A STUDY OF EFFECTS OF VARIOUS DISINFECTANTS ON PROTEUS MORGANI (ATYPICAL) AND PROTEUS MIRABILIS

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A STUDY OF EFFECTS OF VARIOUS DISINFECTANTS ON *PROTEUS MORGANI* (ATYPICAL) AND *PROTEUS MIRABILIS*

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CONTENTS

Introduction

Two species of *Proteus* were obtained from Mrs. Ruth Gunn, of the Poultry Pathology Laboratory, Department of Bacteriology and Public Health, Michigan State College. One of these organisms (T-96-2) was isolated from the liver of a turkey and the other (C-642-3) was isolated from the liver of a chicken. Both apparently caused sickness in turkeys and chickens. Because of the possible pathogenicity of Proteus for poultry, it was thought highly desirable to learn something about the susceptibility of *Proteus* to disinfectants. Therefore, a preliminary study of the effects of various disinfectants on the two species of *Proteus* was conducted. These two species were first identified on the basis of biochemical reactions.

Review of Literature

A review of literature shows that several authors have presented data concerning the pathogenicity of Proteus in infants and poultry, but not very much work has been done on the effects of disinfectants on these organisms.

Booker (1), 1896, who was one of the first to investigate the intestinal flora in summer diarrhea, reported a general increase in the numbers of certain organisms, particularly Proteus and Streptococci.

Metchnikoff (1), 1914, isolated Proteus vulgaris from 204 out of 218 individuals affected with infantile diarrhea.

Jordan and Burrows (2) state that Proteus, both in mixed and pure cultures, has been found to be associated with a variety of pathological conditions. In certain disorders of the digestive tract Proteus has frequently been thought to be the responsible agent. Also in diarrhea stools, especially those of infants, it has often been found in large numbers, and is regarded by many as a cause of infant diarrhea.

According to Felsenfeld and Grant (3) phenol inhibited the growth of Proteus more satisfactorily than benzenes and their derivatives.

A disinfectant which is a member of phenolic group and very commonly used in the laboratory and the household is lysol. Lysol is generally used in a 1-100 dilution. Leavitt (4), 1947, demonstrated that the proper dilution to be used against Eberthella typhosa is 1-600 both in use-dilution method and F.D.A. phenol coefficient method. She also found that the use-dilution of phenol against the same organism is 1-110.

Tonney, Greer, and Danforth (5) found that one part per million of free chlorine will kill Proteus vulgaris in fifteen to twenty seconds.

Chlorine is now the most widely used of all chemical disinfectants, largely due to its almost universal application in the disinfection of water supplies and its power to render sewage less objectionable. Among the chlorine compounds, hypochlorites furnished the most available chlorine for disinfectant use. Although chlorine compounds are not stable, they are still powerful disinfectants which give rapid action by oxidation and chlorination. McCulloch (5) found the phenol coefficient of chlorine, in the form of a hypochlorite solution, to be 139 at 20° C. when tested against Eberthella typhosa by the F.D.A. method. Consequently chlorine exerts a rapid and efficient germicidal action in a high dilution.

In 1935 Domagh (5) reported on the bactericidal action of Roccal. This is a cationic detergent which is produced from cocoanut oil. It is a high molecular alkyl dimethyl benzyl ammonium chloride compound. This cationic detergent is a non-toxic germicide which can be used satisfactorily for dish washing and hand washing. According to McCulloch all the cation detergents are very effective inhibitors of bacterial metabolism at 1-5,000 dilution.

The salts of all the heavy metals are toxic to bacteria. Their mode of action appears to be quite similar, although their effective concentrations vary greatly. Small amounts of arsenous acid is toxic to Proteus vulgaris as reported by McCulloch (5). As early as 1881 Robert Koch observed that very dilute solution of mercury bichloride would prevent the growth of many pathogens, and this led him to consider mercuric chloride solution as a powerful and reliable germicide. Less than ten years later

Geppert (5) demonstrated that the powerful action of mercury bichloride was inhibitory, rather than germicidal. In the laboratory a 1-1,000 solution of mercury bichloride is usually employed as a disinfectant. According to Thomas (5), 1932, the phenol coefficient of mercury bichloride against Eberthella typhosa is 775 (bacteriostatic). Thus, mercury bichloride was chosen as the representative of heavy metals with which to conduct tests on Proteus.

