FURTHER OBSERVATIONS ON THE CLINICAL VALUE OF CERTAIN BACTERIOSTATIC TRIPHENYL- METHANE DERIVATIVES

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The specificity of the action of certain drugs against particular infections has been recognized for a great many years. Perhaps one of the first drugs to be so used was quinin, which was employed in its original state in the treatment of malaria, between the years 1630 and 1640. In 1638, it is said to have proved efficacious in the case of the Countess of Chinchon, whose name was later applied to the bark which the Spanish brought from South America.

After an exhaustive investigation of certain arsenic compounds, Ehrlich¹ arrived at the formula of arsphenamin in the treatment of syphilis. In this case, the toxophore radical of arsphenamin has a greater affinity for the parasite than it has for the body cells of the animal host.

These two classical cases may be cited as opening the way to further knowledge of specific chemotherapy.

CHEMISTRY OF DYESTUFFS

I shall consider first the chemistry of certain dyestuffs, whose action will be discussed in more detail later.

Triphenylmethane, $\text{CH}(\text{C}_6\text{H}_5)_3$, is a crystalline substance and serves as the base for many of the dyes. There are three stages in the formation of the dye: (1) the formation of a (colorless) leukobase; (2) the formation of a (colorless) color base, and (3) the formation of a dye.

Many dyes exist as salts, but on account of their basic reaction they readily dissolve in acid solutions, losing their characteristic color which reappears when an excess of alkali is added to neutralize the acid.

Pararosanilin and rosanilin may have several of the hydrogen atoms of the amino groups replaced by alkyl radicals. The simplest of these dyes are yellow in color, and with the increase in molecular weight, or, in other words, with the increase in the number of alkyl or phenyl groups, the color is changed through orange and red to violet and blue. These substances are crystalline in form, and a great many of them are insoluble in water but highly soluble in alcohol.

1. Ehrlich: Abhandlungen über Salvarsan **1-3**.

It is possible to create certain intensities in color by the addition of certain radicals to the general formula. The addition of methyl groups increases the violet color in direct proportion to the number of methyl groups added. Thus the double salt, pentamethylpararosanilin and hexamethylpararosanilin or "methyl violet" is formed, whose structural formula is a mixture of the following:

By the replacement of one hydrogen atom of each amino group of rosanilin by a phenyl radical, a blue dye may be formed; thus we may make "anilin blue," triphenylrosalin-hydrochlorid. The intensity of the blue color depends here also upon the number of phenyl groups added to the structural formula.

Crystal violet (hexamethyltriaminotriphenylmethane) serves as an excellent example of a phenomenon exhibited by an analogous group of basic substances. When an equivalent amount of alkali is added to any salt of crystal violet, there is no immediate change in the color of the compound. But with the addition of a similar amount of acid, the color base dissolves to form a compound of a different color and not highly water soluble. This color base can, however, be reprecipitated by the addition of an equivalent amount of alkali to the solution, thus bringing back the violet color.

The foregoing will give the reader some idea of the relationship of these compounds to one another and show how the triphenylmethane radical is the fundamental basis of this entire group of compounds.

INHIBITORY PROPERTIES OF DYE STUFFS

Many of these substances have long been known to exhibit inhibitory properties upon the growth of certain organisms in culture mediums. Peabody and Pratt² used malachite green solution as an enriching agent in the isolation of *B. typhosus*.

Churchman,³ found that gentian violet in dilutions of 1:100,000 inhibited the growth of certain types of bacteria while other types were

2. Peabody, F. W., and Pratt, J. H.: On the Value of Malachite Green Media in Differentiating Typhoid and Colon Bacilli with the Description of a New Method of Isolating Typhoid Bacilli from Feces, Boston M. & S. J. **158**: 213, 1908.

