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A PHOTOMETRIC METHOD FOR THE COMPARATIVE EVALUATION OF DISINFECTANTS

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(With 6 Figures in the Text)

CONTENTS

I. INTRODUCTION

In the past twenty years, particularly as far as this country is concerned, the most widely employed test used in the standardization of disinfectants has been the Rideal-Walker Method. This test was first described in 1903 (Rideal & Walker, 1903), and an amended version was adopted as an official method in 1919 by the British Disinfectant Manufacturers' Association. This amended version was based on the recommendations of the Association's Technical Committee. The original authors shortly afterwards published a revised technique (Rideal & Walker, 1921), and still further revision was made when the method was issued as a B.S. specification (B.S.I. 1934), this latter being the technique now employed. In spite of these three revisions and the testing of numerous variations during the process of revision, reproduction of results of the test in different laboratories is not satisfactory, and the possibility of further standardization is again under examination by a committee formed at the request of the B.D.M.A. Further evidence of the widespread nature of the dissatisfaction with the results obtained by the Rideal-Walker method is to be found in the large number of modifications and alternatives which have been suggested (Anderson & McClintic, 1911, 1912; *U.S. Publ. Health Rep.* 1921; Moore, 1926; South African Govt. 1929, 1930, 1931; Jensen & Jensen, 1933; Isaacs, 1938; L.C.C.; G.W. Rly.). Some of these, particularly the Food and Drug Administration Method (Brewer & Reddish, 1929; Ruehle & Brewer, 1931), have attained very considerable importance in other countries. Though American workers have claimed superiority for the F.D.A. method over the Rideal-Walker method, the appointment of a revision committee suggests that results from the former test have shown similar variations to those experienced with the Rideal-Walker method.

All the methods considered possess the common requirement that the disinfectant should kill the test bacteria in the absence of particulate organic matter. This has led to some criticism which had its origin in a paper by Chick & Martin (1908)

advocating the use of dried faeces as organic matter. With the passage of the Diseases of Animals (Disinfection) Order of 1926, the Chick-Martin method was adopted as the test upon which the dilution to be awarded to commercial disinfectants was based. The consequent widespread use of the test speedily revealed the lack of reproducibility inherent in the method and produced widespread dissatisfaction amongst disinfectant manufacturers. Garrod (1934, 1935*a*, *b*) placed the test on a sounder basis when he proposed the substitution of yeast for the objectionable dried faeces, this modification being incorporated in the description of the technique issued by the B.S.I. (1938). Other tests requiring the presence of organic matter at the time of medication are those of the Admiralty (Patterson & Frederick, 1931), the Crown Agents for the Colonies (1934) and the methods suggested by Jensen & Jensen (1938). For some unknown reason the demand for the use of tests made in the presence of organic matter appears to have been to a marked extent limited to this country, and even here such tests have never been widely employed.

One common factor in the aforementioned methods is that they all necessitate the determination of the minimum concentration of the disinfectant necessary to produce the death of all the bacteria present in the medication tube. Consequently these methods all suffer from the defect that the presence of even a single abnormally resistant survivor will produce the same final result as would be obtained where there had been complete failure to kill any of the organisms originally present.

It is believed that the presence of such abnormally resistant organisms is responsible to a large extent for producing a considerable proportion of the test-to-test variations in the minimum lethal concentration of the disinfectant. This point has been referred to in greater detail by Withell (1942*a*, *b*).

Other possible sources for the discrepancies which occur have been suggested by Cade & Halvorson (1934), Cade (1937) and also by Thaysen (1938) and include:

(1) The sampling errors attendant on the use of a platinum loop for the transfer to the subculture tube, the quantity transferred being variable according to: (*a*) the angle of the loop to the surface of the contents of the medication tube at the time of withdrawal; (*b*) the speed of withdrawal; and (*c*) the surface tension of the contents of the medication tube.

(2) Alterations in the resistivity of the bacteria due to variations in the characteristics of the raw materials used in preparing the culture media (Wright, 1917; Brewer, 1943*b*).

Within recent years a different approach to the problem of disinfectant standardization has been suggested by the work of Bronfenbrenner, Hershey & Doubly (1938*a*, *b*, 1939), by Ely (1939) and by Greig & Hoogerheide (1941) with proposals based on the use of respiratory methods, but their methods do not appear to have received any general acceptance, presumably owing to the inherent complexities of manometric measurements.

The technique to be described in this paper originated in an attempt to overcome the defects which had been encountered in the continuous use of the accepted methods. It employs the simplest possible culture medium, permits the use of a measured quantity for the medication-subculture transfer, and makes use of a photo-electric density determination, using the apparatus described in a preliminary communication (Needham, 1946), to indicate the relative number of viable bacteria remaining at the end of the medication period. In addition, it possesses the merit of enabling a result to be obtained in a considerably shorter time than was the case with the older methods.