The Food and Drug Administration Method (6) is most commonly used today for testing disinfectants in this country. Leavitt (4) reported that the F.D.A. phenol coefficient method indubitably gives comparable results when testing coal tar products, but it cannot be used to compare the disinfectant values of a quaternary ammonium compound, a mercurial and a chlorophenol. It is hard to evaluate disinfectants on a comparative basis as we have no single reliable method for comparative testing. Thus the use-dilution method of testing disinfectants was devised in an effort to improve upon the F.D.A. phenol coefficient method, and to evaluate disinfectants under conditions more comparable to those under which they are actually used. Also in 1945 Hanes (7) found that the use-dilution technic is simpler and more practical than the phenol coefficient method.

Experimental Work

A. Identification of the organisms

Morphological studies were made by preparing gram and flagella stains from tryptose agar slants. Motility was determined by the use of a semisolid lactose motility medium(Darby's modification of Difco's motility medium).

Cultural characteristics (8) were studied by action on urea, Kligler's agar, gelatin and the different kinds of sugars. Tests were also made for production of indol and acetyl methyl carbinol.

These two organisms were found to be gram-negative, highly motile rods with peritrichous flagella. The growth on tryptose agar plates spread rapidly over the entire surface of the medium to give rise to the phenomenon of "Swarming".

C-642-3 fermented dextrose, trehalose, galactose, levulose, xylose, and glycerol with acid and gas. Sucrose fermentation was very slow after 5-7 days of incubation. Gelatin was liquefied and indol was negative which is characteristic of Proteus mirabilis. (9).

T-96-2 showed the same biochemical reactions as Proteus mirabilis except that liquefication of gelatin was negative which is characteristic of Proteus morgani (atypical).

The biochemical reactions of these two organisms are shown in Table 1.

Table 1. Biochemical Characteristics

Media	Culture Number	
	T-96-2	C-642-5
Urea (prod. of NH_3	+	+
Kligler's agar (prod. of H_2S)	+	+
Voges Proskauer	-	-
Methyl red	+	+
Indol	-	-
Gelatin liquefication	-	+
Dextrose	AG	AG
Lactose	-	-
Maltose	-	-
Mannose	-	-
Sucrose	AGSW	AGSW
Salicin	-	-
Trehalose	AG	AG
D-galactose	AG	AG
D-levulose	AGSW	AS
L-arabinose	-	-
Xylose	AG	AG
Sorbitol	-	-
Mannitol	-	-
Glycerol	AG	AGW
Dextrin	-	-

\+ Positive reaction

\- No reaction

A Acid production

G Gas production

S Slow acid production

W Weak acid production

B. Effects of various disinfectants

As stated previously, not very much work has been done on the effects of disinfectants on Proteus. For this preliminary study four groups of disinfectants were selected and from each group one was selected as a representative agent. Phenol was used as the standard of comparison and lysol as the representative of the coal-tar products. Roman cleanser (3% sodium hypochlorite) represented the chlorine oxidizing agents. In the cationic agent group Roccal (10% of alkyl dimethyl benzyl ammonium chloride) was chosen as the representative. Mercury bichloride (1-1,000 dilution) represented the heavy metal group.

By using the bacteriostatic titer method, F.D.A. phenol coefficient method, and the use-dilution method the effects of the above representative disinfectants on Proteus morgani (atypical) and Proteus mirabilis were determined.

1. Bacteriostatic properties.

Various dilutions of different disinfectants (such as phenol, lysol, Roccal, Roman Cleanser and mercury bichloride) were prepared. One ml. of each of these dilutions was transferred to separate F.D.A. broth tubes (each tube containing 10 ml). One loopful of the 24 hours broth culture of the organisms to be tested was placed in the F.D.A. broth tubes containing disinfectants and the tubes were then incubated at 37° C. for three days. The effects on the two species of Proteus are shown in the Tables 2, 3, 4, 5 and 6.

