A COMPARATIVE STUDY OF THE GERMICIDAL PROPERTIES OF A SELECTED GROUP OF SKIN DISINFECTANTS

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

Edward Howard Armbruster

1942

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A COMPARATIVE STUDY OF THE GERMICIDAL PROPERTIES OF A SELECTED GROUP OF SKIN DISINFECTANTS

by

EDWARD HOWARD ARMBRUSTER

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

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INTRODUCTION

Man had practiced disinfection long before he understood that such things as bacteria existed. Antony Van Leewenhoek was the man to discover the "wee small animalcules" in 1683, but it took nearly 200 years before the investigators realized the significance of this discovery. It remained for Koch and his method of pure culturing to bring the study out of its chaotic state into that of a science. Koch's experiments with silk threads impregnated with anthrax spores are well known to every student in the field of disinfection. In 1897 Kronig and Paul[1] published a new method by which it was possible to make a quantitative study of disinfection. They demonstrated that disinfection by a germicidal agent was not an instantaneous act, but rather a gradual process that followed an orderly sequence. The influence of temperature on the rate of disinfection was reported by these two men at this time.

Rideal and Walker[2] introduced the use of phenol as a basis of comparison for a "carbolic acid coefficient." This was probably the first attempt to devise a practical method of testing disinfectants because the conditions under which the tests were made were standardized and in doing so, many of the sources of error that had confused earlier workers were avoided. The present F. D. A. method of determining a phenol coefficient is an outgrowth of the Rideal-Walker method.

The phenol coefficient has been used for many years as a measurement of the efficiency of most types of disinfectants without regard to their chemical composition or the application of the compound. Inasmuch as chemical composition varies widely and application may be of totally different natures, it is apparent that no one method of testing could be applicable to all compounds. In these studies, we were interested primarily in compounds used for skin and wound disinfection. The phenol coefficient seemed a poor method of evaluation, first, because the action must take place rapidly on the skin or wound so the time factor of 10 minute test period as used in the phenol coefficient is too long. Second, it has been customary to rate the phenol coefficient on the original concentration of the product as sold and since the strength of the various disinfectants vary one from the other, the phenol coefficient frequently has been high for compounds actually of poor quality as measured by killing properties.

Therefore, the problem resolved itself into one of seeking a new and more practical method of evaluating disinfectants which are used on skin and wounds in order to fairly compare all compounds. The method had to take into consideration the difference in the type of action of the various compounds, the difference in their reaction rates or ability to kill in short intervals, and the ability to penetrate into the bacterial cell to cause death of the organism as well as penetration into the wound to reach the organism.

The compounds selected for this study are listed below

with their original concentrations as well as their source. All compounds, with the exception of the Phemerols were purchased on the market and all compounds are listed in the dilution as sold. The source of each compound is also listed.

*Phemerol 44 (tinct.)	1-1000	Parke, Davis & Co.
*Phemerol 41 (tinct.)	1-500	Parke, Davis & Co.
*Phemerol 45 (tinct.)	1-500	Parke, Davis & Co.
*Phemerol (aqueous)	1-1000	Parke, Davis & Co.
Hexylresorcinol S. T. 37	1-1000	Sharpe & Dohme Co.
Iodine (tinct.)	1-40	Frank W. Kerr Co.
Zepharin (tinct.)	1-1000	Alba Pharmaceutical Co.
Zepharin (aqueous)	1-1000	Alba Pharmaceutical Co.
Mercurochrome	1-20	Frank W. Kerr Co.
Mercresin (tinct.)	1-1000	Upjohn Co.
Merthiolate (tinct.)	1-1000	Eli Lilly & Co.
Merthiolate (aqueous)	1-1000	Eli Lilly & Co.
Metaphen (tinct.)	1-200	Abbot Laboratories
Metaphen (aqueous)	1-500	Abbot Laboratories
Merphenyl Nitrate (aqueous)	1-1500	Hamilton Laboratories
Merphenyl Borate (tinct.)	1-500	Hamilton Laboratories

Phemerol and Zepharin were included as they are two relatively new compounds. Phemerol is designated chemically as a para-tertiary-octyl-phenyl-diethoxy-dimethyl-benzyl ammonium chloride and Zepharin as alkyl-dimethyl-benzyl ammonium chlorides.

*The Phemerols were supplied through the courtesy of Parke Davis & Co.

Since disinfectants must be active against a varied flora as is found on the skin, several types of organisms were used in this study. These organisms were:

Eberthella typhosa	Michigan State College	F. D. A.,
Eberthella typhosa	Parke, Davis & Co.	F. D. A.,
Staphylococcus aureus	Michigan State College	F. D. A.,
Staphylococcus aureus	Parke, Davis & Co.	F. D. A.,
Diplococcus pneumoniae	Parke, Davis & Co.	
Streptococcus hemolyticus	Parke, Davis & Co.	
Pseudomonas aeruginosa	Parke, Davis & Co.	

Eberth. typhosa was chosen as it is the standard Gram negative test organism and Staph. aureus was chosen as it is the standard Gram positive test organism in the F. D. A. phenol coefficient. Dip. pneumoniae and Strep. hemolyticus were chosen as they represent two true pathogenic organisms. Pseud. aeruginosa was selected as the organism to represent the group of bacteria which are intermediates between Gram positives and Gram negative organisms.

Both Parke, Davis & Co. and Michigan State College obtained their original cultures of Staph. aureus and Eberth. typhosa from the Food and Drug Administration.

I. KILLING DILUTIONS

The killing dilution of each disinfectant was obtained by using the F. D. A. Phenol Coefficient Method as presented in the U. S. D. A. circular 198.[3] With Strept. hemolyticus and Dip. pneumoniae a veal glucose infusion broth was used in place of the F. D. A. nutrient broth. This, however, was the only deviation from the standard procedure. Medication temperatures of 20° C and 37° C were used on all organisms to obtain the effect of temperature in disinfection by the various compounds.

The Shippen modification[4] was used on the mercurials to eliminate the bacteriostatic action of the disinfectant. This consisted of making a second subculture in broth. To assure sufficient seeding, four loopfuls of broth from the primary subculture were transplanted to the secondary subculture.

The results obtained are presented in Tables I-VI. The killing dilutions expressed are based on the compound as sold and not on the original dilution of the chemical agent in the preparation.

