(From the Laboratory for Chemo-physical Biology Stanford University.)

The sterilization of protozoa.

By

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Contribution No. 2 in Studies of Protozoa.

By

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As stated in our first paper in this series (8) we have been interested recently in investigating the sterilization of protozoa. Our studies of this problem have been confined to two principal methods, sterilization by washing of the motile organism, and chemical disinfection of the encysted form. With respect to the former we have already indicated that the rhizopod, *Euplotes taylori*, may be rendered free of bacteria by repeated immersion in successive baths of sterile salt solutions (1). The first part of the present communication consists of a more detailed study of this method.

The second part is devoted to an inquiry into the action of simple bactericidal agents on *Euplotes* cysts and the associated bacteria. It has been our hope that this phase of the investigation would reveal a chemical method of sterilization which would be less laborious than the multi-bath technique. In the achievement of this practical end we have thus far met with disappointment, although we have discovered that silver nitrate in concentrated solutions of glucose and sucrose is more toxic towards the bacteria than towards the cysts. Whether or not we shall succeed in so simplifying the conditions of treatment as to permit the substitution

of this method for that of washing the motile organism, is nevertheless uncertain.

We have been somewhat more attracted by several questions of theoretical interest which came to us in the course of the investigation. At the expense of anticipating the presentation of the results themselves we must in the first place point out the need of an extensive inquiry into the action of electrolytes on bactericidal agents. NEERGAARD (10), WERNICKE and MODERN (16), BENEDICENTI and BONINO (1), HOCS (5), EGG and JUNG (2), LEITNER (6), VIOLLE and GIBERTON (15), and many others have made excellent investigations of the well-known effect of electrolytes in suppressing the oligodynamic action of heavy metals; first demonstrated by NÄGELI (9). We suspect however that many other toxic agents are affected by electrolytes. It seems possible, for example, that the very low toxicity exerted by resorcinol and argyrol under the conditions of this investigation may be associated with the high salt content of the medium. A second question deserving of further inquiry is the peculiar specificity of some substances. Thus we observed mercurochrome to be strikingly toxic to *Euplotes* cysts but of very low activity towards the associated bacteria. Again, the mechanism by which concentrated solutions of glucose and sucrose enormously reduce the toxicity of silver nitrate towards the encysted protozoa with much less effect upon the bacteria is not without some theoretical interest. Finally, the means by which several substances display quantitatively the same toxicity towards both cysts and bacteria, suggests itself as a problem of considerable importance. Thus, the behavior of chloramine T, formaldehyde, and phenol, as summarized in Table VIII, seems to indicate that these agents diffuse readily into both cysts and bacteria and there exert a toxic action. The external surfaces of these organisms would, in our judgment, differ so greatly both in structure and electrical charge as to render any equality in behavior of surface-acting bactericidal compounds highly improbable.

Before proceeding with a description of the experiments it should be mentioned that others, from time to time, have reported success in the chemical sterilization of protozoan cysts. Thus FROSCH (3) succeeded in sterilizing old cysts of *Amoeba nitrophila* by immersing them for 3 days at room temperature in 20 per cent sodium carbonate. He also tried concentrated sodium chloride and sucrose. A variety of disinfectants was examined by OEHLER (11) without very encouraging results. SEVERTZOFF (14) investigated

the action of toluene, chlorine and calcium sulphide on amoeba cysts. He considered calcium sulphide to be especially promising and seems to have succeeded in killing the associated bacteria without complete loss of the amoebae. Potassium dichromate was used successfully by GLASER and CORIA in sterilizing *Euglena* (4).

Part I.

Sterilization by Washing.

Experimental.

