A NOTE ON THE VARIATION OF THE RATE OF DISINFECTION WITH CHANGE IN THE CONCENTRATION OF THE DISINFECTANT.

By HERBERT EDMESTON WATSON, B.Sc. (Lond.), A.I.C.

In a recent paper by Miss Chick on "The Laws of Disinfection[1]," it was pointed out that disinfection of bacteria is strictly analogous to a chemical reaction in which individual bacteria play the part of molecules. Thus, if n be the number of bacteria present at any time t during disinfection, $-\frac{dn}{dt} = K \,.\, n$, where K is a constant. Also, if K_1, K_2 are these constants for two different temperatures T_1, T_2, $\frac{T_1 T_2}{T_1 - T_2} \log \frac{K_1}{K_2}$ is also constant, *i.e.* Arrhenius' formula for the temperature coefficient of chemical reactions holds good in the case of bacteria as well. In addition to this, it was found that the relation between the concentration of the disinfectant and the time of disinfection (that is, the time required to reduce the original number of bacteria by a given percentage) might be approximately expressed by the empirical law

$$\frac{1}{C_0 - C} \log \frac{Ct}{C_0 t_0} = \text{a constant},$$

where C is the concentration at time t.

Now it is at once evident that this last expression admits of no physical interpretation, whereas the two former ones may be deduced theoretically, and the meaning of all the constants in them is intelligible. Consequently it seemed probable that some other expression with a physical meaning might be found which would replace the empirical one, and by analogy with chemical reactions it was thought that a law of the form $c^n \,.\, t = \text{a constant}$, might hold, t being the time of disinfection for concentration c of disinfectant, and n being a constant, for it may

[1] This *Journal*, vol. VIII. p. 92, 1908.

https://doi.org/10.1017/S0022172400015928 Published online by Cambridge University Press

be shown that this is the relation when one molecule of one substance reacts with n molecules of a second, the latter being in great excess.

For purposes of calculation, this law may be written

$$n \,.\, \log c + \log t = \text{constant},$$

that is to say, the relation between $\log c$ and $\log t$, is a linear one.

The formula was applied to Miss Chick's results, making use of a graphical method in which $\log c$ was plotted against $\log t$. In every case the result was a straight line from the slope of which n could be calculated[1].

The following tables show the nature of the constant obtained when this value of n was substituted in the formula $n \,.\, \log \,.\, c + \log \,.\, t = \text{const.}$ The first two columns give the relative concentrations and the times of disinfection, and are taken from the most suitable experiments recorded in Miss Chick's paper. Also for the sake of comparison, the values of the constant K obtained by means of the empirical formula $\frac{1}{C_0 - C} \log \frac{Ct}{C_0 t_0}$ are given in the third column.

TABLE I.

Disinfection of B. paratyphosus by phenol. 20° C.

Parts phenol per 1000	Time taken for disinfection	'K'	$5{\cdot}5 \log c + \log t$
8	45 minutes	—	6·62
7·5	75	0·39	6·69
7	105	0·31	6·67
6·5	125	0·24	6·58
6	225	0·29	6·64
5·5	440	0·29	6·71
5	690	0·33	6·68

[1] It may perhaps be here mentioned that in cases of this kind, a graphical method is greatly superior to the usual methods of calculation, provided that the quantities plotted are so chosen that the resulting curve extends well across the paper, and, if possible, approximates to a straight line. Actually in the present case, the advantage is not very great, but in formulae such as $K = \frac{1}{t - t_0} \log \frac{n_0}{n}$, employed by Madsen and Nyman, *Zeitschr. f. Hygiene*, vol. LVII. p. 388, 1907, and H. Chick, this *Journal*, vol. VIII. p. 92, 1908, to express the reaction velocity of disinfection, the calculated value of K may lead to quite erroneous results, firstly because the values t and n may be inaccurate if taken from a single experimental value, as is usually the practice, and secondly, because the effect of a given experimental error on the value of K is greatly exaggerated when $t - t_0$ is small, while the same error when $t - t_0$ is great is almost inappreciable. The magnitude of these errors is, however, at once seen from a suitably drawn curve. Consequently, before making any deductions from values calculated by means of a formula, it is always advisable to examine the graphical solution to see if they are justified.

TABLE II.

Disinfection of Staph. pyogenes aureus by phenol. 20° C.

Parts phenol per 1000	Time taken for disinfection	'K'	5·5 log c + log t
14	4·5 minutes	—	6·95
12·5	2·5	—	6·42
10	25	0·15	6·90
8	95	0·16	6·94
7	186	0·20	6·92
6	395	0·20	6·88
4	1425	0·19	6·47

TABLE III.

