A STUDY OF THE ANTISEPTIC PROPERTIES OF CERTAIN ORGANIC COMPOUNDS.*

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Interest in the selective behavior of dyes dates back to Ehrlich's work with methylene blue. However, Stilling was probably the first to call attention to the antiseptic action of dyes on bacteria. He examined the inhibitive effect of diphenyl- and triphenylmethane dyes and recommended auramine and methyl violet as good antiseptic agents. Penzoldt tested a series of dyes and found that methyl violet, malachite green, etc., were good inhibitors. Von Drigalski and Conradi carried the idea a step further and showed that some aniline dyes inhibited *B. typhosus* less than other bacteria present in feces and recommended the use of crystal violet for the isolation of *B. typhosus* from stools, etc. Loeffler, and later Conradi, studied this phase of the problem more thoroughly, the latter testing about 400 dyes, but since they limited their observations to the action of these substances on *B. typhosus*, they contributed nothing new concerning the specific behavior of the dyes. Churchman, however, developed these observations and showed that certain violet dyes (gentian violet, crystal violet, dahlia) were more inhibitive for Gram-positive than for Gram-negative bacteria. These findings were confirmed and extended by Krumwiede and Pratt, who studied a larger series of this group of dyes.

It is evident from this brief review that the triphenylmethane dyes constitute a group of substances toxic to bacteria and reacting in a partially specific manner in the sense of Bechhold and Ehrlich. But, owing to the fact that the investigators were more absorbed in the practical application of this property, little is known concerning the nature of the action. Since my problem was similar to theirs, and since their extensive investigations resulted in only a partially successful solution, it seemed that a more effective attack might be made possible by a better understanding of the factors concerned in the specific affinity manifested by these dyes.

The noteworthy fact gathered from the literature is that all the dyes used in the isolation of *Bacillus typhosus* (crystal violet, malachite

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green, brilliant green), as well as those studied by Churchman (gentian violet, crystal violet, dahlia), belong to the triphenylmethane group. Diamino or triamino triphenylcarbinol may be considered the basis of these dyes. Upon treatment with acid, these dye bases are converted into dyes themselves, the conversion, as is commonly accepted, being accompanied by a rearrangement to the quinoid form. A series of homologous dyes may be produced by substituting the hydrogen in the NH_2 group by alkyl or aryl radicals. This class of substances promised, therefore, to be a good starting-point for the study of the structural chemical factors involved in the action of dyes on bacteria. A series of representative compounds was selected and their action on a number of typical bacteria studied quantitatively under carefully controlled conditions.[1] It soon became apparent that it would be desirable to extend the list and include for comparison a number of the simpler aromatic amino compounds. Consequently, aniline, toluidine, and some of their alkyl derivatives, and a few other related compounds were tested.[2]

Technique.

Substances Used.—A list of the substances used and their chemical constitution are given in Table I. The compounds are arranged as nearly as possible in the order of their complexity. The list is by no means exhaustive. Other compounds might have been included but were not easily obtainable, while still others were ruled out because of their insolubility.

Method.—The details of the method used in testing the antiseptic property of a given compound are highly important. Although comparable and fairly constant results may be obtained under identical conditions, a variation in one or another of the factors involved will cause decided fluctuations in the results. The important factors to be controlled are the composition and reaction of the medium and the condition of the culture used in the test.

[1] On account of the war, only a limited number of the compounds selected could be obtained.

[2] The dyes used were all Grübler's and were presumably fairly pure. The auramine, as well as the aniline, toluidine, and other compounds tested, was kindly furnished by Dr. W. A. Jacobs of The Rockefeller Institute for Medical Research, and were obtained from either Kahlbaum or Schuchardt.

TABLE I.

List of Compounds Used.

