FLAVINE AND BRILLIANT GREEN, POWERFUL ANTISEPTICS WITH LOW TOXICITY TO THE TISSUES:

THEIR USE IN THE TREATMENT OF INFECTED
WOUNDS.*

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The great majority of chemical substances at present employed as antiseptics exhibit other properties which militate against efficient action in the living body. So much is this the case that many observers have come to regard antiseptic power as almost indissolubly associated with a series of objectionable characteristics, which confine the justifiable application of such bodies to within extremely narrow limits. Cushny seems to sum up justly the state of knowledge up to the present regarding these agents in the following statements: "They have no specific action on the microbes, however, but act as general protoplasm poisons, destroying living tissue of all kinds whenever they come in contact with it." "It is important to note that the ordinary antiseptics do not act more strongly on microbes than on the tissues in which they are embedded." "Their action as irritants arises from the same qualities as their disinfectant power—namely, from their general toxicity to living matter—and it is impossible to dissociate the one from the other and to produce non-irritant effective disinfectants." The defects and vices of the antiseptics in common use have been so clearly emphasized of late that it is necessary here only to enumerate the outstanding points, namely:

1. Antiseptics are fixed by protein material in general; hence, when bacteria are suspended in a fluid containing abundant protein, such as serum, very little of the substance is free to act on the organisms. This explains the well-known fact that the antiseptics in common use, almost without exception, show a reduced potency when tested in serum—that is, under conditions resembling those in which they would be used in the treatment of wounds. For instance, the efficiency of corrosive sublimate is reduced one hundred times by serum.

2. By destroying the life of cells and inhibiting phagocytosis, antiseptics deprive the body of one of its most important weapons in combating local infection.

3. By producing layers of dead tissue they supply a nidus for the proliferation of organisms.

4. Owing to their deficient penetrating power they fail to reach organisms situated in the tissues, even supposing that they act efficiently on those disposed on the free surfaces.

With regard to the last point, the question of penetration seems to us to be extremely complicated, and in the present state of knowledge scarcely able to be completely analysed. Speaking generally, two main factors are of practical importance in hindering effective penetration; one is purely physical, depending on the fact that a wound is often divided into loculi, so that even if a solution is poured into the main cavity there is great probability that it will not reach the surface of the remote crevices. The second factor is the fixation of the antiseptic compound by constituents of the tissues, so that at a short distance below the surface a sufficiency of the substance does not remain available to act on the microbes. Beyond these aspects it is scarcely profitable to carry the discussion further at present.

PROPERTIES OF AN IDEAL ANTISEPTIC.

Important properties of an ideal antiseptic would thus appear to be:

1. Great potency against all micro-organisms in the presence of protein material—for example, serum.

2. No deleterious effect on phagocytosis.

3. Absence of irritant action on living tissues in general, so that it may be applied to delicate surfaces such as mucous membranes.

4. A suitable stimulating effect on connective tissue cells, so as to promote growth of healthy granulation tissue.

5. The compound, when absorbed, must not be highly toxic for any specialized tissue; thus, even if strychnine were the most potent antiseptic known, its effect on the central nervous system would probably entirely preclude its therapeutic use.

We have, in the course of the past year and a half, examined an extensive series of substances, comprising the principal antiseptics in common use and also other compounds, some of which have not hitherto been recognized as antiseptics or applied as such. The series includes carbolic acid, mercury perchloride, iodine, hypochlorites (in the form of "eusol" and also Daufresne's modification of Dakin's solution), chlorine water, malachite green, brilliant green, crystal violet, ethyl violet, and flavine. In this work we have directed attention to the following points, which seem to possess special importance in regard to the use of any substance as an antiseptic for therapeutic purposes, when it must act in the presence of living tissues, as in the treatment of wounds.

Bactericidal Potency in Undiluted Serum as Compared with that in Watery Solution.

If serum produces a marked reduction in efficiency, then this indicates that the antiseptic power will be lowered far out of proportion to the degree of mere dilution which must inevitably occur in a wound. On the other hand, should serum fail to diminish the action of the antiseptic or should it actually enhance its effect, then, other things being equal, this will be a valuable advantage of such a substance.

From the results obtained (see table) it will be seen that we have found a compound, flavine, which is distinctly increased in its effectiveness as an antiseptic by the presence of serum, and further, this among the substances tested is the only one of high antiseptic potency which more than maintains its efficiency in serum. Under these conditions, when tested on staphylococci, flavine is about twenty times more powerful than mercury perchloride, and 800 times more powerful than carbolic acid or chloramine-T as a bactericide.

Effect on Phagocytosis.