Table 2. The bacteriostatic properties of phenol

Dilution	Pr. morgani (atypical)			Pr. mirabilis*		
	24	48	72	24	48	72
1-200	-	-	+	-	-	+
1-500	-	-	+	-	-	+
1-800	-	+	+	-	+	+
1-1,000	+	+	+	+	+	+
1-2,000	+	+	+	+	+	+
1-5,000	+	+	+	+	+	+

* Hours of incubation
\+ Presence of Growth
\- Absence of Growth

Table 3. The bacteriostatic properties of Lysol

Dilution	Pr. morgani (atypical)			Pr. mirabilis *		
	24	48	72	24	48	72
1-10	-	-	-	-	-	-
1-20	-	-	-	-	-	-
1-50	-	-	-	-	-	-
1-100	-	-	-	-	-	-
1-500	+	+	+	+	+	+
1-1,000	+	+	+	+	+	+

Table 4. The bacteriostatic properties of NaOCl

Dilution	Pr. morgani (atypical)			Pr. mirabilis *		
	24	48	72	24	48	72
1-1,000	-	-	-	-	-	-
1-2,000	-	-	-	-	-	-
1-5,000	-	+	+	+	+	+
1-10,000	+	+	+	+	+	+
1-20,000	+	+	+	+	+	+
1-50,000	+	+	+	+	+	+

*Hours of incubation
+ Presence of Growth
- Absence of Growth

Table 5. The bacteriostatic properties of Roccal

Dilution	Pr. morgani (atypical)			Pr. mirabilis*		
	24	48	72	24	48	72
1-1,000	-	-	-	-	-	-
1-2,000	-	-	-	-	-	-
1-6,000	-	+	+	-	+	+
1-10,000	-	+	+	-	+	+
1-20,000	+	+	+	+	+	+
1-50,000	+	+	+	+	+	+

Table 6. The bacteriostatic properties of $HgCl_2$

Dilution	Pr. morgani (atypical)			Pr. mirabilis*		
	24	48	72	24	48	72
1-10,000	-	-	-	-	-	-
1-20,000	-	-	-	-	-	-
1-50,000	+	+	+	-	-	-
1-100,000	+	+	+	+	+	+
1-200,000	+	+	+	+	+	+
1-500,000	+	+	+	+	+	+

* Hours of incubation
\+ Presence of growth
\- Absence of growth

2. Determination of the phenol coefficient by the F.D.A. method.

a. The F.D.A. medium was prepared as follows (6)

Peptone	10 gm.
Beef extract	5 gm.
Sodium chloride	5 gm.
Distilled water	1,000 ml.

This was boiled for 20 minutes and adjusted to pH 6.8 then placed in tubes in 10 ml. amounts and sterilized at 15 pounds for 40 minutes.

b. Test Organisms -The cultures of the two species of Proteus were transferred every 24 hours in the above medium for not more than one month. At the end of each month a fresh transfer was made from the stock culture. The stock cultures were carried on tryptose agar slants and transferred twice a month.

A five percent solution of phenol (by weight) was prepared by using the pure phenol. The solution was kept in an amber-colored bottle in the refrigerator.

c. Procedure -Five ml. quantities of 1-80, 1-90, 1-100 dilutions of phenol in sterile distilled water were placed in seeding pots which were placed into a 20° C. water bath. In the same manner seven dilutions were prepared of the disinfectant to be tested.

Before transferring to seeding pots the 24 hours broth culture of the test organism was shaken for 15 minutes to break up the clumps. Then 0.5 ml. of the 24 hours broth culture was placed into each dilution at intervals of 30 seconds by using a two ml. pipette. At the end of 5 minutes from the time of each inoculation, a loopful was transferred from the proper seeding pot to a tube of F.D.A. broth. Transfers were repeated at the end of 10 and 15 minutes.

When a disinfectant, mercury bichloride, which has marked bacteriostatic property was tested, a Shippen modification method was used. This consisted of making a second set of transfers by using 4 loopfuls from each of the broth tubes just seeded after shaking for 5 minutes. All these tubes were incubated at 37° C. for 48 hours and the phenol coefficient was computed by dividing the killing strength of phenol and the disinfectant being tested at 10 minutes. d. The results are shown in Tables 7, 8, 9, 10, 11, 12, 13 and 14.

