AN INVESTIGATION OF THE LAWS OF DISINFECTION.

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Introduction.

The work of Pasteur, showing the action of preservatives to be due to their toxic effect upon micro-organisms, and the extension of the same explanation, by the work of Lister, to cover the case of disinfectants and disease germs, was followed by a considerable amount of successful work dealing with the disinfectant action of vast numbers of substances upon putrefying matter. (Buchholtz 1875 ; Jalan de la Croix 1881.)

Robert Koch (1886), however, is responsible for the first systematic experiments on disinfection, using pure cultures of bacteria. By means of his "thread" method he investigated the effect on anthrax spores of the then popular disinfectants, carbolic acid and sulphur dioxide, and of many substances previously uninvestigated. He confirmed previous work, demonstrating the powerful disinfectant, and even greater antiseptic, properties of mercuric chloride, and the great reputation of this salt may be said to date from these experiments.

Shortly afterwards much interest was excited by the appearance of the first of many emulsified disinfectants, creolin, and this substance was investigated by many contributors to the study of disinfection, *e.g.*, Esmarch (1887) working with putrefying liquids, and Henle (1889), who worked with cholera and typhoid. The latter also showed that creolin contained higher homologues of phenol (cresols, etc.) which, themselves only slightly soluble in water, are conveniently emulsified by the addition of soap; these higher phenols are at the same time more powerful disinfectants and less poisonous than carbolic acid. Fraenkel (1889) showed that the extra disinfecting power of crude over pure carbolic acid was due also to the admixture of higher phenols; he

investigated these compounds in pure samples and demonstrated their excellent disinfecting properties by means of experiments with anthrax spores.

Geppert (1889 and 1891) published experiments which showed that the disinfecting powers of mercuric chloride had been over-estimated, and criticised previous work and especially Koch's "thread" method, in view of the unavoidable carrying-over of traces of sublimate from the disinfecting fluid into the test cultures, growth being thus inhibited, although the organisms might not have been destroyed at the time of sub-culture. He proved this by adding to his test culture enough ammonium sulphide to precipitate the mercuric chloride carried over, and he worked directly with emulsions of bacteria in place of the silk threads soaked in broth cultures.

Behring (1890) published the results of a number of experiments upon disinfection and vigorously combated Geppert's views in respect of mercuric chloride, but Koch's "thread" method has since been largely abandoned for the above reason.

In 1897 Krönig and Paul published their classic work upon disinfection; their "garnet" method will be referred to later, and by its means they studied most carefully the disinfecting process, using definite species of organisms and disinfectants of every class in widely varying concentration. Their experiments were conducted with the utmost care, and the valuable quantitative results which they obtained form the most important contribution to the subject which has yet appeared.

The more recent work on disinfection has been concerned chiefly with the standardisation and comparison of different disinfectants from a practical point of view. Rideal and Walker (1903) described a method by means of which the value of disinfectants could be expressed as a numerical ratio, using pure phenol as a standard. Their method, with certain modifications, was used in the present investigation and was briefly as follows:—a definite small amount of broth cultures of the same species and age was added to a constant volume of disinfectant solution. By making a series of trials with varying concentrations of the disinfectant to be tested, and of pure phenol under otherwise similar conditions, they determined the relative concentrations of the two fluids required to bring germicidal action to completion in the same time. The ratio of the reciprocals of such concentrations was taken to express the disinfecting value of the unknown disinfectant in terms of carbolic acid as a standard. This ratio was called by them the 'carbolic acid coefficient.'

Madsen and Nyman (1907), using Krönig and Paul's method, have further investigated the progress of the reaction during the disinfection of anthrax spores by mercuric chloride and heat. Their experiments are in many ways parallel to my own and their results will be discussed later.

Three classes of disinfectants have been investigated in the present research.

(1) Dissolved organic disinfectant:—phenol.

(2) Emulsified disinfectants, containing insoluble coal tar derivatives held in very fine suspension in water by means of soap, glue, etc.:—disinfectant "A."

(3) Metallic salts:—mercuric chloride and silver nitrate.

Section I. Reaction Velocity of Disinfection.

Krönig and Paul (1897) published the results of a few careful experiments with anthrax spores, using mercuric chloride of different concentrations. Their method was as follows:—garnets were selected of equal size, carefully cleaned and dipped into an emulsion of sporing anthrax bacilli, which were allowed to dry on their surface in a thin film. The garnets were immersed in the solution of mercuric chloride, and from time to time a definite number was taken, and all traces of sublimate were removed by gentle washing and treatment with ammonium sulphide. These garnets were then well shaken in a measured quantity of water to detach the adherent spores and a constant amount of the washings was plated. The number of germinating organisms was counted. It was shown that by thus shaking in water a fairly constant definite proportion of the total organisms was detached.

From a study of the figures obtained in Krönig and Paul's experiments, it is evident that the disinfection process is a gradual one. No definite conclusions were drawn by the authors themselves, but Ikéda (1897) attempted to explain the course of the reaction and deduced from their results the following formula:—

$$\frac{n_1}{n_2} \times \frac{t_1}{t_2} = \text{constant},$$

where n_1 and n_2 are the numbers of organisms surviving after times t_1 and t_2 respectively.

On plotting the results of Krönig and Paul (see Fig. 1, ordinates representing the numbers of surviving bacteria, and abscissae the corresponding times), the points were found to lie upon fairly smooth

curves. The form of these curves suggested the existence of a logarithmic relation between the two variables, time on the one hand, and the number of surviving bacteria on the other. In fact the curves appeared to be very similar in form to that expressing the course of a so-called "unimolecular reaction," and the equation

$$-\frac{dC}{dt} = KC,$$

or

$$\frac{1}{t_2 - t_1} \log \frac{C_1}{C_2} = K$$

was found to be applicable to the case of disinfection, if, in place of the terms C_1 and C_2 expressing concentration of reacting substance, numbers of surviving bacteria were inserted, thus:—

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K,$$

where n_1 and n_2 are the numbers of bacteria surviving after times t_1 and t_2 respectively.

In their recent work Madsen and Nyman (1907) also discover from Krönig and Paul's figures that, in the disinfection of anthrax spores with mercuric chloride, the reaction proceeds according to the equation given above. A table[1] is given (p. 390) showing the good agreement between the numbers of surviving bacteria actually counted by Krönig and Paul, and those calculated by Madsen and Nyman on the supposition that the above relation holds good. Their own experiments with mercuric chloride and anthrax spores, using Krönig and Paul's methods, have fully confirmed the previous work and have shown that the same law holds good for the destruction of anthrax spores by heat.

It was necessary, first of all, to repeat[2] these enumeration experiments of Krönig and Paul, using a different germicide, and the following experiments were therefore made with anthrax spores, phenol being substituted for mercuric chloride. In order to obtain perfect comparison with further experiments upon a non-sporing organism (*Bacillus paratyphosus*), for which the garnet method was unsuitable, the experiments were made as follows:

An agar culture of anthrax, about a week old, consisting almost entirely of spores, was emulsified with a little distilled water and centrifugalised for a few

[1] This table will be referred to again later (Section II, p. 130).

[2] Some enumeration experiments of Spiro and Bruns (1898) with pyrocatechin and anthrax spores roughly confirm those of Krönig and Paul; they were, however, not made with any very great accuracy, but form an interesting confirmation.

minutes to get rid of any scraps of agar or aggregated masses of spores. The supernatant liquid was pipetted off and heated to 80° C. for about five minutes. This formed the emulsion used for the experiments ; it was preserved in the cold room and was found to suffer very little change.

Everything used in the experiments, tubes, pipettes, etc., was previously sterilised, and the disinfectant, in this case phenol (5 %), was measured into a test tube and placed in a water-bath whose temperature was maintained constant, in most experiments at 20° C. A small amount of the emulsion (depending upon the concentration of spores, determined by a previous control experiment) was added

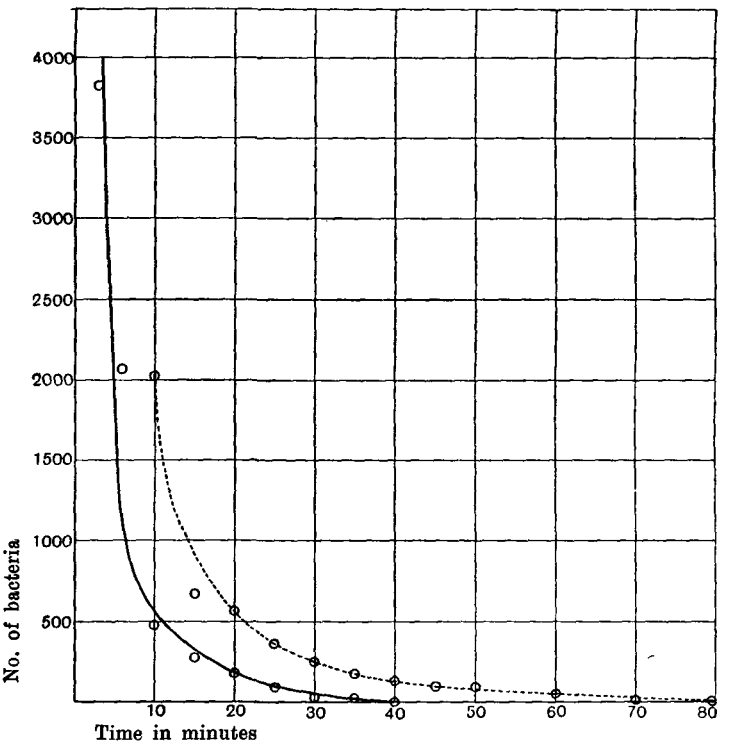

Fig. 1. Illustrating the results of Krönig and Paul's experiments. Continuous curve, disinfection of anthrax spores with 2·1 per 1000 $HgCl_2$. Dotted curve, disinfection of anthrax spores with 1·1 per 1000 $HgCl_2$.

to the disinfectant tube. At definite intervals of time samples were withdrawn, diluted, and immediately plated on agar. These samples were obtained by means of a capillary pipette fitted with a rubber cap and fixed by means of a cotton wool plug into the mouth of the disinfectant tube. These pipettes were specially made so that the external diameter at the mouth was constant in size ; it was then found that the size of the drop yielded was constant also, in the present instance = 0·02 c.c.

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A disadvantage of this method, compared with the "garnet" method, is that the trace of disinfectant adhering to the sample is incubated along with it. In the "garnet" method these traces are removed by washing before incubation. However, in the case of phenol, this drawback was found to be unimportant, owing to the great dilution the sample underwent. At the beginning of the experiments the samples were one drop (= 0·02 c.c.); later, when the number of survivors was very much reduced, they were increased to 4 or 6 drops. Although in the case of anthrax spores the disinfectant, phenol, was very concentrated (5 %), in the sub-culture plates the concentration was reduced to from 1 in 10,000 to 5 in 10,000, a concentration exerting no inhibition upon the development of anthrax spores [1].

TABLE I. (*Exp.* 15. 6. 07.)

Anthrax spores. 5 % phenol. 20·2° C.

Sample No.	Amount of sample	Time elapsing hours	Nos. of bacteria counted (6 plates)	Mean	Mean no. of bacteria present in 1 drop disinfecting mixture	K*, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$ $\left(K=\frac{1}{t_2-t_1}\log\frac{n_1}{n_2}\right)$
1	1 drop	0	441, 491, 489 365, 382	434	434, taken as initial value of $n\,(=n_1)$ in calculating values of K	
2	1	0·5	429, 340, 361 462, 443, 426	410	410	0·049
3	1	1·5	276, 398, 360 351, 341, 379	351	351	0·061
4	1	2·7	310, 322, 338 351, 287, 378	331	331	0·044
5	1	3·92	324, 280, 294 277, 289, 329	299	299	0·041
6	2 drops	5·95	528, 501, 441 495, 451	483	241	0·043
7	4	25·6	61, 77, 149 127, 135, 125	112	28	0·046

Mean value of K, 0·047.

* In Tables I—XV, values of K from expression $\frac{1}{t_2-t_1}\log\frac{n_1}{n_2}$ were calculated with Briggs' logarithms in place of natural logarithms.

In the case of anthrax it was found necessary to use as small an amount of agar as was convenient (*viz.* 8 *c.c.*), so that, after pouring, the film of agar in the petri dish formed as thin a layer as possible, and abundant oxygen was provided for the germination of the spores. The melted agar was poured at a temperature of about 65° C.; this was done to obtain perfect mixing and separation of the spores in the drop sample. For the same reason, the plates were previously warmed to about 40° C., and 0·5 to 1 *c.c.* sterile distilled water placed near the middle of each; the sample from the disinfectant tube was dropped into the water, and this instant was considered the exact moment of sampling. The sample was well stirred with a

[1] In the experiments with *B. paratyphosus* the concentration of disinfectant in the sub-cultures was quite insignificant, *e.g.*, phenol 1 in 200,000 to 1 in 50,000, according to the size of the sample taken. When mercuric chloride was employed it had to be neutralised.

platinum needle, the agar then poured, and the whole well mixed. The correct enumeration of anthrax spores presents some trouble owing to difficulty in separation, and very many failures resulted before the above exact procedure was evolved and adhered to.

Six plates were poured of each sample, they were incubated for 24—48 hours at 37° C., and the mean of the six enumerations taken as the result.

The results of two experiments are given in Tables I and II, the values of "*K*" are calculated, as before, from the mean values of the numbers counted on the plates; the constancy of the value is exceedingly well marked.

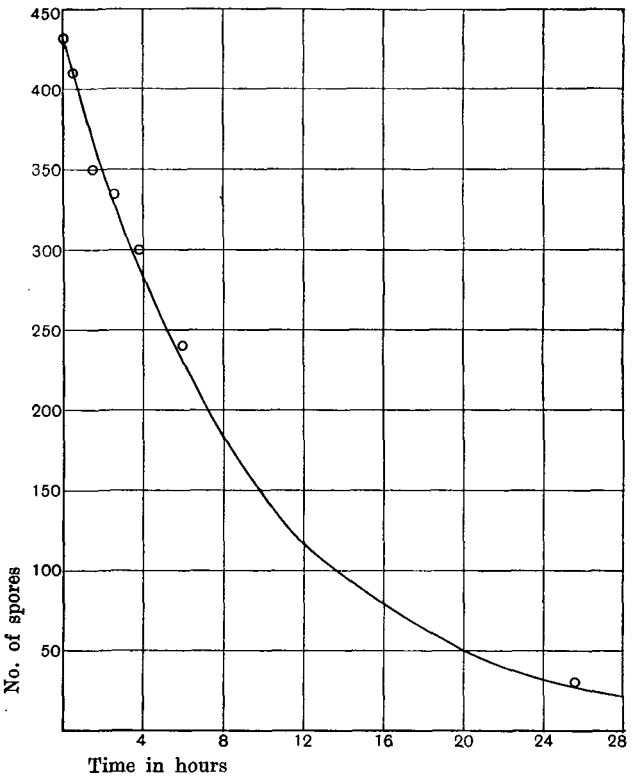

Fig. 2. (Exp. 15. 6. 07.) Showing course of disinfection of anthrax spores with 5% phenol at 20·2° C. (see Table I). The curve is drawn through a series of points found by calculation; the circles show the results of experimental determinations.

The close agreement of the results of these two experiments with the theory is also shown in Figures 2 and 3. In the experiment at 20·2° C. (Table I), the mean value of *K* was found to be 0·047. A curve

TABLE II. (*Exp.* 18. 6. 07.)

Anthrax spores. 5 % phenol. 33·3° C.

Sample No.	Amount of sample	Time elapsing hours	Nos. of bacteria counted, mean of 6 plates	Mean no. of bacteria present in 1 drop disinfecting mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$ $\left(K=\frac{1}{t_2-t_1}\log\frac{n_1}{n_2}\right)$
1	1 drop	0	439	439, taken as initial value of $n\,(=n_1)$ in calculating values of K	
2	1	0·5	275·5	275·5	0·40
3	1	1·25	137·5	137·5	0·40
4	1	2	46	46	0·49
5	2 drops	3	31·6	15·8	0·48
6	2	4·1	10·9	5·45	0·46
7	4	5	13·9	3·6	0·41
8	6	7	3	0·5	0·42

Mean value of K, 0·44.

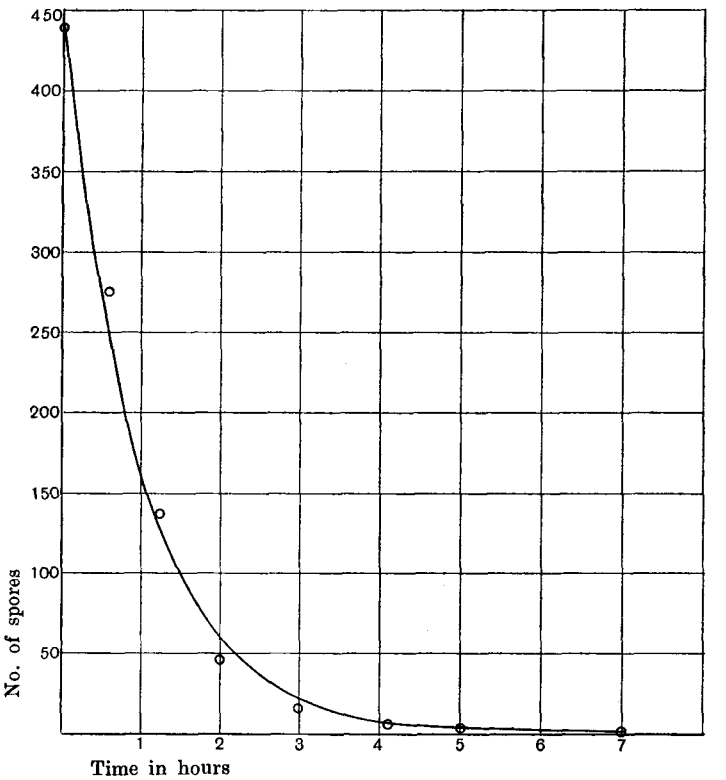

Fig. 3. (Exp. 18. 6. 07.) Showing course of disinfection of anthrax spores with 5 % phenol at 33·3° C. (see Table II). The curve is drawn through a series of calculated points, the circles show the results of experimental determinations.

