed in salt water unless the concentration was equal to or below the maximum direct transfer. *Colpoda aspera* and *Bodo uncinatus* were recovered in 100% artificial sea water inoculated with unsterilized dry hay."

In the present experiments *C steinii* was acclimatized to salinities greater than those recorded by Finley. These differences may be ascribed to two causes: failure by Finley to adapt the ciliate slowly to increased salinities, and failure to provide adequate food. The same differences with other species are found between Finley and Hoare's (1927, 193) work and even more strikingly in the work done on *Tetrahymena pyriformis* (Chatton and Tellier, 1927a, b; Loefer, 1939). Adequate food is essential if the ciliate is to be acclimatized to higher salinities, and the failure of Finley (1930) to acclimatize *Colpoda steinii* to a salinity higher than 1.4% and of Chatton and Tellier (1927a) to acclimatize *Tetrahymena pyriformis* to a salinity higher than 1 8%, may be attributed to inadequate nutrition.

The chief factor, however, is the increment of salinity to which the ciliate will acclimatize at one time. This varies with the species (Finley, 1930), and with the saline concentration (Chatton and Tellier, 1927a, b). My experiments show that excystment is inhibited by hypertonic solutions, but they also show that excystment is dependent not only upon the salinity of the excysting medium but also upon the original salinity of the encysting medium. Cysts formed in cultures of high salinity excyst in solutions of comparatively high salinity, whereas cysts grown in cultures of low salinity do not. This may be interpreted as the acclimatization of the ciliates to increased salinities by increased osmotic tension of the milieu interieur. It is clear that there is a critical salinity above which excystment will not take place. This critical salinity is not absolute but relative, and apparently the essential point is that the osmotic tension of the milieu exterieur must be less than the osmotic tension of the milieu interieur This explains why Chatton and Tellier (1927a, 287) found that there was a proximal and distal limit to which their ciliates could be acclimatized It is obvious that if there is a critical osmotic difference between the milieu interieur and milieu exterieur which can be tolerated by the ciliate, then this difference will be reached at the proximal limit—i e , in the first transfer. Then the osmotic difference is between the ciliate acclimatized to an initially low salinity and subjected to an increased salinity. If the initial concentration is progressively raised, then the distal limit will be progressively increased, as was found by Chatton and Tellier (1927b, 781), until the absolute distal limit—i e , the highest salinity tolerated by the ciliate, is reached. If the ciliate is transferred immediately beyond its proximal limit it will die, because it cannot overcome the osmotic difference between the two milieux. In other words, the acclimatization to increased salinity is interpreted as involving a change in the internal osmotic tension. It should be correlated then with decrease in size and increase in density, and this appears to be the case for Pack's (1919) ciliates. It is also the case with *Tetrahymena pyriformis,* which Loefer (1939, 167) found to average 50 mu in the 1% van't Hoff's solution but only 42 mu in the 35% solution Because of the inhibition of division in *Colpoda* with increased salinities the ciliates were generally larger in higher concentrations than lower, but when the food was exhausted this was not the case At the highest salinity tolerated by *Tetrahymena pyriformis,* Loefer found that the contractile vacuoles did not function This may indicate that there was no osmotic difference between the two milieux

Finley's results may be readily interpreted on this basis He found that the ciliates excysted in direct transfer but not in the ultimate saline concentration This agrees with the present experiments. Cysts formed at low saline concentrations will excyst only within the proximal limit. However, had the cysts been formed at higher salinities, Finley might have found excystment at higher concentrations.

Other results show that increasing salinities slow excystment, slow the division rate, and finally inhibit division leading to unstable cyst formation These results are similar to those of Johnson and Evans (1940) with *Woodruffia metabolica.* They found that increasing salinities slowed excystment and also led to the formation of unstable cysts. The inhibition of division is similar to results with mammalian epithelial tissue (Hughes, 1952), and its significance will be discussed later.

Experiment 1

Transfer of the ciliate to culture media of increasing salinity.

Methods

The salinity of yeast extract prepared with double distilled water was adjusted with sodium chloride to the following concentrations: 0·4% (the standard preparation), 1%, 2%, 2 5%, 3% and 4% NaCl Ciliates (*C. steinii*) were inoculated into progressively increased salinity and their growth and reactions studied

Results

C. steinii was cultured successfully in saline concentrations up to 3% Attempts to culture the ciliate in 4% NaCl were unsuccessful. In the 3% medium the ciliates appeared sluggish, and though a few ciliates divided, growth was restricted and slow with the ciliates forming unstable cysts. Growth was better at 2 5% and 2%, although there was a lag phase before the ciliates began to divide. Growth was normal in 0%, 0 4% and 1% NaCl.