Disinfection of Anthrax spores by mercuric chloride. 18° C.
(Krönig and Paul.)

Relative concentration of Hg^{++} ions	Time taken for disinfection	'K'	4·9 log c + log t
88·5	4·54 minutes	—	10·20
83·0	7·14	0·031	10·26
76·8	12·5	0·032	10·35
69·0	14·4	0·023	10·19
61·0	38·4	0·028	10·33

TABLE IV.

Disinfection of B. paratyphosus by mercuric chloride. 20° C.

Relative concentration of Hg^{++} ions	Time taken for disinfection	'K'	3·8 log c + log t
63	1·5 minutes	—	7·02
57·5	7	—	7·54
42·5	13	0·037	7·31
37	10	0·023	6·95
23	65	0·030	6·99
16·5	230	0·027	6·98

TABLE V.

Disinfection of B. paratyphosus by silver nitrate. 20° C.

Concentration of $AgNO_3$	Time taken for disinfection	'K'	0·86 log c + log t
5000	0·75 minutes	—	3·06
1000	1·5	0·10	2·76
500	2·5	0·11	2·70
100	6·5	0·15	2·53
50	22·5	0·10	2·81
10	56	0·16	2·61
5	140	0·14	2·75
1	>390	—	>2·59

https://doi.org/10.1017/S0022172400015928 Published online by Cambridge University Press

From these figures it will readily be seen that the constant obtained is as good as may be expected when the experimental difficulties are considered, and it may be quite well assumed that for practical purposes, the law $c^n t = \text{constant}$, is true, since it agrees within experimental error with results obtained by the best methods at present known.

It will be seen that in the above tables, n is assigned the following values: 5·5 for phenol, 3·8 for mercuric chloride, and 0·86 for silver nitrate. In all cases the bacteria disinfected are *B. paratyphosus*. The figure for disinfectant 'A' is probably about 8·5, but measurements with this substance are very difficult, and the present experimental data insufficient for more accurate deductions. These numbers are probably constant only at a constant temperature, and may vary with the bacteria disinfected, though the figures for phenol in the cases of *B. paratyphosus* and *Staph. pyogenes aureus* are the same. Information on this point would be interesting, but unfortunately is not at present available. However, from the results already obtained, it does seem possible to derive a little insight into the process of disinfection, and perhaps the following idea is not wholly unreasonable.

Each bacterium is composed of a number of molecular groups, and some or all of these contain a chemical compound which can react chemically with, say, N molecules of poison. When disinfection begins, this substance will start to react. Now, although it is capable of reacting with N molecules of poison altogether, it is evident that the substance, which we may denote briefly by the symbol X, will react initially with a smaller number of molecules and thereby form various compounds. Thus, after a short period of disinfection, there will exist in the bacterium a certain number of molecules of X which have not reacted at all, some molecules of X combined with one molecule of poison, some of X combined with two molecules of poison, and so on up to N. We must suppose however that when X has combined with more than a certain definite number of molecules of poison, it becomes incapable of performing its original function in the bacterium. Thus, for example, the compound $X + 3$ molecules of poison, may be able to play its part in the life processes of the bacterium, while the compound $X + 4$ molecules of poison may not; or perhaps the union of X with only one molecule of poison may be sufficient to destroy its power of reproduction (the criterion of vitality determined in the experiments). In any case, however, the result is the same. Disinfection will proceed gradually until a certain number of molecules, and hence of molecular groups, are, so to speak, incapacitated, and when this number reaches

some definite percentage of the total number existing in the bacterium, the whole bacterium will lose its vitality, or at any rate it will no longer grow. This occurrence enables us to measure the rate of the reaction, for the death of one bacterium indicates that a certain fixed proportion of the molecules of the substance X which it contains has reacted to a definite degree.

The question which now arises is—What is the actual meaning of n as measured above? It has already been mentioned that if the reaction under discussion were an ordinary complete chemical reaction, n would represent the number of molecules of one substance acting with one molecule of another, but the present case is rather different. For instance, suppose the bacterium ceases to grow when 70 % of the molecules of the substance X which it contains have each combined with at least two molecules of poison. When death occurs, there will be 30 % of molecules uncombined or in combination with only one molecule of poison, while 70 % will be combined with two or more molecules, and consequently the reaction is not completed at this stage, although it is the time at which n is measured. Consequently n does not in this case represent the total number of molecules of poison which can combine with one molecule of X, but the average number which have combined when the bacteria become incapable of further growth.