The Phemerol group was the most germicidal of the selected group of compounds. At 20° C Phemerol tincture 44 (1-1000) was able to stand a dilution of 1-50 times against Staph. aureus, 1-40 times against Eberth. typhosa, 1-2 times against Pseud. aeruginosa, 1-10 times against Strept. hemolyticus, and 1-60 times against Dip. pneumoniae and still sterilize in less than 10 minutes. Zepharin which is closely related was slightly less active than Phemerol, but still it was superior in action to the remainder of the group of compounds tested.

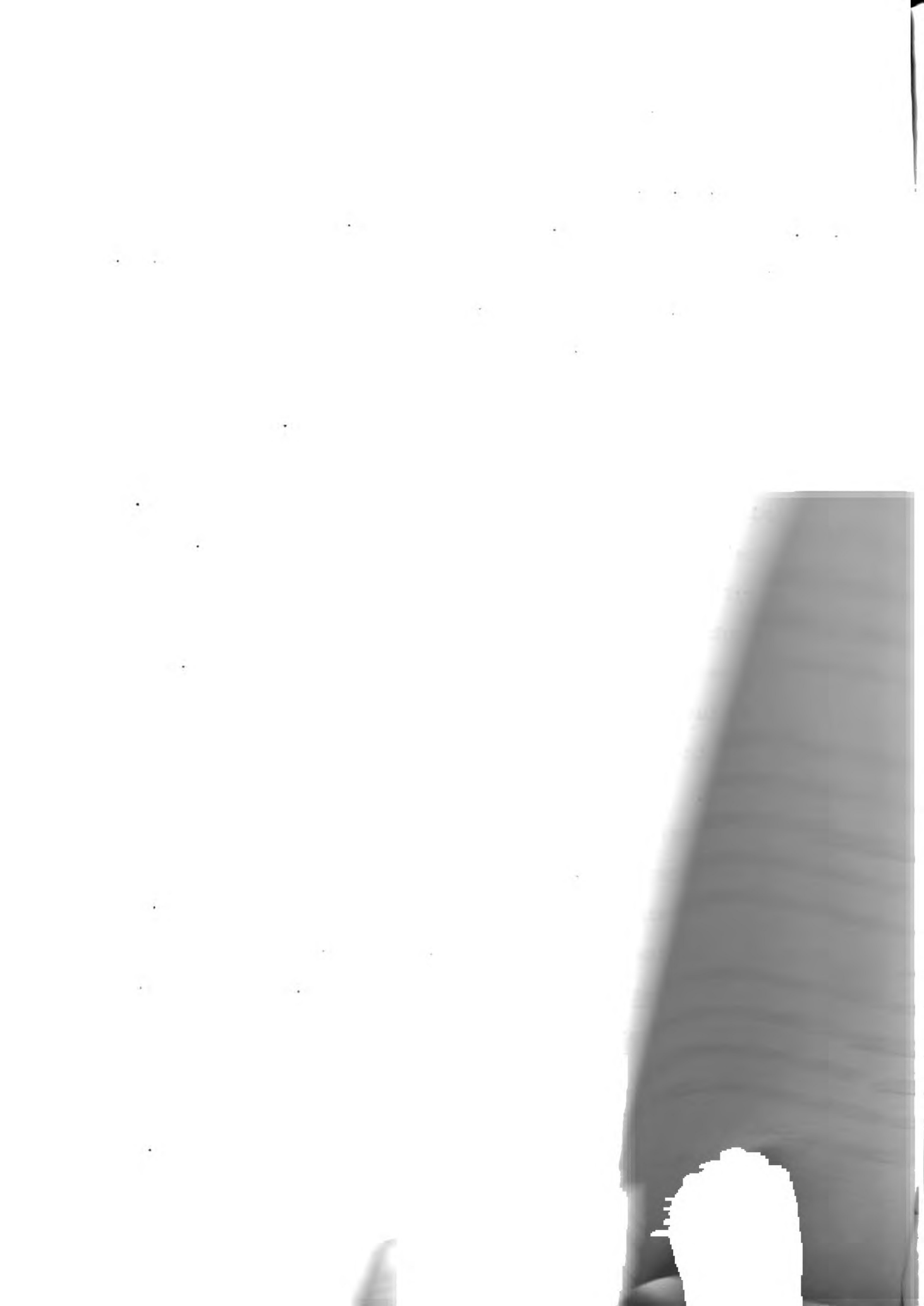
When Hexylresorcinol S. T. 37 (1-1000) was diluted more than 1-5, no evidence of killing action could be shown. Against Pseud. aeruginosa the compound could not stand any dilution and still sterilize in 10 minutes at 20° C.

Tincture of Iodine (1-40) could stand a dilution of from 1-150 against Pseud. aeruginosa to 1-350 against Strept. hemolyticus.

Mercresin was the most consistant germicidal agent among the mercurials exhibiting germicidal properties against all organisms in 10 minutes at 20° C in a diluted form. This was probably due to the addition of cresols to the compound to take care of the Gram positive organisms. Mercresin tincture (1-1000) could stand a dilution of 1-10 against Staph. aureus, 1-70 against Eberth. typhosa, 1-30 against Pseud. aeruginosa, 1-15 against Strept. hemolyticus, and 1-40 against Dip. pneumoniae.

Merphenyl Borate tincture (1-500) showed no killing action against Strept. hemolyticus at 20° C and could stand only a dilution of 1-3 against Staph. aureus before its action was destroyed by dilution. When tested against the Gram negative Eberth. typhosa, this compound could be diluted 1-225 and still kill in 10 minutes.

Mercurochrome (1-20) showed no evidence of kill against Strept. hemolyticus and it had to be used full strength against Staph. aureus. Against the Gram negative Eberth typhosa, this germicide exhibited most action, killing in 10 minutes at 20° C at a dilution of 1-50.



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Aqueous Metaphen (1-500) was another selective agent which sterilized Eberth. typhosa and Pseud. aeruginosa in high dilutions while being unable to kill Staph. aureus, Strep. hemolyticus, and Dip. pneumoniae when diluted. Metaphen tincture (1-200) killed all Gram positive test organisms in a dilution of 1-2 in 10 minutes at 20° C.

Aqueous Merthiolate (1-1000) was the least effective compound tested, showing absolutely no kill on any of the three Gram positive test organisms in the 10 minute period. Against Eberth. typhosa a dilution of 1-2 effected kill while against Pseud. aeruginosa it was impossible to kill if the solution were not used full strength.

Merphenyl Nitrate (1-1500), while showing fair activity against the Gram negative test organisms, was ineffective against the Gram positives.