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(Fig. 2) was then drawn through a series of points obtained by putting $K = 0{\cdot}047$ in the equation given on p. 95, thus:—

$$0{\cdot}047 = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2},$$

n_1, the initial number of organisms, being 434. The circles, Fig. 2, represent the points actually found by experiment. The curve in Fig. 3 was drawn in a similar way using the mean value of K obtained in the experiment at 33·3° C. There is an equally close agreement between the observed and calculated points in this instance also. One may therefore rely upon this method to give as consistent results as the "garnet" method.

In the case of anthrax spores the course of disinfection, either with mercuric chloride or phenol[1], proceeds in a perfectly orderly manner and can be represented by the equation:—

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K.$$

Experiments with B. paratyphosus.

To determine whether the law thus obtained for disinfection of anthrax spores with two different disinfectants was capable of wider application, experiments were made with a non-sporing organism. *B. paratyphosus* was chosen for most experiments because its recognition is at all times facilitated by its property of fermenting dulcite and glucose.

The disinfectants chosen were: (1) phenol, (2) disinfectant "A" (an emulsified preparation of higher coal tar derivatives), (3) mercuric chloride.

A 24 hours' broth culture was used, and in the earlier experiments this broth culture was used directly without dilution, and the samples from the disinfection tubes diluted before plating (Tables III, IV and XI).

[1] Phenol is a weak and uncertain germicide for spores and it was found necessary to use it as strong as possible, viz. 5 %; even then the reaction at 20° C. was not complete after 28 hours. At 33° C. the disinfection proceeded much more rapidly and was nearing completion after 7—8 hours. These results roughly confirm those of previous workers using widely different methods: Koch (1886) showed phenol to be an untrustworthy disinfectant for anthrax spores, Fraenkel (1889) found them to be killed only in 40 days by 5 % phenol, while Behring (1890) found that although at room temperature many days were necessary, at 37·5° C. disinfection was complete in 3 hours. These three workers all used the "thread" method. Krönig and Paul (1897), with the "garnet" method, showed the reaction with 5 % phenol to be incomplete in 24 hours.

Dilution tubes, containing a suitable amount of sterile water, were prepared, and small amounts (1, 2 or 5 drops) added to them from the disinfection tubes by means of the capillary pipettes. From the dilution tubes, small quantities measured with capillary pipettes were plated. At the beginning of an experiment dilution was necessarily large, later on it became possible to plate samples from the disinfection tube undiluted. This method, which is very troublesome in practice and needs many trial experiments before a successful one is obtained, has the additional drawback that, whereas the object of the experiment is to obtain a series of perfectly comparable results, hardly any two of them are obtained by means of an entirely comparable procedure. There appears to be a very considerable error introduced with dilution and the use of capillary pipettes, probably due to a tendency for the suspended bacteria to be attracted to the walls of the pipettes, etc., consequently the drops yielded may not be true samples. However, if the same sampling pipette is always used, and no subsequent dilution takes place, the results are found to be fairly comparable. Later on therefore, the method was changed, the broth culture was extensively diluted before use, and a comparatively small number of organisms was placed in the disinfection tubes, less than a thousand per drop. The sample drops, all removed with the same pipette, were plated undiluted, and the plates, poured from time to time, being perfectly comparable, the results presented much greater consistency.

As has been said before (p. 97), in the case of phenol and disinfectant "A," it was not found necessary to neutralise the trace of disinfectant carried over with the sample into the test cultures, as separate experiments showed the proportion there present to exercise no inhibition upon growth. The sample drop or drops, from disinfection tube or dilution tube, were dropped upon the plate, and melted agar, about 12 c.c. at a temperature not exceeding 45° C., was immediately poured upon the plate, and the whole well mixed. As a rule duplicate plates were poured at the same instant, and this necessitated the help of one or more assistants.

In the case of mercuric chloride, the trace carried over in the sample required precipitation (see p. 123), for mercuric chloride in exceedingly small amount can effectually inhibit subsequent growth even when the organisms may have retained their vitality at the moment of sampling. Accordingly the samples were neutralised with ammonium sulphide, of which a sufficient amount was added to the dilution tube, when diluted samples were plated, or directly placed

upon the plate when drops from the disinfection tube were plated undiluted. In the latter case the sample drop or drops were dropped directly into the ammonium sulphide solution and melted agar immediately poured into the plate and the whole well mixed. The results of these enumeration experiments with all three disinfectants are given in Tables III—X; phenol, Tables III—VII; disinfectant "A," Tables VIII and IX; mercuric chloride, Table X.

The values of K given by the equation

$$K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}$$

are also given, and it will be seen that the value of K does not remain constant as was the case with anthrax spores, but continuously decreases as the experiment proceeds. This continuous fall in the value of K occurred in the case of all three types of disinfectants worked with.

TABLE III. (*Exp.* 6. 2. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in diluted samples plated		Mean no. of bacteria present in 1 drop original disinfection mixture	Values of K, assuming reaction to be in accordance with the equation $(a) -\frac{dn}{dt} = Kn$	$(b) -\frac{dn}{dt} = Kn^{1\cdot5}$	$(c) -\frac{dn}{dt} = Kn^2$
·5	121	× 125	15,000, taken as initial value of n ($=n_1$) in calculating values of K			
1	110	× 50	5,500	—	—	—
2	238	× 25	5,950	—	—	—
3	41	× 25	1,025	0·27	0·009	0·00015
4	173	× 10	1,730	0·27	0·012	0·00024
5	86	× 10	860	0·26	0·012	0·00031
6	140	× 4	560	0·22	0·011	0·00038
7	131	× 4	524	0·22	0·013	0·00042
8	78	× 4	312	0·20	0·012	0·00024
9	76	× 4	304	0·18	0·011	0·00037
10	69	× 4	276	0·15	0·013	0·00056
15	25·5	× 4	102	0·12	0·011	0·00071
20	18	× 4	72	0·08	0·007	0·00048
30	—	—	71	0·06	0·007	0·00050

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TABLE IV. (*Exp.* 1. 2. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in diluted samples		Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming the reaction to be in accordance with the equation $(a) -\frac{dn}{dt} = Kn$	$(b) -\frac{dn}{dt} = Kn^{1\cdot5}$	$(c) -\frac{dn}{dt} = Kn^2$
1	54	× 50	2,700, taken as initial value of n $(=n_1)$ in calculating values of K			
2	12·5	× 50	625	0·63	0·042	0·0012
3	6	× 25	150	0·61	0·062	0·0031
4	5·5	× 25	137	0·42	0·044	0·0025
5	11·5	× 10	115	0·34	0·036	0·0021
7	5·5	× 10	55	0·25	0·038	0·017

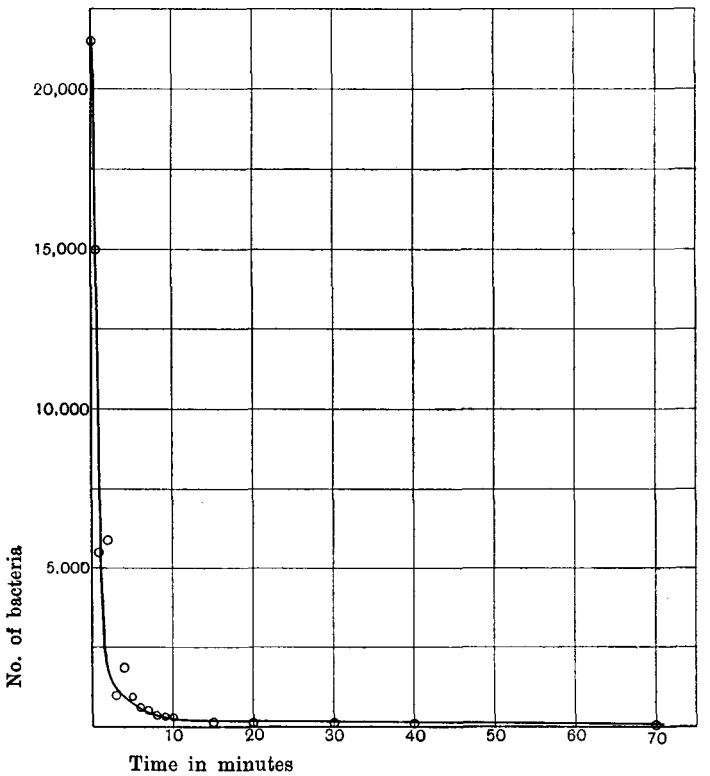

Fig. 4. (Exp. 6. 2. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table III).

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TABLE V. (*Exp.* 11. 7. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000.

	Time mins.	Amount of sample taken	Nos. counted on plates	Mean	Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$
(*a*) 20° C.	1	1 drop	569, 509	539	539, taken as initial value of n ($=n_1$) in calculating values of K	
	2	1	357, 247, 226	276·6	276·6	0·29
	3	1	151, 124	137·5	137·5	0·30
	4	2 drops	140, 167, 174	160·3	80·1	0·28
	6	3	139, 113	126	42	0·22
	8	5	83, 90, 93	88·8	17·8	0·21
	12	7	21, 14, 12	15·7	2·2	0·22
(*b*) 30° C.	1	1 drop	1252, 1480	1368	1368, taken as initial value of n	
	2	3 drops	473, 474, 509	485	162	0·93
	3	6	438, 352, 388	392·6	65·5	0·66
	4·1	6	117, 82, 73	90·6	15·1	0·63
	6	10	8, 13, 25	15·3	1·5	0·59

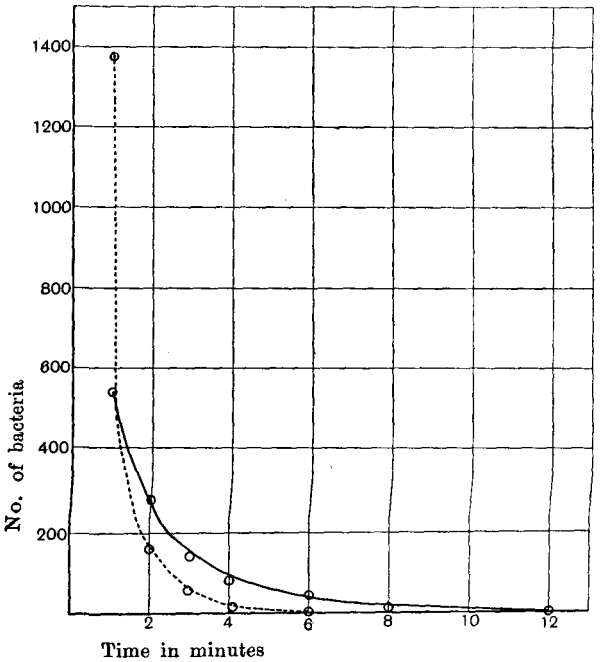

Fig. 5. (Exp. 11. 7. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 20° C., continuous curve: at 30° C., dotted curve (see Table V).

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TABLE VI. (*Exp.* 24. 7. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000.

		Time minutes	Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$
(*a*)	11° C.	0	823, taken as initial value of $n\,(=n_1)$ in calculating values of K	
		1·3	202	0·47
		2·3	80	0·44
		3·3	44·7	0·38
		5·3	14·3	0·33
		7·3	4·35	0·31
		10·3	1·0	0·28
		15·3	0·22	0·23
(*b*)	21° C.	0	974, taken as initial value of n	
		1·2	73	0·94
		2·2	16·3	0·81
		3·3	4	0·72
		4·3	0	—

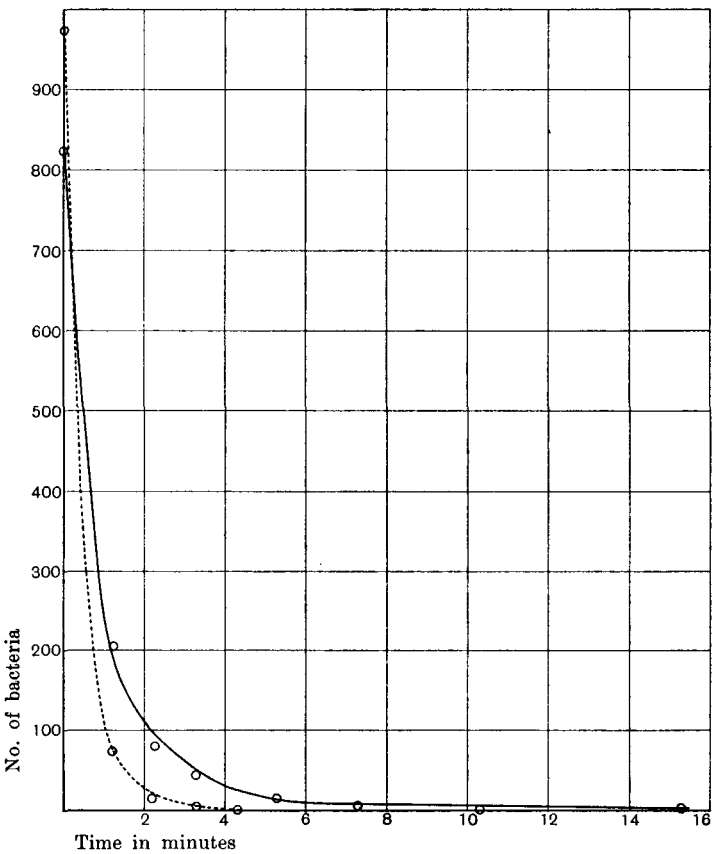

Fig. 6. (Exp. 24. 7. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 11° C., continuous curve: at 21° C., dotted curve (see Table VI).

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TABLE VII. (*Exp.* 23. 7. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000.

		Time minutes	Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$
(*a*)	11° C.	1	765, taken as initial value of n ($=n_1$) in calculating values of K	
		3	496	0·094
		5	271	0·112
		10	92·5	0·102
		20	5	0·062
		44	0·7	0·076
(*b*)	21° C.	1	484, taken as initial value of n	
		3	90	0·36
		5	28·5	0·31
		7	9·3	0·28
		10	3·5	0·24
		20·5	0·15	0·18

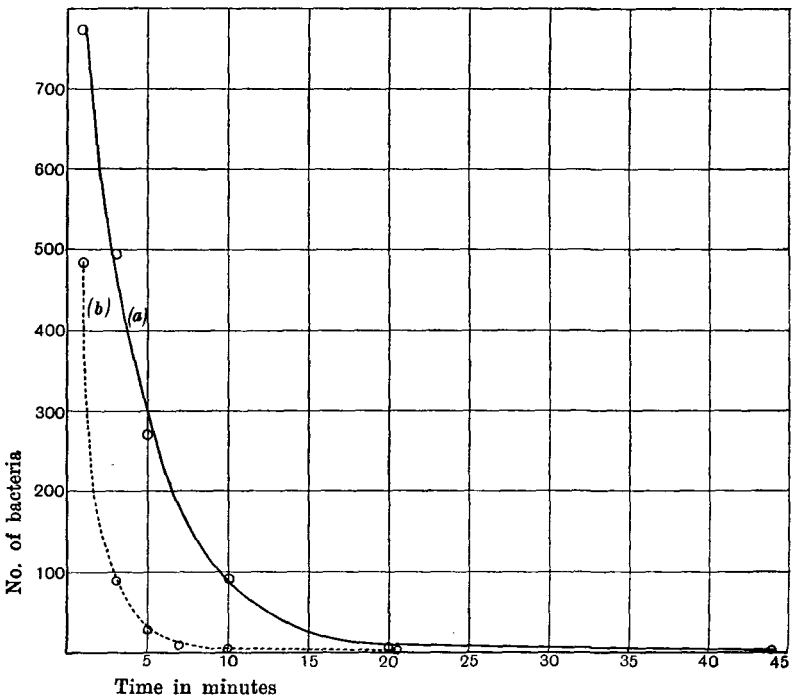

Fig. 7. (Exp. 23. 7. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 11° C., continuous curve: at 21° C., dotted curve (see Table VII).

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TABLE VIII. (*Exp.* 20. 3. 07.)

B. parat. Disinfectant "A," 5·5 per 10,000. 20° C.

Time minutes	Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equations $(a) -\frac{dn}{dt} = Kn$	$(b) -\frac{dn}{dt} = Kn^{1.5}$	$(c) -\frac{dn}{dt} = Kn^{1.7}$	$(d) -\frac{dn}{dt} = Kn^{2}$
0·5	370, taken as initial value of $n\,(=n_1)$ in calculating values of K				
1	85	1·28	0·34	0·081	0·018
2	38·8	0·65	0·15	0·059	0·016
3	15	0·56	0·16	0·077	0·026
4	11	0·44	0·14	0·070	0·025
5·5	8	0·33	0·12	0·061	0·024
7	4	0·30	0·14	0·074	0·038

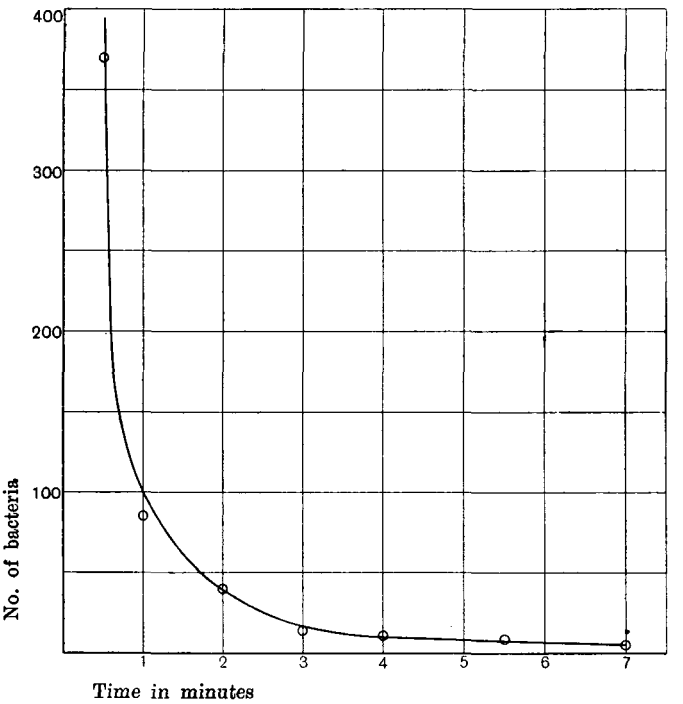

Fig. 8. (Exp. 20. 3. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with disinfectant "A," 5·5 per 10,000, at 20° C. (see Table VIII).