Vigorous specimens of *Euplotes taylori* were selected from stock cultures which had been maintained for many generations in 1 : 1 artificial sea water on a mixture of two strains of bacteria (organism A and B Coli K_{13}) [1]. Groups of between 5 and 50 individuals were passed through 12 to 14 baths of sterile 1 : 1 artificial sea water, each 2 cc. in volume. The vessels and apparatus employed and the details of manipulation are described elsewhere (8). In all but the first four experiments the organisms were permitted to remain 10 to 15 hours in the sixth bath, and 30 or 40 minutes in each of the others. The total time of washing thus extended over 24 hours.

The sterility of the washed protozoa was determined by making a pour plate in nutrient agar of the entire contents of the last bath, protozoa and all. The plates were incubated at 20° and examined daily for colonies. In several instances pour plates were also made of the bath water from the last one, two, or three of the remaining vessels.

As a final precaution, routine sterility tests were regularly made of the artificial sea water used for washing, and of representative specimens of culture vessels and apparatus employed in the procedure.

The results are summarized in Table I p. 258.

We conclude that at least 10 protozoa may be rendered completely free of bacteria by passage through 12 baths under the conditions of this investigation. If the quantity of liquid carried over from bath to bath be small enough [2]) we are of the opinion that even 25 organisms may be washed at one time.

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[1]) For a description of the organisms and culture medium, cf. the first paper of this series (8).

[2]) An average of 0.007 cc. of liquid was carried over with every group of organisms.

Table I.

Sterilization of *Euplotes taylori* by washing.

Number of Euplotes washed	Number of baths	Sterility of washed protozoa	Bacteria remaining in baths. Number of colonies obtained from pour plates of various baths after 6 days			
		Colonies after 6 days	Bath No. 9	Bath No. 10	Bath No. 11	Bath No. 12
6 [1]	14	0				
5 [1]	14	1				
5 [1]	14	1				
5 [1]	14	0				
7	12	0			0	
10	12	0			0	
10	12	0			2	
10	12	0			0	
10 [2]	12	1			1	
10	12	0			0	
10	12	0	many	5	0	
10	12	0				
10	12	0	many	4	0	
25	12	0		7	0	
10	12	0			2	
10 [3]	12	1				
10	12	0				
10	12	0				
10	12	0				
10	13	0				
10 [4]	13	8			many	33
25 [4]	13	35			many	many
50	13	0			many	1
50	13	16				many
50	13	24				many

Euplotes taylori, and perhaps all other rhizopods, are admittedly difficult to sterilize. Much patience and care in manipulation are required. In contrast, our colleague, Mr. J. O. THOMAS, finds that the same procedure applied to *Colpidium colpoda* gives sterile organisms in much less time and with less labor than must be expended on *Euplotes*. Likewise PARPART (12) found that *Paramaecium caudatum* could be sterilized in 10 baths or less.

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[1]) Organisms permitted to remain 3 hours in the sixth bath. Total time in baths was 9 hours.

[2]) Cleaners at work in laboratory.

[3]) Colony did not appear until the 4th day.

[4]) Unusually large amount of liquid transferred with protozoa from bath to bath.

Part II.

Chemical Sterilization of *Euplotes* Cysts.

Experimental.

Thriving cultures of *Euplotes taylori* in 1:1 A. S. W. were caused to encyst by gradual evaporation of the medium. In general,

Table II.

Disinfection of cysts with mercuric chloride and metallic mercury.