It is an unfortunate fact that, for some time now, developments in disinfectants have tended to lead to the preparation of products showing greater organism specificity than was the case with the older disinfectants (Walters, 1917; Leonard, 1931; Fuld, 1937; Brewer, 1943*a*). It is suggested that this increasing specificity will unquestionably complicate the problem of the comparative evaluation of disinfectants, and that the general employment of such disinfectants will necessitate the employment of tests which will give some indication of the ability of the product to kill bacteria under a wider range of circumstances than has been the case with the tests so far employed.

In spite of this complication it would appear that there is, and will always remain, a vital need for a simple routine test for those cases where the only requirement is ability to decide whether a disinfectant of known general characteristics reaches an appropriate and agreed standard. There is little doubt that a considerable proportion of all the tests which are carried out on disinfectants fall inside this classification, and the present paper puts forward a suggested technique for such a routine test. It is probable that the same basic principles may be of help in solving the problems entailed in evolving more complex tests suitable for demonstrating the relative merits of two or more disinfectants of different types. It is proposed to leave this latter problem for description in a future paper.

II. APPARATUS

(*a*) *Compensated photometric nephelometer.* This apparatus is shown diagrammatically in Fig. 1. A 12 V., 24 W. micro-projection bulb (*l*) is fed with a stabilized a.c. of the specified voltage. The current is derived from equipment which reduces the voltage

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fluctuations found in public supply systems to a small fraction of their original magnitude.

The light from the bulb is rendered parallel by passage through a collimating lens, and a pencil beam, $\frac{5}{8}$ in. diameter, obtained by the use of the two diaphragms (d_1, d_2). A shutter (not shown in the diagram) is placed immediately behind the lens and is used in preference to the switch (s_1) whenever it is desired to cut off the beam during a series of measurements. The beam of light is allowed to fall on an angularly placed optically flat glass plate (m) so disposed that it splits the beam, part being reflected on to a photocell (p_1), whilst the remainder passes through the glass plate. The transmitted portion of the beam then passes through a flow-through type of glass cell (c) having two optically flat sides 3 cm. apart. From this optical cell the light falls on a second photocell (p_2), the centre of which is screened with an opaque disk $\frac{3}{4}$ in. diameter, i.e. slightly larger than the beam. The output from this second (screened) photocell is connected across a reflecting galvanometer (g), whilst that from the first photocell is connected across a potentiometer (pt) graduated 0–100, and thence with reversed polarity to the galvanometer.

When the apparatus is required for purposes other than the standardization of disinfectants, the sensitivity of the instrument can be varied absolutely by the use of the variable resistance (r) in the filament circuit, and relatively by the use of neutral filters in the positions marked (f_1, f_2). The purpose of the three switches (s_1, s_2, s_3) should be apparent from the diagram.

(b) *Incubator.* This should be capable of maintaining a temperature of $37 \pm 0{\cdot}5°$ C. With some present-day incubators this will necessitate the use of an internal fan, owing to the temperature variation which exists at different levels.

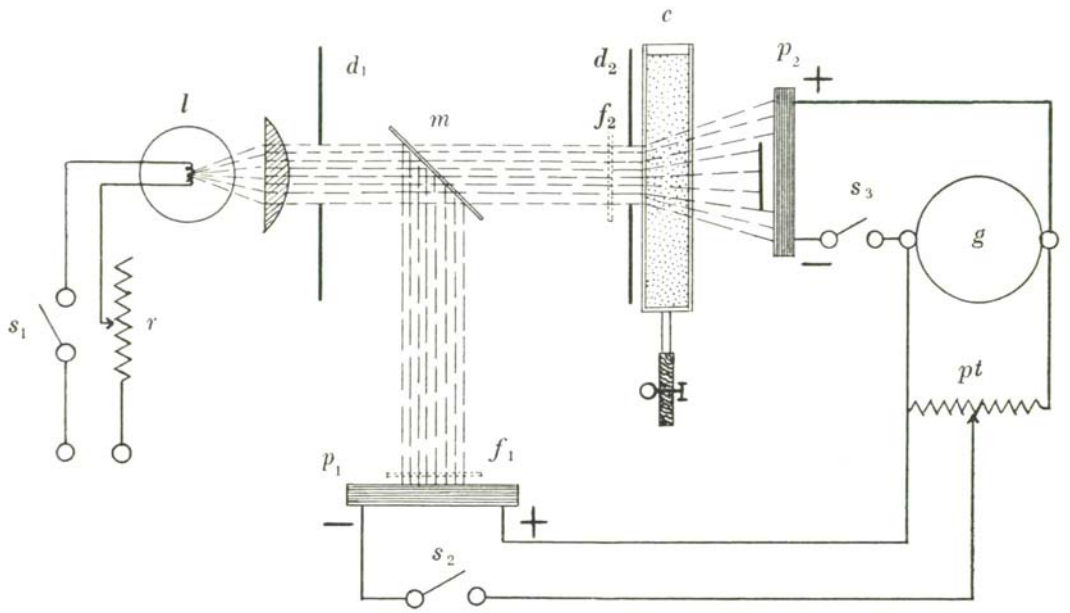

Fig. 1. Circuit employed in compensated photometric nephelometer. c, flow-through optical cell (Whilems Ltd. Type CT1); d_1, d_2, diaphragms having $\frac{5}{8}$ in. diam. apertures; f_1, f_2, positions for the insertion of neutral filters; g, galvanometer (Tinsley and Co.'s S.S. 5, 10 mm. deflexion per μA.); l, 12 V., 24 W. micro-projection bulb; m, optically flat glass plate; p_1, photocell (Evans's Electroselenium Type A, 45 mm. diam.); p_2, screened photocell, identical with p_1, but $\frac{3}{4}$ in. opaque disk fixed to centre of sensitive surface; pt, potentiometer (Cressall and Co.'s Type R. 100, 560 ohms); r, variable resistance (Cressall and Co.'s Type R. 100, 8·5 ohms); s_1, s_2, s_3, on-off switches.

