the thyroids and parathyroids does not result in tetany. Tetany does not supervene because the goat can maintain a normal or nearly normal blood calcium.

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Use of Paramecia for Studying Toxins and Antitoxins (Measles, Scarlet Fever and Diphtheria).

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At the suggestion of Dr. Hektoen the effect of bacterial toxins and antitoxins on paramecia has been studied. Others have used paramecia for testing toxic action. Hamilton[1] observed that normal human serum, at a dilution of 1:5, usually was not toxic to paramecia, while serum from scarlet fever patients was nearly always toxic (85%) and serum from pneumonia patients was toxic in 66%. Takenouchi[2] found that strong diphtheria and tetanus toxins and hemolytic staphylococcus and streptococcus culture filtrates had no effect on paramecia. Cultures of *B. pyocyaneus* caused death of paramecia, but this seemed due to alkalinity of the filtrate and not to any specific pyocyanolysin, because, neutralization of the filtrate caused the effect to be entirely lost. Philpott[3] found that virulent *B. pyocaneus* and *B. enteritidis* were toxic to paramecia. Diphtheria toxin had no appreciable effect on the division or death rate of 3 species of paramecia tested.

In testing the action of filtrates of bacterial cultures on paramecia it is necessary to grow the bacteria in a medium that is itself not toxic for the paramecia. The solutions must be isotonic with the paramecia, which according to Balbiani[1] corresponds to 0.3% solution of common salt. Crane[4] states that paramecia can live 24 hours in any hydrogen ion concentration between pH 5 to 7.6.

One percent dextrose broth, pH 7.6, made with Liebig's beef extract and Witte's peptone and containing 1% sheep blood, was found suitable for the production of streptococcus toxins and was in itself not toxic to paramecia. No sheep blood was added to the dextrose broth for the production of diphtheria toxin. The bacteria were grown 5 days at 36° C. and the cultures filtered through

[1] Hamilton, A., *J. Infect. Dis.*, 1904, i, 211.

[2] Takenouchi, M., *J. Infect. Dis.*, 1918, xxiii, 396.

[3] Philpott, C. H., *J. Morph. and Physiol.*, 1928, xlvi, 85.

[4] Crane, *J. Phar. and Exp. Therap.*, 1921, xviii, 319.

Berkefeld W filters. Antiseptics must not be added to the filtrates. Two cultures of paramecia were used in these experiments with similar results. One culture was obtained from Dr. L. H. Hyman of the University of Chicago and one culture was purchased from a biologic supply house. The paramecia were grown in whole wheat water: a few grains of wheat are added to 15 cc. of tap water in a test tube and the mixture autoclaved. The culture medium must stand open to the air several days to become contaminated with bacteria before the paramecia are added. Actively motile paramecia are essential for the test. Before dying, paramecia begin to move more slowly, rotate, become darker, extrude some of their protoplasm, and finally become motionless.

To test the toxicity of a bacterial filtrate a nearly equal part of the so-called toxin was added to a culture of paramecia in a hollow glass slide. A similar mixture of paramecia and culture medium was used as a control of the activity of the paramecia. Each specimen contained approximately the same number of paramecia. The mixtures were left in a moist chamber at room temperature and examined hourly to find when, if at all, the paramecia in the mixture with toxin were dead and showed no indication of recovering motility. A potent toxin killed all of the paramecia within 5 hours. No toxin tested was found sufficiently powerful to permit dilution. Not all of the bacteria tested produced toxins strong enough to kill paramecia during the period of the experiment.

If a bacterium produced toxin potent for paramecia, an effort was made to determine whether the toxin could be neutralized by the corresponding antitoxin. Normal and immune serum were diluted with wheat water and then to one part of the diluted serum, 4 parts of toxin were added. A similar mixture of toxin and wheat water was made. These mixtures were incubated 1 hour at 36° C. Equal parts of these mixtures were then added to the paramecium culture. The final dilutions of the serum were therefore 10 times the original dilution. If concentrated immune serum was used in a test, a concentrated serum was also used for the control.