Conc'n of mercuric chloride	Solvent	Time of treatment of cysts	Washing fluid	Age of cysts	Sterility of plates inoculated with treated cysts	Behavior of treated cysts after placing in nutrient medium
per cent		minutes		days	colonies after 96 hours	
1	Distilled water	60	saturated NaCl	7	0	all dead
1	dito	60	„	4	0	„
1	„	50	„	4	0	„
1	„	40	„	4	0	„
1	„	30	„	4	0	„
1	„	20	„	4	0	„
1	„	10	„	4	2	„
0.5	Buffered 2.5 percent NaCl	60	„	2	0	None excysted. all dead
0.1	„	60	„	2	0	Few excysted and died
0.01	„	60	„	2	7	All excysted and died
0.1	Bufler sat'd with NaCl	60	Buffer sat'd with NaCl	2	0	All dead
0.01	„	60	„	2	0	„
0.001	„	60	„	2	7	„
0.001	Sat'd $NaNO_3$	60	Sat'd $NaNO_3$	2	0	„
0.0001	„	60	„	2	1	„
0.00001	„	60	„	2	2	„
0.001	„	15	„	2	0	„
0.0001	„	15	„	2	0	„
0.00001	„	15	„	2	4	„
2.5 per cent NaCl in contact with metallic mercury		22 hours	buffered 2.5 percent NaCl	2	many	„
Metallic mercury in contact with artificial Sea water saturated with $NaNO_3$ [1 cc. Hg: 2 cc. solution]		24 hours „	A.S.W. sat'd[1]) with $NaNO_3$ „	2 2	1 0	„ „

[1]) The cysts were washed twice in this solution and once in artificial sea water only.

Table III.

Disinfection of Euplotes cysts with chloramine T, mercurochrome, and argyrol.

Agent	Conc'n	Solvent	Times of treatment of cysts	Washing fluid	Sterility of plates inoculated with treated cysts	Behavior of treated cysts after placing in nutrient medium
	percent		minutes		colonies after 96 hours	
Chloramine T	0.5	bufferet 2.5 percent NaCl	60	buffered 2.5 per-cent NaCl	0	dead
"	0.1	"	60	"	0	"
"	0.01	"	60	"	0	"
"	0.001	"	60	"	5	alive but abnormal
"	0.5	"	60	"	0	dead
"	0.1	"	60	"	0	"
"	0.01	"	60	"	0	"
"	0.001	"	60	"	3	excysted and died
"	0.5	buffer sat'd with NaCl	60	buffer sat'd with NaCl	0	dead
"	0.1	"	60	"	0	"
"	0.01	"	60	"	0	"
"	0.001	"	60	"	2	"
"	0.0001	"	60	"	17	fairly normal
"	0.00001	"	60	"	many	normal
Mercuro-chrome	1	"	60	"	0	dead
"	0.5	"	60	"	0	"
"	0.1	"	60	"	10	"
"	0.01	"	60	"	many	"
"	0.001	"	60	"	"	"
Argyrol	1	"	60	"	"	normal
"	0.5	"	60	"	"	"
"	0.1	"	60	"	"	"

maintenance of the culture in a partly open watch glass at room temperature was sufficient to concentrate the sea water from 2 cc. to 0.1 cc. and produce encystment within 15 hours. The organisms were always freshly fed with A + B coli (8) about 48 hours prior to encystment. As preliminary experiments indicated that resistance of a cyst to a bactericidal agent was somewhat dependent upon age, cysts 2 days old were always employed unless otherwise indicated in the tables.

2 cc. of a bactericidal solution were placed in a watch glass contained within a PETRI dish. 35 to 45 cysts, 2 days of age, were immersed in this solution for a period ranging from 10 minutes to

Table IV.

Disinfection of Euplotes cysts with formaldehyde, hydrochloric acid, sodium hyroxide, sodium carbonate, barium sulphide, copper sulphate, metallic copper, and metallic silver.