In this connection it is interesting to note the high value of n. The 'order of the reaction' is at least the integer next greater than $n + 1$, and so in the case of phenol we have a reaction of the seventh order at least. This result may at first sight seem rather extraordinary, especially when it is remembered that the ordinary chemical reactions which have so far been worked out, are rarely of an order greater than the third, but it must be remembered that in the case of bacterial proteins we are dealing with substances of exceedingly high molecular weight, and there seems to be no reason why such molecules should not react with a great many molecules of another less complex substance.

In the whole of the above it has been assumed that there is only one kind of active substance in the bacterium, but, of course, all that has been said applies equally well however many such substances exist, and unfortunately no clue is to be obtained by this method as to their number or nature.

Finally, a word may be said with regard to practical applications of the formula given above. These may perhaps be best illustrated by two examples.

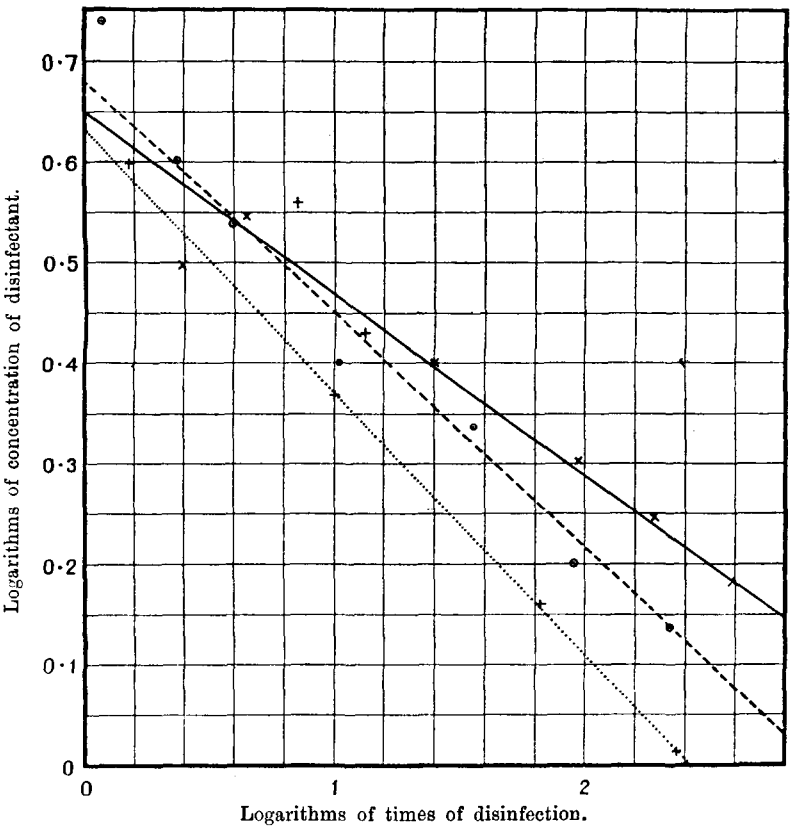
1. A solution of phenol containing 10 parts per 1000, disinfects a culture of *B. paratyphosus* in 25 minutes, another solution takes 35 minutes. What is the strength of the second solution?

Let the strength of the second solution be x.

For phenol $n = 5{\cdot}5$,

therefore, $5{\cdot}5 \log 10 + \log 25 = \text{const.} = 5{\cdot}5 \log x + \log 35$.

From this $x = 9{\cdot}4$.



The above figure shows graphically the results contained in Tables II, IV, and V, the corresponding points being denoted by the signs ×, + and 0 respectively. The straight line drawn in full represents the variations in the case of phenol, the dotted line those of mercuric chloride, and the broken line those of silver nitrate. In order to represent all three lines on the same diagram a constant quantity has been added to or subtracted from some of the values of the logarithms as deduced from the tables. This, of course, does not alter the slope of the lines. In the case of silver nitrate the vertical scale is only one fifth of that in the other two cases, and therefore the true slope is five times as steep as it appears to be.

https://doi.org/10.1017/S0022172400015928 Published online by Cambridge University Press

2. A disinfectant takes 9 minutes to disinfect a given culture when the concentration is 7 parts per 10,000 and 50 minutes when the concentration is 5 parts per 10,000. What concentration would disinfect in 15 minutes?

This is most simply solved graphically. Logarithms of the quantities given are taken and marked on a diagram where concentration is plotted against time. The two points thus obtained are joined by a straight line, and the point where this cuts the line representing time 15 minutes, shows the logarithm of the required concentration. The result thus obtained is 5·7.

Miss Chick for allowing me to use her results, and also for much detailed information concerning them.