Many factors operate in order to bring about the healing of a septic wound, in addition to the killing of such organisms as the antiseptic may come into contact with. A chief place in the earlier processes must be assigned to the action of the phagocytes. After making allowance for reduction in potency in the presence of serum and for dilution by the exudate of the wound, any substance which, in order to restrain or kill organisms, requires to be used in such concentration as to seriously damage phagocytic action must be of very doubtful therapeutic value. Accordingly, phagocytic experiments were performed in which leucocytes, normal serum, and staphylococci were mixed with varying concentrations of the antiseptics. From the results (see table) there emerges the fact that germicidal potency and inhibition of phagocytosis do not necessarily run parallel. In the case of each substance we have determined the strength of antiseptic which is required in order to reduce the phagocytic count to 50 per cent. of the control with serum alone; this has been called the concentration which inhibits phagocytosis. A comparison between the bactericidal power in serum and the effect on phagocytosis shows that in the case of all the antiseptics in common use a concentration which is sufficient to cause death of the organisms is also detrimental to phagocytosis. The measurement of antiphagocytic action is obviously an important point, since it enables "general toxicity to living matter" to be estimated to some extent by test tube experiments. Thus carbolic acid both kills organisms and inhibits phagocytosis at a concentration of 1:250 to 1:500, and mercury perchloride exerts both effects at 1:7,000 to 1:10,000. On the other hand, brilliant green kills cocci at 1:30,000 and only inhibits phagocytosis at 1:2,000. Finally, flavine kills both cocci and *B. coli* at a concentration of 1:100,000, whereas in order to affect

* We have much pleasure in acknowledging our indebtedness to the Royal Society's Scientific Grants Committee and to the Medical Research Committee for grants towards the expenses of this work.

† Inman has also recently determined the effect of ethylhydrocuprein hydrochloride on phagocytosis as a factor in relation to its employment as an antiseptic.—*Journal of the Royal Army Medical Corps*, vol. xxvii, p. 500, 1916.

ANTISEPTIC.		STAPHYLOCOCCUS AUREUS.						BACILLUS COLI COMMUNIS.					
		In Water + 0.7 per Cent. Peptone.		In Serum.		Concentration which Inhibits Phagocytosis.	Therapeutic Coefficient.	In Water + 0.7 per Cent. Peptone.		In Serum.		Therapeutic Coefficient.	
		Lethal Concentration.	Antiseptic Potency.	Lethal Concentration.	Antiseptic Potency.			Lethal Concentration.	Antiseptic Potency.	Lethal Concentration.	Antiseptic Potency.		
Chloramine-T (content in "available" Cl = 25 per cent.)	Concentration of substance	1:2000	8	1:250	1	1:625	0.4	1:2000	8	1:250	1	0.4	
	Concentration of "available" Cl	1:8000	32	1:1000	4	1:2500		1:8000	32	1:1000	4		
Eusol (content in "available" Cl = 0.34 per cent.)	Dilution of standard solution	1:13	0.05	1:3.25	0.0125	1:13	0.25	1:13	0.05	1:3.25	0.0125	0.25	
	Concentration of "available" Cl	1:4000	16	1:1000	4	1:4000		1:4000	16	1:1000	4		
Dakin's Solution (modified by Daufresne). (Content in "available" Cl = 0.22 per cent.)	Dilution of standard solution	1:9	0.036	1:2.25	0.01	1:9	0.25	1:9	0.036	1:2.25	0.01	0.25	
	Concentration of "available" Cl	1:4000	16	1:1000	4	1:4000		1:4000	16	1:1000	4		
Chlorine Water	Concentration of Chlorine	1:2500	10	(1:1000 not sufficient)	—	1:1500	—	1:2500	10	(1:1000 not sufficient)	—	—	
Carbolic Acid		1:250	1	1:250	1	1:500	0.5	1:500	2	1:500	2	1	
Mercury Perchloride		1:1,000,000	4000	1:10,000	40	1:7000	1.4	1:1,000,000	4000	1:10,000	40	1.4	
Iodine (in KI)		1:10,000	40	1:700	2.8	1:3500	0.2	1:5000	20	1:800	3.2	0.2	
Brilliant Green Sulphate		1:10,000,000	40,000	1:30,000	120	1:2000	15	1:130,000	520	1:3500	14	1.7	
Brilliant Green Oxalate		1:10,000,000	40,000	1:100,000	400	1:7000	14	1:200,000	800	1:3500	14	0.5	
Malachite Green, Oxalate and Sulphate		1:10,000,000	40,000	1:40,000	160	1:7000	6	1:20,000	50	1:1000	4	0.15	
Crystal Violet		1:4,000,000	16,000	1:400,000	1600	1:7000	57	1:8000	32	1:8000	32	1.1	
Ethylhydrocuprein Hydrochloride		1:660	2.6	1:2000	8	—	—	1:660	2.6	1:660	2.6	—	
Flavine		1:20,000	80	1:200,000	800	1:500	400	1:1300	5.2	1:100,000	400	200	

Lethal Concentration = that which just suffices to kill organisms—as tested by subculture.

Therapeutic Coefficient = Ratio $\frac{\text{Concentration which reduces Phagocytosis by 50 per cent.}}{\text{Lethal Concentration in Serum.}}$

Antiseptic Potency = Ratio $\frac{\text{Lethal Concentration of Substance in question.}}{\text{Lethal Concentration of Chloramine-T in Serum (taken as 1).}}$

Potassium iodide in the concentration employed in the solution of iodine by itself produced no bactericidal action.

"Available" chlorine was estimated by titration with sodium thiosulphate.

EXPLANATION OF TABLE.

The figures shown in the table give the general results of many repeated tests of each substance.