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TABLE IX. (*Exp.* 26. 3. 07.)

B. parat. Disinfectant "A," 5·5 per 10,000. 20° C.

Time minutes	No. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$
0	25,250, taken as initial value of $n\,(=n_1)$ in calculating values of K	
0·5	540	3·74
1	305	1·92
2·1	97	1·15
3·1	50	0·87
4·1	24	0·74
5·2	10	0·65
7	2	0·57

TABLE X. (*Exp.* 23. 3. 07.)

B. parat. Mercuric chloride, 2 per million. 20° C.

Time minutes	Mean no. of bacteria present in diluted sample		Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$
1·1	85	× 250	21,250, taken as initial value of $n\,(=n_1)$ in calculating values of K	
2·1	82	× 250	20,500	*
3	68	× 250	17,000	*
4	52	× 250	13,000	0·074
5	53	× 250	13,250	0·050
7	62	× 250	15,500	0·023
10	97	× 125	12,125	0·027
15	96	× 125	12,000	0·017
20	69	× 100	6,900	0·026
30	139	× 50	6,950	0·018
42	305	× 25	7,625	0·011
51	279	× 25	6,975	0·010
60	276	× 10	2,760	0·015
80	215	× 10	2,150	0·012
93	130	× 10	1,300	0·013
140	—	—	150	0·015
180	—	—	49	0·015
210	—	—	29	0·013
245	—	—	29	0·012

* The values of n at these times are not sufficiently accurate to give consistent values for K.

The following hypotheses are advanced to account for the facts recorded above, viz. that in the case of anthrax spores, disinfection

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proceeds in accordance with the equation $-\frac{dn}{dt} = Kn$, while in the case of *B. paratyphosus* this equation cannot be applied.

Let us assume that during disinfection the germicide operates upon the bacterium by so altering the constitution of its protoplasm (by chemical combination or otherwise), as to render it unfit for the continued vitality of the organism. The disinfectants, mercuric salts and phenols, used in this investigation form chemical compounds with proteins. It would not therefore be surprising that disinfection should conform to a law known to govern many chemical processes, and we may employ one of the well known interpretations as to why these reactions should be gradual and not sudden, viz., that at a particular time only a proportion of the molecules (of the bacteria in the case of disinfection) are temporarily in such a state as to permit of the combination[1]. With *B. paratyphosus* we must presuppose the existence of another factor in addition. In this case it would appear that certain individuals are permanently more sensitive to the reaction than others, or, in other words, possess less resistance to the disinfecting process. The less resistant organisms would then be killed in greater proportion than the more resistant during the earlier stages of the reaction, and the value of K would progressively diminish The nearer one could approach to uniformity of resistance, the more nearly constant would the value of K become (this being very nearly realised in the case of an emulsion of anthrax spores, especially after they have been subjected to a temperature of 80° C.).

In the case of a 24 hours' culture of *B. paratyphosus* permanently different resistances to disinfectants may conceivably be bound up with variation in age. In any collection of bacteria of differing resistance the velocity of disinfection will be proportional to the number of low resistant individuals present. This number, if age is the condition determining resistance, will again be a function of the total number present[2], and the course of the reaction will be expressed thus:—

$$-\frac{dn}{dt} = Kn \times f(n),$$

[1] This theory has received substantial support in the case of some radioactive substances, whose decay proceeds in accordance with the unimolecular law (see Rutherford, *Radio-activity*, pp. 182 and 338, 4th ed., 1904).

[2] The decrease in the value of K in the case of *B. paratyphosus* is a regular and orderly one. If values of K are plotted against numbers of surviving individuals, a continuous curve is obtained, showing that the value of K is altering in accordance with some law and bears some relation to the number of surviving bacteria.

instead of

$$-\frac{dn}{dt} = Kn \left(\text{or, on integration, } K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}\right),$$

which is the equation referring to the case where the individuals are of uniform resistance.

These hypotheses were confirmed by experiment.

If $f(n) = n^0$, the first equation becomes equal to the second one, and for this, as has been seen, the value of K in every case progressively decreased.

If $f(n) = n^1$, the equation becomes $-\frac{dn}{dt} = Kn^2$, and on integration we get $K = \frac{1}{t_2 - t_1}\left(\frac{1}{n_2} - \frac{1}{n_1}\right)$; applying this equation, the value of K was found in every case to progressively increase (see Tables III, VIII and XI).

Between the two values $f(n) = n^0$ and $f(n) = n$ a large series was tried, where $f(n)$ was placed equal to n raised to a series of powers between 0 and 1, *e.g.*, $n^{0\cdot2}$, $n^{0\cdot4}$, $n^{0\cdot5}$, $n^{0\cdot7}$, etc. In many cases the value of K was found to remain fairly constant when $f(n)$ was made equal to $n^{0\cdot5}$ (*e.g.*, Tables III, IV and XI). The differential equation then has the form

$$-\frac{dn}{dt} = Kn^{1\cdot5},$$

and on integrating

$$K = \frac{1}{0\cdot5} \cdot \frac{1}{t_2 - t_1}\left(\frac{1}{(n_2)^{0\cdot5}} - \frac{1}{(n_1)^{0\cdot5}}\right).$$

In some cases (*e.g.*, Table VIII), a constant value for K was obtained when $f(n) = n^{0\cdot7}$, *i.e.*

$$-\frac{dn}{dt} = Kn^{1\cdot7},$$

and

$$K = \frac{1}{0\cdot7} \cdot \frac{1}{t_2 - t_1}\left(\frac{1}{(n_2)^{0\cdot7}} - \frac{1}{(n_1)^{0\cdot7}}\right).$$

In some cases (Tables V and VII) the value of $f(n)$ lay between n^0 and $n^{0\cdot5}$; putting $f(n) = n^{0\cdot5}$ one obtained an ascending value for K.

The exact value of $f(n)$ was found to depend on details of the materials used, on the exact admixture of the different resistances in any collection of individuals. It depended upon the age of the culture and age of the seed material used for making the culture, and upon the exact period of the disinfection process which was under examination. The value, for example, may be different if K is calculated with a trustworthy control enumeration as n_1, or if the initial number for calculation is the amount surviving after some time has elapsed.

This reasoning was further tested as follows: if the above hypotheses are true, then if by any means a number of *B. paratyphosus* organisms of exactly the same age could be obtained, the truth should again be expressed by the original formula,

$$-\frac{dn}{dt} = Kn,$$

or

$$K = \frac{1}{t_2 - t_1} . \log \frac{n_1}{n_2}.$$

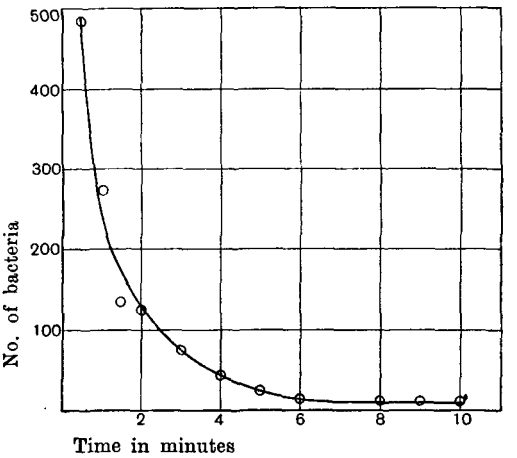

Fig. 9. (Exp. 6. 5. 07.) Disinfection of a 3 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table XI).

TABLE XI. (*Exp.* 6. 5. 07.)

B. parat. 3 hours' culture. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in 1 drop of disinfection mixture	Values of K, assuming reaction to be in accordance with the equations		
		(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1\cdot5}$	(c) $-\frac{dn}{dt} = Kn^2$
0	1000, taken as the initial value of n ($=n_1$) in calculating values of K			
0·5	484	0·62	0·056	0·0021
1	272	0·56	0·058	0·0026
1·5	136	0·58	0·072	0·0042
2	128	0·46	0·056	0·0034
3	72	0·38	0·058	0·0043
4	46	0·33	0·058	0·0052
5	24	0·32	0·078	0·0081
6	16	0·30	0·072	0·0085
8	13	0·23	0·062	0·0095
9	10·5	0·22	0·062	0·0105
10	11	0·20	0·054	0·0090

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This condition cannot be satisfied completely, but approximations can be attempted by using very young cultures. An experiment made with a 3 hours' culture (see Table XI) inoculated in the ordinary way,

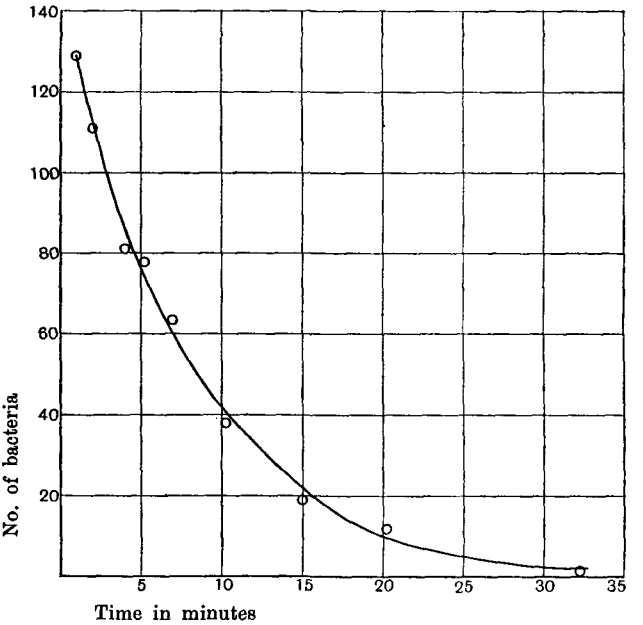

Fig. 10. (Exp. 30. 5. 07.) Disinfection of young individuals of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table XII).

TABLE XII. (*Exp.* 30. 5. 07.)

B. parat. 3rd generation of young cultures. Phenol, 6 per 1000. 20° C.

Time mins.	Amount of sample taken	Numbers counted on plates	Mean	Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equations (a) $-\frac{dn}{dt}=Kn$	(b) $-\frac{dn}{dt}=Kn^{1.5}$
1	1 drop	147, 142, 99	129	129, taken as initial value of n $(=n_1)$ in calculating values of K		
2	1	114, 106, 112	111	111	0·066	0·014
3	1	94, 99, 97	97	97	0·062	0·013
4	1	100, 91, 57	83	83	0·064	0·015
5·1	2 drops	157, 182, 132	157	78	0·053	0·012
7	3	186, 215, 177	193	64	0·051	0·012
10·1	5	177, 190, 196	188	38	0·058	0·016
15	5	102, 141, 48	97	19	0·059	0·020
20·3	10	145, 154, 55	118	11·8	0·054	0·021
32·3	10	55, 42, 15	37	3·7	0·049	0·028

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showed no essential difference from those made with 24 hours' cultures. In the next attempt the relatively large number of veterans added in the seed culture was avoided by passing the organism through several generations in broth, and sub-culturing with small amounts every three hours. It was thus possible to work with young cultures of the third and fourth generation.

The results of experiments with phenol are given in Tables XII—XVI, and it will be seen that the value of K given by the expression

$$K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2},$$

although still decreasing in value during the experiments, does not decrease in value nearly so rapidly as was the case with 24 hours' cultures. The alternative equation

$$K = \frac{1}{0{\cdot}5} \cdot \frac{1}{t_2 - t_1} \left(\frac{1}{(n_2)^{0{\cdot}5}} - \frac{1}{(n_1)^{0{\cdot}5}} \right), \text{ integral of } -\frac{dn}{dt} = Kn^{1{\cdot}5},$$

applicable in many of the latter instances gave values of K which increased in value as the experiment proceeded (see Tables XII, XIII, XIV and XV *a*). The experiment in Table XII is given in detail, in XIII, XIV and XV *a*, results only are stated.

It is therefore clear that the age of the bacterium is a factor in determining its resistance to the action of a disinfectant. In the experiments (Tables XII—XV) made with young individuals, the value of the velocity constant is comparatively small, and approximates to that obtained towards the end of the reaction in more mixed cultures (*e.g.*, compare Table XII, initial value of $K = 0{\cdot}066$, and falling to 0·049, with Table III, initial value of $K = 0{\cdot}27$, falling to 0·06: concentration of phenol in both experiments the same, 6 per 1000: temperature in both instances, 20° C.).

This shows that it is the younger individuals which possess the higher resistance for disinfectants.

TABLE XIII. (*Exp.* 7. 5. 07.)

B. parat. 2nd generation of young cultures. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in 1 drop of disinfecting liquid	Values of K, assuming reaction to be in accordance with the equations: (*a*) $-\frac{dn}{dt} = Kn$	(*b*) $-\frac{dn}{dt} = Kn^{1{\cdot}5}$
1	1125, taken as initial value of $n\,(=n_1)$ in calculating values of K		
2	418	0·43	0·038
3	212	0·36	0·038
5	47	0·34	0·058
7	20	0·29	0·064

TABLE XIV. (*Exp.* 23. 5. 07.)

B. parat. 3rd generation of young cultures. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria in 1 drop of disinfection mixture	K, assuming reaction to be in accordance with the equations: (a) $-\frac{dn}{dt}=Kn$	(b) $-\frac{dn}{dt}=Kn^{1\cdot5}$
1	166, taken as the initial value of $n(=n_1)$ in calculating values of K		
1·9	125	0·13	0·026
3·0	95	0·13	0·028
5·3	39	0·15	0·038
7·1	33	0·11	0·032
10	16	0·10	0·034
28	1·2	0·08	0·06

TABLE XV. (*Exp.* 27. 6. 07.)

B. parat. 4th generation of young cultures. Phenol, 6 per 1000. 20° C.

	Time minutes	Mean no. of bacteria present in 1 drop of disinfection mixture	K, assuming reaction to be in accordance with the equations: (a) $-\frac{dn}{dt}=Kn$	(b) $-\frac{dn}{dt}=Kn^{1\cdot5}$
(a) 20° C.	1	135, taken as initial value of $n(=n_1)$ in calculating values of K		
	3	93	0·081	0·018
	5	61	0·086	0·020
	7·5	33·6	0·093	0·026
	10·2	28·3	0·072	0·022
	12·3	25·2	0·065	0·020
	15	16·6	0·065	0·022
	20	10·3	0·059	0·024
	25·3	11·6	0·044	0·017
	30	8	0·042	0·022
	45	0·5	0·055	0·060

TABLE XV. (*Continued.*)

B. parat. 4th generation of young cultures. Phenol, 6 per 1000. 30° C.

	Time minutes	Mean no. of bacteria present in 1 drop of disinfection mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$
(b) 30° C.	1	341, taken as the initial value of $n(=n_1)$ in calculating values of K	
	2	166·5	0·31
	3	76	0·33
	5·4	8·4	0·38

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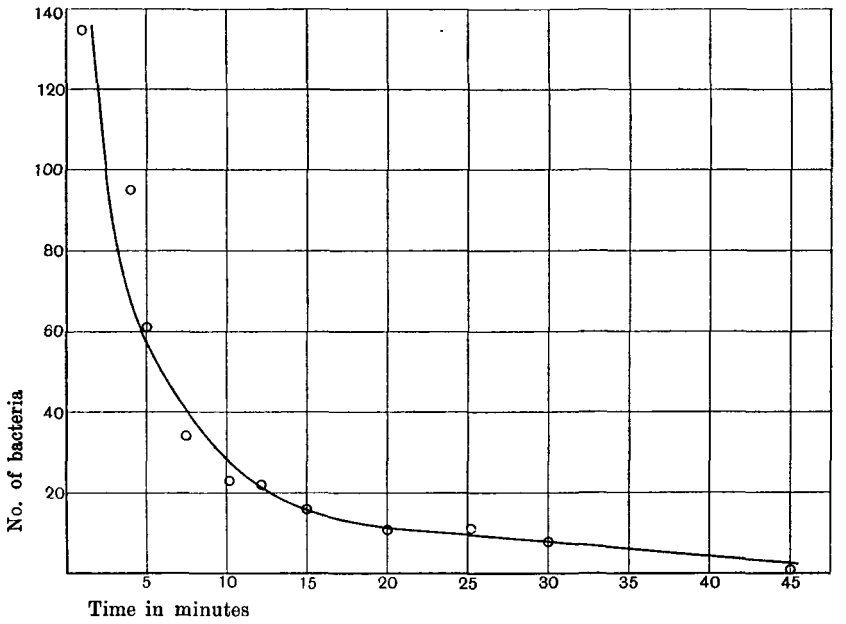

Fig. 11. (Exp. 27. 6. 07.) Disinfection of young individuals of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table XV *a*).

