STANDARDISATION OF DISINFECTANTS.

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THE steadily increasing demand for disinfectants, due in great measure to the fact that the general public are acquiring a better knowledge of the true cause of zymotic disease, has so stimulated invention that many chemical products of undoubted value have been introduced in recent years, but the makers of these genuine articles have to compete with others whose preparations are not only worthless but actually dangerous, since the use of such products gives rise to a false sense of security. There can be no doubt that the public should know that disinfection—that is the killing of disease germs—is by no means a simple operation, and that the control of disease by this means should form part of the routine work in every sanitary area under the direction of the Medical Officer of Health. At the same time it is absolutely necessary that the efficiency of all disinfectants sold as such should have, if not a Government guarantee, at least a maker's warranty which is capable of being checked. As the Medical Officer of Health can only generally control the area under his jurisdiction, the choice of a disinfectant will at times rest with the medical man in charge, the nurse, or the householder, and they require such directions as will ensure freedom from error, and such descriptions of strength as will render the operations as certain as possible. When it is recollected that one case of infectious disease improperly treated is liable to become the source of many others, and that differences of practice in regard to notification obtain in different parts of the kingdom, it will be recognised that this subject is one of more than professional interest.

It is a notorious fact that at present there exists no official control over the sale of disinfectants, with the exceptions of those set forth in the Privy Council Orders of July 27th, 1900, and June 5th, 1902.

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The first of these permits the sale, without control, of liquids containing less than 3% of carbolic acid or its homologues, as disinfectants, on the ground that such fluid is not a poison within the meaning of the Pharmacy Act of 1868; and this has the direct effect, obviously, of placing a premium on inefficiency. In the subsequent order it is stated, liquid disinfectants containing scheduled poisons (which for present purposes are practically confined to carbolic acid and its homologues in solutions of more than 3%, and corrosive sublimate) shall be sent out in bottles rendered distinguishable by touch from ordinary medicine bottles, with a label giving notice that the contents of the bottle are not to be taken internally. Formaldehyde, and presumably potassium mercuric iodide, which are both efficient disinfectants, can be advertised and sold as such by anyone without control. Biniodide of mercury has been added to the schedule of poisons in Ireland, but not in England.

It has been frequently suggested that the provisions of the Food and Drugs Act should be extended to such articles. In other countries (*e.g.*, Massachusetts, U.S.A.) such legislation exists, and we hope as an outcome of the present discussion that some Parliamentary action which will effectively deal with this particular problem may be taken in this country.

The absence of a thoroughly reliable test may in a great measure account for this apparent apathy on the part of the authorities. It is obvious that a bacterial, rather than a chemical determination of efficiency is required, as although the strength of a preparation of carbolic acid and its homologues can be ascertained with great accuracy, it is a well-known fact that creolin, for example, does not depend for its remarkable efficiency as a bactericide on the content of these acids. Again, much depends upon the conditions, both chemical and physical, in which the disinfectant is employed. Thus we find that a disinfectant containing 10% of tricresol *in emulsion* is equivalent in bactericidal value to one containing 30% *in solution*, when tested against a virulent culture of B. typhosus.

There is, therefore, a great need for a bacteriological test: but unfortunately, investigations by different bacteriologists have led to glaring discrepancies. These differences are not so much due to the personal equation as to variations in the mode of procedure adopted by the different workers. We feel sure that many of these differences have no real scientific explanation, and that workers would willingly accept any uniformity of procedure which would ensure their results being comparable. We do not think it necessary to draw further attention to the impossibility of reconciling the values given in any two published

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reports at the present time, as we feel sure that this is obvious to anyone who has attempted to make the comparison; it is not too strong a statement to make that no two results obtained by different investigators may be compared with any satisfaction, unless all the details of procedure have been agreed upon beforehand. In selecting any particular process to be employed as a standard method for the examination of disinfectants, we submit that the following are some of the factors to be considered, in order that the difficulties we have pointed out may be overcome:—

1st. —Time.
2nd.—Age of Culture.
3rd.—Choice of Medium. Reaction of same.
4th.—Temperature of Incubation.
5th.—Temperature of Medication.
6th.—Variations in vital resistance of same species.
7th.— „ „ different species.
8th.—Proportion of culture to disinfectant.