3. Churchman, J. W.: The Relative Bactericidal Action of Gentian Violet, J. Exper. M. **16**:221, 1912. Churchman, J. W., and Michael, W. H.: The Selective Action of Gentian Violet and Closely Related Bacterial Stains, J. Exper. M. **16**:822, 1912.

not affected by its presence. Those organisms which grew in the presence of gentian violet solution were of the gram-negative type, whereas those which were inhibited belonged to the gram-positive group. Churchman⁴ used a diagram to illustrate the effect of gentian violet upon gram-negative and gram-positive organisms. He found that out of 182 strains of gram-positive organisms, only seventeen strains were unaffected, and out of 136 strains of gram-negative organisms, only fifteen were unaffected by the dye.

In an earlier publication, he used the terms "violet-positive" and "violet-negative" for the action of the dye in its inhibition as associated with gram-positive and gram-negative organisms. This nomenclature was objected to on the ground that the selective action of the dye is not at all dependent upon the color of the dye but rather upon some other factor. Consequently, he modified the terms in regard to the use of gentian violet to "gentian-violet-positive" and "gentian-violet-negative" in regard to its selective action upon the growth of bacteria.

Since, however, these terms are only adaptable to the one dye mentioned above, they could hardly be applied to other dyes of the same type. Better terms would be perhaps "triphenylmethane-positive" and "triphenylmethane-negative" in regard to the action of this whole group of chemicals in their selectivity upon the growth of gram-positive and gram-negative organisms.

Crossley⁵ also noted that the inhibition of gram-positive organisms by crystal violet took place when other members of the triphenylmethane group were used.

He assumed that there are two factors at work which control the action of the dye: (1) the composition of the dye, and (2) the nature of the organism. He further suggested that there must be certain groupings inherent in the dye which combine with certain "reactive groups" in the structure of the organism, and thus produce bacteriostasis.

This selective action appears to be related in some way to the basic nitrogen, and substitution of methyl grouping for the aminohydrogen groupings causes an increase in bacteriostasis on the part of the compound. He called attention to the value of this feature in the treatment of infections, and suggested that further work be done on allied compounds.

It was Churchman⁶ who called attention to the fact that dahlia, parafuchsin, magenta, pararosanilin, rosanilin, crystal violet, methyl

4. Churchman, J. W.: Gentian Violet in the Treatment of Purulent Arthritis, *J. A. M. A.* **75**:583 (Aug. 28) 1920.

5. Crossley, M. L.: Gentian Violet, Its Selective Bactericidal Action, *J. Am. Chem. Soc.* **41**:2, 2083, 1919.

6. Churchman, J. W.: The Selective Bactericidal Action of Stains Closely Allied to Gentian Violet, *J. Exper. M.* **17**:373 (April) 1913.

violet, all have one thing in common, that is, their selective action upon bacteria, and he assumes that since each compound is a derivative of triphenylmethane, the power of bacteriostasis is inherent in this triphenylmethane radical.

Churchman,⁷ in explaining the mechanism of this parallelism, states that "the factor which determines the relation of an organism to the gram process of staining is not the same as the factor which determines its relation upon divided gentian violet plates or after staining with the dye."

Nevertheless, combined with the gram stain, a "gentian" positive or "triphenylmethane" positive dye contains certain groups possibly related, as Crossley states, to the substituted alkyl radicals for the aminohydrogen of the basic nitrogen. These combinations when added to the "reactive groups" of the structure of the organism combine with them and later are fixed by the iodine solution which acts as a mordant and prevents the redissolution of the dye. This process definitely takes place in fixed or dead organisms. The same reaction may occur in the instance of living organisms, save that here the combination of dye with the "reactive groups" of the organism results not in mere staining of the parasite, but prevents further metabolism and division on the part of the bacterium by effecting some change in the tissue structure of the organism. At any rate, this idea must be kept in mind when considering the action of this group of substances.

Krumwiede and Pratt⁸ not only verify the statements of Churchman, but state that occasionally there is found a particular strain of a gram-negative organism, which will not grow upon gentian violet mediums. This strain of organism was found to differ from other strains of the same organism by agglutination tests, which may account for the noninhibition in the same dilution of dye.