Experiment 2

Increasing the salinity of the culture medium by the addition of NaCl.

Methods

The tests were prepared in duplicate. To one dish was added 0.4% NaCl, and to the other 4% NaCl.

Results

The ciliates in the 0 4% NaCl medium grew normally and formed resistant cysts on the exhaustion of food. Ciliates in 2 5% to 3% medium occasionally divided after a lag period of 48 hours, but generally formed unstable cysts. If the salinity was further increased division was entirely inhibited, and the ciliates all formed unstable cysts. Dilution of these cultures with distilled water resulted in excystment and division

Experiment 3

Effect of salinity on the division rate.

Methods

Hanging drop preparations were made of ciliates in yeast autolysate of different salinities—viz., 0 4% and 2% NaCl.

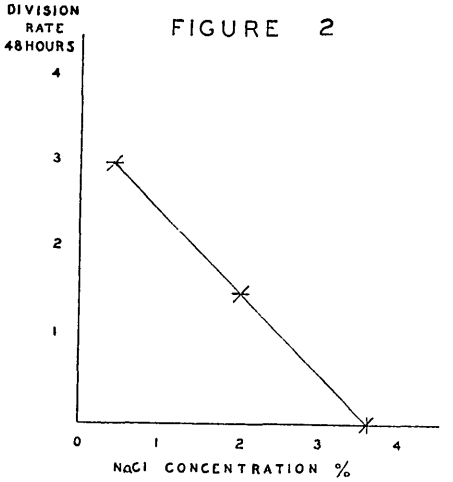

FIGURE 2

FIG. 2.—Division Rate of *Colpoda steinii* as a function of salinity.

Results

Time (Hours)	0	48	D R.(48 hrs.)
2% NaCl	3 active	15 active (3 tetragenic cysts)	3
0.4% NaCl	3 active	5 active (3 monogenic cysts)	1.6

The results are shown diagrammatically in Figure 2, where the relation between the division rate and the salinity is assumed to be linear.

Experiment 4

Effect of salinity on excystment.

Methods

Resistant cysts were placed in Syracuse watch glasses and washed with double glass distilled water, 0·4% NaCl, 1% NaCl, and 2% NaCl.

Colpoda steinii

K. Cysts taken from 0.4% NaCl completion culture

Salinity	Time (Hours)	0	4	% Excystment
0.4%	Active	—	450	90
	Cysts	500	28	
1%	Active	—	—	0
	Cysts	400	400	

L Cysts taken from 2.4% NaCl completion culture.

Salinity	Time (Hours)	0	5	24	% Excystment
0%	Active	—	175	200	100
	Cysts	200	—	—	
1%	Active	—	19	22	71
	Cysts	30		8	
2%	Active	—	6	5	10
	Cysts	50			
3%	Active	—	—	—	0
	Cysts	50			
4%	Active	—	—	—	0
	Cysts	400			

M. Cysts from centrifuged 0.4% NaCl completion culture.

Salinity	Time (Hours)	0	5	% Excystment
0%	Active	—	90	90
	Cysts	100	6	
0 4%	Active	3	200	100
	Cysts	200	—	

N. Cysts from 3% NaCl completion cultures.

Salinity	Time (Hours)	0	22 30	% Excystment
0%	Active	—	6	100
	Cysts	5	—	
1%	Active	1	28	100
	Cysts	30	—	

O. Cysts from 2.4% NaCl completion culture.

Salinity	Time (Hours)	0	6	% Excystment
0%	Active	6	200	98
	Cysts	200	5	
0.4%	Active	4	200	100
	Cysts	200	—	
1%	Active	—	280	98
	Cysts	300	10	
2%	Active	1	5	15
	Cysts	30	27	
3%	Active	—	—	0
	Cysts	25	25	

P. Cysts from 2% NaCl completion culture

Salinity	Time (Hours)	0	3 40	22	% Excystment
1%	Active	—	20	32	90
	Cysts	35	15	3	
2%	Active	3	7	32	25
	Cysts	120	120	90	

Q. Cysts from 0.4% NaCl completion culture

Salinity	Time (Hours)	0	5	% Excystment
0%	Active	—	30	50
	Cysts	60	30	
0%	Active	—	15	50
	Cysts	30	15	
1%	Active	—	—	0
	Cysts	25	25	
1%	Active	—	—	0
	Cysts	35	35	

Colpoda inflata

R Cysts from 0.4 NaCl completion culture.