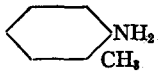
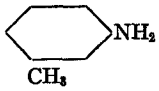
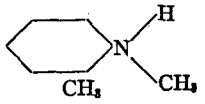
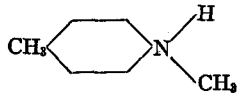
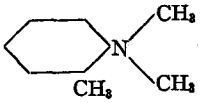
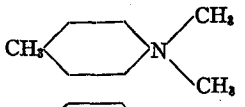
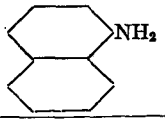
No.	Name.	Formula.
1	Methylamine (mono-).	$NH_2.CH_3.HCl$
2	" (di-).	$NH(CH_3)_2.HCl$
3	" (tri-).	$N(CH_3)_3.HCl$
4	Methyl alcohol.	$CH_3.OH$
5	Ethyl "	$CH_3.CH_2.OH$
6	Ethylamine.	$NH_2.C_2H_5$
7	Diethylamine.	$NH(C_2H_5)_2$
8	Aniline.	$C_6H_5.NH_2$
9	Methyl aniline.	$C_6H_5.NH.CH_3$
10	Dimethyl "	$C_6H_5.N(CH_3)_2$
11	Ethyl "	$C_6H_5.NH.C_2H_5$
12	Diethyl "	$C_6H_5.N(C_2H_5)_2$
13	*o*-Toluidine.	$C_6H_4.CH_3.NH_2$ 
14	*m*- "	$C_6H_4.CH_3.NH_2$ 
15	*p*- "	$C_6H_4.CH_3.NH_2$ 
16	N-Methyl *o*-toluidine.	$C_6H_4.CH_3.NH.CH_3$ 
17	N- " *p*- "	$C_6H_4.CH_3.NH.CH_3$ 
18	N-Dimethyl *o*- "	$C_6H_4.CH_3.N(CH_3)_2$ 
19	N- " *p*- "	$C_6H_4.CH_3.N(CH_3)_2$ 
20	α-Naphthylamine.	$C_{10}H_6.NH_2$ 

TABLE I—*Continued.*

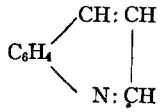
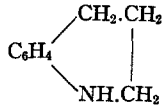
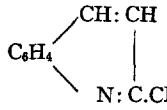
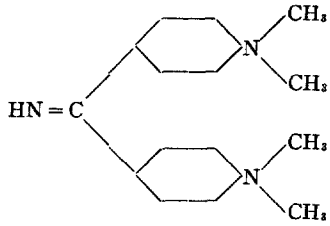
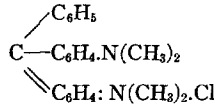
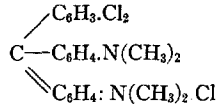
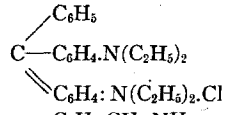
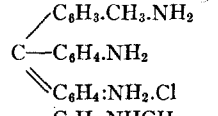
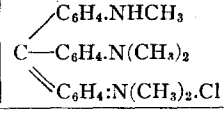
No.	Name.	Formula.
21	Quinoline.	
22	Tetrahydroquinoline.	
23	Quinaldine.	
24	Auramine.	
25	Malachite green.	
26	Victoria "	
27	Brilliant "	
28	Fuchsin (acid).	
29	Methylviolett B.	

TABLE I—*Concluded.*

No.	Name.	Formula.
30	Crystal violet.	$C_6H_4.N(CH_3)_2$ / C—$C_6H_4.N(CH_3)_2$ \ C_6H_4: $N(CH_3)_2.Cl$
31	Gentian "	Mixture of crystal and methyl violet plus dextrin.
32	Methyl green.	$C_6H_4.N(CH_3)_2.CH_3Cl$ / C $C_6H_4.N(CH_3)_2$ \ C_6H_4: $N(CH_3)_2.Cl$
33	Aniline blue.	$C_6H_3.CH_3.NH.C_6H_4$ / C—$C_6H_4.NH.C_6H_5$ \ C_6H_4: $NH.C_6H_5.Cl$
34	Dahlia.	$C_6H_3.CH_3.NH.C_2H_5$ / C—$C_6H_4.NH.C_2H_5$ \ C_6H_4: $NH.C_2H_5.Cl$

