the clinical department. Whether on this side or on that, a beginning means much and could not fail to issue in larger ambitions.

Again, the clinical laboratory ought to be made a centre of practical interest to the practitioner, and the ideal scheme would enable him to attend in order actually to see for himself the results of the tests applied in connexion with cases for which he was responsible. Too many of us are content in this matter to accept reports for which we can supply no personal verification, and it may perhaps be asked whether such a proceeding is capable of ethical defence, seeing that in the relation between practitioner and patient there is implied on the part of the practitioner the guarantee of a personal responsibility.

A third possibility of the establishment of an organic union between the local hospital and the neighbouring profession would be the institution of a medical reference library with obvious advantages to all concerned. There exist a number of admirable atlases illustrative of various departments of clinical work, but their expense forbids their wide distribution, and they remain of little general service. To be effective these illustrations need to be displayed, and a clinical picture gallery constructed from these and other sources, and in every hospital, would mean within reach of the majority of practitioners an attractive and stimulating centre for the diffusion of clinical knowledge. Hospitals are usually very keen, and rightly so, on the creation of a pathological museum. But the pictorial side of clinical work has little recognition paid to its educational values, and many contributions of this order, save for some special reference, remain isolated from the interests they are well fitted to serve. The creation of an opportunity for their exhibition widely throughout the profession would be an educational advance of high merit.

Such, then, are some of the developments which might be expected were it once recognized that our hospitals ought to be, not merely centres of benevolence, but also centres of organized clinical enterprise and progress in which members of the local profession would find their share, their interest, and their pride. Before such a position can be reached there must probably be an educational campaign directed both to the profession and to the lay public, and especially perhaps to those among the latter who form the governors of our hospitals. We have recently had a demonstration of what can be secured when our hospital authorities are convinced that the institutions over which they preside have large public and educational interests to serve, and no one now objects to the institution of special hospital clinics for the treatment of diseases which but yesterday were held to be outside the range both of sympathy and of help. It can hardly be doubted that a similar welcome would wait on such a scheme as is here proposed when the responsible authorities were satisfied, as they surely could be, that its adoption would mean both an increased area of hospital usefulness and a greater measure of helpfulness for the individual patient.

I now go further and say that along such lines as are here defined may be found a large contribution to a question of the highest importance—namely, the provision of ready opportunities for post-graduation medical education and study. Most of us in our younger, more flexible, and more impecunious days—that is, in the days when we most need such opportunities—cannot afford to leave our practices for a number of weeks and months to visit one or other of the great educational centres; and even at the best the necessary brevity of such visits puts an inevitable limitation on their values. If post-graduation medical education is to become effective and widely spread, it must offer itself in more sustained opportunities and must secure centres not very far away from the scene of the practitioner's daily duty. My contention is that the local hospital ought to be such a centre, and its wide and continuous service makes it all the more appropriate to this end, seeing that the aim of post-graduation study is not to tempt the practitioner to forsake the wide horizons of general practice for some narrow and restricted sphere of work, but to raise the all-round proficiency of those who are the chief agents for carrying the help of medicine into the homes and lives of the people. The benefits of such an arrangement would not be all in one direction. On the contrary, the scheme here outlined would not only make available larger and more systematized clinical opportunities to the great body of the profession, but it would exercise a helpful and stimulating pressure on our hospital staffs. It would urge them both to raise their routine work to the highest possible level and also to equip themselves with the latest developments of clinical doctrine and practice and to be agents for the communication of these to their professional brethren. Related to this is the suggestion that all such educational schemes should include opportunities for lectures or demonstrations by those whose original work had become wedded to clinical service as well as by others who had attained a recognized position of professional leadership and authority. These ends are perhaps for the fullness of time, but an opportunity for beginnings is ready to hand and wisdom will not despise it.

SUMMARY.

To sum up my suggestions in brief form I would say: (1) That the general efficiency of the profession may be promoted by the organization of co-operative clinical study; (2) that for such organization appropriate centres already exist in the shape of our local hospitals and kindred institutions; (3) that an effective scheme of this order demands the co-ordination of the work of the hospital with the interests of the local profession; and (4) that by movement in this direction are to be secured ready, sustained, and distributed opportunities for the cultivation and development of post-graduation medical education and study.

I submit these suggestions with great respect to the attention of the Branch.

THE ANTISEPTIC PROPERTIES OF ACRIFLAVINE AND PROFLAVINE, AND BRILLIANT GREEN;

*WITH SPECIAL REFERENCE TO SUITABILITY FOR WOUND THERAPY.**

BY

C. H. BROWNING, R. GULBRANSEN,

AND

L. H. D. THORNTON.

(From the Bland-Sutton Institute of Pathology, the Middlesex Hospital.)