(c) *Pipettes.* It is advantageous to employ pipettes graduated between two points and having a reasonably wide-bore tip, in order to keep the times required for filling and emptying to a minimum.

(d) *Medication tubes.* These should be capable of holding 20 ml. in approximately half their capacity, i.e. $6 \times \frac{3}{4}$ in. or larger diameter.

(e) *Culture bottles.* These should be approximately 8 oz. capacity and should be closed by aluminium screw caps and fitted with rubber washers.

(f) *Subculture bottles.* These should be similar to the above but 4 oz. capacity.

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(*g*) *Medication bath.* A water-bath set and maintained at 20 ± 0·2° C., with provision to hold twenty medication tubes, in two rows of ten, the tubes being immersed to not less than the level of the contents. Where a number of tests is likely to be carried out at any one session, considerable time can be saved by having the bath large enough to accommodate two, or even three, sets of tubes. In the absence of automatic control a larger bath is also advantageous in providing better temperature constancy during the medication period.

The particular water-bath employed for the experimental work measured 24 by 18 in. with a depth of 10 in. It provided for three sets of tubes and was equipped with a mechanical agitator and a thermo-regulated heating device. No provision was made for automatic cooling, as this was not found to be necessary under the usual laboratory conditions.

III. REAGENTS

(*a*) *Broth:*

'Oxoid' peptone	10 g.
Sodium chloride (A.R.)	5 g.
Distilled water	1000 ml.

The solids are dissolved in the water and the reaction adjusted by the addition of caustic soda to show a *p*H of 7·2 at a temperature of 20° C. After filtration through filter paper, the broth is bottled in quantities of 100 ml. if required for primary cultures or in quantities of 50 ml. if for subcultures. Both size bottles are sterilized at 15 lb. pressure for 15 min.

(*b*) *Phenol.* A standardized 5 % solution in distilled water is prepared from analytical grade phenol. Suitable directions for the preparation will be found in B.S.S. 808: 1938.

(*c*) *Bacteria.* The organism employed is *Bacterium typhosum*, Lister strain, a suitable strain of which can be obtained from the Curator of the National Collection of Type Cultures.

IV. METHOD

(*a*) *Maintenance of culture.* A portion of the growth on the slope received from the National Collection is inoculated into 100 ml. of broth, which has been kept at 37° C. overnight. After inoculation, the culture is incubated at 37° C. for 24 hr. and then a single 4 mm. loop is used to inoculate a further 100 ml. of broth which has also been kept at 37° C. overnight. This subculture procedure is repeated each day.

The culture used for the test should have been through not less than four, and not more than eighteen daily incubation periods and should have been incubated at 37° C. for not less than 23, and not more than 25 hr. For every test to be carried out, 5 ml. of culture should be placed in each of ten medication tubes, these tubes being then placed in alternate rows in the medication bath.

Where it is desirable to maintain the stock culture in the laboratory, this should be done by growing it for 24 hr. at 37° C. in a medium consisting of the specified broth, solidified by addition of 1·5 % of agar. After incubation for 24 hr., the culture should be kept at room temperature for not more than 1 week before being subcultured on a fresh agar slope.

(*b*) *Dilutions of disinfectant.* From stock dilutions of convenient strength, a total of ten dilutions, each of 20 ml., should be prepared, the dilutions being such that each succeeding one is a fixed proportion of the preceding one. If a 10 % diminution is desired, this can be done conveniently by making up 200 ml. of the strongest required dilution, withdrawing 20 ml. and replacing with the same amount of sterile distilled water. The withdrawal and replacement are repeated until the required number of dilutions has been prepared. For a 5 % series diminution, 400 ml. of the first dilution should be prepared, again withdrawing and replacing 20 ml. Where it is desired to include both the known and unknown disinfectants in a single test, the proportion of dilutions of the known to those of the unknown is left to the operator's discretion.

Each set of ten dilutions is placed in the medication bath behind a corresponding set of culture-containing tubes.