The method of the tests was as follows: The substance to be tested, in a volume usually not exceeding 0.1 c.cm., was added to 1 c.cm. of the culture medium, which consisted in one series of 0.7 per cent. of peptone water and in the other of undiluted serum, and then 0.1 c.cm. of a 1:20,000 dilution in saline of a twenty-four hour peptone water culture was added. A control was made with peptone water or serum without antiseptic; one loopful of this mixture when streaked immediately on agar yielded twenty or more colonies of staphylococcus or *B. coli*. The tubes were then placed at 37° C., and were examined at the end of twenty-four to forty-eight hours in order to determine the concentration of antiseptic which killed the organisms introduced; for this purpose subcultures were made on agar and also in peptone water. The results of both methods of subculture corresponded in general; but it was sometimes found that cultures containing antiseptic, which showed no turbidity after incubation and in which, therefore, little or no multiplication of organisms had occurred, still contained living bacteria.

Ox serum heated at 57° C. was used throughout; experiments were also performed with human serum and rabbit serum. A certain amount of variation was obtained with different specimens of serum; but the results corresponded in general with those quoted in the table. Fresh rabbit serum gave practically the same result as serum heated at 57° C.

B. coli frequently grew very poorly in serum; this difficulty was overcome by adding 0.25 c.cm. of 10 per cent. peptone water to each 1 c.cm. of serum.

In a number of experiments the time rate of sterilization was ascertained by making subcultures from the antiseptic mixtures at intervals of two, five, and twenty-four hours. Mercury perchloride, carbolic acid, chloramine-T, and flavine were compared in this way, and it was found that the maximum action of the first three substances was exerted within a shorter time than was the case with flavine; thus after two hours' exposure the results with mercury perchloride and with flavine were practically equal, and the effect of flavine became enhanced subsequently.

The effect of the presence of red blood corpuscles in the mixture with antiseptic and serum was tested as Emery has suggested. Thus, defibrinated rabbit's blood was employed as the medium, and it was found that staphylococci were killed in defibrinated

blood by flavine 1:40,000, whereas mercuric chloride 1:2,000 failed to sterilize the mixture.

Pus from an empyema, which on centrifuging yielded half its volume of sediment and in culture gave a growth of staphylococci and streptococci, was just sterilized in twenty-four hours by the addition of mercury perchloride to a concentration of 1:1,000; carbolic acid 1:500; brilliant green 1:10,000, and flavine 1:2,000.

The result obtained with chloramine-T indicates a much weaker action than that found by Dakin, Cohen, Daufresne, and Kenyon (*Proc. Roy. Soc.*); the explanation of this lies in the fact that the medium used by us contained a small amount of peptone. We adopted this procedure advisedly, since we found by plating that in the absence of peptone a proportion even of staphylococci might cease to be viable when suspended in salt solution or water for several hours. In control experiments with *Staphylococcus aureus* suspended in water we obtained precisely the same results as Dakin, Cohen, Daufresne, and Kenyon—namely, sterilization after exposure for two hours to chloramine-T in 1:500,000 and survival in 1:1,000,000.

In addition, *Streptococcus faecalis* (enterococcus) and *Streptococcus pyogenes* were tested with mercury perchloride, brilliant green, and flavine; the enterococcus proved the more resistant, and with it the following results were obtained:

	Lethal concentration in Peptone water.		Serum.	
Mercury perchloride	1:200,000	...	1:10,000	...
Brilliant green	1:150,000	...	1:10,000	...
Flavine	1:10,000	...	1:40,000	...

Do Organisms Develop Resistance to Flavine and Brilliant Green?

—Were such resistance to develop to an appreciable extent, as described in the case of trypanosomes by Ehrlich and Browning, and, as Richet suggests, may occur in the case of the organisms in wounds treated by other substances, the value of the antiseptics would be seriously limited. Variations obtained in the sterilizing dose of these substances with the same organism at different times suggested that a certain degree of variation in resistance did exist; but prolonged attempts to produce highly resistant strains by growth in media containing flavine or brilliant green over six months have not led to any remarkable elevation of resistance. Thus, the development of insuscepti-

EXPLANATION OF TABLE (continued).

bility to the antiseptics is not to be feared under practical conditions of wound treatment.

Phagocytic Experiments.—Equal quantities of human serum, washed human "leucocyte cream," emulsion of organisms and appropriate dilutions of antiseptic were mixed together, and then incubated in capillary tubes for twenty minutes at 37° C. At the end of this time the mixtures were spread on slides, stained, and the number of organisms ingested by fifty leucocytes estimated in each case; the control contained normal saline in place of the antiseptic. The serum and corpuscles were on every occasion obtained from the same subject. The

organism employed was a twenty-four hour agar slope culture of *Staphylococcus aureus* emulsified in normal saline to give an homogeneous emulsion of about two thousand million organisms per cubic centimetre; the control then gave an average of twenty-five cocci per leucocyte. The dilutions of the various antiseptics were in all cases made with normal saline. The figures given in the table were confirmed by the results of at least three experiments with each antiseptic; a number of antiseptics were investigated simultaneously so as to avoid attaching importance to any differences in the results due merely to individual variations.