(*A Report to the Medical Research Committee.*)

In view of the attention which has recently been directed to the employment of "flavine" compounds and other basic benzol derivatives in the treatment of infected wounds, it appears desirable that certain features of these bodies, and especially of the flavine group of antiseptics, should be dealt with in greater detail than hitherto, in order that those who intend to employ these substances clinically may have more fully at disposal the indications for their use afforded by laboratory investigations. Subsequent trials along similar lines have fully substantiated the reports already published on the use of these antiseptics, but it is not proposed at present to enter further into clinical results until continued tests on an extensive basis enable a decision to be reached as to the best mode of application and range of usefulness. The powerful bactericidal properties of acridine dyes were pointed out by Browning and Gilmour, who also investigated brilliant green and other triphenylmethane compounds.† They observed in the case of acridine compounds that, in marked contrast to most other antiseptics, their action was enhanced by the presence of serum. With the exception of carbolic acid, we do not know of any other antiseptic which is not greatly weakened in its action by admixture with serum, nor are we aware that this property has been claimed for any powerful bactericidal substance. A further study of compounds belonging to this group was undertaken with a view to determining their suitability for therapeutic use in infected wounds (Browning, Gulbransen, Kennaway,

* We have much pleasure in acknowledging our indebtedness to the Medical Research Committee for a grant toward the expenses of this work.

† These are all "elaborate compounds of which most are brilliantly coloured and are used as dyes"; such characteristics are inseparable from the compounds in question, but they have been suggested for use clinically on account of outstanding properties which are likely to render them valuable in the treatment of infected wounds—at present so urgent a problem—and which are not shared by any colourless substances thus far known.

and Thornton). The substances investigated included 3.6-diamino-acridine derivatives with substituted methyl groups in the amino side-chains, or in the benzol rings, or in both positions; also the unsubstituted 3.6-diamino-10-methyl-acridinium compound—"flavine," now called "acriflavine"—prepared by Benda for Ehrlich, and originally named "trypaflavin," on account of its therapeutic action in trypanosome infections.*

The term "flavine" compounds will continue to be applied to the acridine group of compounds as designating them in relation to their use as antiseptics. The results of clinical trials in the case of acriflavine and allied flavine bodies have been reported by Colonel Pilcher, D.S.O. (see Browning, Gulbransen, Kennaway, and Thornton, also James), and by Ligat, and Thornton and Walker from the wards of the Middlesex Hospital. Since the publication of the above work the unsubstituted 3.6-diamino-acridine sulphate ("proflavine")† has also been examined more completely than had been done in connexion with the base by Browning and Gilmour.

It was found that while, in the case of all the diamino-acridine compounds tested, the antiseptic effect was enhanced by the presence of serum, especially for *B. coli*, diamino-methyl-acridinium chloride ("acriflavine"), and diamino-acridine sulphate ("proflavine") exerted the least degree of inhibitory effect on phagocytosis.

The effect on phagocytosis was in the first instance tested according to Wright's method, whereby 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; a number of antiseptics were investigated simultaneously so as to avoid attaching importance to any differences in the results due merely to individual variations.

Observations made by Colonel C. J. Bond on the effect of these substances (acriflavine and proflavine) on leucocytes *in vivo* have yielded similar results to our own.‡ He estimates the influence of antiseptics on leucocytes in two ways: (1) By dressing a wound with gauze soaked in the antiseptic with protection against drying; at the next dressing the leucocytes entangled in the gauze are examined in normal saline with reference to their capacity to phagocytose starch granules previously dusted on the wound. Dead leucocytes lose this property. Or the pus cells from such a wound may be incubated with carmine granules in a plasticine cell. Colonel Bond finds that in spite of slight damage leucocytes in pus from a wound irrigated or dressed with acriflavine are capable of ingesting pigment granules when incubated. (2) A drop of blood from the finger is mixed with antiseptic in saline solution and then incubated in a hermetically sealed chamber. The leucocytes which emigrate from the clot and collect on the slide are gently washed in saline and then treated with 1 per cent. iodine solution, and their capacity to give the "iodophil reaction" is so tested. Any definite degree of damage to the vitality of the leucocytes hinders the elaboration of "iodophil substances" during the period of incubation. Hence the presence of the latter may be taken as a delicate method for detecting injury to the white cells. The "iodophil reaction" was obtained to a considerable degree with acriflavine and proflavine 1 : 1,000 in normal saline when employed in this manner.

These compounds are comparatively little toxic for the body as a whole, and although proflavine is slightly the more inhibitory towards phagocytosis, its general toxicity for mice, as tested by subcutaneous injection, and also the irritating effect of concentrated solutions on the conjunctiva, are markedly less than that of acriflavine. The bactericidal concentration of proflavine is practically the same as that of acriflavine—that is, for *Staphylococcus aureus* and *B. coli* in serum 1 : 100,000 to 1 : 200,000. In the case of streptococcus (*pyogenes* and enterococcus) and the bacillus of malignant oedema it is also equal to acriflavine. The concentration which reduces phagocytosis of *Staphylococcus aureus* to 50 per cent. of the control is 1 : 500 in the case of acriflavine, and proflavine is only slightly inferior in this respect. The concentration of proflavine which produces slight irritation of the conjunctiva (rabbit) is 1 : 50 as compared with 1 : 150 in the case of acriflavine when applied for three minutes. The methods of test were those previously described.

The Parts Played by Phagocytosis and Antiseptics in Overcoming Local Infection.