These data show that the mercurials, as a group, exert a strong selective action against the Gram negative organisms while they remain ineffective or only weakly effective against the Gram positive organisms. The Merthiolates showed their action to be more like that of a bacteriostat than that of a germicide.

In no instance did the Shippen subcultures show growth where the primary subculture did not when a Gram negative test organism was used. This was not the case when Gram positive test organisms were used. This would seem to indicate that the Shippen modification cannot be applied to the testing of mercurials with Gram negative test organisms and the high

values obtained by the method are due in part to bacteriostasis. It may be that the disinfectant is adsorbed on the organisms and held there very tightly. In this way the organisms would be prevented from multiplying but at the same time not be killed. If this hypothesis is true, the F. D. A. method cannot be used to compare the killing dilutions of mercurials and non-mercurials against Gram negative organisms.

A second explanation for this failure of the Shippen modification would be the possibility that the germicide kills the majority of the organisms and only a very few viable organisms are carried into the primary subculture. This would make it extremely improbable for the second subculture to receive any organisms from the primary, even by using four loopfuls as in transfering by the Shippen technique. This possibility was shown to be false in the rate reaction tests which follow.

If these compounds were to be compared on the basis of the F. D. A. phenol coefficient, Iodine, instead of Phemerol would be considered the most desirable compound. The phenol coefficient of Iodine against Staph. aureus at 20° C is 2.9 and the phenol coefficient of Phemerol 44 under comparable conditions is 0.7. This would indicate falsely that Iodine is 4 times more active than Phemerol 44, while on the basis of effective dilution it can be seen that the Phemerol 44 is diluted 50,000 times and Iodine only 8000 times which makes Phemerol over 8 times more active as a germicidal agent.

The mere fact that one manufacturer puts his compound up in a 1-1000 dilution while his competitor retails his at a

1-500 dilution is no criterion of the germicidal effectiveness or reserve strength of the compound. This is proven by the results against Strept. hemolyticus at 20° C where Merphenyl Borate (1-500) was unable to kill in 10 minutes while Phemerol 44 (1-1000) could stand a dilution of 1-10 and still kill.

A bactericide which is to be used in skin and tissue disinfection must be effective against all types of organisms in a diluted form if it is to be considered a good germicide. Mercurochrome, Metaphen, Merphenyl Nitrate, Merphenyl Borate, and Merthiolate do not possess this property as they are unable to kill Strept. hemolyticus in a diluted form. The only instance where a selective compound could be safely used is in the case of a specific instance where the type of organism causing the infection is known and a selective acting compound can then be used if it is effective. This is also the reason why a series of different types of organisms must be used for determining the germicidal properties of a compound.

Killing in a diluted form is another vital factor in tissue disinfection. In a wound the compound may be diluted several times by the serum present. A compound like Merthiolate, while a good bacteriostat, may prevent further multiplication of the organism but it would have insufficient killing action when diluted with the serum and unless constantly applied, the infection could become active once more.

When the temperature under which the killing dilution tests were made was raised to 37° C, all compounds increased

in their activity with the exception of Iodine, which remained at the same level as in the 20° C test.

TABLE I

THE KILLING DILUTIONS OF VARIOUS DISINFECTANTS IN 10 MINUTES ON STAPHYLOCOCCUS AUREUS
(P. D. 02482)

Disinfectant	Orig. Conc.	Killing Dilution 20°C	37°C
Phenol		1-70	1-80
Phemerol 44 (tinct.)	1-1000	1-50	1-130
Phemerol 41 (tinct.)	1-500	1-60	1-225
Phemerol 45 (tinct.)	1-500	1-80	1-300
Phemerol (aqueous)	1-1000	1-50	1-150
Hexylresorcinol S. T. 37	1-1000	1-4	1-5
Iodine (tinct.)	1-40	1-200	1-200
Zepharin (tinct.)	1-1000	1-20	1-90
Zepharin (aqueous)	1-1000	1-20	1-90
Mercurochrome	1-20	1-1	1-2
Mercresin (tinct.)	1-1000	1-10	1-10
Merthiolate (tinct.)	1-1000	1-1	1-2
Merthiolate (aqueous)	1-1000	no kill*	1-1
Metaphen (tinct.)	1-200	1-2	1-5
Metaphen (aqueous)	1-500	1-1	1-3
Merphenyl Nitrate (aqueous)	1-1500	no kill*	1-1
Merphenyl Borate (tinct.)	1-500	1-3	1-5

Shippen modification used on all compounds showing bacteriostatic properties.

*No kill indicates original concentration failed to sterilize.

The literature reveals that discrepancies in the F. D.A. Phenol coefficient method have been observed in numerous instances. Among the authors reporting are Meyer and Gathercoal[5], and Vicher, Meyer, and Gathercoal[6]. They reported that they were unable to secure uniform results in the F. D. A. Phenol coefficient using Staph. aureus as the test organism. The latter authors report a study in which 19 strains of Staph. aureus exhibited a day to day variation in resistance.

Table VI shows the difference in resistance of the F. D. A. test culture of Staph. aureus at 20° C. Both Michigan State College and Parke, Davis and Co. obtained their culture of the standard test organism from the Food and Drug Administration. It can readily be seen that the college strain is much less resistant to all compounds tested. The Parke, Davis strain of Staph. aureus shows the Phemerol to be only 2/3 as effective as shown by the college strain, and the Zepharins are shown to be only 1/2 as effective whereas, on Phenol the resistance was approximately the same. Reddish[7] stated that weak cultures will give results different from those obtained with cultures of normal resistance as based on phenol. The question now arises as to which culture is to be considered as normal. Phenol cannot be relied on to determine normalcy of an organism in relation to its germicidal resistance unless the compounds are closely allied structurally to phenol. It was observed on Eberth. typhosa F. D. A. test culture obtained from the college laboratory,

that the 10 minute killing dilution on phenol was 1-100 and the 10 minute killing dilution on aqueous Phemerol (1-1000) was 1-20. Several days later it was noticed that the same organism had become more resistant to phenol, requiring a 1-90 dilution to kill in 10 minutes. A check was made at this time on the aqueous Phemerol (1-1000) which should kill at 1-50. As the resistance of the organism increased toward phenol, it decreased in relation to Phemerol. This action was noted primarily on the Phemerols and the Zepharins. This is another indication that the Phenol coefficient cannot be applied to all types of disinfectants. Reddish[8] has stated that the Phenol coefficient should be confined to compounds closely allied to phenol and that unrelated compounds could not be compared with any degree of accuracy by this method.

