THE INFLUENCE OF CHANGES IN CONCENTRATION OF SODIUM HYDROXIDE UPON ITS BACTERICIDAL ACTIVITY

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Although the literature contains a number of reports regarding the bactericidal efficiency of certain specified concentrations of sodium hydroxide against various test organisms, very little of this information is presented in a manner suitable for mathematical analysis. To obtain the data required for analysis, an experimental study was made of the bactericidal efficiency of sodium hydroxide. This study was conducted along the lines of a previously described study of the bactericidal efficiencies of some of the phenols and alcohols (Tilley, 1939).

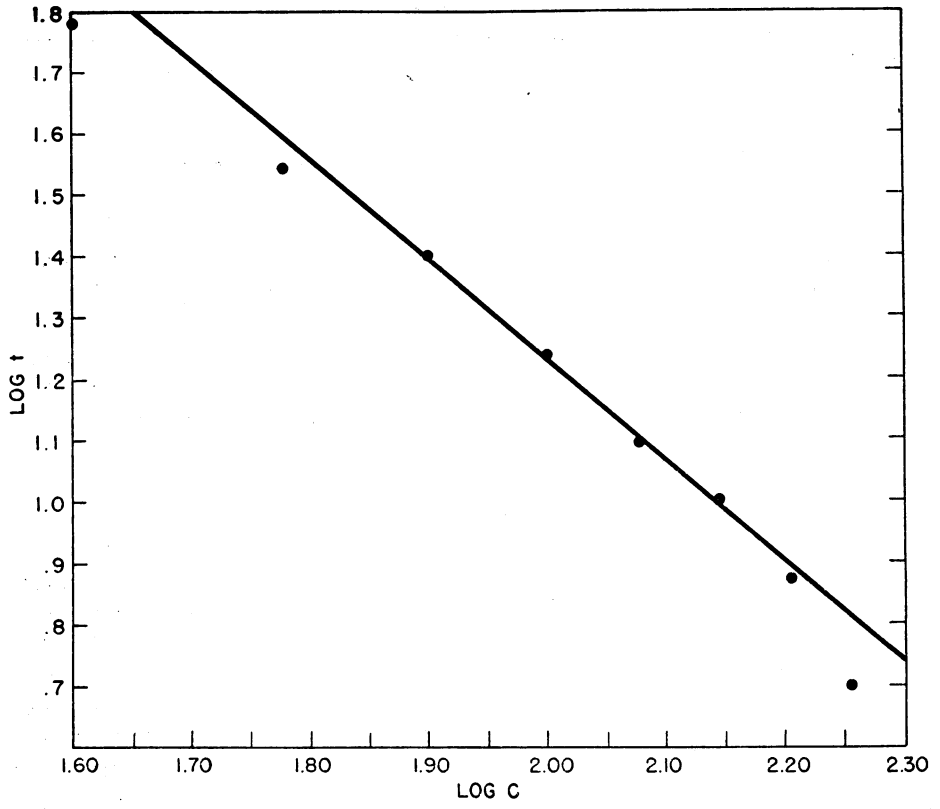
EXPERIMENTAL PROCEDURE

Bactericidal efficiency was determined by the Rideal-Walker technique, modified as follows: Either infusion broth or FDA beef extract broth was used instead of R-W standard broth; instead of a standardized dropping pipette a 1-ml pipette graduated into tenths was used to measure out the 0.5 ml of culture required for the test; medication temperatures were sometimes 20 C and sometimes 30 C instead of 15 C to 18 C; dilutions were made in sterile Erlenmeyer flasks instead of in cylinders; fixed amounts of the stock solution of disinfectant were added to varying amounts of distilled water, all measured with sterile standardized pipettes; the time of exposure was extended beyond 15 minutes, subcultures being made at 2.5-minute intervals when exposure varied from 2.5 to 30 minutes, at 5-minute intervals when exposures varied from 30 to 80 minutes, and at 10-minute intervals when exposures were beyond 80 minutes; and *Staphylococcus aureus* and *Escherichia coli* were used as test organisms instead of *Eberthella typhosa*.

The bactericidal efficiency of sodium hydroxide was determined by the technique described above, and from the experimental data thus obtained values of the concentration exponent, n, were calculated as described in a previous paper (Tilley, 1939) by the equation, $n = \frac{\log t_2 - \log t_1}{\log C_1 - \log C_2}$ etc., for results presented in tabular form, and by the equation, $n = \frac{y_2 - y_1}{x_1 - x_2}$ for results presented graphically.