An ideal case of disinfection, such as that of anthrax spores, may therefore be supposed, with experimental support, to proceed in accordance with the equation $-\frac{dn}{dt} = Kn$, an equation exactly similar in form to that expressing the course of a unimolecular reaction, or reaction of the first order. This latter equation is deduced directly from the Law of Guldberg and Waage: that the velocity of any reaction is at any moment proportional to the active mass of reacting substance present at that moment. For the case of disinfection one may then formulate a similar law: that the velocity of disinfection at any instant is proportional to the number (or weight) of living bacteria present.

An interesting parallel with the case of disinfection is found in such a reaction as that of the inversion of cane sugar with a dilute acid. This reaction is obviously dimolecular, so that the velocity, at any instant, should be proportional to the product of the active mass of the two reagents concerned, sugar and water. Inversion of cane sugar is, however, ordinarily so arranged that one reacting substance, the water, is present in so great an excess that one may consider its concentration

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to remain constant during the course of the reaction, and the reaction velocity at any moment to be determined only by the active mass of the second reagent, the sugar, and hence to follow the law deduced for a unimolecular reaction. In a similar way in these experiments with disinfection the one reagent, the disinfectant, is present in an excess extremely great in comparison with the weight of bacterial substance present. The latter therefore governs the velocity of the process which in its nature shows an interesting analogy with that of a unimolecular reaction.

In the case of a non-sporing organism such as *B. paratyphosus*, experiments show that the reaction cannot be represented by this simple law, but that a permanent difference in resistance exists amongst individuals of different ages. The younger individuals possess greater resistance. When the conditions are so modified as to operate with individuals of approximately the same age the divergence largely disappears.

The gradual course of the disinfection process has been considered[1] to be completely explained by permanent variations of resistance in the bacteria employed, but it seems more probable that these differences in resistance are less significant than this, but sufficient to modify a process which would otherwise follow the above definite and constant logarithmic law.

Summary of Section I.

1. Disinfection is a process showing close analogy with a chemical reaction, the disinfectant representing one reagent, and the protoplasm of the bacterium the second.

2. It is a gradual process, without any sudden effects, and if the disinfectant is sufficiently dilute to admit of a reasonable time being taken for the process, the reaction velocity can be studied by enumerating the surviving bacteria at successive intervals of time.

3. In the case of disinfection of anthrax spores the reaction proceeds according to the well known equation for a unimolecular reaction embodying Guldberg and Waage's law. In this case "numbers of

[1] G. Bellei (1904), in the course of experiments on disinfection, repeatedly found that the greater the number of bacteria (*Staph. pyog. aureus*) disinfected, the longer was the time taken by the process. He explained the phenomena as due entirely to differences in resistance. One would rather explain it by considering it in the light of a reaction, where if more of the less abundant reagent be employed the time taken to complete the reaction will naturally be greater also.

surviving bacteria" are inserted in place of "concentration of reacting substance," *i.e.*, the reaction velocity at any moment is proportional to the number of organisms surviving at that moment,

$$-\frac{dn}{dt} = Kn. \quad \text{Values of } K\left(=\frac{1}{t_2 - t_1}\log\frac{n_1}{n_2}\right),$$

calculated from the results of experiments, remained constant during the whole time of disinfection. This was the case with values calculated from experiments of

(*a*) Krönig and Paul, working with mercuric chloride, and using the "garnet" method.

(*b*) Madsen and Nyman, working with mercuric chloride and heat, and using Krönig and Paul's method.

(*c*) The present work, using phenol as disinfectant, a simple emulsion of spores, and the method described in the present work.

4. The process, although really involving two "reagents," follows the law of a unimolecular reaction, because the second reagent, the disinfectant, is present in so great an excess, comparatively, that its concentration may be regarded as unaltered during the process. An interesting analogy is thus offered with the case of the inversion of sugar, which, in reality a dimolecular process, obeys the laws relating to a unimolecular reaction for a similar reason.

5. Experiments with cultures of *B. paratyphosus* show a departure from this simple law, the reaction velocity diminishing during disinfection more rapidly than is accounted for by the fall in number of the surviving bacteria. This was the case with each of the three types of disinfectant used.

This divergence is due to differences in resistance between individuals of the various ages contained in such cultures.

SECTION II. THE EFFECT OF VARYING THE CONCENTRATION OF A DISINFECTANT UPON ITS GERMICIDAL ACTION.

Perhaps the best method to employ in investigating the effect of varying concentrations of disinfectant would be the comparison of the velocity coefficients of a series of different concentrations, using exactly similar groups of bacteria. This method is, however, almost impossible, owing to the labour and length of time that would be occupied by any one set of experiments. Another difficulty is that the reaction velocity, in the case of vegetative forms of bacteria, is changing in value through-

out the course of the disinfection, so that the velocity coefficients for a particular part only of the process, would be comparable. Accordingly, the method was adopted of measuring the time taken for almost complete disinfection of a constant number of bacteria, this time being considered to be inversely proportional to the mean reaction velocity of the process. This method justified itself by the concordant results which were obtained and has the practical advantage of yielding information specially with regard to the more resistant organisms of the culture. Exactly comparable experiments with a series of different strengths of disinfectant were made. The observations were made simultaneously and the same culture was used.

The method of experiment, except for a few details, was very similar to that of Rideal and Walker. One important difference was the introduction, as unit of measurement, of the drop from the standard pipettes (see p. 96) and the employment of much larger samples in the test cultures (4 drops = 0·08 c.c.). The experiments, moreover, extended over all lengths of time, whereas those of Rideal and Walker were confined to concentrations giving disinfection within fifteen minutes.

The same three types of disinfectants were again investigated and the organism generally used was *B. paratyphosus*, though a few results were also obtained with *Staphylococcus pyogenes aureus*.

A culture of standard resistance was obtained as follows: stock cultures upon sloped agar were maintained at room temperature, and from these a standard loopful was inoculated into 6 c.c. of standard broth and incubated exactly 24 hours at 37° C. This culture was immediately cooled to about 10° C. and then used for experiment.

5 c.c. of disinfectant solution was always employed and to this was added from a standardised pipette 5 drops (0·085 c.c.) of the broth culture, or about 30,000,000 organisms. A series of tubes were prepared containing different concentrations of disinfectant. These were inoculated as nearly as possible at the same time and maintained in a thermostat at constant temperature, viz., 20° C.

At suitable intervals of time samples, 4 drops from the standard pipettes (0·08 c.c.), were withdrawn, added to tubes of dulcite or glucose broth and at once incubated at 37° C. If the sample had contained any living organisms, growth (as evidenced by acid and gas production in either medium in the case of *B. paratyphosus*, and acid production in glucose broth in case of *Staph. pyogenes aureus*) was usually apparent in 24 hours; the tubes were, however, always kept for four days.

The sample taken (·08 c.c.) represented about 1/60 of the total liquid present in the disinfection tube; a negative result in the test culture indicated therefore that about 30,000,000 bacteria had been reduced to less than 60 individuals. The times measured refer therefore to this practical end-point, which represents almost complete disinfection.

TABLE XVI.

Phenol. *B. paratyphosus.* 20° C. 5 drops (·085 c.c.) of a 24 hours' broth culture (about 30,000,000 bacteria) added to 5 c.c. disinfectant solution.

	Parts phenol per 1000 $= C$	Time taken for disinfection, *i.e.* reduction in number of about 30,000,000 individuals to less than 60 $= t$	Values of expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$ *, where initial concentration of phenol, $C_0 = 12$, initial time, $t_0 = 3{\cdot}25$
Exp. 21. 6. 06	12	3·25 minutes	—
	11	5·5	0·19
	10	8·5	0·17
	9	20·5	0·22
	8	68	0·28
	7	126	0·27
	6	240	0·26
	4·5	?19 hours	—
	4	?19	—
	3	75	0·28
Exp. 6. 6. 06	8	45 minutes	$C_0 = 8$; $t_0 = 45$
	7·5	75	0·39
	7	105	0·31
	6·5	125	0·24
	6	225	0·29
	5·5	440	0·29
	5	11·5 hours	0·33

* These and all subsequent values of the expression are calculated with Briggs' logarithms.

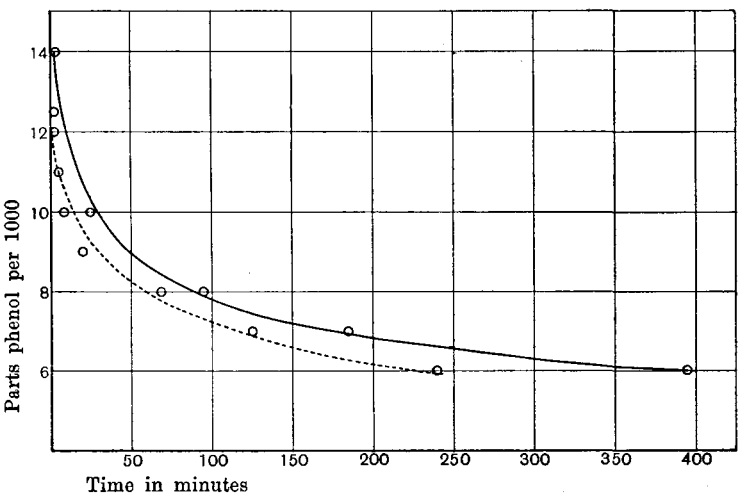

Fig. 12. Effect of varying the concentration of phenol upon the time taken for disinfection of (*a*) *B. paratyphosus*, dotted curve (Exp. 21. 6. 06, Table XVI): (*b*) *Staph. aureus*, continuous curve (Table XVII).

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(1) *Phenol.*

The results of experiments with phenol are given in Table XVI (*B. paratyphosus*) and Table XVII (*Staph. pyogenes aureus*) and shown graphically in Fig. 12. The disinfection times given in the tables are the means between the time of last positive and first negative sub-culture. In some cases, where skipping occurred, each end-point was separately determined and the result given is the mean of the separate determinations. In these and similar tables where more than one experiment is given they were always made at different times with other but quite similar cultures.

TABLE XVII.

Phenol. *Staphylococcus pyogenes aureus.* 20° C.

Phenol parts per 1000 $= C$	Time taken for disinfection $= t$	Values of expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial value of C, $C_0 = 14$, initial value of t, $t_0 = 4{\cdot}5$
14	4·5 minutes	—
12·5	2·5	—
10	25	0·15
8	95	0·16
7	186	0·20
6	395	0·20
4	23·7 hours	0·19

It is evident that within the range observed small variations in concentration of disinfectant caused large differences in the time of disinfection. The relation between these concentrations and the corresponding times when plotted (Fig. 12, ordinates = concentrations of disinfectant: abscissae = times of disinfection) make continuous curves of similar form in the case of either organism. The curves suggested a logarithmic relation and the following equation, arrived at with the kind assistance of Dr J. C. G. Ledingham, was found to apply with very fair accuracy:

$$(\text{a constant})^C = Ct,$$

or

$$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0} = \text{a constant},$$

where t_0 and t_n are times for disinfection corresponding to concentrations C_0 and C_n. This equation was obtained in an empirical manner; its marked symmetry, however, suggests that it also has some physical significance.

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The values of this expression calculated from the experiments were approximately constant (see Tables XVI and XVII). Considering the difficulty in accurately determining end-points in a process so gradual towards the end as disinfection was shown to be in Section I, the agreement with the above formula is maintained too well to be explained by mere coincidence.

TABLE XVIII.

Disinfectant "A." *B. paratyphosus.* 20° C.

	Parts "A" per 10,000 $= C$	Time taken for disinfection $= t$	Values of expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial value of C, $C_0 = 10$ and initial value of t, $t_0 = 1{\cdot}5$
Exp. 13. 9. 06	10	1·5 minutes	—
	8	2·5	—
	7	9	0·21
	6	12·5	0·17
	5	162	—
	2·5	more than 4 days	—
	1	,, ,, ,,	—
Exp. 18. 9. 06	5	44 minutes	—
	5 (simultaneous experiments)	210	—
	5 (simultaneous experiments)	75	—
	5 (simultaneous experiments)	105	—
Exp. 19. 9. 06	5·5	110 minutes	—
	5	205	—
	4·5	365	—
	4	500	—
	3	more than 5 days	—

TABLE XIX.

Disinfectant "A," another sample. *B. paratyphosus.* 20° C.

Parts "A" per 10,000 $= C$	Time taken for disinfection $= t$	Values of expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial value of C, $C_0 = 10$, initial value of t, $t_0 = 1{\cdot}25$
10	1·25 minutes	—
8	1·75	—
7	5	0·15
6	30	0·26
5	84	—

(2) *Disinfectant "A."*

In the case of disinfectant "A" (see Tables XVIII and XIX) there was only a small range of concentrations over which the corresponding

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times of disinfection could be conveniently measured, *e.g.*, a concentration of 10 in 10,000 disinfected in 1·5 minutes, while 2·5 in 10,000 disinfected in 4 days.

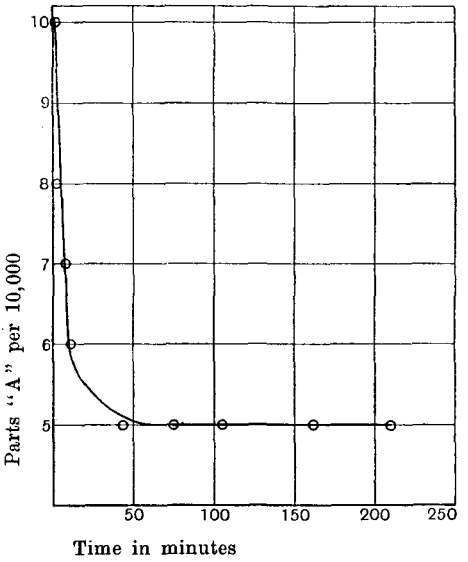

Fig. 13. (Exp. 13. 9. 06 and 18. 9. 06.) Effect of varying concentration of disinfectant "A" upon the time taken for disinfection of *B. paratyphosus* (Table XVIII).

The relation between concentration and time taken is different from that obtaining in the case of phenol. With disinfectant "A" (see Fig. 13) dilution has comparatively little effect upon the course of disinfection up to a certain point (*e.g.*, "A" 10 per 10,000 to "A" 5 per 10,000), then suddenly the curve becomes horizontal and the end-points of the reaction are in consequence difficult to determine accurately (see Table XVIII, Exp. 18. 9. 06). Two different samples of "A" were examined and both showed the same properties.

The number of determinations that can be made with any accuracy are necessarily limited, but if the portion of the curve between the two asymptotes be examined the values of the expression

$$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$$

remains fairly constant in value (Table XVIII, Exp. 13. 9. 06, and Table XIX).

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(3) *Metallic Salts* (mercuric chloride $HgCl_2$, and silver nitrate $AgNO_3$).

Salts of the heavy metals, even when present in a nutrient medium in extremely small amount, exert an inhibitory influence upon the growth of bacteria. This fact was observed by Koch (1881), Behring (1890), Geppert (1889 and 1891) and others. The extent of this inhibition may be gathered from the fact that in my own experiments with mercuric chloride a concentration of 1 in 1,000,000 in the culture medium was found to prevent growth when about 20 individuals (*B. paratyphosus*) were added; a concentration of silver nitrate of 2 in 1,000,000 prevented growth when about 40 individuals were added.

In the case of phenol and the disinfectant "A" this phenomenon of inhibition by low concentrations of the disinfectant was found to be negligible. Control experiments, which were undertaken to test this point, showed that in the case of the most concentrated solutions employed, no error was introduced by the carrying-over of the disinfectant in making the test culture.

In the case of mercuric chloride, or silver nitrate, however, it is essential that the metal be thrown out of solution at the moment of sampling, and, with all but the most dilute solutions, it was necessary to add a large excess of precipitant over and above the amount actually required for the reaction. This fact, noticed by Geppert (1889 and 1891) is well shown in Table XX, where the time taken for disinfection by a definite concentration of mercuric chloride is seen to vary in proportion to the excess of precipitant employed.

TABLE XX.

Effect of adding varying excess of ammonium sulphide to neutralise mercuric chloride on sampling.

Concentration of mercuric chloride in disinfection tube	Amount of AmHS necessary to neutralise $HgCl_2$ carried over	Excess AmHS added over and above * necessary amount	Apparent time taken for disinfection
1 in 1000	0·4 drop	1·00 drop	less than 7 minutes
1 in 1000	0·4	2·00 drops	25 minutes
1 in 10,000	0·04	0·96 drop	less than 5 minutes
1 in 10,000	0·04	1·96	29 minutes
1 in 10,000	0·04	2·96 drops	56 ,,
1 in 10,000	0·04	3·96	56 ,,

* An even number of drops was actually added in each case, the AmHS solution being suitably diluted when necessary.

In addition to the inhibitory action upon the growth of bacteria exercised by traces of metallic salts, there is another phenomenon

exhibited by this class of disinfectant. If bacteria are subjected to the action of 1 in 1000, 1 in 10,000, or even weaker solutions of mercuric chloride, there is an interval during which some at least of them may be resuscitated by the timely administration of an antidote (in this case a sulphide solution), but if this antidotal treatment is not employed, no amount of dilution beyond the limits where inhibition occurs can prevent the death of the organism. It would seem that the mercuric salt has been already absorbed by the bacterium and possibly formed some combination with its substance, not, however, to a sufficient extent to prevent recovery if a large excess of the sulphide solution be employed.

The following two experiments illustrate this phenomenon:

Experiment I. 20. 3. 07. (*a*) A 24 hours' broth culture of *B. paratyphosus* was so diluted that in one standard drop there were 20 organisms. One such drop was added to tubes containing 10 c.c. glucose broth together with varying small amounts of mercuric chloride; inhibition took place when concentration of mercuric chloride in the culture tube reached 1 in 1,000,000.

