THE DISINFECTION OF THE ORAL MUCOSA WITH CRYSTAL VIOLET AND BRILLIANT GREEN¹

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CONTENTS

I. Introduction	22
II. The general plan, method, and results of the author's experiments	23
1. First series. Results with alcohol, acetone, ether and tincture of iodine...	23
2. Second series. Results with dyes	25
A. Historical	25
B. Technical	26
C. Results with brilliant green (saturated solution in alcohol)	27
D. Results with brilliant green (1 per cent solution in 50 per cent alcohol)	28
E. Results with brilliant green and crystal violet in solution <i>together</i> (1 per cent of each in 50 per cent alcohol) applied for 1.5 minute ...	28
F. Results of tests similar to those in the last preceding section (E), with additional bacteriological precautions	30
G. Results of tests with brilliant green and crystal violet in solution <i>together</i> (1 per cent of each in aqueous solution) compared with hydro- quinone (1 per cent aqueous solution)	33
H. Results of a repetition of the tests with brilliant green and crystal violet in solution <i>together</i> in alcohol (section E), with an extension of the period of application to the gum (from 1.5 minute) to 2 minutes	33
I. Results of a repetition of the tests with brilliant green and crystal violet <i>together</i> in alcohol, in the last preceding section (H), after previous thorough flushing of the mouth with an alkaline wash and brushing of the teeth	38
3. General discussion of the results of the first two series of tests	38
4. Third series. Clinical findings	39
Discussion of the clinical findings	40
III. Summary of general conclusions	41
IV. Literature cited	41

¹ According to Churchman (*Journal of the American Medical Association*, January 17, 1920), the term "disinfection" when applied to the action of dyes, is a misnomer. He prefers the term "*bacteriostasis*," not only from an etymological point of view, but also from the standpoint of accuracy of description. We believe, with Churchman, that the term "*bacteriostasis*" is a more suitable one than "disinfection," in this relation.

I. INTRODUCTION

In the study of the bacteriology of apical and periapical infections, there is great variation in the cultural technique of different investigators. Since the relative value of the results obtained by different experimenters depends upon an unbroken chain of aseptic procedures in each case, it is important to ascertain what technical conditions must be established for the sterilization of the oral mucosa, and also to determine the relative efficiency for this purpose of the available antiseptics. From a consideration of some of the many methods used to obtain material for bacteriological study, especially material in the vicinity of the gingival border, it is apparent that the technique described is not free from criticism. To such an extent does this fact obtain that opinions, rather than scientific facts, appear to have influenced, if not actually to have served as a foundation for, the results reported.

Gilmer and Moody (7) state that "the field to be incised was made as nearly aseptic as possible by thorough washing with 50 per cent alcohol." Schamberg (21) says: "We removed the tooth and from it got a pure culture of *Streptococcus viridans*." Hartzell and Henrici (10) state that it is "almost an impossibility to extract a healthy tooth and find it free from viridans unless that tooth has been rendered free from bacteria by rubbing the tooth with iodine and subsequently burning the gingival field with the actual cautery." Head and Roos (11) "washed the neck of the tooth with alcohol until it was considered to be practically free from living bacteria. It was then painted with tincture of iodine three times at short intervals so that it would be practically impossible for any organisms on the neck of the tooth to be alive." Howe (12) uses two solutions: (a) silver nitrate, 3 gm.; distilled water, 1 cc.; ammonium hydroxid (28 per cent), 2.5 cc. and (b) formalin (25 per cent). These two solutions, mixed, make everything absolutely sterile, according to Howe, and destroy every type of microorganism. Rosenow (20) does not give any technique for obtaining his material but states that the danger of contamination, with the mouth flora, in the removal of teeth, "becomes slight in proper hands."

Kelsey (13) tested a great variety of substances in the culture of organisms to ascertain their value as disinfectants. He concluded that "tincture of iodine is the most useful disinfectant for use in the mouth in treating lesions where strepto-bacillus is present." Kelsey gives no clinical evidence with the results of his laboratory work, but assumes that his laboratory findings apply in vivo as well as in vitro.

II. THE GENERAL PLAN, METHOD, AND RESULTS OF THE AUTHOR'S EXPERIMENTS

In view of the uncertainty revealed by these quoted statements, we sought to find an antiseptic which would be efficient and yet which would be relatively innocuous to the normally sensitive mucous membrane of the oral cavity. In the light of the suggestion by Meyer (19), the problem was attacked from a chemotherapeutic view-point. Accordingly, observations were made upon the therapeutic and chemical effects produced by various drugs ordinarily used in clinical practice, as well as upon suggested changes in concentration of the antiseptics. The mode of application was the same in all instances, namely, by means of cotton pellets moistened with the different fluids. Application was always made on the gum of the maxilla near the frenum.