Salinity	Time (Hours)	0	19	42	% Excystment
1%	Active	—	5	25	0 5
	Cysts	1000	1000	1000	
0 4%	Active	—	13	65	50
	Cysts	26	14	6	
0 4%	Active	—	42	300	80
	Cysts	60	12		
0%	Active	—	23	160	95
	Cysts	24	1	1	

Experiment 5

Effect of salinity on excystment.

Methods

Cysts from 3% NaCl completion cultures were placed in watch glasses and bacteria and yeast added.

Results

No excystment was obtained either with bacteria or yeast Addition of distilled water resulted in excystment and division.

iv. Temperature

Cysts of *Colpoda* survive indefinitely whether in the wet or dry state (Taylor and Strickland, 1936), but they react differently to high temperatures. Wet cysts

are much more sensitive to high temperatures than dry cysts. The lethal temperature for wet cysts is 44° C. for a one hour exposure and for dry cysts is 106° C. Similar figures are given by Bodine (1923). Using liquid air, Taylor and Strickland found that the cysts could survive a temperature of about –180° C.

Active ciliates are killed at about 40° C.—i.e., about the same temperature as the wet cysts. The ciliates can be gradually acclimatized to higher temperatures (Bodine, 1923). Excystment takes place within a temperature range of about 8° to 32° C. (Thimann and Barker, 1934; Bodine, 1923; Goodey, 1913). The rate of excystment, or rather the speed, varies directly with the temperature with an optimum about 25° to 28° C. Johnson and Evans (1939) working with *Woodruffia metabolica* found that cysts were formed only at temperatures from 10° to 37½° C., and that temperatures of 4° C. and 38½° C. were lethal to the active ciliates and no cysts were formed.

In my experiments encystment of *Colpoda* was not affected by temperature. However, both at high and low temperatures, (37° C. and 1° C.), division was inhibited and unstable cysts were formed. The ciliates continued to feed, move, and grow at these temperatures. On return to room temperature division and excystment take place. Ciliates kept at 20° F. for 24 hours survive, provided the culture does not freeze. At this temperature the ciliates are inactive and settle at the bottom of the dish. Ciliates kept just above freezing point move and feed, though very sluggishly.

Compared with other ciliates—e.g, *Tetrahymena* (Phelps, 1947), the effect of temperature on the division rate of *Colpoda* is very similar. Failure of *Colpoda* to divide at 37° C. indicates that growth will be inhibited at human body temperatures. For this reason, *Colpoda* could not successfully parasitize warm blooded animals, although it is able to parasitize cold blooded animals (Reynolds, 1936). Moreover, it seems unlikely that cysts would remain viable after passing through the alimentary canal of man. Certainly the ciliates would not excyst at such temperatures. It must be concluded, therefore, that *Colpoda* is not physiologically adapted to human parasitism, and the records of Schulz (1899), Yakimoff and Kolpakoff (1921) and Demianov (1943) must be discounted.

Robertson founded his theory of allelocatalysis largely on his study of the growth of *Colpoda*. His theory is generally rejected (Richards, 1941). In my experiments, the highest division rate of *Colpoda* was recorded with a hanging drop inoculated with a single ciliate (Experiment U), contrary to Robertson's evidence.

Methods

Hanging drops of *C. steinii* were prepared, some with bacteria and others with yeast extract. They were maintained at room temperature (ca. 18° C.), at ca. 14° C., and at 28° C. They were also placed in an incubator at 37° C.

Results

Ciliates placed in the incubator at 37° C. continued to swim normally and to feed for several hours. They then encyst prior to division, but at this temperature division is entirely inhibited, and, consequently, the ciliates form unstable cysts. If they are returned to room temperatures, division and excystment take place. The effect of temperature on the division rate is shown diagrammatically in Figure 3.