Composition of the Medium.—That the presence of different amounts of colloidal or organic matter influences the action of dyes is well known. This is equally true of other antiseptics. An idea of the effect of different media on the antiseptic action of chemicals is obtained from the data shown in Table II. In these tests the only variable was the composition of the test media; a special peptone broth and standard beef extract broth were used. It is clear from the results that although the characteristic action of the drug is not modified by the medium, the effective concentration is appreciably altered. It is important, therefore, in testing a large number of drugs, to use a medium subject to as little variation in composition as possible. The following medium proved satisfactory:[3]

	per cent
Peptone	1.0
Potassium phosphate (dibasic)	0.5
Sodium chloride	0.5
Glucose	0.1

[3] Fairchild's peptone was used throughout the investigation. The salts and sugar were chemically pure.

TABLE II.

Action of Antiseptics in Peptone and Nutrient Broth Respectively.

Test substance.	Dilution.	Test media.	Test cultures.*				
			2	6	17	12	14
Dibromo-β-naphthol.	12,500	Nutrient broth.	−†	−	−	+++	+++
	25,000	" "	+	−	++	+++	+++
		Peptone "	−	−	−	+++	+++
	40,000	" "	+	+	++	+++	+++
Hexamethylenetetramine quaternary salt of *p*-chloroacetylaminotetraethyl *p'*, *p"*- diaminotriphenylmethane.	15,000	Nutrient broth.	+	+	+	++	+++
		Peptone "	−	−	−	+	+
	30,000	Nutrient "	+	+	+	+++	+++
		Peptone "	−	−	−	+	++
Hexamethylenetetramine quaternary salt of chloroacetylethylamine.	10,000	Nutrient broth.	−	−	−	+	−
	20,000	" "	+	±	±	+++	++
		Peptone "	−	−	±	++	−
	40,000	" "	+	+	+	+++	++

* The numbers of the cultures correspond with those given in Table IV.

† − indicates no growth; ±, +, ++, +++ indicate the relative amount of turbidity at the end of 24 hours.

This culture fluid varies little, if at all, is easily prepared, and serves as a favorable substratum for all the cultures used in these tests.

Reaction of the Medium.—The reaction of the test medium in terms of hydrogen ion concentration is of even greater importance. This fact has been entirely overlooked until recently (Wright, 1917). A few preliminary tests showed that not only was the antiseptic power affected, but the specific behavior towards different organisms was also modified. Typical results are given in Table III. The medium given above eliminated this factor, because it had a constant pH of 7.1, the reaction usually favorable for growth of bacteria; also, since it required no adjustment, it was not subject to variation on that account.

TABLE III.

Effect of the Reaction of the Test Medium on the Action of Antiseptics.

Test substance.	Dilution.	Reaction of test medium.*	Test cultures.†				
			2	6	17	12	14
		pH					
		6.2	±	±	±	+	±
Caffeine.	1: 100	7.4	±	±	±	±	−
		8.2	±	∓	±	±	−
Hexamethylenetetramine quaternary salt of *p*-chloroacetylamino-leukomalachite green.	1: 10,000	6.2	−	−	−	+++	±
		7.4	+	+	±	+++	++
		8.2	+	+	+	+++	++
Dibromo-β-naphthol.	1: 12,500	6.2	−	−	−	++	++
		7.4	−	−	−	+++	++
		8.2	−	−	+	+++	+++
Hexamethylenetetramine quaternary salt of chloroacetylethylamine.	1: 10,000	6.2	−	−	−	−	−
		7.4	−	−	−	+	−
		8.2	−	−	−	++	±

* Nutrient broth was used in all these tests. A quantity of broth was prepared and tubed and N acid or alkali added sterilely to give the desired pH.

† The cultures and symbols are the same as in Table II.