Occasionally normal serum neutralized toxins in low dilutions and some serums were in themselves toxic in low dilutions to paramecium. Often immune serums, not toxic in themselves, did not neutralize the toxins until diluted 100 or 200 times ("prozone"?). For these reasons it was found best to dilute the normal or immune serum from 1:100 to 1:1600 or higher.

Measles Toxin. Formerly rabbits have been used in testing the neutralizing effect of anti-measles-diplococcus horse and goat serum on measles antigens. Since all rabbits do not react to measles anti-

gen and many are sensitive to horse and goat serum, it has been troublesome to find rabbits suitable for these tests. Paramecia were found to be sensitive to measles toxin, being killed in 1 to 2 hours. This toxic action for paramecia was neutralized by anti-measles-diplococcus horse or goat serum, and consequently this method is considered useful in standardizing these serums. The goat serum was not concentrated, while the horse serum was. The serums neutralized measles toxin in final dilutions of 1:1600 to 1:16,000. The unconcentrated goat serum neutralized the toxin in as high dilutions as concentrated horse serum (Table I).

TABLE I. *Action of Measles Toxin and Antitoxin on Paramecia.*

Paramecium Suspension Parts		Parts		Parts	Paramecia Living After		
					1 Hr.	2 Hrs.	3 Hrs.
5	Culture Medium,	4	Wheat Water,	1	+++	+++	+++
5	Measles Toxin,	4	" "	1	+++	0	0
5	" "	4	Normal Goat Serum 1:100,	1	++	0	0
5	" "	4	" " " 1:200,	1	+++	++	0
5	" "	4	" " " 1:400,	1	+++	+	0
5	" "	4	" " " 1:800,	1	++	0	0
5	" "	4	Antidiplococcus Goat Serum[1] 1:100,	1	+++	+++	+++
5	" "	4	" " " 1:200,	1	+++	++	++
5	" "	4	" " " 1:400,	1	+++	+	++
5	" "	4	" " " 1:800,	1	+++	0	0
5	" "	4	" " " 1:1600,	1	++	0	0

+++ = All paramecia alive.
++ = About half of paramecia alive.
+ = Almost all paramecia dead.
0 = All paramecia dead.

[1] Serum of goat immunized with coccus from measles.

Scarlet Fever Toxin. Strains of hemolyzing streptococci were tested by the opsonic method[5] to determine whether or not they were scarlatinal streptococci. For these tests unconcentrated serum from a horse immunized with scarlet fever streptococci by a modification of the Dochez method, was furnished by Dr. Benjamin White of the Laboratory of the Massachusetts Department of Health. This serum was specific for scarlet fever streptococci. Toxins were prepared with these streptococci and their effect on paramecia studied. Neutralization experiments with concentrated scarlet fever antitoxin (Dr. White) were made with toxic strains. One commercial scarlet fever antitoxin was tested also.

Eighteen strains of scarlatinal streptococci were studied, 12 were toxic, 4 partially toxic and 2 not toxic for paramecia. Four strains of hemolytic streptococci not belonging to the scarlet fever group

[5] Tunnicliff, R., *J. Am. Med. Assn.*, 1926, lxxxvi, 625; *J. Infect. Dis.*, 1928, xli, 272.

were toxic to paramecia. Two of these strains were from the blood from patients with septicemia, one from a patient with pyemia and one from the skin in erysipelas. Ten nonscarlatinal strains from patients with sore throat, acute rhinitis and *otitis media* were found to be not toxic to paramecia.

The toxins from the scarlet fever streptococci were all neutralized by the Massachusetts antitoxin in final dilutions of 1:2000 to 1:32,000 and not by the concentrated normal serum used for control. The scarlet fever toxins which were only partially toxic for paramecia were also neutralized by this serum, but experiments with such toxins do not give convincing results unless the differences between the specimens treated with normal and immune serum are clear and distinct.