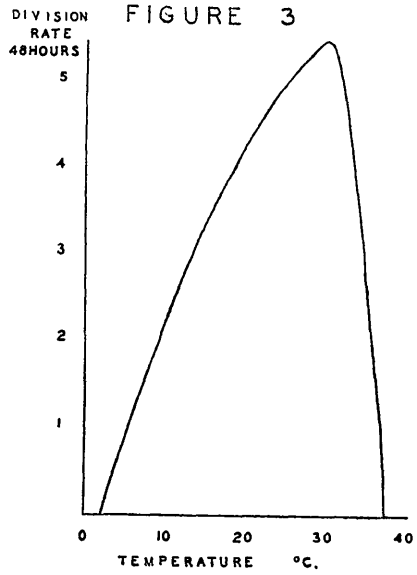

FIGURE 3

FIG 3.—Division Rate of *Colpoda steinii* as a function of temperature

	Hanging Drops With Yeast Autolysate			
S. Time (Hours)	0	26.30	48	D R.
13° C	2 active	4 active	16 active	3 (48hrs.)
18° C	1 active	2 active	16 active	4 (48hrs.)
28° C	1 active	8 active	(2 division cysts)	3 3 (26hrs.)

The hanging drops in this experiment (Experiment S.) kept at 13° C. and 28° C. were transferred to the incubator at 37° C After 26 hours there were 20 unstable cysts in the first and 11 unstable cysts in the second. There were no active ciliates. The second was returned to air temperature (18° C.) and, after 24 hours, there were 23 active ciliates, one division cyst and 5 resting cysts No change had taken place in the hanging drop kept in the incubator at 37° C.

T Time (Hours)	0	24	72	D.R. (72hrs.)
12° C	8 active	7 active (1 division cyst)	80 active 5 resting cysts	3 5
18° C	7 active	12 active	150 active 35 resting cysts	4 4
28° C	4 active	13 active (3 tetiagenic cysts)	200 active 40 resting cysts	5 9

	Hanging Drops With Bacteria		
U Time (Hours)	0	19.30	D R (19 30hrs)
18° C	1 active	4 active (10 division cysts)	4
28° C.	1 active	220 active (22 division cysts)	8

Ciliates can be kept for comparatively long periods at temperatures just above freezing point. At this temperature they are extremely sluggish but do feed and form unstable cysts, prior to division. Division at this temperature is entirely inhibited. If the cysts are returned to room temperature, division and

excystment take place. If they are not returned to room temperatures, they become resting cysts (Padnos *et al.*, 1953b, 18). The following are typical experiments.

Hanging Drops of *C. steinii* With Yeast Autolysate.

Temperature	1° C.			18° C.		
V. Time (Hours)	0	67.30	140	0	26	31
Active	10	7	3	3	150 and div. cysts	
Cysts	10	13	17	17	—	
Active	10	8	3	3	25	
Cysts		1	3	3	—	
Active	1	1				4
Cysts			1	1	1 tetragenic cyst	

In the third test the encysted ciliate still showed cyclosis after 140 hours at 1° C. but after four hours at room temperature cyclosis had ceased and 24 hours later division had taken place, later to be followed by excystment

It was also found that ciliates survived 24 hours at 20° F., provided the culture did not freeze. At this temperature the ciliates are inactive and settle at the bottom of the culture dish. There is no division or encystment.

III. Discussion

The effect of the various environmental factors on *Colpoda,* considered in retrospect, indicates · first, that the primary effect of all these factors is differential, suppressing one physiological process and not another; and secondly, that the response of *Colpoda* is similar to the response of other types of plant and animal. The first phenomenon is known as dissociation (Needham, 1942) and the two systems dissociated by these factors are the "activity" and "resting" systems (McElroy, 1947).

Recent study has confirmed that the "activity" system is generally associated with the aerobic carbohydrate metabolism, and in particular oxidative phosphorylation. Inhibitors, therefore, which are most effective in suppressing the "activity" system are those which interfere with the carbohydrate cycle, and it is these inhibitors, and these inhibitors alone, which prevent cells entering the prophase of division (Hughes, 1950). Naturally oxidative phosphorylation will be inhibited in the absence of oxygen and further the reduced carbohydrates of the Krebs cycle will accumulate as with *Tetrahymena* (Thomas, 1942). The effect of oxygen lack, then, can be attributed simply to the suppression of phosphorylation which normally takes place under aerobic conditions. Anaerobes must either have an alternative system of phosphorylation or else division must be independent of such organic phosphates.