Agent	Conc'n	Solvent	Time of treatment of cysts	Washing fluid	Sterility of plates inoculated with treated cysts	Behavior of treated cysts after placing in nutrient medium
	per-cent		minutes		colonies after 96 hours	
Formaldehyde	1	buffered 2.5 percent NaCl	60	buffered 2.5 percent NaCl	0	dead
"	0.5	"	60	"	0	"
"	0.1	"	60	"	few	"
"	1	buffer sat'd with NaCl	120	buffer sat'd with NaCl	0	"
"	0.5	"	120	"	0	"
"	0.1	"	120	"	2	"
"	0.01	"	120	"	15	fair
"	0.5	"	60	"	0	dead
"	0.1	"	60	"	3	"
"	0.01	"	60	"	19	fair
HCl	1	water	60	sat'd NaCl	many	disintegration
NaOH	1	"	60	"	"	"
Na_2CO_3	1	"	60	"	"	swollen and dead
"	1	"	120	"	"	much swollen and dead
BaS	1.5	buffered 2.5 percent NaCl	60	cysts distintegrated during treatment		
$CuSO_4 \cdot 5H_2O$	2	water	60	buffered 2.5 percent NaCl	12	dead
"	1	"	60	"	34	"
"	0.5	"	60	"	many	"
"	0.1	"	60	"	"	"
"	2	buffered sat'd with NaCl	60	buffer sat'd with NaCl	3	"
Sat'd NaCl in contact with metallic copper			20 hours	sat'd NaCl	many	normal
Sat'd NaCl in contact with metallic silver			20 hours	"	"	"
Sat'd NaCl in contact with metallic silver			42 hours	"	"	"

42 hours (usually 1 hour). Approximately 30 of the cysts were then passed through 3 portions of washing fluid, each of 2 cc., to rinse off the bactericidal agent. 12 or 15 were then used in the sterility test. For this purpose, either the pour plate method with 10 cc. of nutrient agar was employed, or, as in a few of the experi-

Table V.

Disinfection of cysts with phenol, toluene, and resorcinol.

Conc'n of disinfectant	Solvent	Time of treatment of cysts	Washing fluid	Sterility of plates inoculated with treated cysts	Behavior of treated cysts after placing in nutrient medium
percent		minutes		colonies after 96 hours	
phenol 1	2.5 percent NaCl, 0.1 percent $CaCl_2$	60	2.5 percent NaCl, 0.1 percent $CaCl_2$	0	dead
0.5	"	60	"	19	"
0.1	"	60	"	40	fairly normal
0.01	"	60	"	many	normal
1	distilled water	60	sat'd NaCl	0	disintegration
1	"	60	"	0	"
0.5	"	60	"	3	a few excysted and died
0.1	"	60	"	many	fairly normal
0.01	"	60	"	"	normal
1	buffered 2.5 percent NaCl	60	buffered 2.5 percent NaCl	0	dead
0.5	"	60	"	0	"
0.1	"	60	"	many	fairly normal
0.01	"	60	"	"	
0.5	buffered sat'd with NaCl	60	buffer sat'd with NaCl	0	dead
0.1	"	60	"	23	fairly normal
0.01	"	60	"	many	normal
Artificial sea water Saturated with toluene		2 hours	buffered 2:5 percent NaCl	"	dead
Artificial sea water Saturated with toluene		22 hours	"	3	"
Resorcinol 1	buffered 2.5 percent NaCl	60	"	many	fair
0.5	"	60	"	"	"
0.1	"	60	"	"	"
1	"	60	"	"	"
0.5	"	60	"	"	"
0.1	"	60	"	"	"

ments, the organisms were transferred to nutrient broth. The remaining 12 or 15 cysts were placed in 2 cc. of 1 : 1 A. S. W. with organisms A and B coli as food. If the protozoa excysted and behaved in movement and reproduction like untreated controls they were regarded as normal.

For control purposes, a second group of 35 to 45 cysts was run through exactly the same treatment with the single exception that

Table VI.

Disinfection of Euplotes cysts with silver nitrate in sodium chloride, sodium nitrate, and sucrose solutions.