1. First series. Results with alcohol, acetone, ether, and tincture of iodine

The results obtained with alcohol, acetone, ether, tincture of iodine, and *dilute* tincture of iodine, are summarized in *table 1*. It will be noted that, in the use of either 60 per cent alcohol or 95 per cent alcohol, there was variation in the degree of dehydration of the gum. When the application of alcohol was followed, in turn, first by ether, second by acetone, finally by a mixture of the three in equal proportions, the results varied in the degree of dehydration induced, as well as in the degree of sterility of the tissue. In each case there was a definite dehydrating action on the gum by the alcohol, an effect that renders the use of alcohol of these strengths unsuitable for all practical purposes of oral disinfection, even though in some tests

sterility was obtained in 100 per cent of the tests. Tincture of iodine, also dilute tincture of iodine (1:10), was used. With the former sterility was obtained, but only at the expense of blistering the gum surface. With the latter practically no sterility was obtained.

TABLE 1

Data on the efficiency of alcohol, ether, acetone, and iodine in efforts to obtain sterility in the buccal cavity.

DRUGS TESTED	PERIOD OF APPLICATION	NUMBER OF CASES	BACTERIOLOGICAL RESULT	EFFECTS ON THE GUMS
	<i>minutes</i>			
Alcohol, 95 per cent	2	5	Sterility in but one case	Gums badly dehydrated
Alcohol, 95 per cent	3	5	Sterility in four cases	Gums badly dehydrated
Alcohol, 60 per cent	2	7	No sterility	Gums not dehydrated
Alcohol, 60 per cent	3	5	No sterility	Gums slightly dehydrated
Alcohol (95 per cent), ether, and acetone, in one-two-three-order	1, for each substance	5	No sterility	Gums badly dehydrated
Alcohol (95 per cent), ether, and acetone, in one-two-three order	2, for each substance	5	Sterility in but two cases	Gums badly dehydrated
Alcohol (95 per cent), ether, and acetone, mixed in equal proportions	2	4	Sterility in but two cases	Gums badly dehydrated
Tincture of iodine	1	3	Sterility in three cases	Gums dehydrated and "burned"
Tincture of iodine, diluted with water (1:10)	1	3	No sterility	Gums apparently not "burned"
Tincture of iodine, diluted with water (1:10)	2	3	Sterility in one case	Gums "burned"
Total.....		45	Sterility in thirteen cases	

Seelig and Gould (22) as early as 1911, definitely proved that alcohol is a good antiseptic in direct proportion to its concentration. The results of our experiments show that the utility of alcohol as an antiseptic, in the strengths referred to (60 per cent and 95 per cent),

is impaired, for oral purposes, by its undesirable dehydrating action on the mucous membranes—one of the effects we have endeavored to overcome. Such dehydration of the oral mucous membranes is objectionable because blistering or burning of the gum follows it, and the ensuing lowered local resistance and subsequent superficial necrosis delay the process of healing and favor the further ingress of microorganisms.

2. *Second series. Results with dyes*

A. Historical

Leitch (14) used brilliant green in dilute aqueous solution. He found it non-toxic to normal tissue, though antiseptic in action. He also observed that this dye inhibited the growth of staphylococci to a greater extent than it did that of streptococci. The same dye was used, as a paste for injection into wounds, by Short (23) who obtained equally good results. Browning (5) found that brilliant green, 1:2000 in water, is five hundred times as toxic for staphylococci as is Dakin's solution. He also found (4) that it stimulates healthy granulation tissue as well as acts as an antiseptic. Webb (24) and Ligot (15) obtained similar results with the dye.

Browning (2, 1, 3) found that a mixture of crystal violet and brilliant green kills gram-positive cocci chiefly, whereas *B. coli*, resists its action to a greater extent. He used a solution of 1 per cent of crystal violet and 1 per cent of brilliant green, together in 50 per cent alcohol, with excellent results, and suggested the use of the dye as an antiseptic for mucous membranes.

Graham-Smith (8) found that the toxicity or inhibitory power of crystal violet, as well as various flavines [acriflavine and proflavine, which are acridine dyes, derivatives of acridine, a base found in coal tar (9)], depends to a great extent upon the degree of alkalinity of the solution to which the dye is added—that is, the greater the alkalinity, to a certain degree, the greater is the antiseptic power. For example, crystal violet inhibits the growth of *Staphylococcus aureus* under the conditions indicated on the next page:²

²The ratio signifies, in each case, that one part of the dye in the indicated number of parts of aqueous solution, with the reaction stated, is the greatest dilution of the dye, in the particular circumstances, that will completely prevent growth of the organism named.