Test Cultures.—The condition of the test culture was the third important factor that had to be considered. Variations are likely to occur either because of lack of adaptation to the test fluid or on account of the inherent fluctuations of the organism itself. To eliminate the former, the cultures were grown in the test broth for at least 3 days, daily subcultures being made; the latter were partly controlled by using wherever possible recently isolated organisms and more than one strain of each type. The strains used, together with their origin and some descriptive remarks, are given in Table IV.

Procedure.—The procedure given below was followed throughout the work. The peptone broth was put up in flasks and autoclaved. 5 cc. portions were then pipetted out sterilely into sterile test-tubes and incubated in a saturated incubator over night. Solutions of the substance to be tested were made up in sterile water and graded amounts added to the broth to give the desired concentration. The stock so-

TABLE IV.

Culture No.	Name.	Source.	Remarks.
123	*B. aerogenes.*	American Museum of Natural History.	Indol −; Voges-Proskauer reaction +; typical.
14	*B.* "	Isolated from stool, 1916.	Indol +; Voges-Proskauer reaction −; not typical; behaves like *B. aerogenes.*
24	*B. cloacæ.*	American Museum of Natural History.	Indol −; Voges-Proskauer reaction +; does not liquefy.
11	*B. coli (communis).*	Isolated from stool, 1916.	Indol +; sucrose −.
13	*B.* " "	" " " 1916.	" +; " −.
12	*B.* " *(communior).*	" " " 1916.	" +; " +.
15	*B.* " "	" " " 1916.	" +; " +.
17	*B. typhosus.*	" " Patient O., 1916.	Agglutinated with typhoid serum. Culturally typical.
19	*B.* "	Isolated from Carrier L., 1916.	
20	*B.* "	Isolated from Carrier C., 1916.	
2	*B. dysenteriæ* Flexner.	Old Institute laboratory stock.	Maltose − (Hiss-Russell).
24	*B.* " "	Isolated by Dr. Smillie, 1916.	Maltose + (Flexner).
26	*B.* " "	Isolated by Dr. Smillie, 1916.	Maltose − (Hiss-Russell).
6	*B.* " Shiga.	Institute stock strain (Gay).	Reacts typically.
27	*B.* " "	Isolated by Dr. Smillie, summer, 1916.	" "
30	*B.* " "	Albany stock 114 F.	" "
21	*B. proteus.*	Isolated from stool, 1916.	
106	*B. subtilis.*	American Museum of Natural History.	
347	*Staphylococcus aureus.*	American Museum of Natural History.	

lutions were made up of such strength that no more than 1 cc. or less than 0.1 cc. had to be added to give the proper dilution. The cultures to be inoculated were filtered through sterile cotton filters, diluted with broth to give uniform turbidity, and a large standard loop was inoculated into the broth tubes. The tubes were then incu-

bated for 24 hours and the growth was recorded in terms of degree of turbidity. This gave the inhibitive power of the substance in 24 hours. In a few cases the killing power in 2 and 24 hours respectively was ascertained by subculturing the broth tubes to agar slants. This procedure was not carried out systematically because of the time consumed and since for the purposes of the study it was sufficient to determine the inhibitive property in a constant time limit.

RESULTS

The results are given in Table V. The compounds used are arranged in the order of their increasing antiseptic power, and the results recorded in terms of the highest inhibiting dilution (first row) and the dilution which just failed to inhibit (second row). These two figures indicate the limits of the zone in which the inhibiting dilution lies. Whenever the difference between the two figures was too great, additional tests were made to reduce the gap. When more than one test was made, the average of the results was taken, and when more than one strain of a given organism was used, the average for the type is given. On the whole, individual strains of the typhoid, dysentery, or *aerogenes* bacilli varied but little, while more decided fluctuations were obtained with *Bacillus coli*. The repeated tests checked fairly closely with the original ones.