II. AGAR CUP PLATE METHOD

Penetration must play a major factor in the destruction of organisms on the skin or in a wound. It is obvious that to kill an organism, the compound must first penetrate the cell to be an effective germicide, therefor the property of penetration must be determined for a comparative study of disinfectants.

The Food and Drug Administration presents a procedure known as the Agar-cup-plate method for the measurement of penetration of germicides.

In this study, the method as outlined by the U. S. D. A. circular 198 was slightly revised to obtain more accurate results. A flask containing 500 ml. of plain nutrient 1.5 per cent agar was seeded with 2.5 ml. of a 24 hour culture of the test organism and distributed aseptically in Petri dishes using approximately 40 ml. to each dish. When the agar had solidified, a layer of paraffin was poured over its surface so as to seal it from air. This was done to prevent errors resulting from the compound volatilizing into the atmosphere above the agar and later redissolving in the moisture on the surface of the agar.

After the paraffin had solidified, a cup 1.5 cm. in diameter was cut aseptically from the center of the dish. This cup was then filled to the top of the paraffin level with seeded agar in such a manner, as to seal the interface between the paraffin and the agar. In the center of this fill a cup 1 cm. in diameter was cut so as to leave a collar

of agar to prevent capillary seepage between the agar-paraffin interface. The bottom of the cup was sealed with a drop of agar to prevent capillary seepage at this point. Under these conditions, the penetration measured would be from the cup out towards the edge of the dish rather than from the surface of the agar to the bottom. Into each cup, 0.3 ml. of disinfectant was placed and the dishes then incubated for 48 hours. At this time the paraffin was removed and the plates observed.

TABLE VII

TESTS MADE ON VARIOUS DISINFECTANTS BY THE AGAR CUP METHOD

Staphylococcus aureus
(P. D. 02482)

Disinfectant	Orig. Conc.	Zone produced in centimeters	
		Plain Agar	Dextrose Agar
Phemerol 44 (tinct.)	1-1000	1.1	0.4
Phemerol (aqueous)	1-1000	1.0	0.4
Phemerol 45 (tinct.)	1-500	1.3	0.4
Hexylresorcinol S. T. 37	1-1000	0.5	0.4
Iodine (tinct.)	1-40	1.0	1.0
Zepharin (tinct.)	1-1000	1.8	0.6
Zepharin (aqueous)	1-1000	1.8	0.6
Merthiolate (tinct.)	1-1000	complete	1.1
Merthiolate (aqueous)	1-1000	complete	1.0
Mercurochrome	1-20	3.2	0.6
Metaphen (tinct.)	1-200	3.3	1.2
Metaphen (aqueous)	1-500	3.3	1.2
Merphenyl Nitrate (aqueous)	1-1500	3.4	1.2
Merphenyl Borate (tinct.)	1-500	complete	1.2
Mercresin (tinct.)	1-1000	3.8	1.2
Control		0	0

TABLE VIII

TESTS MADE ON VARIOUS DISINFECTANTS BY THE AGAR CUP METHOD

Eberthella typhosa
(Lab. strain)

Disinfectant	Orig. Conc.	Zone Produced in Centimeters Plain Agar
Phemerol 44 (tinct.)	1-1000	0.7
Phemerol (aqueous)	1-1000	0.6
Phemerol 45 (tinct.)	1-500	0.8
Hexylresorcinol S. T. 37	1-1000	0.2
Iodine (tinct.)	1-40	1.0
Zepharin (tinct.)	1-1000	1.1
Zepharin (aqueous)	1-1000	1.2
Merthiolate (tinct.)	1-1000	3.2
Merthiolate (aqueous)	1-1000	3.2
Mercurochrome	1-20	2.6
Metaphen (tinct.)	1-200	2.5
Metaphen (aqueous)	1-500	2.5
Merphenyl Nitrate (aqueous)	1-1500	2.0
Merphenyl Borate (tinct.)	1-500	3.2
Mercresin (tinct.)	1-1000	3.1
Control		0

As can be seen from Tables VII and VIII, there is no correlation between the killing dilutions and the penetration as measured by this method. One would conclude that the Merthiolates and Merphenyl Borate are the superior compounds because they have a higher rate of penetration than any of the remainder of the disinfectants. This assumption must be false because Merthiolate was unable to kill Staph. aureus even in concentrated form in the dilution test, while here it completely prevented growth of the organism.

Phemerol 44 (1-1000) produced a zone of 1.1 centimeters from the cup and Zepharin produced a zone of 1.8 centimeters against Staph. aureus. As these are related compounds of a non-mercurial nature, the element of bacteriostosis may be eliminated. The killing dilution studies would indicate that Phemerol 44 should be the more active in penetration than the Zepharin. Again this was not borne out.

Further evidence that this test does not measure penetration is the fact that the mercurials show less activity against the Eberth. typhosa than Staph. aureus in this test, while the opposite was shown to be true in the killing dilution studies.

Harris and Prout[9] believe that the agar cup-plate method shows diffusion and recommend this test as a means of determining the degree of diffusion of a compound.

It has been observed that the addition of dextrose to a gel will retard diffusion through the gel[10]. Table VII shows a comparison between plain nutrient agar and 0.2 per

cent dextrose agar, with Staph. aureus being used as the test organism.

The zone of diffusion by Phemerol was decreased from 1.1cm. on plain agar to 0.4 cm., Zepharin from 1.8 cm. to 0.6 cm., Merthiolate from a complete zone to 1.0 cm., Metaphen from 1.3 cm to 1.2 cm., Mercresin from 3.8 cm. to 1.2 cm., and Merphenyl Borate from a complete zone to 1.2 cm.

These data substantiate the assumption that the agar cup-plate method is a measure of the diffusion and not penetration; that is the property to go into a water phase and diffuse through a colloidal medium rather than the measure of the property of a compound to penetrate organic matter.

III. RATE REACTIONS

The problem now was to determine a procedure to supplant the agar cup-plate method for measuring penetration and this was done by the rate reaction test.

Anderson[11] stated that the rate of kill could also be taken as a measure of penetration. From a study of rate reactions it is possible to compute the reaction rate, temperature rate, and dilution rate of a germicide. It is possible to determine killing action in a time interval of 15 seconds, which makes this method especially applicable to the evaluation of skin disinfectants, in which a short exposure period is essential.

The knowledge of the rate of reaction of a disinfectant is important as this will limit its application. A compound to be used on the skin or in a wound must have a rapid rate of reaction in order to exert killing power in the limited time it is in contact with the organisms it must destroy.