Table 7. Effect of Lysol on Proteus morgani (atypical)

Phenol	Time of exposure in minutes		
	5	10	15
1-80	-	-	-
1-90	+	-	-
1-100	+	+	+
Lysol			
1-200	-	-	-
1-300	-	-	-
1-400	-	-	-
1-500	+	+	-
1-600	+	+	+
1-700	+	+	+
1-800	+	+	+

Phenol coefficient = 5

Table 8. Effect of Lysol on Proteus mirabilis

Phenol	Time of exposure in minutes		
	5	10	15
1-80	-	-	-
1-90	+	-	-
1-100	+	+	+
Lysol			
1-200	-	-	-
1-300	-	-	-
1-400	-	-	-
1-450	-	-	-
1-500	-	-	-
1-600	+	-	-
1-700	+	+	+

Phenol coefficient = 6.66

Table 9. Effect of NaOCl on Proteus morgani (atypical)

Phenol	Time of exposure in minutes		
	5	10	15
1-90	+	-	-
1-100	+	+	+
1-110	+	+	+
NaOCl			
1-5,000	-	-	-
1-10,000	-	-	-
1-12,000	+	-	-
1-15,000	+	-	-
1-20,000	+	+	+
1-25,000	+	+	+
1-30,000	+	+	+

Phenol coefficient = 166.6

Table 10. Effect of NaOCl on *Proteus mirabilis*

Phenol	Time of exposure in minutes		
	5	10	15
1-80	+	-	-
1-90	+	-	-
1-100	+	+	+
NaOCl			
1-5,000	-	-	-
1-8,000	-	-	-
1-10,000	-	-	-
1-12,000	+	-	-
1-15,000	+	-	-
1-18,000	+	+	+
1-20,000	+	+	+

Phenol coefficient = 166.6

Table 11. Effect of Roccal on Proteus morgani (atypical)

Phenol	Time of exposure in minutes		
	5	10	15
1-80	+	-	-
1-90	+	-	-
1-100	+	+	+
Roccal			
1-3,000	-	-	-
1-6,000	-	-	-
1-10,000	-	-	-
1-15,000	+	-	-
1-20,000	+	+	+
1-25,000	+	+	+
1-30,000	+	+	+

Phenol coefficient = 166.6

Table 12. Effect of Roccal on *Proteus mirabilis*

Phenol	Time of exposure in minutes		
	5	10	15
1-80	-	-	-
1-90	+	-	-
1-100	+	+	+
Roccal			
1-4,000	-	-	-
1-6,000	-	-	-
1-8,000	+	-	-
1-10,000	+	-	-
1-12,000	+	+	+
1-15,000	+	+	+
1-18,000	+	+	+

Phenol coefficient = 111.1

Table 13. Effect of $HgCl_2$ on Proteus morgani (atypical)

Phenol	Time of exposure in minutes					
	5		10		15	
1-80	-		-		-	
1-90	+		-		-	
1-100	+		+		+	
$HgCl_2$	F.D.A. Method	Shippen Method	F.D.A. Method	Shippen Method	F.D.A. Method	Shippen Method
1-10,000	-	-	-	-	-	-
1-30,000	-	-	-	-	-	-
1-50,000	-	-	-	-	-	-
1-80,000	+	+	-	-	-	-
1-100,000	+	+	+	+	+	+
1-120,000	+	+	+	+	+	+
1-150,000	+	+	+	+	+	+

Phenol coefficient = 888.8

5. The use-dilution method of testing disinfectants.

a. Medium - F.D.A. broth.

b. Same test organisms as in F.D.A. method.

c. Procedure - Sterile glass rods, one inch in length and $\frac{1}{4}$ inch in diameter, having a loop at one end for handling, were dipped in a 24 hour broth culture of the organism to be tested and were laid on sterile filter paper to dry for 30 minutes at room temperature. Four dilutions of the disinfectants were placed in 1 x 3 inch medication pots in 10 ml. amounts and placed in a 20° C. water bath with eight tubes of sterile saline (10 ml. in each). The four rods coated with the culture were picked up with a sterile platinum wire and dropped simultaneously into one of the above dilutions in the water bath. At the end of 1, 5, 10 and 30 minutes a rod was removed from the disinfectant by a sterile transfer wire and immersed in a tube of sterile saline. At the end of one minute the rod was transferred from the saline to a tube of F.D.A. broth. All the broth tubes were shaken vigorously, after the introduction of the glass rods, to release the organisms from the rods and were then incubated for 48 hours at 37° C.

d. The results are shown in Tables 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24. The use dilution is one in which organisms are killed in 10 minutes and not in 5 minutes.