Viewed as a source of possible confusion this list appears formidable enough, yet most of the difficulty is at once removed by the introduction of a standard control as used by us in the method about to be described.

The standard which we recommend is pure phenol. We are well aware that the so-called pure crystals contain at times as much as 7 or 8 per cent. of water, but if the operation of standardising a solution by means of bromine be considered too tedious the B. P. article will be found to answer the purpose admirably. 110 parts by weight of B. P. carbolic acid contain 100 parts by weight of pure phenol. In many respects this disinfectant has marked advantages over a metallic salt or other standard.

Our method may now be described briefly as follows:—To 5 c.c. of a particular dilution of the disinfectant in sterilised water add 5 drops of a 24 hours blood-heat culture of the organism in broth; shake and take sub-cultures every $2\frac{1}{2}$ minutes up to 15 minutes. Incubate these sub-cultures for at least 48 hours at 37° C. Allowing 30 seconds for each act of medication and the same time for making each sub-culture, four different dilutions of the disinfectant under examination, together with one standard control, may be tested against the same culture, under conditions which make the results strictly comparable. If preferred, the field may be extended and divided into intervals of 5 minutes: but we contend that no table is complete which does not show a positive result in the first column, and a negative result in the last. The strength or efficiency of the disinfectant is expressed in multiples of carbolic acid performing the

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same work :—*i.e.*, when we have obtained a dilution of the disinfectant which does the same work as the standard carbolic acid dilution, we divide the former by the latter, and so obtain a ratio which we call the "carbolic acid co-efficient."

The following table shows the degree of refinement to which this test may be carried with a little care :—

TABLE I.—*B. Typhosus (Kral)*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

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

Carbolic Acid Co-efficient $\frac{7}{80} = 0{\cdot}87$.

We are now in a position to discuss the influence of the various factors enumerated.

1st. *Time.*—One of the greatest mistakes made in estimating the relative values of disinfectants is introduced in connection with this factor. We refer to the practice of taking *strength of disinfectant* as the constant and *time* as the variant. A glance at tables II. and IIA. will convey some idea of the extent of the error due to the adoption of this method. It is therefore evident that to obtain regular and consistent results, time must be taken as the constant and strength as the variant.

TABLE II.—*B. Coli*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Disinfectant No. 1 (containing 19·5 % Cresols)	1 : 200	×	×	.	.	.	.	48 hours	37° C.
Disinfectant No. 2 (containing 10·1 % Cresols)	1 : 100	×	×	.	.	.	.	"	"

True relative values = 2 : 1.

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TABLE IIA.—*B. Coli, 24 hours Broth Culture at 37° C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		5	10	45	50	55	60	Period of Incubation.	Temperature.
Disinfectant No. 1 (containing 19·9 % Cresols)	1 : 200	×	.	.	.	.	.	48 hours	37° C.
Disinfectant No. 2 (containing 10·1 % Cresols)	1 : 200	×	×	×	×	×	.	,,	,,

Apparent relative values = 11 : 1.

2nd. *Age of Culture.*—This is a factor which is often ignored. In the usual trial with a non-sporing organism where a primary culture in nutrient broth is prepared and small quantities are mixed with the diluted disinfectant, a variation in the incubation period undergone by the primary culture will make a difference in the valuation of the disinfectant. As an example of this we have prepared a culture of B. typhosus in nutrient broth incubated at 37·5° C. After 16 hours incubation 0·1 c.c. of this culture was removed with a sterile pipette and added to 3 c.c. of diluted disinfectant fluid. The remainder of the primary culture was then incubated for a further 8 hours; 0·1 c.c. was then removed and treated with the disinfectant as before, and this was again repeated after a further incubation of 16 hours. The actual number of typhoid organisms present in the culture was carefully determined at the end of each period. The results were as follows:—

TABLE III.—*B. Typhosus, Broth Culture at 37·5° C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Period of Incubation. Primary culture.	No. of Organisms per c.c. after 48 hours incubtn.	Temp.
		2½	5	7½	10	12½	15			
									millions	
Disinfectant A	1 : 95	×	×	×	.	.	.	16 hrs.	212	37·5°C.
,, ...	,,	×	×	×	.	.	.	24 ,,	233	,,
,, ...	,,	×	×	×	×	×	×	48 ,,	90	,,

