THE PRINCIPLES INVOLVED IN THE STANDARDISATION OF DISINFECTANTS AND THE INFLUENCE OF ORGANIC MATTER UPON GERMICIDAL VALUE.

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https://doi.org/10.1017/S0022172400016016 Published online by Cambridge University Press

CHAPTER I. INTRODUCTION.

THE importance of the standardisation of disinfectants is universally admitted but no unanimity of opinion has been arrived at regarding the conditions under which germicidal value should be determined It is the precise conditions under which the observations are to be made, upon which agreement is so necessary, but so difficult to obtain. An agreement is essential for the reason that the efficiency of every different class of disinfectant is differently affected by alteration in the circumstances of its use. Standard conditions for testing purposes have been difficult to define because in practice disinfectants are used as germicides under a variety of conditions, of which the following afford typical instances :—

(1) For the chemical sterilisation of surgical instruments and the hands of the operator, and for various antiseptic lotions and washes, in which case they are mixed with water and are not subjected to the possibly disadvantageous admixture of other substances.

(2) For the purpose of preserving sera, vaccines, etc. against possible bacterial contamination, in which case they act in the presence of a considerable amount of soluble organic matter.

(3) For the disinfection of utensils, soiled linen, closet pans, dejecta, etc. and for the washing of walls and articles of furniture.

By far the greatest quantity of disinfectants is sold for one or other of the purposes detailed under No. 3.

The determination in water of the germicidal value of a preparation will give a serviceable indication if it is to be used for some purposes, but will not necessarily be a reliable guide to its value when mixed, for example, with blood serum or dusty water or the dejecta of a typhoid patient.

It is impracticable to standardise disinfectants under a great variety of circumstances, but it is important to ascertain the effect upon various classes of disinfectants of such conditions as those under which they are commonly employed, and it is likely that the determination of the value of disinfectants in the presence of some definite amount of organic material, both particulate and in solution, will afford a more useful guide to their value from a hygienic point of view.

The work published during the last few years upon the nature of the disinfection process and the laws it obeys (Krönig and Paul (1897), Madsen and Nyman (1907), H. Chick (1908)) has made it possible to formulate the fundamental conditions which must be satisfied in the comparison of germicidal values. These conditions and means suggested for satisfying them are first dealt with in the present paper.

Experiments have been made with three types of disinfectants commonly employed, viz. phenol, mercuric chloride and two emulsified disinfectants[1], referred to as disinfectants "A" and "B." Each of these types has been studied with sporing organisms, *B. subtilis* and *B. anthracis*, with *B. typhosus* and *B. paratyphosus* and with *Staphylococcus aureus* and *B. pestis*. The influence of the virulence or otherwise of the strain of organism has also been considered.

Particular attention has been given to the influence of various forms of organic matter both in suspension and solution upon the germicidal action of these types of disinfectant, experiments being made upon some or all of the above organisms.

The results of our experiments have led us to suggest some modification in the methods commonly employed for the standardisation of disinfectants, and at the end of the paper we put forward a scheme for the standardisation of disinfectants intended for use upon dejecta, or for other purposes involving the presence of a considerable amount of suspended organic matter.

Historical. Systematic experiments comparing the values of different substances to hinder putrefaction were made as long ago as 1750 by *Pringle* (1750). *Buchholtz* (1875) made extensive experiments with a great variety of reputed disinfectants, using an infusion of tobacco leaves, and *Jalan de la Croix* (1881) covered much the same ground, using broth made from meat and the organisms naturally occurring in it. *Baxter* (1875) used vaccine lymph and glanders nodules as test materials, and emphasized the influence of associated organic matter in diminishing the value of the disinfectant.

Robert Koch (1881), however, was the first to compare the germicidal value of various disinfectants upon *pure* cultures of bacteria. He worked with emulsions of anthrax spores dried upon silk threads and estimated the time of survival in solutions of the then known disinfectants, with the result that mercuric chloride has since been justly regarded as the most efficient disinfectant where spores are concerned. Koch's thread method was also used by *Behring* (1890) in a number of

[1] The active principles in these emulsified disinfectants were derivatives of higher tar acids; the greater part of these acids distilled over between 210 and 300° C. Disinfectant A contained glue, and Disinfectant B resin and soft soap as emulsifiers. Both A and B formed extremely fine emulsions when diluted with distilled water.

experiments in which comparison was made of the germicidal value of a series of disinfectants upon anthrax spores; Behring also published a number of results of experiments made with many vegetative forms, e.g. anthrax bacilli, *B. cholerae*, *B. typhosus*, streptococci and staphylococci. *Geppert* (1889 and 1891) confirmed Koch's results, but criticised the thread method in view of the unavoidable carrying-over of traces of disinfectant into the test culture, these traces preventing growth of the organisms adherent to the thread, although they might not have lost their vitality at the moment of subculture. On adding to the test culture sufficient ammonium sulphide to precipitate the traces of mercuric chloride that were thus carried over, when working directly with emulsions of bacteria in place of the silk threads soaked in broth cultures, Geppert found that the organisms might still grow[1], and concluded that the germicidal value of mercuric chloride, though doubtless preeminent where spores are concerned, had been overestimated. *Gruber* (1891) advocated working with emulsions of bacteria and supported Geppert's view as regards the overcarriage of $HgCl_2$, the misleading results of which he avoided by successive dilution of the test sample.

About the same time Creolin, the first of many emulsified disinfectants, received a great deal of attention from many workers; its germicidal value was compared with that of the older disinfectants, various organisms and different methods being used. *Esmarch* (1887) found it to be far superior to carbolic acid for the disinfection of emulsions of *B. cholerae* and streptococci, and less efficient for disinfection of anthrax spores, using the thread method. *Henle* (1889), also working with emulsions of bacteria, obtained excellent results with *B. typhosus*, while *Fraenkel* (1889), working with anthrax spores and the thread method, demonstrated the high disinfecting power of cresols and higher phenols, to which the germicidal value of creolin as well as that of crude carbolic acid is due.

Krönig and Paul (1897) devised a method for estimating germicidal value of disinfectants with anthrax spores, which had none of the drawbacks of Koch's thread method. Garnets were selected of similar size, carefully cleaned and dipped into an emulsion of sporing anthrax bacilli which was allowed to dry on their surface in a thin film. The garnets were then immersed in the solution of the disinfectant in question; from time to time a definite quantity was taken out, the disinfectant carried over was removed by gentle washing with water and, if necessary, with a precipitant (e.g. ammonium sulphide in the case of mercuric chloride). The garnets were then vigorously 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 having been shown that by vigorous shaking in water a fairly constant proportion of the total organisms was detached. By this method comparison could be made of the velocity of disinfection in the case of different disinfectants under different conditions. Krönig and Paul published a large number of very careful experiments, made with disinfectants of every class and in widely varying concentrations. They were the first to realize that the relative value of disinfectants depends very largely upon the conditions under which they work, and laid down the general laws that in any comparison of disinfectants close regard must be paid to the following conditions:

[1] The effect of treatment with a sulphide is not entirely due to the neutralisation of the $HgCl_2$ carried over in solution (*vide* p. 668).

(1) Constancy of number and species of bacterium used.
(2) Constancy of temperature.
(3) Constancy of nutrient medium for test cultures.
(4) Absence of other organic matter during disinfection.

They scrupulously observed all of these conditions, thus making their very exhaustive set of experiments the most valuable work which has yet appeared upon the subject of disinfection. A full description of the method as applied to the practical determination of the germicidal value of disinfectants was afterwards published by *Paul* (1901), anthrax spores and mercuric chloride being recommended as standard organism and disinfectant respectively.

Rideal and Walker (1903) published a method for the standardisation of disinfectants. They also realized the importance of the conditions of experiment. In their method (known as the "drop" method) a definite small amount of a broth culture of constant species and age was added to a constant volume of disinfectant solution. *B. typhosus* was chosen as the standard organism, and pure phenol as the standard disinfectant. By a series of trials with different concentrations both of disinfectant and pure phenol, under otherwise similar conditions, these authors determined the relative concentrations necessary to complete germicidal action in the same time. The ratio of the reciprocals of these concentrations was called the "carbolic acid coefficient" and was taken to express the germicidal value of the disinfectant in terms of carbolic acid as a standard.

This method has come into very general use, and been widely commended, among others by *Firth and Macfadyen* (1906) who found it to be superior in accuracy and convenience both to a method based on the "garnet" method of *Krönig and Paul* (1897) and the "thread" method of *Robert Koch* (1881).

The method of Rideal and Walker, in common with all preceding methods of standardisation, has been considered, in the opinion of many, to suffer from the disadvantage that whereas, in the majority of cases of practical disinfection, organic matter of some sort is present, there is no attempt to realize this condition during standardisation. Accordingly Kenwood and Hewlett (1906) and Wynter Blyth (1906) have suggested modifications of the Rideal and Walker method in which organic matter of different kinds is introduced; these modifications will be described later, in the section dealing with the effect of the presence of organic matter upon the efficiency of disinfectants.

Chapter II. Conditions which must be complied with in comparing the germicidal value of disinfectants, and the means suggested to satisfy them.

Disinfection has been shown by Madsen and Nyman (1907) and H. Chick (1908) to be a process exhibiting many analogies with a chemical reaction, one reagent being represented by the

bacterium and the second by the disinfectant. When the disinfectant is present in considerable excess the process proceeds in accordance with a definite law, the number of living bacteria per unit volume progressively and regularly decreasing with increase of time in a logarithmic ratio. This law is expressed by the equation

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

or

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

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

In determining the relative germicidal value of disinfectants, whatever the procedure adopted, the temperature at which the disinfection takes place and the number in unit volume and resistance of the bacteria employed for the test must, as was pointed out by Krönig and Paul (1897, p. 3), be constant. If an end-point method is used, such as that of Rideal and Walker, the nutrient medium into which the test samples are withdrawn must not be altered during the progress of the experiment, and it is also necessary to maintain uniformity in the time allowed for the disinfectant to act.

The reasons which make constancy in these several points a necessity are discussed below.

1. *Temperature.* The velocity of disinfection has been shown to be influenced by temperature in accordance with the equation of Arrhenius. In the case of mercuric chloride and anthrax spores disinfection velocity was found to be increased 3 times for every 10° C. rise in temperature (Madsen and Nyman, 1907); in the case of disinfection of *B. paratyphosus* with metallic salts it was influenced to the same extent, but with phenol and emulsified disinfectants the velocity was increased 7—8 fold for every 10° C. rise in temperature (H. C. 1908, pp. 146 and 148). In consequence it is necessary that during any determination of germicidal value standard and disinfectant should be maintained at the same temperature, and germicidal values cannot be directly compared unless the temperature at which the determination was made is the same.

The general effect of rise in temperature in assisting disinfection has been known for some time (Koch (1881), Henle (1889), Behring (1890), Heider (1892)), and the necessity of keeping temperature constant during standardisation was insisted upon by Krönig and Paul (1897)

and by Paul (1902). In our own work 20° C. was fixed as a convenient temperature, and one approximating to the conditions of practical disinfection, and all experiments were made in a bath kept within half a degree of this temperature.

2. *Number of bacteria.* It follows from the law of disinfection formulated above that even when the disinfectant is present in great excess the greater the number of bacteria present in a unit volume the longer will be the time required for their disinfection. This effect may be seen from the instances in Table I below.