phagocytosis a concentration greater than 1 : 500 is required. Clearly, the higher the germicidal power and the lower the point of phagocytic inhibition the more valuable the antiseptic becomes. In order to furnish a numerical representation of the relationship existing between bactericidal power and interference with phagocytosis in the case of each antiseptic, we have, therefore, calculated the ratio of the one value to the other, which we may call the Therapeutic Coefficient (TC), and by means of this figure we may compare the actual value of different antiseptics as judged from both the above standpoints.* Thus—

$$TC = \frac{\text{concentration of antiseptic which reduces phagocytosis to 50 per cent. of control}}{\text{concentration of antiseptic just sufficient to kill staphylococcus in serum}}$$

If TC is 1, then this means that a concentration of antiseptic which just suffices to kill off the organisms exerts a distinctly harmful effect on phagocytosis. On the other hand, a high value of TC shows that the lethal concentration of the antiseptic has little or no inhibitory action on phagocytosis; therefore, if we find an antiseptic which possesses a high TC value and which at the same time is powerfully bactericidal in the presence of serum, then we know that, as compared with another compound yielding a low TC value, the former substance can be employed in a much higher concentration *qua* bactericidal efficiency, without at the same time damaging the natural defensive mechanism of phagocytosis. As has been seen, flavine satisfies these conditions to a degree far exceeding any of the common antiseptics; brilliant green and crystal violet also surpass these as regards their effect on cocci.

Effect on Tissues.

It must be recognized, however, that the susceptibility of different tissues toward any given chemical compound may vary greatly, just as the susceptibility of different types of organisms toward an antiseptic may show wide variations. The susceptibility of different tissues requires much more investigation, but we considered that a further valuable indication would be afforded by the behaviour of a delicate epithelial membrane, such as the conjunctiva, toward different dilutions of the antiseptics. The results were as follows:

Three or four drops of solution applied to the conjunctiva (rabbit) for three minutes.

Mercury perchloride ...	...	...	(1 : 500 -
Carbolic acid ...	...	...	(1 : 250 +
Chloramine-T ...	...	...	1 : 50 ±
Iodine ...	...	...	1 : 25 -
Iodine ...	...	...	1 : 500 ±
Brilliant green ...	...	...	1 : 1,000 ±
Flavine ...	...	...	(1 : 200 -
Flavine ...	...	...	(1 : 100 +

+ Indicates production of definite irritation.

- Indicates production of no irritation.

± Indicates production of irritation in about half the cases.

Clinical observations on the effect of brilliant green and flavine on other tissues are recorded in the section headed Clinical Observations.

Summary of Results.

Taking the whole of the results in conjunction, the following points emerge: Of all the substances examined, the compound *flavine* stands out as possessing the highest sum of desirable properties for therapeutic purposes—that is, great antiseptic power, along with relative non-toxicity toward phagocytosis and lack of irritating effect on epithelium. The triphenylmethane compounds, especially

brilliant green and crystal violet, are extremely active in the case of cocci, but toward *B. coli* their bactericidal value is considerably lower. On the other hand, flavine is much the most potent of all substances investigated toward both groups of organisms. In the case of *B. coli* suspended in serum, flavine is approached only by mercuric chloride (although the latter is about ten times weaker); but the disadvantage of the mercury salt lies in the serious effect which it has in interfering with phagocytosis. This action of mercuric chloride *in vitro* affords a striking parallel to its well-known toxic effect on living tissues. Thus, as compared with the other substances under examination, a much higher effective concentration of flavine can be brought safely into contact with the tissues; in this way, allowing for dilution in the wound, there is the best prospect of dealing efficiently with organisms which may be present.

Flavine is diamino-methyl-acridinium chloride (see formula), which was originally prepared by Benda at the desire of Ehrlich, and was found to have a very marked therapeutic effect in trypanosome infections. Attention was first drawn by Browning and Gilmour to the powerful action of this substance on bacteria. On account of its trypanocidal action the compound was called *trypanflavine*, but, as its range of use promises to be much wider, we shall refer to it simply as *flavine*. It is a fairly stable substance, and solutions may be boiled or heated up to 120° C. in the autoclave.

The property of exerting a more powerful bactericidal action in serum than in watery solutions has been found by us to reside in a number of acridine dyes analogous to flavine. Colonel Pilcher, D.S.O., has reported that a mixture of such compounds has given very favourable results in the treatment of septic wounds under his care at the Queen Alexandra Military Hospital, Millbank, and this has also been the experience of Mr. Ligat and others in the Middlesex Hospital; the results obtained by one of us (L. H. D. T.), along with Dr. J. Leeming Walker, in the treatment of over two thousand casualty cases in the outpatient department of the Middlesex Hospital are summarized in an appendix. Hitherto the pure flavine has not been available in sufficient amount for extended clinical trial, but while of approximately equal antiseptic power to the other acridine compounds, it is much less harmful to phagocytosis than the latter.† This fact corresponds well with the statement that it is also less toxic than the other acridine compounds in the animal body (see Benda). It can be administered intravenously, and does not exert toxic action on any special organ. Solutions more concentrated than 1 in 1,000 should not be administered, however, owing to the danger of causing agglutination of the red blood corpuscles.