Krumwiede⁹ recently made a brilliant green agar for the isolation of typhoid bacilli. Brilliant green, a diaminotri-phenylmethane derivative, they found not only inhibited gram-negative organisms but also many types of gram-positive bacteria as well. It exerted a specific differential action upon the colon-typhoid group. *B. lactis aerogenes*

7. Churchman, J. W.: The Cause of the Parallelism Between the Gram Reaction and the Gentian Violet Reaction, *Proc. Soc. Exper. Biol. & M.* **18**: 17, 1920.

8. Krumwiede, Charles, and Pratt, J. H.: Observations on the Growth of Bacteria on Media Containing Various Aniline Dyes, *Proc. New York Path. Soc.* **13**:43, 1913; Dahlia Agar als Unterscheidungs Mittel Zwischen Cholera und Anderen Vibrionen, *Centralbl. f. Bakteriologie*, **58**:585, 1913.

9. Krumwiede, Charles: Footnote 8. Hiss, P. H., and Zinsser, H.: *Text-book of Bacteriology*, New York, D. Appleton & Co., 1919.

and the paratyphoid organisms are not affected, and *B. typhosus* is inhibited only in lower dilutions, whereas dysentery and the other colon groups are inhibited.

APPLICATION OF SPECIFIC CHEMOTHERAPY TO INFECTED WOUNDS

Such information as this stimulated many clinical workers to investigate the possible application of specific chemotherapy to cases of infected wounds.

Consequently, Leitch¹⁰ used brilliant green 1:1,000 in aqueous solution and in normal saline solution as an antiseptic for infected wounds. He very soon observed that vital tissue under the action of the dye retained its normal appearance and color, whereas dead or necrotic tissue was deeply stained with the dyestuff. He also noted that the dye appeared to exert a more selective action upon staphylococci than upon streptococci.

A short while later, Short¹¹ reported upon the injection of a paste, composed of boric acid, French chalk, and brilliant green 1:200 in liquid petrolatum, into the sinuses of infected wounds with excellent results. They injected a 1:200 aqueous solution of brilliant green into the wound just before injecting the paste. They also maintained that there was no tissue destruction on the part of the dye and asserted that it was mildly stimulating to granulation tissue.

Browning, Gulbransen and Thornton¹² tested acriflavine, proflavine and brilliant green with especial reference to their suitability to wound therapy. They found in the test tube that brilliant green 1:2,000 aqueous solution is 500 times as toxic for a twenty-four hour broth culture of *Staphylococcus aureus* as is Dakin's solution, and it is 700 times as toxic as Dakin's solution for *B. coli*. Consequently, they used repeated irrigations of 1:2,000 aqueous solution of brilliant green to infected wounds and obtained excellent results. Furthermore, they found that it was absolutely innocuous to vital tissues and also that the dye is not reduced or hampered in any way in its activity by tissue juices and wound secretions. They did note, however, that blood serum in the test tube does reduce the activity of brilliant green in its toxicity for bacteria.

Later on, Browning¹³ ascertained that brilliant green will kill gram-positive cocci in dilutions of 1:100,000. He also says that in dilutions

10. Leitch, A.: Brilliant Green as an Antiseptic. *Brit. M. J.* **1**:236 (Feb. 1) 1916.

11. Short, A. R.; Arkle, J. S., and King, C.: Brilliant Green Paste in Wounds, *Brit. M. J.* **2**:506 (Oct. 20) 1917.

12. Browning, C. H.; Gulbransen, R., and Thornton, L. H. D.: Acriflavine, Proflavine and Brilliant Green with Reference to Suitability to Wound Therapy, *Brit. M. J.* **2**:70, 1917.