EXPERIMENTAL AND CALCULATED RESULTS

Experimental results obtained with *Staphylococcus aureus* as the test organism are shown in table 1 and graph 1. When calculations are based on results with concentrations between 40 and 160 g in 10,000 ml, the value of the concentration exponent, n, is 1.58 when calculated from results shown in table 1, and 1.62 when calculated from results shown in graph 1. The result obtained



GRAPH 1. BACTERICIDAL EFFICIENCY OF SODIUM HYDROXIDE AGAINST STAPHYLOCOCCUS AUREUS, $n = 1.62$

TABLE 1

Bactericidal efficiency of sodium hydroxide against Staphylococcus aureus in ordinary beef infusion broth

C G IN 10,000 ML	t KILLING TIME IN MINUTES	LOG C	LOG t	LOG C_1 − LOG C_2 ETC.	LOG t_2 − LOG t_1 ETC.	n
180	5	2.25527	0.69897			
160	7.5	2.20412	0.87506	5115	17609	3.44*
140	10	2.14613	1.00000	5799	12494	2.15
120	12.5	2.07918	1.09691	6695	09691	1.44
100	17.5	2.00000	1.24304	7918	14613	1.84
80	25	1.90309	1.39794	9691	15490	1.59
60	35	1.77815	1.54407	12494	14613	1.17
40	60	1.60206	1.77815	17609	23408	1.33
Average						1.58

Experiment conducted at 20 C.

* Anomalous and therefore omitted in calculating average.

TABLE 2

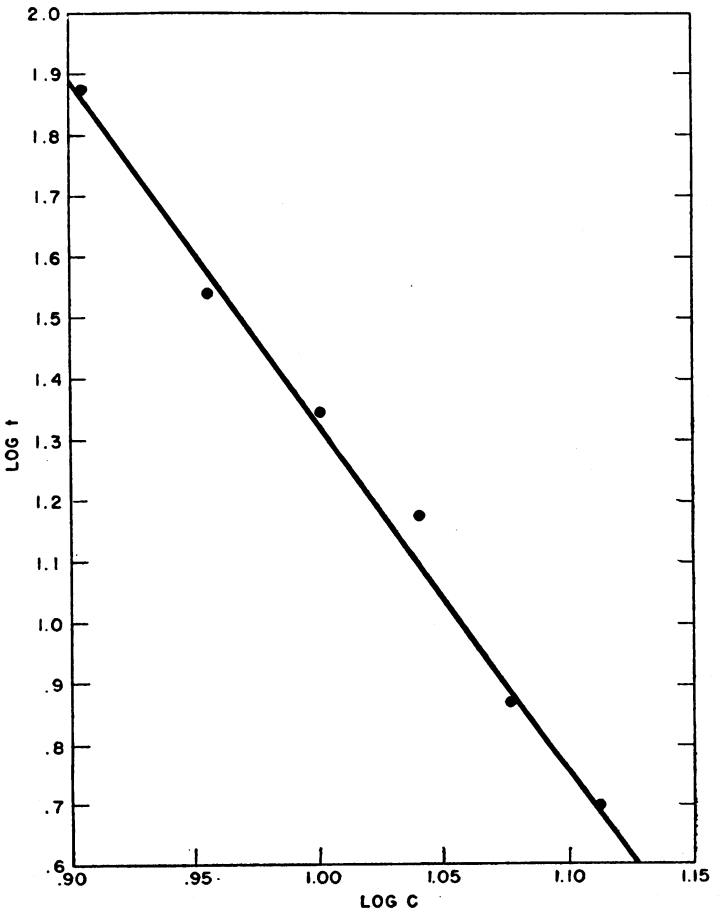
Bactericidal efficiency of sodium hydroxide against Escherichia coli grown in ordinary beef infusion broth

C G IN 10,000 ML	t KILLING TIME IN MINUTES	LOG C	LOG t	LOG C_1 − LOG C_2, ETC.	LOG t_2 − LOG t_1, ETC.	n	LOG A
13	5.0	1.11394	0.69897				6.93
12	7.5	1.07918	0.87506	3476	17609	5.00	6.92
11	15.0	1.04139	1.17609	3779	30103	7.96	7.00
10	22.5	1.00000	1.35218	4139	17609	4.25	6.95
9	35.0	0.95424	1.54407	4576	19189	4.19	6.89
8	75.0	0.90309	1.87506	5115	33099	6.47	6.93
7	*	0.84510					
6	†	0.77815					
Average						5.6	6.93

Experiment conducted at 30 C.

* Calculated disinfection time was 159 minutes. Observed time was between 24 and 48 hours.

