THE KINETICS OF THE TOXIC ACTION OF ANTISEPTIC AGENTS TOWARD PARAMECIUM CAUDATUM[1]

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Introduction

Bancroft[2] has recently revived the colloidal coagulation theory of disinfectant action. The expression

$$c^n t = k$$

where **c** is the concentration of the toxic agent
t is the time required for disinfection
n and **k** are constants

has been shown by a number of workers[3,4,5,6,7] to govern the kinetics of toxic action. This equation is analogous to the Ishizaka[8] equation

$$k = ac^n$$

for the relation between the velocity constant of a coagulation, **k,** and the concentration of the coagulating agent, **c, a** and **n** being constants. The complete form of the Ishizaka equation is:

$$t = \frac{1}{ac^n(1+b)}\left[\frac{2.303}{1+b}\log\frac{b+x}{b(1-x)} + \frac{x}{1-x}\right]$$

where **x** is the fraction coagulated in time **t, c** is the concentration of KCl, and **a, b,** and **n** are constants. If the time to a given degree of coagulation, t_s, is taken, the Ishizaka equation reduces to the form

$$c^n t_s = k'$$

Assuming that a micro-organism dies suddenly at a given degree of coagulation of its protoplasm, the correspondence

1 Taken from the thesis presented by Leo J. Geppert in partial fulfillment of the requirements for the degree of Master of Arts, University of South Dakota, May, 1937.

2 W. D. Bancroft and G. H. Richter, **J. Phys. Chem., 35,** 511-530 (1931)

3 H. Watson, **J. Hyg., 8,** 537 (1908)

4 Wo. Ostwald, **Arch. Ges. Physiol. (Pfluger's), 120,** 19-30 (1908)

5 T. Paul, G. Birstein and A. Reuss, **Biochem. Z., 29,** 202-247 (1911)

6 E. B. Phelps, **J. Infect. Dis., 8,** 27-38 (1911)

7 E. Bateman, **U. S. Dept. Agr. Tech. Bull., 346,** 1-53 (1933)

8 N. Ishizaka, **Z. Physik. Chem., 83,** 97-125 (1913)

between the kinetic expressions for toxicity and for coagulation reinforces the Bancroft coagulation theory of death.

This work was carried out with the hope that the collection of data on the kinetics of toxicity for a closely related group of meta-substituted phenols would show some regularities that would shed some further light on the mechanism of toxic action. A group of unrelated compounds was run for comparison.

Experimental

The duration of life in seconds of individual paramecia was measured at pH 7.6, at 25±0.5°C., and at various concentrations of the antiseptic agents. The temperature was maintained in a stage thermostat, whose temperature was maintained by the balance between a heating unit and the flow of water through the apparatus, the water serving also as a heat filter in the light path. The dilution series was based on the reciprocals of the powers of the eighth root of ten, giving a spread of eight equal logarithmic steps in the decade. These dilutions were made by mixing three parts of the previous dilution and one part of water. From microburettes, 0.015 ml. of antiseptic agent were added to 0.005 ml. of paramecium culture contained in a small depression slide with a hemispherical concavity. The 0.005 ml. of paramecium culture contained from one to three organisms. Following the addition of the antiseptic agent, the mixture was stirred with the tip of the microburette and observed in the stage thermostat. Time of death was taken when motility ceased. There were usually simultaneous morphological changes that indicated that the organisms were dead, particularly a darkening and coagulation of the protoplasm. The individual death times, 14 to 20 observations at each concentration, were averaged and the probable error of the mean calculated statistically. In order to prove that the results had statistical significance, a distribution curve was drawn in each case from the probable error of the distribution, and compared with the standard distribution curve.

From the average death times, **t** in seconds, at each concentration, **c** in mols per liter, plots were made with **log c** as the ordinate and **log t** as the abscissa. From the slope of this line, **log t** divided by **log c,** the contant **n** in the equation

$$c^n t = k$$

was calculated. In the case of each compound, two separate runs were made, using different cultures and sets of dilutions of the antiseptic agent, so that two values of **n** were obtained, which were statistically averaged. From the average value of **n**, **k** was calculated for each point in each curve. All the values of **k** for a given antiseptic agent were then statistically averaged. Using the average values of **n** and **k**, the **n**th root of **k** was calculated for each compound. The **n**th root of **k**, equivalent to the concentration required to kill in unit time, is suggested as a comparative measure of antiseptic efficiency. It might be called the Toxicity Coefficient.