13. Browning, C. H., et al.: Brilliant Green in Infected Wounds, *Brit. M. J.* **1**:73 (Jan. 20) 1917.

of 1:500, it stimulates phagocytosis. Similar results were obtained by Webb¹⁴ and by Ligat,¹⁵ both of whom used a 1:1,000 solution as irrigations in infected wounds. J. E. Adams,¹⁶ in the treatment of erysipelas, used a 5 per cent. aqueous solution of brilliant green, picric acid and tincture of iodine in separate, yet comparable, cases, and concluded that the dye gave the most satisfactory results. It was painted upon the affected skin surface once or twice a day, according to the severity of the infection. No dressing was necessary, save to protect the clothing.

J. W. Churchman⁴ used dilutions of gentian violet in the treatment of purulent arthritis. He first tapped the joint to ascertain the type of infection. The joint was then lavaged and stained as outlined in a previous article.¹⁷ He obtained a cure with good functional results in these types of joint infection: *Staphylococcus aureus*, two cases; pneumococcus, one case, and, gonococcus, four cases.

One lavage and staining was sufficient to procure an uneventful recovery in each case.

He¹⁸ also treated several infected wounds of the skin and serous membranes with excellent results. He suggested the use of solutions of dye injected into the gums in the treatment of infections deeper in the tissues as well as superficial gingivitis. He prefers the term "bacteriostasis" to "bactericidal" in speaking of the action of dyes upon organisms.

Mackie¹⁹ endeavored to clean up diphtheria carriers by using a spray of an aqueous solution of brilliant green (1:250 or 1:500) into the mouth and pharynx. He sprayed, three times daily, for one week, and asserts that he obtained negative cultures after such a spray.

This work was repeated by Kolmer,²⁰ who also used a 1:250 solution of brilliant green. He found that the disappearance of the diphtheria bacilli was merely temporary. He did not note permanent disappearance of the organisms.

14. Webb, C. H.: The Value of Brilliant Green as an Antiseptic, *Brit. M. J.* **1**:870 (June 30) 1917.

15. Ligat, D.: Brilliant Green and Flavine in Infected Wounds, *Brit. M. J.* **1**:78 (Jan. 20) 1917.

16. Adams, J. E.: A Note on the Treatment of Cutaneous Erysipelas with Brilliant Green, *Brit. M. J.* **2**:779 (Nov. 20) 1920.

17. Churchman, J. W.: The Treatment of Acute Infections of the Joint by Lavage and Direct Medication, *J. A. M. A.* **70**:1047 (April 13) 1918.

18. Churchman, J. W.: Selective Bacteriostasis in the Treatment of Infections with Gentian Violet, *J. A. M. A.* **74**:145 (Jan. 17) 1920.

19. Mackie, H., cited by Browning, C. H.: *Applied Bacteriology*, Oxford University Press, London, 1918.

20. Kolmer, J. A.; Woody, S. S., and Yogle, E. M.: The Influence of Brilliant Green on the Diphtheria Bacillus, *J. Infect. Dis.* **26**:179 (Feb.) 1920.

Graham Smith,²¹ who was investigating the value of acriflavine, proflavine (acridin dyes, derivatives of acridin, a base found in coal tar ²²) and crystal violet, found that the toxicity or inhibitory power depends to a great extent upon its alkalinity. Thus crystal violet inhibits *Staphylococcus aureus* as follows: 0.15 per cent. tenth normal hydrochloric acid, 1:1,500,000; 0.08 per cent. tenth normal sodium hydroxid, 1:3,500,000, and 0.58 per cent. tenth normal sodium hydroxid, 1:5,000,000.

From this it can be seen that the dye is three times as toxic for *Staphylococcus aureus* in alkaline solution as it is in acid solution, within certain limits.