<i>Dye tested and medium used</i>	<i>0.15 n/10 hydrochloric acid</i>	<i>0.08 n/10 sodium hydroxid</i>	<i>0.58 n/10 sodium hydroxid</i>
	1:1,500,000	1:3,500,000	1:5,000,000
	<i>Organisms studied</i>		
	<i>Staphylococcus</i>	<i>B. coli</i>	<i>B. pyocyaneus</i>
Crystal violet			
Agar.....	1:3,500,000	1:10,000	1:10,000
Broth.....	1:10,000,000		
Brilliant red			
Agar.....	1:3,250,000	1:1,000	1:1,000
Broth.....	1:500,000	1:10,000	1:10,000
Quinone			
Agar.....	1:10,000	1:95,000	1:30,000
Broth.....	1:60,000	1:100,000	1:10,000
Hydroquinone			
Agar.....	1:60,000	1:40,000	1:10,000
Broth.....	1:20,000	1:20,000	1:10,000
Homoflavine			
Agar.....	1:14,000	1:9,000	1:2,000
Broth.....	1:300,000	1:250,000	1:30,000

Thus we note that in alkaline media, the dye tends to be more efficient as an antiseptic, at least so far as *Staphylococcus aureus* is concerned.

According to Marshall (18) the hydrogen ion concentration of saliva varies not only in different individuals, but also in the same individual from time to time. This variation, as has been pointed out by other investigators, may be due to nervous impulses or reflexes which appear to be correlated with such factors as diet, and with the pathology of the oral and gastric mucosae and the teeth.

A small series of experiments was undertaken by us to determine the hydrogen ion concentration (P_H) of saliva by the colorimetric method (with phenolsulfonephthalein as the indicator), according to the procedures of Clark and Lubs (6) and of MacLeod (17), with the following results for P_H in six different specimens: 7.3, 7.3, 7.8, 7.6, 7.3, 7.5; average, 7.43.

The average for P_H , 7.43, indicates a fair degree of alkalinity, which appears to be sufficient to enhance the value of the dye (crystal violet) as an antiseptic.

B. Technical

The solutions to be tested were applied to the gum of the upper jaw between the mucosa of the lip and of the gum beside the frenum, because of the ease with which one may eliminate saliva from the field

by packing with sterile cotton. The dye was applied by means of pledgets of cotton saturated with the dye solution of various strengths and for different periods. Bacterial cultures were made in glucose-veal bouillon, and on glucose-blood agar slants or plates, by the use of sterile swabs, kept in the individual test tubes in which they were sterilized.

C. Results with brilliant green (saturated solution in alcohol)

A saturated solution of brilliant green³ in 95 per cent alcohol or in 60 per cent alcohol, was applied, as indicated above, and the swab cultured after one minute of application. A second test was subse-

TABLE 2
Data on the efficiency of alcoholic solutions of brilliant green in efforts to obtain sterility in the buccal cavity

SOLUTION OF BRILLIANT GREEN	PERIOD OF APPLICATION	NUMBER OF CASES	BACTERIOLOGICAL RESULT	EFFECTS ON THE GUMS
	<i>minute</i>			
Saturated, in 95 per cent alcohol	1	5	Sterility in five cases	Gums badly dehydrated
Saturated, in 60 per cent alcohol	1	5	Sterility in five cases	Gums only slightly dehydrated
Saturated, in 95 per cent alcohol	1	5	Sterility in five cases	Gums very badly dehydrated
Followed by washing with 95 per cent alcohol	0.5			
Saturated, in 60 per cent alcohol	1	5	Sterility in five cases	Gums only slightly dehydrated
Followed by washing with 60 per cent alcohol	0.5			
Total.....		20	Sterility in twenty cases	

quently made in which the excess of the dye remaining on the gum was removed by washing with alcohol of the same strength as that used originally to dissolve the dye. The part so treated was then tested for sterility in the usual manner. Sterility was obtained in all cases,

³ Thus far only Grüber's dyes have been used in this work.

but the alcohol exerted too great a dehydrating action upon the mucous membrane to permit of its routine application. The results are summarized in *table 2*.

D. Results with brilliant green (1 per cent solution in 50 per cent alcohol)

In this group of tests the technique was similar to that of the tests in section C, except that we used a 1 per cent solution of brilliant green in 50 per cent alcohol. Sterility was obtained in 78.5 per cent of the cases and there was only a slight degree of harmful effect of the alcohol upon the gums. The results are shown in *table 3*.

