The bacteriological examination of disinfectants / by William Partridge ; with a preface by C.E.P. Fowler.

THE BACTERIOLOGICAL EXAMINATION OF DISINFECTANTS

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BY

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WITH A PREFACE BY

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PREFACE.

The author has asked me to look over the proof sheets of these pages and write a short preface, giving my views on the present position of the question of disinfectant standardisation. I can add little to the knowledge, which may be gleaned from a perusal of these pages, in which the question is discussed in a fair and unbiassed manner. The reader will understand how extremely difficult it is, at the present time, to form any hard-and-fast rules regarding standardisation.

The old method of chemical standardisation is done with; as by it we can obtain no clue to the germicidal action of disinfectants, and this is the information required in order to obtain their real working value. Various methods have been employed with this end in view, the best known of which are the "Garnet," the "Thread," and the "Rideal-Walker" Details of these will be found in the text. It is sufficient here to state that the "Garnet" has been generally discarded as untrustworthy. The "Thread" has some adherents, but the technique required is extremely elaborate. There remains the "Rideal-Walker," which has been very largely used since its introduction some three years ago. The technique is simple, and the conclusions are rapidly arrived at. Lately the method has undergone severe criticism, on the ground that it may show false values for disinfectants when tested against organisms in distilled water only; but this same criticism must apply to whatever method is employed, and every one is now agreed that the introduction of some form of organic matter, in carrying out experimental work, is absolutely essential. Opinions regarding the

quality and quantity which it is necessary to employ have been, and are still, very greatly at variance. Taking a general review of the situation, the case stands thus:—

A moderate amount of solid organic matter, such as 1 per cent. or 2 per cent., is found amply sufficient to lower the possibly false values of disinfectants, obtained against a pure culture in distilled water, and yet to allow a varying range to others, which are stable in the presence of this amount of organic material.

A large quantity of solid organic matter, such as 10 per cent. or 15 per cent., is found to bring all disinfectant values to almost one dead level.

In practical disinfection, what have we to deal with? A moderate or an excessive amount of organic material in which the organisms are embedded? On the answer to this question lies the *rationale* of adapting our experimental work. Personally, I am of opinion that the moderate amount is the right figure to make use of, as by it we obtain the true relative values, without obscuring these altogether by an overwhelming addition of organic matter. Again, it can by no means be claimed that practical disinfection always has to be carried out in the presence of such a vast amount of organic material; in medical practice the reverse is certainly the case.

To sum up the technique which it is recommended to employ when asked to pass an opinion on a disinfectant, it may be stated as follows:—

Ascertain the value by the "Rideal-Walker" method against a pure culture, making the dilutions with distilled water, then confirm this value by carrying out the same technique, but employing 1 or 2 per cent. of some solid organic material in making the dilutions, as recommended in the Sommerville-Walker modification of the test.

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Introduction.

WHETHER it be employed as a curative or prophylactic agent, a disinfectant is used with the intention of killing the various forms of micro-organisms which may, or may not be pathogenic, but whose existence in either an active or dormant state is not desired. The synonym "germicide" exactly expresses what the function of a true disinfectant should be—a killer of germs.

There is curiously, but most unfortunately, a widely prevalent idea that the word antiseptic is synonymous with disinfectant. This fallacy is soon revealed when we learn that the utmost expected of a preparation of an antiseptic nature is that it should inhibit or retard the growth of micro-organisms. For example, two of the antiseptics most commonly used in the preparation of alimentary substances are sugar and salt. These, for a time at any rate, prevent the multiplication and growth of fungi and putrefactive organisms, but they cannot be credited with any germicidal powers.

The third class of preparations with which we have any concern here are the "deodorants"; these may or may not be disinfectants. There are two considerations which appear to account for the widespread belief in the beneficial action of these substances. The first is a purely æsthetic desire, which demands that odours objectionable to our olfactory organs should be eliminated, though it is to be feared that many preparations used for this reason possess odours scarcely preferable to those of decomposing organic matter. The second and perhaps the more prevalent reason is due to the delusion that the bad smells *themselves* cause the disease, and that by overcoming the smells the danger of infection is removed. This is

a half-truth, inasmuch as bad smells are undoubtedly a provision of Nature to drive people away from their neighbourhood; but it is conceivable to all who understand anything of the acquirement of disease from bacterial sources, that stopping a smell or overwhelming it with a stronger smell does not necessarily indicate the coincident destruction of the pathogenic power of the disease-producing organism. A point in the indictment of deodorants that are not germicides, therefore, is that they engender a false sense of security, which for obvious reasons must be highly detrimental to the public health.

CHAPTER I.

The Ideal Disinfectant.

In connection with the query as to what is an ideal disinfectant, we must consider the several virtues which a germicide may possess, and which, although not all of equal import, are often adduced in favour of alleged disinfectants as though they were.

(*a*) *It should possess a high germicidal power.* This is undoubtedly by far the most important qualification, as it is obvious that should a disinfectant not possess marked germicidal properties, any other laudatory characters that might be pleaded on its behalf will not warrant its claim to the appellation of disinfectant. Germicidal efficiency must therefore be a *sine quâ non.*

(*b*) *It should be capable of being used in the presence of organic matter without any great diminution of its germicidal power.* A disinfectant which becomes a mere placebo in the disinfection of fæces and urine may be eminently suitable for disinfecting the hands; but it is evident that such a preparation will have but a limited application when compared with a disinfectant against which this objection cannot be sustained. The inability to conform to this requirement is a very strong point against chloride of lime and other oxidising agents, and against mercuric chloride, the employment of which, in some instances, affords a farcical display of ignorance on the part of those responsible. Mercuric chloride is precipitated by albumin, soap, &c., and is, moreover, readily converted into an insoluble sulphide when brought in contact with organic matter containing sulphur, which is quite inert so far as germicidal action is concerned. In this connection it is advisable to emphasise the desirability of having a disinfectant which is capable of being used with soap. Floors and other articles, as well as the skin, are often washed with soap before disinfection,

and where it is necessary to get rid of all traces of soap before applying the disinfectant, precious time is often wasted. Moreover, it is not always possible to obtain sufficient hot water with which to remove the soap before applying the disinfectant—in district nursing this want is felt most acutely.

(*c*) *It should be homogeneous and capable of retaining its homogeneity.* A preparation which in a cask will separate out into what has been aptly described as "a thin serum at the top and something like putty at the bottom," carries its own condemnation.

(*d*) *It should yield a solution or emulsion in all proportions.* This is too obvious to require further comment.

(*e*) *It should be innocuous to man and the higher animals.* Fatal cases are constantly being reported owing to the accidental or wilful ingestion of such toxic substances as carbolic acid and corrosive sublimate. In many cases poisonous disinfectants are handled and administered by persons unacquainted with their toxic nature; and in the interests of humanity it is desirable that when poisonous disinfectants can be replaced by innocuous ones, the latter should be employed.

(*f*) *It should be free from corrosive action on the skin and on metals.* A disinfectant having a corrosive action on metallic surfaces is obviously inadmissible for disinfecting instruments or metallic objects, and cannot be stored in metallic receptacles or used in sprayers, &c., having metal fittings. The objection to a disinfectant having any caustic action on the skin is patent, and it is preferable to choose one that, further, has no action on the mucous membrane.

(*g*) *It should have the power of penetration.* A disinfectant that may easily kill bacteria when the latter are isolated, and yet has not the property of so acting when the bacteria are more or less enveloped in a covering of animal matter, obviously fails, and it is because of their efficiency in this respect that disinfectants of a saponaceous nature have become so popular of recent years.

(*h*) *It should be reasonable in price when diluted to a working solution.* There appears to be a great demand among certain sanitary authorities for a "concentrated" (*sic*) disinfectant "at a shilling a gallon," and it is pro-

bable that a disinfectant at this price that could not be diluted with four times its volume of water would be received by these authorities with greater favour than one at four shillings a gallon that would bear dilution with four hundred volumes of water!

(*i*) *It should be a deodorant.* The best way to eradicate an evil is to remove the cause, and this applies to smells as well as to other evils. A deodorant that is not a disinfectant is worthless from a utilitarian standpoint, and is useful only to allay fastidious qualms. A deodorant such as permanganate which deodorises by an oxidation action has been shown to oxidise the organic matter *before* exercising its germicidal action, and therefore its employment necessitates the use of a larger quantity of the disinfectant than is necessary to oxidise all the organic matter. Other deodorants such as formalin and sulphur dioxide have an irritating action on the mucous membranes, which for many purposes renders them objectionable. Where a disinfectant is employed in the form of a powder, the base of such a preparation should be composed of lime, which has a high capacity for absorbing sulphuretted hydrogen and other gases having an offensive smell. Unfortunately, the use of this base is inadmissible with carbolic acid, but it can be employed with certain coal tar disinfectants.

CHAPTER II.

The Fallacy of Chemical Methods.

No sane person will deny the need for efficiency in disinfectants, and all who have attempted to compare the relative values of such preparations are agreed as to the desirability of adopting a standard method of test; but, unfortunately, there is a wide diversity of opinion as to the exact nature of the method to be adopted. Some authorities still adhere to methods based on chemical analysis, whilst others favour processes of a bacteriological nature.

Unquestionably, certain disinfectants of definite chemical composition, and answering to well-known chemical tests, can be quickly assayed by chemical means. The available chlorine of chloride of lime, the valuation of sulphurous acid, the available oxygen of permanganate compounds, and the estimation of mercuric chloride, can be determined accurately by chemical analysis. But it is now generally admitted that the data obtained by such tests are of little value in assisting us to solve the problems of practical disinfection.

Carbolic acid and its preparations, together with disinfectants containing substances allied to carbolic acid, comprise a very large and important class of germicides. The assay of such preparations by chemical methods, by processes which were quite permissible and legitimate when true carbolic acid comprised the whole, or nearly the whole, of the commercial article, is quite as unreliable as those above referred to; for the demand for carbolic acid has increased to such an extent that the commercial article, as sold for disinfecting purposes, is, as a rule, quite innocent of true phenol, its place being taken by other tar acids. These tar acids have not all the same germicidal power, and are only slightly soluble in water. What can be the value from a disinfecting standpoint of "carbolic

acid " that is scarcely soluble in 500 times its weight of water ?