Churchman has utilized chiefly gentian violet, which, as he says, is but 90 per cent. effective in its selectivity of gram-positive organisms, and only 10 per cent. effective against gram-negative organisms. He fails altogether to recognize the value of such dyes as brilliant green, whose sphere of selectivity is the gram-negative type of micro-organisms. Thus, in using a mixture of brilliant green and crystal violet

TABLE 1.—COMPARISON OF ACTION OF GENTIAN VIOLET AND CRYSTAL VIOLET

Location	Hours	No. Cases	Sterile
Abdominal skin.....	6	22	22
Peri-anal skin.....	3	20	Growth in two of four streaks in one case; growth in one of four streaks in two cases.

one would have a therapeutic agent whose selectivity was not only 100 per cent. effective against gram-positive organisms, but against gram-negative ones as well.

Browning²³ compared the action of gentian violet and crystal violet and found them to be practically identical in action. In sterilizing the skin, he used a mixture of 1 per cent. each of brilliant green and crystal violet in 50 per cent. alcoholic solution. He washed the skin off with the solution and applied a compress soaked with it. He obtained the results given in Table 1.

In order to ascertain what becomes of the dye in the animal body, much work has been done. Perhaps the most authoritative of which was done by Churchman.⁴ He injected solutions of gentian violet into the marginal vein of a rabbit's ear, and one and one-half hours

21. Graham-Smith, G. S.: Some Factors Influencing the Action of Dyes and Allied Compounds on Bacteria, *J. Hygiene* **18**:1, 1919.

22. New and Nonofficial Remedies, *J. A. M. A.* **73**:1443 (Nov. 2) 1919.

23. Browning, C. H.: Footnote 20, second reference. Browning, C. H., and Bonney, V.: Sterilization of the Skin and Other Surfaces with a Mixture of Crystal Violet and Brilliant Green, *Brit. M. J.* **1**:562 (May 18) 1918 and Footnote 4.

later, when blood was drawn from the ear, no dye could be demonstrated by its bacteriostatic power. This was not due to mere contact with the blood as he proves, nor was it due to oxidation on the part of the blood. Venous blood and arterial blood proved the same in their actions.

When the dye was taken up by epithelial cells it did not break down so rapidly—the change was much more gradual. There was no tissue destruction on the part of the dye when used in dilute solutions. At the same time, the dye persisted in the tissues unchanged for sufficient time to produce bacteriostasis before it was finally disposed of. This valuable property is not found in other bacteriostatic substances.

Strasznor²⁴ found that methylene blue was reduced by (—SH) sulfhydryl groups in the tissues. This activity on the part of the tissues is reduced by heating them, but is restored again by the addition of tissue containing (—SH) sulfhydryl groupings. It is entirely possible that the sulfhydryl grouping may be responsible for the reduction in vital tissue of members of the triphenylmethane derivatives as well.

BACTERIOSTATIC VALUE OF BRILLIANT GREEN AND CRYSTAL VIOLET

As a result of these findings we have undertaken to ascertain the bacteriostatic value of brilliant green and crystal violet when acting together in solution, and to standardize, if possible, the technic of application of such solutions.

In the following experiments Grübbler's dyes were used except where specially indicated.

We have repeated in part the experiments of Browning, by first swabbing the skin of the abdomen and about the anal orifice with a mixture of 1 per cent. brilliant green and 1 per cent. crystal violet in 50 per cent. alcohol. Cultures were made of a series of cases by scratching the skin with a sterile needle and stroking an agar plate. Four strokes were made in each case, the needle being flamed between strokes. Cultures were made of another series after painting the skin surface as above, but in addition, applying a compress soaked in the dye and later taking cultures in the manner just described (Table 2).

A third series of experiments was conducted thus: The skin was washed with soap and water and rinsed with 4 per cent. sodium bicarbonate solution. Following this a compress of aqueous solution of 1 per cent. each of brilliant green and crystal violet was applied. Cultures were made of areas so treated as heretofore described.

24. Strasznor, Walter: Die Reduzierenden Wirkungen des Gewebes, *Biochem. Ztschr.* **29**:295, 1910.

The time element in each case was five hours in the following series of cases, from which one would conclude that it is necessary to apply the compress about the anus, in order to obtain sterility in 100 per cent. of the cases (Table 3).