The dilution rate is also essential as it must be known as to whether or not the compound will destroy organisms in a short period of time even in a diluted form such as exists when in contact with the serum in a wound.

The temperature rate is a third factor of major importance in testing germicides. While a high temperature rate is a desirable property in a compound which is used on skin or wound , it may also be an undesirable property if the compound is to be used on a cold surface such as metal. In the latter instance the concentration of the disinfectant

would have to be increased in order to sterilize due to its decrease in activity at lower temperatures, while in the former case it may be possible to dilute the compound and secure disinfection because of the greater activity at elevated temperatures.

Lastly, the rate reaction would be a measure of bacterial penetration, as a compound which kills the offending organisms must penetrate the cell in order to destroy them.

In testing by the rate reaction method, 10 ml. of the dilution of disinfectant was pipetted into a medication tube and one ml. of a 24 hour test culture was then added. Prior to the addition of the test culture, it was filtered through a sterile cotton filter to remove clumps of organisms. At intervals of 15, 30, 45, 60, 90, 120, and 180 seconds and 4, 5, and 10 minutes from initial seeding, a 0.5 ml. sample was pipetted into a 99.5 ml. dilution blank. This blank contained 1 percent peptone and 0.85 percent sodium chloride in distilled water. The special peptone dilution blank was used to aid in overcoming the bacteriostatic action of the disinfectants.

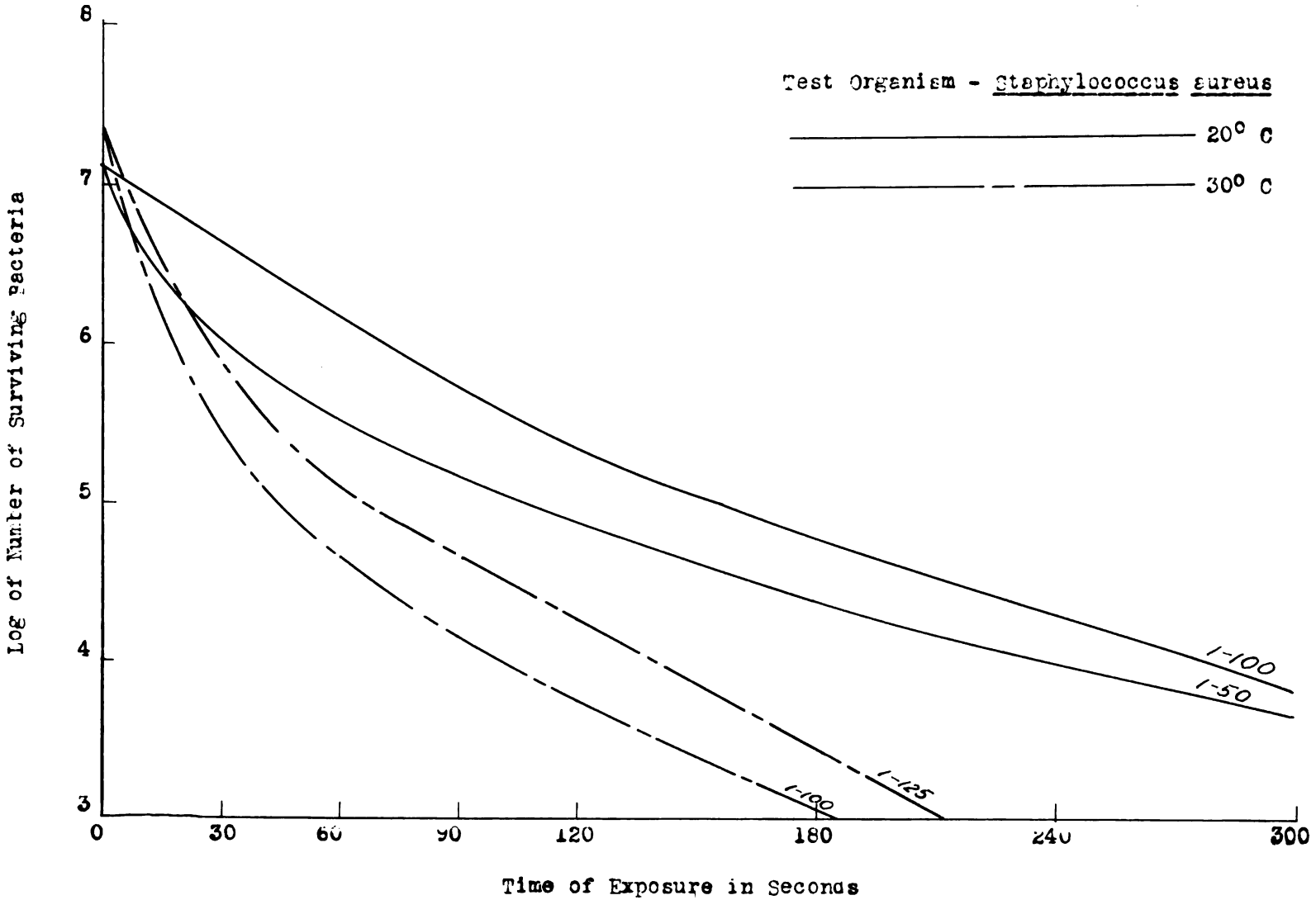
Further dilutions were made in sterile saline and plated on 1.5 per cent tryptose agar. To determine the number of organisms present at zero time, a medication tube of 10 ml. sterile water was seeded in a similar manner as the disinfectant tube. One sample was plated out as before and all plates were incubated at 37° C for 48 hours before counting.

Staph. aureus and Eberth. typhosa obtained from Parke,

Davis and Co. were used as the test organisms. Medication temperatures of 20° C and 30° C were used to determine the effect of temperature on the speed of action.