Recent observations of Bond, Rous and Jones, and others, suggest that organisms may be protected rather than destroyed as a consequence of being ingested by leucocytes. This may bear somewhat the same relationship to the cellular aspect of immunity which anaphylaxis does to the humoral, but in view of Metchnikoff's classical observations it is difficult to relinquish the conclusion that phagocytosis is, in general, part of an important defensive mechanism. We have extended our previous work by studying the effect of prolonged contact of a solution of antiseptic in serum on the phagocytic power of leucocytes, when subsequently staphylococci are added. The mixtures of one volume each of "leucocyte cream," serum, and antiseptic solution were incubated in capillary tubes at 37° C. for two hours. The control contained saline instead of antiseptic. At the end of this time one volume of staphylococcus suspension was added, and after thorough mixing the whole was again incubated for twenty minutes in order to permit of phagocytosis occurring. Films were then made in the usual fashion. It was found on comparing in this way the flavine compounds with mercuric chloride that 1 : 10,000 of the flavine antiseptics, after two hours' contact with the leucocytes at 37° C. had little effect on the phagocytic power, whereas this concentration of the mercury salt reduced the phagocytic count to below 50 per cent. of the control. Now it is to be remembered that, so far as bactericidal action is concerned, 1 : 10,000 represents practically the limiting concentration of mercuric chloride, any further dilution of which with serum abolishes antiseptic action; on the other hand, such highly bactericidal concentrations of the flavine antiseptics after prolonged contact leave the leucocytes still capable of phagocytic action. Accordingly, both the flavine compounds are recommended for trial in the prevention and treatment of septic infection in wounds.§

Properties Desirable in a Therapeutic Antiseptic.

The question as to what constitute the most advantageous properties of an antiseptic depends almost entirely on the particular purpose for which it is used. Thus, the sterilizing of material outside the human body, as in disinfecting garments, instruments, etc., is a matter entirely different from sterilizing a wound; in the former practically the sole necessity is to destroy the organisms, but in the latter the properties of the antiseptic must be so adjusted as to ensure efficient action on the bacteria while at the same time giving rise to a minimum of tissue destruction or interference with those protective and proliferative functions upon which healing depends. A cogent illustration of the point in question is the harmlessness of flavine compounds when solutions of 1 : 1,000 strength are brought into contact with the peritoneum. It appears most likely that in wounds, especially when treated early, actual destruction of the bacteria through the sole agency of the antiseptic is not essential, it being necessary merely to ensure that no significant multiplication of the implanted pathogenic organisms takes place; in other words, a concentration powerful enough merely to inhibit proliferation

* In our previous work the abbreviation "flavine" was applied to the latter compound, but since this name might give rise to confusion owing to its use in connexion with other substances in commerce, the product here referred to will in future be called "acriflavine," as suggested by the Medical Research Committee.

† The specimen of this substance required for our investigations was prepared by Drs. Barger and Ewins in the Department of Biochemistry and Pharmacology of the Medical Research Committee, and we have pleasure in expressing our indebtedness to them for the valuable facilities which were thus placed at our disposal. The name "proflavine" has been suggested to us by the Medical Research Committee. As it is in the form of a salt (sulphate) that 3.6-diamino-acridine is recommended for use the name is, therefore, to be applied to the sulphate.

‡ We are indebted to Colonel Bond for permission to record these results (see also BRITISH MEDICAL JOURNAL, July 7th, 1917).

§ Proflavine represents an earlier stage in the production of acriflavine, hence it would be cheaper in use. The solubility of proflavine is similar to that of acriflavine in normal and hypertonic NaCl solution, and along with 0.5 per cent. sodium citrate; the addition of alkali to the neutral solution must be avoided, however, in the case of proflavine, as this causes precipitation. Clinical observations indicate that proflavine has haemostatic action.

is sufficient. The efficient prophylactic effect of flavine compounds in the treatment of contaminated wounds in civil practice before suppuration had set in bears out this view. Accordingly, it is far preferable to succeed in inhibiting organisms in a wound, while at the same time avoiding damage to tissues or diminution in their resistance, than to aim at obtaining bacterial sterility by means of reagents which produce necrosis and throw out of action protective mechanisms such as phagocytosis. Although for sterilizing instruments, etc., the more rapidly an antiseptic works the better, for wounds it is equally efficacious, from the point of view of healing, to destroy the organisms in ten minutes or in ten hours, provided that their virulence is efficiently controlled.

In connexion with the use of "bacterial charts," on which Carrel relies as a criterion for basing the prognosis of behaviour in a wound destined for suture which is undergoing treatment by frequent periodic flushing with hypochlorite solution, it must be remembered that diminution in numbers of the organisms is considered in this case to be practically synonymous with loss of power to cause pathogenic action. The fact that the effects which follow inoculation with bacteria depend very largely on the size of the dose is, of course, well established; but it is also, in general, impossible to determine by microscopic inspection the virulence of a given organism. Accordingly, the evidence accumulated by the bacterial charts must be clearly understood so far to apply under a special set of circumstances. There is no proof that the standards established in connexion with hypochlorite solutions will apply precisely to wounds treated by other antiseptics. Information on this point in connexion with flavine compounds and other substances must be interpreted without bias derived from a knowledge of the significance of the findings under the particular conditions studied by Carrel.