The facts brought out in Table V are difficult to summarize. In general, it is clear that on starting with aniline or its mono- or dimethyl derivatives, the introduction of a methyl group in the nucleus, as seen in the behavior of the corresponding toluidine derivatives, results in a definite increase in the inhibitive power of the compound. This is also evident from the contrast between quinoline and quinaldine. Similarly, the antiseptic property is enhanced by the substitution of either methyl or ethyl radicals in place of the hydrogens in the NH_2 group. The amount of increase, up to a certain point at least, depends on the number and character of the alkyl radicals introduced. A second alkyl produces a more marked rise than the first, while an ethyl group is more effective than a methyl radical. This general phenomenon is also observed among the dyes. Beginning with fuchsin there is a progressively increasing antiseptic action which

TABLE V.

Inhibition of Growth of Bacteria by Certain Chemical Compounds.

Antiseptic compound.	Gram-negative.							Gram-positive.	
	B. aerogenes.	*B. coli* A.	*B. coli* B.	*B. typhosus.*	*B. dysenteriæ* F.	*B. dysenteriæ* S.	*B. proteus.*	*B. subtilis.*	*S. aureus.*
Methyl alcohol.	10*	10	5	10	10	10			
	20	20	10	20	20	20			
Ethyl alcohol.	15	15	10	20	25	25			
	25	25	20	25	40	40			
Aniline.	300	250	250	350	450	450	350	500	1,000
	350	300	300	400			400	1,000	5,000
o-Toluidine.	475	475	475	475	600	600			
	550	550	550	550	1,000	1,000			
p-Toluidine.	475	475	450	550	600	600			
	550	550	475	600	650	650			
Methyl aniline.	650	600	600	650	950	950	650	1,000	6,000
	700	650	650	700	1,000	1,000	700	5,000	10,000
Ethyl aniline.	1,000	1,000	1,000	1,100	1,350	1,350	1,100		
	1,100	1,100	1,100	1,200	1,500	1,500	1,200		
Methyl *o*-toluidine.	1,100	950	950	1,100	1,400	1,400	1,100		
	1,350	1,100	1,100	1,350	1,600	1,600	1,350		
Methyl *p*-toluidine.	1,100	950	950	1,100	1,400	1,400	1,100		
	1,350	1,100	1,100	1,350	1,600	1,600	1,350		

Dimethyl aniline.	1,350 1,500	1,350 1,500	1,350 1,500	1,500 1,750	1,750 2,000	1,750 2,000	1,100 1,350		
Dimethyl *o*-toluidine.	1,550 1,750	1,550 1,750	1,550 1,750	1,750 2,100	2,100 2,500	2,100 2,500	1,350 1,550		
Dimethyl *p*-toluidine.	2,500 2,650	2,650 2,900	2,500 2,650	2,900 3,200	3,500 5,000	3,500 5,000	2,500 2,650		
Diethyl aniline.	4,500 5,400	4,500 5,400	4,500 5,400	6,000 7,000	7,000 8,500	7,000 8,500	4,000 4,500		
Quinoline.	1,400 1,600	1,100 1,400	1,100 1,400	1,600 2,100	1,600 2,100	1,600 2,100	1,100 1,400		
Tetrahydroquinoline.	1,600 2,100	1,400 1,600	1,400 1,600	1,600 2,100	1,600 2,100	2,100 5,000	1,400 1,600		
Quinaldine.	2,600 3,400	2,100 2,600	2,100 2,600	2,600 3,400	2,600 3,400	3,400 5,000	2,100 2,600		
α-Naphthylamine.	2,600 3,100	2,600 3,100	2,100 2,600	2,600 3,100	3,100 7,500	3,100 7,500			
Auramine.	1,800 2,200	2,200 2,700	2,200 2,700	2,700 3,500	2,700 3,500	4,200 5,200	1,500 1,800	6,000 10,000	10,000 25,000
Fuchsin.	 8,000	8,000 12,000	12,000 15,000	12,000 15,000	100,000 110,000	110,000 200,000	12,000 150,000	500,000 1,000,000	300,000 500,000
Malachite green.	 20,000	40,000 45,000	45,000 50,000	30,000 35,000	250,000 500,000	500,000 1,000,000	35,000 40,000	4,000,000 5,000,000	1,000,000 2,000,000

* The numbers indicate dilutions; the first row the inhibiting dilution, the second the one which failed to inhibit.