TABLE II. *Action of Scarlet Fever Toxin and Antitoxin on Paramecia.*

Paramecia Suspension Parts			Parts				Parts	Paramecia Living After 1 Hr.	2 Hrs.	3 Hrs.
5	Culture	Medium,	4	Wheat	Water,		1	+++	+++	+++
5	Scarlet	Fever Toxin,	4	"	"		1	++	0	0
5	"	" "	4	Normal	Horse	Serum, 1:100,	1	+	0	0
5	"	" "	4	"	"	" 1:200,	1	+	0	0
5	"	" "	4	"	"	" 1:400,	1	++	0	0
5	"	" "	4	Scarlet	Fever	Antitoxin[1] 1:100,	1	+++	+	+
5	"	" "	4	"	"	" 1:200,	1	+++	+++	+++
5	"	" "	4	"	"	" 1:400,	1	+++	+++	+++
5	"	" "	4	"	"	" 1:800,	1	++	0	0

+++ = All paramecia alive.
++ = About half of paramecia alive.
+ = Almost all paramecia dead.
0 = All paramecia dead.

[1] Obtained from the Massachusetts Antitoxin and Vaccine Laboratory

The Massachusetts antitoxin was specific for scarlet fever streptococci and did not neutralize nonscarlatinal toxins. The commercial scarlet fever antitoxin was not specific, neutralizing not only the scarlet fever toxin, but also the erysipelas toxin and the toxins from the nonscarlatinal streptococci isolated from the blood.

Diphtheria Toxin. A filtrate of the Park-Williams No. 8 strain of diphtheria bacilli killed paramecia in 5 hours. Normal horse serum neutralized this toxin in only low dilutions, while diphtheria antitoxin neutralized it completely at a final dilution of 18,000 and partially at a dilution of 32,000 at the end of 24 hours. Eight other strains of diphtheria bacilli were tested, 7 of which were toxic for paramecia in from 3 to 5 hours. Two-tenths cc. of 5 day cultures of the Park-Williams organism and the same amount of the strain non-virulent for paramecia was injected intradermally into a normal guinea pig weighing 250 gm. In 24 hours the Park-Williams strain

produced a reddened induration, one cm. in diameter, which was followed by desquamation. The strain which was nontoxic for paramecia produced no effect in the skin of the guinea pig. This experiment indicated that the toxic and non-toxic strains acted similarly on paramecia and guinea pigs (Table III).

TABLE III. *Action of Diphtheria Toxin and Antitoxin on Paramecia.*

Paramecium Suspension Parts		Parts		Parts	Paramecia Living After		
					3 Hrs.	5 Hrs.	24 Hrs.
5	Culture Medium,	4	Wheat Water,	1	+++	+++	+++
5	Diphtheria Toxin,	4	" "	1	++	0	0
5	" "	4	Normal Horse Serum 1:200,	1	++	++	0
5	" "	4	" " " 1:400,	1	++	0	0
5	" "	4	" " " 1:800,	1	++	0	0
5	" "	4	Diphtheria Antitoxin[1] 1:200,	1	+++	+++	+
5	" "	4	" " 1:400,	1	+++	+++	+++
5	" "	4	" " 1:800,	1	+++	+++	+++
5	" "	4	" " 1:1600,	1	+++	+++	+++
5	" "	4	" " 1:3200,	1	+++	+++	++

+++ = All paramecia alive.
++ = About half of paramecia alive.
+ = Almost all paramecia dead.
0 = All paramecia dead.

[1] Each cc. contained 2000 units of antitoxin. The lowest dilution of antitoxin in the mixtures contained approximately 0.29 units of antitoxin and the highest dilution contained approximately 0.0127 unit.

Summary: These experiments show that paramecia can be used to determine toxin production of certain bacteria (diphtheria bacillus, measles diplococcus, scarlet fever streptococcus) in a crude way only because the toxins are not sufficiently potent to be diluted, but that paramecia may be helpful in determining the strength of antitoxins.

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Experiments in Filtration of the Virus of Avian Molluscum.*

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There are conflicting statements in the literature regarding the filterability of the virus of *Avian molluscum.* Some observers reported the presence of the virus in Berkfeld but not in porcelain

* These experiments were done in part at the Lister Institute, London, under the direction of Dr. J. C. G. Ledingham, whose advice is gratefully acknowledged.