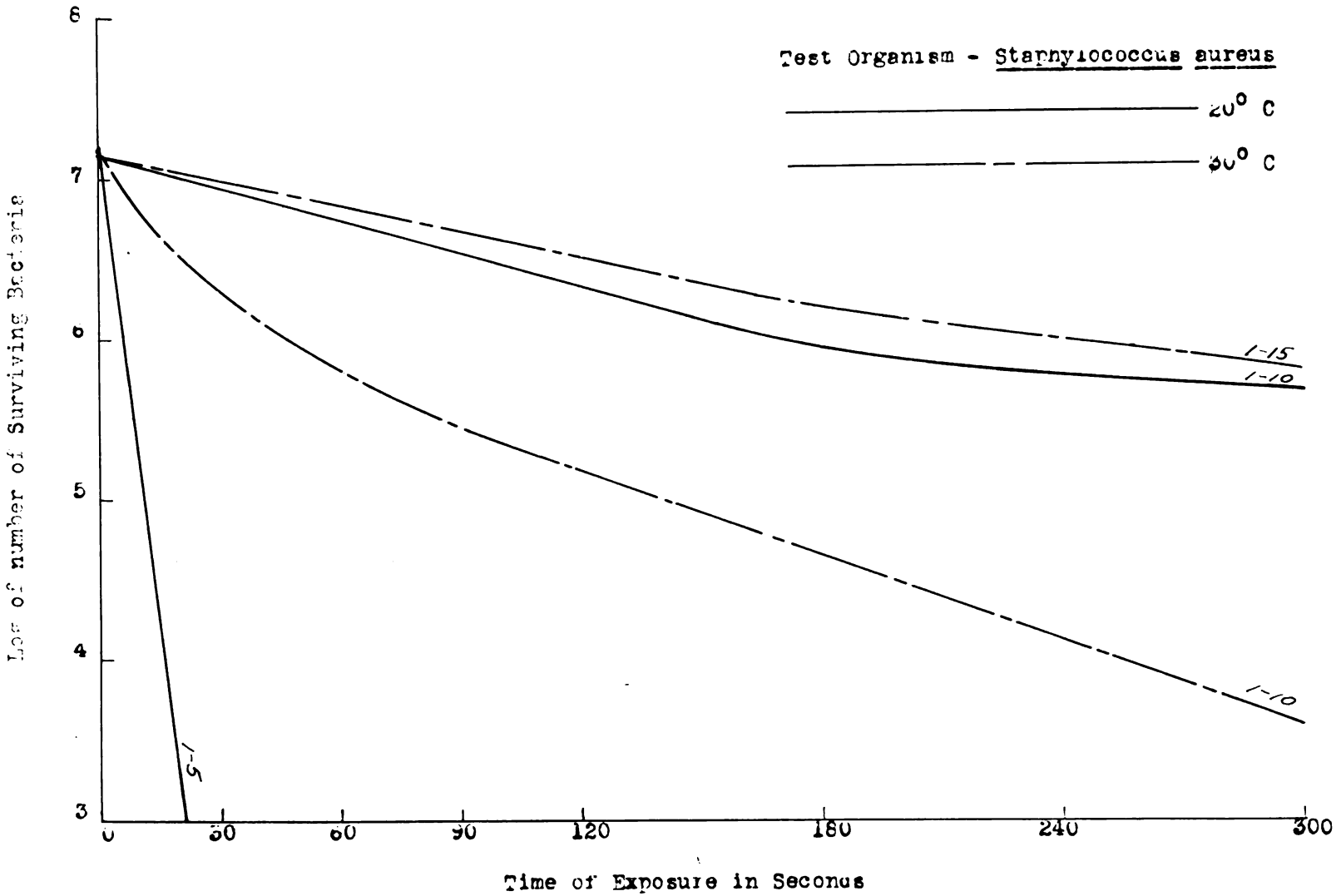
In these tests, the initial count of organisms present was always between 15 and 25 million organisms. A base line of 1000 organisms was chosen so that the effect of bacteriostasis would be minimized in the plating of samples; reduction from 15 million organisms to 1000 organisms being taken as a criterion of kill.