The greatest objection to chemical tests is the fact that they are inapplicable to the assay of disinfectants whose germicidal powers are dependent on physical conditions as well as on chemical composition. Rideal and Walker found, for example, that a disinfectant containing 10 per cent. of tricresol *in emulsion*, was equal in bactericidal power to one containing 30 per cent. *in solution*, when tested against B. typhosus. Chemical analysis is, therefore, not to be depended on, and the only process by which we can hope to ascertain in the laboratory the possible efficiency of a disinfectant in the sphere in which it is intended to be used, will be one based on the determination of the strengths of the disinfectant that will kill bacteria in a reasonable time—a bacteriological process in fact.

CHAPTER III

The Carbolic Acid Coefficient.

It is a matter of no small difficulty to those purchasing disinfectants on a large scale to ascertain the relative values of the various disinfectants with which they are acquainted. It may be argued that this difficulty is easily overcome by calculating the cost of a certain quantity of the disinfectant when diluted ready for use, the amount of dilution being that prescribed by the manufacturers of the disinfectant. If the amount of dilution recommended were based on bacteriological examinations carried out in a systematic manner, such a procedure would be quite admissible; but we are all too familiar with the confusion arising out of the erroneous interpretations of results obtained by the tests that have done duty in the past.

If, however, all disinfectants be compared with reference to their germicidal powers, with some standard disinfectant such as carbolic acid, and their germicidal properties be expressed in terms of same, we shall have at our disposal a ready means of comparing the different disinfectants. Such an expression of germicidal activity has been proposed, and is known as "the carbolic acid coefficient." The figure expressing this is arrived at by dividing the strength of a disinfectant which will kill a certain organism in a given time, by the strength of carbolic acid solution required to kill the same organism, in the same time, and under exactly similar conditions. For example, if a 1 in 250 solution of a certain disinfectant "X" will kill a certain strain of the typhoid bacillus in ten minutes, and a 1 in 100 solution of carbolic acid will kill the same strain of bacillus in the same time, the carbolic acid coefficient of "X" will be $\frac{250}{100} = 2{\cdot}5$. Similarly, when dealing with a disinfectant of smaller bactericidal power than carbolic acid, if a 1 in 70

dilution will perform the same task as a 1 in 100 solution of carbolic acid in the same time, against the same organism and under the same conditions, the carbolic acid coefficient of this disinfectant will be $\frac{70}{100} = 0{\cdot}70$.

The method of using the carbolic acid coefficient in practice will best be shown by the following table of results; the conclusions to be drawn from these results will be obvious:—

Sample.	Organism.	Carbolic Acid Coefficient.	Price per gal. or 10½ lb.	Cost of Disinfectant equivalent to one gallon of Carbolic Acid.
			s. d.	£ s. d.
A	B. typhosus	0·02	3 6	8 15 0
B	,,	0·30	5 6	0 18 4
C	,,	15·00	4 0	0 0 3
D	,,	8·00	4 0	0 0 6
Carbolic acid	,,	1·00	1 0	0 1 0
F	,,	2·50	8 0	0 3 2
G	,,	1·40	2 6	0 1 9
H	,,	0·30	157 6	26 5 0
I	,,	0·10	18 9	9 7 6
J	,,	0·10	15 0	7 10 0
K	,,	0·90	7 6	0 8 4
L	,,	2·50	3 6	0 1 5
M	,,	0·03	40 0	66 13 4

The cost of disinfectant equivalent to one gallon carbolic acid—*i.e.*, capable of performing the work of one gallon carbolic acid—is obtained by dividing the price per gallon by the carbolic coefficient.

CHAPTER IV.

Principal Factors to be Recognised in the Rideal-Walker Method.

In the choice of a bacteriological process there are several points that must be considered, and it was failure to recognise these factors which led to so many discrepancies in the work of different bacteriologists in the past. It will readily be understood on a consideration of the subject that the process must be performed under certain conditions, which, although they may appear to be, or may really be, arbitrarily chosen, are absolutely necessary, owing to the intricate and delicate nature of the investigation. In the original process these conditions were chosen as the outcome of many experiments, and were formulated to ensure uniformity of result with the minimum of labour. These conditions appear at first sight to render the process an extremely elaborate one; but a brief consideration will show that recognition of the standard conditions entails but little extra trouble. A bacteriological process often calls for greater attention from the investigator than is customary with a chemical process, since the investigator cannot afford to disregard the peculiarities of the micro-organisms, which vary so greatly, even in different strains of the same species.

The following are the principal factors to be recognised in the Rideal-Walker method, which has recently been recommended for general purposes by the Disinfectant Standardisation Committee of the Royal Sanitary Institute :—

1. Time.
2. Age of Culture.
3. Choice of Medium. Reaction of same.
4. Temperature of Incubation.
5. Temperature of Medication.
6. Variations in vital resistance of same species.

7. Variations in vital resistance of different species.
8. Proportion of culture to disinfectant.

1. *Time.*—In the Rideal-Walker process, *time* is taken as the constant and the *strength of disinfectant* as the variant. Should this procedure be reversed, and time be taken as the variant and strength of disinfectant as the constant, totally erroneous results will be obtained. This statement will be realised by reference to Tables I. and II., in which *time* is taken as the constant in the former and as the variant in the latter.

2. *Age of Culture.*—Tables III. and IIIA show the influence of this factor, as also the manner in which it is controlled by the standard solution of carbolic acid. In the absence of the latter the disinfectant in Table III. would appear stronger than that in Table IIIA, although in reality they are one and the same. This difference is entirely due to the fact that a broth culture of B. typhosus is more vigorous after 48 hours than one grown in 24 hours, and emphasises the necessity for the introduction of a standard control disinfectant. In working with organisms such as diphtheria bacilli, which do not grow rapidly, a 48-hour culture may be desirable, but for the typhoid bacillus, the colon bacillus, staphylococcus pyogenes aureus, and others, a 24-hour culture is invariably used.

3. *Choice of Medium, Reaction of same.*—The choice of medium for the cultivation of an organism growing at blood-heat is practically restricted to broth and agar. In agar cultures part of the growth is taken up on the point of an inoculating needle and rubbed up in sterilised water. The culture thus prepared is not so convenient to use as a broth culture, and, moreover, gives a higher coefficient. (See Tables IV. and IVA.) But the reaction of the medium is a factor of even greater importance, as will be seen by reference to Table IVB, in which the reactions of the media correspond to $+0{\cdot}6$ and $-0{\cdot}2$ respectively. The American Public Health Association in 1898 adopted a reaction of $+1{\cdot}5$ per cent. as the best for general work, and this is the reaction recommended in the Rideal-Walker

* The tables given in this chapter are reproduced from the original paper by Dr. Rideal and Mr. Ainslie Walker, and through the kindness of these gentlemen I am permitted to reproduce them here.

TABLE I.—*B. Coli*, 24 *Hours Broth Culture at* 37 *deg. Cent.*—Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
									Deg. C.
Disinfectant No. 1 (containing 19·5 per cent. cresols)	1 : 200	×	×	.	.	.	.	48 hours	37
Disinfectant No. 2 (containing 10·1 per cent. cresols)	1 : 100	×	×	.	.	.	.	,,	,,

True relative values = 2 : 1.

TABLE II.—*B Coli*, 24 *Hours Broth Culture at* 37 *deg. Cent.*—Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		5	10	45	50	55	60	Period of incubation.	Temperature.
									Deg. C.
Disinfectant No. 1 (containing 19·9 per cent. cresols)	1 : 200	×	.	.	.	.	.	48 hours	37
Disinfectant No. 2 (containing 10·1 per cent. cresols)	1 : 200	×	×	×	×	×	.	,,	,,

Apparent relative value = 11 : 1.

TABLE III.—*B. Typhosus*, 24 *Hours Broth Culture at* 37 *deg. Cent.*—Room temperature, 15·1 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		$2\frac{1}{2}$	5	$7\frac{1}{2}$	10	$12\frac{1}{2}$	15	Period of incubation.	Temperature.
									Deg. C.
Fluid W[1]	1 : 90	.	.	.	.	.	.	48 hours	37
Fluid W[1]	1 : 100	×	.	.	.	.	.	,,	,,
Carbolic acid...	1 : 90	.	.	.	.	.	.	,,	,,
Carbolic acid...	1 : 100	×	.	.	.	.	.	,,	,,
Carbolic acid...	1 : 110	×	×	×	.	.	.	,,	,,

Carbolic acid coefficient = 1·0.

TABLE IIIA.—*B. Typhosus*, 48 *Hours Broth Culture at* 37 *deg. Cent.*—Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Fluid W1	1 : 90	×	.	.	.	.	.	48 hours	Deg. C. 37
Fluid W1	1 : 100	×	×	×	.	.	.	,,	,,
Carbolic acid...	1 : 90	×	.	.	.	.	.	,,	,,
Carbolic acid...	1 : 100	×	×	×	.	.	.	,,	,,
Carbolic acid...	1 : 110	×	×	×	×	×	×	,,	,,

Carbolic acid coefficient = 1·0.

TABLE IV.—*B. Coli*, 24 *Hours Broth Culture at* 37 *deg. Cent.*—Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Disinfectant A	1 : 1000	×	×	.	.	.	.	48 hours	Deg. C. 37
Disinfectant Z	1 : 800	×	×	.	.	.	.	,,	,,
Carbolic acid...	1 : 100	×	×	.	.	.	.	,,	,

Coefficients { A = 10·0. Z = 8·0.

TABLE IVA.—*B. Coli*, 24 *Hours Agar Culture at* 37 *deg. Cent.*—Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cul ures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temper-ature.
Disinfectant A	1 : 1500	×	×	.	.	.	.	48 hours	Deg. C· 37
Disinfectant Z	1 : 1200	×	×	.	.	.	.	,,	,,
Carbolic acid...	1 : 120	×	×	.	.	.	.	,,	,,

Coefficients { A = 12·5. Z = 10·0,

TABLE IVB.—*B Typhosus*, 24 *Hours Broth Culture at* 37 *deg. Cent.*—Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
									Deg. C.
Carbolic acid[1]	1 : 90	×	×	.	.	.	.	48 hours	37
Carbolic acid[2]	1 : 120	×	×	.	.	.	.	,,	,,

[1] Culture grown in broth, acid to phenolphthalein 100 c.c. = 0·6 Normal NaHo.
[2] Culture grown in broth, alkaline to phenolphthalein 100 c.c. = 0·2 Normal HCl.
N.B.—Both were alkaline to Litmus paper.

process, and, except where otherwise stated, is the one adopted in the tables appearing in this volume. It is scarcely necessary to indicate that this acid broth is only recommended for use in cases where the typhoid bacillus is the test organism. In those cases where such organisms as the cholera vibrio and the bacillus of diphtheria are employed, neutral or alkaline broth must be substituted. The directions for making the nutrient broth are given in a later chapter on "Apparatus and Materials."