TABLE I.

Showing the effect upon the time taken for disinfection by phenol of varying the number of bacteria per unit volume.

B. paratyphosus 21° C.

Concentration of phenol parts per 1000	No. of organisms in unit volume	Time taken for disinfection, mins.
10	440,000	0·75
10	66,000,000	6·0
8	187,000	2·25
8	440,000	4·5
8	56,000,000	32·75
8	66,000,000	34·5
6	110,000	17·5
6	16,000,000	141

To obtain a constant result with a particular disinfectant, it is essential to work always with the same concentration of organisms, but in comparative experiments this is not necessary as long as they are consistent. Therefore, if the comparison of germicidal value is always made against a standard, the standard being tested at the same time against the same amount of culture, no inaccuracy results, even if the number of organisms should vary from time to time. Such variations are, however, extremely inconvenient, as they make it difficult to arrange beforehand experiments to yield the necessary negative and positive results.

A broth culture containing about 250 to 500 millions of *B. typhosus* per c.c. was obtained by sowing 6 c.c. of broth with a loopful from a fresh agar culture, and incubating for 24 hours at 37° C. Such a seeding

is an excessive one, so that although the number of bacilli sown may vary considerably, there are always enough added to obtain within 24 hours the maximum growth the medium can sustain.

3. *Resistance of the culture.* Any variation in the resistance of the culture although not introducing any error in experiments where disinfectant and standard are simultaneously tested is also inconvenient for the reason discussed in the preceding paragraph. It is therefore very important to obtain cultures of a standard resistance.

The difference in resistance between a culture freshly isolated and one grown for some time upon culture media will be referred to later (p. 677), but even in the case of a stock laboratory culture of *B. typhosus*, it was found that its resistance to phenol was considerably modified by its previous history. If such a stock culture were successively subcultured and maintained at 37° C., its resistance to disinfection was found to progressively increase; when kept in the cold room, after growth had taken place at room temperature, its resistance was found to be gradually lessened although in both cases the culture actually used for the experiment had been grown at 37° C.[1] It was found most satisfactory to grow and maintain the stock culture on sloped agar at room temperature (16—20° C.), subculturing about every two weeks. From such growths a standard loopful was sown in broth and cultivated for 24 hours at 37° C. By these means cultures of moderately constant resistance were obtained.

4. *Culture media.* It was found convenient, though not indispensable for accuracy in a comparative experiment (see above), to maintain the culture medium as constant as possible.

The exact nature of the medium employed for the test cultures, though not affecting the coefficient if both disinfectant and standard were simultaneously tested, was found to influence very much the actual result obtained. If as is the case with end-point methods, few, and possibly somewhat damaged, organisms be transferred to the culture tubes, the chances of growth are considerably greater in a more favourable medium. Thus the germicidal value of any disinfectant will apparently vary with variation in the medium employed for the test cultures, although the value relative to that of the standard phenol will remain constant. This is well shown in Table II.

[1] Rideal and Walker (1903, p. 431) concluded that increased temperature of incubation raised the resistance of *B. typhosus* to disinfectants. The data given are, however, insufficient, as no mention is made of the number of organisms, per unit volume being the same in the two experiments cited.

To obviate the variation due to unavoidable differences in beef broth, Brand's "Meat Juice" was substituted for fresh beef broth, and the following medium was adopted:

Brand's Meat Juice	10 c.c.	in 1 litre tap-water.
Salt	5 gr.	
Peptone	10 gr.	
Glucose	10 gr.	

The medium had a reaction of +6 to +7 to phenolphthalein according to the notation of Eyre.

A large stock of the "Meat Juice" was put up in quantities of 10 c.c. and sterilised. The medium made with this preparation is not quite as favourable to the growth of *B. typhosus* as that made with fresh beef extract, but has been found to work well in practice.

TABLE II.

Influence of the suitability of the culture medium into which test samples are seeded upon the apparent germicidal value. The experiments were made with B. typhosus and all other conditions were identical.

	Disinfectant	Medium	Concentration required to kill in 15 minutes: Disinfectant parts per 1000	Concentration required to kill in 15 minutes: Pure phenol parts per 1000	Carbolic acid coefficient of the disinfectant
Exp. 23. 1. 08	I	Broth A	4·4	10·5	2·4
,, 31. 1. 08	,,	Broth B	5·2	12·7	2·4
,, 23. 1. 08	II	Broth A	3·5	10·5	3·0
	,,	Broth B	4·4	12·7	2·9
,, 17. 1. 08	III	Broth A	8	10·5	1·3
	,,	Broth B	10	13	1·3
,, 23. 1. 08	IV	Broth A	3·5	10·5	3·0
,, 7. 2. 08	,,	Broth B	4·8	13	2·7

5. *Time.* It has been shown by one of us (H. C. 1908) that a logarithmic relation exists between concentration of disinfectant and velocity of disinfection. This was found to be true in the case of such different organisms as *B. paratyphosus*, *Staphylococcus pyogenes aureus* and spores of *B. anthracis*, and such disinfectants as phenol, an emulsified disinfectant containing higher coal tar derivatives, and mercuric chloride (when the concentration of the metallic ions is taken into account). It follows that variations in concentration of disinfectant correspond to greater differences in the time taken for disinfection.

It was also found that the effect upon the time taken to kill of varying concentration is not the same with different classes of disinfectants. Two concentrations of two disinfectants, which are equally efficient, if allowed to operate for 5 minutes, do not necessarily bear the same relationship to one another as do two other concentrations of the same two disinfectants, which are equally efficient, if the time chosen for the test is 20 minutes. It follows that in comparing germicidal value in two cases where the effect of varying concentration upon the velocity of disinfection is very different (e.g. metallic salts and phenoloid bodies) completely different phenol coefficients will be obtained according to the time taken for the test, see Table III. If however, in comparing the germicidal value of two disinfectants, they are such, that the effect

TABLE III.

Showing the carbolic acid coefficients of mercuric chloride and silver nitrate, with variation in the time during which the disinfectant is allowed to act.

B. paratyphosus 20° C.

Disinfectant	Time of disinfection minutes	Concentration of disinfectant grm. per 1000	Carbolic acid coefficient
Mercuric chloride	2·5	0·88	13·6
Phenol	2·5	12	
Mercuric chloride	10	0·06	173
Phenol	10	10·4	
Mercuric chloride	30	0·018	550
Phenol	30	9·9	
Silver nitrate	2·5	0·068	176
Phenol	2·5	12	
Silver nitrate	10	0·015	693
Phenol	10	10·4	
Silver nitrate	50	0·009	922
Phenol	50	8·3	

of altering concentration upon the time taken for disinfection is about the same, it does not signify very much what time is taken for the test. An example of this is seen in the comparison of germicidal values of phenol and emulsified disinfectant "A" towards *B. paratyphosus* (see Table IV). On reference to a previous paper by one of us (H. C. 1908, pp. 119 and 122) it will be seen that the curves obtained by plotting concentration against time of disinfection are very similar for phenol and the coal tar disinfectant "A," and in the table

below (Table IV) the coefficients obtained by comparing the respective concentrations are the same within the error of such experiments.

TABLE IV.

Showing the constancy in the value of the carbolic acid coefficient of disinfectant 'A' with variation in the time during which the disinfectant is allowed to act.

B. paratyphosus 20° C.

Disinfectant	Time of disinfection minutes	Concentration of disinfectant per 1000	Carbolic acid coefficient of disinfectant 'A'
'A'	2·5	0·8	15
Phenol		12	
'A'	12·5	0·6	17
Phenol		10·2	
'A'	40	0·5	16
Phenol		8·5	

It is obligatory, however, in any method for the standardisation of disinfectants, which shall be applicable to germicides of every class, to choose an arbitrary time, during which the disinfectant shall be allowed to act. What time shall be employed must be decided by considerations of hygiene, and convenience in working the test. Provided these conditions be satisfied, the time chosen should be such that the test be not unduly prejudicial to any particular class of disinfectant. As previously shown, as far as the relationship between the germicidal efficiency of phenol and the emulsified disinfectants containing higher tar acids is concerned, it is immaterial whether the comparison be made after 10 minutes or 30 minutes, but the shorter time is greatly to the disadvantage of mercury and silver salts. We are of the opinion that for practical purposes it is desirable that a disinfection be completed in about half an hour, and this time is quite a convenient one for the laboratory tests. The employment of a shorter interval for the tests would be too unfavourable to metallic salts such as those of mercury and silver, which are undoubtedly amongst the most powerful germicides we possess.

Metallic salts are effective in very small concentration (1000 times less than phenol) and have the peculiarity that, if the concentration is increased, although the velocity of disinfection is increased also, this does not occur to nearly the same extent as with other disinfectants (H. C. 1908, Tables XVII, XIX and XXII). In the case of $HgCl_2$ this

is partly but not altogether[1] accounted for by the fact that the Hg^{++} ions are the real disinfecting agent and owing to peculiarities in the ionisation of mercuric salts, changes in concentration of the salts influence the concentration of the ions comparatively little.

Compared with phenol, the action of $HgCl_2$ upon bacteria is slow. For example, it was found that *B. paratyphosus* (6,000,000 per c.c.) could withstand the action of 5 % $HgCl_2$ for more than four minutes (H. C. 1908, p. 126), and *Staphylococcus pyogenes aureus* in similar concentration for more than 15 minutes (see Table VI, below) if immediately treated with a sulphide solution. This appears to be due to delay in $HgCl_2$ actually "getting to work" upon the contents of the bacterium, and will be discussed later.

Activity in high dilution is a valuable characteristic in a disinfectant. From this point of view mercury and silver are preeminent amongst chemical disinfectants, and it is advisable to choose a time for the standardisation which shall to some extent measure this property. When we embarked upon this enquiry we did not realise to what extent the metallic salts would suffer by comparison when short times were allowed for disinfection, and for most of our work 15 minutes was the time during which the disinfectant was allowed to act. We think however that, for the consideration discussed above, the time should be extended to 30 minutes.

CHAPTER III. MODIFICATION OF RIDEAL-WALKER METHOD NECESSITATED BY ADOPTING A CONSTANT TIME.

The introduction of an arbitrary time, which is necessitated by the considerations dealt with above, involves a modification in the method recommended by Rideal and Walker; unfortunately this modification makes the determination more tedious to perform.

The procedure we adopt is as follows:

Everything used in the experiment, tubes, pipettes, etc., being previously sterilised, a series of tubes containing 5 c.c. of the disinfectant in different concentrations are placed in a water bath at 20° C. When the tubes have taken the temperature of the bath, they are one after another inoculated with five drops of 24 hours' culture of *B. typhosus* from a standard pipette[2], the time being registered by a chronograph.

[1] Watson (1908). Constant K for $HgCl_2$, when Hg^{++} ions are reckoned as disinfectant, $=3{\cdot}8$, for phenol $K=5{\cdot}5$.

[2] H. C. 1908, p. 96.

Exactly one minute is allowed to pass between each inoculation. When 30 minutes have elapsed since the first tube was inoculated, samples in duplicate are taken from it with a platinum loop[1], and sown in 10 c.c. glucose broth containing litmus (see p. 662). One minute later the second tube is sampled and so on. These test cultures are incubated at 37° C. and always kept four days under observation.