After an exhaustive review of carbon dioxide toxaemia, Chang and Loomis (1945) attributed its inhibitory effect to the formation of a hydrogen bond linkage perhaps with the one-alpha-amino nitrogen of a protein. The coagulation of the cytoplasm indicates that molecular structure has been affected. In other words, the effect of carbon dioxide is more fundamental than anoxia in that the enzyme itself is reversibly affected. Kavanau (1950), in discussing the effect of pH and temperature on enzyme kinetics, also suggests that the inactivation of the enzyme system is caused by the formation of intramolecular hydrogen bridges. Kavanau considers that there is a thermodynamic equilibrium between catalytically active and catalytically inactive forms of the enzyme. The active

configuration of the enzyme is possessed in only a relatively narrow temperature band, being lost at both high and low temperatures. Increasing the temperature from the point of low temperature inactivation results in the equilibrium between inactive and active configuration of the enzyme changing from the inactive to the active form. This continues until the optimum temperature is reached, after which the equilibrium changes from the active to the inactive form. The rate of biological processes can then be explained in terms of an absolute reaction rate with a changing equilibrium of active and inactive enzyme. This theory accounts satisfactorily for the curves illustrating biological activity such as division or excystment in *Colpoda*. While this interpretation may be accepted it is important to stress the differential aspect of these factors, for it is only the enzymes of the "activity" system that are affected by temperatures outside the range 8° C. to 37° C. Other enzyme systems function quite normally, such as those connected with encystment and ciliary movement.

It has been emphasised that an organism will tolerate only a limited osmotic difference between its own cytoplasm and the surrounding medium. In most fresh water animals adaptation to increasing salinity cannot be taken past the point where the medium is hypotonic to the organism. It is probable that euryhaline organisms are exceptions to this rule, and this view is strengthened for protozoa by the fact that the contractile vacuole may cease to function in higher salinities. It is apparent that *Colpoda steinii* is stenohaline and that increasing salinity seriously affects its physiology. It is difficult to attribute the inhibition of division in this case either to metabolic interference or to inactivation of the protein. A simpler explanation is that as the salinity approaches the distal limit the increasing inhibition of division is due to the decreasing osmotic difference of organism and medium and the increasing difficulty of the organism to absorb water against this gradient (Hughes, 1952).

It is now necessary to consider the action of the excystment promoting substances on the metabolism of *Colpoda*. It is apparent that the activity of the potassium ion and alcohol is due to their mobility and is not due to any metabolic significance, and this is confirmed by Garnjobst (1947) who found that the basic growth medium did not stimulate excystment. The activity of the K ion in cells is well known. It is known to stimulate respiration and in particular there is an uptake of K following fertilization of sea urchin eggs accompanying increased acidity and increased oxygen uptake (Oddo and Esposito, 1951). It is considered that the role of K in these exchanges is the replacement of H ions. If this is so the effect of the potassium ion in accelerating excystment in *Colpoda*, a phenomenon known to be comparable to division, may be interpreted in terms of Kavanau's theory. During the encysted period respiration is depressed, but it rises rapidly in response to the excystment stimulus, and this respiratory increase is cyanide sensitive (van Wagtendonk and Taylor, 1942; Thimann and Commoner, 1940). It is not unreasonable to presume that the increasing metabolic activity is due to increasing activity of the enzymes connected with the "activity" system. That the increased respiration is due to increased enzyme activity and not to a new substrate is shown by the fact that distilled water may stimulate excystment, although it is patently not a metabolic substrate. If, then, there is a change-over between the encysted ciliate before and after stimulation, comparable to the difference between the unfertilized and fertilized sea urchin egg, and this change-over is due, as in the case of the sea urchin egg, to the intro-