TABLE 3
Additional data on the efficiency of brilliant green in alcoholic solution in efforts to obtain sterility in the buccal cavity

SOLUTION OF BRILLIANT GREEN	PERIOD OF APPLICATION	NUMBER OF CASES	BACTERIOLOGICAL RESULT	EFFECTS ON THE GUMS
	<i>minute</i>			
1 per cent in 50 per cent alcohol	1	7	Sterility in five cases	Gums very slightly dehydrated
1 per cent in 50 per cent alcohol	1	7	Sterility in six cases	Gums very slightly dehydrated
Followed by washing with 50 per cent alcohol	0.5			
Total.....		14	Sterility in eleven cases (78.5 per cent)	

E. Results with brilliant green and crystal violet in solution *together* (1 per cent of each in 50 per cent alcohol) applied for 1.5 minute

In this series of cases, the dye employed was a mixture of brilliant green and crystal violet, 1 per cent of each in 50 per cent alcohol, applied for 1.5 minute. The action of the dye upon the organisms normally present was measured, in this instance, by taking cultures (a) from the gum-surface before applying the dye, and (b) from the relative numbers of organisms computed to be present in smears of the broth culture, as well as (c) from the number of colonies on plates of enrichment media.

TABLE 4

Data on the efficiency of crystal violet and brilliant green together (1 per cent of each), in 50 per cent alcohol, in efforts to obtain sterility in the buccal cavity. Period of application to the gum: 1.5 minute. (In this, and succeeding tables, the signs, x, xx, and xxx, indicate the relative numbers of organisms in the cultures. Where no such sign is recorded, a pure culture was involved)

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED	RESULTS AFTER THE EXCESS OF THE DYE HAD BEEN REMOVED BY WASHING WITH ALCOHOL	TYPE OF CASE
17	Streptococci xxx Diplostreptococci x	Sterile in 48 hours	Same	Pyorrhea
18	Diplostreptococci xxx Streptococci, short chain x Staphylococcus albus x	Sterile in 48 hours	Same	Pyorrhea
19	B. acidophilus xxx Diplostreptococci xx Streptococci x	Diplostreptococci present	Same	Pyorrhea
20	Diplostreptococci xxx Streptococci xx Staphylococcus albus x	Sterile in 36 hours	Same	Pyorrhea
21	Diplostreptococci xxx Staphylococcus albus x Streptococci x	Sterile in 36 hours	Same	Pyorrhea
22	Diplostreptococci xxx Streptococci x	Sterile in 36 hours	Same	Pyorrhea
23	Diplostreptococci xxx Staphylococcus albus x	Sterile in 36 hours	Same	Pyorrhea
24	Diplostreptococci xxx Streptococci x	Sterile in 36 hours	Same	Pyorrhea
25	Streptococci, long chain xxx Staphylococcus albus xxx	Streptococci, long chain present	Same	Pyorrhea
26	Streptococci xxx Diplostreptococci x	Sterile	Same	Pyorrhea
27	Staphylococcus albus xxx Streptococci, short chain xxx	Sterile in 48 hours	Same	Pyorrhea

TABLE 4--*Concluded*

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED	RESULTS AFTER THE EXCESS OF THE DYE HAD BEEN REMOVED BY WASHING WITH ALCOHOL	TYPE OF CASE
28	B. acidophilus xx Streptococci, short chain xx Staphylococcus albus x	Gram-rod-like organism present	Same	Pyorrhea
29	Diplostreptococci xxx Streptococci, short chain xx	Sterile in 48 hours	Same	Pyorrhea
30	Streptococci, long chain xx Staphylococcus aureus x	Sterile in 36 hours	Same	Pyorrhea
Total number of cases, 14. Sterility in the number of cases indicated below:				
		11 cases; 78.5 per cent	10 cases; 75 per cent	

By comparing these results with those obtained *after* swabbing the gum-surface with the dye, and after the subsequent removal of the excess of the dye with 50 per cent alcohol, a direct determination of the sterilizing action of the fluids could be made. The cases were all diagnosed as pyorrheal. Sterility was obtained in 75 per cent of them. The results are given in *table 4*.

F. Results of tests similar to those in the last preceding section (E), with additional bacteriological precautions

In addition to the previous precautions and technique, this series of experiments was further controlled by planting the last swab, used in culturing the gum-surface, in glucose-veal broth. In this series of tests we obtained sterility in 80 per cent of the cases. The findings are given in *table 5*.

TABLE 5

Additional data on the efficiency of crystal violet and brilliant green together (1 per cent of each), in 50 per cent alcohol, in efforts to obtain sterility in the buccal cavity. Period of application to the gum: 1.5 minute.