4. *Temperature of Incubation.*—If the culture of the test organism be incubated at a temperature at which it has been found to grow best, it is only to be expected that a more vigorous growth will be obtained at this temperature than if it were grown at a lower temperature. Nevertheless, this will not alter the carbolic acid coefficient, although it may be necessary to alter the strength of the standard control to suit the altered resistance of the organisms.

It will be noticed in Tables V. and VA, which are given to illustrate this point, that the culture grown at 22 deg. Cent. was allowed 48 hours' incubation, as against 24 hours in the case of the other culture, and would, but for the unsuitability of the temperature, have shown greater resistance than that grown at 37 deg. Cent.

5. *Temperature of Medication.*—It is a recognised fact that the higher the temperature (within certain limits) at which a disinfectant acts on the test organism the greater its efficiency, and this renders the recognition of this factor a matter of prime importance. It will be seen from Tables VI. and VIA that carbolic acid is at least 50 per cent. more efficient at 37 deg. Cent. than at 16 deg. Cent. It is therefore necessary to fix on some temperature which is generally easy of attainment, and for this reason the range from 15 deg. Cent. to 18 deg. Cent. has been adopted in the process. The investigator is at liberty to perform the test at any temperature he pleases, as the carbolic acid control will be a sufficient safeguard. The range from 15 deg. to 18 deg. Cent. is suggested more for the convenience of the operator, since if the temperature varies from day to day to the extent of 5 deg. or 10 deg. he will find that what was a suitable dilution of carbolic acid for the one day's test, will be entirely useless on the next.

6. *Variations in Vital Resistance of the same*

TABLE V.—*B. Typhosus* (*S.S*), 48 *hours Broth Culture at* 22 *deg. Cent.* Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Fluid W²	1 : 90	×	.	.	.	.	.	72 hours	37·5° C.
Carbolic acid...	1 : 120	×	.	.	.	.	.	,,	,,

Carbolic acid coefficient = 0·75.

TABLE VA.—*B. Typhosus* (*S.S*), 24 *hours Broth Culture at* 37 *deg Cent.* Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Fluid W²	1 : 90	×	×	×	.	.	.	72 hours	37·5° C.
Carbolic acid...	1 : 110	×	.	.	.	.	.	,,	,,
Carbolic acid...	1 : 133	×	×	×	×	×	×	,,	,,

Carbolic acid coefficient = 0·75 (average of 0·82 and 0·67).

TABLE VI.—*B. Coli* (*Escherich*), 24 *hours Broth Culture at* 37 *deg. Cent.* Room temperature, 16 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Carbolic acid.	1 : 70	.	.	.	.	.	.	48 hours	37° C.
,, ,, ...	1 : 80	×	.	.	.	.	.	,,	,,
,, ,, ...	1 : 90	×	×	×	.	.	.	,,	,,
,, ,, ...	1 : 100	×	×	×	×	×	×	,,	,,
,, ,, ...	1 : 110	×	×	×	×	×	×	,,	,,

TABLE VIA.—*B. Coli (Escherich)*, 24 *hours Broth Culture at* 37 *deg. Cent.* Room temperature, 37 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Carbolic acid...	1 : 70	.	.	.	.	.	.	48 hours	37° C.
,, ,, ...	1 : 80	.	.	.	.	.	.	,,	,,
,, ,, ...	1 : 90	.	.	.	.	.	.	,,	,,
,, ,, ...	1 : 100	.	.	.	.	.	.	,,	,,
,, ,, ...	1 : 110	.	.	.	.	.	.	,,	,,

TABLE VII.—*Staph. p. aureus*, 24 *hours Broth Culture at* 37 *deg. Cent.* Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Carbolic acid[1]	1 : 70	×	×	.	.	.	.	96 hours	37° C.
Carbolic acid[2]	1 : 90	×	×	.	.	.	.	,,	,,

[1] Culture obtained from Major Firth. [2] Culture obtained from Dr. Klein.

TABLE VIIA.—*B. Typhosus*, 24 *hours Broth Culture at* 37 *deg. Cent.* Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Carbolic acid[1]	1 : 70	×	×	.	.	.	.	48 hours	37° C.
Carbolic acid[2]	1 : 100	×	×	.	.	.	.	,,	,,

[1] Culture obtained from Major Firth. [2] Culture obtained from Dr. Ridea

TABLE VIII.—*Staph. p. aureus*, 24 *hours Broth Culture at* 37 *deg. Cent.* Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Disinfectant W³	1 : 80	×	×	×	.	.	.	48 hours	37°
Carbolic acid...	1 : 80	×	×	×	.	.	.	,,	,,

Carbolic acid coefficient = 1·0.

TABLE VIIIA.—*B. Typhosus* (*Kral*), 24 *hours Broth Culture at* 37 *deg. Cent* Room temperature, 15-18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
Disinfectant W³	1 : 250	×	×	.	.	.	.	48 hours	37° C.
Carbolic acid...	1 : 80	×	×	.	.	.	.	,,	,,

Carbolic acid coefficient = 3·1.

TABLE IX.—*B. Typhosus* (*Kral*), 24 *hours Broth Culture at* 37 *deg. Cent.* (*Taking* 5 *c c. Diluted Disinfectant* + 5 *c.c. Broth Culture*). Room temperature, 15 deg.–18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		$2\frac{1}{2}$	5	$7\frac{1}{2}$	10	$12\frac{1}{2}$	15	Period of incubation.	Temperature.
	*								
Disinfectant $A^2/03$	1 : 450	×	×	.	.	.	.	48 hours	37° C.
,, ,, ...	1 : 485	×	×	×	.	.	.	,,	,,
,, ,, ...	1 : 525	×	×	×	×	×	.	,,	,,
Carbolic acid	1 : 75	×	×	×	.	.	.	,,	,,

Coefficient $\frac{4.8.5}{7.5} = 6{\cdot}5$. * Allowing for extra diluent introduced with culture.

TABLE IXA.—*B. Typhosus* (*Kral*), 24 *hours Broth Culture at* 37 *deg. Cent.* (*Taking* 5 *c.c. Diluted Disinfectant* + 5 *drops Broth Culture*). Room temperature, 15 deg.–18 deg. Cent.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2¼	5	7½	10	12½	15	Period of incubation.	Temper. ture.
Disinfectant A²/03	1 : 700	.	.	.	.	.	.	48 hours	37° C.
,, ,, ...	1 : 800	×	.	.	.	.	.	,,	,,
,, ,, ...	1 : 900	×	×	×	.	.	.	,,	,,
Carbolic acid	1 : 80	×	×	×	.	.	.	,,	,,

Coefficient $\frac{900}{80} = 11 \cdot 2$,

Species.—If a broth culture of an organism, such as Bacillus typhosus, be made from an agar culture of the organism, and a sub-culture be made 24 hours later from the first broth culture, the second broth culture will show a higher resistance to a disinfectant than the first; should a third sub-culture be made from the second when the latter is 24 hours old, this will be stronger than the second, and so on, to a certain point, after which no appreciable increase in resistance will be observed. Although this difference in resistance will be shown and controlled by the standard solution of carbolic acid, it is necessary to recognise it, in order to facilitate the choice of the particular strength of the control to be employed in the test. Further, cultures obtained from different sources may have different powers of resistance. This is exemplified in Tables VII. and VIIA.

7. *Variations in Vital Resistance of Different Species.*—Most disinfectants show different coefficients when tested against different organisms. For example, a well-known disinfectant has the following carbolic acid coefficients for the organisms specified:—

B. typhosus	11·0
Staphylococcus pyogenes aureus	9·3
B. pestis	34·0
B. tuberculosis	11·0
B. dysenteriæ	10·0
B. diphtheriæ	20·0
Vibrio choleræ	32·3
B. mallei	15·0

Tables VIII. and VIIIA. show the carbolic acid coefficients of a coal tar disinfectant for Staphylococcus pyogenes aureus and Bacillus typhosus respectively.

8. *Proportion of Culture to Disinfectant.*—As will be seen in Tables IX. and IXA., no uniformity of result can be obtained where this factor is neglected. The proportion adopted in the Rideal-Walker method is one drop of culture to every c.c. of disinfectant solution; thus, working with 3 c.c. of disinfectant solution, we should employ three drops (each approximately 0·1 c.c.) of the culture.

CHAPTER V.

Apparatus and Materials for Rideal-Walker Test.

Nutrient Broth.—As a general rule, nutrient broth is used both for the culture of the test organism and for the cultivation of the organism after exposure to the disinfectant. For the former, as mentioned in a previous chapter, an agar growth rubbed up in sterile distilled water is employed by some workers. The reason given for preferring this mode of procedure is that the use of broth is liable to unfavourably affect those disinfectants which would be depreciated by the organic matter of the broth. But as these organic substances, or similar ones, are common in nature, the employment of broth will only accentuate the value of the test by showing the value of the disinfectant in the presence of a slight amount of organic matter. A meat extract is used in place of meat in the preparation of the broth, because this ensures a more constant composition. Different brands of meat extract will be found to give different results, and for this reason it is desirable to employ one brand alone, and experience has shown Lemco to be in all respects very suitable for the purpose. It is prepared in the following manner:

Lemco	20 grammes.
Peptone (Witte's)	20 grammes.
Salt (sodium chloride)	10 grammes.
Distilled water	1 litre.