(*c*) *Test proper.* The test should be started, preferably at some easily recorded time period, after the culture and dilution tubes have been allowed to stand in the medication bath for approximately 15 min. Fifteen seconds before the chosen zero time, 5 ml. of diluted disinfectant is withdrawn from the first dilution tube by means of a pipette and added to the contents of the first culture tube under such conditions that the time taken for the addition is evenly divided about the chosen zero time. The resulting mixture of culture and disinfectant is shaken vigorously for 10 sec. before being returned to the bath. Thirty seconds after zero, the second disinfectant dilution tube is taken from the bath and the pipette is twice rinsed out with this dilution before being employed to add 5 ml. of disinfectant to the second culture-containing tube, the addition being made exactly 1 min. after the comparable addition to the first tube. This cycle of operations is repeated with the third and subsequent dilutions until 5 ml. from all ten dilutions have been added to their appropriate culture tubes.

Twenty-five seconds before the completion of the 10 min. contact period, the first medication tube is taken from the bath and shaken for 10 sec. Immediately thereafter 0·5 ml. of the culture/disinfectant

mixture is withdrawn and added to 50 ml. of broth under such conditions that the addition is evenly timed about the zero plus 10 min. mark. The broth should have been maintained at 37° C. overnight and only removed from the incubator immediately before use. At 1 min. intervals the remaining medication tubes are treated in order in a similar manner, whereupon the ten subculture bottles must be replaced in the incubator and allowed to incubate for exactly 5 hr. from the time of the inoculation of the first subculture.

(*d*) *Measurement of bacterial density.* Some 3–5 min. before the conclusion of the incubation period, the nephelometer light (*l*) is switched on (s_1) and 2 min. allowed for it to reach a state of equilibrium before any adjustments are made. The intensity of the light is checked with the transmitted portion of the beam cut off by the interposition of a solid disk in position (f_2) and with the screened photocell (p_2) isolated from the galvanometer by means of its switch (s_3). With the potentiometer (*pt*) adjusted to the 75 mark and a 66 % transmission filter in the reflected beam (f_1), the light is adjusted by means of the variable resistance (*r*) until the galvanometer (*g*) shows a deflexion corresponding to 10 μA. The light beam is now completely cut by closing the shutter fixed to the lens mounting: the disk is removed from the transmitted beam and the switch (s_3) closed, thereby rendering both photocells usable. Immediately the specified incubation period has expired, the first subculture is run into the optical cell and the light shutter opened. This causes the galvanometer to swing according to the relative response of the two photocells to the amount of light which is falling on their sensitive surfaces. The potentiometer is now adjusted until a null-point deflexion is obtained, the balancing potentiometer reading being recorded. The flow-through cell is now emptied by opening the pinch cock and allowing the contents to run into some suitable and conveniently sterilizable receptacle. The second subculture is then placed in the cell and the circuit brought to balance, the contents of the cell being subsequently discarded. This cycle is repeated until all the ten subcultures have been examined.

(*e*) *Calculation of results.* In spite of disadvantages, as a standard for the comparison of disinfectants, it is more desirable that phenol should continue to be used than any other single alternative. An exception would be in the case of large-scale contracts where a manufacturer might be expected to deposit a reference sample with the purchaser, thereby permitting side-by-side comparison with actual deliveries.

To determine the relative efficiency of two disinfectants, graphs are prepared in which the experimentally determined potentiometric balances are plotted against the corresponding dilutions of the disinfectants. The ratio is then obtained by comparing the mean of the three dilutions of the unknown which would have produced a balanced circuit with the potentiometer adjusted to give readings of 25, 50 and 75 and the corresponding mean for the control. When phenol is employed as the reference standard, the result of this calculation could conveniently be referred to as the 'Phenol ratio'. Typical examples of the curves obtained are given in Figs. 2–5. The phenol ratios to be derived from these curves are given in Table 1.

Table 1

Disinfectant	Dilution corresponding to potentiometer reading of: 25	50	75	Mean	Phenol ratio
Phenol (%)	0·795	0·740	0·705	0·747	—
Black fluid (%)	0·0365	0·028	0·023	0·029	25·5
White fluid (%)	0·052	0·047	0·043	0·047	15·9
Chlor-phenolic fluid (%)	0·115	0·10	0·090	0·102	7·3

V. DISCUSSION

The departures which the described technique makes from those previously employed, coupled with the complex nature of the problems involved in the comparative evaluation of disinfectants, make it desirable to treat the problems and points of difference on an individual basis.

(*a*) *Bacteria*

General experience with other methods over a number of years by numerous workers has shown the undesirability of using a culture which has been grown in broth for only one or two 24 hr. periods, as such cultures tend to show abnormality in their response to the action of disinfectants. Similar discordant results have been obtained when the culture has been maintained in liquid media beyond the third week, and these considerations have led to the stipulation that the culture employed should have been through not less than four and not more than eighteen 24 hr. incubation periods.