The Rate of Progress of Sterilization.

In view of these considerations as to what an efficient therapeutic antiseptic should accomplish, it appeared unnecessary that for therapeutic purposes in wounds there need be great rapidity of the lethal action exerted by antiseptics such as the flavines, which, so far from suffering diminution in potency through admixture with serous secretions, are most active in the presence of serum, and hence must continue to act for a long period, and which, in comparison with their bactericidal potency, are relatively harmless to the tissues. Dakin and his co-workers would also seem not to regard great rapidity of action as essential in antiseptics to be used for treatment, since they adopted two hours as the duration of contact between antiseptic and organisms in their experiments. Many observers, however, following more or less the classical procedure advocated by Rideal and Walker, estimate the bactericidal value of a compound according to the effect produced by contact of the antiseptic with the organisms for a comparatively brief period—up to fifteen minutes. It is difficult to determine from the results of such short contact experiments what conclusion, if any, may be drawn regarding the action of greater dilutions acting over longer periods, as would almost necessarily be the case in wounds unless the antiseptic is immediately fixed or inactivated.

Browning and Gilmour, following the procedure of Churchman, originally tested the antiseptic power of a substance by incorporating it in melted agar medium, and then, after cooling, they inoculated the surface by stroking with a loopful of a dilute suspension of a 24-hour culture. In later work the method adopted by us was to test the power of inhibiting growth and killing the organisms in fluid medium.

The substance to be tested, in a volume usually not exceeding 0.1 c.cm., was added to small test tubes containing 1 c.cm. of the culture medium, which consisted in one series of 0.7 per cent. 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 peptone water culture was added. A control was made with peptone water or serum without antiseptic: one loopful of this mixture when stroked 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; the development of turbidity, of course, indicated the occurrence of definite proliferation of the bacteria, but subcultures were made also on agar and 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.

We selected the quantity of bacteria employed for the inoculation dose because, when added to the standard volume of fluid used in our tests (1 c.cm.), a loopful yielded a convenient number of colonies for estimating subsequent increase or decrease. The impression may arise, however, that this minute quantity was employed because the flavine antiseptics could not withstand a test involving the rigour of a larger inoculation. Hence we add the following experiment:

ESTIMATION OF BACTERICIDAL CONCENTRATION OF ACRIFLAVINE FOR B. COLI IN OX SERUM (57° C.).

The tests were carried out as previously described; but three parallel series were set up, which differed only in the amount of organisms used for inoculation. Of a twenty-four hour peptone water growth of *B. coli* (Escherich) each tube (1 c.cm. volume) received respectively, in Series A, 0.1 c.cm. 1 : 20,000 dilution; Series B, 0.1 c.cm. 1 : 1,000; Series C, 0.1 c.cm. undiluted culture. The mixtures were placed at 37°, and subcultures made on agar at intervals. The controls contained no acriflavine.

TABLE I.

Time of Subculture.		Dilution of *B. coli* Culture used for Inoculation. A. 1 : 20,000.	B. 1 : 1,000	C. Undiluted.	Controls.
At once ...	+	1 : 100,000	1 : 10,000	1 : 10,000	15 colonies in loopful from Series A; ∞ from C.
	−	?	?	?	
2 hours ...	+	1 : 100,000 D	1 : 40,000 D	1 : 40,000 D 1 : 10,000 D	No increase as compared with "at once."
	−	?	1 : 20,000	?	
5 hours ...	+	1 : 100,000 D	1 : 100,000 D	1 : 40,000 D	Increase.
	−	?	1 : 40,000	1 : 20,000	
24 hours ...	+	1 : 400,000 D	1 : 400,000 D	1 : 100,000 D	Increase.
	−	1 : 100,000	1 : 40,000	1 : 40,000	

+ = Growth. − = No growth. D = Decrease in number of colonies as compared with control made from same series "at once." ? = Concentration not determined.

Thus, a twenty-thousand-fold increase in the inoculating dose causes only about a two-and-a-half-fold increase in the amount of antiseptic necessary to produce sterilization.*

Experiments on the *rate of progress of sterilization* were carried out as an essential part of the work preliminary to our first report and the results were briefly referred to, but it appeared then to be unnecessary to enter into details on this point. It seems, however, that observers may experience some difficulty in dissociating the conception of *potency* of antiseptic and bactericidal action from that of *rapidity* of sterilization. Further, sufficient emphasis, perhaps, has not been attached to the fact that, in order to secure the best performance of the flavine antiseptics, it is essential that they should act in a serous medium. Therefore the experimental results are now published more fully—the method of the tests was that already described. The progress of sterilization has been investigated by making subcultures from the antiseptic mixtures at intervals of two, five, and twenty-four hours. The subcultures were made by inoculating a loopful of the mixture into 10 c.cm. of peptone water, and also by stroking agar and then incubating for forty-eight hours at 37° C. The following table (II) gives the results of representative experiments.