These results are also of interest in showing that the resistance of a culture to the action of a disinfectant is by no means dependent on the actual *number* of organisms present. In the above instance the typhoid

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had reached its maximum degree of multiplication apparently after 16 to 24 hours, and with a further incubation at the optimum temperature the number of organisms diminished; their power of resistance to the action of the disinfectant was, however, decidedly increased. The explanation of this result may be found in the slight clotting of the culture which takes place during protracted incubation. The bacilli in this way appear to be to a great extent protected from the action of the disinfectant by each other, and by their own products. But no false or misleading results can be obtained with an attenuated culture, either by design or through ignorance, where a carbolic acid control is introduced (cf. tables IIIA. and IIIB.).

TABLE IIIA.—*B. Typhosus*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Fluid W [1]	1 : 90	.	.	.	.	.	.	48 hours	37° C.
„ „	1 : 100	×	.	.	.	.	.	„	„
Carbolic acid	1 : 90	.	.	.	.	.	.	„	„
„ „	1 : 100	×	.	.	.	.	.	„	„
„ „	1 : 110	×	×	×	.	.	.	„	„

Carbolic Acid Co-efficient = 1·0.

TABLE IIIB.—*B. Typhosus*, 48 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation,	Temperature
Fluid W [1]	1 : 90	×	.	.	.	.	.	48 hours	37° C.
„ „	1 : 100	×	×	×	.	.	.	„	„
Carbolic Acid	1 : 90	.	.	.	.	.	.	„	„
„ „	1 : 100	×	×	×	.	.	.	„	„
„ „	1 : 110	×	×	×	×	×	×	„	„

Carbolic Acid Co-efficient = 1·0.

3rd. *Choice of medium: Reaction of same.*—For most practical purposes the choice of media is restricted to broth and agar. The behaviour of cultures of B. coli obtained from both is shown in the following tables:

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TABLE IV.—*B. Coli*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

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	37° C.
Disinfectant Z	1 : 800	×	×	.	.	.	.	,,	,,
Carbolic acid	1 : 100	×	×	.	.	.	.	,,	,,

Co-efficients { A = 10·0. Z = 8·0.

TABLE IVA.—*B. Coli*, 24 *hours Agar Culture at* 37° *C.*

Room Temperature 15°–18° C.

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 : 1500	×	×	.	.	.	.	48 hours	37° C.
Disinfectant Z	1 : 1200	×	×	.	.	.	.	,,	,,
Carbolic acid	1 : 120	×	×	.	.	.	.	,,	,,

Co-efficients { A = 12·5. Z = 10·0.

In both cases the higher factor is obtained when using agar cultures, which are prepared by taking up part of the growth on the point of a needle and distributing it evenly in sterilised water; the resulting emulsion may be used in the place of the broth culture, but we recommend the latter as being much more convenient to handle.

The reaction of the medium used for primary cultures is a factor of greater interest.

TABLE IVB.—*B. Typhosus*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

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 : 90	×	×	.	.	.	.	48 hours	37° C.
Carbolic acid [2]	1 : 120	×	×	.	.	.	.	,,	,,

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

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These media corresponded therefore to reactions of +0·6 and −0·2 respectively. The American Public Health Association in 1898 adopted a reaction of +1·5 °/₀ as the best for general work, and we have used this in all tests referred to.

We think that the fresh meat recommended in many of the text books might be replaced by Liebig's extract, to the greater convenience of the operator; but the *reaction* of the resulting broth is a detail which will not admit of any latitude.

4th. *Temperature of incubation.*—The extent to which this factor will affect the power of resistance of a culture to the action of disinfectants is clearly shown in the following tables:—

TABLE V.—*B. Typhosus* (*S.S.*), 48 *hours Broth Culture at* 22° *C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Fluid W [2]	1 : 90	×	.	.	.	.	.	72 hours	37·5° C.
Carbolic acid	1 : 120	×	.	.	.	.	.	,,	,,

Carbolic Acid Co-efficient = 0·75.