The triphenylmethane group also contains extremely potent bactericidal substances, as Dreyer, Kriegler, and Walker have pointed out in the case of the triamino-triphenylmethane compound methyl violet; the members of this series, *hexamethylviolet* ("crystal violet"), and *hexaethylviolet* are especially active toward staphylococci. Of the diamino-triphenylmethane group *brilliant green*

* This represents an estimation, under quantitative conditions *in vitro*, of certain of the functions of leucocytes, which Colonel Bond has investigated by new and highly ingenious methods *in vivo*.

† The preparation of flavine has been worked out by Drs. Barger and Ewins in the chemical laboratory of the Department of Biochemistry and Pharmacology of the Medical Research Committee, to whom we are indebted for the products. Arrangements have been made for the commercial production of the substance on a larger scale. The products will be tested biologically at the Bland-Sutton Institute of the Middlesex Hospital, and will be available for trial on application to Dr. Browning on behalf of the Medical Research Committee.

satisfies best the desiderata for therapeutic use; malachite green is more inhibitory toward phagocytosis.

Commercial malachite green is commonly the oxalate, and we have found that oxalic acid also when combined with the brilliant green base leads to a compound which is much more harmful to phagocytosis than is the sulphate (commercial brilliant green).

The results show that *iodine* in the presence of serum is an antiseptic of only moderate potency; at the same time, it is markedly inhibitory to phagocytosis and highly irritating.

In this connexion we would note also that the great value of the *chloramine* and *hypochlorite* group of antiseptics in the case of wounds appears to rest to a considerable extent on their action as solvents of necrotic tissue (Dakin, Cohen, Daufresne, and Kenyon), and on their property of destroying toxic substances, as emphasized by Lumière, rather than on their high bactericidal potency, since, as Dakin has also shown, their bactericidal action in the presence of serum is comparatively weak (for example, chloramine-T is 800 times weaker than flavine for staphylococci and 400 times weaker for *B. coli*); at the same time they interfere seriously with phagocytosis.

THERAPEUTIC APPLICATIONS.

One of the foremost necessities of the present time is the furtherance of wound healing by procedures which shall combat or prevent septic infection. It is not proposed to discuss here the general question of wound treatment; there is a great measure of agreement that the removal of dead tissue and the conversion of the wounded area into a free surface by incision and excision are essential factors. Under actual conditions, however, it is seldom practicable or expedient by these means to remove the whole site of bacterial infection; the problem then remains how to overcome as rapidly as possible the persisting bacterial contamination. For the attainment of this object the mechanical detergent action of a more or less continuous flow of fluid plays an important part; this can be accomplished by irrigation with some antiseptic solution, as in Carrel's procedure, or by the lymphagogue action of hypertonic saline solution according to Wright. The advantages which would be gained by the employment, in addition, of an efficient antiseptic are admitted practically by all; but to the mind of many the substances available are so deficient in antiseptic effect and so abounding in harmful action on the tissues that any benefit attending on their use is more than counterbalanced by the harm which they do. Hence appears further the *rationale* of the hypertonic method, which, by inducing a continuous flow of lymph from the vessels, should maintain a supply of substances important for the natural defensive mechanisms of the tissues, while at the same time avoiding the action of chemicals inimical to these processes. Our observations have shown how the manifold effects of chemical antiseptics may to a considerable extent be standardized by experimental methods, and how a basis may be established for comparing the local therapeutic properties of different bactericidal substances. The comparative inefficiency of the common antiseptics under practical conditions has been paralleled by their behaviour in these tests; on the other hand, the acridine compound flavine has exhibited outstanding characters, especially high bactericidal power, which is enhanced rather than diminished by the presence of serum, combined with very little inhibitory effect on phagocytosis. The recommendation of this substance for clinical use, based on the results of the laboratory tests, has appeared to be well justified; a 1:1,000 solution (equivalent on the basis of bactericidal concentration to an 80 per cent. solution of carbolic acid in the case of staphylococci) has been applied to the surface of wounds without causing pain or local irritation, and the skin has not been harmed by its use. In addition, there have been no local or general toxic effects, even when quantities of the solution have been kept in contact with the tissues for a considerable time, or when it has been injected into the tissues. The granulations which form in the presence of the acridine compounds are both of good colour and texture, and the whole process of healing advances rapidly. Especially significant in regard to the

action of these substances has been the retrogression observed in wounds when applications of acridine compounds have been temporarily replaced by some other agent; on reverting to their use the wounds have again become markedly less septic and the advance of healing has been resumed. Leitch has recorded highly satisfactory results from the use of brilliant green in septic wounds, and Captain C. H. S. Webb reports that from his experience at a casualty clearing station he regards brilliant green as greatly superior to any of the antiseptics in common use. A striking feature of this substance is the abundant formation of bright red granulations which follows its use.

Prevention of Sepsis.