Supposing the value of the disinfectant to be tested is totally unknown, the first series of observations must be scattered over a wide range, e.g. concentrations from 1 in 10 to 1 in 10,000. Having ascertained that the concentration necessary to kill in 30 minutes is between, say, 10 in 1000 and 1 in 1000 the second series is arranged to narrow it down to between, say, 4 and 5 per 1000, and a third series may determine the necessary concentration as between 4·2 and 4·5 per 1000. At this last trial a series of tubes, containing various strengths of pure phenol, are simultaneously tested.

The Rideal-Walker method, when employed to test disinfectants containing the higher coal tar derivatives, has been criticised (Gruber, (1891), Proskauer (1907), Seligmann (1907)), in view of the carrying-over of traces of disinfectant with the test samples, which may cause inhibition of growth in the test cultures. When disinfectants containing metallic salts are in question this carrying-over is of great importance and the traces thus added to the test cultures have to be neutralised. This is conveniently done by the addition of sulphides (see below, p. 668 where the subject is treated in detail). In the case of phenol and coal tar disinfectants there is, however, no suitable neutralising agent, but special experiments made for the purpose showed that there was no error introduced in carrying-over small quantities of these disinfectants. For example, a concentration of 1 in 1000 phenol in the test culture did not inhibit growth either of 10,000 or of 40 organisms in 10 c.c. In the case of disinfectant "B" a concentration of 1 in 10,000 was necessary to cause inhibition of the same concentration of organisms; 1 in 100,000

[1] A platinum loop of standard size was used for sampling. It is important to make the sample as large as possible to avoid errors in sampling, consequently the loop was bent horizontally and removed parallel to the surface of the liquid; it was found that the sample was much larger than when the plane of the loop was removed at right angles to the surface of the liquid. According as the sample taken is large or small, the determination is concerned with more or less complete disinfection. The two loopfuls removed (=about 0·0086 gr.) constitute about $\frac{1}{800}$ of the total volume, 5 c.c., and, if the test sample shows no growth, this indicates that the original number, about 30 millions, is reduced to less than 300 (cf. H. C. 1908, p. 118, where a larger sample was withdrawn (0·08 c.c.) and no growth indicated that about 30,000,000 per 5 c.c. had been reduced to less than 60 per 5 c.c.). For practical purposes disinfection may be regarded as complete in either case.

caused no inhibition in either case (see Table V below). These concentrations are higher than any attained in the test cultures in the course of a standardisation experiment.

CHAPTER IV. SPECIAL CONSIDERATIONS INVOLVED IN THE CASE OF DISINFECTANTS CONTAINING MERCURY.

In the standardisation of disinfectants containing mercuric chloride and some other salts of heavy metals some complications are encountered which are not met with in the case of the organic disinfectants. Disinfection by salts of mercury exhibits the following two peculiarities.

1. *Mercuric chloride is capable of acting as a germicide even in high dilution* (1 *in* 1,000,000) *if sufficient time be allowed for the action.*

Robert Koch (1881) found that 3 in 1,000,000 mercuric chloride inhibited growth of anthrax spores; Geppert (according to Behring) found that a concentration of 1 in 2,000,000 was sufficient to inhibit growth of anthrax spores; Behring himself found that 1 in 400,000 mercuric chloride was needed to prevent growth of anthrax spores.

TABLE V.

Showing the comparative inhibiting power of disinfectants upon the growth of some organisms.

Date of exp.	Test organisms	Disinfectant	Concentration inhibiting growth, parts per million	Concentration not inhibiting growth, parts per million	No. of organisms sown in 10 c.c.
15. 1. 07	*B. paratyphosus*	Mercuric chloride	1	0·1	1020
30. 6. 08	,,	,,	—	1	5000
28. 5. 08	*Staphylococcus*	,,	34	17	5000
	pyogenes aureus		34		3,000,000 to 4,000,000
28. 5. 08	*B. pestis*	,,	1·7	—	2000
15. 3. 07	*B. paratyphosus*	Silver nitrate	1·4	0·14	40 to 80
20. 7. 08	*B. typhosus*	Phenol	—	1000	40 or 10,000
20. 7. 08	,,	Disinfectant B	100	10	40 or 10,000

We have made some determinations of these amounts with different test organisms and a few results with different disinfectants are arranged in Table V, for purposes of easy comparison. The results with mercuric chloride are roughly in accord with those of Koch and Geppert, and also

show the influence of the quantity of bacteria introduced upon the result. This difference may be due to selection; the more bacteria introduced, the greater will be the number of specially robust individuals present. Or, there may be an exhaustion of the mercuric chloride owing to the number introduced. Possibly the result may be due to a combination of both effects.

In consequence of this germicidal action in high dilution it is necessary, as originally insisted upon by Geppert, to precipitate the mercury carried over with samples withdrawn during an experiment, as this would otherwise prevent growth in the test cultures. A sulphide is the common precipitant employed and it has been found necessary to add an excess over and above the amount calculated for combination with the mercury carried over (H. C. 1908, p. 123, Tab. XX). The reason for this excess will be discussed when dealing with a second peculiarity of disinfection by mercuric salts. This treatment with sulphide means a little extra trouble, for such precipitants have themselves an inhibiting action upon the growth of bacteria, and it is important not to add enough to interfere with subsequent growth. A saturated solution of hydrogen sulphide[1] in distilled water was found to be the most satisfactory precipitant and 0·2 c.c. added to 10 c.c. of broth (see H. C. 1908, pp. 123—125) was found ample for neutralisation and not sufficient to interfere with subsequent growth.

2. *Organisms submitted to the action of mercuric salts and subsequently washed free of the disinfectant, may not grow if planted directly into broth, but are not necessarily damaged irretrievably, and if treated with a sulphide solution, a certain proportion can be resuscitated.*

These facts have already been recorded by one of us (H. C. 1908, p. 132), and it was suggested that they were due to the formation of a mercury addition-compound with the substance of the bacterium, which prevented the organism manifesting its vitality by growth, until it was decomposed by the action of a sulphide.

The following series of experiments demonstrates the correctness of this interpretation.

To an emulsion of *Staphylococcus aureus* sufficient $HgCl_2$ was added to make a concentration of 1 %. The mixture was immediately decanted into a series of tubes and placed in a rapidly rotating centrifuge ($t = 18 - 20°$ C.). After 13 minutes the supernatant fluid was removed from the tubes and the deposited bacteria thoroughly

[1] The H_2S solution must be fresh.

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washed with distilled water in the centrifuge. The water was withdrawn and the tube filled with nutrient broth and incubated. In no case was growth observed. In a second tube the bacteria, after removal of the mercury solution, were at once treated with 1/10th saturated H_2S water. After ten minutes the sulphide solution was removed and the tube filled with broth; on incubation growth occurred. In the case of five other tubes, varying intervals up to five hours elapsed before the sulphide was added, with the result that the addition of sulphide was found to be effective up to 77 minutes after the treatment with mercuric chloride. The addition of the sulphide blackened the washed bacterial deposit, showing that some mercuric salt had been absorbed by the bacterium and formed a compound with its substance. With the mercury attached, subsequent manifestation of life by multiplication is in abeyance unless a large excess of sulphide be applied as antidote.

From the above observations it follows that in determining the germicidal value of $HgCl_2$ (and the same holds for preparations containing salts of the heavy metals) we are confronted with the difficulty of deciding when the bacterium is to be considered for practical purposes dead[1]. Bacteria which have been subjected to the action of dilute solutions of $HgCl_2$ will not multiply unless they come into contact with SH_2 or soluble sulphides. When used for surgical purposes, where there is little chance that the action of the $HgCl_2$ may be reversed by contact with sulphides, mercuric chloride will prove effective even in very great dilution. Should, however, the disinfected material subsequently come in contact with decomposing organic matter, animal dejecta, etc., mercuric chloride will prove less efficient.

It is impossible to institute an accurate comparison between the value of metallic salts and organic disinfectants of the type of phenol. If, in estimating the germicidal value of mercury or silver salts, we omit to treat the sample of liquid containing the bacteria with a sulphide at the expiration of the time allowed for disinfection, we shall form a greatly exaggerated conception of their efficiency, whereas their value will be somewhat underestimated if this procedure be adopted. The latter method is now commonly employed. This escapes the

[1] Most divergent results have been obtained with mercuric chloride. R. Koch (1881), who was the first to lay stress upon the powerful germicidal value of mercuric chloride, obtained an exaggerated idea of its worth because he employed no precipitant to neutralise the traces of sublimate carried over into his test cultures. Geppert (1889 and 1891) pointed this out, but it is probable that he also overestimated the germicidal properties of $HgCl_2$ because, although he neutralised the traces of sublimate carried over, he did not employ a sufficient excess of sulphide.

grosser error but affords a severe judgement upon the value of metallic salts when used under certain conditions.

Summary of Chapters II, III, and IV.

1. In any method of standardisation it is necessary that the test shall be carried out at a constant temperature, as the disinfection process has a high temperature coefficient. For any method to be of general application, it is also necessary that the temperature selected shall be adhered to in all determinations, since the temperature coefficient of disinfection varies for different disinfectants. The temperature adopted was 20° C.

2. To avoid unnecessary labour, the number per unit volume and resistance of the bacteria employed for the test should be kept as constant as possible, and the culture medium used both for the original culture and for the test subcultures should be maintained of constant composition.

Neglect of these precautions does not affect the value found for the carbolic acid coefficient, if disinfectant and phenol are simultaneously tested, but leads to much trouble and inconvenience in the practice of standardisation.

3. It is necessary to arbitrarily select a fixed time during which the disinfectants shall be allowed to act.

A logarithmic relation exists between concentration of disinfectant and velocity of disinfection, and the effect of varying concentration upon the time taken to kill, is different for different disinfectants. Different phenol coefficients may be obtained according to the time adopted in the test.

The time suggested is 30 minutes.

4. In the case of metallic salts, a sulphide must be employed to neutralise the traces of disinfectant carried over with the test sample. With other disinfectants neutralisation was found to be unnecessary.

5. In the case of mercuric chloride, it was found necessary to add a large excess of sulphide over and above that required for combination with the mercury carried over, in order to decompose a compound formed between the mercuric salt and the substance of the bacterium. The presence of this compound prevents manifestation of vitality on the part of the organism unless an excess of soluble sulphide be administered as an antidote.

CHAPTER V. CHOICE OF TEST ORGANISM.

An important advance, made by Koch (1881) upon the method of his predecessors, Pringle (1750), Baxter (1875), L. Buchholtz (1875), and Jalan de la Croix (1881), was the use of pure cultures of bacteria for the determination of germicidal values of disinfectants instead of mixtures of putrefactive bacteria. He employed the spores of *B. anthracis*. Spores are much more resistant to disinfection than most vegetative forms, and many disinfectants which may usefully be employed for the latter are practically valueless against the former, so that Koch's test demanded a high standard of efficiency.

The question arises whether, for practical purposes, such a high standard is necessary. Rideal and Walker's method, which is largely used in this country, and which adopts carbolic acid and *B. typhosus* as standard disinfectant and test organism respectively, assumes that this question may be answered in the negative.