CASE NUM- BER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED	RESULTS AFTER THE EXCESS OF THE DYE HAD BEEN REMOVED BY WASHING WITH ALCOHOL	RESULT IN THE CULTURE OF THE LAST SWAB	TYPE OF CASE
31	{ Streptococci xxx Diplostreptococci x Staphylococci x	Present: Streptococci xx Diplostrep- tococci x	Sterile	Sterile	Pyorrhea
32	{ Streptococci xx Staphylococci x	Sterile in 48 hours	Staphylo- coccus al- bus pres- ent	Staphylo- cocci present	Pyorrhea
33	Diplostreptococci xxx	Sterile in 48 hours	Same	Same	Pyorrhea
40	Streptococci, long chain xx	Sterile in 36 hours	Same	Same	Pyorrhea
41	{ Streptococci, long chain xxx Diplostreptococci x	Sterile in 36 hours	Same	Same	Pyorrhea
42	{ Gram-rod (B. coli) xxx Staphylococci xx	B. coli pres- ent	B. coli pres- ent	B. coli pres- ent	Pyorrhea
43	{ Streptococci, short chain xxx Staphylococci x Streptococci, long chain x Diplostreptococci x	Sterile in 48 hours	Same	Same	Pyorrhea
46	{ Staphylococcus albus xx Streptococci x	Sterile in 48 hours	Same	Same	Pyorrhea
47	{ Diplostreptococci xx Streptococci, short chain x	Sterile in 48 hours	Same	Same	Pyorrhea
49	{ Diplostreptococci xxx Streptococci x Staphylococcus albus x	Sterile in 48 hours	Streptococci present	Sterile in 48 hours	Pyorrhea
Total number of cases, 10. Sterility in the number of cases indicated below:					
		8 cases; 80 per cent	7 cases; 70 per cent	8 cases; 80 per cent	

TABLE 6

Data on the comparative efficiency of crystal violet and brilliant green together (1 per cent of each) and of hydroquinone (1 per cent) in aqueous solution, in efforts to obtain sterility in the buccal cavity. Periods of application to the gum: (a) crystal violet and brilliant green, 2 minutes, or 2.5 minutes; (b) hydroquinone, 2.25 minutes. Type of cases: all pyorrhea

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED FOR 2 MINUTES	RESULT IN THE LAST CORRESPONDING SWAB CULTURE	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED FOR 2.5 MINUTES	RESULT IN THE LAST CORRESPONDING SWAB CULTURE	RESULTS AFTER THE HYDROQUINONE SOLUTION HAD BEEN APPLIED FOR 2.25 MINUTES
60 {	Streptococcus xxx Diplostreptococcus x	Same	Same	Same	Same	Same
61 {	Streptococcus xx Diplostreptococcus x Staphylococcus albus x	Streptococcus present	Present: Streptococcus xx Diplostreptococcus x	Streptococcus present	Streptococcus present	Same as control (first column)
62 {	Streptococcus x Diplostreptococcus x	Same	Same	Same	Same	Same
63 {	Staphylococcus xxx Streptococcus xx	Streptococcus present	Present: Streptococcus xx Staphylococcus x	Streptococcus present	Streptococcus present	Same as control (first column)
64 {	Streptococcus xx B. coli x	Same	Same	Same	Same	Same
65	Streptococcus	Same	Same	Same	Same	Same
66 {	Streptococcus xx Staphylococcus xx Diplostreptococcus x	Present: Streptococcus x Staphylococcus x	Present: Streptococcus x Staphylococcus x Diplostreptococcus x	Present: Streptococcus x Staphylococcus x	Same	Same as control (first column)
67 {	Staphylococcus xx Streptococcus x	Same	Same	Same	Same	Same

TABLE 6—*Concluded*

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED FOR 2 MINUTES	RESULT IN THE LAST CORRESPONDING SWAB CULTURE	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED FOR 2.5 MINUTES	RESULT IN THE LAST CORRESPONDING SWAB CULTURE	RESULTS AFTER THE HYDROQUINONE SOLUTION HAD BEEN APPLIED FOR 2.25 MINUTES
68	Diplostreptococcus xx Streptococcus x	Same	Same	Same	Same	Same
69	Streptococcus xx B. coli xx Diplostreptococcus x Staphylococcus x	Same	Same	Present: Streptococcus xx B. coli xx Diplostreptococcus x	Same	Same as. control (first column)

G. Results of tests with brilliant green and crystal violet in solution *together* (1 per cent of each in aqueous solution) compared with hydroquinone (1 per cent aqueous solution)

At this stage we considered it advisable to ascertain the relative value of an *aqueous* solution of the dyes compared with an aqueous solution of hydroquinone. Accordingly we made a solution of brilliant green and crystal violet (1 per cent of each, together), and another of hydroquinone (1 per cent), in sterile distilled water. The bacteriological technique was the same as that outlined for the tests in section F.

The results, as shown in *table 6*; were wholly unsatisfactory, from an experimental point of view, for practically no sterility was obtained under the conditions of the tests.