Boil the mixture for 30 minutes, filter, neutralise with normal sodium hydrate solution, using phenol-phthalein as an indicator. In order to avoid contaminating the broth with phenol phthalein, and also because it is a cumbersome performance to titrate so large a quantity as one litre, it is better to take an aliquot part of the hot filtered broth (say 10 c.c.), and titrate this with decinormal sodium hydrate, and then calculate the amount of normal sodium hydrate necessary for the

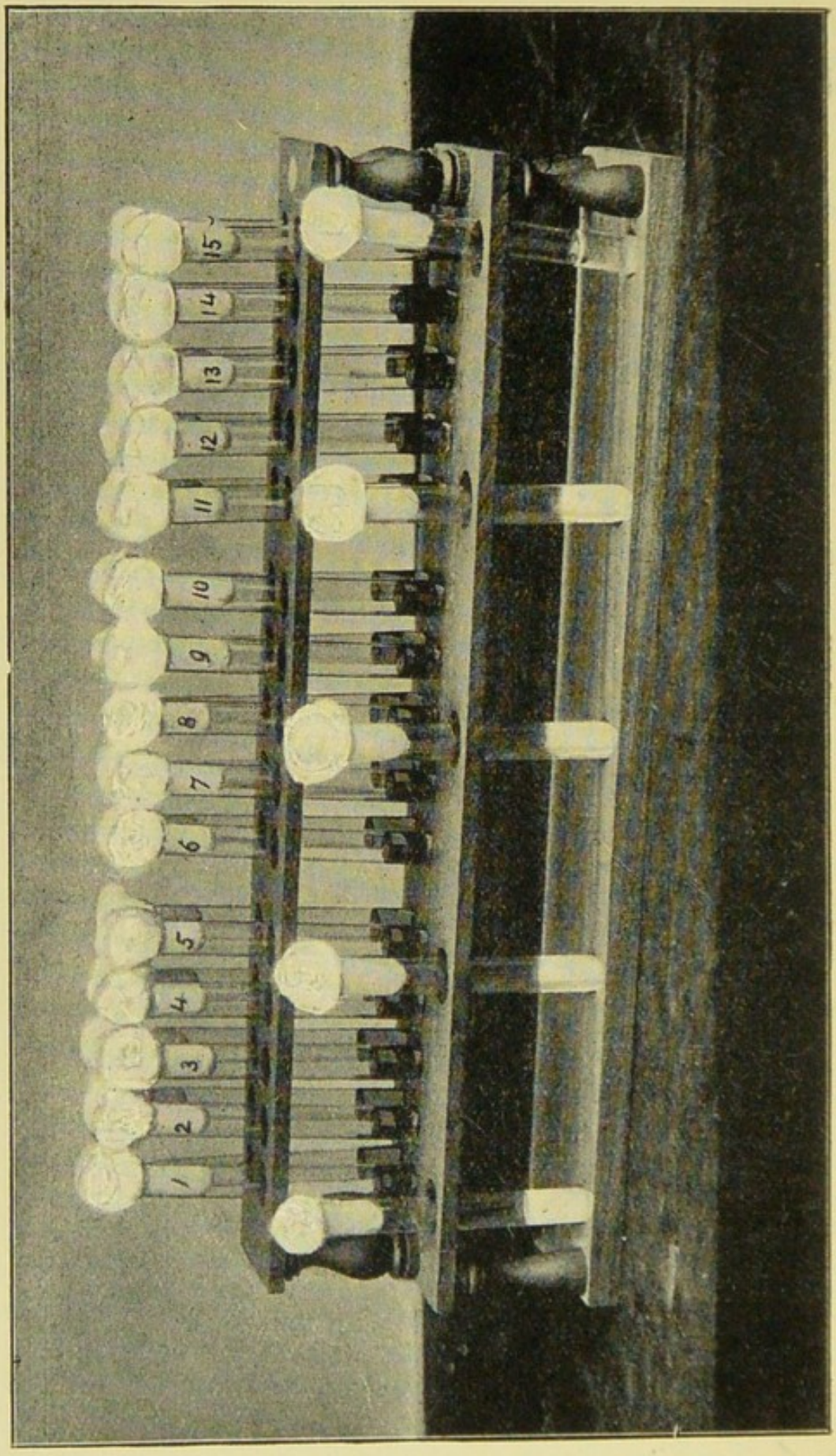

Fig. 1.—SHOWING GROUPING AND ARRANGEMENT OF TUBES.

neutralisation of the remainder of the broth. Add, when quite neutral, 15 c.c. of normal hydrochloric acid. This will give the broth a reaction of + 1·5 per cent. (the reaction recommended by the Standardisation Committee). The broth is then made up to a litre, and sterilised. About 5 c.c. are run into sterile test tubes, which, after plugging with sterile cotton wool, are placed in a steam steriliser for half an hour or so.

Standard Carbolic Acid.—The carbolic acid of the British Pharmacopœia contains 100 parts of pure phenol in every 110 parts of the acid, but it is very desirable to make up and standardise by titration with bromine a stock solution containing 5 per cent. carbolic acid. From this solution the working strengths are made up by diluting some comparatively large quantity, such as 100 c.c., to the desired volume. Such a procedure practically eliminates the error introduced by measuring out small quantities of strong acid.

Test Tube Rack.—A special rack (made by Baird and Tatlock) is used for this process (Fig. 1). The rack contains two tiers, the upper having spaces for 30 test tubes in two rows, and each row contains three sets of five holes, each of the latter corresponding to one of the five holes in the lower tier. The upper tier is for the unused broth tubes, each test tube being numbered by marking with a grease glass pencil. The lower tier is for four disinfectant dilutions and the control, the latter being placed in the fifth hole. This rack is rendered necessary owing to the fact that not more than 30 seconds are at the disposal of the worker for each inoculation. The test tubes being numbered in rotation, it will be seen that the medication tube (the tube containing disinfectant) in the first hole will be used for inoculating tubes 1, 6, 11, 16, 21, and 26, the second medication tube for tubes 2, 7, 12, 17, 22, and 27, and so on.

The Inoculating Needle (Fig. 2).—The needle used should be composed of thin aluminium rod, with a short piece of thin platinum wire passed through and twisted round an eye in the end of the rod. When working with liquids, as in this case, the platinum wire is made into a loop at the end, and bent down in the centre to allow of a fair-sized drop being taken up for each inoculation (Fig 2c). Satisfactory results cannot be expected when one broth tube is inoculated with a

fair-sized drop, as shown in the figure, and a mere film is introduced into another. After a little practice it is easy to obtain a satisfactory drop on the needle, which is got by dipping the needle into the medicated culture and bringing it out with a little jerk, not sufficient to throw the drop off the loop. The size of the loop used is about 4 mm. in diameter.

Test Tubes.—The test tubes should be of fairly strong glass, so as to minimise as far as possible the risk of breakage. A size 5in. × $\frac{5}{8}$in. will be found very suitable for use. The cotton wool plugs for both the medication tubes and broth tubes should be well-made, so that they can be withdrawn and reinserted with the minimum loss of time.

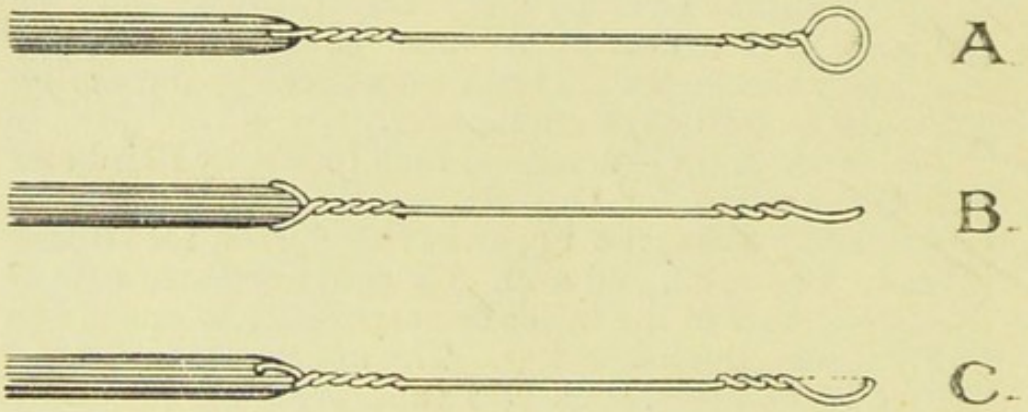

Fig. 2.

a Needle viewed from above.
b Needle viewed from side.
c Showing the relative size of loop and drop.

Dilution of the Disinfectant.—The Standardisation Committee recommend the employment of sterilised distilled water as the diluent for the disinfectant. As a corollary to this they add later, "Where special conditions exist which may interfere with the activity of the disinfectant, the consumer should be advised to call for the same conditions to be embodied in the test." That this recommendation is well advised is evident from the fact that although permanganate of potash has an extremely high carbolic acid coefficient when the test is conducted with distilled water, its employment would not be advisable in the presence of organic matter. Further instances will be given in a later chapter ("The Introduction of Organic Matter"). It is sufficient to remark here that in cases where a

disinfectant is to be used in conjunction with soap, a standard soap solution may be employed as the diluent, and that mucin and albumen may similarly be used.

The Broth Culture.—It is advisable to make a sub-culture of B. typhosus every 24 hours, when possible, from the previous 24-hour culture, even if on many days no test is to be performed. If in addition the same loop be used for making the daily cultures of the organism, and the same number of loopfuls of culture be used for the purpose (such as two), a culture not varying much from day to day in resistance to disinfectants will be obtained, and it will consequently be much easier to estimate the strength of phenol solution to be used as a control, than it would if the culture from which the 24 hours' growth is obtained were of older growth on one occasion than on another. The same broth as is used for sub-cultures from the medication tubes should be used ; it is, perhaps, unnecessary to remark that the sub-culture for the following day should be made from the culture before the latter is used for the test.

The process of measuring out the quantity of the concentrated disinfectant for dilution should be performed with a wash-out pipette having the bulb at the end (when dealing with a thick liquid at least 5 c.c. should be measured), and the nose of the pipette should be wiped with a piece of sterile filter paper, to remove any disinfectant clinging to the same before allowing the contents to run into the graduated cylinder. The graduated cylinders, and also the flask containing the sterile diluting fluid, should be sterilised, with inverted beakers (with cotton wool at the bottom) placed over the mouth of the flask and cylinder, which are, of course, also closed by cotton wool plug and stopper respectively.

The pipette for pipetting out the drops of the culture should be plugged at the top with cotton wool, and should, except when actually in use, be kept in a sterile test tube plugged at the mouth with cotton wool. It is useful to have the cotton wool plug fastened on to the pipette with wire, and the same remark applies to the 5 or 3 c.c. pipettes used for measuring out the disinfectant dilutions, which should also be of the form having the bulb at the end.