Table 19. Effect of NaOCl on Proteus morgani (atypical)

NaOCl	Time of exposure in minutes			
	1	5	10	30
1-5,000	+	-	-	-
1-10,000	+	-	-	-
1-15,000	+	+	-	-
1-18,000	+	+	+	-

Use-dilution = 1-15,000

Table 20. Effect of NaOCl on Proteus mirabilis

NaOCl	Time of exposure in minutes			
	1	5	10	20
1-5,000	-	-	-	-
1-10,000	-	-	-	-
1-15,000	+	-	-	-
1-18,000	+	+	+	-

Use-dilution > 1-15,000 < 1-18,000

For the determination of the resistance to drying of the two species of Proteus the glass rods were coated with the organisms and put on sterile filter paper in a Petri dish. At the end of 20, 30, 60, 90, and 120 minutes respectively one rod was placed into a tube of F.D.A. broth and the tube was shaken vigorously, then incubated at 37° C. for 24 hours. The results are shown in the Table 25 and indicate that these two species of Proteus were quite resistant to drying.

Table 25. Resistance of Proteus morgani (atypical) and Proteus mirabilis to drying.

Drying Period	Pr. Morgani(atypical)	Pr. mirabilis
20 minutes	+	+
30 minutes	+	+
60 minutes	+	+
120 minutes	+	+

+ Growth

4. Comparative study on F.D.A. method and use-dilution method.

The comparative results obtained from the two methods are shown in the Table 26. For phenol the killing strength of the dilutions was slightly higher by the use-dilution method than by the F.D.A. method. Almost the same results were obtained by these two methods for Lysol and sodium hypochlorite. It requires a higher concentration of Roccal to kill the organisms when testing by the use-dilution method than when employing the F.D.A. method. The same is found when testing the killing power of solutions of heavy metals.

Table 26. Comparative results obtained by the F.D.A. method and the use-dilution method (Dilution of killing strength)

Disinfectant	*Proteus morgani* (atypical)		*Proteus mirabilis*	
	F.D.A. method	use-dilution method	F.D.A. method	use-dilution method
Phenol	1-90	1-100	1-90	1-100
Lysol	1-450	>1-400 < 1-500	1-600	>1-500 < 1-600
Sodium Hypochlorite	1-15,000	1-15,000	1-15,000	>1-15,000 < 1-18,000
Roccal	1-15,000	>1-1,000 < 1-2,000	1-10,000	1-2,000
Mercury Bichloride	1-80,000	>1-10,000 < 1-50,000	1-80,000	>1-50,000 < 1-80,000

Discussion

The organism, T-96-2, which was isolated from a turkey differs from Proteus morgani biochemically in that it does not produce indol but ferments sucrose. Aside from the fact that it liquefies gelatin, its biochemical characteristics closely resemble those of Proteus mirabilis. Because of the incompleteness of available literature, considerable difficulty was encountered in our efforts to identify this organism.

Little information is available on the effectiveness of disinfectants against Proteus. Therefore the choice of the compounds used in this study was not made on the basis of previous experimental work or practical experience, except in-so-far as it concerns general information on the subject of disinfection. One representative was chosen from each of the four groups of disinfectants and these were tested by three different methods. The results show that all the compounds used were effective in killing these members of the genus Proteus in the absence of organic matter. Sodium hypochlorite, considering all factors involved, would seem to be the most effective, Roccal ranting next. Mercury bichloride, which exhibiting great killing power in these experiments, is not desirable for several well known reasons.

Sodium hypochlorite is very easily oxidized in the presence of air, so the dilution used should be lower than that found to be effective in these experiments.

The bacteriostatic titer method is very good for finding out the range of dilutions which is effective against an organism when a new

chemical is to be tested. Results obtained by the F.D.A. method when testing phenol compounds and sodium hypochlorites were quite regular, but wild positive results (growth) often appeared in testing Roccal and heavy metal compounds. In such cases the use-dilution method gave more consistent results.

These two organisms failed to grow on plain agar but showed abundant growth on tryptose agar plates, spreading rapidly over the whole surface. Therefore, plate counts were not made when employing the use-dilution method.

Conclusion

1. The effective dilutions of the various disinfectants when used against Proteus were:
   (1) Phenol 1-90
   (2) Lysol 1-500
   (3) Sodium hypochlorite 1-10,000
   (4) Roccal 1-2,000
   (5) Mercury bichloride 1-50,000
2. The use-dilution method proved to be a more reliable method of testing the killing power of quaternary ammonium chloride compounds and heavy metals when dried organic matter (in this case dried organisms) is present on the surface to be disinfected.

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