H. Results of a repetition of the tests with brilliant green and crystal violet in solution *together* in alcohol (section E), with an extension of the period of application to the gum (from 1.5 minute) to 2 minutes

We repeated the technique of the tests in section E (with 1 per cent each of brilliant green and crystal violet together in solution in 50 per cent alcohol), but allowed a little more time for the action of the

TABLE 7

Additional data on the efficiency of crystal violet and brilliant green together (1 per cent of each), in 50 per cent alcohol, in efforts to obtain sterility in the buccal cavity. Period of application to the gum: 2 minutes

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED	RESULT IN THE CULTURE OF THE LAST SWAB	TYPES OF CASES
65/2	{ Streptococcus xx Diplostreptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
66/2	Streptococcus	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
67/2	{ Diplostreptococcus xx Streptococcus	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea and caries
68/2	{ Staphylococcus albus xx Streptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
69/2	Streptococcus	Sterile in 48 hours	Sterile in 48 hours	Edentulous
70	{ Diplostreptococcus x Staphylococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
71	{ Diplostreptococcus xx Streptococcus x Staphylococcus albus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
72	{ Streptococcus, long chain xx Streptococcus, short chain xx Staphylococcus x Diplostreptococcus x	Streptococcus, long chain, present	Streptococcus, long chain, present	Pyorrhea
73	Staphylococcus albus	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
75	{ Streptococcus x Diplostreptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
76	{ Diplostreptococcus xx Staphylococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
77	{ Diplostreptococcus xxx Staphylococcus x Streptococcus, long chain x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea

TABLE 7—Continued

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED	RESULT IN THE CULTURE OF THE LAST SWAB	TYPE OF CASES
78	Streptococcus xxxx Diplostreptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea and caries
79	Streptococcus xx Staphylococcus albus x Diplostreptococcus x	Staphylococcus albus present	Staphylococcus albus present	Pyorrhea
80	Streptococcus, long chain	Sterile	Sterile	Edentulous
81	Diplostreptococcus xx Streptococcus x	Sterile	Sterile	Advanced caries
85	Staphylococcus albus x Diplostreptococcus x	Sterile	Sterile	Pyorrhea
86	Streptococcus xxx Diplostreptococcus x	Sterile	Sterile	Pyorrhea
87	Diplostreptococcus xx Streptococcus x Staphylococcus albus x	Sterile	Sterile	Pyorrhea
88	Staphylococcus albus xx Streptococcus x	Sterile	Staphylococcus albus present	Pyorrhea
89	Streptococcus	Sterile	Sterile	Pyorrhea
90	Diplostreptococcus xx Streptococcus x	Sterile	Sterile	Pyorrhea
92	Streptococcus, long chain xx Streptococcus, short chain x Staphylococcus albus x	Sterile	Sterile	Fairly clean mouth
93	Diplostreptococcus xxx Streptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
94	Diplostreptococcus	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea

TABLE 7—*Concluded*

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED	RESULTS IN THE CULTURE OF THE LAST SWAB	TYPES OF CASES
95	{ Streptococcus xxx Staphylococcus xx Diplostreptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
96	{ Streptococcus x Diplostreptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
97	{ Staphylococcus albus xx Streptococcus x	Sterile in 48 hours	Sterile in 48 hours	Pyorrhea
98	{ Streptococcus xxx Diplostreptococcus xx	Streptococcus present	Streptococcus present	Pyorrhea
99	{ Diplostreptococcus xx Streptococcus x	Streptococcus present	Streptococcus present	Pyorrhea
101	{ Streptococcus x Staphylococcus albus x	Streptococcus present	Streptococcus present	Pyorrhea
102	{ Streptococcus	Streptococcus present	Streptococcus present	Pyorrhea
103	{ Streptococcus xx Diplostreptococcus x	Streptococcus present	Streptococcus present	Pyorrhea
104	{ Diplostreptococcus xxx Staphylococcus albus x Streptococcus x B. coli xx	B. coli present	B. coli present	Pyorrhea
105	{ Streptococcus xx Staphylococcus albus x	Sterile	Sterile	Pyorrhea
106	{ Streptococcus xxx Staphylococcus albus xx	Sterile	Sterile	Pyorrhea
108	{ Diplostreptococcus xxx Streptococcus x	Sterile	Sterile	Pyorrhea

dye, namely, 2 minutes instead of 1.5 minute. The results in *table 7* are summarized below:

	Number	Per cent
Total number of cases cultured.....	37	
Sterile after application of the dye for two minutes.....	33	89.2
Sterile swabs.....	32	86.9
Staphylococcus persisted after the application of the dye.....	1	2.8
Streptococcus persisted after the application of the dye.....	1	2.8