At the time when Koch made his experiments the number of pathogenic organisms, which formed spores, was overestimated. At the present time it is known that the germs of the great majority of diseases to which man is liable are produced by vegetative forms. It must however be remembered that there are a number of diseases about the etiology of which we have no data (e.g. typhus, scarlet fever, measles, etc.) and also several others, common to man and animals, which are known to be caused by spore-bearing organisms (e.g. tetanus, anthrax, malignant oedema, etc.). A disinfectant may at any time be required to obviate risk of infection from any of such diseases, and the departure from the high standard of Koch (mercuric chloride and anthrax as standard disinfectant and test organism respectively) and the adoption of a low standard, such as that of Rideal and Walker (carbolic acid and *B. typhosus*) may give rise to a false sense of security unless it is clearly understood what has been done.

Variation in the resistance of different vegetative forms to three different types of disinfectant.

The types of organisms chosen were (1) *B. typhosus* and *B. paratyphosus*, (2) *Staphylococcus pyogenes aureus*, (3) *B. pestis*. In each case 5 drops (·086 c.c.) from a standard pipette[1] taken from a 24 hours'

[1] See H. Chick (1908, p. 96).

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culture at 37° C. (30° in the case of *B. pestis*) were added to 5 c.c. disinfectant solution.

The disinfectants used were phenol, emulsified disinfectant B, mercuric chloride, and oxychinoline sulphonate of potassium ("chinosol").

In each case determination was made of the concentration of disinfectant required to kill the amount of culture added in 15 minutes. The details of the method, which is the one ultimately adopted for the standardisation of disinfectants, has been described in detail (p. 666); it will be sufficient here to give the results obtained (Table VI).

TABLE VI.

	Test organisms	Concentration, parts per 1000, required for disinfection in 15 minutes					
		Pure phenol	Disinfectant B	Relative conc. of Hg^{++} ions	Mercuric chloride[1]	Oxychinoline sulphonate of potassium	Carbolic acid coefficient
Exp. 20. 3. 08	*B. typhosus*	7·9	0·51	—	—	—	15·5
,, 11. 3. 08	,,	8·2	0·54	—	—	—	15·2
,, 22. 5. 08	*Staphylococcus pyogenes aureus*	9·0	2·0	—	—	—	4·5
,, 28. 5. 08	,, ,,	10·5	2·4	—	—	—	4·4
,, 19. 5. 08	*B. pestis*	8·5	0·2	—	—	—	40
,, 3. 6. 08	,,	7·25	0·18	—	—	—	40·3
,, 14.11.07	*B. paratyphosus*	8·00	—	about 42·5	about 0·1	—	about 80
,, 21. 5. 08	*B. typhosus*	7·75	—	46·0	0·15	—	81
,, 2. 6. 08	*Staphylococcus pyogenes aureus*	10·5	—	more than 107·5	more than 50	less than	0·21
,, 2. 6. 08	,, ,,	10·5	—	do.	do.	— ,,	0·21
,, 16. 6. 08	,, ,,	10·5	—	do.	do.	— ,,	0·21
,, 16. 6. 08	,, ,,	9·5	—	do.	do.	— ,,	0·19
,, 3. 6. 08	*B. pestis*	7·25	—	33·5	0·035	—	207
,, 22. 8. 07	*B. typhosus*	6·5	—	—	—	26	0·25
,, 23. 9. 07	*Staphylococcus pyogenes aureus*	9·0	—	—	—	10	0·9

It is very noticeable that, whereas the strengths of carbolic acid required to kill the three types of organism chosen varies comparatively

[1] H_2S was used for neutralising traces of $HgCl_2$ carried over with the sample into the test culture at the end of the 15 minutes.

https://doi.org/10.1017/S0022172400016016 Published online by Cambridge University Press

little (see column 2, Table VI), *B. pestis* to a large degree and *B. typhosus* to a less degree show themselves more susceptible to the action both of emulsified disinfectant "B" and mercuric chloride. In the case of "chinosol," the reverse is true, *Staphylococcus aureus* being found more susceptible than *B. typhosus* to the disinfectant.

The determinations of concentration of $HgCl_2$ necessary for disinfection in 15 minutes (Table VI column 5), and therefore its carbolic acid coefficients are merely approximate. This is owing to the fact that it is not the concentration of $HgCl_2$ as such which is operative, but the concentration of mercuric ions (Krönig and Paul (1897), H. Chick (1908)). The ionisation of mercuric salts is peculiar and 100% increase in concentration of the salt, e.g. from 1 to 2 parts per 10,000, only increases the concentration of ions by 15%, an amount which does not greatly exceed the experimental error of the determination. The numbers representing the concentration of mercuric ions corresponding to various concentrations of mercuric salt, given in Table VI, were obtained from a curve constructed by means of the results of Luther (1904) and Kahlenberg (1901).

Whereas very low concentrations of mercuric chloride are effective against *B. typhosus*, *B. paratyphosus* or *B. pestis* (see Table VI), with the method employed, viz.—treatment with H_2S at the end of 15 minutes—it was found impossible to kill *Staphylococcus pyogenes aureus* by treatment with 5% $HgCl_2$ for this length of time. 5% of mercuric chloride is a nearly saturated solution, so that a determination of the carbolic acid coefficient of mercuric chloride could not be made.

Comparative resistance to disinfectants of spores and vegetative forms.

It is notorious that spores are much less readily destroyed by disinfectants than vegetative forms, and we have tried to ascertain the relation between the strengths of some disinfectants necessary for the destruction of one and the other.

In order to compare by direct experiment the relative strengths of any given disinfectant required to kill (*a*) spores and (*b*) vegetative forms it would be necessary that approximately the *same number per unit volume* of individuals of either class should be killed in the same time. The relative strengths of disinfectant in the two cases would then be an indication of the relative resistances of the particular spore and vegetative form. It would however be extremely difficult to make

such an experiment, the necessary conditions could only be obtained by accident and this never has occurred in the course of an extensive series of experiments. The result could however be calculated from the relation which has been shown to exist between the concentration of disinfectant and time taken for disinfection. Unfortunately we have no available data of our own except in the case of phenol.

Experiments with phenol. B. paratyphosus and anthrax spores. Experiments have been published by one of us in which anthrax spores were destroyed by phenol, and the progress of the reaction was studied by enumerating the spores surviving in unit volume at successive intervals of time. In one such experiment with 5 % phenol at 20° C. (H. C. 1908, p. 97) it was found that 434 anthrax spores in unit volume (a standard drop) of the solution were reduced to 28 in 25·5 hours.

Exactly similar experiments were made with phenol and *B. paratyphosus,* at the same temperature. In one such experiment with 0·6 % phenol it was found that 484 bacteria in unit volume were reduced to 28·5 in 4 minutes (H. C. 1908, p. 106); in a second similar experiment 484 were reduced to 24 in 4·5 minutes (p. 111). It is therefore not very inaccurate to say that in the case of *B. paratyphosus* and 0·6 % phenol, 434 bacteria per unit volume would be reduced to 28 in about 4·25 minutes.

In these three experiments the concentration of bacteria was almost identical. If however this had not been so, the actual time necessary for a reduction in numbers of *B. paratyphosus* similar to that of the anthrax spores could have been obtained from the drawn curves showing rate of decrease of numbers of *B. paratyphosus* during disinfection with 0·6 % phenol (see figs. 7 and 9, H. C. 1908, p. 105) or from the formula expressing the same relation (p. 95).

The two series of experiments are still not directly comparable, for the time of disinfection of the anthrax spores was 25·5 hours and that of the similar reduction in number of *B. paratyphosus* was 4·25 minutes. It is necessary to find out what concentration of phenol would perform the disinfection of the same number of *B. paratyphosus* in 25·5 hours.

The logarithmic relation existing between the concentration of a disinfectant and the time taken for disinfection has recently been suitably expressed by H. E. Watson (1908) by the equation.

$$K \log C + \log t = \text{constant},$$

where K is some constant depending on the nature of the disinfectant

and in some cases also of the organism employed. For carbolic acid and *B. paratyphosus*,

$$K = 5{\cdot}5,$$

and we have the equation

$$5{\cdot}5 \log C + \log t = \text{constant}.$$

Therefore $5{\cdot}5 \log 0{\cdot}6 + \log 4{\cdot}25 = 5{\cdot}5 \log x + \log 1530$

from which $$x = 0{\cdot}206,$$

and the concentration is $0{\cdot}206\,\%$. The ratio of the concentration required to kill anthrax spores to that required in the case of *B. paratyphosus* is as 5 to 0·2, that is 25 times as strong.

Staphylococcus pyogenes aureus and anthrax spores. Krönig and Paul (1897) and Paul and Prall (1907) have made enumeration experiments with phenol upon anthrax spores and *Staphylococcus pyogenes aureus* respectively, and among these we have found two sets of observations where the reduction in numbers of the two species happens to be almost identical.

It is, strictly speaking, not allowable to institute a comparison between two such experiments, as no data are to be obtained as to the concentration of the bacteria during disinfection. Both sets of authors employed the garnet method (Krönig and Paul, 1897, pp. 7—11), in which the organisms are submitted to the action of the disinfectant in a thin film dried upon the surface of the garnets. For any series of experiments, in which the same emulsion of organisms is used for preparing the garnets in the first instance, it may be concluded that the concentration of bacteria during disinfection will be constant[1] also. It is less permissible to compare experiments made with different organisms, and at different times, even though the details of the method remain the same. At the same time, the probability is that, since in both cases the concentration of bacteria approached a maximum, we shall not obtain very erroneous results by comparing the two experiments.

In the one case (Krönig and Paul, 1897, p. 93) about 1000 anthrax spores were reduced to 260 in 25 hours by 5 % phenol at 18° C.; in the other (Paul and Prall, 1907, p. 116) 1040 staphylococci were reduced to 205 in $2\frac{1}{2}$ minutes by $0{\cdot}94\,\%$ phenol at 18° C.

[1] That this is the case is shown by the fact that the laws shown to govern the disinfection process have been as accurately deduced from experiments using the garnet method (Krönig and Paul (1897), Madsen and Nyman (1907)) as from those in which the constancy of the concentration of bacteria was assured (H. C. 1908).

By calculation from the equation $5{\cdot}5^{1} \log C + \log t = \text{constant}$, it appears that 0·29 % carbolic would reduce 1040 staphylococci to 205 in 25 hours, so that the relative concentration is approximately as 17 to 1. This figure is in fair agreement with the calculated result in the case of paratyphoid, for, as previously shown (Table VI), staphylococci possess a somewhat greater resistance towards phenol.

Mercuric chloride. It has not been possible to obtain any perfectly comparable data as to the comparative resistance of spores and vegetative forms towards mercuric chloride. However, with the reservation mentioned above, we propose to use again two sets of experiments with the garnet method in which anthrax spores and *Staphylococcus pyogenes aureus* respectively were disinfected.

TABLE VII.

	Test organism	Concentration of disinfectant B necessary to kill in 15 minutes, parts per 1000
Exp. 28. 5. 08	*Staphylococcus pyogenes aureus* A, old laboratory culture	2·0
,, 22. 5. 08	*Staphylococcus pyogenes aureus* B, virulent culture	2·4
,, 4. 6. 08	*B. typhosus* A, old laboratory culture ...	0·55
,, 4. 6. 08	*B. typhosus* B, which had then recently been passaged	0·80
,, 15. 7. 08	*B. typhosus* A	0·59
,, 15. 7. 08	*B. typhosus* B after 41 days' culture upon artificial media	0·56

In the former case 82 anthrax spores were reduced to 19 in 2 minutes by 1·69 % mercuric chloride at 18° C. (Krönig and Paul, 1897, p. 24). In the latter case, from three experiments (Paul and Prall, 1907, pp. 103, 104, and 105) 98 staphylococci were reduced to about 22 by 0·053 % mercuric chloride, also in 2 minutes at 18° C.