TABLE VA.—*B. Typhosus* (*S.S.*), 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Tempe a-ture
Fluid W [2]	1 : 90	×	×	×	.	.	.	72 hours	37·5° C.
Carbolic acid	1 : 110	×	.	.	.	.	.	,,	,,
,, ,, ...	1 : 133	×	×	×	×	×	×	,,	,,

Carbolic Acid Co-efficient = 0·75 (average of 0·82 and 0·67).

It is interesting to note that in spite of this difference in resistance the carbolic acid co-efficients found for the disinfectant tested are practically identical. The culture grown at 22° C. was allowed twice the incubation period of that grown at blood heat, and should therefore, but for the influence of temperature, have shown the greater power of resistance.

5th. *Temperature of medication.*—No scheme can be considered satisfactory which does not take into account the temperature during medication. Slight variations in temperature of one or two degrees do not

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seriously affect the results obtained by our method, but if the standardising of disinfectants is to become a question of international interest—and we trust it may—then a standard temperature will require to be established. In our own practice we have adopted 15° C. to 18° C. as the range of temperature most easy of attainment throughout the year. The influence of this factor is felt in two different directions: In the first place emulsions prepared from certain disinfectants at 5° C. will be found to yield a very much lower co-efficient than those made from the same disinfectant at 15° C., the explanation in this case being that the ultimate globules of the disinfectant at the higher temperature are in a much finer condition and more evenly distributed. Further it will be seen from tables VI. and VIa. that the bactericidal action of carbolic acid is *at least* 50 per cent. greater at 37° C. than at 16° C. The influence of temperature may be less marked in the case of other disinfectants, but we think that the example given will serve to prove that this is a factor of the first importance.

TABLE VI.—*B. Coli* (*Escherich*), 24 *hours Broth Culture at* 37° *C.*

Room Temperature 16° C.

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° *C.*

Room Temperature 37° C.

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	.	.	.	.	.	.	„	„

6th. *Variations in vital resistance of the same species.*—That cultures of the same organism obtained from different sources show marked variations in resistancy is a fact too patent to all investigators to require further

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corroboration. We content ourselves therefore by calling attention to tables VII. and VIIA.

TABLE VII.—*Staph. p. aureus*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

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] „ „ Dr. Klein.

TABLE VIIA.—*B. Typhosus*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

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] „ „ Dr. Rideal.

It will be noted that this is a factor which ceases to be of interest in the presence of a standard control.

7th. *Variations in vital resistance of different species.*—Here again we have a fact which must be well known to all investigators. It may not be so generally known, however, to what extent these variations may work out in practice.

In tables VIII. and VIIIA. will be found the carbolic acid co-efficients of an ordinary coal-tar disinfectant for *Staph. p. aureus* and *B. typhosus.*

TABLE VIII.—*Staph. p. aureus*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Disinfectant W [3]	1 : 80	×	×	×	.	.	.	48 hours	37° C.
Carbolic acid	1 : 80	×	×	×	.	.	.	„	„

Carbolic Acid Co-efficient = 1·0.

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TABLE VIIIA.—*B. Typhosus (Kral)*, 24 *hours Broth Culture at* 37° *C.*

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Disinfectant W[3]	1 : 250	×	×	.	.	.	.	48 hours	37° C.
Carbolic acid	1 : 80	×	×	.	.	.	.	,,	,,

Carbolic Acid Co-efficient = 3·1.

8th. *Proportion of culture to disinfectant.*—This is perhaps the most important factor of all, and yet it is the one which is most frequently ignored. When the casual reader learns that a certain disinfectant is capable of destroying certain organisms within a specified period of time he accepts the statement as a valuable contribution to our knowledge of the article in question. Yet the absence of this factor entirely robs the statement of any value it might otherwise have. It is in cases such as the following that we find the strongest argument in favour of a standard control.