From the data given in Tables 2 and 3 it may be readily seen that aqueous solutions are just as satisfactory as alcoholic solutions when applied over longer periods of time.

Considerable success has been encountered in treating secondarily infected wounds of the skin and subcutaneous tissue. The first consideration is to insure free drainage and then to apply a compress soaked in aqueous solution of 1 per cent. each of brilliant green and crystal violet. This dressing should be changed two or three times a day depending upon the amount of drainage. Care should be taken to get the dye in contact with all of the wound surfaces.

The application of alcoholic solutions of mixed dye to chronic leg ulcers caused each one so treated to heal remarkably fast, whereas

TABLE 2.—COMPARATIVE RESULTS WHEN COMPRESSES WERE APPLIED AND WHEN NOT APPLIED

Location	—No Compress—		—Compress Applied—	
	No. Cases	Sterility	No. Cases	Sterility
Abdominal skin.....	10	6	16	16
Peri-anal skin.....	18	8	12	12

TABLE 3.—RESULTS OF APPLICATION OF COMPRESS ABOUT THE ANUS

Location	No. Cases	Sterile
Abdominal skin.....	13	13
Peri-anal skin.....	13	13

other cases, quite similar but treated otherwise, showed no sign of closing over.

Similar results have been obtained in the treatment of chancroidal infections.

Mr. E. W., single, aged 53, had large varicose veins of both legs with an ulceration 3.5 cm. in diameter on the lower third of the right tibia. The ulcer has been present and draining for seven months without a sign of closing over. The patient, without stopping his regular work, was treated with daily applications of 1 per cent. brilliant green and 1 per cent. crystal violet in 50 per cent. alcohol for five days. At this time, the discharge had practically stopped and healthy granulations had formed at the base, epithelium growing in from the edges. The dye therapy was continued three times a week for two weeks more, when the ulcer had entirely healed.

The hydrogen ion concentration (p_H) of saliva varies greatly in different individuals, and also in the same individual from time to time. This change may be brought about by changes in diet, appetite and

general health, by psychic changes, and by a pathologic condition of the oral and gastric mucosa and teeth. Bloomfield²⁵ finds that the saliva from normal people varies between p_H 6.0 and p_H 7.3²⁶ and that 80 per cent. of the specimens fell between p_H 6.6 and p_H 7.1. Persons who were suffering from a variety of diseases had a slightly wider range (p_H 5.8 to p_H 7.5) than was found in the normal group.

I²⁷ likewise examined a series of specimens of saliva of normal people and found that the average was p_H 7.43.²⁶ This is fairly alkaline and is sufficient to enhance the value of crystal violet and brilliant green when acting in the oral cavity. It has been demonstrated in previous work that these dyestuffs are more effective in alkaline than in acid mediums.

For this reason, I recommended the use of a mouth wash of 4 per cent. sodium bicarbonate solution after carefully brushing the teeth with an alkaline tooth powder. Then a mixture of 1 per cent. each of brilliant green and crystal violet in 50 per cent. alcohol was applied to the surface of the gums. Three minutes later, cultures were taken from this area and proved sterile. Further observations showed that the alcohol alone did not account for the sterility obtained. Aqueous solutions of the dye used over a very limited period of time (2.5 minutes) did not give the same result, and it was concluded, in order to obtain the result sought in the shorter period of time, that the alcohol was necessary to carry the dye. No experiments were undertaken at that time to test the value of aqueous solutions over longer periods.

Infected maxillary sinuses are thoroughly irrigated with sodium bicarbonate solution by means of a trocar passed through the mesial wall below the inferior turbinate, and then injected with from 2 to 3 c.c. of aqueous solution of the mixed dye. The treatment may be repeated every day for several days, depending upon the severity of the case. In all, twenty patients have been treated in this manner, with excellent results in twelve. The cases of six of the remaining eight patients were complicated by further sinus infection and were only temporarily relieved, and two patients had open sinuses into the oral cavity, reinfection taking place very rapidly afterward.