We would especially emphasize the possibilities in the direction of prevention of sepsis which would be likely to attend the use of flavine or brilliant green in recent wounds applied during that dangerous period between the infliction of the wound and the establishment of an adequate local defensive mechanism (granulation tissue, etc.). It is precisely at this period that the employment of an efficient antiseptic will prove of value by leading to the destruction of virulent organisms before they have obtained a foothold and have multiplied extensively. The stimulation of tissue cells is a highly important factor at this stage, and there is reason to believe that such stimulation can be attained by the application of flavine or brilliant green. Of course, such treatment is to be used in addition to operative procedures necessary for obtaining free access. It has become the custom at the out-patient department of the Middlesex Hospital to treat all casualty cases by washing out with a 1:1,000 solution of flavine, and the conclusion has been justified that a wound so treated will in all probability heal by first intention (see Appendix). Of course there is a wide difference between such cases and many of the wounds of war, but the results of an extensive series are sufficiently noteworthy to merit their being placed on record. It is not too much to hope that even in severe cases of injury, with extensive and highly contaminated wounds, the onset of the phenomena of sepsis and anaërobie infection may be aborted to a great extent, provided that energetic treatment is begun at the earliest possible moment. We would suggest that in such cases, after suitable surgical procedure and before packing with gauze soaked in flavine, the solution might also be injected into the ends of the injured muscles and into the intramuscular planes and subcutaneous tissues. Such treatment could be best initiated in the casualty clearing station zone.

Flavine in a concentration of 1:1,000 is not precipitated by the addition of NaCl to 5 per cent. strength or 0.5 per cent. sodium citrate, nor by a mixture of hypertonic salt and citrate. Thus this substance is available as an addition to the "physiological" method of treatment.

Antiseptics with the properties of those now described open up many therapeutic prospects, such as the problems of the treatment of intestinal and urinary infections, which are already in course of investigation. We hope also to be able later to offer an explanation of the remarkable effect of serum in enhancing the bactericidal action of compounds of the acridine group.

SUMMARY.

1. A substance belonging to the acridine group, *flavine*, has been found to possess extremely powerful bactericidal and antiseptic properties, which are enhanced rather than diminished by admixture with serum. In this respect flavine differs from all the powerful antiseptics in common use.
2. In the presence of serum flavine is the most potent bactericide of all those investigated for both staphylococcus and *B. coli*, and it is equally efficient for the enterococcus and for anaërobes such as *B. oedematis maligni*.
3. Flavine, in relation to its bactericidal power, is very much less detrimental to the process of phagocytosis and less harmful to the tissues than the other substances; hence much higher effective concentrations can be employed without damaging the tissues or interfering with the natural defensive mechanisms. Brilliant green also compares most favourably with the other antiseptics in these respects.

* We are indebted to the Imperial College of Science for the preparation of a series of salts of the brilliant green base—namely, hydrochloride, mucate, oxalate, tartrate.

4. Clinical results have substantiated the estimate of the therapeutic value of flavine and brilliant green based on the characters above noted.

APPENDIX ON CLINICAL OBSERVATIONS.

During the past fourteen months brilliant green and flavine have been used on a considerable scale as antiseptics in the casualty department of the Middlesex Hospital, and also in a certain number of cases in the wards. Between two and three thousand patients have been treated, approximately half with each substance; there has been no selection of cases.

The cases fall into two main categories—those which already exhibit manifest infection with more or less abundant pus formation; and those which have come up for treatment within a few hours of receiving an injury and before suppuration has had time to occur.

Cases with Suppuration.

These belong to the group of minor affections, such as local abscesses and infections of the hand, which although not menacing to life are often the source of considerable economic loss. Especially in the cases of hand injuries, a method of treatment which preserves the part and hastens on the healing confers a great advantage on the patient. Such conditions appear to respond especially well to these antiseptics.

After free incision, the affected parts have always been syringed or swabbed out with a 1:1,000 concentration of brilliant green or flavine in 0.8 per cent. NaCl solution; the injured area has then been covered with gauze soaked in the solution and a piece of "protective" placed over the whole to prevent evaporation; swabbing or syringing with the antiseptic has been carried out once or twice daily, according to the acuteness of the condition. Between two and three hundred cases of septic hand and finger affections have been so treated, and on the average marked improvement as regards both reduction of inflammatory swelling and discharge of pus has been evident in two days; even in the most severe cases healing has been complete in fourteen days.

Two cases are especially noteworthy as illustrating the efficiency of the treatment, since in both of them the condition was most unpromising, and it appeared inevitable that death of a considerable portion of tissue must ensue. The first was of two weeks' duration, and had been under medical care during that time. The third finger was at least twice its normal diameter, livid, the skin shiny, and with two sinuses on dorsal and palmar aspects. It appeared to us certain that the finger would be lost. Treatment by free incisions and thorough irrigation with flavine reduced the condition, and within fourteen days there was no trace of suppuration, and the finger had resumed its normal size.

In the second case, septic infection of the hand had been under other treatment for three weeks. The hand was as thick as it was broad, and there was a sinus in the palm; this was enlarged, incisions were made on the back of the hand, and flavine was syringed in twice a day, followed by boric fomentations. Within three days the hand resumed its normal size; within ten days all pus had disappeared, and the infected areas had granulated up.