TABLE IX.—*B. Typhosus (Kral)*, 24 *hours Broth Culture at* 37° *C.*

(*Taking* 5 *c.c. diluted Disinfectant* + 5 *c.c. Broth Culture.*)

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Disinfectant A²/03.....	* 1 : 450	×	×	.	.	.	.	48 hours	37° C.
,, ,, ...	1 : 485	×	×	×	.	.	.	,,	,,
,, ,, ...	1 : 525	×	×	×	×	×	.	,,	,,
Carbolic acid	1 : 75	×	×	×	.	.	.	,,	,,

Co-efficient $\frac{485}{75}$ = 6·5.

* Allowing for extra diluent introduced with Culture.

TABLE IXA.—*B. Typhosus (Kral)*, 24 *hours Broth Culture at* 37° *C.*

(*Taking* 5 *c.c. diluted Disinfectant* + 5 *drops Broth Culture.*)

Room Temperature 15°–18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Disinfectant A²/03.....	1 : 700	.	.	.	.	.	.	48 hours	37° C.
,, ,, ...	1 : 800	×	.	.	.	.	.	,,	,,
,, ,, ...	1 : 900	×	×	×	.	.	.	,,	,,
Carbolic acid	1 : 80	×	×	×	.	.	.	,,	,,

Co-efficient $\frac{900}{80}$ = 11·2.

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In conclusion, we venture to think that the facts to which we have drawn attention show the great need that exists for a reliable bacteriological test; and we are further of opinion that the time is now ripe for a general discussion of the whole subject, with a view to obtaining such a consensus of opinion as may result in the establishment of a standard method, which, if brought out under the ægis of The Sanitary Institute, might the more readily meet with official adoption.

Dr. Rideal moved the following resolution, which was passed:—

"That the Council of The Sanitary Institute do appoint a Committee to inquire into the desirability of establishing a standard bacteriological method for determining the efficiency of disinfectants, and take such steps as may be necessary for ensuring the legal control of disinfectants."

NOTE.—Resolutions passed at meetings of the Institute can only be in the form of recommendations to the Council, to whom they must be submitted for consideration and approved before they can be considered as the official opinion of the Institute.

Notes of the decisions of the Council upon any resolution are published in the page preceding the Law Reports.

DR. RIDEAL (London) said he should like to draw the attention of the meeting to some facts concerning the use of carbolic acid. The Regulations of the Army Medical Service for 1897, the last report giving their rules in time of peace, recommended carbolic acid as a useful disinfectant for tuberculous sputa. They stated that it might also be employed as a disinfectant for cholera dejecta, and if available in the crystal form a five per cent. solution should be used; so that the War Office thought a five per cent. solution efficient for the two purposes. But the Regulations did not state the time of contact, nor the volume to be added to any particular tuberculous sputa or to cholera dejecta, so that in these two particulars it was left perfectly vague as to whether it was to be a long contact with a small quantity, or a very large quantity for a short time. Further, the Order said that the crude commercial form of the acid was weaker and needed to be employed in much larger quantity. Now that statement was not true, for the commercial forms, which contained cresols, were really stronger. Then there was another point in regard to chloride of lime. The War Office Regulations said, "dissolve four ounces of chloride of lime in one gallon of water." Turning to the Local Government Board Regulations he found that they adopted a different method entirely, so that the authorities of the War Office were at variance with the Local Government Board. The Local Government Board Regulations, which were issued three years after those of the War Office, said,

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"chloride of lime one pound, water ten gallons," which was about one and half ounce per gallon, whereas the War Office recommended four ounces per gallon *for ten minutes*. The Local Government Board did not mention the time of contact. It was interesting to note other differences. For example, the Local Government Board recommended with regard to chloride of lime that the use of non-metallic vessels should be enjoined for liquids of that sort, whereas there was no corresponding Regulation under the War Office. The Navy printed no Official Regulations, but he found that they used a variety of disinfectants. It was very curious that izal happened to be a disinfectant in use in the Navy, and particulars of the use of izal were also given by the War Office, but other proprietary articles were omitted from the list. This was singular, because he was certain that there were many other proprietary articles which could be usefully employed if their strengths could be declared.