Thus, it is clear that, as compared with the other substances tested, the maximum bactericidal action of the flavine compound is developed comparatively slowly (proflavine behaves similarly), whereas mercury perchloride, chloramine-T and phenol, all exert their maximum effect within two hours and no significant change occurs subsequently. The flavine compound has by that time exerted only a restraining effect on the multiplication of organisms in the higher dilutions, which will become sterile later on; this is shown by the relative scantiness of growth when a subculture is made on agar from the tubes containing those particular concentrations. The progressive action of the flavine compound is evident both in the watery (peptone)

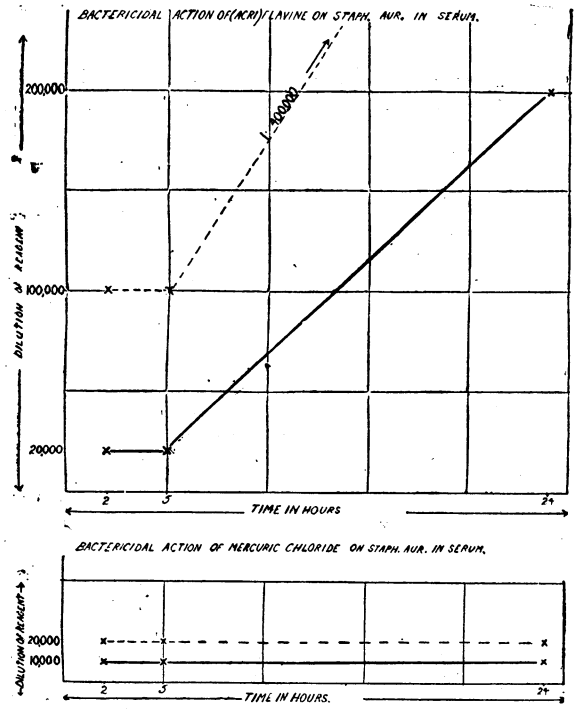
* Similar results were obtained with *Staphylococcus aureus*, but with the heavy inoculation distinct proliferation preceded the lethal action.

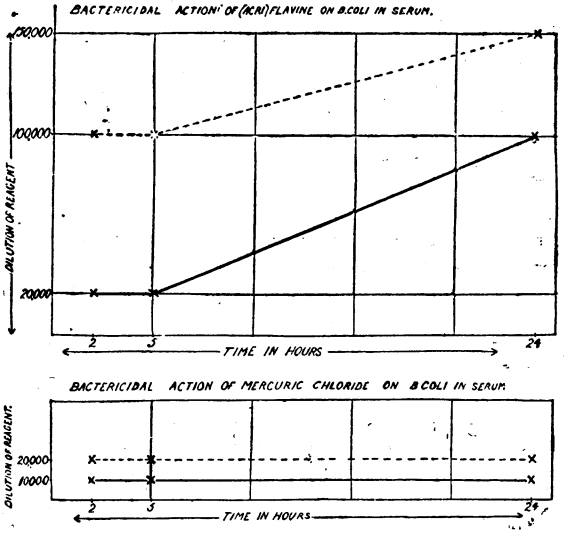
TABLE II.—*Rate of Progress of Sterilization with Flavine Compounds and Other Antiseptics.*

Time in Hours.	Acriflavine.		Chloramine-T.		Mercury Perchloride.		Phenol.	
	0.7 per cent. Peptone Water.	Serum.	0.7 per cent. Peptone Water.	Serum.	0.7 per cent. Peptone Water.	Serum.	0.7 per cent. Peptone Water.	Serum.
B. coli.								
2 hours ... ... { +	1 : 1,000	1 : 100,000	1 : 10,000	1 : 5,000	1 : 4,000,000	1 : 20,000	1 : 500	1 : 500
2 hours ... ... { −	?	1 : 20,000	1 : 5,000	1 : 1,000	1 : 2,000,000	1 : 10,000	?	?
5 hours ... ... { +	1 : 2,000	1 : 100,000						
5 hours ... ... { −	1 : 1,000	1 : 20,000						
24 hours ... ... { +	1 : 2,000	1 : 150,000	Ditto as above	Ditto as above	Ditto as above	Ditto as above	Ditto as above	Ditto as above
24 hours ... ... { −	1 : 1,000	1 : 100,000						
48 hours ... ... { +	1 : 10,000	1 : 1,000,000						
48 hours ... ... { −	1 : 2,000	1 : 200,000						
Staphylococcus aureus.								
2 hours ... ... { +	1 : 10,000	1 : 100,000	1 : 10,000	1 : 1,000	1 : 1,000,000	1 : 20,000	1 : 250	1 : 250
2 hours ... ... { −	?	1 : 20,000	1 : 5,000	1 : 500	1 : 660,000	1 : 10,000	?	?
5 hours ... ... { +	1 : 10,000	1 : 100,000	1 : 10,000	1 : 500			1 : 500	1 : 250
5 hours ... ... { −	?	1 : 20,000	1 : 5,000	?	Ditto as above	Ditto as above	1 : 250	?
24 hours ... ... { +	1 : 100,000	1 : 400,000	1 : 10,000	1 : 500			1 : 500	1 : 500
24 hours ... ... { −	1 : 20,000	1 : 200,000	1 : 5,000	?			1 : 250	1 : 250