Conc'n of silver nitrate	Solvent	Time of treatment of cysts	Washing fluid	Sterility of plates inoculated with treated cysts	Behavior of treated cysts after placing in nutrient medium
percent		minutes		colonies after 96 hours	
1	buffer sat'd with NaCl	60	buffer sat'd with NaCl	0	dead
0.5	"	60	"	0	"
0.1	"	60	"	0	poor
0.01	sat'd $NaNO_3$	60	sat'd $NaNO_3$	0	dead
0.001	"	60	"	0	"
0.0001	"	60	"	3	"
0.00001	"	60	"	15	"
0.001	"	60	"	0	"
0.0001	"	60	"	4	"
0.00001	"	60	"	2	"
0.01	"	60	"	0	dead
0.001	"	60	"	2	"
0.0001	"	60	"	5	"
0.00001	"	60	"		"
0.01	40 percent $NaNO_3$	60	40 percent $NaNO_3$	0	dead
0.001	"	60	"	2	"
0.0001	"	60	"	3	"
0.1	88 percent sucrose	60	buffer sat'd with NaCl	0	dead
0.01	"	60	"	0	normal
0.001	"	60	"	many	"
0.1	"	60	88 percent sucrose	0	dead
0.01	"	60	"	5	poor
0.001	"	60	"	many	normal
Sucrose alone	(88 percent)	24 hours	2 baths of 88 percent sucrose 1 bath of A. S. W.	12	"
"	"	"	"	8	dead
"	"	"	"	10	normal

the bactericidal agent was not added to the 2 cc. of fluid in which the cysts were first immersed.

Finally to make certain that the cysts were alive at the time of use, a third group of 12 or 15 was transferred directly from the encystment vessel to 1 : 1 artificial sea water containing organisms A and B coli as food.

One of the greatest technical difficulties was found to be the rigid control of encystment. Owing to changes in the laboratory temperature and humidity it was not always possible to attain the

Table VII.

Disinfection of *Euplotes* cysts with silver nitrate in 44 percent glucose solutions.

Conc'n of $AgNO_3$	Time of treatment of cysts	Washing fluid	Sterility of plates inoculated with treated cysts	Behavior of treated cysts after placing in nutrient medium
percent	minutes		colonies after 96 hours	
1	60	buffered 2.5 percent NaCl	0	dead
0.5	60	"	0	dead
0.1	60	"	0	fairly normal
0.01	60	"	3	normal
0.1	60	buffer sat'd with NaCl	0	dead
0.01	60	"	0	fairly normal
0.001	60	"	many	"
0.01	60	44 percent glucose	0	fair
0.01	60	"	1	"
0.01	60	"	0	excysted and died
0.01	60	"	0	pour
0.01	60	"	0	normal — 1.5 hrs. required for excystment
0.01	60	"	2	normal — 1.5 hrs. required for excystment
0.001	60	art. sea water	7	normal — 3 hrs. required for excystment
0.001	60	"	6	normal — 3 hrs. required for excystment
0.01	60	"	[1]) clear and sterile	dead
0.01	60	"	cloudy (infected)	"
0.01	60	"	clear and sterile	fair
0.01	60	"	cloudy	fair
0.01	60	"	clear and sterile	normal
0.01	60	"	"	"
0.01	60	"	"	"
0.01	60	"	"	"
0.01	60	"	cloudy	fair
0.01	60	"	clear and sterile	normal

same degree of concentration of the sea water during the 15 hour encystment period. It was also frequently observed that some organisms which failed to encyst during the period of 15 hours did so during the next 48 hours, even though the vessel had been sealed and further concentration minimized. In consequence of these variations the cysts actually employed in the experiments were not uniformly 2 days old. A few were more nearly $2^1/_2$ days of age and some may have been little more than 24 hours old. It

[1]) The remaining information in this column pertains to nutrient broth preparations used in place of agar plates, to test the sterility of the treated cysts.

Table VIII.

Lethal concentrations.