PHEMEROL 44



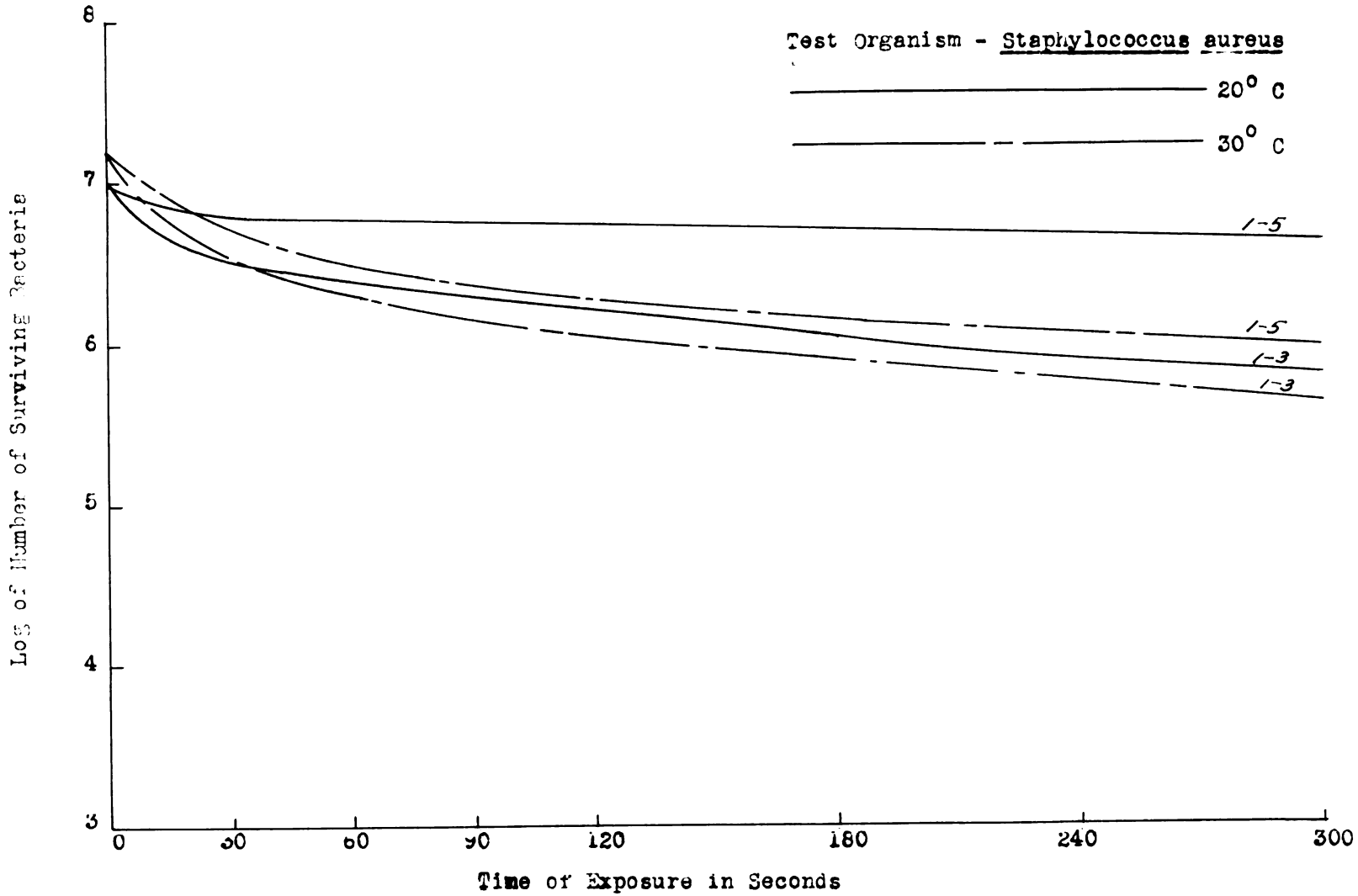
RATE REACTION GRAPH I

HEXYLRESORCINOL S. T. 37

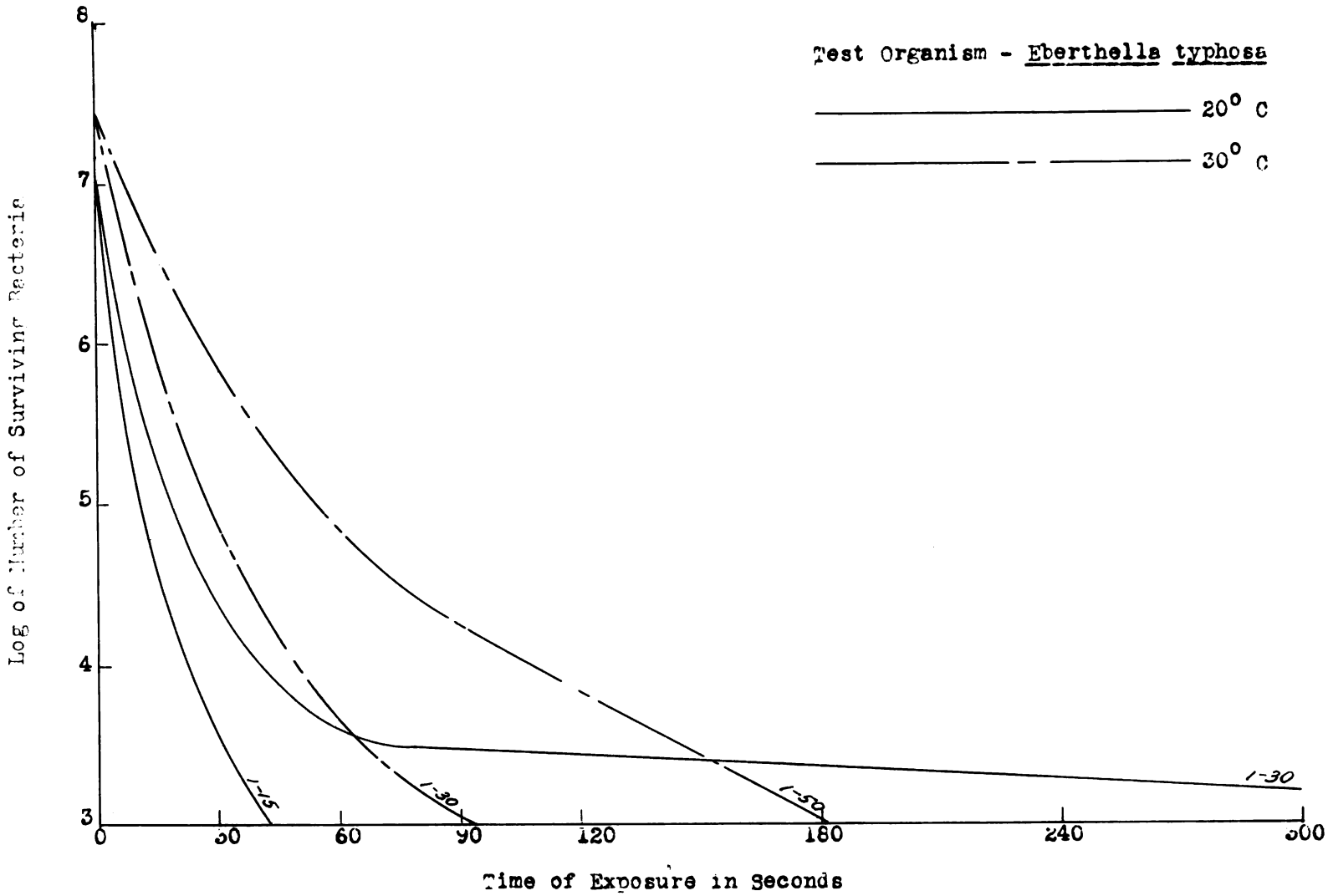


RATE REACTION GRAPH III

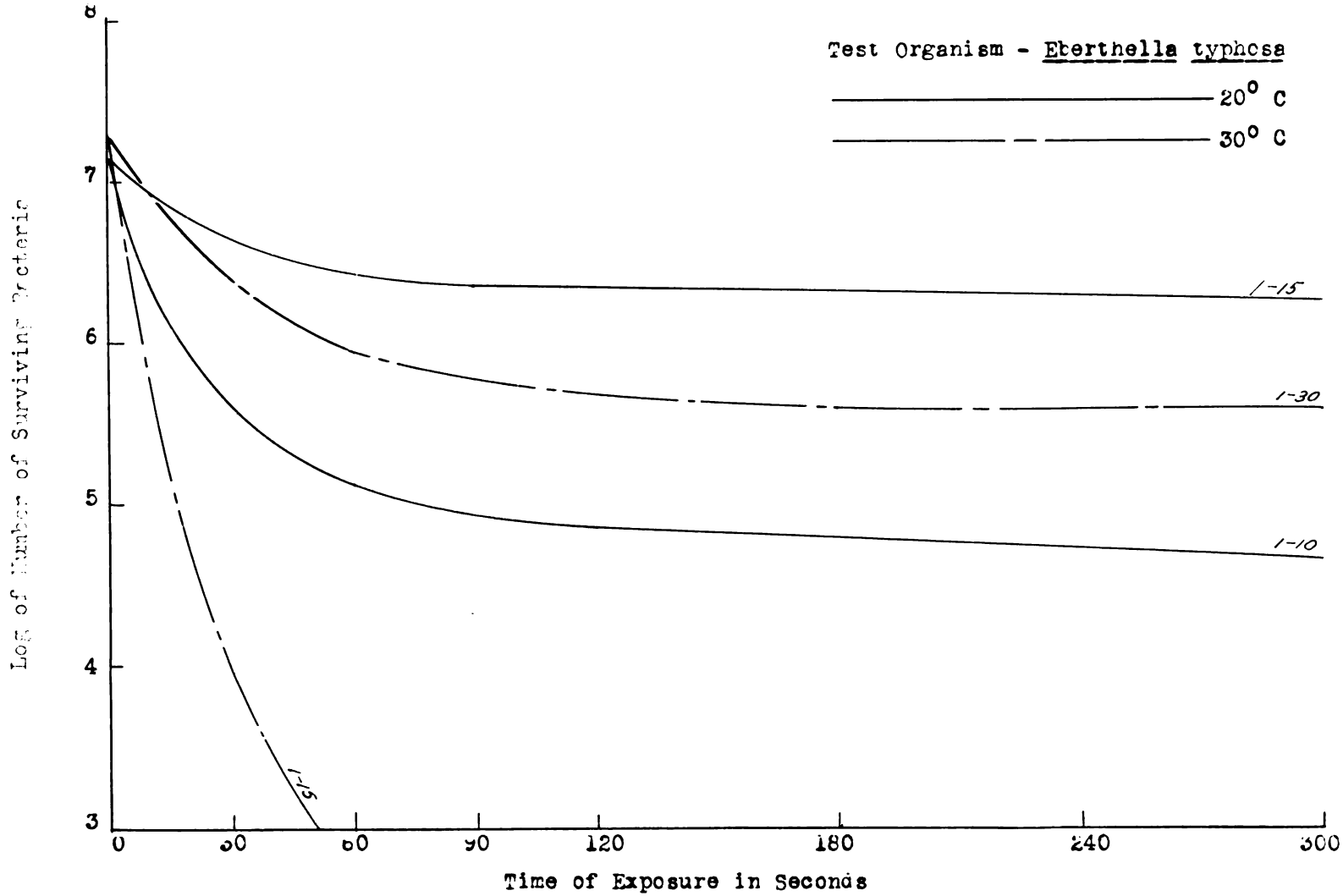
METAPHEN (aqueous 1-500)



PHENEROL 44


Test Organism - Eberthella typhosa
20° C
30° C
Log of Number of Surviving Bacteria
8
7
6
5
4
3
0
30
60
90
120
180
240
300
1-15
1-30
1-50
1-30
Time of Exposure in Seconds

HEXYLRESORCINOL S. T. 37



RATE REACTION GRAPH VII

RATE REACTION GRAPH VIII

By analizing these graphs, it can readily be seen that Phemerol tincture 44 (1-1000) was the most effective compound tested. Against Staph. aureus (P. D. strain) Phemerol tincture 44 was diluted 100 times and still showed a more rapid reduction of organisms in 5 minutes than did Hexylresorcinal S. T. 37 (1-1000) diluted 1-10, Merthiolate tincture (1-1000) diluted 1-2, and aqueous Metaphen (1-500) diluted 1-3. When the same series of compounds were tested at 30° C against Staph. aureus, Phemerol tincture 44 could be diluted to 1-125 and still far surpass the rate of reaction of Hexylresorcinal S. T. 37 diluted 1-10, Merthiolate tincture diluted 1-3, and aqueous Metaphen diluted 1-3. Phemerol tincture 44 has such a high temperature coefficient that the speed of reaction at 30° C is more effective than a dilution of 1-50 at 20° C.

When Eberth. typhosa (P. D. strain) was used as the test organism, aqueous Metaphen showed the highest effective dilutions. Phemerol tincture 44 could be diluted 15 times and still kill Eberth. typhosa in less than one minute at 20° C. At 30° C it killed at a dilution of 1-50 in three minutes. Hexylresorcinal S. T. 37 at a dilution of 1-10, and Phemerol tincture 44 at a dilution of 1-30 have rapid initial rates of reaction but their power was soon exhausted and the rate of reaction became extremely slow. Merthiolate could stand no more than a 1-3 dilution before its action became dissipated.

When these compounds were tested at 30° C, all showed an increase in disinfecting properties, aqueous Metaphen being the most pronounced.

From these data, it can readily be seen that Phemerol penetrates the bacterial cell more rapidly than any of the other compounds tested.

SUMMARY

It can readily be seen that the quaternary ammonium salts are superior in disinfecting powers to the other types of compounds which are on the market.