\+ = Growth in subculture in peptone water. − = No growth. ? denotes that the lethal concentration was not determined.

medium and also in serum. The following graphic representations (all to the same scale) of the results in serum demonstrate strikingly the difference between the progress of sterilizing action of diamino-methyl-acridinium chloride (acriflavine) on the one hand, and mercury perchloride on the other; in this respect the mercury salt behaves similarly to the other antiseptics tested, although, of course, much higher concentrations of the others are required, as shown in the table. After two hours' action the difference between the lethal concentration of mercury perchloride and of diamino-methyl-acridinium chloride is practically negligible, as we have previously noted; subsequently the effect of the flavine compound becomes at least ten to twenty times more potent than that of the mercury salt, and even a further increase in the sterilizing action has been observed to occur from the twenty-fourth to the forty-eighth hour (see table). Brilliant green (sulphate of tetra-ethyl-diamino-triphenylmethane carbinol), which was first employed in the treatment of infected wounds by Leitch,* is also slow in action, a feature which has been observed in the case of the allied methyl-violet compounds by various observers. Accordingly a fifteen minutes' test on the Rideal-Walker plan, especially in a watery medium with the flavine compounds, will reveal so low a "coefficient" as to discourage those who would seek to estimate the therapeutic possibilities of a substance according to this criterion.





× Indicates actual observations. The heavy line shows lethal concentrations. The broken line shows non-lethal concentrations.

The Importance of a Serum Medium in Intensifying the Antiseptic Action of Flavine Compounds.

The results shown by the table also illustrate exceedingly well the fact which has been repeatedly remarked upon—namely, that serum is essential in order that the full bactericidal activity of the flavine compounds should develop. This is especially true for *B. coli*, but also holds for the other organisms tested—namely, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *faecalis* (enterococcus), and the bacillus of malignant oedema.

Relative Bactericidal Potencies for B. coli (see our previous report)—

of acriflavine in water*	1
,, ,, in serum	80
of mercuric chloride in water*	800
,, ,, ,, in serum	8

* Containing 0.7 per cent. peptone.

Hence, other things being equal, the most satisfactory therapeutic results are likely to be obtained from these antiseptics when the conditions of the wound dressing are so arranged that the antiseptic is acting in a serum medium.

* In our previous report the experimental basis for the conclusion that brilliant green is preferable to malachite green as a therapeutic agent, has been fully stated.

In order to test this point further, as far as possible *in vitro*, the sterilizing action in varying concentrations of serum has been estimated, and it has been found with staphylococcus* and *B. coli* that even 25 per cent. serum causes a ten to forty-fold increase in the bactericidal action of "acriflavine" and "proflavine" as compared with the effect in 0.7 per cent. peptone water (higher amounts of peptone—that is, 5 per cent.—diminished the bactericidal power).

Investigations have not yet decided upon what factor this intensifying action of serum depends. Preliminary experiments along with E. L. Kennaway have shown that the fluid derived from boiled serum, which contained a trace of protein, on some occasions intensified the action, while other specimens lacked this effect. A specimen of human cerebro-spinal fluid containing only a trace of protein also exhibited the intensifying action on acriflavine as compared with the effect in 0.7 per cent. peptone water, and in this specimen of cerebro-spinal fluid the bactericidal effect was equal to that in undiluted serum. Thus it is possible that the flavine compounds may have an application in the treatment of cerebro-spinal infections when introduced intraspinally. But it is essential that clear indications of the suitable dosage should first be obtained experimentally, since the cerebro-spinal system represents a locus in which intense and peculiar pharmacological effects occur. As regards cerebro-spinal meningitis, it has been reported that "the use of flavine by intraspinal injection has been distinctly unfavourable" (Gray); but no details of dosage are given.

Therapeutic Application of Brilliant Green.

Antiseptics which are diminished in their activity by serum ought clearly to be renewed frequently in a wound in order that a concentration lethal to the bacteria may be maintained (unless complete sterilization could be effected almost immediately by a single application!). The danger attending this procedure is, of course, the possibility of toxic action exerted by the substance on the tissues, and it is for this reason that carbolic acid and mercuric chloride, which are poisonous both locally and also after absorption, are generally avoided; on the other hand, the neutral hypochlorite solutions are rapidly converted into innocuous compounds. Among antiseptics of the type inactivated by serum, brilliant green possesses the advantage of being an extremely potent bactericide—far exceeding the flavines in watery solution—while at the same time it is comparatively harmless to phagocytosis, as well as to the tissues locally, and when applied to a wound it is devoid of general toxic action on the body.