Results of treatment of large superficial infected wounds have been highly satisfactory, the noteworthy points being the very rapid formation of healthy granulations and also the great rapidity with which the epidermis covered the area. Thus, a large raw surface left after removal of a breast became infected, the whole surface discharged abundant pus and showed much sloughy tissue. The area was swabbed with brilliant green 1:1,000, and kept covered with gauze soaked in this solution. Six days' application led to a clean surface, covered with bright red granulations. The green was then no longer applied and dry dressing was used. Within three days the surface was as septic as before. Creolin fomentations were then used for four days, but no improvement resulted. Green was again applied, and one week's treatment sufficed to again clean the wound, which subsequently healed satisfactorily.

Where brilliant green or flavine is used the granulations which form are of healthy appearance and very rapid growth, the former producing granulations of an extremely exuberant bright red type, while with flavine they are not so bulky and of a pale pink colour. This contrasts with the grey sodden character of the granulation tissue which has often been observed to follow prolonged use of the hypochlorite solutions. With both flavine and brilliant

green healing is very rapid, and in our opinion requires only about half the time which might be expected with other methods of treatment.

Four infected amputation stumps have been treated with brilliant green. In these some infection of the bone has always been present. With one exception, all the cases have granulated up within a month, separation of sequestra being notably rapid. The exception was an old man whose feeble state made it unlikely that any marked tissue reaction would be obtained. He eventually died after further operative procedures had failed.

We have seen no marked benefit in localized peritonitis following appendix abscess, and in tuberculous infections of joints and cold abscesses which had become secondarily infected. In both of these conditions we have washed out once daily, in some cases with brilliant green, in others with flavine, but although the discharge has usually been greatly lessened for a few days, there has on the whole been no very definite permanent improvement. There is always the doubt as to whether the antiseptic obtains free access to all the crevices of such abscess cavities, and it is evident that more continuous application of the antiseptics will be required before coming to a conclusion as to their action in this type of case.

As regards toxicity, we have never observed any ill effects following the use of these substances. A boy with subperiosteal abscess of the femur was treated with brilliant green after incision had been made down to the bone and pus evacuated. For four weeks about four ounces of brilliant green 1:1,000 were poured daily into the wound, and left in contact with the soft tissues. No harmful effect of this procedure was observed either generally or locally, and eventually the sinus granulated up completely. Unfortunately the medullary cavity of the bone had not been laid open at the operation, and the infection spreading along within the bone led to death.

In the case of flavine, its low toxicity is well shown by the fact that 300 c.cm. of a 1:1,000 solution has been injected intravenously into a man without any ill effect. It has commonly happened that 5 or 10 c.cm. of 1:1,000 flavine have been injected directly into the tissues—subcutaneous tissue, muscle, etc.—for prophylactic purposes in cases of recent injury. We should not at present, however, recommend the introduction of brilliant green into closed spaces.

Cases Treated before Suppuration had Occurred.

Neither of these antiseptics causes pain when applied to cut surfaces. In the case of brilliant green, some slight irritation of the skin around a wound has been observed after use over a period of fourteen days or more. This has never occurred in the case of flavine. As regards prophylactic use, we have made it our custom to swab out every recently inflicted wound brought into the casualty department with flavine or brilliant green. Many of the injuries thus treated were heavily contaminated with road dirt, hair, clothing, etc. We have observed that, almost without exception, wounds so treated may be closed by suture and confidently expected to heal by first intention.

Especially noticeable has been the success obtained with scalp wounds, of which we have now treated some thirty without any septic infection resulting, healing always taking place by first intention. One such case stands out as worthy of mention. It was that of a man injured in a street accident; a piece of scalp the size of the palm of a man's hand being stripped up and the wound heavily contaminated with mud and hair. It was thoroughly swabbed out with flavine, about an ounce being left in the cavity, and sutured by eighteen horsehair sutures, no drainage being allowed for, and a pad of gauze soaked in flavine applied. After the third day all inflammatory swelling had subsided, no signs of suppuration were present and the edges of the wound were in firm apposition. On the fifth day all eighteen sutures were removed; firm union had taken place, and the patient was discharged.

These results are quite in line with those obtained by treatment of similar conditions in other parts of the body. We consider that less than 1 per cent. of the minor cuts and wounds sufficiently severe to necessitate suture proceed to suppuration when treated promptly in this manner. During the past six months we have seen some half-dozen cases of minor wounds, such as those above described, which

have suppurated after receiving similar prophylactic treatment with iodine. The number of cases in which iodine has been used has been small, considerably less than one hundred.

Several more severe cases, such as compound fractures, have been similarly treated with brilliant green or flavine, and the results have in all cases been equally satisfactory. Of course, free access of the antiseptic to the injured parts is essential.

From the above considerations it is our definite opinion that the results of treatment of septic conditions with flavine and brilliant green show a marked superiority over those obtained by other methods in common use. We consider that the duration of such infections is in general reduced to at least a half, that the stimulus to connective tissue to form granulations is a very outstanding feature, and that these antiseptics can be applied in considerable amounts with the minimum of interference with the normal tissue functions.

We would most particularly emphasize the important advantages to be gained by the prophylactic use of flavine in all injuries of recent origin before there has been time for the evidence of infection to appear, and we believe that almost all such injuries will heal by first intention provided thorough cleansing on these lines be employed.