In this instance temperature of disinfection, the number (and presumably the concentration) of bacteria disinfected and the time taken all being the same, comparison of the concentration of disinfectant in the two cases gives direct indication of the relative resistance to mercuric chloride of anthrax spores and *Staphylococcus pyogenes aureus*; this ratio is as 32 to 1[2].

[1] The constant K happens to be the same in the case of phenol for both *B. paratyphosus* and *Staphylococcus pyogenes aureus*.

[2] In the case of mercuric chloride, it has been shown that mercuric ions are the real disinfecting agent (Krönig and Paul, 1897; H. Chick, 1908; Watson, 1908). Figures

Differences in resistance to disinfectants between virulent and avirulent strains of the same organism.

After cultivation upon artificial media a certain loss of resistance towards disinfectants was noticed in the case of *B. typhosus* and *Staphylococcus pyogenes aureus*, when compared with freshly isolated virulent strains. The experimental results are set forth in Table VII above.

Summary of Chapter V.

1. In the case of vegetative organisms a disinfectant varies in efficiency as much as ten times according to the organism against which it is tested. Some disinfectants are more efficient against one vegetative species of bacteria, others against another.

2. In the case of spores, metallic salts are the most efficient germicides. The action of phenol and emulsified disinfectants is too feeble for practical use.

3. The ratio between the concentration of phenol required to kill the same number per unit volume of sporing and vegetative forms in the same time varied according to the particular organism employed between 17 and 25 to 1.

4. A virulent strain of any particular species is generally somewhat more difficult to kill than a non-virulent strain.

5. Owing to the want of constancy as regards disinfection shown by various species, it is necessary to fix upon one particular organism for use in testing the germicidal value of disinfectants.

6. *B. typhosus* occupies an intermediate position in many respects, and has come to be employed in this country for the standardisation of disinfectants. It possesses the advantage of forming a fairly uniform suspension in broth culture and it is an organism which it is frequently desired to destroy in practice.

7. There is no particular reason why a virulent strain of *B. typhosus* should be employed in standardisation, and its use for the purpose would entail an unnecessary amount of danger.

CHAPTER VI. INFLUENCE OF ORGANIC MATTER.

(1) *Introductory.*

The practical value of the results obtained by any method of standardising disinfectants may justly be questioned if the comparison is

expressing the relative concentration of Hg^{++} ions, corresponding to concentrations of the salt in the above experiments, were obtained from the results of Kahlenberg (1901) and Luther (1904); the ratio of the concentrations of Hg^{++} ions in the two cases is as 1·5 to 1.

made in the absence of organic matter, since in practice disinfectants are commonly used in its presence. The importance of this is evident when it is realised that, although presence of organic matter lessens the efficiency of every chemical disinfectant with which we have worked, the interference is to a different degree in every special case, depending (1) on the chemical nature and physical condition of the disinfectant, and (2) on the nature and condition of the organic matter.

Kenwood and Hewlett (1906) showed that in the presence of urine and faeces the value of phenol (Rideal-Walker method) was barely impaired, while emulsified disinfectants were reduced to two-thirds and one-half of their original value. These authors therefore suggest that the Rideal-Walker coefficient may in certain cases give a misleading value and that in any useful method of standardisation organic matter should be introduced. The sum of opinion, as the result of a long controversy[1] lasting over the last few years, is that it is desirable to introduce organic matter into any standard method of comparing the germicidal value of disinfectants, but great difficulty has been experienced in selecting the most suitable material.

It is impossible to arbitrarily select some convenient organic material without doing injustice to one or other class of disinfectant, so that one has to fall back upon some form which the disinfectant may encounter in practice. The use of faeces has the advantage of an approximation to the conditions of disinfection in many cases; it has, however, been objected to by Somerville and Walker (1906, I and II) as being too irregular in composition and therefore too uncertain in its effect upon disinfection to be adopted. Somerville and Walker suggest in a later paper (1907) the introduction of some standard organic matter, such as protein or gelatin, *in solution.*

In the case of many disinfectants, however, the physical condition of the organic matter present is an important factor in its effect upon the disinfectant, so that any method of standardisation which introduced organic matter only in solution would be inadequate, as in practice contaminating organic matter is partly in a particulate form. Wynter Blyth (1906) considers the question of organic matter both in solution and suspension, and notes the especial effect of the latter (e.g. faeces) in reducing the efficiency of emulsified disinfectants. He considers faeces, however, to be too inconstant in composition to be included in any standard test, but finds that a similar effect is produced

[1] Full abstract is to be found in *Public Health Engineer*, May—Aug. 1906, and in the *Local Government Officer*, Sept. 1906 to May 1907.

by the addition of milk and recommends that milk should be adopted as a standard organic matter on the score of its convenience and approximate constancy in composition.

We have made experiments to determine the effect of the presence of various kinds of organic matter both in solution and in suspension upon the germicidal value of a variety of disinfectants.

These experiments were undertaken with the view to ascertain how far the efficiency of disinfectants was modified by the conditions obtaining when they are added as a second line of defence to sera, vaccines, etc.; or when employed to disinfect dejecta.

Two methods were employed for estimating the effect of organic matter upon the germicidal value of disinfectants.

Method 1. In these experiments the time of disinfection was constant. Two concentrations (C_1 and C_2) which disinfected in 15 minutes, in distilled water and in presence of organic matter respectively, were directly determined, and the relative efficiency of the disinfectant in the two cases expressed as the ratio $\frac{C_2}{C_1}$.

Method 2. In the case of mercuric chloride we had not sufficient data available for an adequate investigation of the effect of serum in different concentrations and a second method was adopted which was less direct and involved rather more calculation.

The concentration (C_1) of a disinfectant was kept constant and the time (t_1) of disinfection of a certain number of organisms in unit volume was measured, when no organic matter was present, and the time (t_2) was also measured, when organic matter was present. It is possible to estimate the concentration C_2 of disinfectant, which would kill in the time t_2, if no organic matter had been added, by means of the curves showing the relation between concentration and time taken (H. C., 1908), or the result can be calculated by means of the formula $K \log C + \log t = K$, which has been shown by Watson to express these curves. The ratio $\frac{C_2}{C_1}$ expresses the efficiency of the disinfectant in presence of the organic matter if the efficiency in distilled water is reckoned as unity.

The following is an example of the calculation of relative efficiency in a case where the addition of organic matter was in the form of 10 % blood serum. In an experiment with phenol and *B. paratyphosus* it was found that 10 per 1000 phenol disinfected 30 millions of *B. paratyphosus* in 5 c.c. in 3 minutes. When 10 % serum was present the time taken was 12·5 minutes. Watson (1908) has shown that in the experiments of one of us with phenol and *B. paratyphosus* $5{\cdot}5 \log C + \log t = \text{constant}$.

Therefore $5{\cdot}5 \log 10 + \log 3 = 5{\cdot}5 \log C_2 + \log 12{\cdot}5$, where C_2 = concentration of phenol which would disinfect in 12·5 minutes if no serum had been present. Solving this equation we obtain $C_2 = 7{\cdot}7$, and the relative efficiency of phenol with and without 10 % blood serum, $\frac{C_2}{C_1} = 0{\cdot}77$.

(2) *Organic matter in solution.*

Blood serum was chosen as a type of soluble organic matter which would be present in certain cases of disinfection. Its influence upon the germicidal value of disinfectants when present in concentrated form is also important, because disinfectants are frequently added to antitoxic sera as an additional precaution against contamination.

Experiments with 10 % *blood serum.* The effect of 10 % blood serum upon the efficiency of disinfectants was investigated by the two methods already described.

The effect of serum was determined in the case of phenol, mercuric chloride and the emulsified disinfectant "B," using *B. paratyphosus* and *B. typhosus* as test organisms. The results obtained are set forth in the tables below.

TABLE VIII.

Showing relative efficiency of various disinfectants with and without the addition of 10 % *blood serum.*

Experiments made with Method 1.

Date of exp.	Test organisms	Disinfectant	Concentration required to kill in 15 minutes, parts per 1000		Relative efficiency, (concentration required in distilled water = 1)
			In distilled water	In presence of 10 % serum	
12. 11. 07	*B. paratyphosus*	Phenol	8	9	0·89
14. 11. 07	,,	,,	7·75	8·25	0·94
26. 5. 08	,,	,,	8·75	10·5	0·83
21. 5. 08	*B. typhosus*	,,	7·75	9·5	0·87
13. 11. 07	*B. paratyphosus*	Disinfectant "B"	0·7	1·0	0·70
15. 11. 07	,,	,,	0·625	0·85	0·77
15. 11. 07	,,	Mercuric chloride *	about 0·05	about 0·45	about 0·11
	,,	,,	,, 0·10	,, 0·40	,, 0·25

* For reasons explained above, p. 673, the error in the determination of the germicidal value of mercuric chloride is 100 %.

https://doi.org/10.1017/S0022172400016016 Published online by Cambridge University Press

TABLE IX.

Showing the relative efficiency of mercuric chloride when employed as disinfectant in the presence of various concentrations of blood serum.

Experiments made with Method 2.

Organism	Initial concentration of $HgCl_2$ parts per 1000	Nos. expressing initial concentration of Hg^{++} ions	Concentration of blood serum %	Time taken for disinfection, minutes	Nos. expressing effective concentration of Hg^{++} ions	Relative efficiency in terms of concentration of Hg^{++} ions	Effective concentration of $HgCl_2$, parts per 1000	Relative efficiency in terms of concentration of $HgCl_2$
Exp. 9. 11. 07.								
B. paratyphosus	0·5	57·5	—	7·2	57·5	1·0	0·5	—
	0·5	57·5	5	10	52·7	0·91	0·30	0·60
	0·5	57·5	10	14·2	48·1	0·84	0·18	0·36
	0·5	57·5	20	39	36·9	0·64	0·05	0·10
	0·5	57·5	30	62	32·6	0·56	0·03	0·06

In the case of phenol the efficiency of the disinfectant is reduced about 12 % in the presence of 10 % blood serum, and with the emulsified disinfectant "B," the reduction is rather greater. The results with corrosive sublimate (see Table IX) require a few words of explanation. In calculating the concentration of $HgCl_2$, which in the absence of serum would disinfect in the time observed, it is necessary to express the original concentration of sublimate in terms of concentration of Hg^{++} ions. This was done by means of a curve constructed from the figures of Luther and Kahlenberg already referred to (p. 677 footnote). The calculated concentration, which in the absence of the serum would operate in the same time, was therefore also in terms of concentration of Hg^{++} ions and had to be again translated back into concentration of $HgCl_2$ by the same means. The effect of adding blood serum to a solution $HgCl_2$ is to precipitate some of the mercuric salt as an albuminate which thereby lowers the concentration of mercuric chloride, but, owing to the peculiar ionisation of this salt, the concentration of Hg^{++} ions varies but slowly with alteration in concentration of the salt.