TABLE V.—*Concluded.*

Antiseptic compound.	Gram-negative.							Gram-positive.	
	B. aerogenes.	*B. coli* A.	*B. coli* B.	*B. typhosus.*	*B. dysenteriæ* F.	*B. dysenteriæ* S.	*B. proteus.*	*B. subtilis.*	*S. aureus.*
Victoria green.		60,000	70,000	40,000	700,000	700,000	40,000	5,000,000	1,000,000
	40,000	70,000	80,000	50,000	1,000,000	1,400,000	50,000	10,000,000	2,000,000
Dahlia.		55,000	65,000		110,000	110,000	55,000	2,000,000	800,000
	45,000	65,000	75,000	45,000	150,000	150,000	65,000	4,000,000	1,000,000
Methylviolett B.		70,000	75,000	60,000	175,000	175,000	85,000	4,000,000	800,000
	60,000	75,000	85,000	65,000	250,000	250,000	95,000	5,000,000	1,000,000
Gentian violet.	25,000	85,000	90,000	50,000	100,000	100,000			
	50,000	95,000	100,000	60,000	200,000	200,000			
Crystal violet.		85,000	100,000	85,000	400,000	400,000	200,000	4,000,000	1,000,000
	60,000	95,000	110,000	95,000	800,000	800,000	250,000	5,000,000	2,000,000
Brilliant green.	100,000	675,000	550,000	510,000	1,500,000	2,000,000	675,000	15,000,000	4,000,000
	175,000	800,000	600,000	550,000	2,000,000	3,000,000	800,000	20,000,000	5,000,000
Methyl green.	1,200	1,200	1,200	1,200	8,000	8,000	2,700	50,000	30,000
	2,200	2,200	2,200	2,200	15,000	15,000	3,500	100,000	50,000
Aniline blue.									
	1,200	1,200	1,200	1,200	1,200	1,200	1,200		

runs parallel with the increase in the number of methyl or ethyl groups. The triethyl derivative is about as effective as the hexamethyl, while the tetraethyl is the most active of the series. The behavior of the *o*- and *p*-dimethyl toluidines indicates that position may be a factor in determining the degree of antiseptic action. The effect of the introduction of chlorine into the benzene nucleus is seen from the differences between malachite green and victoria green. This effect on the introduction of halogen has been observed before in other classes of compounds. It is also interesting to note that the substances containing two aromatic nuclei, namely naphthylamine, quinoline, quinaldine, and the diphenylmethane dye, auramine, are more potent than the corresponding monophenyl derivatives, whereas the triphenyl derivatives are the most active of the substances tested.

It would seem, then, that the inhibiting effect of these substances is due on the one hand to aniline with the benzene nucleus as its basis, and on the other to the number of these nuclei. The effect is consistently enhanced by the addition of alkyl radicals, either to the nucleus, or to the amino group. The number and character of these radicals also determine the degree of effectiveness.

An exception to the general phenomenon of the increase of inhibitive action produced by the increase in the number of alkyl groups is seen in the anomalous behavior of methyl green. This substance is identical with crystal violet, with the exception that one of the tertiary nitrogens of this dye has been changed as a quaternary salt by the addition of methyl chloride. Contrary to the expectation of an increase in antiseptic power, this dye is almost inert. It is also noteworthy that in the case of the triphenyl derivative of rosaniline, aniline blue, in which the hydrogens are substituted by phenyl groups, there is a decided reduction in inhibitive action.

In some respects, these results are in accord with those obtained by other workers with other classes of compounds. The well known difference between phenol and cresol and the observations of Jacobs, Heidelberger, and Bull of the progressively increasing bactericidal action, on proceeding from the dimethyl to the diethyl and dipropyl derivatives of certain quaternary salts of hexamethylenetetramine, may be cited as instances.