25. Bloomfield, A. L., and Huck, J. G.: The Fate of Bacteria Introduced Into the Upper Air Passages. IV. The Reaction of Saliva, *Bull. Johns Hopkins Hosp.* **31**:118 (April) 1920.

26. According to the colorimetric method of Clark, U. M., and Lubs, H. A. (The Colorimetric Determination of Hydrogen Ion Concentration and Its Application to Bacteriology, *J. Bacteriol.* **2**:191, 1917) and of MacLeod, J. J. R., (The Diagnosis of Acidosis, *J. Lab. & Clin. M.* **4**:315 [March] 1919).

27. Berwick, C. C.: The Disinfection of the Oral Mucosa with Brilliant Green and Crystal Violet, *J. Dental Res.* **2**:21, 1920.

A large ulcer on the posterior pharyngeal wall had remained unchanged for a long time under treatment with ordinary drugs. After three applications of the dye in alcoholic solution at twenty-four hour intervals, granulations formed at the base of the ulcer and it healed in a very short time.

Acute pharyngitis and tonsillar ulcers are likewise benefited by frequent applications of alcoholic solution of mixed dyes.

The following is an outline of procedure for cleaning up gross infections in the mouth preparatory to surgical operations in the mouth, nasopharynx, larynx or trachea. First, the teeth are thoroughly cleansed by carefully brushing all surfaces with a hard, small, baby's tooth brush, using a mixture of equal parts of talc and sodium bicarbonate. Care must be taken not to brush the gums, for then tissue destruction will take place at a faster rate and the infection will be the more difficult to eradicate. This brushing should be done carefully and systematically, taking at least from ten to fifteen minutes to the entire mouth. The mouth is then washed out with an alkaline wash, either a 4 per cent. solution of sodium bicarbonate or the ordinary liquor alkalinus antisepticus solution. Following this, the gums and necks of the teeth should be painted with a 50 per cent. alcohol solution containing 1 per cent. each of brilliant green and crystal violet. Care should be taken to get the dye in between each tooth, and where it may enter the pyorrheal pockets about the necks of the teeth.²⁸

This procedure should be carried out once the first day, twice on the next day, if possible, and once several hours preceding the operation.

This technic has been successfully carried out in many cases of oral and dental surgery, as well as in three cases of laryngectomy and several cleft palate operations.²⁹

After operation in mouth cases, it is of benefit to swab the mouth and wound surface with alcoholic solutions of the dye at frequent intervals. This not only hastens the healing process but also serves to lessen the pain to a great extent, as illustrated by the case herewith reported.

Mr. J. A. P., aged 39, had osteomyelitis of the left mandible. The technic just described was carefully carried out before operation, and at operation the infected portion of the jaw was removed and drained. The wound was dressed

28. Considerable work is still being done to determine the effect of dyes on pyorrheal infections (*J. Dental Research* 2:21, 1920) and we may state only that so far our observations tend to show that the dyes exert their action by merely temporarily lessening the symptoms caused by the infecting organisms. The tendency toward tissue destruction remains more or less unchanged.

29. This technic has been adopted as the standard in the University of California Hospital, standing orders for cleaning up of gross infection in the oral cavity, preparatory to oral and neighboring operations.

daily with gauze packs soaked in 1 per cent. each of brilliant green and crystal violet (aqueous). In six days, there was no more discharge, and the packs were no longer inserted. In ten days the soft tissue had entirely healed over. Roentgenograms later revealed no local pathologic condition.

For rectal cases, this method gives excellent results: Repeated soapsuds enemas are given until they return perfectly clear on the night previous to operation. The operative field, if about the anus, is shaved and painted with 1 per cent. brilliant green and 1 per cent. crystal violet in 50 per cent. alcoholic solution. Then about 20 c.c. of a 1 per cent. aqueous solution of brilliant green and crystal violet solution in aqueous solution is injected into the rectum and sterile dressings applied. On the morning of operation, the injection of aqueous dye into the rectum is repeated and the sterile dressings reapplied. When colostomy has been performed, the dye may be injected into the descending loop of the bowel before performing the Kraske or similar operations.