All compounds showed a marked variation in germicidal activity against the test organisms; Phemerol and Zepharin being the only compounds consistently killing in a diluted form. Against Staph. aureus, Phemerol and Zepharin showed greater activity than against Eberth. typhosa, while Merthiolate, Mercresin, Metaphen, Merphenyl Borate, and Merphenyl Nitrate showed just the reverse. When a selective agent is used in wound disinfection, it should be determined as to whether or not the compound is active against the specific organism to be destroyed.

The phenol coefficient is a poor means of evaluating a skin disinfectant because the time interval of the test is too long, the method of calculating the coefficients is unfair as it does not take into account the original concentration of the germicide, phenol cannot be relied on to determine normalcy of the test organism, and not enough different types of test organisms are used to simulate conditions which would be found on the skin or in a wound.

The killing dilution tests partially answers their faults and they tell exactly how much reserve strength the manufacturer has put into his compound.

A rate reaction method is a better means of evaluation

as this procedure will determine a temperature rate, a reaction rate, and a dilution rate, in addition to the penetrative powers of the disinfectant.

Rate reaction graphs I - VIII show that Phemerol acted in a matter of seconds against both Eberth. typhosa and Staph. aureus, even in diluted concentrations. The mercurials were shown to have very marked bacteriostatic powers so that they act for long periods of time. In this way they resemble antiseptics and are of little value for the rapid killing of bacteria.

The agar cup-plate method, while designed to measure penetration, fails in its objective and is a measure of the diffusion of a compound through a gel giving the extent of diffusion rather than the degree of penetration.

CONCLUSIONS

The F. D. A. phenol coefficient is an unreliable and unfair means of testing the efficiency of disinfectants.

The Agar cup-plate method determines a degree of diffusion and not a rate of penetration.

The Rate Reaction method is a superior method of evaluating disinfectants as it determines penetration, temperature rates, dilution rates, and reaction rates.

The new types of compounds, quaternary ammonium salts such as Phemerol and Zepharin, are better skin disinfectants because of their rapid action and general effectiveness against all types of organisms.

The mercurials may be classed as a group of slow acting compounds capable of killing over a long period of time, their action being more like that of a bacteriostat.

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