A letter from Dr. Klein to Mr. Ainslie Walker was read as follows:—

> I have read your paper with the very greatest interest, and I quite agree with your argument and the recommendations; in fact, I have for years practically worked on principles in many respects similar to those so ably described in your paper. I have made three annotations indicated on the first page of your proof: (1) Avoid using the word "virulent" in respect of "culture of B. typhosus," because it may lead some people to attach a meaning different from what you intend to indicate. I should think you mean "actively growing" culture (Mr. Walker said the word was overlooked in reading the proof. The word they wished to use was "vigorous", not "virulent."). (2) I recommend that subcultures of the medicated microbes be made both in broth and on agar; the latter controls the former in this way: if you make your subcultures in broth only, and growth takes place—shown by turbidity—you cannot be quite sure that this growth is due to your medicated microbe having caused it, but if at the same time you have made an agar surface culture you are at once better able to judge of it, because the growth on agar would in most instances denote the true character of the microbe which you employed. The broth serving for the subculture should be about ten c.c., because in the case of a disinfectant of great potency a big loopful of the disinfectant introduced into, say, only about five c.c. of broth might cause inhibition, or at least retardation of growth taking place. If, however, the subculture be made in broth only, then it is necessary to make a further, second subculture on agar from the medicated growth, in order to be certain that you were dealing with the survival of the employed microbe. (3) I should also recommend that the subcultures be incubated seventy-two hours, in order to make sure that no growth occurs. Your insisting on the subcultures being incubated at 37 C. is very important. Experience has taught me that a medicated microbe might fail to develop at 20°—21° C. when it would develop at 37 C. Your tables are highly instructive.

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DR. ROBERTSON (Sheffield) said the subject of the paper was one in which he had been interested for a number of years, and he had worked on lines somewhat similar to those which had been described, though his work had given somewhat different results. One might take it that there were two classes of users of disinfectants. (*a*) Local authorities and Government departments who bought disinfectants in large quantities and gave specific instructions as to their use, and (*b*) the general public who bought disinfectants and used them in a more or less valueless manner, getting practically no good results from their use. He had dealt with the matter from the local authorities' point of view; the question to which he set out to find an answer being, what was the best disinfectant for a local authority buying large quantities for specific purposes and what were the best directions to give for its use? About five years ago his colleague, Dr. Hector, and himself carried out a long series of experiments on the subject extending over a couple of years. They got disinfectants which were commonly used by local authorities over England. They also purchased samples from makers and obtained information from the makers as to their value. But they very soon found that in order to test one disinfectant against another it was impossible to adopt one standard alone. Everybody must admit that there was no one standard by which all the properties of disinfectants could be tested. His remarks applied to one test alone, the poison to kill the typhoid organism in a typhoid motion. Instead of taking carbolic acid they took acid corrosive sublimate solution, of which an accurate solution could easily be obtained by everybody. They worked out the amount of corrosive sublimate required to completely kill the typhoid bacillus in such a motion and then estimated the cost of 1,000 gallons of such a solution, one could get results that were absolutely uniform, and then they tested other disinfectants against the corrosive sublimate standard and worked them out on a cost basis. The cost basis they took was the cost of 1,000 gallons of efficient disinfectant. The disinfectants were to be efficient for the disinfection of their standard typhoid stool in half an hour, and they found that acid corrosive sublimate solution was quite the cheapest disinfectant on the market. It cost, speaking from memory, about 6½d. per 1,000 gallons, that was buying the corrosive sublimate in large quantities from the makers. The prices of disinfectants tested against it went up to £128 per 1000 gallons in the case of one very much advertised disinfectant. That was to say the public were paying £128 for what could be got, just as good and efficient, for 6½d. After the corrosive sublimate solution came the various hypochloride solutions which cost from 1s. 3d. to 2s. per 100 gallons. Then there was a long interval, the next disinfectants in the sale (the tar oils) costing about £2 10s., £2 15s., and £3 per 1000 gallons. He thought the cost basis, so far as any one particular quality of disinfectant was concerned, was the best to go on, but it must be tested, as Mr. Walker said, against a standard, and if one tested a number of disinfectants he thought it would be found that acid corrosive

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sublimate standard would give clearer and more accurate results than the carbolic acid one. He had rejected 'absolute' phenol as a standard on account of the difficulty of getting a definite "end point" in the reactions and also because no two samples of 'absolute' phenol gave quite the same results.