† Calculated disinfection time was 372 minutes. Growth occurred in subcultures after 7 days' exposure.



GRAPH 2. BACTERICIDAL EFFICIENCY OF SODIUM HYDROXIDE AGAINST ESCHERICHIA COLI, $n = 5.6$

with a concentration of 180 g in 10,000 ml was anomalous and was not used in calculating this value. With this exception, the results shown indicate that, within the concentration range employed, the process of disinfection follows an approximately logarithmic course.

TABLE 3

Bactericidal efficiency of sodium hydroxide against Escherichia coli grown in beef extract broth

C G IN 10,000 ML	t KILLING TIME IN MINUTES	LOG C	LOG t	LOG C_1 − LOG C_2, ETC.	LOG t_2 − LOG t_1, ETC.	n
10	5	1.00000	0.69897			
9	7.5	0.95424	0.87506	4576	17609	3.84
8	10	0.90309	1.00000	5115	12494	2.44
7	17.5	0.84510	1.24304	5799	24304	4.19
6	27.5	0.77815	1.43933	6695	19629	2.93
5	60	0.69897	1.77815	7918	33882	4.29
Average						3.54

Experiment conducted at 30 C.

TABLE 4

Bactericidal efficiency of sodium hydroxide against Escherichia coli grown in special beef infusion broth

C G IN 10,000 ML	t KILLING TIME IN MINUTES	LOG C	LOG t	LOG C_1 − LOG C_2, ETC.	LOG t_2 − LOG t_1, ETC.	n	LOG A
14	5	1.14613	0.69897				7.72
13	7.5	1.11394	0.87506	3219	17609	5.47	7.70
12	12.5	1.07918	1.09691	3476	22185	6.38	7.71
11	20	1.04139	1.30103	3779	20412	5.40	7.68
10	35	1.00000	1.54407	4139	24304	5.87	7.67
9	75	0.95424	1.87506	4576	33099	7.23	7.72
8	160	0.90309	2.20412	5115	32906	6.43	7.74
7	*	0.84510					
6	†	0.77815					
Average						6.13	7.71

Experiment conducted at 30 C.

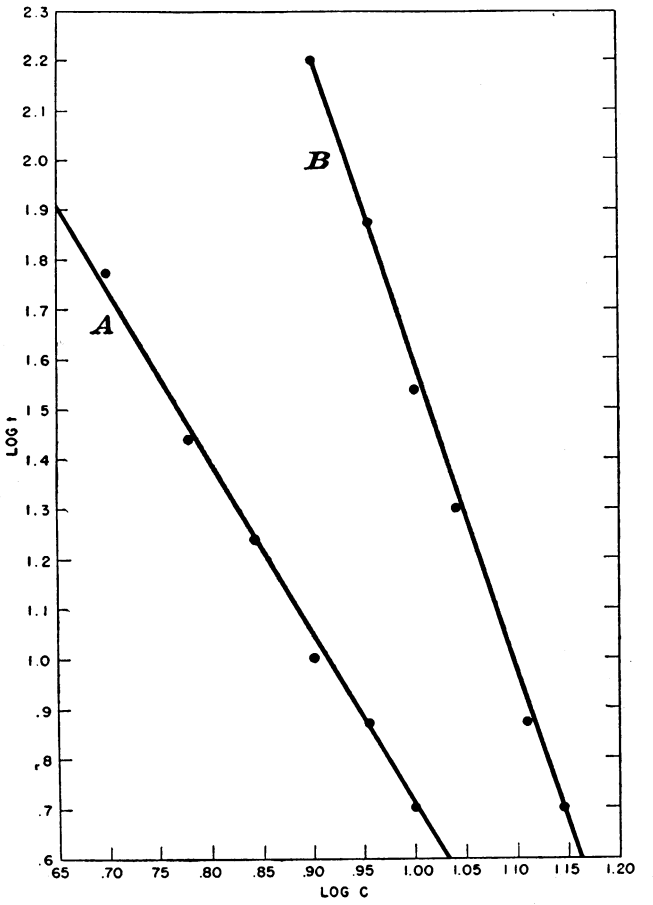
* Time calculated by equation, $\log t = \log A - n \log C$ with the values of n and $\log A = 339$ minutes. Observed time was between 24 and 48 hours.

† Time calculated as above was 871 minutes. Growth occurred in subcultures after 7 days' exposure.