Brilliant green 1 : 2,000 in water represents a bactericidal concentration for staphylococcus and *B. coli* which is respectively 500 and 7 times that of Dakin's solution; at the same time it is much less harmful to phagocytosis than is hypochlorite. Hence it might be anticipated that brilliant green would prove valuable when employed according to Carrel's approved method of intermittent irrigation at two-hourly intervals. Lieut.-Colonel Hull has applied brilliant green in this manner, and we are indebted to him for an unpublished communication to the effect that "brilliant green 1 : 2,000 has proved superior to Dakin's solution for use according to Carrel's method with two-hourly flushing." Brilliant green has previously been used in the form of wet dressings with very favourable results by Leitch, and also by Ligat, who, by this method, however, found flavine compounds greatly superior; it is clear from what has been already stated why this should have been Ligat's experience. Webb also considers brilliant green superior to "eusol." As regards further modes of application of brilliant green, Lieut.-Colonel Hull reports having used a 1 : 2,000 solution in the form of hot stupes with exceptionally good results in the case of acute septic compound fractures and amputation stumps. In *burns*, brilliant green 1 : 2,000 was also used to irrigate the burn, which was then treated by the paraffin method, the paraffin containing 1 : 2,000 brilliant green. This treatment of burns in Colonel Hull's hands proved superior to any tried method.

Suggested Applications in Wound Treatment.

The sterilizing action of flavine compounds—acriflavine and proflavine—progresses gradually as compared with that of mercury perchloride, phenol, or chloramine-T. Thus, after two hours' contact in the presence of serum, mercuric chloride is practically equal to acriflavine in its lethal effect on staphylococcus and *B. coli*. But by this time the effective action of the mercury salt on the bacteria has come to an end, and a concentration which has then failed to kill the organisms exerts subsequently little or no inhibitory effect on the proliferation of the survivors. On the other hand, concentrations of the flavines which at this period have merely inhibited multiplication, later on prove bactericidal, so that finally the flavine compound is ten to twenty times more lethal than corrosive sublimate. Experiments show that such concentrations of flavine, while effectively controlling the bacteria, do not interfere with phagocytosis. The clinical evidence which has already been obtained indicates that when the flavine compounds are used for therapeutic purposes in the treatment or prevention of septic infection in wounds this slow progressive action is not a disadvantageous feature, since, on the one hand, the tissues are not harmed by the concentration of the antiseptic employed, and, on the other, admixture with the serous secretions of the wound enhances the sterilizing effect instead of bringing it to an end. It is therefore suggested that, in estimating the value of antiseptics for *therapeutic* purposes, a test such as that associated with the names of Rideal and Walker, which involves only a brief contact between antiseptic and organisms, is not likely by itself to afford satisfactory indications, although it has been found invaluable as a means of determining the relative potencies of quickly acting bactericidal agents designated for sterilizing instruments, clothes, polluted fluids, etc.

There is a clear indication that for the treatment of wounds it is advisable to avoid too frequent flushing with watery solutions of the flavine compounds.† The explanation is in all probability a twofold one. In the first place, the maximum antiseptic effect of flavine is obtained in a serous medium. Further, since the antiseptic properties—and also most probably other actions of these bodies—are not rapidly neutralized by the tissue secretions, too frequent additions of fresh doses may lead to undesirable cumulative effects on the tissues locally. This would account for unsatisfactory results following two-hourly irrigations with acriflavine solution used merely as a substitute for the approved hypochlorite. However, brilliant green has proved of great value when employed in watery solution by Carrel's approved method of frequent intermittent flushing, which is to be explained by the fact that it acts best in watery solution, and is applicable in a highly bactericidal concentration without causing harmful effects to the tissues, either locally or generally.

Should it be desired to adopt the method of introducing flavine compounds into wounds by means of tubes, in place of packing with gauze soaked in the 1 : 1,000 solution once or twice daily, it is suggested that the best results may be obtained by introducing small amounts of fluid, the frequency of the irrigations not exceeding three or four in the twenty-four hours. There is, however, no reason so far for suggesting that the method of irrigation will prove superior to packing adequately applied; in fact, the evidence now at disposal points definitely in the other direction. It is hardly necessary to comment on the numerous advantages which would attend a method necessitating only one or two manipulations of the patient daily.

Once the infection has been practically overcome considerably weaker solutions of flavine bodies than 1 : 1,000—for example, 1 : 5,000—may be subsequently employed with advantage, or in late stages the application of flavine may be intermitted for a day every few days, dry dressing being substituted in the intervals, or one may use "stimulating" applications such as Leitch first demonstrated brilliant green (1 : 1,000) to be. Since individual variations in the behaviour of the tissues of different patients must

* For some unascertained reason the bactericidal potency of flavine compounds for staphylococcus in dilute peptone water shows considerable variations in an extended series of experiments, whereas with serum the results are remarkably constant, hence the "lift up" due to the serum is caused to vary.

† We are indebted to Dr. Carrel for a preliminary account of observations on this point in the case of acriflavine.

play an important part in the process of healing it is important to discover at this stage what compound will secure the best progress.*

Prevention of Sepsis.

Special attention is directed to two further points—first, the possibility of preventing sepsis by the use of flavine compounds in early wounds at a period before the evidence of infection has developed. It may be doubted whether it is advisable to practise immediate suture of war wounds in the fashion which has been so successful in civil practice after thorough surgical cleansing and pouring in as much acriflavine 1 : 1,000 as the tissues will contain; possibly an interval of some days should be allowed before suturing, and if success follows this procedure the time may be shortened. It may be noted here that there is every indication that proflavine will be at least as harmless to the tissues as acriflavine when injected subcutaneously and between muscle planes.† Secondly, attention is directed to the promising results which have attended the use of flavine compounds with the purpose of preventing recrudescence of acute septic manifestations when operating in an area already infected, for example, in secondary amputations.