Effect of blood serum in high concentration. A few experiments were made with *B. paratyphosus*, but this organism was abandoned because it was found to be particularly susceptible to the bactericidal action of horse serum. This was still very marked after the serum had been heated to 60° C. for one hour.

The effect of serum in high concentration upon the germicidal value of phenol was therefore studied with *Staphylococcus pyogenes aureus.*

In spite of the fact that the presence of the serum lessens the efficiency of the disinfectant, it was found that 0·25 % carbolic acid disinfected a liberal seeding (6,000,000 per c.c.) of staphylococcus in the presence of serum (previously heated to 60° C.) in less than two weeks at laboratory temperature (Table X). The disinfection was rendered much more rapid at 37° C., and in this case the staphylococci added were killed in two or three days.

TABLE X.

Showing the germicidal effect of dilute solutions of phenol upon staphylococcus pyogenes aureus in the presence of 90—95 % *blood serum previously heated to* 60—70° C.

Experiments made with Method 1.

Date of exp.	Temperature, degrees centigrade	Concentration of disinfectant parts per 1000	Concentration of blood serum %	Time taken for disinfection in presence of serum	Time taken for disinfection in distilled water
18. 11. 07	20	5	90	2 days	3 hours
	20	2·5	95	9 days	3 days
27. 11. 07	20	5	90	5·5 days	2 hours
	20	2·5	95	more than 12 days	23 hours
27. 3. 08	20	2·5	95	11 days	8 days
	37	2·5	95	30 hours	greater than 8, less than 24 hours
30. 3. 08	20	2·5	95	10·5 days	8 days
	37	2·5	95	50 hours	30 hours

(3) *Experiments with particulate organic matter of various kinds.*

a. Animal charcoal. As might be expected the influence of animal charcoal varies very considerably both according to the nature of the germicide and its condition, viz. whether in solution, e.g. phenol, or in suspension, as in the case of emulsified disinfectants.

50 c.c. of 1 % phenol was shaken up with 1 grm. of charcoal and the filtrate was found to contain 0·74 %[1]. The same amount of charcoal reduced the concentration of 50 c.c. of 5 % phenol to 3·85 %, the amount removed by the charcoal being nearly proportional to the concentration of the original solution. This suggests that the process is one of adsorption[2].

[1] Estimated with bromine.

[2] The first portion of adsorption curves are not uncommonly nearly linear, so that observations confined to a small range of low concentrations show an approximation to proportionality.

With emulsified disinfectants, the proportion removed is much greater. 0·6—0·8 grm. charcoal completely removed the tar-acids from 50 c.c. of an emulsion of disinfectant "A," containing 5 parts in 1000 (2·5 parts per 1000 of tar-acids) so that the filtrate was perfectly clear. In the case of disinfectant "B" 1 grm. charcoal added to 25 c.c. of 5 in 1000 disinfectant (3 per 1000 of tar-acids) gave a filtrate with only a trace of opalescence. In these observations the germicidal value of the filtrates was also directly determined, and it was found that in the case of "B" loss of germicidal power accompanied loss of opacity. Disinfectant "A" contained kresols in addition to higher tar-acids, and in this instance the clear filtrate still possessed one-tenth of its original potency.

The above facts suggest that the removal of an emulsion of tar-acids by animal charcoal might be a case of adsorption. This action of animal charcoal was therefore made the subject of special study and the quantitative relationships between the amount removed and the original concentration determined. The proportion of animal charcoal used was comparatively small, so that a considerable proportion of emulsified tar-acids were left unadsorbed at the end of the experiment. The residual tar-acids after treatment with animal charcoal were estimated. The following method was tested and found to yield sufficiently accurate results.

0·5 grm. animal charcoal (Kahlbaum's purest) was added to 20 c.c. of a series of concentrations of emulsified tar-acids varying from 0·23 to 1·39 %. The mixtures were allowed to remain in contact 3 hours in a shaking apparatus. At the end of this period they were filtered and a small proportion (3 to 10 c.c.) of the filtered solution was taken for analysis in each case. The sample was placed in a small separating funnel, acidified with hydrochloric acid, and shaken with ether, in which tar-acids are readily soluble. The watery layer was drawn off and the ether extract was dried by the addition of calcium chloride and withdrawn into a weighed beaker. The calcium chloride was washed with dry ether and the washings added. The ether was evaporated, first on a water-bath and finally on a warm plate at a temperature of 60°—70° C., and the residue weighed.

The results of these experiments, given in Table XI, show that the amount of emulsified tar-acids removed by animal charcoal is not constant. It is at first nearly proportional to the original concentration of tar-acids, but, as this concentration increases, its influence becomes progressively less and the amount adsorbed rapidly reaches a maximum. The maximum amount that the animal charcoal took up was 20 % of its own weight.

TABLE XI.

Adsorption of emulsified tar-acids of different concentrations by animal charcoal.

Date of exp.	Concentration of animal charcoal %	Initial concentration of tar-acids %	Final concentration of tar-acids %	Quantity adsorbed by 1 grm. animal charcoal grms.
8. 10. 08	2·5	1·39	0·88	0·202
,,	2·5	1·16	0·75	0·200
,,	2·5	0·70	0·23	0·185
7. 10. 08	2·5	0·64	0·21	0·169
8. 10. 08	2·5	0·46	0·11	0·144
,,	2·5	0·35	0·043	0·122
,,	2·5	0·23	0·031	0·080

The amounts adsorbed by 1 gram of charcoal plotted against either the initial or final concentration of the emulsion give points which lie upon a regular curve of the same essential form as has been obtained in the case of other adsorption phenomena[1] (cf. Schmidt (1894); Bayliss (1906)).

b. Dust. The dust was obtained from the tops of cupboards and contained 39 % organic matter and 61 % inorganic residue. The effect of dust in reducing efficiency was tested upon an emulsified disinfectant only.

The results of experiments using method 2 (p. 679), are given in Table XII and it will be seen that with 3 % dust and concentrations of emulsified disinfectant "B" ranging from 1·5 to 4 parts per 1000, there is a reduction of efficiency to about one half, independent of the original concentration of the disinfectant.

With a bacteriological method the observations were necessarily limited to a small range of low concentrations, when, as shown by observations with animal charcoal, the amount of adsorption is approximately proportional to the concentration. This indicates that the action is of a physical and not of a chemical nature.

In an experiment made with *B. typhosus* and the disinfectant "B" at 20° C., using method 1, it was found that when 3 % dust was present a concentration of 1·4 per 1000 was required to disinfect in 15 minutes, whereas in distilled water 0·47 per 1000 was adequate, the effective concentration being reduced to 0·34 of the original. At the same time an experiment was made using 3 % dust which had previously been ignited to free it of all organic matter, and in this case 0·45 parts per 1000 of disinfectant "B" were required for 15 minutes' disinfection,

[1] See this *Journal*, p. 702.

the strength of the disinfectant not being impaired by the addition of the ignited dust. This experiment shows that it is the organic part of dust which alone is responsible for lessening the efficiency of emulsified disinfectants.

TABLE XII.

Effect of presence of dust upon emulsified Disinfectant "B."

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

	Concentration of dust %	Concentration of disinfectant "B," parts per 1000	Time taken for disinfection	Calculated * effective concentration of disinfectant "B," parts per 1000	Relative efficiency (original concentration = 1)
Exp. 31. 10. 07	3	4	0·75 min.	2	0·50
	,,	3	2·75 mins.	1·13	0·38
	,,	2	6 ,,	0·92	0·46
	,,	1·5	27 ,,	0·75	0·50
	,,	1·0	76·5 ,,	—	—

* Experiments were made determining time of disinfection for a series of concentrations in distilled water; from the results of these experiments curves were drawn, and the concentrations given in column 4, corresponding to the times in column 3, were determined from the curves.

c. Coagulated horse serum. Coagulated horse serum was used in the form of a very fine precipitate. The serum was coagulated by heat, after dilution with distilled water in such a proportion that the concentration of the suspension was 0·66 %. 15 c.c. of this suspension was mixed with 5 c.c. of disinfectant "B" (2 per 1000). The resulting fluid yielded therefore a suspension of 0·1 grm. proteid in 20 c.c. of an emulsion of disinfectant "B" in the strength of 0·5 in 1000. After centrifuging, the mixture was found to be completely freed from all opacity by the addition of this small quantity of coagulated proteid.

In another experiment 20 c.c. of 0·5 in 1000 disinfectant "B" was completely cleared by the presence of ·07 grm. of the same coagulated serum.

The disinfectant "B" contained 39·2 % of water so that in the above cases the finely divided coagulated albumen had appropriated at least 8 % of its own weight of tar-acids, and this under circumstances which from analogy with the results with animal charcoal (Table XI) must be regarded as the least favourable.

d. Bacteria, dead and alive. A similar phenomenon was observed when suspensions of bacteria, dead or alive, were mixed with solutions

of emulsified disinfectants. While a control tube containing the emulsified disinfectant showed little or no settlement on centrifuging, the tube containing bacteria was cleared of all opacity and disinfectant properties if the relative quantities of disinfectant and bacteria were suitably arranged. For example, when 1 c.c. of an emulsion of *B. paratyphosus* was mixed with 1 c.c. of a solution of disinfectant "B," containing 0·5 part per 1000, the resulting fluid after centrifuging was clear.

In experiments, in which the quantitative relations were studied, the same general results were obtained as with animal charcoal, viz. the amount of tar-acids removed by the bacteria was at first proportional to the concentration and then rapidly reached a maximum. It was found that bacteria could under favourable circumstances remove 39 % of their own dry weight of tar-acids from an emulsion.

These facts are capable of explaining the high efficiency of this class of disinfectant and are dealt with in detail in a separate communication[1].

(4) *Influence of the presence of a suspension of faeces upon the various types of disinfectants.*

The bulk of the disinfectants manufactured are destined for the disinfection of excreta, drains, etc. where they have to operate in the presence of more or less faecal matter. It is therefore of interest to learn to what extent the action of various classes of disinfectants may be affected by the presence of faeces.

The fact that the germicidal value of emulsified disinfectants is seriously reduced in the presence of faeces has been pointed out by Kenwood and Hewlett (*loc. cit.*) and Wynter Blyth (*loc. cit.*). Fowler (1906) also made some experiments with a 5 % extract of fresh faeces in equal parts of urine and water. The effect upon phenol and emulsified disinfectants of this very dilute watery extract of faeces was found to be negligible. As however all particulate matter was filtered off through paper, Fowler's results do not really bear upon the matter at present under discussion.

The following table (Table XIII) shows the effect upon the efficiency of different classes of disinfectants of the addition of faeces. The concentration of various disinfectants, which killed 6,000,000 per c.c. *B. typhosus* in 15 minutes at 20° C., was determined (*a*) in distilled

[1] This *Journal*, p. 698.

https://doi.org/10.1017/S0022172400016016 Published online by Cambridge University Press

TABLE XIII.