In this series of tests, and in those of the preceding groups, we noted that *B. coli* was not affected by the dye to any appreciable extent. We endeavored, then, to ascertain the comparative effects of the dye on *B. coli* and on a streptococcus. See the experiment described in the footnote.⁴

⁴ The supernatant fluid from a 100 cc. glucose-blood veal-broth culture of a streptococcus isolated from an apical infection was carefully pipetted off and centrifuged. The organisms thus obtained were suspended in normal salt solution and half their number, 500 billions, injected into the ear veins of a rabbit. The rabbit was sacrificed in ten minutes, immediately placed in 1:1000 lysol solution, and carefully autopsied. Portions of the liver, lungs, heart, and kidneys were obtained aseptically and placed in sterile petri dishes. Several pieces of each of these tissues, 0.5 x 0.5 x 1 cm. in size, were dipped into a suspension of *B. coli*. One of these pieces was then immersed for five seconds in paraffin oil at 180°C., when it was macerated in a sterile mortar in broth and sand, and plated in glucose-blood agar; also in Endo's medium according to the method of McMaster (16). Others of the pieces treated with *B. coli* were dipped in a solution of brilliant green and crystal violet together (1 per cent each) in 50 per cent alcohol for 1.5 minute and the excess of the dye washed off with 50 per cent alcohol for 0.5 minute. The tissue was finally macerated as in the case of the pieces immersed in paraffin oil, with the following results:

TISSUE TREATED WITH <i>B. COLI</i>	AFTER IMMERSION IN PARAFFIN OIL AT 180°C. FOR 5 SECONDS		AFTER APPLICATION OF THE DYE SOLUTION — CRYSTAL VIOLET AND BRILLIANT GREEN TOGETHER (1 PER CENT OF EACH) IN 50 PER CENT ALCOHOL — FOR 1.5 MINUTE FOLLOWED BY WASHING WITH 50 PER CENT ALCOHOL FOR 0.5 MINUTE	
	Organisms in Endo's medium	Organisms in the glucose-blood broth	Organisms in Endo's medium	Organisms in the glucose-blood broth
Heart.....	No growth	Streptococci	<i>B. coli</i>	Streptococci and <i>B. coli</i>
Kidney.....	No growth	Streptococci	<i>B. coli</i>	Streptococci and <i>B. coli</i>
Lungs.....	No growth	Streptococci	No growth	Streptococci
Liver.....	No growth	Streptococci	No growth	Streptococci

These tests show that in the second case, i.e., with the dye, there was no growth of *B. coli* in the cultures from the lungs and liver, possibly because of the better penetration of the dye into the lungs and liver, as a consequence of the peculiarities of the anatomical structure of these tissues.

I. Results of a repetition of the tests with brilliant green and crystal violet *together* in alcohol, in the last preceding section (H), after previous thorough flushing of the mouth with an alkaline wash and brushing of the teeth

After carefully flushing the mouth with an alkaline wash and brushing the teeth, the technique for the tests in section H was used, with the results shown in *table 8*.

TABLE 8

Additional data on the efficiency of crystal violet and brilliant green together (1 per cent of each), in 50 per cent alcohol, in efforts to obtain sterility in the buccal cavity. Period of application to the gum: 2 minutes (after flushing the mouth with an alkaline wash and brushing the teeth)

CASE NUMBER	ORGANISMS ON THE GUM-SURFACE BEFORE THE DYE WAS APPLIED	RESULTS AFTER THE DYE SOLUTION HAD BEEN APPLIED	RESULT IN THE CULTURE OF THE LAST SWAB	TYPES OF CASES
110	Streptococcus	Sterile	Sterile	Pyorrhea
111	Streptococcus	Sterile	Sterile	Pyorrhea
112	Staphylococcus	Sterile	Sterile	Pyorrhea
113 {	Streptococcus x	Sterile	Sterile	Pyorrhea
	Diplostreptococcus			
114	Streptococcus	Sterile	Sterile	Pyorrhea
115	Diplostreptococcus	Sterile	Sterile	Pyorrhea
116 {	Diplostreptococcus xx	Sterile	Sterile	Extensive caries
	Streptococcus x			
117	Diplostreptococcus	Sterile	Sterile	Pyorrhea
118	Streptococcus	Streptococcus present	Sterile	Pyorrhea
119 {	Diplostreptococcus xx	Sterile	Sterile	Pyorrhea
	Streptococcus x			
120	Staphylococcus	Sterile	Sterile	Pyorrhea
121 {	Streptococcus xxx	Sterile	Sterile	Pyorrhea
	Diplostreptococcus x			
122	Streptococcus	Sterile	Sterile	Pyorrhea
123 {	Streptococcus, long chain x	Sterile	Sterile	Pyorrhea
	Streptococcus, short chain x			

Total number of cases: 14; sterility in 13 cases, or 92 per cent; absolute sterility in 14 cases or 100 per cent.