As regards the comparative merits of brilliant green and flavine, we consider that the latter is both the more efficient and the more rapid in its action. Especially is this the case in infections accompanied by considerable inflammatory swelling; it is not uncommon to find that the part has resumed its normal shape and size after twenty-four hours' treatment with flavine, whereas with brilliant green the process is more gradual. Flavine has the added merit of being highly antiseptic toward all the common pyogenic organisms, whereas we have seen cases of *B. coli* infection which have proved intractable to brilliant green. Brilliant green stimulates the formation of exuberant but well vascularized granulation. This is a useful property, and suggests that brilliant green might be advantageously employed in order to promote the filling up of large cavities; but we have found that the final result is as satisfactorily and as rapidly attained by the use of flavine.

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FLAVINE AND BRILLIANT GREEN IN THE TREATMENT OF INFECTED WOUNDS.

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THE sharp division of opinion which exists as to the advisability of employing antiseptics in the treatment of infected wounds is probably to be explained by the fact that most of the substances in general use fall far short of the ideal in their therapeutic action. Either they are highly irritating to the tissues, or they are rendered practically inert owing to admixture with the secretions of the wound. Any substance which would act as a powerful bactericidal agent in wounds, and which at the same time would neither damage the reparative powers of the fixed tissue nor inhibit normal protective functions, such as phagocytosis, would satisfy a felt want in wound treatment.

The recent investigations carried out in the Bland-Sutton Institute of the Middlesex Hospital by Browning, Gulbransen, Kennaway and Thornton have indicated that there are chemical compounds with powerful antiseptic action which possess the qualifications for therapeutic use

in a degree greatly exceeding those commonly employed. Thus, the acridine compound flavine and also brilliant green are very strong antiseptics; at the same time, concentrations which are powerfully bactericidal, do not harm the tissues or interfere with phagocytosis. A special feature of flavine is that its action suffers no weakening in the presence of serum, and it is powerfully bactericidal against all the common pathogenic organisms—for example, staphylococci, streptococci, both of the *pyogenes* and enterococcus types, and *B. coli*.

For over a year I have employed flavine compounds and brilliant green in septic cases under my care in the Middlesex Hospital. Upwards of 150 cases have been treated, about 50 per cent. of these being war wounds. They may be divided into the following groups: (1) Wounds already thoroughly septic, showing an abundant discharge of pus. (2) Injuries newly inflicted which have not yet had time to suppurate.

In the case of suppurating wounds the procedure followed has been to secure adequate drainage by free incision when necessary, and then to irrigate with a 1:1,000 solution of the antiseptic in normal saline; finally the wound is covered with gauze soaked in the solution, and protective applied to prevent evaporation. Where there has been a cavity it has been packed lightly with gauze soaked in the solution.

As to the results seen by this method of treatment, a considerable diminution in the discharge of pus may be expected at the end of twenty-four hours. In wounds of moderate severity in which no foreign body is present and in which free drainage is secured, the suppurative process may be expected to come to an end after four or five days' treatment. Any difficulty in obtaining free access for the antiseptic to the infected area was overcome as far as possible by syringing the solution into the wound cavity under slight pressure, and in some cases flavine 1:1,000 was injected into the tissues and muscle planes by a cannula; no subsequent damage to the parts was observed.

A noticeable feature in the use of these antiseptics is the stimulus to early formation of granulation tissue. Within forty-eight hours the more superficial parts of the wound begin to show small firm granulations, and by the end of a week the whole wound may be expected to be lined by granulations. In the case of brilliant green these are large, nodular, and bright red in colour, while after the use of flavine they are small and of a healthy pink colour. In both cases they are firm, do not bleed readily, and grow very rapidly. Concurrently with this the epithelium grows over the raw area, and almost from the first day may be seen creeping in from the edges of the wound. It is my experience that actual healing of the wound is markedly expedited under this treatment, the majority of cases requiring only about half the time to be anticipated by other methods.

This was well illustrated by a case of nephrectomy. An infected kidney was removed, a large drainage tube inserted, and the wound left open; secondary infection of the wound cavity followed, with very copious discharge of pus. Creolin fomentations were applied for four days, but no improvement resulted. Treatment was then carried out with brilliant green, the wound being washed out and packed with gauze soaked in the antiseptic twice a day. In four days all discharge had ceased and bright red granulations were visible covering the infected surface. The cavity, which was originally large enough to contain a man's fist, completely filled in by granulation within three weeks.

On several occasions I have been able, after treatment with flavine, to undertake successfully secondary operations on infected wounds.

A case of gunshot wound of the arm necessitated operation in France, a circular amputation being performed, and no skin flaps provided. When the case came under my care the stump was heavily infected. Treatment with flavine for one week sufficed to remove all discharge; I then reamputated immediately above the old stump, cutting skin flaps from tissues which had previously been infected; the stump has since healed healthily without suppuration.

In another case the leg had been removed elsewhere, and the patient came under my care with a very septic stump. It had been treated with eusol for three weeks, but showed no definite improvement in the suppurative condition; a few granulations were present at the edges,