(*b*) 5 c.c. of 1 in 1000 mercuric chloride solution was placed in the thermostat at 20° C. and inoculated with about 16,000,000 organisms (5 drops of broth culture). The complete disinfection tested by means of sub-cultures precipitated with H_2S, was found to take at least 5 minutes. Between 0·5 and 1·25 minutes after the start, samples of 4 drops (·08 c.c.) were added to

(1) 900 c.c. glucose broth,

(2) 900 c.c. glucose broth, containing 0·15 c.c. H_2S solution.

In (2), 24 hours' incubation showed abundant formation of acid and gas; in (1) no growth was apparent after many days incubation.

Experiment II. (*a*) A drop from a diluted culture, containing 25 bacteria (*B. paratyphosus*), was added to 900 c.c. glucose broth to which 4 drops (·08 c.c.) $HgCl_2$ 1 in 1000 had previously been added. The result on 24 hours' incubation was abundant acid and gas formation. A similar experiment with 50 organisms gave the same result.

(*b*) About 16,000,000 bacteria of the same culture, *B. paratyphosus*, were added to 5 c.c. of 1 in 1000 mercuric chloride at 20° C. The disinfection was found to take 7·5 minutes. About one minute after the start 4 drops (·08 c.c.) were added from the disinfection tube to

(1) 900 c.c. glucose broth,

(2) 900 c.c. glucose broth, containing ·15 c.c. H_2S solution.

The result was just the same as that of Exp. I: (1) remained sterile, while in (2) acid and gas were apparent after 24 hours' incubation.

In these two experiments, the dilution of 0·08 c.c. mercuric chloride (1 in 1000) by 900 c.c. of broth produced a concentration of mercuric chloride in the broth of 1 in 11,000,000, a concentration which did not produce inhibition (Exps. I (*a*) and II (*a*)) even when very few bacteria

were present. After a sojourn of one minute in 1 in 1000 mercuric chloride, exactly similar conditions of sub-culture failed to reveal the existence of any living bacteria (Exps. I (*b* 1) and II (*b* 1)), although the sub-cultures received a comparatively large number of organisms, which could have recovered their vitality if sulphide had been administered as an antidote (Exps. I (*b* 2), II (*b* 2)).

A difficulty in using large excess of these sulphide precipitants is that they themselves are, to a certain degree, inhibitive to bacterial growth. The following plan was therefore adopted in every case, whatever the concentration of mercuric chloride. A constant amount of precipitant was used, viz., the amount shown by separate experiments (with very few, about twenty, organisms) to be the maximum that could be safely employed without fear of inhibition.

The two sulphides generally used were yellow ammonium sulphide and hydrogen sulphide water. Solutions of these sulphides are unstable, hence they were always titrated before use (AmHS with $HgCl_2$ solution, lead acetate paper being used as an indicator; H_2S water directly with standard iodine solution). Sodium and potassium sulphides were also tried, but proved unsuitable owing to the solubility of mercury sulphide in the excess necessarily employed. According to the precipitant used, whether yellow ammonium sulphide or hydrogen sulphide, the disinfecting power of mercuric chloride was found to be very different in amount, and it appeared a question whether the power of the precipitant to decompose the bacteria-mercury compound was being determined rather than the disinfecting power of the original mercuric chloride.

Tables XXI and XXII show the effect of varying the concentration of mercuric chloride upon the time taken for disinfection, when the precipitants used are yellow ammonium sulphide and hydrogen sulphide respectively, and it will be seen that the germicidal value of mercuric chloride is different in the two instances. Yellow ammonium sulphide (Table XXI), *in a certain condition*, would appear to be a more effectual antidote for sublimated bacteria than hydrogen sulphide (Table XXII). It is, however, very difficult to obtain ammonium sulphide in precisely the right condition and it is very unstable. Possibly a certain amount of polysulphide should be present so that the small amount of dissolved mercury sulphide may contain no Hg^{++} ions. On account of this uncertainty ammonium sulphide was abandoned and hydrogen sulphide adopted as a standard precipitant. A saturated solution in distilled water ($= \frac{1}{2}$ normal) was easily prepared and kept in small stoppered bottles in the dark. 0·15 c.c. of this solution to 10 c.c. glucose broth

was found to be a sufficient excess to precipitate any of the strengths of sublimate worked with and to exercise no inhibition (tested with 10—20 organisms) on its own account.

TABLE XXI.

Mercuric chloride. *B. paratyphosus.* 20° C. Precipitant, yellow ammonium sulphide.

	Parts $HgCl_2$ per 1000	Time taken for disinfection
Exp. 12. 12. 06	50	4·5 minutes
	10	17
	1	27
	0·1	>127
Exp. 14. 12. 06	10	17
	1	56

TABLE XXII.

Mercuric chloride. *B. paratyphosus.* 20° C. Precipitant, H_2S (0·15 c.c. saturated solution added to each sub-culture tube containing 10 c.c. glucose broth).

	Parts $HgCl_2$ per 1000	Time taken for disinfection $= t$	Nos. expressing concentration of Hg^{++} ions (Luther & Kahlenberg)	$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial concentration of Hg^{++} ions, $C_0 = 63$, initial time, $t_0 = 1·5$
Exp. 27. 2. 07	1	1·5 mins.	63	—
	0·5	7	57·5	*
	0·1	13	42·5	0·037
	0·05	10	†37	0·023
	0·01	65	†23	0·030
	0·005	230	†16·5	0·034
Exp. 2. 3. 07	1	2·5	63	$C_0 = 63$, initial time, $t_0 = 2·5$
	0·5	7	57·5	*
	0·1	9·5	42·5	0·020
	0·05	22	†37	0·027
	0·03	57	†33	0·036
	0·01	>150	—	—
	0·005	347	†16·5	0·034
	0·001	>450	—	—

* Numbers obtained were not concordant, probably due to errors in measuring short times of disinfection.

† Numbers obtained by extrapolation.

The examination of the results obtained (see Tables XXI and XXII) shows that when the method of the present research is employed, lower values are obtained for the germicidal value of $HgCl_2$ than those of Koch (1881), Behring (1890), and Geppert (1889). From Table

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XXI the astonishing fact is discovered that in a 24 hours' culture of *B. paratyphosus* some individuals at least are able to manifest vitality after contact with 5% mercuric chloride for 4 minutes, when ammonium sulphide is immediately applied as an antidote. When hydrogen sulphide is used, mercuric chloride is also seen (Table XXII) to have a considerably lower germicidal value than has usually been attributed

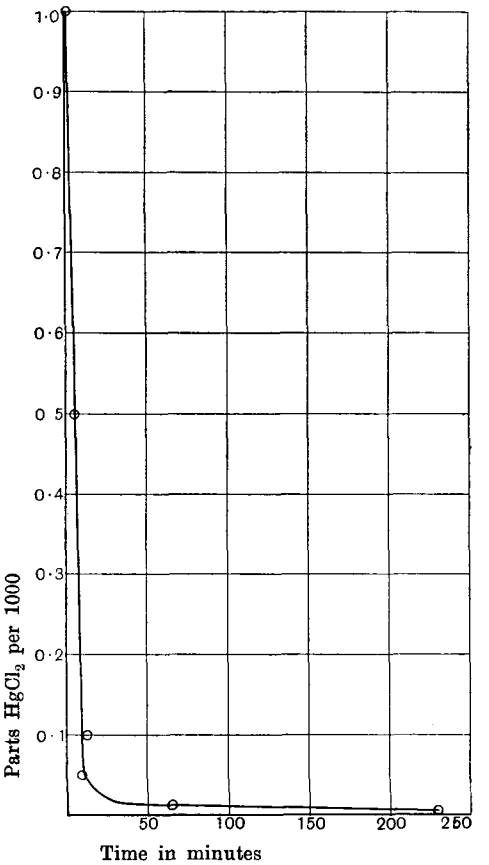

Fig. 14. (Exp. 27. 2. 07.) Times taken for disinfection of *B. paratyphosus* with varying concentrations of mercuric chloride, H_2S being used as precipitant (Table XXII).

to it. The difference in the results shown in Tables XXII and XXI is due to the different antidotal powers of hydrogen sulphide and the particular sample of ammonium sulphide employed. This again is doubtless due to different efficiency of the two reagents in decomposing a fairly stable compound formed by mercuric chloride and some con-

stituent of the bacterium. The value of corrosive sublimate as a practical disinfectant, however, remains unassailed.

The effect of altered concentration upon the time taken for disinfection when hydrogen sulphide is the precipitant is graphically shown in Fig. 14. The curve is very different in form to that obtained for phenol, and the formula $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0} = \text{constant}$, was found to be quite inapplicable. Confirming an idea originally put forward by Dreser (1893), Krönig and Paul (1897) and Paul and Prall (1907) have published experiments showing that in the case of mercury salts, the mercuric ion[1], rather than the salt, is the real disinfecting agent. These workers employed *Staphylococcus pyogenes aureus* and anthrax spores and showed: (*a*) Mercuric salts, when arranged in order either of their electrolytic dissociation or of their disinfecting value, form two similar series. (*b*) Any procedure which diminishes the dissociation of mercuric chloride, such as the addition of sodium chloride or the substitution of alcohol for water as solvent, also diminishes the disinfecting power.

It therefore seemed probable that the difference in the relation between concentration and reaction velocity in the case of phenol and mercuric chloride might disappear if the mercuric ions were regarded as the real disinfecting agent. I am indebted to Dr N. T. M. Wilsmore of University College for figures representing the concentration of Hg^{++} ions corresponding to various concentrations of mercuric chloride, obtained from the results of Luther (1904) and Kahlenberg (1901). As the determinations of the latter worker were made at 95° C. they were reduced to the required temperature. The combined results of both workers were then plotted and from the curve values were obtained corresponding to the concentrations of mercuric chloride here employed; unfortunately only extrapolation values were available for the lower concentrations. In Table XXII values of the expression $\frac{1}{t_n - t_0} \log \frac{C_n t_n}{C_0 t_0}$ are given, where numbers representing concentrations of mercuric ions are substituted for concentrations of mercuric chloride, and it will be seen that a fair constancy is maintained, showing that disinfection by mercuric chloride is not very unlike that by phenol when the real agent is taken into account.

[1] That H^+ ions are the real toxic agents in disinfection by the mineral acids appears probable from the experiments of Bial (1897 and 1902) and Winslow and Lockridge (1906).

Silver nitrate. The validity of the arguments given above can also be tested by investigating the relation between concentration and rate of disinfection in the case of such a metallic salt as silver nitrate. Here the electrolytic dissociation in dilute solution is so complete that the concentration of metallic ions will be in proportion to the concentration of the salt.

In working with silver nitrate the procedure had to be modified owing to precipitation of the silver by the trace of broth introduced with the bacteria. An emulsion of bacteria in distilled water was therefore substituted for the drops of broth culture and it was obtained as follows: from the stock culture a broth culture was inoculated with a standard loopful and incubated for 24 hours at 37° C.; from the broth culture a standard loopful was inoculated upon sloped agar tubes and also incubated for 24 hours at 37° C. This agar culture was emulsified with 2 c.c. of distilled water, filtered through muslin and centrifugalised for about 45 minutes. The water was removed, the deposit again emulsified with 2 c.c. distilled water, again filtered and shaken with glass beads. This emulsion when diluted fifteen times with distilled water was found to contain a concentration of bacteria about equal to that of the standard 24 hours' broth culture. Five standard drops were used for each experiment and found to contain 20—40 million bacteria.

The silver salt carried over in the test cultures was found to be most conveniently precipitated as sulphide; 0·15 saturated solution of H_2S in water was added to each tube of glucose broth containing 10 c.c.

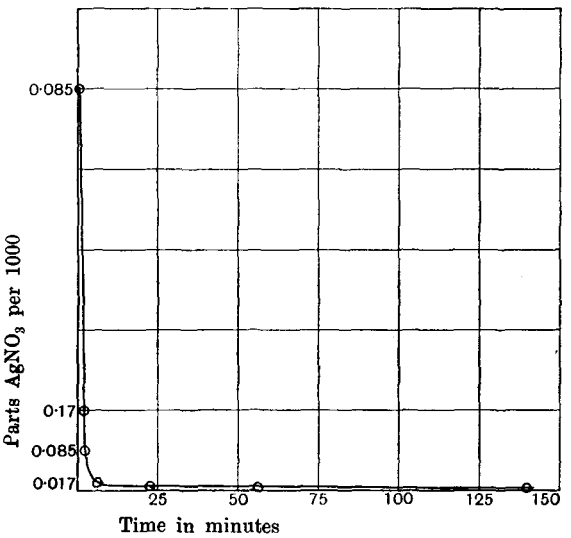

Fig. 15. (Exp. 24. 4. 07.) Times taken for disinfection of *B. paratyphosus* with varying concentrations of $AgNO_3$, H_2S being used as precipitant (Table XXIII).

The results are given in Table XXIII, where also are shown the values of the expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, and these show a very constant value. This constancy, in a case where the concentration of Ag^+ ions may be taken as approximately proportional to the concentration of the silver salt, is an interesting confirmation of the theory substantiated in the case of mercuric chloride, viz., that in cases of disinfection by metallic salts it is the metallic ion that is the real disinfecting agent.

TABLE XXIII.

Silver nitrate. *B. paratyphosus*. 20° C. About 30,000,000 washed bacteria added to 5 c.c. $AgNO_3$ solution.

	Concentration $AgNO_3$			
	Parts per 1000	Proportional nos. (0·17 per 1000 = 1) = C	Time taken for disinfection = t	$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$ *, where initial concentration, $C_0 = 5$, initial time, $t_0 = 0{\cdot}75$
Exp. 24. 4. 07	0·85	5	0·75 min.	—
	0·17	1	1·5	0·10
	0·085	0·5	2·5	0·11
	0·017	0·1	6·5	0·15
	0·0085	0·05	22·5	0·10
	0·0017	0·01	56	0·16
	0·00085	0·005	140	0·14
	0·00017	0·001	more than 6·5 hrs.	—
Exp. 29. 3. 07	0·85	5	0·75	$C_0 = 5$, $t_0 = 0{\cdot}75$
	0·017	0·1	6·5	0·15
	0·0085	0·05	21	0·11
	0·0017	0·01	69	0·15
	0·00085	0·005	122	0·16

* The values of the expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$ are here all negative in sign, the time taken for disinfection increasing so slowly with decreasing concentration of $AgNO_3$.

The above logarithmic formula was also found applicable to the case of disinfection of anthrax spores with mercuric chloride on further examination of the figures of Krönig and Paul (1897), already referred to. In their recent paper Madsen and Nyman (1907) give a table (p. 390), in which values of K, the velocity constant, are deduced for a series of Krönig and Paul's experiments. The velocity constant of any reaction being inversely proportional to the time taken for the completion of the whole or a definite fraction of the reaction, the above expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$ may be also written $\frac{1}{C_0 - C_n} \log \frac{C_n K_0}{C_0 K_n}$.

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In Table XXIV are given the values of K (from Madsen and Nyman) corresponding to different concentrations of mercuric chloride. Numbers expressing the corresponding concentrations of Hg^{++} ions were again deduced from the results of Luther and Kahlenberg; fortunately there was no need of extrapolation in this case. In column 4 are given values of the expression $\frac{1}{C_0 - C_n} \log \frac{C_n K_0}{C_0 K_n}$, numbers representing concentration of Hg^{++} ions being inserted for those of $HgCl_2$, and very good constancy is maintained.

TABLE XXIV.

Mercuric chloride. Anthrax spores. 18° C. Krönig and Paul's experiments.

Parts $HgCl_2$ per 1000	Value of velocity constant $K\left(=\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}\right)$ (Madsen & Nyman)	Nos. expressing concentration of Hg^{++} ions (Luther & Kahlenberg)	$\frac{1}{C_0 - C_n} \log \frac{C_n K_0}{C_0 K_n}$, where initial concentration of Hg^{++} ions, $C_0 = 88{\cdot}5$, initial value of K, $K_0 = 0{\cdot}22$
16·9	0·22	88·5	- -
8·4	0·14	83·0	0·031
4·2	0·08	76·8	0·032
2·1	0·07	69·0	0·023
1·1	0·026	61·0	0·028

As regards the effect of concentration of disinfectant upon disinfection velocity, there is no longer the close analogy shown in Section I to exist between disinfection and a chemical process involving two reagents which conforms to the unimolecular type, owing to the presence of one constituent in large excess. Whereas in the latter instance a simple proportionality exists between reaction velocity and concentration of reagent, the relation between velocity of disinfection and concentration of disinfectant was found to be a logarithmic one. The range of concentrations studied was necessarily limited to the cases where the corresponding times of disinfection could be measured with accuracy; many of these concentrations were approaching the limit of efficient action, where any chemical process involved is possibly reversible. It is conceivable that a simple proportion might still have been found to exist with higher concentrations; such an investigation is experimentally impossible, owing to the very short time that would be occupied by the reaction. Against this view, however, one may cite the experiments of Krönig and Paul, with anthrax spores and more concentrated solutions of mercuric chloride, which are discussed in the preceding paragraph.

Summary of Section II.

1. When phenol is used as a disinfectant a logarithmic relation exists between its concentration and the time taken for disinfection (the expression $\frac{1}{t_n - t_0} \log \frac{C_n t_n}{C_0 t_0}$ remaining constant in value). This was found to be true in the disinfection both of *B. paratyphosus* and of *Staphyl. pyogenes aureus.*

2. The same relation exists in the case of an emulsified disinfectant and *B. paratyphosus*, but only within narrow limits of concentration.