Limits must be placed on the age of the culture owing to the rapidity of development which takes place in the medium employed, otherwise considerable variation in the number, age and, presumably,

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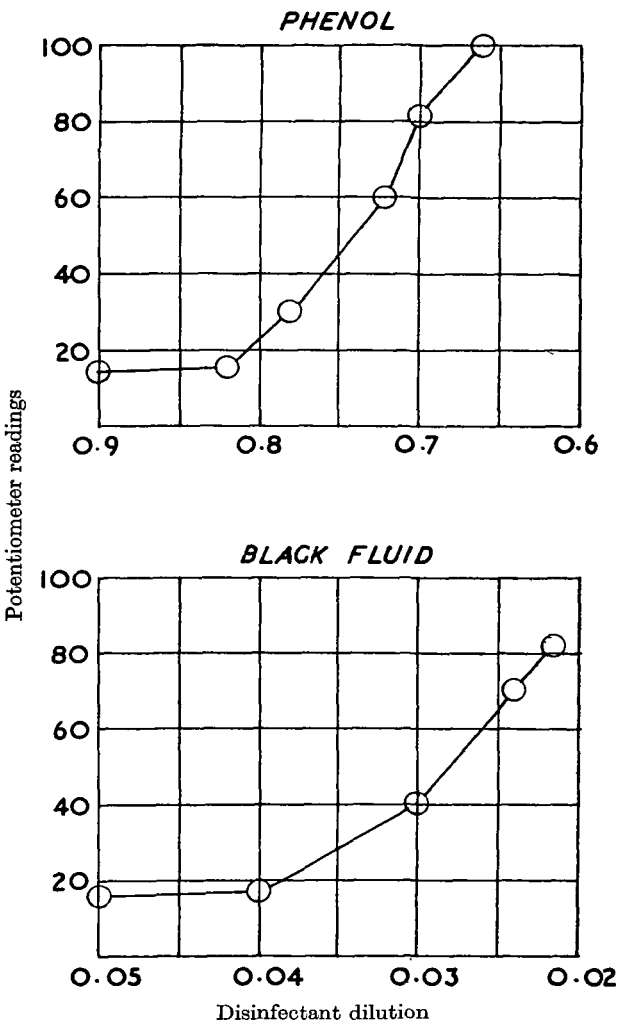
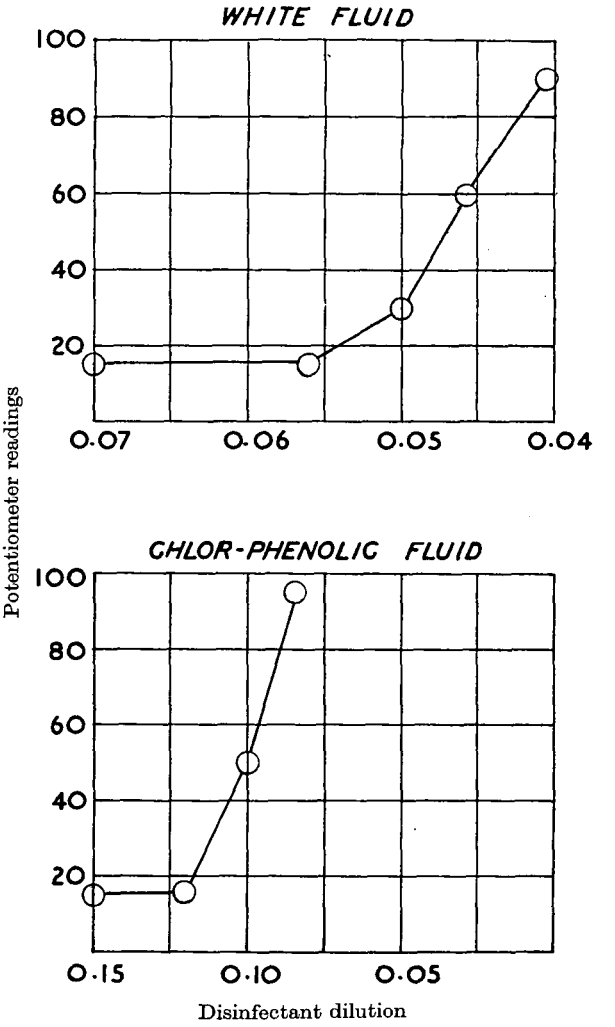
the vitality of varying percentages of the organisms present is to be expected.

There is little doubt that most of our knowledge of the behaviour of disinfectants has been based on the use of *B. typhosum* as a test organism and, after so many years' general acceptance, it would seem



Figs. 2 and 3.



Figs. 4 and 5.

impossible to find reasons for total abandonment of *B. typhosum* in favour of some other organism. There is ample evidence of the necessity for the use of standard strains in any comparative tests if reasonable uniformity is to be attained. Unfortunately, the four standard strains of *B. typhosum* so far employed in disinfectant standardization, 'Hopkins', 'Lister', 'Rawlings' and 'S', have all been criticized at some time or another, mainly on the score of roughness.

In the technique already described, I have advocated the use of the Lister strain, but it is felt that this should be in the nature of a short-term measure, and that steps should be taken by some competent body to examine the position with a view to the possible substitution of a more typical strain as an alternative to the four mentioned. Concurrently, consideration might be given to the advisability of employing two or more individual strains, separate 24 hr. cultures of which should be pooled immediately before the start of the test. It is felt that the adoption of this latter suggestion might be

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an advance in the right direction, as, given suitable strains, its use should help to avoid the unfortunate results occasioned by a particular strain exhibiting abnormal susceptibility to the action of certain specific types of disinfectants.