The apparatus for the experiment should be arranged

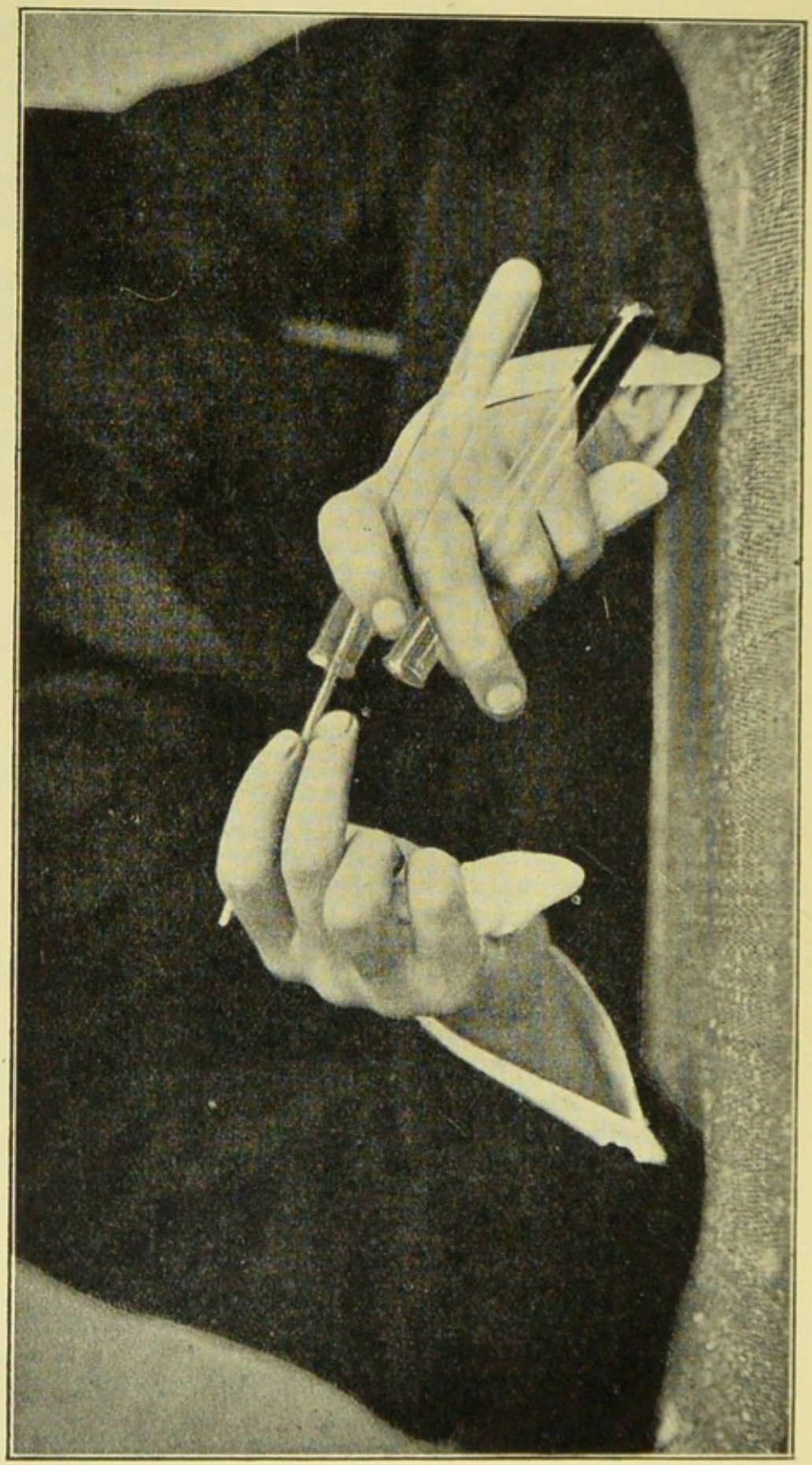

Fig. 3.—SHOWING METHOD OF MANIPULATION.

with the test tube stand containing the disinfectant dilutions and control in the front five holes, and the broth tubes on the upper tier, in front of the operator. Then on the right should come the wire basket for the reception of the broth tubes after inoculation, slightly tilted forward to enable the tubes to be put in expeditiously, and finally, still farther to the right, the Bunsen flame, which should be placed as far as possible from the rack, as the heat of the flame if too near is sufficient to make the temperature of the contents of the control medication tube (usually placed in the fifth hole) higher than the others, which would, of course, give an erroneously low coefficient.

Method of Manipulation.—It is, of course, open to the worker to adopt any position of holding the tubes and plugs which he considers suitable. We give the method used by many workers, and one which the author has found to be very satisfactory, as it admits of a rapid inoculation. The medication tube is taken from the rack, and the contents gently agitated for a second to ensure the even distribution of the bacilli in the disinfectant, and the plug having been taken out and held in the left little finger, the tube is held between the fore and second fingers, as shown in Fig. 3. The corresponding broth tube is taken up by the right hand and transferred to the left, between the second and third fingers, and the plug is extracted and held by the little finger of the right hand, the thumb being placed under this tube. The tubes being now in a position for inoculation, the inoculating needle, which should have been sterilised before the tubes were touched, is now introduced into the medication tube and a loopful is taken and inoculated into the broth tube. The needle is sterilised in the flame and the plugs are replaced, the medication tube going back to the rack, while the broth tube is subjected to a gentle agitation and placed in the wire basket on the right of the rack. Another method of inoculation adopted by Smith and Somerville is shown in Fig. 4.

The basket having been filled, the paper containing particulars of the dilutions, temperature of room, temperature of incubation of test organism, &c., is placed in as well, and the whole is put in the incubator.

Finally, it may not be out of place to mention the self-evident fact that to ensure success all pipettes, &c.,

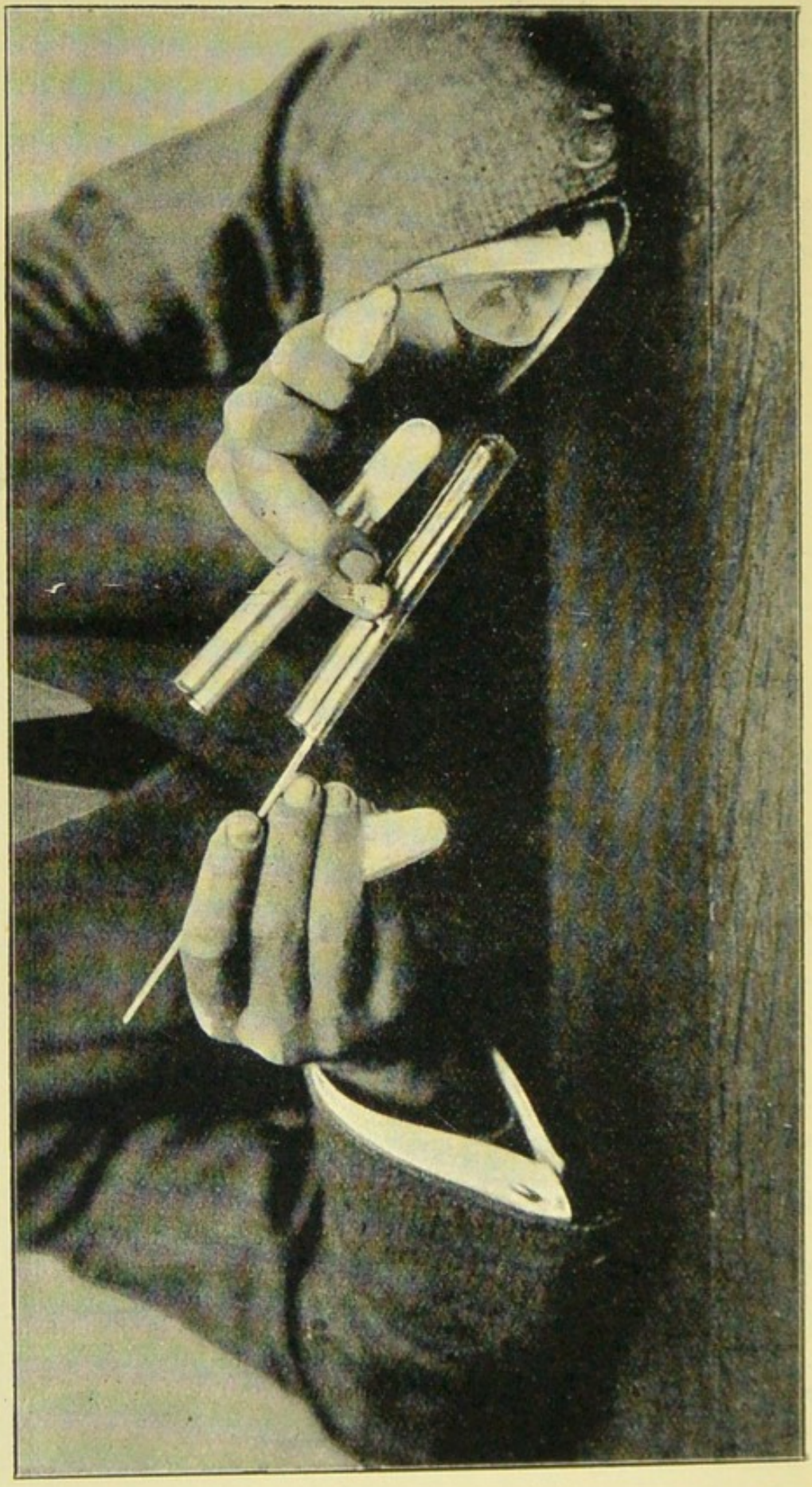

Fig. 4.—SHOWING METHOD OF MANIPULATION.

must be rigorously sterilised before use, and that the utmost care must be taken to guard against aërial contamination. The test should be conducted in a room free from draughts, and speech during inoculation should be reduced to a minimum. The instructions and cautions given in this chapter may seem trivial and unimportant, but it is probable that neglect to recognise the same has been responsible for the rather extraordinary results obtained by those using the process for the first time. In some laboratories it is the practice to wash the benches and floors with some non-volatile disinfectant before starting the experiment, and this is a wise precaution to take.

CHAPTER VI.

Method of Conducting the Rideal-Walker Test.