3. With silver nitrate and *B. paratyphosus* the above logarithmic relation holds good. In the case of mercuric chloride it is also true, if, in place of concentration of mercuric chloride, numbers are inserted representing concentration of Hg^{++} ions. This is shown in the case both of anthrax spores and *B. paratyphosus.* These facts lend further confirmation to the theory that, in the case of disinfection by metallic salts, the metallic ion is the real disinfecting agent.

4. The relation, expressed in 1, between velocity of disinfection and concentration of disinfectant, forms a marked contrast to the simple proportionality obtaining in the case of a chemical reaction of the unimolecular type, with which otherwise (see Section I) disinfection shows a close analogy.

5. Very small traces of salts of the heavy metals were found to prove inhibitive to bacterial growth. Bacteria which had been immersed in such solutions were therefore treated with sulphides when making test cultures. Large excess of sulphide was found indispensable, and, in the case of mercuric chloride, this is probably needed for the splitting up of some compound between the metal and the substance of the bacterium, which will prevent all further growth, however great dilution with culture medium be employed. If, however, a large excess of sulphide is administered subsequently as an antidote, the bacterium may recover its vitality.

Section III. The Influence of Temperature upon the Velocity of Disinfection.

Robert Koch (1881) showed that the disinfection of anthrax spores with carbolic acid vapour was much more quickly completed if the temperature were raised; Henle (1889) showed the same to be true in the case of disinfection of *B. typhosus* with both phenol and creolin, and

Behring (1890) published the results of a few similar experiments made by Hünermann with mercuric chloride. Heider (1892) was the first to make a series of systematic experiments upon the subject, but as the temperatures employed lay chiefly between 40° C. and 80° C., it is probable that a mixed effect was being investigated, and that some at least of the disinfection was due to heat alone. Madsen and Nyman (1907) find that the velocity of disinfection of anthrax spores by mercuric chloride increases with rise of temperature in accordance with the well-known equation of Arrhenius, the increase being about 2·5-fold for a rise in temperature of 10° C. between the temperatures 25° C. and 45° C.

With the exception of a few experiments with anthrax spores, the work about to be described was all done with *B. paratyphosus*. The method generally employed was similar to that used in Section II, viz., a comparison of the times taken for (almost) complete disinfection at the different temperatures in question. In some cases, however, a comparison of the velocity constants of disinfection at various temperatures was made. This involves series of enumeration experiments, which are exceedingly laborious, and by means of which one can compare the reaction velocities corresponding to, at most, two temperatures. Some of the enumeration experiments in Section I were made simultaneously at different temperatures, and can be made use of in this particular.

Anthrax spores. In Section I (Tables I and II), the velocity constant during disinfection of anthrax spores has been shown to remain constant in value throughout the whole reaction, hence the values found at any two temperatures are perfectly comparable. Two experiments were made with 5 % phenol at temperatures 20·2° C. and 33·3° C. respectively, and the velocity constant of disinfection at 20·2° C. (Table I) was ·047, and at 33·3° C. (Table II) 0·44. Assuming that the velocity of disinfection of anthrax spores with phenol increases in a regular manner with rise of temperature, we get a coefficient for a rise in temperature of 10° C. equal to 5·5, a higher figure than that obtained by Madsen and Nyman for disinfection with mercuric chloride. These workers used the "garnet" method of Krönig and Paul (1897), and obtained their coefficient also by comparison of velocity constants.

B. paratyphosus. A direct comparison of velocity constants in the case of *B. paratyphosus* is much complicated by the fact that, as was shown in Section I, the value of these constants decreases progressively during the course of the disinfection. It is possible, on the other hand, to compare the average velocities of disinfection at different temperatures

by determining the times taken for the same number of exactly similar bacteria to be reduced in number by exactly the same amount. This was done by plotting the results of enumeration experiments at two different temperatures, and drawing smoothed curves. Similar points on these curves were subsequently read off and compared. It was essential, for justifiable comparison, that the material disinfected should be exactly similar in either case. The individuals in cultures of *B. paratyphosus* were shown in Section I to possess different resistances to the action of disinfectants. It was therefore necessary that the experiments at different temperatures[1] should be made with the same initial number of bacteria from the same culture, *i.e.*, exactly the same combination of the different resistances.

Two experiments in Section I fulfil these conditions (see Tables VI and VII). The same results are tabulated again in Table XXV *a* and *b* to show the effect of temperature upon disinfection, the times given being obtained from the smoothed curves in Figs. 6 and 7 respectively. The velocity of disinfection of *B. paratyphosus* with 6 per 1000 phenol

TABLE XXV.

Phenol, 6 per 1000. *B. paratyphosus*. 11° C. and 21° C.

No. of bacteria present in one standard drop of disinfection mixture	Time elapsing at 21° C. minutes	Time elapsing at 11° C. minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 24. 7. 07. (*a*).	(See Table VI and Fig. 6.)		
900	0	0	—
100	1·0	2·2	2·2
50	1·6	3·3	2·1
10	2·7	6·2	2·3
		Mean	2·2
Exp. 23. 7. 07. (*b*).	(See Table VII and Fig. 7.)		
*	0	0	—
300	1·2	5	4·2
200	1·75	6·5	3·9
100	3	9·5	3·2
10	7·5	20	2·7
		Mean	3·3

* The initial number of organisms was not determined but was the same for experiments at either temperature.

[1] With an organism as sensitive as *B. paratyphosus* to temperatures above 45° C., the range over which experiments can be made is very limited. At temperatures above 20° C., even with dilute disinfectants, enumeration experiments have to be made with uncomfortable rapidity.

is shown to be increased in the one experiment 2·2 times, in the other 3·3 times, when the temperature is raised from 11° C. to 21° C.

The majority of the experiments were, however, made with the "end-point" method, described in Section II (p. 118), by means of which a comparatively large number of experiments at different temperatures could be made simultaneously.

A series of water baths was maintained at different convenient temperatures, tubes containing 5 c.c. disinfectant solution were placed in them, and inoculated in the usual way with five standard drops of a 24 hours' culture. The disinfection tubes were sampled from time to time, exactly as described in Section II, by withdrawing four standard drops (0·08 c.c.) and adding them to glucose or dulcite broth; the end-points thus determined indicated a reduction of the total number of bacteria to less than 60.

To ascertain whether this less laborious "end-point" method could be substituted for that of comparing the velocity constants at different temperatures, simultaneous comparative experiments were made with exactly similar groups of bacteria. In the one case a comparison was made between the times elapsing at two temperatures before the total number of bacteria was reduced to less than 60, in the other the average reaction velocities at the same two temperatures were compared.

An "end-point" determination was made with a similar culture and similar number of organisms to those of Exp. *a*, Table XXV; unfortunately it was not made simultaneously. Phenol, 6 per 1000, was used, and at a temperature of 21° C. the total number of bacteria was reduced to less than 60 organisms in 6·75 minutes, being the mean of three concordant determinations; at 11° C. the time was 12·5 minutes, the same result being given by three experiments. The coefficient of increase in disinfection velocity for a rise of 10° C. is therefore 1·9. The figure obtained from the enumeration experiment was 2·2 (Table XXV, Exp. *a*).

To test this point further, experiments (Table XXVI) were made employing the two different methods simultaneously upon the same number of bacteria from the same culture. Some difficulty was experienced with the enumeration experiments at 21° C., and only one or two very rapid enumerations were possible before the number of surviving bacteria became too small to be counted with accuracy. The numbers inserted in Table XXVI, 3rd column, are those actually counted at 21° C. The times taken at 11° C., for a corresponding reduction in numbers, were derived from curves drawn through the points obtained by experiment. Figs. 17 *a* and 18 show the course of the latter part

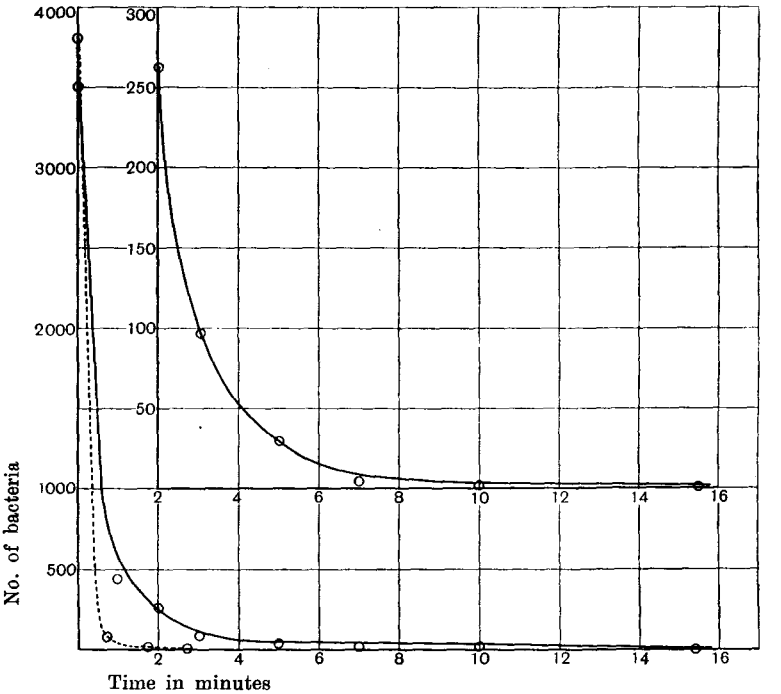

Fig. 17. (Exp. 24. 8. 07.) Disinfection of *B. paratyphosus* with phenol, 8 per 1000, at 11° C., continuous curve: at 21° C., dotted curve.

Fig. 16*a*. (Exp. 24. 8. 07.) Showing course of the latter part of disinfection of *B. paratyphosus* with phenol, 8 per 1000, at 11° C. (Table XXVI, Exp. (*a*)).

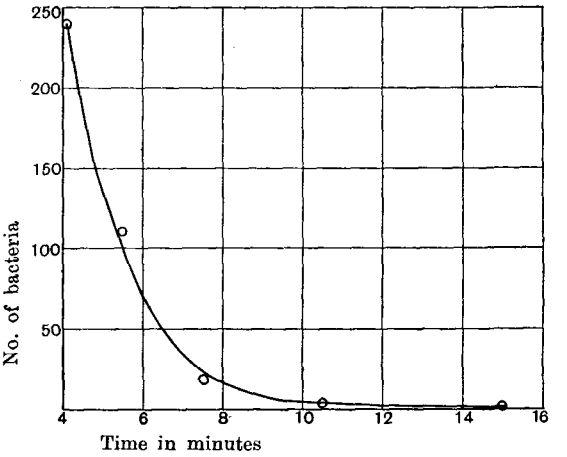

Fig. 18. (Exp. 27. 8. 07.) Showing course of the latter part of disinfection of *B. paratyphosus* with phenol, 8 per 1000, at 11° C. (Table XXVI, Exp. (*b*)).

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of the disinfection at 11° C. in the case of Exps. (*a*) and (*b*) respectively, and were used to obtain the times given in Table XXVI, column 4. From this table it will be seen that the numbers obtained by means of the two methods showed close agreement, so that the subsequent employment of the "end-point" method was justified.

TABLE XXVI.

Phenol, 8 per 1000. *B. paratyphosus*. 11° C. and 21° C.

Method of experiment employed	Total no. of bacteria	No. of bacteria present in one standard drop of disinfection tube	Time elapsing at 21° C. minutes	Time elapsing at 11° C. minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 24. 8. 07. (*a*).					
	950,000	3,650	0	0	—
"End-point"	60	—	6·5	24·5	3·8
"Enumeration"	—	79	*0·75	†3·35	4·4
Exp. 27. 8. 07. (*b*).					
	3,000,000	13,000	0	0	—
"End-point"	60	—	13·5	41	3·0
"Enumeration"	—	66	*1·75	†6·1	3·5 } 3·05
,,	—	11·4	*3·25	†8·5	2·6 }

* Times of direct enumeration experiments.

† Times obtained from drawn curves, Figs. 17*a* and 18.

Experiments with B. paratyphosus, illustrating the effect of temperature upon reaction-velocity, in which the "end-point" method was employed.

In these experiments a much larger number of organisms was employed than were used in those in which a direct enumeration was made. The material disinfected was a 24 hours' culture. The increase in the number of organisms produced, when working with phenol, an unexpected rise in the value of the temperature coefficient. This variability according to the number of organisms used was found to be consistent, and the interpretation will be discussed later (p. 148).

1. *Metallic Salts.*

Mercuric chloride. The experiments were carried out exactly as those in Section II, with 20—40 million bacteria from a 24 hours' culture of *B. paratyphosus*. The precipitant used was sulphuretted hydrogen water, 0·15 c.c. of a saturated solution being added to each sub-culture tube containing 10 c.c. glucose broth.

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TABLE XXVII.

Mercuric chloride, 1 in 1000. *B. paratyphosus.*

	Temperature degrees centigrade	Time taken for disinfection	Values of A $\left(=\frac{T_0 T_n}{T_0 - T_n}\log\frac{t_2}{t_1}\right)$*, where initial absolute temperature $T_0 = 293$, and initial time $t_0 = 2{\cdot}5$
Exp. 9. 3. 07	42	$< 0{\cdot}5$ min.	—
	29·7	$< 0{\cdot}5$	—
	20	2·5	—
	12·6	4·5	2890
	7·9	6	2590
	0	41·5	4880
			Mean = 3420
			Initial temp. $T_0 = 293$, ,, time $t_0 = 1{\cdot}5$
Exp. 12. 3. 07	26·8	1·5	—
	20	1·5	—
	13·2	3·5	4540
	7·2	11·5	5670
			Mean = 5100

* These, and all subsequent values of this expression, are calculated with Briggs' logarithms.

TABLE XXVIII.

Mercuric chloride, 1 in 10,000. *B. paratyphosus.*

	Temperature degrees centigrade	Time taken for disinfection	Values of A $\left(=\frac{T_0 T_n}{T_0 - T_n}\log\frac{t_n}{t_0}\right)$, where initial absolute temperature $T_0 = 314{\cdot}2$, initial time $t_0 = 1{\cdot}5$
Exp. 14. 3. 07	41·2	1·5 min.	—
	29·9	2·5	*
	19·8	21	4930
	11·1	65	4850
	6·7	41	3690
	0	124	3990
			Mean = 4360
			$T_0 = 314{\cdot}6$, $t_0 = 0{\cdot}75$
Exp. 6. 3. 07	41·6	0·75	—
	30·7	2·5	4580
	19·8	11·5	5010
	13·9	36	5480
	6·8	50	4610
	0	101	4390
			Mean = 4810

* Figure is not concordant, probably due to inaccuracy in measuring the short intervals of time.

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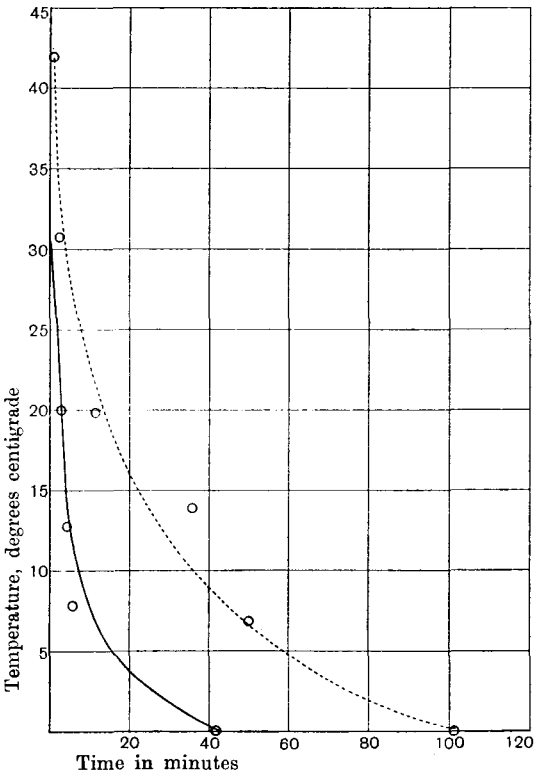

Fig. 19. Influence of temperature upon the time taken for disinfection of *B. paratyphosus* with mercuric chloride. Continuous curve, $HgCl_2$ 1 in 1000 (Table XXVII, Exp. 9. 3. 07): dotted curve, $HgCl_2$ 0·1 in 1000 (Table XXVIII, Exp. 6. 3. 07).

TABLE XXIX.

Mercuric chloride. *B. paratyphosus.*

	Concentration of mercuric chloride parts per 1000	Temperature degrees centigrade	Time taken for disinfection (derived from curve in Fig. 19) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 9. 3. 07, Fig. 19	1·0	25	1·2	
		20	2·7	3·3
		15	4	2·6
		10	7	4·0
		5	16	5·8
		0	41	
				Mean value = 3·9
Exp. 6. 3. 07, Fig. 19	0·1	40	1·2	
		30	4·0	3·3
		20	13·5	3·4
		10	38	2·8
		0	102	2·7
				Mean value = 3·0

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The results of two experiments with $HgCl_2$ 1 in 1000 are given in Table XXVII, and of two experiments with $HgCl_2$ 1 in 10,000 in Table XXVIII. Fig. 19 (abscissae = time taken for disinfection: ordinates = temperature of disinfection) shows the results of one set of experiments with either concentration, and the points found by experiment are seen to lie on or near to continuous curves, the velocity of disinfection increasing with rise of temperature in a very orderly manner (see Table XXIX).

Silver nitrate. An emulsion of washed bacteria was used as material to be disinfected in place of the usual drops from the broth culture, and dilutions were so arranged (see p. 129) that each experiment dealt with the disinfection of about 20—40 million bacteria.