duction of an alternative respiratory mechanism, then its activity will be governed by the principles outlined by Kavanau. Namely, with increasing temperature, up to an optimum, there will be increased acceleration, as the equilibrium increasingly favours the active form of the enzyme. Past the optimum acceleration will decrease, and this is known to be the case (Thimann and Barker, 1934). Accepting this theory, the activity of the K ion can be attributed to the displacement of the hydrogen bond which characterises the inactive form of the enzyme (Seifriz, 1942). With increased concentrations of the K ions up to an optimum there will be increased acceleration of excystment, and this is also known to be the case (Haagen-Smit and Thimann, 1938; Strickland and Haagen-Smit, 1947). However, this initial activation of the enzyme system must be separated from the ultimate respiration of this system for the two are separable, the second is absolutely dependent upon oxygen and the first is not. The effect of alcohol is less certain (Strickland and Haagen-Smit, 1948). It may be that the hydroxl group is active in accelerating the formation of active enzyme, and that its role is complementary to that of the K ions. As the role is performed by a large number of substances, although with greatest efficiency by alcohol, it is difficult to define precisely what this action is (Thimann and Haagen-Smit, 1937; Haagen-Smit and Thimann, 1938). These metabolic changes are accompanied by a marked uptake of water, and as we have seen such uptake is possible only if there is a sufficient gradient of osmotic tension. This water uptake which is definitely dependent upon excystment metabolism is comparable to the water uptake during division which is also dependent upon a favourable osmotic gradient. The fact that considerably lesser salinities are required to inhibit excystment than those required to inhibit division strengthens the view already proposed that this water uptake is not dependent upon absolute, but upon relative tensions, within certain limits.

There remains one phenomenon still to be explained—namely, the difference of high temperature on dry and wet cysts. The former are irreversibly affected at 106° C. and the latter at 44° C. Taylor and Strickland (1936) assume that the temperature of coagulation of a protein in colloidal solution varies with the amount of free water, but they do not discuss why this should be so. It may be, that, as with erythrocytes, with increasing temperature and increasing rate of diffusion there is increased loss of cations, such as K, which is naturally prevented in the dry cyst. The loss of cations, if indeed it takes place, may cause irreversible denaturation of the proteins before the critical temperature is reached. Considering the importance and mobility of the K ions such an explanation is not improbable. It is interesting to note that the lethal limit of the active ciliates is much the same as the lethal limit of the wet cysts (Bodine, 1923). Encystment, per se, does not therefore raise the resistance of the ciliate to high temperatures.

IV. Summary

Work on the life history of the Colpodidae is reviewed and the problems arising therefrom. The formation of " unstable" cysts in *Colpoda,* similar to the "unstable" cysts of *Woodruffia,* is described, and it is shown that their formation is due to the inhibition of normal division by environmental factors. Removal of the inhibiting factor results in divison and excystment. Resting cyst formation is not affected by the same environmental factors.

Colpoda can survive several days in the absence of oxygen and feeds and moves freely. On the exhaustion of food the ciliates will encyst. Rarely "unstable" cysts are formed. Division is totally inhibited The division rate is greater in pure oxygen than in atmospheric air. Reduced oxygen tensions appear to stimulate excystment, but absence of oxygen inhibits excystment.

C steinii and *C. inflata* are far more resistant than *C. cucullus* to carbon dioxide and show recovery after prolonged exposure to an atmosphere of pure carbon dioxide *C. steinii* survived twenty-four hours in a mixed oxygen-carbon dioxide atmosphere moving slowly but not feeding or dividing.

C. steinii grew over a wide range of pH, although growth was inhibited by a tartrate buffer of pH 3·5 and a borate buffer of pH 9 2. Both encystment and excystment are independent of pH.

C steinii can be acclimatized to salinities of 3% NaCl, but growth and division is progressively inhibited with increasing salinity and leads to the formation of "unstable" cysts. Adaptation to higher salinities is achieved by increased osmotic tension of the milieu interieur. Excystment will not take place unless the osmotic tension of the mileu exterieur is less than that of the milieu interieur. My experiments support the belief that the first critical reaction in excystment is osmotic diffusion.

C. steinii divides and excysts only within the temperature range 8° to 35° C. At 37° C both division and excystment are inhibited and "unstable" cysts are formed. Similarly at 1° C. the ciliates move and feed but neither excyst nor divide, and "unstable" cysts are formed. "Unstable" cysts so formed divide and excyst when returned to room temperatures. In this case a second critical reaction is inhibited by temperature.

The dissociation of division and excystment from other physiological processes is discussed in relation to the "activity" and "resting" systems identified in other organisms. It is stressed that the mechanism of inhibition is distinct for each factor and possible interpretations are offered in the light of recent theories.

ACKNOWLEDGMENTS

The author is indebted to Professor L. R. Richardson for helpful criticism during the present studies which form part of a thesis submitted to the University of New Zealand in partal fulfilment of the requirements for the Degree of Doctor of Philosophy.

The author is also indebted to many workers for literature, and in particular to Dr. J O. Thomas, who kindly made available a copy of his unpublished thesis.

N.Z. Industrial Gases Ltd. which supplied the gases used in these experiments also loaned the necessary control valves.

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