Postoperatively, the injection of from 10 to 20 c.c. of aqueous solution of dye into the rectum is of great benefit in the healing process, and the alleviation of pain is quite marked in many cases so treated.

Excellent results were obtained in four cases of resection of the rectum for carcinoma as well as in eight cases of hemorrhoidectomy.

Injection of the aqueous solution into rectal fistulas is of benefit in their excision, when they do not heal after repeated injections of dye into them. These fistulas may be easily located at the time of operation by the dark blue-violet color of their walls.

Ischiorectal abscesses, when irrigated with aqueous solutions of dye, heal more rapidly than with other substances. Of three cases so treated, the alleviation of pain was one of the marked features of the treatment, whether it was due to the cutting down of the infection or not could not be definitely determined.

Equally good results have been obtained in the treatment of unopened secondarily infected tuberculous abscesses in the ileopsoas muscle. The abscess cavity is aspirated of its contents by the use of an aspirating needle or small trocar. The cavity is then irrigated with a 4 per cent. solution of sodium bicarbonate until the fluid returns clear. A small amount of the soda solution is left in the cavity in order to maintain its alkalinity, and a small quantity of 1 per cent. aqueous solution of brilliant green and crystal violet is injected into it. The amount injected varies according to the size of the cavity. In a short time (from twelve to forty-eight hours), depending upon the nature of the infection and the size of the cavity, it will be found that the necrotic material is sterile. It then remains to permit the cavity to granulate in, with occasional lavages administered to remove whatever tissue debris remains in the cavity.

This method has been successful in six cases of *Staphylococcus albus* infection and one of *B. coli* infection of deep muscular tissues. In four cases of psoas abscess, the treatment was successful so far as it produced sterile cultures, but it was not possible to get at the source of the infection in the lumbar vertebrae. In three of these cases, the abscess cavity broke through the line of puncture and drained for a long time. The secretion not only was sterile upon ordinary enrichment mediums; but guinea-pig inoculation was negative for tubercle bacilli. These patients all died some time later from pulmonary involvement. No effort was made to ascertain the effect of the dye upon tubercle bacilli either clinically or in vitro, other than the above.

It has also been our observation that dead or necrotic tissue takes a deep stain and maintains it for a considerable period of time. This does not wash off with either alcohol or water to the extent that the dye does from vital tissue. Living tissue does not keep this heavy stain for so long a period of time, but the dye gradually becomes less intense until one can notice no stain remaining. This may be due, as Strasznor²⁴ has shown, to certain sulphhydrid groups inherent in the living cell. This activity is perhaps lost when the tissues undergo the process of decomposition, either by a catabolic or destructive action on the part of infective organisms.

ADVANTAGES AND DISADVANTAGES

The advantages of the use of dyes as antiseptics may be briefly listed thus: (1) specific selective action; (2) nontoxicity to vital tissues; (3) high alcohol and water solubility; (4) great penetration, and, (5) ease of application.

The possible objectionable feature of the dyes as antiseptics is the almost excessive staining quality. Considerable difficulty is experienced at first by those who use the dye, in trying to prevent staining of contiguous clothing and skin when not desired. With experience, however, one accustoms himself to its use, and the staining of other areas becomes less objectionable. This dye may be removed by immediate washing with alcohol, or acid alcohol, or by applications of, first, oxalic acid solution, and then water, repeated alternately until the stain is entirely removed. Hypochlorite solution is also of value in removing these stains. The same is true of the removal of dye from undyed cloth.

SUMMARY

There is a definite place for certain dyes in the treatment of wound infections. These dyes are extremely valuable on account of their selective bacteriostatic action, their low tissue toxicity and their ease of application.