The results of experiments with two concentrations of silver nitrate are given in Table XXX, and shown graphically in Fig. 20.

TABLE XXX.

Silver nitrate. *B. paratyphosus.*

	Concentration $AgNO_3$ parts per 1000	Temperature degrees centigrade	Time taken for disinfection	Values of A $\left(=\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}\right)$, where initial absolute temperature $T_0 = 313{\cdot}2$ and initial time $t_0 = 0{\cdot}67$
Exp. 25. 4. 07	0·017	40·2	0·67 min.	—
		30·7	2·6	5900
		20	13·5	5930
		12·25	17·5	4530
				Mean = 5450
				$T_0 = 314{\cdot}1$, $t_0 = 4{\cdot}5$
Exp. 21. 6. 07	0·0017	41·1	4·5	—
		33·3	35	*
		20·1	72·5	5280
		16·3	167·5	5740
		1·8	329	4090
				Mean = 5040

* Figure obtained was not concordant.

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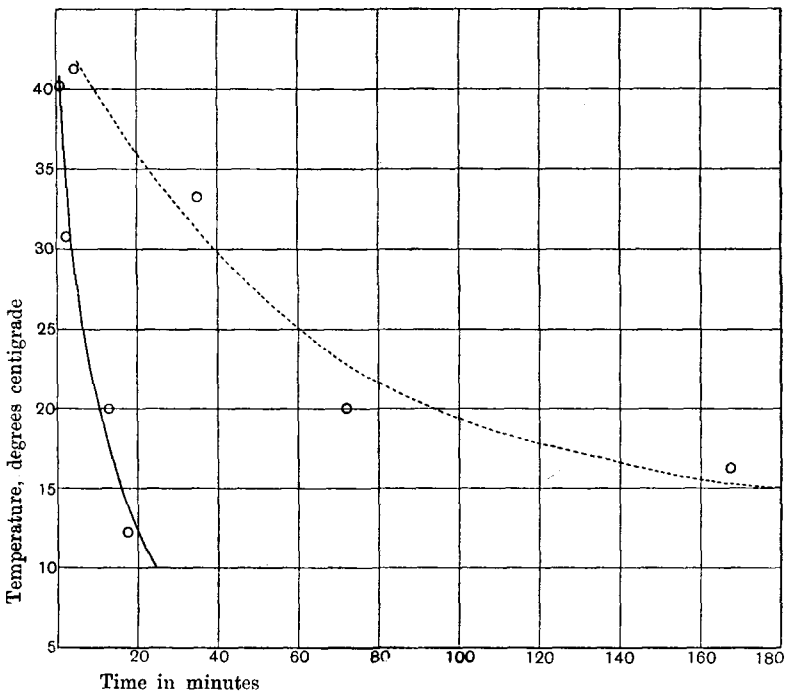

Fig. 20. Influence of temperature upon the time taken for disinfection of *B. paratyphosus* with silver nitrate. Continuous curve, $AgNO_3$ 0·017 per 1000 (Table XXX, Exp. 25. 4. 07): dotted curve, $AgNO_3$ 0·0017 per 1000 (Table XXX, Exp. 21. 6. 07).

TABLE XXXI.

Silver nitrate. *B. paratyphosus.*

	Concentration of $AgNO_3$ parts per 1000	Temperature degrees centigrade	Time taken for disinfection (derived from curves in Fig. 20) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 25. 4. 07, Fig. 20	·017	40	1·2	
		30	4	3·3
		20	10·7	2·9
		10	24·5	2·3
				Mean value = 2·8
Exp. 26. 1. 07, Fig. 20	·0017	40	9·7	
		35	23·5	3·9
		30	39	2·6
		25	60·5	2·4
		20	94·5	3·0
		15	180	
				Mean value = 3·0

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The apparently consistent effect of rise of temperature upon the velocity of disinfection points again to a close analogy with the ordinary chemical reaction. The formula of Arrhenius, derived from the more complicated expression of Van t' Hoff, has been found applicable to many chemical reactions, viz., $A = \frac{T_0 T_n}{T_0 - T_n} \cdot \log \frac{K_0}{K_n}$, where A is a constant, and K_0 and K_n are the velocity constants of the reaction in question corresponding to the absolute temperatures T_0 and T_n respectively. Since the time taken for completion of a reaction may be considered as inversely proportional to the velocity of the reaction, one may rewrite the equation thus:—$A = \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$, where t_n and t_0 are the times taken to complete[1] the reaction at absolute temperatures T_n and T_0 respectively.

This formula, found by Madsen and Nyman (1907) to apply to the disinfection of anthrax spores with mercuric chloride, is also applicable to disinfection of *B. paratyphosus* with both mercuric chloride and silver nitrate. Values of A, calculated from the results of experiment, are inserted in Tables XXVII, XXVIII and XXX, and are found to be approximately constant. A certain amount of discrepancy does exist, but difficulties doubtless arise from the small amount of inhibition, which cannot be avoided when working with metallic salts.

The increase of reaction velocity with rise of temperature is conveniently expressed as the relative increase per 10° C. rise in temperature. From Figs. 19 and 20 (which like all the subsequent curves in this section were constructed by drawing smoothed curves to include as nearly as possible all the points determined by experiment), numbers were obtained giving a series of values for this temperature coefficient in the case of disinfection by mercuric chloride and silver nitrate. These figures are given in Tables XXIX and XXXI respectively. Values of 2·8 and 3·0 were obtained for two concentrations of silver nitrate, and 3·9 and 3·0 were the values obtained in the case of two different concentrations of mercuric chloride, figures of the same order as those obtained for ordinary chemical reactions.

2. *Phenol.*

In Tables XXXII, XXXIII, XXXIV and XXXV is shown the

[1] In theory the reaction is completed only after an infinite time. In this instance the reaction is considered at an end, when less than 60 individual bacteria remain undisinfected, *i.e.*, about $\frac{1}{60000}$ of the total number.

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TABLE XXXII.

Phenol, 12·5 per 1000. *B. paratyphosus.*

	Temperature degrees centigrade	Time taken for disinfection	Value of $A\left(=\frac{T_0 T_n}{T_0 - T_n}\log\frac{t_n}{t_0}\right)$, where initial abs. temp. $T_0=294$ and initial time $t_0=2{\cdot}5$
Exp. 25. 10. 06	21	2·5 minutes	—
	14·6	8	*
	2·6	216	8530
	0	460	8640
	− 3	more than 8·25 hours	—
			Mean = 8580

* Figure obtained was not concordant.

TABLE XXXIII.

Phenol, 10 per 1000. *B. paratyphosus.*

	Temperature degrees centigrade	Time taken for disinfection	Value of $A\left(=\frac{T_0 T_n}{T_0 - T_n}\log\frac{t_n}{t_0}\right)$, where initial abs. temp. $T_0=303{\cdot}5$ and initial time $t_0=1{\cdot}5$
Exp. 24. 9. 06	30·5	1·5 minute	—
	27·5	3·5 minutes	*
	21·3	9	8090
	16·5	27	7870
Exp. 26. 9. 06	15·8	23·5	7130
	6	226	7530
Exp. 25. 10. 06	0	17·5 hours	7910
			Mean = 7710

* Figure obtained was not concordant.

TABLE XXXIV.

Phenol, 8 per 1000. *B. paratyphosus.*

	Temperature degrees centigrade	Time taken for disinfection	Value of $A\left(=\frac{T_0 T_n}{T_0 - T_n}\log\frac{t_n}{t_0}\right)$, where initial abs. temp. $T_0=306{\cdot}6$ and initial time $t_0=3{\cdot}5$
Exp. 25. 9. 06	42·7	< 1 minute	—
	33·6	3·5 minutes	—
	29	9	8250
	21	47·5	8100
	16·3	94	7330
			Mean = 7890
Exp. 27. 9. 06	21	52·5	—
	16	101	—
	3	more than 23 hours	—

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influence of temperature upon disinfection of *B. paratyphosus* in the case of four different concentrations of phenol. The same results are shown graphically in Fig. 21, where the four curves corresponding to the four different concentrations are all seen to be similar in form. The curves have a different slope from those expressing effect of temperature upon disinfection with metallic salts, the influence of temperature being here very much greater. The equation of Arrhenius was, however, applicable to this instance also; values of the expression $\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$ are given in Tables XXXII—XXXV, and remain very constant. The

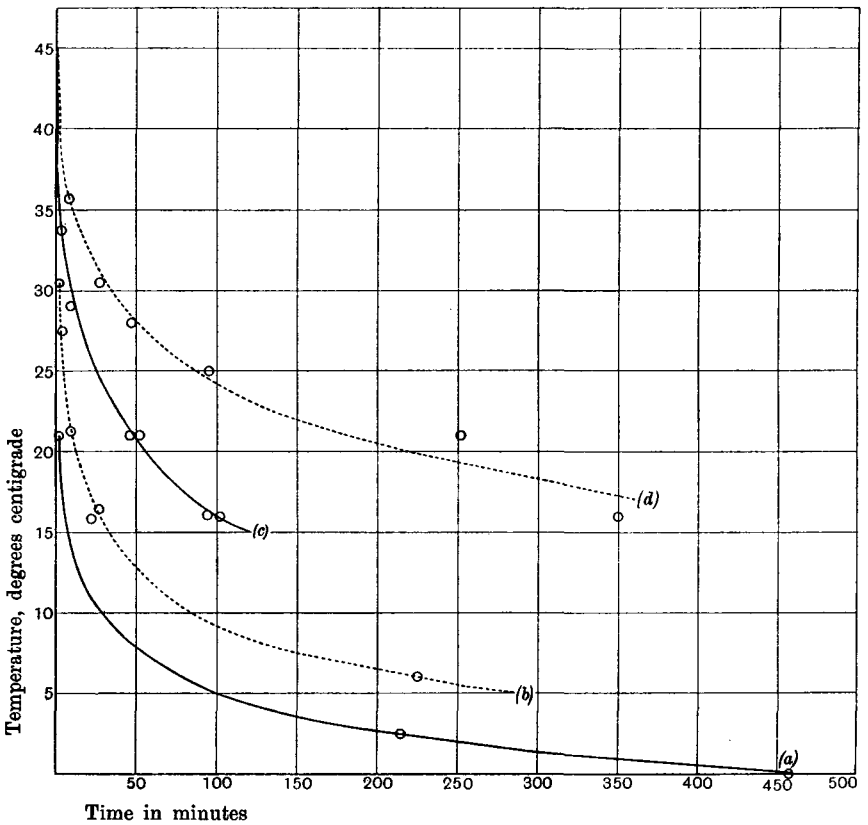

Fig. 21. Influence of temperature upon the time taken for disinfection of *B. paratyphosus* with phenol. Curve (*a*), phenol 12·5 per 1000 (Table XXXII, Exp. 25. 10. 06): curve (*b*), phenol 10 per 1000 (Table XXXIII, Exp. 24. 9. 06 and 26. 9. 06): curve (*c*), phenol 8 per 1000 (Table XXXIV, Exp. 25. 9. 06 and 27. 9. 06): curve (*d*), phenol 6 per 1000 (Table XXXV, Exp. 27. 9. 06 and 1. 10. 06).

TABLE XXXV.

Phenol, 6 per 1000. *B. paratyphosus.*

	Temperature degrees centigrade	Time taken for disinfection	Value of $A\left(=\frac{T_0 T_n}{T_0 - T_n}\log\frac{t_n}{t_0}\right)$, where initial abs. temp. $T_0 = 308{\cdot}7$ and initial time $t_0 = 9$
Exp. 27.9.07	44	< 1 minute	—
	35·7	9 minutes	—
	30·5	27·5	8650
Exp. 1.10.06	28	47·5	8660
	25	95	8750
	21	251	8890
	16	350	7180
	6	more than 10 hours	—
			Mean = 8430
		Phenol, 3 per 1000.	
Exp. 1.10.06	43	131 minutes	—
		Phenol, 1·2 per 1000.	
	44	more than 24 hours	—

TABLE XXXVI.

Phenol. *B. paratyphosus.*

	Concentration of phenol parts per 1000	Temperature degrees centigrade	Time taken for disinfection (derived from curves in Fig. 21) minutes	Relative increase in reaction velocity for a rise in temperature of 10° C.
Fig. 21, curve *a*	12·5	20	2	
		15	7	14·0
		10	28	14·3
		5	100	16·4
		0	460	
				Mean value = 14·9
Fig. 21, curve *b*	10	30	2	
		25	4	6·0
		20	12	8·1
		15	32·5	7·1
		10	85	8·8
		5	285	
				Mean value = 7·5
Fig. 21, curve *c*	8	35	3	
		30	9	8·3
		25	25	6·2
		20	56	4·8
		15	120	
				Mean value = 6·2
Fig. 21, curve *d*	6	35	11	
		30	34	7·9
		25	87	6·5
		20	220	
				Mean value = 7·2

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discrepancies occurring in some cases at the higher temperatures are doubtless due to the impossibility of measuring with accuracy the corresponding short intervals of time.

The temperature coefficients of the reaction velocity for an exact difference of 10° C. are given in Table XXXVI, where the times given in the 3rd column were obtained from the curves in Fig. 21. The value obtained for the highest phenol concentration appears to be abnormally high. In the case of the other concentrations the temperature coefficients are more nearly alike and the mean value, 7, is about twice as large as that obtained for disinfection with metallic salts.

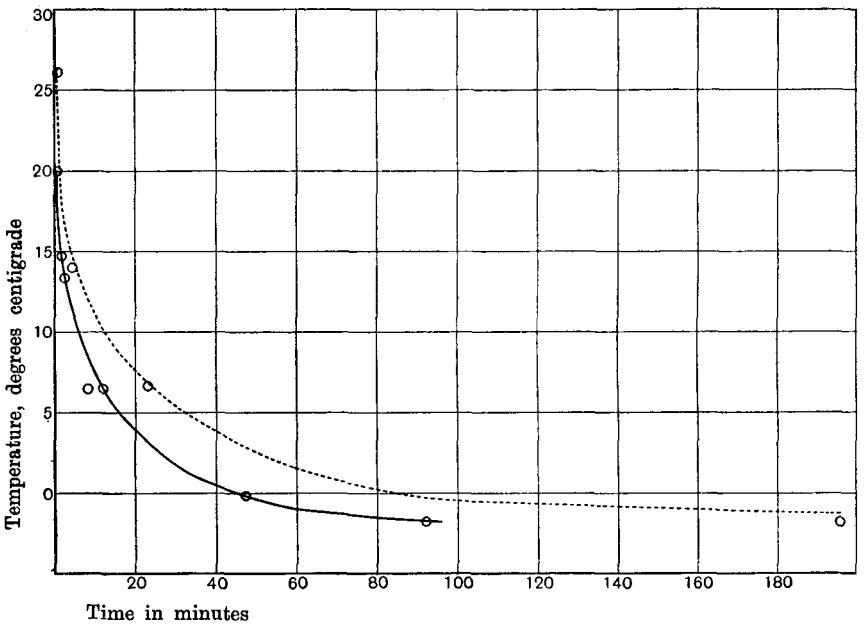

Fig. 22. Influence of temperature upon the disinfection of *B. paratyphosus* with disinfectant "A." Continuous curve, "A" 10 per 10,000 (Table XXXVII, Exp. 24. 1. 07 and 26. 1. 07): dotted curve, "A" 8 per 10,000 (Table XXXVIII, Exp. 2. 2. 07).

3. *Disinfectant "A."*

With regard to the effect of temperature, the disinfectant "A" falls into the same category as phenol. The results of five experiments with two different concentrations of "A" are given in Tables XXXVII and XXXVIII. The formula of Arrhenius was found applicable in this instance also, the expression $\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$ remaining constant in value.

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TABLE XXXVII.

Disinfectant "A," 10 per 10,000. *B. paratyphosus.*

	Temperature degrees centigrade	Time taken for disinfection minutes	Value of $A\left(=\frac{T_0T_n}{T_0-T_n}\log\frac{t_n}{t_0}\right)$, where initial abs. temp. $T_0=293$ and initial time $t_0=0{\cdot}75$
Exp. 24. 1. 07	20	0·75	—
	13·4	2·6	6870
	6·7	12	7250
	−0·3	47·5	7090
			Mean = 7070
			$T_0=293$, $t_0=0{\cdot}87$
Exp. 19. 1. 07	20	0·87	—
	7·9	15	9010
	−1·3	85	7440
			Mean = 8220
			$T_0=293$, $t_0=1{\cdot}25$
Exp. 26. 1. 07	20	1·25	—
	14·7	1·5	—
	6·6	8·5	7830
	−1·7	92	8710
			Mean = 8270

TABLE XXXVIII.

Disinfectant "A," 8 per 10,000. *B. paratyphosus.*

	Temperature degrees *centigrade*	Time taken for disinfection *minutes*	Value of $A\left(=\frac{T_0T_n}{T_0-T_n}\log\frac{t_n}{t_0}\right)$, where initial abs. temp. $T_0=292{\cdot}9$ *and initial time* $t_0=6$
Exp. 29. 1. 07	*19·9*	*6*	—
	14	18·5	6970
	6	140	7860
	−0·1	200	6080
			Mean = 6970
			$T_0=293{\cdot}3$, $t_0=0{\cdot}75$
Exp. 2. 2. 07	26·1	0·75	—
	20·3	0·75	—
	14	4·5	10400
	6·6	23·25	8920
	−1·7	>171	—
			Mean = 9660

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The mean reaction velocity (see Fig. 22 and Table XXXIX) was found to increase 7 to 8-fold for a rise in temperature of 10° C., a figure approximating in value to that found for phenol, and again about twice as great as the number obtained in the case of disinfection by metallic salts.

Disinfection by phenol and the disinfectant "A" being thus equally influenced by temperature is evidence of the similarity of the actual process in the case of the two disinfectants.