Dr. Christopher Childs (London) said he thought they were much indebted to Dr. Rideal and Mr. Walker for working out and bringing that subject before the Congress. There was a great difference of opinion at the present time with regard to the relative value of disinfectants, owing to the different methods of investigating the question, and other causes. He would be prepared to support the resolution placed at the end of the paper.

Mr. Wolf Defries (London) said that the authors had put forward a more practical and definite proposition than he had previously seen for dealing with a very serious difficulty, and he thought that Dr. Rideal had done a considerable service in bringing forward his resolution. The authors had expressed themselves strongly, though not at all more strongly than the facts warranted, as to the chaotic condition of much of the literature of disinfection. It was indeed rare to find two papers of which the results were strictly comparable; much more so than to find individual papers in which the results were rendered useless through some defect in the process of examination, or through the absence of information as to some essential detail. The fullest sympathy was therefore due to the first practical proposal towards remedying this state of things; but it must be clearly recognised that the proposal entailed an enormous amount of labour and critical consideration. The deliberate, systematic, and long continued work which was necessary could not be obtained from the hours of leisure of any man or men; and the first condition of any sort of success was therefore that the investigation should command the services of competent observers who would be paid on a professional basis. The paper having been only just put into the hands of members, it was necessary to take from the experiments of the authors themselves any illustrations to justify this view of the extent of work which remained to be done. In doing so, it was, he hoped, needless to disclaim any desire to pick holes in this admirable paper. The object of pointing out such discrepancies as had struck him in reading the paper was to illustrate how in existing conditions it was difficult, even in the best work, to avoid discrepancies; and had time allowed the reference, it would have been as easy to have taken the illustrations from papers on the subject which had become classical. In the first place, the method of inoculation which seemed to have been used would be liable in some cases to give quite misleading results. The broth culture used for test was introduced into disinfectant of known strength in the proportion of five drops to 5 c.c. Assuming that the broth was thoroughly mixed with the disinfectant, this gave a strength of $\frac{100}{105}$ of the original dilution; and of this

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it appeared that 0·1 c.c. was sub-cultured into 5 c.c. of broth, giving a strength of 1 : 52·5 of the original dilution. Quite a considerable number of disinfectants introduced in this proportion into the sub-culture would be strong enough antiseptically to prevent growth, and thus entirely to frustrate the object of the sub-culture, even when they had wholly failed to disinfect. If, for instance, the original dilution had been a 2 per cent. formaldehyde solution, the sub-culture would be a good deal stronger than a 1 : 3000 solution; a strength which is much more than is usually necessary for prolonged or even permanent antisepsis, although organisms might continue alive and grow readily when removed from it into favourable conditions. The case might be still less satisfactory if, instead of a homogeneous solution, the disinfectant was presented as an emulsion. If bacteria were observed under a high power in a drop containing a solid or an oily particle, it would often be found that they more or less loosely attached themselves to such particle; and there seemed to be nothing to exclude the possibility of the organisms transferred into the sub-culture being temporarily fixed in this way to the floating particles, and being thus restrained from growth by an even stronger antiseptic atmosphere than was present in other parts of the sub-culture, or even than the average strength of the original disinfectant dilution. The attachment of bacteria to such particles being certainly not permanent or trustworthy, effects of this kind cannot be reckoned upon in practice, and when they occur in experiment they represent the disinfectant as of a totally different efficiency to that which it really possesses. The authors had, for instance, recorded the extremely interesting result that the same disinfectant appeared to possess in emulsion three times the strength it exhibited in solution: and it certainly seemed at least as likely that this appearance was due to an antiseptic effect exercised in the sub-culture by the transferred particles as that it arose from so wide a divergence of behaviour in the disinfectant itself. The authors again stated that cultures of the same organism of different ages might vary in resistance to disinfection, but that the ratio of their resistance against one disinfectant to their resistance against carbolic acid would remain constant. A good deal of evidence would be required to support so general a proposition; and either a misprint had occurred in tables III. and IIIA. or the "carbolic-acid co-efficient" of the disinfectant W was higher in the 24 hour culture than in the 48 hour culture. A further point of enormous practical importance was the influence of the natural environments of the organism upon the disinfectant, which he gathered had been considered to some extent in Dr. Robertson's experiments; such a reaction, for instance, as had been studied in the case of perchloride solutions. Other illustrations could be found in the paper, both in what was said and what was omitted, to show that the problem of standardising disinfectants was a huge work, not to be undertaken without means adequate to provide for a prolonged enquiry. If The Sanitary Institute could organise such an enquiry it would confer a very great benefit on this country, and indeed be doing a work of international importance. If, on