Before commencing the test—the apparatus having been arranged as recommended in the previous chapter—the culture of the test organism (if a broth culture is to be employed) is agitated and allowed to rest for 15 minutes to settle out the clumps. The same object may be attained by passing the culture through a sterilised paper filter.

The dilutions of the disinfectant are made up with sterile distilled water, or whatever diluting fluid it is proposed to employ, and three or five c.c. of each dilution introduced into the sterile medication tubes, which are placed in the holes of the lower tier of the rack. The plug of the culture is now replaced by the culture pipette, which has a plug fixed on to it with wire at such a height that when the plug fits easily into the mouth of the culture tube, the point of the pipette is half-way down the broth—*i.e.*, clear of the clumps. The first of the five medication tubes is now inoculated with either three or five drops of the culture, according as to whether it contains three or five c.c. of the disinfectant dilution. At intervals of half a minute each of the other medication tubes is inoculated in turn. By the time the fifth tube has been inoculated the organism in the first will have been exposed to the action of the disinfectant for two minutes, and after the next half-minute a loopful of the latter is inoculated into the first broth tube, and loopfuls from the other medication tubes are in turn inoculated into their respective broth tubes at the rate of one every 30 seconds. By the time the fifth broth tube has been inoculated from the fifth medication tube, the disinfectant in the first medication tube will have acted on the test organism for four and a half minutes, and after the next 30 seconds a loopful is introduced into broth tube six, and so on. The actual test, therefore, occu-

pies 17 minutes. No line in the table can be considered in the result which does not show a life in the first column and a death in the last. Firth and Macfadyen go still farther, and give their opinion that no "coefficient should be deduced from any particular experiment unless there are at least two negative results in the phenol or comparative line."

It may be remarked that the employment of three c.c. of disinfectant and three drops of culture is preferable to the use of larger quantities of each, as it is more readily manipulated.

If B. typhosus be used as a test organism, the sub-cultures should be incubated for at least 48 hours at blood heat before the results are finally read off. A moment's consideration of the manner in which the test has been conducted will suffice to indicate where the results of each sub-culture should be placed in the table.

After the completion of the inoculations, the culture and pipette are sterilised, together with the medication tubes.

CHAPTER VII.

The Choice of a Test Organism.

Some workers, instead of using an artificial culture of a single organism for the purpose of testing disinfectants, have recommended the employment of mixtures of various organisms as they occur in nature, *i.e*, instead of using a pure culture of an organism, they substitute for it some natural product—the excretions of the human system being those most favoured; for example, the fæces from pathological conditions and urine.

Rideal and Walker, in their original paper, recommend the use of pure cultures of B. typhosus in standard broth—a recommendation which has been adopted by the Admiralty, the War-office, the Metropolitan Asylums Board, and many other public bodies, as will be seen by the following extracts taken from tender forms issued during the present year, calling for supplies of disinfectants :—

War-office.

> "The contractor guarantees that tested against B. typhosus, taking carbolic acid as the unit, the (disinfectant tendered for) will give a coefficient of not less than —— (The contractor to insert coefficient)."

Metropolitan Borough of Islington.

> "Any disinfectant fluid may be tendered for provided that its bactericidal efficiency is expressed in terms of absolute phenol (100 per cent.) as determined by the Rideal-Walker method, when working with vigorous cultures of B. typhosus, and that it is miscible with water. The coefficient must be given in the blank space left for the purpose."

Westminster, City of.

"The contractor guarantees that tested against B. typhosus, taking carbolic acid as the unit, the disinfectant tendered will give a coefficient of not less than ——."

Metropolitan Asylums Board.

Disinfecting Fluid: "Here state the name of the fluid quoted for under this schedule number, and its carbolic acid coefficient by the Rideal-Walker method."

Ilford Urban District Council.

"Disinfectant Fluid: N.B.—State guaranteed carbolic acid coefficient on B. typhosus."

Broadstairs and St. Peter's Urban District Council.

"The soluble disinfectant fluid is to be entirely free from sediment, and shall run freely from the cask at all times, and when tested against B. typhosus, taking carbolic acid as the unit, it shall give a coefficient of not less than 10·00."

Barking Town Urban District Council.

"And I, or we, the undersigned, guarantee that, tested against B. typhosus, taking carbolic acid as the unit, the disinfectant fluid above referred to will give a coefficient of not less than—— (The contractor to insert coefficient)."

Whitehaven Corporation.

"Tenderers must state the guaranteed carbolic acid coefficient for B. typhosus."

The County Borough of Brighton.

"A Liquid Disinfectant miscible with water in all proportions to form a homogeneous emulsion and guaranteed to have a bactericidal efficiency of at least (*) times that of pure crystallised phenol when tested against a vigorous culture of Bacillus Typhosus."

* Tenderer to fill in this figure.

Borough of Beccles.

"Disinfecting fluid (soluble, non-poisonous). State guaranteed carbolic acid coefficient by the Rideal-Walker method."

Borough of Darlington.

"Note.—Insert full particulars of component parts of the powder, and state carbolic acid coefficient of the fluid."

Kettering Urban District Council.

"Disinfecting Fluid. State name, strength, and germicidal efficiency."

Metropolitan Borough of Fulham.

"Disinfecting Fluid. State carbolic coefficient on bacillus typhosus."

City of Truro.

"Disinfectant Fluids. The contractor guarantees that tested against B. typhosus, taking carbolic acid as the unit, the disinfectant tendered will give a coefficient of not less than —— (cofficient to be inserted by contractor)."

Sutton (Surrey) Urban District Council.

"The contractor must state the name of the fluid quoted for, and its carbolic acid coefficient by the Rideal-Walker method."

Corporation of Harrogate.

"The Disinfectant Fluid when tested against B. typhosus, taking carbolic acid as the unit, shall give a coefficient of not less than —— (The contractor to insert coefficient)."

Cape Government Railways.

The Stores Contract Form specifies the inclusion of the carbolic acid coefficient for either B. typhosus or B. coli, as determined by the Rideal-Walker method. A clause is also inserted to the effect that: "Tests will be made of each delivery of disinfectants under this contract, and if the result of any test shows that the strength is not equal to that guaranteed, a proportionately reduced price will be paid for the total consignment from which such sample was selected. The cost of all tests will be borne by the Department, provided the results show that the strength is not less than that guaranteed by the contractor. Should the results show that the strength is less than that guaranteed, the cost of such test will be charged to the contractor."

Some criticism has of late been directed against this practice by certain writers who, whilst admitting that "the method of standardisation proposed by Rideal and Walker is a valuable suggestion upon which it may be found possible to base a practical test," still declare that "it does not supply us with a useful indication of the strength at which a disinfectant must be employed in actual practice, because it does not take into account the influence of the associated organic matter upon the potentialities of a disinfectant."

The above quotations are taken from a paper which appeared in the February number of *Public Health*, 1906, and another paper dealing with this subject from the opposite point of view was published in the same number by Dr. Sommerville and Mr. Ainslie Walker. In the latter the authors state that "exception has been taken to the 'carbolic acid coefficient' on the ground that the results obtained are of academic interest only, and cannot serve as a guide in practical disinfection. But far from this being the case, the method, when slightly modified to meet special requirements, is capable, we think, of elucidating problems of the greatest interest."

They then proceed to show how at least one of the "conditions which approximate as closely as possible to those of practice" (to quote once more from the former) can be embodied in the Rideal-Walker test by the simple substitution of sterilised urine for sterilised distilled water. By this means they show how the carbolic coefficient of electrolysed sea water, containing one gramme of available chlorine to the litre, practically vanishes when sterilised urine is used as the diluent, whereas with sterilised distilled water, it is demonstrated to be 0·2 (*i.e.*, equal to 20 per cent. pure phenol). Elsewhere (*Public Health*, March, 1906, page 392) the same authors remark "where special conditions call for the introduction of organic matter, by all means let the purchaser impose the special condition when asking for the test (but this must still remain a laboratory test, the only variable introduced being the diluent); just as in taking the flash point of a railway axle grease the Abel test must be modified to meet the exigencies attaching to the high temperature required to produce the inflammable vapour." This recommendation has

since received the approval of the Sanitary Committee on Disinfectant Standardisation, appointed by the Royal Sanitary Institute, as will be seen from the following, which is taken from the official report recommending the Rideal-Walker method of test "for general purposes":—"Where special conditions exist which may interfere with the activity of the disinfectant, the consumer should be advised to call for the same conditions to be embodied in the test." For further information on this question the reader is referred to the valuable discussion on "Standardisation of Disinfectants," which appeared in the February, March, April, and May numbers of *Public Health*, 1906.

Much still remains to be done before we can tell with precision the absolute values of different disinfectants under all the various conditions existing in practice, but judging from the present state of our knowledge, the most reliable information is to be obtained by the use of the Rideal-Walker method, as modified in the above recommendations.

CHAPTER VIII.

The Introduction of Organic Matter.

In 1896, Pearmain and Moor, in their "Applied Bacteriology," drew attention to the desirability of testing disinfectants in the presence of extraneous matter. "It is indispensable to treat separately the various factors which in practice combine to affect the action of the disinfectant. Thus, in practical experience, organisms are seldom found without some particles to which they are attached, or by which they are surrounded. The presence of these particles is liable to exercise an important action upon the disinfectant, but their absence still leaves the organism undestroyed. The real question in the examination of a disinfectant is therefore the strength and time of exposure which will enable it to kill organisms in the presence of a relatively definite proportion of standard extraneous matter."

In a previous chapter—"The Choice of a Test Organism"—reference is made to experiments with fæces, as performed by Major Fowler, and Drs. Kenwood and Hewlett, in which the organisms to be destroyed by the disinfectant occur naturally in the fæces, and are not artificially cultivated apart from the fæculent matter.

More recently Drs. Smith and Prausnitz (*Journal of Preventive Medicine*, December, 1906) suggest that the efficiency of the disinfectant should be tested in the presence of sputum, strained fæces, or fæces—urine emulsions. They are of opinion that the disinfectant should not be required to penetrate through hard scybala of fæces, but that, on the other hand, the removal of all clumps (by passing the fæcal emulsions through filtering paper, as carried out by Firth, Macfadyen, and Fowler) appears to be rather too easy a condition. Other investigators have worked on slightly different lines to those mentioned above, employing sterile fæcal emul-

sions, sterile milk, sterile urine, and other forms of animal organic matter as the diluent for the disinfectant, and estimating the value of the mixture of disinfectant and organic matter on pure cultures of various organisms.