The question of the use of organisms additional to *B. typhosum* will be dealt with in a subsequent paper covering the wider application of the basic method.

five other well-known makes. The organism employed in this comparison was *B. typhosum*, Lister strain, and a uniform inoculum from a culture which had been grown on a mixed broth was employed to inoculate a constant volume of the six broths. This particular test was carried out with the nephelometer adjusted to a considerably lower degree of sensitivity than that suggested for the disinfectant test. The necessity of making provision for such a

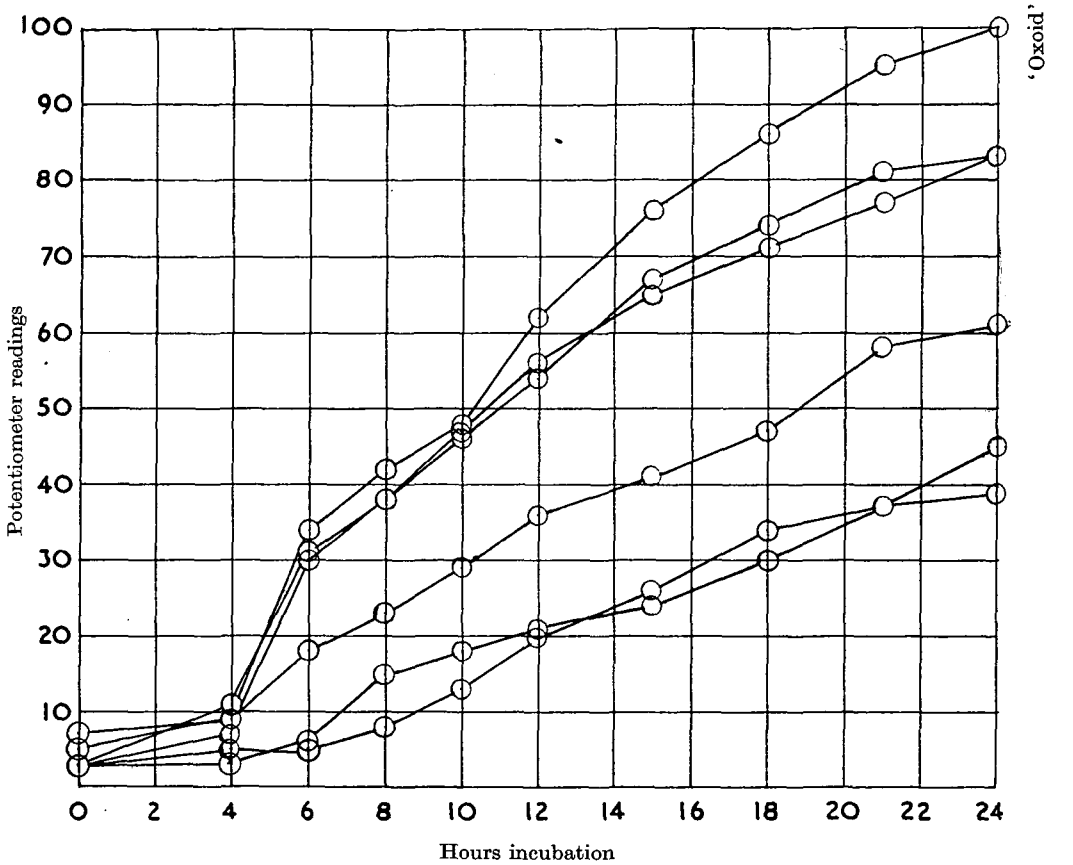

Fig. 6. Comparison of growth obtained with 'Oxoid' peptone and that given by five other well-known brands.

(*b*) *Broth*

It is probable that more time has been devoted to the search for a culture medium possessing all the desired attributes than has been spent on any other single aspect of disinfectant standardization.

The formula now put forward is exceedingly simple, this simplification being possible only because of the superiority of 'Oxoid' peptone over the peptones previously employed. This superiority is well displayed in Fig. 6, where the growth given by 'Oxoid' peptone is compared with that given by wide variation in the turbidity of the broths required other departures from the described technique.

Comparative tests have indicated that, where 'Oxoid' peptone is employed, there is little advantage to be derived from the addition of the customary meat extract. The deletion of this material not only simplifies the broth but also reduces the number of steps necessary for its preparation. The net result of these alterations is a very considerable reduction in the possible variation

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which is likely to occur when the broth is made up in different laboratories.

The *p*H of 7·2 was chosen after it had been shown that the broth gave its maximum growth between *p*H 6·8 and 7·6, the falling away in the amount of growth with broths outside this range being more marked than that shown by a corresponding *p*H difference within the stated range. This phenomenon of limited variation of the growth characteristics within the *p*H range 6·8–7·6 was also experienced with a number of other broths prepared from different raw materials and to different formulae.