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the other hand, it was unable to secure such an investigation, it would be merely adding to the mass of inconclusive results which already encumbered the literature, and by investing them with an appearance of authority and finality would run the risk of doing more harm than good.

Dr. S. G. Moore (Huddersfield) said the subject was one of great interest to sanitarians. He took it that from the time when they first devoted themselves to the question of disinfection they had had in their minds, more or less constantly and prominently, the need of getting some standard for disinfectants. But he thought it was equally true that they had not hitherto considered the subject from a thoroughly scientific standpoint. Dr. Robertson, some years ago, commenced a line of inquiry which led him to consider the subject, but it had not been brought prominently forward as it had been to-day. But what he wished to say particularly was, that it seemed from the course of the discussion that we relied for our disinfection and for our freedom from certain forms of disease upon chemical disinfectants more or less absolutely. After all, chemical disinfection was nothing better than a makeshift. The idea that by the employment of one or other substance we could do away with the cause of disease was much too prominent in the minds of the uninstructed public. The ordinary householder believed that by scattering more or less of a powder in the vicinity of his or her drains, or by adding a certain volume of fluid to a foul ashpit, that they had been disinfected. That of course was very regrettable, and he thought it was worth while to point out that, as he had said, the use of chemical disinfectants was only a makeshift, and that we ought rather to rely on the prompt removal and prompt destruction of infected material, and all organic refuse, and attempt to dispel the common delusion that by disguising one stink by another we had removed innocuous dangerous accumulations of decomposing material.

Mr. J. H. Worrell (Sheffield) said it was easy to criticise a paper like that, which had been read, on points of detail, yet it was a fact, which none of those interested in disinfection could lose sight of, that there ought to be some standard to which to refer. As one who had been privileged to discover a disinfectant which had been widely used, and speaking from the standpoint of a chemist, it might be thought that he would favour chemical examination, but he considered that Dr. Rideal's method was far preferable to any mere chemical test that could be applied to determine disinfecting power. If one took a series of tests of bodies derived from a known source, then a chemical test would measure very fairly the disinfecting power of the bodies dealt with, but when the disinfectant was taken from many various sources a chemical test could by no means measure satisfactorily the disinfecting power. Therefore he thought that Dr. Rideal's paper was very opportune. Some observations had been made on the variation in the virulence of certain disease-germs. Dr. Andrews, in a very interesting paper read before the Pathological Society of London the previous year, pointed

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to the fact that he had obtained a virulent strain of Staphylococcus pyogenes aureus which actually resisted mercuric perchloride 1 in 500 for twenty minutes, whereas carbolic acid, 1 in 40, actually destroyed the same in about two minutes, and another disinfectant, 1 in 400, destroyed it in less than one minute. This was a very striking illustration of the necessity of working with a standard, not only from the standpoint of disinfectant-action, but also from that of alteration of germ-virulence. As to the disinfectant which should be taken as a standard, this was of course a matter open to argument. He might point out, however, that, where there was any chance of albumen being present, mercuric chloride was apt to give very uncertain results indeed, and since it was easy to obtain a definite carbolic acid (as in the B. P., or, better still, by standardising) he thought that, as a commencement, one could scarcely choose a better disinfectant than the latter to which to refer the others.

DR. RIDEAL (London), replying on the discussion, said the advantage of mercuric chloride as compared with carbolic acid as a standard was a question of pro and con. As to the Government taking the matter up, he was afraid that if they waited for them they would have to wait a good many years. The Sanitary Institute had done good work in various directions. and he thought it was a direction in which more of such work might be done. If they could get two or three men like Dr. Klein to take an interest in the matter, he thought they would get something that would be useful to them in practical work, and which might ultimately lead to the adoption of a legal standard.

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