Similar experimental results with *Escherichia coli* as the test organism are shown in table 2 and graph 2. When calculations are based on concentrations between 13 and 8 g in 10,000 ml, the value obtained for n is 5.6, and the process of disinfection appears to follow an approximately logarithmic course. However, it will be noted in table 2 that there is a sharp break between a concentration

of 8 g in 10,000 ml and a concentration of 7 g in 10,000 ml. The calculated disinfection times shown in the table were obtained by using the values n = 5.6 and log A = 6.93 in the equation log t = log A − n log C.

Tables 3 and 4 present the results of experiments with cultures of *Escherichia coli* grown in FDA beef extract broth and in a special beef infusion broth contain-



GRAPH 3. BACTERICIDAL EFFICIENCY OF SODIUM HYDROXIDE AGAINST ESCHERICHIA COLI

A. Beef extract broth. n = 3.5
B. Special beef infusion broth. n = 6.2.

ing 2 per cent peptone, respectively. The results with both media are also shown in graph 3. It is evident that even in the concentration ranges where the process of disinfection follows an approximately logarithmic course, the rate of disinfection is markedly affected by the amount of organic matter present. Likewise a comparison of results in tables 2 and 4 shows that a comparatively small increase in organic matter causes an appreciable increase in the disinfection

time. The results shown in these tables also indicate that the effective concentration range is sharply limited at 6 g per 10,000 ml.

Data presented in table 5 show the effect of varying amounts of organic matter upon the pH of sodium hydroxide solutions and upon their bactericidal efficiency against *Escherichia coli*. The pH values of the original solutions were determined with the Beckman pH meter, using a glass electrode especially designed for use in solutions containing high alkali concentrations. The pH values of the test mixtures were usually determined with the pH meter after standing 24 hours. When growth was noted in subcultures from test mixtures, the pH values of these mixtures were determined colorimetrically, using the standard

TABLE 5

Effect of varying amounts of organic matter upon the pH and upon the bactericidal efficiency of sodium hydroxide solutions against Escherichia coli

NaOH G IN 10,000 ML	ORIGINAL pH VALUES	A		B		C	
		Final pH values	Killing time in minutes	Final pH values	Killing time in minutes	Final pH values	Killing time in minutes
14	12.50	11.7	5.0	10.5	60		
12	12.45	11.4	12.5	10.4	100		
11	12.40	11.1	20.0	10.3	*		
10	12.35	11.0	35.0	10.2	*	11.85	5.0
9	12.30	10.6	75.0	10.1	*	11.75	7.5
8	12.25	10.3	160.0	9.0	‡	11.55	10.0
7	12.15	9.7	†			11.30	17.5
6	12.10	9.5	‡			11.00	27.5
5	12.00					10.50	60.0

Experiment conducted at 30 C.

A and B test cultures grown in special beef infusion broth. A—no additional organic matter. B—20 per cent serum added. C—test cultures grown in beef extract broth; no serum added.

* Growth after 3 but not after 24 hours.

† Growth after 24 but not after 48 hours.

‡ Growth after 7 days.

solutions of Kolthoff and Vlesschouwer as described by Clark (1928). When these standard solutions were freshly prepared, there was good agreement between the results obtained with them and the results obtained with the glass electrode.

The results shown in table 5 indicate that with *E. coli* as the test organism the original concentrations and pH values of sodium hydroxide solutions are not significant. It is the final pH of the test mixtures that should be taken into consideration. These results, as well as the results of other unreported experiments, indicate that when the final pH falls below 10, bactericidal activity disappears. The results obtained with *Staphylococcus aureus* indicate that this statement is also true for that organism.

SUMMARY

The bactericidal efficiency of sodium hydroxide against *Staphylococcus aureus* and *Escherichia coli* was determined by a modified Rideal-Walker technique. From the resulting experimental data, values for the concentration exponent n were calculated by the formula, $n = \frac{\log t_2 - \log t_1}{\log C_1 - \log C_2}$ for results presented in tabular form, and by the formula, $n = \frac{y_2 - y_1}{x_1 - x_2}$ for results presented in graphical form.

When test cultures were grown in ordinary beef infusion broth, the values obtained for n were approximately 1.6 for *Staphylococcus aureus* and 5.6 for *Escherichia coli*.

Within a comparatively limited range of concentrations the bactericidal action of sodium hydroxide against both test organisms followed, with either small or large amounts of organic matter, an approximately logarithmic course. However, the slope of the curve was related to the organic matter present. Bactericidal efficiency was correlated with the final pH of the test mixtures. When the pH fell below 10, efficiency suddenly dropped, and at a pH between 10 and 9 disappeared altogether.

REFERENCES

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TILLEY, F. W. 1939 An experimental study of the relation between concentration of disinfectants and time required for disinfection. J. Bact., 38, 499–510.