Experiments have shown that a slightly heavier growth could be obtained by employing 2 % of the specified peptone, and that a still further slight increase could be obtained by the substitution of the mixture of salts employed in Ringer's solution for the sodium chloride. This latter suggestion complicates the preparation of the medium to the extent that it necessitates the filtering of the precipitate which is thrown down on heating. The advantages to be derived from an increase in the percentage of the peptone do not appear to be of worth-while significance for the specific purpose here concerned.

The problem of the production of a synthetic medium has received very considerable attention (Klarmann & Wright, 1945; Peterson & Peterson, 1945; Wolf, 1945), and has, from the theoretical aspect, much to commend it. To date, however, the behaviour of even the best of the synthetic media has left much to be desired, and, in view of the number of relatively rare chemicals which must be employed in small quantities, it would appear to be a debatable point whether such artificial media could be expected to show less batch to batch variation than would be encountered in the simple medium described. At the same time, there is little doubt that investigations into the problem of synthetic media justly deserve all the attention they can receive.

The reason for specifying that the temperature of the medium at the time of inoculation must be 37° C. rests on the fact that larger volumes are employed than has been customary. If this precaution were to be omitted, the possibility would arise of varying results being obtained through different incubators giving different temperature gradients.

(*c*) *Disinfectant dilutions*

The method employed is that originally put forward by Garrod (1935*a*), who calls attention to its advantages over the alternative methods. Because of the reduction in concentration which takes place when the disinfectant is mixed with the bacteria, the strength of the prepared dilutions should be twice that which it is desired to have present during the medication period.

(*d*) *Compensated photometric nephelometer*

The majority of the different systems which have been proposed for the measurement of the degree of turbidity present in a liquid necessitate the determination of the relative amount of light which can pass through some prescribed depth of the liquid. When such methods call for the use of a photocell as a means of measurement of this amount of light (Alper & Stern, 1933; Bonét-Maury & Walen, 1945; Pulvertaft & Lemon, 1933), the maximum amount of light falls on the photocell in the absence of any turbidity, so that, where such a method is applied to the measurement of bacterial growth, the greatest amount of light falls on the photocell when a sterile broth is being examined. It is known that photocells can be subject to fatigue, particularly if called upon to respond to high light intensity. When fatigue occurs the response of the cell to any given light standard tends to fall, with the result that in the methods advocated by the above authors, the operator may be left in a state of uncertainty as to whether slight changes in the response of the photocell should be ascribed to bacterial growth or fatigue of the cell. This possible defect does not arise in the particular circuit which has been employed. It is a modification of that described by Libby (1938) and, as a result of the screening of the centre of the photocell (p_2), shows the minimum response to sterile medium, an increased reading being shown upon the initiation of bacterial growth. Accordingly, fatigue can only occur with the examination of a very strong 'positive' and, should it occur, will only result in returning a slightly less 'positive' answer than would have been the case if no fatigue had occurred. There are a number of additional reasons why the method chosen is less prone to exhibit fatigue than is the case with the alternative method. Another and more important advantage attaching to the chosen system is that, for a given optical depth, it is far more sensitive than is the case with comparable equipment employing the direct beam system.

It will have been noted that a second photocell has been included which was not present in Libby's circuit. This step was originally taken in the expectation that its employment would counteract the effects of residual variation in the intensity of the light source, subsequently found to be due to changes in frequency being outside the scope of the constant voltage equipment then in use. Experience with the two-cell circuit showed that the second photocell could be used to perform an even more important function, namely, to act as a reference standard, thereby permitting the routine

adjustment of the light to a constant intensity before any turbidity measurements were made.

The optical depth chosen (3 cm.) is that which has been found experimentally to be the most satisfactory for the measurement of the desired turbidity range and, if the apparatus was to be used for other purposes, other depths might be found to be preferable.

Criticism could be directed to the need for transferring cultures from the bottles in which they have been incubated to the optical cell, and it is realized that this stage of the technique calls for the use of reasonable care. The fact that the organism specified is probably by now non-pathogenic has some significance in this connexion.

At the conclusion of a series of measurements, the cell is first rinsed with water and then sterilized by means of an alcoholic solution of phenol. Before being used on a subsequent occasion, the cell is given a short immersion in chromic acid followed by careful rinsing with distilled water.

Selenium photocells have been employed, these cells having the merit of being sufficiently sensitive to give adequate response under the particular operating conditions, without requiring the use of any complicated electrical amplification system.

The galvanometer is a self-contained reflecting instrument with a scale 20 cm. long, the sensitivity being such that full-scale deflexion is given for 20 μA. For use in the test the instrument is adjusted to come to rest in the centre of the scale. A special advantage attaching to the use of the particular make of galvanometer employed is that it does not require the use of any elaborate anti-vibration precautions.

The actual apparatus employed in the experimental work leading to the adoption of the technique now described embodies parts obtained from a number of manufacturers and, in a number of cases, these have been modified to obtain the desired characteristics. It appeared desirable to obtain a more standardized set-up and the manufacture of such an instrument by Messrs Casella and Co. (Regent House, Fitzroy Square, London, W. 1) has been undertaken.