3. General discussion of the results of the first two series of tests

Alcohol and iodine should be rejected as oral antiseptics on account of their harmful effects on the delicate mucous membrane despite their apparently good qualities as antiseptics elsewhere.

Since neither crystal violet nor brilliant green is much affected by the oral secretions, and as the action of each is enhanced by the slight alkalinity of the saliva, we regard these dyes as excellent oral antiseptics, in accord with the findings recorded in sections D, E, F, H, and I above.

The results of the tests described in section I, show that advantage may be derived from a thorough cleansing of the mouth and teeth before applying the antiseptic. The alkaline mouth-wash employed in the tests for this purpose appears to have been responsible for the improvement of the results (I).

4. Third series. Clinical findings

The results of the foregoing experiments show the efficiency of dyes as antiseptics from the laboratory view-point. Consequently, we endeavored to ascertain their value from the clinical and therapeutic aspects.

The following case histories have been selected from a very large number to *illustrate* the effects of practical application, in dental surgery, of crystal violet and brilliant green in solution together (1 per cent of each in 50 per cent alcohol), referred to below as "the dye."

Case 1. Mr. J. A. An extensive bone cyst in the mandible. It was opened, curetted, drained, and treated with the dye. The discharge, which had been profuse and purulent, became scanty and serous after two applications with a one-day interval. Granulations began to grow inward from all sides. The wound gradually closed up in four weeks of this treatment.

Case 2. Mr. M. S. A rather large granuloma, and surrounding infected bone, was incised, curetted, drained, and treated with the dye. Granulations grew rapidly and healing was by first intention.

Case 3. Mr. J. F. An osteomyelitis of the lower third of a fibula. It was opened, drained, and treated with camphor-phenol for one month without improvement and with a continuing profuse, purulent, foul discharge. The dye was applied and the discharge soon became scanty, serous and odorless. Two small sequestra were recently removed. Healing began promptly after the operation and is proceeding rapidly.

Case 4. Mrs. J. A surgical removal of granulomata and the surrounding necrotic bone. The wound was treated with the dye. Healing took place promptly, with no discharge save in one area, which is yielding rapidly to treatment with the dye.

Case 5. Mr. J. H. A severe case of pyorrhea. Teeth were removed. The wounds were treated with the dye three times. There has been no history of pain or soreness. Healing progressed at a rapid rate.

Case 6. Mrs. B. Extensive apical and periapical infections. Teeth were surgically removed. The wounds were treated once weekly, for three weeks, with the dye. Healing was complete with almost no discharge. Dentures were constructed in the third week after the extractions.

Case 7. Mr. R. A superior first molar was removed surgically under local anesthesia. A severe case of eburnated bone. Treated twice with the dye. Healing was complete in five days, with no discharge.

Case 8. Miss C. Granulomata and necrosis about lower first and second molars. These were removed under local anesthesia, with extensive curettage. The wounds were treated daily, for a week, with the dye. There were slight, scanty, serous discharges, but no pain. The wounds were completely healed in three weeks.

Many other cases might be mentioned, in which the results were quite similar. In the research laboratories and clinics of the dental department a large number of patients have been treated with the dye. The histories of these cases appear to confirm the general deduction stated above regarding the antiseptic value of the dye. The improvement in cases of chronic gingivitis has been very marked. Injection of the dye into pyorrheal pockets is of only temporary benefit, however, for the tendency toward tissue degeneration is not noticeably altered.

Discussion of the clinical findings

Solutions of crystal violet and brilliant green may be easily applied to the gums and even deeper structures in the oral cavity. The diffusion and penetration of the dye is considerable. The clinical data reported above are wholly in keeping with the results that various observers have obtained with dyes as antiseptics for other parts of the body. In every instance, in our experience, the case was benefited by treatment with the dye. Extractions, old discharging sinuses and old infections, yield completely to treatment.

Solutions of crystal violet and brilliant green appear to have no lasting beneficial effect upon pyorrhea. The only result we obtained was merely a temporary diminution in the severity of the symptoms.

III. SUMMARY OF GENERAL CONCLUSIONS

Brilliant green and crystal violet, in solution together (1 per cent of each in 50 per cent alcohol), are very efficient, mild antiseptics. This combination has very little, if any, harmful effect on the delicate mucous membrane of the mouth.

Clinical observations indicate that these dyes together in alcohol act not only as an antiseptic, but also as a mild stimulant to newly forming granulations, both by keeping down contamination by oral organisms and by either destroying or inhibiting the growth of organisms initially present.

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