Disinfectant	Active principle	Concentration required to disinfect about 6,000,000 per c.c. *B. typhosus* in 15 minutes at 20° C. (*a*) In distilled water, parts per 1000	(*b*) In presence of 3% dried faeces, parts per 1000	Relative efficiency in presence of 3% faeces as reckoned by ratio $\frac{\text{concentration } a}{\text{concentration } b}$	Remarks
(1) $HgCl_2$ ...	... ...	5	50	0·10	—
(2) Commercial disinfectant	$HgCl_2$ ...	0·13	1·5	0·09	—
(3) Commercial disinfectant "Cresol" No. 3	Cresols and higher tar-acids	0·55	6·0	0·09	Contains resin soap as emulsifier. Fine emulsion on dilution with water.
(4) Commercial disinfectant "B"	Higher tar-acids mostly boiling between 210—260° C.	0·75	4·5	0·17	Contains resin and soft soap as emulsifier. Fine emulsion on dilution with water.
(5) Commercial disinfectant "Cresol" No. 2	Cresols and higher tar-acids	2·5	8·5	0·29	Contains resin soap as emulsifier. Coarse emulsion on dilution with water.
(6) Commercial disinfectant "Cresol" No. 1	Cresols and higher tar-acids	5·0	15·0	0·33	Contains resin soap as emulsifier. Coarse emulsion on dilution with water.
(7) Commercial carbolic acid	91% Cresols ...	2·2	4·2	0·52	1 grm. almost completely soluble in 100 c.c. water.
(8) Commercial carbolic acid	95% Cresols ...	3·2	4·8	0·66	1 grm. almost completely soluble in 100 c.c. water.
(9) Phenol ...	... ...	8·0	9·25	0·86	—

water, (*b*) in water containing 3 per cent. by weight of a sample of dried sterile faeces.

The faeces were obtained from an individual upon an ordinary mixed diet. They were dried at 105° C., powdered and passed through a fine sieve and the appropriate quantity weighed out and added to the tubes.

The fact that the presence of organic matter with which it can combine should seriously reduce the disinfecting power of mercuric chloride requires no comment. Phenol, a completely soluble disinfectant, is reduced in value about 10% by the presence of 3% faeces. The large effect upon disinfectants, in which the active principles (cresols and tar-acids) are in the form of an emulsion, demands some explanation. The germicidal value of tar-acids is, other things being equal, dependent in some way upon the excellence of the emulsion, and, from the results in Table XIII above, it appears that the amount of interference by faeces

is also related to the fineness of the emulsion; in other words, *the higher the quality of the disinfectant in distilled water due to fineness of emulsion, the more it is interfered with in the presence of particulate organic matter.*

The clue to an interpretation of the deterioration of these emulsified disinfectants in the presence of faeces is afforded by the similar effect of charcoal, dust, coagulated albumen and bacterial suspensions upon this class of disinfectant.

From these observations it appears to be the particulate nature of the organic matter which is responsible for this deleterious influence upon emulsified disinfectants, and the quantitative experiments indicate that the process which takes place is one of adsorption of the fine particles of the emulsion.

Experiments were made to ascertain what quantitative relation existed between the reduction in germicidal value and the original concentration of the emulsion, when the amount of faeces was kept constant. The results of these experiments are set forth in Table XIV and show that the same percentage reduction is produced with concentrations of disinfectant varying between 3 and 7 per 1000.

The results with faeces thus fall into line with those obtained with charcoal, dust and bacteria for similar strengths of emulsion and can also be explained as due to adsorption. Since, however, the emulsified tar-acids in disinfectants "A" and "B" are soluble in olive oil and the sample of faeces used contained 24·3 % ether extract, it was conceivable that some portion of the action of faeces might be due to removal of the emulsion by its solution in the fat of the faeces. If this occurred, proportionality between amount removed and the concentration of disinfectant would also be maintained. Accordingly, experiments were made with faeces from which the fat had been extracted and it was found that the action of the extracted faeces was, weight for weight, only slightly less marked than that of the normal faeces (Table XIV). This action of faeces is therefore only due in small part to the solvent action of the contained fat.

It was possible that the loss might be partly or wholly due to a de-emulsifying action of the soluble organic matter present in faeces. Accordingly the dark coloured fluid obtained from a 3 or 6 % suspension of faeces in distilled water was tested for any de-emulsifying action upon the disinfectant "B." This fluid was mixed with a dilution of "B," but no settlement was detected either upon standing or after centrifuging.

TABLE XIV.

Quantitative relationship between original concentration of emulsion and depreciation in germicidal value due to presence of faeces.

Date of exp.	Concentration and nature of faeces	Initial concentration of emulsion, parts per 1000	Time taken for disinfection, 5 drops of 24 hrs. culture of *B. paratyphosus* in 5 c.c., minutes	Calculated effective concentration, parts per 1000	Relative efficiency, initial concentration =1
(1) 26. 10. 07	3 % dried faeces	6	2·5	1·2	0·20
		5	3	1·15	0·23
		4	13	0·87	0·22
(2) 26. 10. 07	3 % dried fat extracted faeces	5	1·5	1·4	0·28
		4	2·5	1·2	0·30
		3	13	0·87	0·29
(3) 26. 10. 07	2 % dried faeces	7	—	1·00	0·14
		6	—	0·9	0·15
		5	—	0·74	0·15
		4·1	—	about 0·55	0·13

Experiments 1 and 2 were made with Disinfectant "B," Experiment 3 with Disinfectant "A."

The action of faeces in reducing the strength of emulsified disinfectants must therefore be attributed principally to a surface action between the particles of the emulsion on the one hand and those of the faeces on the other, resembling closely the action between the other three types of particulate matter investigated, dust, animal charcoal and coagulated blood serum.

Summary of Chapter VI.

1. The presence of 10 % blood serum reduces the efficiency of 1 % phenol about 12 %. The effect upon emulsified disinfectants is somewhat greater. With mercuric chloride the reduction was much greater, a 0·5 % solution being reduced to from 0·6 to 0·06 of its original value as the concentration of serum was increased from 5 to 30 %.

2. Experiments made with more concentrated serum showed that thirty million staphylococci added to 5 c.c. of the serum containing 0·25 % phenol were killed in less than two weeks at a temperature of 20° C. and in 30 to 50 hours at a temperature of 37° C.

3. The presence of particulate organic matter—animal charcoal, dust, finely precipitated coagulated albumen, bacteria, and faeces—affect

the germicidal value of emulsified disinfectants containing tar-acids to a much greater extent than that of solutions of phenol.

4. The whole of the emulsified tar-acids can be removed by a suitable addition of any of the above forms of particulate organic matter.

5. The amount of tar-acids removed by animal charcoal from an emulsified disinfectant is at first proportional to the original concentration of tar-acids, but, as this concentration increases, the proportion removed rapidly diminishes. The amounts removed by a given quantity of charcoal from different strengths of emulsion increase at first rapidly and then more and more gradually until, with an emulsion of 1·4 % tar-acids, the maximum is nearly reached. The curve drawn from the observations, tabulated in Table XI, presents the usual form of an adsorption curve (see this *Journal*, p. 702, Fig. 1).

6. The removal of an emulsion of tar-acids by bacteria obeys the same quantitative laws as obtain in the case of animal charcoal.

7. The removal of tar-acids by dust was only investigated over a limited range of initial concentration and was found to take place in accordance with the same general law.

8. The effect of a 3 % suspension of finely powdered dried faeces upon the efficiency of phenol was to reduce it about 10 %; upon commercial cresols a reduction of 30 to 50 % was produced, depending upon the completeness with which the preparation dissolved. The freer the sample was from higher and insoluble homologues, the less depreciation in the original value occurred.

9. The effect upon various emulsified disinfectants containing higher tar-acids was to reduce their efficiency to a value varying from $\frac{1}{3}$rd to $\frac{1}{11}$th of the original. The finer emulsions were more seriously reduced in value than the coarser ones.

10. Within the limits of concentration of disinfectant employed, the amount of emulsion removed from the liquid by powdered faeces was nearly proportional to the concentration of disinfectant. In this case, as with the other forms of particulate organic matter, the removal was shown to be principally due to adsorption of the emulsion upon the surfaces of the fine particles.

CHAPTER VII. A METHOD OF STANDARDISING DISINFECTANTS IN THE PRESENCE OF FAECES.

It might be imagined that faeces would prove an impossible material to be included in a standard test on account of inconstancy in

composition. This has been contended by Wynter Blyth (*loc. cit.*) and Somerville and Walker (*loc. cit.*), and is certainly true if the faeces are employed in their natural condition, but has not been found by us to be the case if they are dried and powdered. Our experience on the contrary has shown that, when dried and ground to a fine powder, faeces from different individuals upon an ordinary mixed diet display a surprising uniformity with regard to the extent to which they influence the germicidal power of a disinfectant.

The particulate matter in human faeces consists largely of bacteria and portions of undigested food, together with varying quantities of fat and other bodies soluble in ether. In addition, a considerable amount of the pigment hydrobilirubin is present in an insoluble form, combined with calcium. These together account for about 70 % of the total dry weight. In the preceding chapter we have shown that the reduction of the efficiency of emulsified disinfectants by faeces is caused by the solid particles contained therein and is principally due to adsorption, hence depending as much upon the physical condition of the faeces as upon their chemical composition.

The following experiments (see Tables XV and XVI) illustrate the effect of 15 different samples of faeces, dried at 105° C. and ground to a fine powder, upon the germicidal value of emulsified disinfectants. In the experiments with *B. typhosus* (Table XVI) the variation caused by the different samples of faeces is seen to be within the error of the method, for the divergence between the results obtained upon the same day with faeces from different individuals was not greater than those obtained with the same sample of faeces upon different days.

TABLE XV.

Emulsified disinfectant "B" with various samples of faeces.

Disinfection of about 6,000,000 *B. paratyphosus* per c.c. (5 drops from a 24 hours' culture at 37° C.).

Sample of faeces	Concentration of "B" required for complete disinfection in 15 mins. at 20° C. in presence of 3 % faeces, parts per 1000
I	5·5
II	5·5
III	5·0
IV	4·5
V	5·0

TABLE XVI.

Emulsified disinfectant "B" with various samples of faeces.

Disinfection of about 6,000,000 *B. typhosus* per c.c. (5 drops from a 24 hours' culture at 37° C. in 5 c.c.).

Concentration of "B" required for complete disinfection in 15 minutes at 20° C., in presence of 3 % faeces, parts per 1000.

Sample of faeces	Date of exp.: 21. 3. 08	28. 3. 08	29. 4. 08	5. 5. 08	7. 5. 08	8. 5. 08	9. 5. 08
II	—	—	—	—	—	4·0	—
V	—	—	—	—	—	—	4·5
VI	—	—	—	4·8	—	—	—
VII	—	—	4·4	—	—	4·0	—
VIII	—	—	—	—	—	4·6	—
C	5·2	4·8	—	4·3	4·0	more than 6	—
D	—	—	—	—	—	4·0	—
E	—	—	—	—	4·6	—	—
F	—	—	—	—	—	—	4·3
G	4·4	4·1	—	—	—	—	—
H	4·1	—	—	—	—	—	4·9
I	—	—	—	4·5	4·3	—	4·3

TABLE XVII.

Mercuric chloride with various samples of faeces.