Substance	Time of treatment	Lethal concentrations	
		To bacteria	To cysts
	minutes	percent	percent
Chloramine T in NaCl	60	0.001	0.001
Formaldehyde in NaCl	60	0.1 to 0.5	0.1 to 0.5
Phenol in NaCl	60	0.5 to 1.0	0.5
Mercurochrome in NaCl	60	0.5	< 0.001
Copper sulphate	60	> 2.0	< 0.1
Hydrochloric acid	60	> 1.0	< 1.0
Sodium hydrocide	60	> 1.0	< 1.0
Sodium carbonate	60	> 1.0	< 1.0
Mercuric chloride in NaCl	60	0.001 to 0.01	< 0.001
„ „ „ $NaNO_3$	15 to 60	0.00001 to 0.0001	< 0.00001
Metallic mercury in NaCl	22 hours	not lethal	lethal
Silver nitrate in $NaNO_3$	60	0.0001 to 0.001	< 0.00001
Toluene in A. S. W.	2 to 22 hrs.	not lethal	lethal
Argyrol in NaCl	60	> 1.0	> 1.0
Resorcinol in NaCl	60	> 1.0	> 1.0
Metallic copper in NaCl	20 hrs.	not lethal	not lethal
„ silver in NaCl	20 to 42 hrs.	„	„
Silver nitrate in conc'd' glucose	60	0.01 „	0.1 „

was usually possible, however, to exclude the very young cysts which differed from the older ones by being larger and more brilliant. Actually, only the small, dark, specimens were selected for use.

Although numerous bactericidal agents were tried, most of the experiments were performed with mercuric chloride, silver nitrate, phenol, and chloramine T. Formaldehyde, resorcinol, toluene, mercurochrome, argyrol, hydrochloric acid, sodium hydroxide, sodium carbonate, barium sulphide, copper sulphate, and metallic copper, silver, and mercury were used in a few instances.

The well-known toxicity of metallic copper, silver, and mercury to bacteria prompted their use in this investigation. As will be indicated later, however, these metals were found to be completely inert under the conditions of this research. The most reasonable explanation of this loss of toxicity would seem to be the suppression of oligodynamic activity induced by the high electrolyte content of the medium (1, 6, 10, 15). The action of the heavy metal chlorides on *Amoeba proteus* has been studied by Reznikoff (13), while the toxicity of various other salts of the heavy metals to bacteria and other organisms has been demonstrated by many. The use of hydrochloric acid, sodium hydroxide, sodium carbonate, and toluene in

the sterilization of cysts has already been referred to in the introduction.

With respect to the solvent employed for the bactericidal agents, it should be pointed out that the osmotic pressure of the medium in which the cysts are immersed for treatment ought to be great enough to prevent excystment. In a few of the experiments reported in this paper distilled water was used as the solvent. This was soon abandoned for though actual excystment was not observed during treatment, the cysts occasionally swelled and showed changes indicative of the first stages of excystment. In consequence, 2.5 per cent sodium chloride, or saturated solutions of sodium chloride were most frequently used. These were generally buffered by the addition of a mixture of the two acid phosphates of sodium in a final concentration of 0.02 M. The phosphates were so proportioned as to give a p_H of about 7.0, although it is well known that high concentrations of electrolytes affect materially the p_H of buffer systems and, in addition, render difficult the precise determination of p_H. Since the sign and magnitude of the charge on the cyst and bacterial surfaces would necessarily affect the reaction with toxic agents, whether they be in the form of simple ions or substances in colloidal dispersion, it seemed desirable to exercise as much as possible this control of the hydrogen ion concentration. In a few cases, 0.1 per cent calcium chloride was added, in the hope that it might reduce the permeability of the cyst to the toxic compound. In a large number of experiments, mostly those with silver nitrate, high concentrations of glucose, sucrose, or sodium nitrate were employed as solvent for the disinfectant. This change was clearly necessary in order to escape the difficulties that would otherwise have entered through precipitation of silver chloride.

The washing fluid used for rinsing of the treated cysts was generally of the same composition as that employed for dissolving the bactericidal agent. Exceptions to this generalization are noted in the Tables. Where artificial sea water was used as washing fluid following treatment with silver nitrate, the cysts were given a preliminary rinsing in a single bath of 44 per cent glucose to minimize the trouble caused by silver chloride precipitation in the sea water. The washing fluids were always sterilized before use by filtration through sterile candles.

The complete results of the experiments are presented in Tables II to VIII.