(*e*) *Medication*

(1) *Temperature.* A temperature of 20° C. has been chosen for the medication bath for the reason that this corresponds with that now employed for the majority of laboratory standards and also because its use to some extent simplifies the problem of maintaining the required temperature, no simple, yet at the same time efficient, method of reducing a bath temperature being available.

(2) *Amount of culture employed.* The older tests have called for the measurement of very small amounts of culture, which had usually to be dropped on the surface of the disinfectant dilutions. The possible measurement errors attaching to the use of such small quantities were correspondingly great. In addition, should any of the culture touch the side of the tube, there existed the possibility that bacteria which, at best, had been exposed to the action of the disinfectant for less than the stipulated period, might be present in the sample transferred to the subculture medium. To avoid these possibilities 5 ml. of culture was employed, and this was placed in the tube before the addition of the disinfectant.

(3) *Length of medication period.* Ten minutes was chosen for the length of the medication period as being as representative of practical conditions as any other one period can be. No serious objection could be made against an extension of this period, whereas a reduction in the medication time would be open to the criticism that it would only permit the testing of a lower number of disinfectant dilutions.

As described, the technique is based on the use of 60 sec. intervals between tubes, but there is nothing to prevent this interval being reduced to either 50 or even 40 sec. In this event the number of possible dilutions would be increased to 12 and 15 respectively. Should either of these shorter periods be chosen, it would be found advantageous to make use of some timing device capable of giving one complete revolution for the actual period employed, so as to avoid the necessity of making rapid mental calculations whilst performing the test.

(4) *Transfer at conclusion of medication period.* Reference has already been made to the many factors which can alter the amount of material which, in the case of earlier methods, will be present in the loop at the time it is plunged into the subculture medium. I have chosen to employ a quantity which should be measurable with considerably greater uniformity, and this in its turn has dictated the amount of culture medium which must be employed to prevent the possibility of bacteriostatic action being confused with bactericidal action. Under the conditions chosen the ratio of culture media to disinfectant-culture mixture is 100 to 1, and it is considered that this ratio will be found to be adequate for the majority of disinfectants. In dealing with a product having a very high bacteriostatic/bactericidal ratio, special precautions appropriate to the particular case must be employed. Such precautions might include the addition of inactivating agents to the culture medium or the use of considerably larger volumes of broth for the subcultures.

(5) *Incubation period.* It has been shown experimentally that if a number of flasks containing the same volume of broth are inoculated with varying

levels of inoculum and then incubated for varying periods at 37° C., the turbidities due to the growth of the bacteria first of all show increasing differences with the different inoculum levels. Subsequently these differences become reduced and finally disappear, all the cultures ultimately attaining the same density levels. To make use of this phenomenon, it was necessary to choose a period within the time range showing the greatest turbidity variations for the different inoculum levels, and this period of maximum variation appeared to occur between 4 and 8 hr.

Consideration of the length of the normal working day dictated a preference towards the shorter time period, and 5 hr. was accordingly chosen. With this incubation period and the nephelometric apparatus adjusted to the described sensitivity, an inoculum, equal to the survival of 1 in 300 of the number of organisms employed in the test, produces a detectable effect and, under the same conditions, the equivalent of 6 % survivors gives sufficient turbidity to produce the maximum readable deflexion. It would be possible to increase the percentage of survivors necessary for the production of full-scale deflexion by removing the neutral filter from the compensating circuit, but practical tests failed to show that any advantage was to be derived from the adoption of such a procedure.

Tests carried out with the operating conditions adjusted to permit the determination of the disinfectant concentration required to produce 50 % mortality did not appear to be as reproducible as were tests carried out by the described technique.

(*f*) *Calculation of results*

This new method gives far more information on the behaviour of a disinfectant than has hitherto been obtainable with any single method, and it has already shown that two disinfectants giving comparable results by one of the older methods may still show considerable differences in the slope of the curve given by the method here described. It is suggested that this difference in slope may be yet another part of the explanation of some of the cases where different laboratories have reported widely varied results for one and the same disinfectant. This variation of slope is likely to be of even greater importance when a comparison is being made between two disinfectants of totally different types and, for that reason, the suggested procedure of taking the mean of the dilutions giving potentiometer readings of 25, 50 and 75 (corresponding approximately to the growth produced by 0·75, 2 and 3 % survivors) is considered to be superior to comparisons made at a single level. Where greater accuracy of comparison is required, statistical treatment of the complete curve would provide the required data.

VI. SUMMARY

1. Attention is called to the widespread dissatisfaction with the standard of reproducibility attained with the existing disinfectant tests.

2. It is suggested that this lack of reproducibility is inherent in any 100 % mortality test.

3. The trend in all recent disinfectant developments appears to be towards greater specificity than was the case with the older products.

4. Where a comparison is being made to ascertain the true relative efficiency of two or more disinfectants, this possible specificity necessitates their examination under a wider range of conditions than has been catered for in the past.

5. A technique is suggested for use in those cases where a more limited examination will provide all the information that is required. It employs a simple culture medium, uses a measured quantity for the transfer from the medication tube, and makes use of a compensated photo-electric circuit to determine the amount of growth produced by the organisms which have survived the contact with the disinfectant.

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