Klein (*Public Health*, October, 1906), following the experiments of Sommerville and Walker (*Public Health*, February, 1906), has tested the efficiency of an oxidising disinfectant (in this case chloros) in the presence of urine, obtaining very similar results. In one series of experiments Klein allowed the mixture of equal parts of sterile urine and chloros dilution to stand for one hour before adding the broth culture (*B. typhosus*); the carbolic coefficient obtained was 0·8. In a previous paper by Klein (*Public Health Engineer*, June 9th, 1906) the carbolic coefficient of chloros was shown to be 21·0 when tested in the absence of organic matter. Mr. M. Wynter Blyth prefers to introduce organic matter in the form of milk (*Analyst*, May, 1906), on the ground that its composition is easily determined, and that it contains three important classes of organic matter—proteids, fat, and carbohydrates. He found the influence of 2·5 per cent. of milk sugar on coal tar disinfectants to be so small as to be negligible. A similar result attended his experiments on the ash of 5 c.c. of milk in 10 c.c. of sterile water. He found also that serum-albumin and gelatin had a depreciating influence. The fat of milk was shown to produce a pronounced fall, which Mr. Blyth thinks is due to the emulsified oils in the disinfectant being dissolved by the fat, and thus removed from contact with the organism. Unfortunately, in Mr. Blyth's experiments a modification of the Rideal-Walker method was employed; even when using distilled water as the diluent he got very different results from those obtained by other observers, who have adhered strictly to the conditions laid down by the authors of this test, which renders comparison with other work out of the question.

Mr. Blyth also read a paper before the London section of the Society of Chemical Industry on December 3rd, 1906, in which he suggested that a mixture of whole milk and skimmed milk would form a satisfactory substitute for fæces, as in one instance his experiments had led him to the same result in the case of both forms of organic matter. He admitted that fæces gave discordant figures. In the discussion following this paper, Pro-

fessor Martin pointed out that milk would be inadmissible for use where mercuric chloride was employed as a standard, and Mr. Kingzett and other speakers laid stress on the fact that fæces seldom called for chemical disinfection.

Mr. Blyth claimed, as the result of his experiments, that the efficiency of the coal tar disinfectants falls as the percentage of solid matter increases, until a certain point is reached (about 10 per cent.), when the increase of organic matter does not further lower the efficiency of these disinfectants. In the case of potassium permanganate, however, the efficiency continues to fall off precisely as the organic matter increases (see also Blyth's article in THE SANITARY RECORD, December 20th, 1906).

Sommerville and Walker (THE SANITARY RECORD, November 29th, 1906) describe how they "attempted to grow *B. typhosus* in various forms of sterile organic matter, such as urine, blood, blood serum, solution of mucin, gelatin, pus, solution of casein, solution of albumoses and peptones, and then to add the various disinfectants in suitable dilutions, to measured quantities of these organic media, and finally to plant out in broth as per the original method." They were not successful, however, and were forced for the time being to relinquish all media for the production of the test organism except the original one (nutrient broth) owing to the alteration or enfeebling of the growth, which rendered it impossible to obtain uniform results. They then proceed to show how organic matter may be introduced into the Rideal-Walker test to give uniform results by substituting 1 per cent. solutions of gelatin, mucin, peptone, &c., for the water employed in the original method to dilute the disinfectants under test.

In allowing the disinfectant to remain in contact with the organic diluent for one hour before adding the test organism, the authors remark :—"There are many conditions in practice in which infection is constantly added to a limited area, whilst a disinfectant is only occasionally applied. In the latrine, spittoon, on the public-house floor, &c., disinfectants of the nature of chloros, potassium permanganate, &c., rapidly expend their germicidal properties on the organisms and associated organic matter, and in such exhausted condition can offer no further opposition to infected material received

later. The following tests, designed to throw light on such problems, by allowing the disinfectants to remain in contact with the organic diluents for one hour, clearly prove, we think, that the coal tar preparations best meet the requirements."

The disinfectants were first diluted with water in the proportion recommended by the manufacturers (*e.g.*, cyllin 1 : 400) or sanctioned by use (as in the case of potassium permanganate — K Mn O_4—1 : 20) all further dilutions being made with the standard organic solutions. "The serum consisted of sterile sheep's serum diluted with sterile distilled water. The blood was prepared by drawing off 10 c.c. sheep's blood into 90 c.c. of a 1 per cent. solution of citrate of potassium, and afterwards making up to the required dilution with sterile distilled water. The mucin was obtained from Merck, of Darmstadt, and dissolved in a 1 per cent. watery solution of sodium carbonate. The casein was derived from the same house, and dissolved in a 1 per cent. watery solution of sodium carbonate. The peptone was the ordinary commercial preparation of Witte dissolved in a 1 per cent. watery solution of sodium carbonate. The gelatin was prepared in distilled water in the usual manner."

Through the courtesy of the authors I am able to reproduce the accompanying table showing the results obtained by this modification of the Rideal-Walker test.

The action of gelatin in raising the coefficients of Izal and Cyllin is very curious; but the authors offer no suggestion as to the probable reason for this increase. The coefficient of "Sanitas," as the authors remark, is such as to remove all claim to oxidising properties.

In reviewing the various suggestions that have been put forward in connection with the Rideal-Walker method, with the object of so modifying the conditions of the test as to make them conform as closely as possible to those existing in practice, we must not lose sight of the fact that chemical disinfection has its limitations. It is absurd, for instance, to suggest that a disinfectant should be required to kill organisms when protected in such a manner that contact with the disinfectant is impossible.

The disinfection of fæces by chemical means is doubtless highly desirable, but the complete disinfection of such material is better accomplished by other means, and probably the utmost a disinfectant should

Diluent.	Chloros, 1 in 10.	Formalin, 1 in 10.	Izal. 1 in 400.	Cyllin, 1 in 400.	K Mn O_4, 1 in 20.	Sanitas, pure.
Water	21·0	0·3	10·0	14·0	50·0	0·02
Urine	0·9	0 25	7·0	12·5	0·8	0·02
1 per cent. serum	1·6	0·2	7·5	13·5	17·0	0·02
1 per cent. mucin	1·1	0·3	6 0	13·0	3·0	0·02
1 per cent. peptone	0·2	0·3	8 0	13·5	1·3	0·02
1 per cent. casein...	0·3	0·2	6·5	13·0	1·7	0·02
1 per cent. gelatin	0·4	0·2	10·5	14·5	7·5	0·02
1 per cent. blood	0·6	0·25	8·0	13·5	6·2	0·02

be required to do in this connection is *to lessen the risk of infection.*

As a source of organic matter in any standard method of test, the fæces must be reduced to an emulsion with water or urine—if not actually filtered—a condition which bears no resemblance to that existing in practice. Then, again, the characters of fæces vary to an enormous extent, being dependent on so many factors—the food taken, the powers of digestion, the products of the decomposition of food, bile residues, &c.

Certain medical men, whose opinions are to be treated with respect, request the inclusion of this variable material as a routine procedure. It is only natural that a pathologist should desire to know the disinfectants that will best prevent infection from the excreta, but this is a very narrow view to take. It is in spheres other than the isolation hospital that the bulk of commercial disinfectants is employed, and the low degree of dilution permissible when dealing with the disinfection (*sic*) of excreta, if adhered to for purposes of general disinfection, would result in much unnecessary loss. It is quite conceivable that a disinfectant which would be useless for the disinfection of fæces might with economy be employed to destroy bacteria on floors, walls, instruments, &c. Further, it must be recollected that the composition of fæces varies considerably, and, as Dr. Sommerville points out (*Journal of the Society of Chemical Industry*, December 31st, 1906) :—"In a particular typhoid stool there were often sloughs and portions of intestinal mucous membrane, and in another there might be a large hæmorrhage resulting in coagulated blood; while dysentery stools often contained 90 per cent. of mucin."

The use of milk, as proposed by Mr. Wynter Blyth, is open to the objection that milk is never met with in practice as a material requiring to be disinfected by chemical means, and it cannot be said to approximate very closely to the organic matter usually offered for disinfection. The state in which fat exists in milk is never met with in any form of organic matter calling for practical disinfection. The chief fault with the employment of such a diluent for the disinfectant is that it has a very levelling effect, more even than pus or fæces; in other words, the highly efficient disinfectant is brought by its agency nearer to those of acknowledged

inferiority. It thus causes an illusory comparative value to be placed on the disinfectants under test.

The experiments of Sommerville and Walker showing results obtained when working with diluents containing 1 per cent. of various forms of organic matter have been criticised by Wynter Blyth, who objects to the percentage of organic matter used. Defries, commenting on this criticism (THE SANITARY RECORD, January 10th, 1907), remarks:—"It must be remembered, however, that these experiments are wholly relative, and that the quantity of organic matter must be considered in relation to the quantity of disinfectant which is opposed to it. Take, for example, the experiment with cyllin 1 : 400 diluted further with 1 per cent. peptone solution, and giving a coefficient of 13·5. If the experimenters were working with a 1 per cent. phenol solution, this would mean that the 1 part of cyllin was diluted with 950 parts of peptone solution in addition to the 399 parts of water—that is to say, that the quantity of organic matter was nine and a half times as much as that of the disinfectant which came in contact with it."

The matter is one that cannot at the present juncture be settled by dogmatic assertion. The subject bristles with difficulties preventing the universal acceptance of any one form of organic matter in preference to another; for the disinfectants of to-day are used in connection with many forms of matter which cannot be introduced into a laboratory test.

A great amount of highly valuable research has been carried out during the past three years, and we are now in possession of facts relating to disinfectants which anyone who has followed the subject can with benefit apply in practice.

Much still remains to be done before we can so modify the test as to formulate a set of conditions acceptable to all workers. In the meantime, Sanitary Authorities and others charged with the selection of efficient disinfectants for public use will continue to call for the Rideal-Walker coefficient if they are well advised.