Discussion of results.

A fairly close parallelism was observed in three instances in the responses of *Euplotes* cysts and the associated bacteria to toxic agents. Thus the lethal concentration of cloramine T was approximately the same for both the cysts and bacteria. A similar equality in behavior was found in the experiments with phenol and formaldehyde.

Generally, however, the cysts were more easily killed than the bacteria. The most striking instance of this was observed with mercurochrome which was much more toxic to the encysted protozoa than to the bacteria. So also copper sulphate, hydrochloric acid, sodium hydroxide, sodium carbonate, mercuric chloride and metallic mercury were more toxic to the cysts than to the bacteria. Solutions of silver nitrate in sodium nitrate exerted a similar preferential action.

In only one instance was the reverse phenomenon observed. Silver nitrate in concentrated solutions of glucose or sucrose was invariably more toxic to the bacteria than to the cysts. In several cases complete sterilization was effected without impairment of the protozoa. Although there were several failures, we are satisfied that further work will establish the conditions under which chemical sterilization with silver nitrate may consistently be obtained. We propose to investigate the action of other bactericidal agents in concentrated solutions of glucose, in addition to the prolonged exposure of cysts to glucose alone. From the work of Liese (7) it would appear that chinosol (dihydroxy quinoline sulphate) would be worth examination for Liese reports that some protozoa are much more resistant to this substance than bacteria were found to be.

It should be pointed out at this time that the control experiments were in complete agreement in showing that mere manipulation of the cysts and their immersion for designated times in the various solvents and washing fluids employed were not sufficient to reduce appreciably the numbers of associated bacteria nor impair the health of the cysts. After treatment with sodium nitrate and glucose, excystment sometimes proceeded quite slowly but the resulting motile organisms behaved normally in every respect.

Perhaps the most interesting results were obtained with the heavy metals and their salts. The most toxic compound was mercuric chloride dissolved in saturated sodium nitrate. A distinct lowering of its toxicity was observed in sodium chloride, probably

due to suppression of ionization of the mercuric chloride through a common ion effect. BENEDICENTI and BONINO (1) have also shown that the activity of the mercuric ion is greatly reduced in solutions of sodium chloride. This is insufficient in itself, however, to explain the chloride lowering of toxicity observed by us, since concentrated solutions of sodium nitrate ought also to reduce in similar fashion the activity of the mercuric ion. Silver nitrate dissolved in saturated sodium nitrate was also of great toxicity, both to the bacteria and the cysts but copper sulphate, on the contrary, was of remarkably little toxicity to the former. It is not surprising, therefore, that metallic copper failed to kill either the bacteria or the cysts. The great toxicity of mercuric chloride led us to expect that metallic mercury would likewise be quite poisonous, but, in fact, the protozoa alone were killed. Possibly the concentration of mercuric ions to which the oligodynamic properties of the metal are due was reduced to a sub-lethal level by the high electrolyte content, or there was a reduction of mercuric ion activity (1). LEITNER (6) suggests that electrolytes in high concentration reduce the surface charge of the bacteria and, in consequence, lessen the electrostatic adsorption of the metallic ion. Still less is it to be expected that metallic silver in sodium chloride would be toxic. In fact, neither the bacteria nor cysts were killed by it.

We were interested also by the observation that neither resorcinol nor argyrol in the highest concentrations employed were toxic to either the bacteria or cysts.

Summary.

1. Cysts of *Euplotes taylori*, two days old, were found less resistant to disinfectants than the associated bacteria.

2. In high concentrations of glucose and sucrose, silver nitrate was more toxic to the bacteria than to the cysts, thus permitting sterilization of the latter.

3. The lethal concentrations of the following substances were determined both for the cysts and the associated bacteria: chloramine T, formaldehyde, phenol, resorcinol, argyrol, toluene, mercurochrome, copper sulphate, mercuric chloride, and silver nitrate. Metallic copper, silver, and mercury were also employed.

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