While it is possible to point to the factors concerned in the enhancement of the germicidal power, it is difficult to account for the specific behavior of these substances. All the compounds tested are decidedly more active against the Gram-positive than the Gram-negative bacteria. This is true as well of aniline and its methyl derivative as of auramine and the triphenylmethane dyes. It is interesting to note that while aniline and its derivatives are more active against *Staphylococcus aureus* than *Bacillus subtilis*, the converse is true of the dyes.

Partial specificity becomes most marked in the triphenylmethane dyes. This is particularly evident in their more potent action against the Gram-positive organisms and the dysentery bacilli. Though the dysentery bacilli are exceedingly sensitive to the action of these substances, they are decidedly less so than the Gram-positive bacteria. These dyes are also markedly inactive against *Bacillus aerogenes* and, with the exception of fuchsin, are more inhibitive for *Bacillus coli* than for *Bacillus typhosus*.

The fact that all these dyes behave alike, irrespective of the number and character of the alkyl radicals, indicates that the molecule as a whole is concerned with the partial specific action. This is in accord with the fact that three of these dyes have been used in the isolation of *Bacillus typhosus*.

Mention should be made of the side-light which the behavior of these organisms towards this group of substances throws on their possible relationship. *Bacillus typhosus* and *Bacillus aerogenes* (also *Bacillus paratyphosus* B) are more sensitive to the simple aniline derivatives and less so to the dyes than *Bacillus coli*. The latter, as well as *Bacillus dysenteriæ*, is relatively much more sensitive to the dyes than to the aniline compounds. The extreme sensitiveness of the dysentery bacilli to the action of the dyes is especially interesting. While there was little difference between the behavior of the two classes, the Flexner cultures invariably showed a greater tolerance for fuchsin than did the Shiga bacillus. Their extreme sensitiveness to this class of chemical compounds renders it unlikely that any representative of the group may be found that will be of service in isolating them from polluted materials.

CONCLUSIONS.

This study of the inhibitive effect of aniline and some of its derivatives and of the triphenylmethane dyes on certain bacteria warrants the following tentative conclusions:

1. The composition and reaction of the medium exert a marked influence on the behavior of the antiseptic. The higher the concentration of organic nitrogenous compounds (peptone) in the medium, the lower is the effective concentration of the dye. The reaction of the medium modifies the specific action of the antiseptic, owing probably to an alteration in the bacterial cell.

2. The germicidal action of the compounds is a function of the benzene nucleus, the added elements or radicals, their number, and, in the case of the dyes, probably the quinoid structure of the nucleus.

3. As far as tested, the increase in the number of alkyl radicals increases the antiseptic power. Methyl green is an interesting exception to this rule, for the change of one of the nitrogens to the quaternary salt is accompanied by an almost complete loss in inhibitive action.

4. The antiseptic power is enhanced to a greater extent by an ethyl than a methyl group, and the second alkyl produces a proportionately greater increase than the first. It appears that the relative position of the introduced group may be a factor in determining the relative improvement in the effectiveness of the compound.

5. The introduction of a methyl group in the nucleus consistently enhances the inhibitive action of the compound and its alkyl derivatives. This is evident from a comparison of the action of aniline and its derivatives with that of toluidine and its corresponding derivatives.

6. The simple aniline derivatives, as well as the dyes, are more toxic for the Gram-positive than the Gram-negative bacteria. Of the former, *Bacillus subtilis* is more sensitive to the dyes than *Staphylococcus aureus*, while the reverse is true in the case of the aniline compounds.

7. The most marked specific selective effect is manifested by the triphenylmethane dyes. *Bacillus aerogenes* and *Bacillus typhosus* possess a higher resistance to these substances than *Bacillus coli* or

Bacillus dysenteriæ. The last is exceedingly sensitive. This partial specificity is apparently a function of the molecule as a whole.

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