As the Medical Officer of Health for the Cape of Good Hope, in his report for the year 1905, remarks:—"It (the Rideal-Walker test) is the best—in fact the only—method of accurately expressing in figures the relative germicidal power of disinfectants under an agreed upon set of conditions. The introduc-

tion of fæces, urine, and other complicated and little understood organised mixtures into this simple and reliable test is nothing short of vandalism. We do not believe that Messrs. Rideal and Walker ever intended the method to be a test for penetrative power of necessarily variable albuminous envelopes. If such experiments are required they should be made on entirely different lines, and should be supplementary to the ordinary estimation of the killing power of the disinfectant under examination."

And as Dr. Rideal has pointed out, "the method, although only introduced so recently as three years ago, is already in use in Public Health laboratories all over the world." Further argument in favour of the adoption of this test, pending such time as a better one is forthcoming, is to be found in the opinion recently expressed by Wolf Defries—an opinion which is shared by many earnest workers, and which therefore forms a fitting conclusion to this chapter:—

"Personally, I should be very sorry if at the present time makers of disinfectants were to cease to use the plain Rideal-Walker coefficient obtained on pure cultures, as it would deprive the art of disinfection of the one datum which can be expressed with certainty. That this datum must in practice be qualified by others which cannot be determined with the same certainty is an unfortunate fact; but it is better to look it in the face, and use a factor of safety which is avowedly wide of the bare requirements of the case, than to muddle up the relatively exact phenol coefficient on pure cultures with the uncertain allowance to be made for chemical decomposition, and deprive one's coefficient of any substantial foundation in fact whatever."

CHAPTER IX.

Further Methods of Bacteriological Examination.

The old method of testing disinfectants, which is even now employed by more conservative workers, was to add cultures of bacteria to known dilutions of the disinfectant, and at the end of stated times to make subcultures therefrom, to ascertain whether the organism was alive at the time of subculture. That such results had a certain value cannot be denied, but that this value was small, and for the purposes of comparison with other results practically worthless, will be evident. In the first place, the time of exposure of the bacteria to the disinfectant varied, according to the taste of the worker, from two or three minutes to the same number of hours, or even days. Then the variation in resistance to disinfection of different cultures was ignored, and while one investigator would be using a weak culture another would be employing a vigorous one, and obtaining, of course, very different results. The proportion of culture to disinfectant, and the other factors which influence this work so considerably, were lost sight of, and in view of these facts it cannot be doubted that the results, when not actually misleading, were of little value. The chief advantage of such tests may be found in the fact that their performance called for very little skill on the part of the worker, and the results obtained by a hospital nurse who had had a few elementary lessons were as worthy of consideration as those obtained by learned bacteriologists.

There are, however, two processes which in their original or modified forms have certain claims to our consideration. In many respects they are similar to the Rideal-Walker method, and vary only in points of technique.

In the "Garnet" method of Kronig and Paul the culture of the test organism is dried on garnets, which are submitted to the action of the disinfectant, washed

in sterile distilled water, and transferred to broth subculture tubes. A carbolic acid or mercuric chloride control is introduced. The chief source of error in this method is found in the washing of the garnets in which the organism, as well as the disinfectant, is liable to be washed away. The opinion of many who have worked with this method is embodied in Firth and Macfadyen's statement that "the results are too irregular to justify its adoption as a means of obtaining any comparative figure of disinfecting efficiency."

The "Thread" method (of which a full description will be found in the *Journal of the Royal Sanitary Institute*, February, 1906, p. 18) is similar to the "Rideal-Walker" or "Drop" method, but differs in the following respects:—An emulsion of an agar culture is made in sterile distilled water, and after filtration thirty silk threads are soaked in the filtered emulsion for an hour and then dried. For the medication test tubes of the Rideal-Walker method, watch glasses containing the disinfectant dilutions are substituted, with this further difference—that in place of one test tube for each dilution, as in the Rideal-Walker method, six watch glasses are employed, an impregnated culture thread is placed in each, and at the end of each two and a half minutes one is taken out, washed with water (or, in the case of mercuric chloride, with ammonium sulphide), and placed in a broth subculture tube. The above remarks with reference to washing away disinfectant in the Garnet method apply with even greater force to the Thread method; for to those familiar with the structure of the cells of the silk fibre the absurdity of attempting to wash out the disinfectant without removing the soluble surface coating of organisms will at once be apparent. The following opinion of the thread method is taken from the Report of the Standardisation Committee of the Royal Sanitary Institute:—"We consider the technique of the 'Thread' method to be so elaborate that, no matter what may be its merits, its adoption as a standard procedure seems impossible. It is eminently unfitted for working with micro-organisms at all sensitive to desiccation."

APPENDICES.

Standardisation of Disinfectants.

In October of 1903 the Council of the Royal Sanitary Institute appointed a Special Committee, consisting of Dr. A. Wynter Blyth, Dr. C. Childs, Colonel R. H. Firth, and Dr. Samuel Rideal, to inquire into the desirability of establishing a standard bacteriological method for determining the efficiency of disinfectants. The following additional experts were subsequently added to the Committee: Dr. F. W. Andrewes (St. Bartholomew's Hospital), Professor Sheridan Delepine (Manchester), Mr. Walter Hills (representing the Pharmaceutical Society), Dr. E. Klein (St. Bartholomew's Hospital), Dr. Allan Macfadyen (Lister Institute of Preventive Medicine), Dr. James Ritchie (Oxford), Professor C. Hunter Stewart (Edinburgh), Professor G. Sims Woodhead (Cambridge), and Colonel J. Lane Notter, R.A.M.C., Chairman of Council, *ex officio*.

During the last two years this Committee have had a large number of meetings to consider the question referred to them, and numerous experiments have been carried out under the direction of the Committee to aid the Committee in coming to a decision. In the *Journal* of the Institute, Vol. XXVII., No. 1, is published a report of experiments made on behalf of the Committee and submitted to them by Colonel Firth and Dr. Allan Macfadyen; and it is proposed to publish in No. 5 a contribution from Professor Delepine on the "Thread Method."

The following report of the Committee has been approved and adopted by the Council:—

REPORT OF THE DISINFECTANT STANDARDISATION COMMITTEE.

(1) The Committee are of opinion that no one method of testing disinfectants can indicate their relative values under

every possible condition. These must be specially determined for the given case required, and where penetration is important a "Thread Method" is indicated.

(2) For general purposes, on account of its simplicity of working, the Committee recommend the "Drop Method" as described in the *Journal* of the Royal Sanitary Institute, Vol. XXIV. (1903), page 424.*

The test to be carried out with pure broth cultures of the *B. typhosus*, using sterilised distilled water as the diluent of the disinfectant.

All nutrient broth to have a constant reaction of + 15.

Where special conditions exist which may interfere with the activity of the disinfectant, the consumer should be advised to call for the same conditions to be embodied in the test.

(Signed)

April 9th, 1906. A. Wynter Blyth, Chairman.

* The "Drop Method" referred to is that of Rideal and Walker.

The Rideal-Walker Coefficient.

A letter from the authors of the Rideal-Walker Test to the *British Medical Journal* of April 6th, 1907, is appended, which fully explains the reasons for altering the term "Carbolic Acid Coefficient" to "Rideal-Walker Coefficient."

This abuse was inevitable, and it is a pity that the authors of the test did not foresee it. It is only in the fitness of things, and with many unfortunate precedents, that the authors should find it necessary to associate their names with the expression of this factor.

To the Editor of the British Medical Journal.

SIR,—We ask a few lines of your space to state that it has been found necessary to substitute the term "Rideal-Walker Coefficient" for that originally introduced by us, viz., "Carbolic Acid Coefficient," owing to the abuse of the latter on the part of unscrupulous manufacturers and vendors.

The necessity for adhering strictly to the *modus operandi* prescribed in our test has been strongly emphasised in the last two or three years since we published our method, and that this caution is necessary is shown by the fact that many disinfectants are advertised as having a certain "carbolic acid coefficient" when the figure given has obviously been obtained by methods having little or nothing in common with the test as described in the *Journal of the Royal Sanitary Institute*,* thus introducing confusion and robbing the term of the value it originally possessed.

Much of the success of our method is due to the fact that we provide for the postulant and control being tested not only *in* the same time but *at* the same time. As the resistance of the test organism, even when sub-cultured from the same stock, varies from day to day, it is unfair and misleading to offer a "coefficient" obtained by testing the postulant on one occasion and the phenol control on another.

Similarly, when the "Thread," "Garnet," or any modification of the "Rideal-Walker" test has been employed,

*Vol. XXIV., Part III., 1903.

information that such a method has been used should accompany the "coefficient," and the latter should certainly never be advertised without this information.

It is therefore most desirable that bacteriologists when reporting on the germicidal value of a disinfectant should specify *precisely* the method employed. Great discredit has been thrown on the Rideal-Walker test by the publication of results obtained by the worker modifying the technique—sometimes to a very large extent—without notifying the modification, and many of the discrepancies referred to by critics are directly attributable to this fact.—We are, Sir, yours truly,

SAMUEL RIDEAL.
J. T. AINSLIE WALKER.

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To H.M.  The King.

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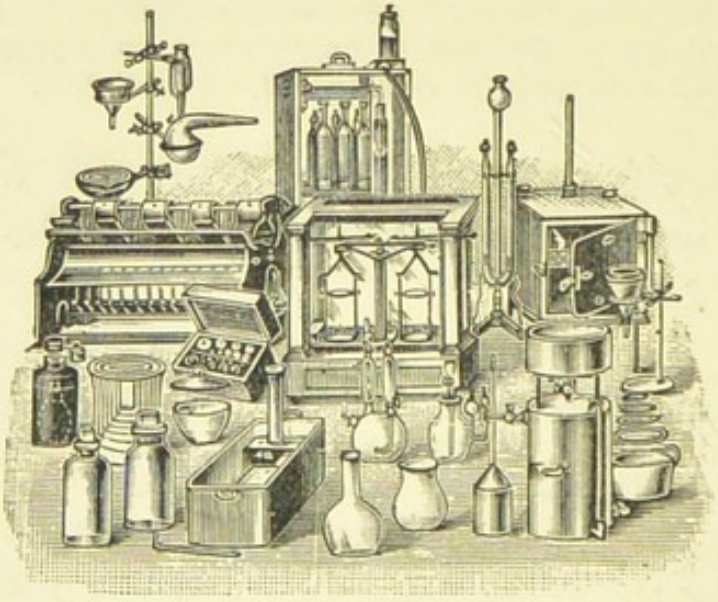

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