A summary of the experimental data is given in Table I.

Table I

The Constants **n**, **k**, and the **n**th Root of **k**

Compound	n	k	nth root of k
Mercuric chloride	2.64±0.01	$8.77\pm0.14\times10^{-16}$	1.931×10^{-6}
m-Hydroxybenzoic acid	5.41±0.10	$1.22\pm0.04\times10^{-13}$	0.004112
m-Chlorophenol	3.37±0.02	$2.50\pm0.11\times10^{-7}$	0.01095
m-Nitrophenol	2.63±0.03	$2.01\pm0.05\times10^{-5}$	0.01644
m-Cresol	5.71±0.02	$1.80\pm0.02\times10^{-10}$	0.01962
Resorcinol mono-methyl ether	2.73±0.07	$2.44\pm0.09\times10^{-4}$	0.04754
Phenol	4.99±0.01	$1.84\pm0.05\times10^{-7}$	0.04770
Resorcinol	3.42±0.03	$9.99\pm0.23\times10^{-4}$	0.1328
m-Aminophenol	4.15±0.03	$3.99\pm0.30\times10^{-3}$	0.2646
Sodium chloride	1.48±0.01	2.93±0.07	2.061

It is probable that death in these cases was due to coagulation, since definite coagulation of the protoplasm was observed at death, and since the kinetics of the process obeys the Ishizaka equation that governs the coagulation of inorganic colloids. With the substituents arranged in increasing order of the **n**th root of **k**, as above, it is interesting to note that Labes and Jansen[9] arranged the substituents in phenol

[9] R. Labes and E. Jansen, **Arch. exptl. Path. Pharmakol., 158,** 1-28 (1930)

in the following order of decreasing ability to coagulate serum albumin, chloro, nitro, methyl, hydroxyl, indicating a definite parallelism between antiseptic efficiency and ability to coagulate proteins. It is interesting to note that surface tension could not have played a predominant role as the cause of death, since the surface tension of the strongest concentration of each antiseptic was higher than the surface tension of the oat straw infusion in which the paramecia were grown.

In the case of saponin, the toxic action was definitely lytic in character, death being usually accompanied by rupture of the membrane and dispersion of the cytoplasm. The equation

$$t = \frac{22.7}{c^{0.5}} + \frac{0.0056}{c^{4}}$$

fitted the data, as can be seen from Table II.

Table II

Toxicity of Saponin to Paramecium Caudatum pH 7.6 25°C.

Concn. gms./100 ml.	Death Time in Seconds			Surface Tension dynes per cm.
	Run I	Run II	Calc.	
1.001	19.59± 0.52	18.67± 0.46	22.71	49.5
0.7500	25.77± 0.91	24.62± 0.41	26.23	50.3
0.5625	33.31± 0.32	28.42± 0.59	30.33	50.3
0.4219	39.36± 0.79	35.19± 0.78	35.14	49.2
0.3164	42.28± 1.21	38.95± 0.23	40.93	51.0
0.2373	46.65± 0.76	44.50± 0.39	48.39	51.4
0.1780	56.70± 0.32	53.67± 0.54	59.43	53.0
0.1335	82.37± 2.18	83.45± 1.89	79.87	53.0
0.1001	131.0 ± 4.8	139.2 ± 3.3	127.8	53.3
0.0750	211.2 ± 7.8	206.7 ± 5.3	260.0	54.5
0.0563	736.1 ±17.9	681.1 ± 9.7	655.7	54.0
0.0422	1797 ±33	1689 ±29	1882	57.0
0.0316	—	3223 ±53	5728	57.5

Summary

1. The Watson formula, $c^n t = k$, for the kinetics of the process of disinfection is found to hold in the case of Paramecium caudatum for the action of mercuric chloride, m-hydroxybenzoic acid, m-chlorophenol, m-nitrophenol, m-cresol, resorcinol monomethyl ether, phenol, resorcinol, m-aminophenol, and sodium chloride.

2. An analogy is shown between the Watson formula for the kinetics of disinfection and the Ishizaka formula for the kinetics of coagulation of colloids.

3. Support has been given for the concept of determining the values of **n** and **k** in comparing antiseptics. The **n**th root of **k** is suggested as a useful factor for comparing antiseptic activity. It might be called the Toxicity Coefficient.

4. Death by saponin is lytic and does not follow the Watson formula.