TABLE XXXIX.

Disinfectant "A." *B. paratyphosus.*

Concentration of "A" parts per 10,000	Temperature degrees centigrade	Time taken for disinfection (derived from curves in Fig. 22) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
10	20	0·8	
	15	1·5	6·9
	10	5·5	10·7
	5	16	8·2
	0	45	
			Mean value = 8·6
8	25	0·7	
	20	1·5	6·1
	15	4·3	8·3
	10	12·5	7·6
	5	32·5	6·8
	0	85	
			Mean value = 7·1

This high temperature coefficient in the case of phenol was surprising in view of the results obtained from the enumeration experiments given in detail at the beginning of the present section (Tables XXV and XXVI). In those experiments, the mean velocity of disinfection was found to increase two or three, or, at most, four times with a rise in temperature of 10° C. To explain the discrepancy by a criticism of the "end-point" method was not possible, because, as shown above, perfectly concordant results were obtained from parallel experiments using the two methods, the same lower coefficient being obtained in either case (Table XXVI). The only difference between the earlier enumeration experiments and those just described is in the number of bacteria disinfected. In the former case, thousands only of bacteria were taken, in the latter case 20—40 million bacteria were disinfected in each experiment. Simultaneous parallel experiments were therefore made with the "end-point"

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method, using exactly similar organisms from the same culture, but varying the total number disinfected, and, to make the comparison of results easier, water baths were maintained at temperatures differing exactly 10° C. from one another. The results are given in Table XL, each separate experiment showing the result of two sets of determinations, made over the same range of temperature, and concerned with the disinfection of exactly similar material, the quantity only of bacteria being different in the two cases. It was found to be invariably true that where more bacteria were disinfected, the temperature coefficient was higher (Exps. I, II, III and IV, Table XL).

TABLE XL.

Phenol. *B. paratyphosus.*

	Concentration phenol parts per 1000		No. of organisms added (approximately)	Temp. degrees centigrade	Time of disinfection (reduction of nos. in Col. II to less than 60) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. I, 28. 8. 07	10	(*a*)	1,000	21	0·75	3·3
			,,	11	2·5	
		(*b*)	750,000	21	2·5	6·0
			,,	11	15	
Exp. II, 2. 8. 07	8	(*a*)	6,600	21	2·75	8·2
			,,	11	22·5	
		(*b*)	1,030,000	21	25	10·3
			,,	11	257	
Exp. III, 31. 8. 07	8	(*a*)	440,000	21	4·5	6
			,,	11	27·5	
		(*b*)	76,000,000	31	4·5	7·7
			,,	21	34·5	
			,,	11	>283	>8·2
Exp. IV, 2. 8. 07	6	(*a*)	6,600	31	3·5	4·3
			,,	21	15	
		(*b*)	750,000	31	20	10·4
			,,	21	208	

In Section I, it was shown that among the individuals of a 24 hours' culture of *B. paratyphosus*, there existed permanent differences in resistance to disinfection, and that the younger organisms possessed the greater resistance. It therefore seemed possible that, among other differences, there might also be a different temperature coefficient for disinfection of bacteria of different resistances. The greater the total number of bacteria the greater also will be the number of more resistant forms. In any "end-point" method the properties of the more resistant

individuals alone are investigated; if then the disinfection of the latter should have a higher temperature coefficient than that of the less resistant individuals, disinfection experiments made with a large number of bacteria would show a higher temperature coefficient than those made with comparatively few[1]. For in this latter instance the presence of a few of the more resistant bacteria with higher temperature coefficient will not be made evident by the experimental method.

TABLE XLI.

Phenol. *B. paratyphosus.*

Concentration of phenol parts per 1000	Nature of culture employed	No. of organisms added (approximately)	Temperature of disinfection degrees centigrade	Time of disinfection (reduction of nos. in Col. III to less than 60) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 6. 9. 07.					
8	24 hrs.' culture	187,000	21	2·25	5·5
	,,	,,	11	12·5	
	,,	56,000,000	21	32·75	12·2
	,,	,,	11	401	
	Young culture	81,500	21	0·8	13·7
	3rd generation	,,	11	11	
Exp. 3. 9. 07.					
6	24 hrs.' culture	110,000	31	3·2	
	,,	,,	21	17·5	5·5
	,,	,,	11	67·5	3·9
					Mean = 4·7
	,,	16,000,000	41	1·75	
	,,	,,	31	13·75	7·8
	,,	,,	21	14·1	10·3
					Mean = 9·05
	Young culture	8,850	31	1·5	
	3rd generation	,,	21	12·25	8·1
	,,	,,	11	83	6·8
					Mean = 7·45

Special experiments were made to investigate the effect of temperature upon the disinfection of young organisms which were shown to possess the higher temperature coefficient. The necessary cultures were obtained by repeatedly sub-culturing in broth after intervals of

[1] It seems probable that in the case of disinfection with metallic salts, no such difference exists; a comparatively low temperature coefficient was obtained when large numbers of bacteria were disinfected. Whether this coefficient would be reduced in this case also, when small numbers were disinfected, could only be ascertained by further experiment.

about 3 hours. For purposes of comparison, simultaneous experiments were made with 24 hours' cultures, themselves taken from the same stock culture as the series of young growths (see Table XLI). Two concentrations of phenol were employed, and, in both cases, it was found that, whereas in the case of bacteria from a 24 hours' culture the high temperature coefficient was given only when a large number was present, an equally high coefficient is shown when only a few younger individuals were being disinfected. This result, combined with the theoretical considerations in the foregoing paragraph, yields additional evidence that, in a culture of *B. paratyphosus* the younger organisms offer the greater resistance to disinfection.

The fact that the younger and more resistant individuals in a culture of *B. paratyphosus* become relatively less resistant if the temperature is raised, is of great practical importance, and suggests an additional advantage in using hot or even warm solutions when disinfecting. In the case of spore-bearing organisms, such as *B. anthracis*, there probably exists no such complication, for the velocity coefficient of disinfection at constant temperature is the same for all individuals and no such difference in resistance can be detected. The temperature coefficients will doubtless be the same also for all the organisms.

TABLE XLII.

Results of Ballner's experiments with saturated steam and anthrax spores.

Temperature degrees centigrade	Time taken for complete sterilisation minutes	Values of $A\left(=\frac{T_0 T_n}{T_0 - T_n}\log\frac{t_n}{t_0}\right)$, where initial abs. temp. $T_0=378{\cdot}3$ and initial time $t_0=0{\cdot}43$
105·3	0·43	—
104·5	0·66	—
103·3	0·75	16480
102·3	0·9	16110
101·2	1·14	14610
100·7	1·7	18390
99·7	2·5	19190
98·4	2·8	16570
97·45	3·16	15460
96·4	3·3	13890
95·2	4·5	14070
94·2	5	13200
93·3	9	15260
92·7	8·7	14330
91·2	14	14780
90·4	14·7	14150

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Some interesting parallel experiments upon disinfection by heat should be mentioned here. Ballner (1902) measured the time taken to kill an approximately constant number of anthrax spores, using saturated steam at different temperatures, and obtained a valuable series of figures (Table XLII). The object of his investigation was a purely practical one, concerning laboratory sterilisation at high altitudes. The formula of Arrhenius was, however, applied to his figures, and will be found to fit them very well, when the error inseparable from the measurement of very short intervals of time is taken into account (see values of "A," Table XLII). Ballner's results are expressed graphically in Fig. 23, where a smooth curve is drawn to include as many experimental points as possible. The velocity of disinfection is seen to be increased about tenfold (see Table XLIII) for a rise in temperature of 10° C., a very high temperature coefficient.

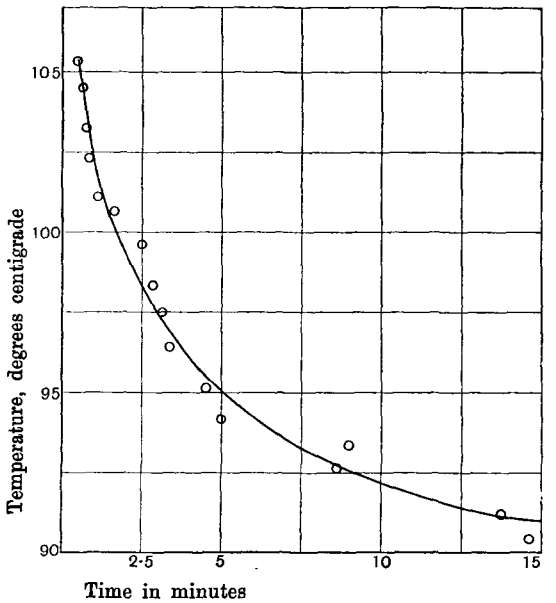

Fig. 23. Sterilisation of anthrax spores with saturated steam at different temperatures, from Ballner's results (see Table XLII).

Similar experiments by Meyer (1906) gave a much lower figure for the disinfection of the spores of *B. subtilis* and *B. robur*, the velocity of disinfection increasing only 4—5 times for 10° C. rise in temperature. Meyer examined his results for theoretical significance and found a logarithmic ratio to exist between the temperature of the experiment

and the time taken to kill. The series of temperatures formed terms of an arithmetical progression, while the corresponding times of disinfection (reciprocals of mean velocities of disinfection) were in geometrical progression. Meyer's relation may be expressed thus:

$$\frac{1}{t_1 - t_2} \log \frac{v_1}{v_2} = \text{constant},$$

where v_1 and v_2 are the reaction velocities of disinfection at temperatures t_1 and t_2 respectively.

The formula of Arrhenius has reference to the absolute temperatures involved, thus:

$$\frac{T_1 T_2}{T_1 - T_2} \log \frac{v_1}{v_2} = \text{constant},$$

where v_1 and v_2 are the reaction velocities of disinfection at absolute temperatures T_1 and T_2 respectively.

TABLE XLIII.

Results of Ballner's experiments derived from curve drawn in Figure 23.

Temperature degrees centigrade	Time of disinfection minutes	Relative increase in the mean velocity of disinfection for a rise in temperature of 10° C.
105	0·55	—
104	0·70	—
103	0·80	—
102	1·05	—
101	1·30	—
100	1·65	—
99	2·15	—
98	2·65	—
97	3·27	—
96	4·05	—
95	5·10	9·3
94	6·40	9·2
93	8·10	10·1
92	10·50	10·0
91	14·75	11·3
		Mean = 10·0

The simpler relation is included in the expression of Arrhenius and applies perfectly to the results of Ballner, and also to all those obtained in the present investigation with chemical disinfectants. The values of both constants were calculated in the case of most experiments and equally concordant results were obtained. The question as to which formula should be applied could only be settled by means of experiments

over a much larger range of temperature, and this is impossible from the nature of the organism employed. For a range of temperature of only about 40° C., the value of the product T_1T_2 in Arrhenius' expression remains throughout very much the same in value, and the two formulae become almost identical. It might be instructive to make similar investigations with thermophilic bacteria.

Effect of Temperature upon Inhibition.

Behring (1890) showed that the influence of temperature upon inhibition was the exact opposite of that upon disinfection. He found that the growth of anthrax bacilli at room temperature was inhibited when 1 in 400,000 mercuric chloride was present; at 37° C. a concentration of 1 in 100,000 was necessary to inhibit growth. A similar effect was shown by Brooks (1906) in the case of fungi and various poisons.

These somewhat anomalous facts were confirmed by some experiments made during the present work with dilute phenol solutions and *B. paratyphosus*. The concentration of phenol had been reduced to 4 per 1000, in order to obtain a slower rate of disinfection, so that the effect of temperature might be more easily studied with enumeration experiments. The temperatures chosen were 20° C. and 30° C., and the very unexpected result was obtained that disinfection progressed more rapidly at the lower than at the higher temperature, the average value of the velocity constant being about 1·5—3·0 times as great at 20° C., as at 30° C. (Table XLIV).

TABLE XLIV.

Phenol, 4 per 1000. *B. paratyphosus.*

Temperature of experiment	Time elapsing minutes	Mean no. of bacteria present in one standard drop of disinfection mixture	K, assuming reaction to be in accordance with equation $-\frac{dn}{dt}=Kn$
Exp. 16. 7. 07 20° C.	1	685, taken as initial value of n	
	5	143	0·17
	11	175	0·16
	20	3	0·14
30° C.	1	661, taken as initial value of n	
	3	437	0·09
	5	238	0·11
	11	69	0·10
	22	0·66	0·14
Exp. 18. 7. 07 20° C.	1	459, taken as initial value of n	
	2	143	0·51
	3	27·3	0·61
	4	4·7	0·66
	5	1·7	0·56
30° C.	1	519, taken as initial value of n	
	2	464	0·05
	3	274	0·14
	4·4	124	0·18
	5·2	75	0·20
	7	28·5	0·21
	10	6·1	0·20
	15	0·96	0·19

In Section II (Table XVI), it is seen that a concentration of 4 per 1000 phenol took about 20 hours to complete disinfection when 6 per 1000 required only 4 hours. Phenol in the lower concentration may be said to be altering in character from that of a disinfectant to that of a solution able only to produce inhibition.

When bacteria are exposed to the action of weak antiseptics, it is evidently a very great point in their favour if the temperature should happen to be at or near that of their own growth optimum. In the case of strong solutions of disinfectants, the times involved are altogether too short for any effort after growth on the part of the bacteria to be apparent. But in the case of weak disinfectant solutions the organisms are able to make a struggle and are much assisted if the temperature is in the neighbourhood of their own growth optimum. Probably the results shown in Table XLIV would be reversed if the temperatures chosen were say 40° C. and 50° C.; unfortunately such experiments would be useless because at 50° C. temperature itself becomes harmful to such an organism as *B. paratyphosus*.

Summary of Section III.

1. The reaction velocity of disinfection increases with rise of temperature in a manner similar to that of a chemical reaction. The formula of Arrhenius is applicable also to the case of disinfection, the expression $\frac{T_1 T_2}{T_1 - T_2} \log \frac{t_2}{t_1}$ (where t_1 and t_2 represent times taken for disinfection, reciprocals of mean reaction velocities, at absolute temperatures T_1 and T_2 respectively), remaining approximately constant in value.

2. The mean reaction velocity of disinfection with metallic salts increases 2—4-fold for a rise in temperature of 10° C. In the case of phenol and the disinfectant "A" the temperature coefficient is much higher, usually between 7 and 8. These experiments were made with about 20—40 million bacteria from a 24 hours' culture of *B. paratyphosus*.

3. The value of the temperature coefficient for disinfection of *B. paratyphosus* with phenol was found to vary with the number of individuals disinfected. The younger, and more resistant, individuals possess a higher temperature coefficient than the less resistant forms. The value of the coefficient varied from 2 to 10, according to the nature of the bacteria used for the experiment.

4. Inhibition is also influenced by temperature, but apparently in a different manner, being lessened or increased according as the particular temperature is near to or remote from that of optimum growth for the organism used.

General Summary.

1. A very complete analogy exists between a chemical reaction and the process of disinfection, one reagent being represented by the disinfectant, and the second by the protoplasm of the bacterium.

2. Three classes of disinfectants were studied, (*a*) metallic salts ($HgCl_2$ and $AgNO_3$), (*b*) phenol, and (*c*) emulsified disinfectants (disinfectant "A"). *B. paratyphosus* and spores of *B. anthracis* were chosen as types of vegetative and spore-bearing organisms respectively.

3. In the case of anthrax spores, the disinfection process proceeds in obedience to the well-known equation for a unimolecular reaction, if numbers expressing "concentration of reacting substance" are replaced by "numbers of surviving bacteria."

4. Experiments with *B. paratyphosus* show a departure from the simple law owing to permanent differences in resistance to disinfectants among the individual organisms. The younger bacteria were proved to be the more resistant.

5. The process of disinfection is influenced by temperature in an orderly manner, and the well-known equation of Arrhenius can be applied.

(*a*) Disinfection of *B. paratyphosus* by metallic salts is influenced by temperature to about the same degree as most chemical reactions, the reaction velocity being increased about three-fold for a rise in temperature of 10° C.

(*b*) For disinfection of *B. paratyphosus* by phenol and the disinfectant "A" there was a much higher temperature coefficient, viz., seven to eight. In the case of phenol the effect of temperature was again found to be complicated by the want of uniformity among the individual bacteria. Disinfection of the younger, more resistant bacteria, was found to possess a higher temperature coefficient than that of the less resistant forms, the coefficient varying from ten to three, or two according to the age and number of the bacteria disinfected.

6. It follows from (5) that there is a very great advantage in the use of warm solutions for practical disinfection.

7. Experiments, made with varying concentrations of disinfectant, and using similar groups of bacteria from cultures of *B. paratyphosus*, showed a definite logarithmic relation, between the concentration of disinfectant and the mean reaction velocity of disinfection, to exist in the case of phenol and the disinfectant "A."

8. In the case of silver nitrate, the same relation existed, but, in the case of mercuric chloride, numbers representing concentration of the salt had to be replaced by those representing concentration of the metallic ion. This confirms the theory that in disinfection with metallic salts the metallic ion is the real disinfecting agent.

9. This logarithmic relation is surprising in view of the simple proportionality existing in the case of chemical processes running the course of a unimolecular reaction, with which disinfection shows a close analogy.

10. Some evidence was obtained that, in disinfection with mercuric chloride, a toxic compound is formed between the metal and the substance of the bacterial cell. This compound prevents all further growth, but vitality can be restored by the administration of a large excess of soluble sulphide as an antidote.

I am glad to have this opportunity of expressing my great indebtedness to Dr C. J. Martin, at whose suggestion the work was undertaken, and who has helped me throughout, not only with most valuable advice, but also with practical assistance in many of the experiments.

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