	Samples of faeces	Concentration of mercuric chloride required for disinfection of about 6,000,000 per c.c. *B. typhosus* in presence of 3 % faeces at 20° C. in 15 minutes, parts per 1000	Nos. expressing concentration of mercuric ions
Exp. 23. 7. 08	VII	1·0	63
	VIII	1·0	63
	IX	1·5	68
	II	1·5	68
	I	2·0	71
	I	2·0	71

The same uniformity of effect was apparently not maintained when the experiments were made with mercuric chloride, but here again the discrepancy is really within the experimental error. As shown in Table XVII the concentration necessary to disinfect 6,000,000 per c.c. *B. typhosus* in 15 minutes varied between 1 and 2 parts per 1000. This is not however of material significance, because the error of determining the germicidal value of mercury in this concentration is about 100 %, owing to the fact that, although the concentration of mercuric

https://doi.org/10.1017/S0022172400016016 Published online by Cambridge University Press

chloride changes from 1 to 2, the concentration of mercuric ions only varies as 63 to 71.

Description of the method employed.

The essential features of the method which we have been in the habit of using are those of the Rideal-Walker process, but a constant time, 30 minutes, is allowed for the disinfectant to act. The introduction of the faeces somewhat increases the experimental error which if we exclude one aberrant result in Table XVI is now less than 20 %, whereas in distilled water (see p. 665) we could rely upon an error not exceeding 10 %.

The faeces used were dried first in a water bath and subsequently at 105° C., ground to a fine powder, and passed through a fine sieve with a mesh of 130 to the inch. Quantities of 0·15 grm. were weighed out and placed in test tubes. To each test tube 2·5 c.c. distilled water was added and the tube sterilised in the autoclave (10 minutes at 120° C.). The tubes were covered with indiarubber caps and kept in jars with greased lids to prevent evaporation.

At the time of the experiment different amounts of a suitable dilution of the disinfectant were added to each tube together with enough distilled water to make the total volume up to 5 c.c. The tubes then contained different concentrations of the disinfectant in question in the presence of 3 % faeces[1]. The tubes were inoculated and sampled in exactly the same way as when the test was made in distilled water (see p. 665).

In the case of an unknown disinfectant one or two preliminary trials are necessary to ascertain a suitable range of concentrations to give a useful result. The exact procedure may be made clearer by an actual example.

Six tubes each containing 0·15 gr. faeces in 2·5 c.c. of distilled water having been prepared, to each is added varying quantities of 2 % disinfectant and water as in Table XVIII below, so arranged that the tubes contain 0·5 c.c. of a series of concentrations of disinfectant in the presence of 3 % faeces. In this experiment the approximate concentration necessary to disinfect in 15 minutes has been determined previously, and the actual concentration required to disinfect in the time is found to be between 4 and 4·6 parts per 1000.

[1] Three per cent. faeces was chosen as representing the amount of solid matter present if a liquid stool (containing 10 % solids) were mixed with twice its volume of the disinfectant.

An exactly similar experiment with pure phenol is simultaneously made, so that the carbolic acid coefficient of the disinfectant can be directly determined.

TABLE XVIII.

Faeces I. 3 %, and a constant number of *B. typhosus* per c.c.

	No. of c.c. of 2% disinfectant	No. of c.c. distilled water added	Total volume added, c.c.	Concentration of disinfectant in the tube, parts per 1000	Result of experiment + = acid formation in test cultures* in glucose broth
Exp. 9. 5. 08	0·9	1·6	2·5	3·6	+ +
	1·0	1·5	2·5	4·0	+ +
	1·15	1·35	2·5	4·6	– –
	1·3	1·2	2·5	5·2	– –
	1·5	1·0	2·5	6·0	– –
	1·7	0·8	2·5	6·8	– –

* Test cultures were always made in duplicate.

This method in one way departs from the methods of practical disinfection. A disinfectant is in practice added to a mixture of organic matter and bacteria; in the method above the disinfectant is previously mixed with the organic matter and the bacteria are added subsequently. It was conceivable that the latter order of mixing might impose a harder test upon the disinfectant than was necessary. Comparative experiments were therefore made using either method, in the one case the test was carried out as already described, in the other case the bacteria were introduced into the solution of faeces and the disinfectant added to this mixture. No significant difference was found between experiments using the two methods.

	Disinfectant added before culture. Concentration necessary for 15 minutes disinfection, parts per 1000	Disinfectant added after culture. Concentration necessary for 15 minutes disinfection, parts per 1000
Exp. 16. 7. 07	5·0	4·4
Exp. 19. 7. 07	4·8	4·8

We are not particularly enamoured with the method of testing a disinfectant in the presence of three per cent. dried faeces. In many respects it departs from the ideal, but by its means the germicidal value of a disinfectant can be approximately measured under conditions such as it may not unlikely encounter in practice. We realise that disinfectants are usually employed in a very casual way and quite as commonly as deodorizers as with a view to the sterilisation of the germs of disease. But there are times when the medical man or sanitarian

wants to know what reliance he can place upon this or that disinfectant for a particular purpose, and we think that a determination of the value of a disinfectant made in the presence of three per cent. faeces will, for the majority of purposes, be more valuable than when made in distilled water. Everyone, we presume, would agree as to the absurdity of forming an estimate of the utility of an oxidising disinfectant, such as permanganate of potash, by determining its germicidal value upon a few bacteria in distilled water, or to accept the statement that 1 in 10,000 parts of mercuric chloride would be a reliable disinfectant for pus, because this concentration will suffice in distilled water to destroy 6,000,000 *B. typhosus* per c.c. in 15 minutes at 20° C. Except on a few occasions, for surgical purposes, a disinfectant will have to act in the presence of more or less particulate organic matter, and we have chosen that form which will be present in one of the common cases for which disinfectants are employed.

Our observations have clearly shown that just as the value of oxidisers will, from their nature, be depreciated in the presence of oxidisable matter other than the bacteria, and mercurial preparations by the presence of proteid or other material with which mercury combines, so emulsified disinfectants from their very nature will be diminished in value by the adsorption of the emulsion of tar-acids upon a great variety of particles of organic matter, if such be present.

BIBLIOGRAPHY.

BAXTER (1875). *Report of the Medical Officer of the Local Government Board*, New Series, no. 5, 1875, Appendix, pp. 216 to 256.

BAYLISS (1906). On some aspects of adsorption phenomena, with especial reference to the action of electrolytes and to the ash constituents of proteins. *Biochemical Journal*, vol. I. p. 175.

BEHRING (1890). Ueber Desinfection, Desinfectionsmittel und Desinfectionsmethoden. *Zeitschr. f. Hygiene*, vol. IX. p. 396.

BLYTH, WYNTER (1906). The Standardisation of Disinfectants. *Analyst*, May. *Journ. Soc. Chem. Industry*, vol. XXV. Dec.

BUCHHOLTZ (1875). Antiseptica und Bakterien. *Archiv f. experiment. Path. u. Pharm.*, vol. IX. p. 1.

CHICK, H. (1908). An Investigation of the Laws of Disinfection. *Journ. of Hygiene*, vol. VIII. p. 92.

DE LA CROIX (1881). Das Verhalten der Bakterien des Fleischwassers gegen einige Antiseptica. *Archiv f. experiment. Path. u. Pharm.*, vol. XIII. p. 175.

ESMARCH (1887). Das Creolin. *Centralb. f. Bakteriologie*, I. Abt. vol. II. pp. 295 and 329.

EYRE. Bacteriological Technique.

FIRTH and MACFADYEN (1906). Report of the Disinfectant Standardisation Committee. *Journ. of Roy. Sanitary Inst.*, vol. XXVII. p. 17.

FOWLER (1906). The working efficiency of some disinfectants. *Journ. Roy. Army Medl. Corps*, vol. VI. p. 6.

FRAENKEL (1889). Die desinficirenden Eigenschaften der Kresole, ein Beitrag zur Desinfectionsfrage. *Zeitschr. f. Hygiene*, vol. VI. p. 521.

GEPPERT (1889). Zur Lehre von dem Antisepticis. *Berl. klin. Wochenschr.* 1889, pp. 789 and 819.

—— (1891). Zur Desinfectionsfrage. *Deutsch. med. Wochenschr.*, no. 25, pp. 797, 829 and 855.

GRUBER (1891). Transactions of the VII. Internat. Congress of Hygiene and Demography, vol. II. p. 255. *Centralb. f. Bakt.* I. Abt. vol. XI. 1892, p. 115.

HEIDER (1892). Ueber die Wirksamkeit der Desinfectionsmittel bei erhöhter Temperatur. *Archiv f. Hygiene*, vol. XV. p. 341.

HENLE (1889). Ueber Creolin und seine wirksamen Bestandtheile. *Archiv f. Hygiene*, vol. IX. p. 188.

KAHLENBERG (1901). The Theory of Electrolytic Dissociation as viewed in the light of facts recently ascertained. *Journ. of Phys. Chem.*, vol. V. p. 349.

KENWOOD and HEWLETT (1906). Some observations upon the Practical Standardisation of Disinfectants. *Journ. of the Roy. San. Inst.*, vol. XXVII. p. 1.

—— (1906). The Practical Standardisation of Disinfectants. *Public Health.* April, p. 462.

KOCH (1881). Ueber Desinfection. *Mittheil. a. d. kaiserl. Gesundheitsamte*, vol. I.

KRÖNIG and PAUL (1897). Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. *Zeitschr. f. Hygiene*, vol. XXV. p. 1.

LUTHER (1904). Die Hydrolyse des Quecksilberchlorids. *Zeitschr. f. physik. Chem.*, vol. XLVII. p. 107.

MADSEN and NYMAN (1907). Zur Theorie der Desinfektion. *Zeitschr. f. Hygiene*, vol. LVII. p. 388.

PAUL (1901). Entwurf zur einheitlichen Werthbestimmung chemischer Desinfektionsmittel. Berlin, 1901.

PAUL and PRALL (1907). Die Werthbestimmung von Desinfectionsmittel mit Staphylococcen die bei der Temperatur der flüssigen Luft aufbewahrt wurden. *Arbeit. aus dem kaiserl. Gesundheitsamte*, vol. XXVI. p. 73.

PRINGLE (1750) I. Some Experiments on Substances resisting Putrefaction. *Phil. Trans.*, vol. XLVI. p. 480.

—— (1750) II. A Continuation of the Experiments on Substances resisting Putrefaction. *Phil. Trans.*, vol. XLVI. p. 525.

PROSKAUER (1908). Einheitliche Regelung der Prüfungsmethodik für Desinfektionsapparate und Desinfektionsmittel. *Ber. v. d. XIV. Internat. Kongr. f. Hyg. u. Dem.* Berlin, 1907, vol. II. p. 973.

RIDEAL and WALKER (1903). The Standardisation of Disinfectants. *Journ. of the Roy. San. Inst.*, vol. XXIV. p. 424.

SCHMIDT (1894). Ueber Adsorption. *Zeitschr. f. physikal. Chemie*, vol. XV. p. 56.

SELIGMANN (1908). Discussion upon Proskauer's paper above. *Ber. v. d. XIV. Internat. Kongress f. Hyg. u. Dem.* Berlin, 1907, vol. IV. p. 373.

SOMERVILLE and WALKER (1906) I. The Rideal-Walker method of standardising disinfectants. *Public Health.* March, 1906.

—— (1906) II. Standardisation of Disinfectants. *Public Health.* May, p. 526.

—— (1906) III. Note on the standardisation of disinfectants in the presence of organic matter. *Sanitary Record.* Nov. 29th, 1906.

—— (1907). The Standardisation of Disinfectants in the presence of organic matter. *Sanitary Record.* May 9th, 1907.

WATSON (1908). *Journ. of Hygiene*, vol. VIII. p. 536.