The Importance of Preliminary Operative Procedure.

It must be emphasized again that the usual surgical procedures are an essential preliminary to the use of flavine or brilliant green, in order that these antiseptics may be enabled to act effectively. As we have stated in our previous communication in connexion with the necessity for procedures which shall combat or prevent septic infection, "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"; and, again, in relation to the prophylactic application of flavine, "of course such treatment is to be used in addition to operative procedures." No quantity of flavine bodies, nor of any other feasible antiseptic so far available, seems likely to prove efficacious when there is failure to remove considerable portions of necrotic tissue or foreign bodies. Intimate contact of the antiseptic with the infected tissue must be secured.‡ The fact should not be overlooked that in our first publication we expressly emphasized such considerations. On the other hand, it would require the greatest optimist to assert that operative procedures in themselves achieve all that could be desired in the treatment of infected wounds. Thus, there is little doubt that the appropriate employment of efficient therapeutic antiseptics, combined with operative measures, can effect an almost incalculable saving; such benefit will be especially evident if the antiseptics may be applied simply and quickly.

It has been the purpose of our work thus far to examine certain little-known antiseptics, like brilliant green, or substances like the flavine bodies, whose almost unique characters as potent bactericides do not seem even to have been suspected. Laboratory tests as well as clinical trials have demonstrated, *inter alia*, that these compounds are devoid of harmful side-actions which might prejudice their use. Accordingly, we venture to urge most strongly their employment on an extensive scale, as we believe that their outstanding properties can be utilized practically to great advantage, and will lead to general recognition of the fact that they constitute a notable addition to the treatment of infected wounds and other accessible localized infective lesions. Work is in progress with a view to the development of substances suited for special uses, and it is hoped that further advance may be effected if those interested in special clinical aspects will formulate the requirements which they desiderate in substances to be employed for their particular purposes.

SUMMARY.

1. Flavine compounds and brilliant green are antiseptics which exert a slowly progressive bactericidal action. Concentrations of these substances which at first inhibit and finally kill bacteria, are without harmful effect on phagocytosis or on the tissues locally or generally; hence they are specially suited for therapeutic purposes in infected wounds. Flavine compounds may be applied to the peritoneum with safety.

2. Flavine compounds (acriflavine and proflavine) are enhanced in their bactericidal potency by the presence of serum; brilliant green, in common with most other antiseptics, is reduced in its activity by serum.

3. The most suitable method of application of an antiseptic for therapeutic purposes must depend very greatly on its behaviour in the presence of serum. When the antiseptic is inactivated by serum, frequent renewal of the watery solution is indicated as in Carrel's procedure; this, of course, is only permissible provided that the substance is not in itself toxic.

4. Brilliant green satisfies the requirements for application by repeated irrigation, as a powerfully bactericidal solution (1 : 2,000) in water is practically innocuous to the tissues. On the other hand, since flavine compounds are most bactericidal in serum, the indication is to arrange the wound dressing so that these antiseptics may act in a serum medium; also, since these bodies are not rapidly thrown out of action by serum accumulative deposit should be prevented by avoiding too frequent additions of considerable quantities of the antiseptic solution. Clinical experiences have substantiated these conclusions, and the evidence at disposal points to the application of flavine bodies by means of gauze packing or some appropriate modification of this procedure as likely to yield the best results. Thus there is evidence that, by taking full advantage of the properties of flavine bodies, a relatively simple technique may be followed.

5. The application of the flavine compounds especially for the purpose of preventing the onset of septic manifestations in early wounds is emphasized; also their use for preventing exacerbations after operating in areas already infected.

6. Operative measures are an essential preliminary to the effective use of therapeutic antiseptics in wounds, since the antiseptic can act only when brought into intimate contact with the infected tissues.

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* In certain cases the prolonged application of flavine dressings to the skin has caused the appearance of a vesicular eruption, which has rapidly disappeared on interrupting the treatment.

† The absorption of flavine compounds is followed by an appearance of green fluorescence in the urine, but no toxic action has been found to result therefrom. Observations on intravenous injection will be recorded elsewhere.

‡ Several clinical observers have applied flavine compounds incorporated in a paste with a view to securing "dépôt" action (see Bond, BRITISH MEDICAL JOURNAL, July 7th, 1917).

MEDICAL AND SURGICAL NOTES FROM MESOPOTAMIA.

BY

G. GREY TURNER, MAJOR R.A.M.C.(T.).

PART II.*

HEAT-STROKE.

THE cases coming under the heading "Effects of Heat," though interesting, were depressing and disappointing, for so very often treatment was unavailing. I shall not soon forget the streams of patients in June and July who were hurried to the bath-house, nor the occasion when nearly a dozen stertorous patients lay in the open air along the side of the creek, many of whom went to make up the sixteen funerals that left our hospital the next day. These men were not weaklings, but often big strong fellows who might be expected to be the last to go under. Nearly every case illustrated the importance of prophylaxis and of early treatment, and those that developed in hospital often recovered as the result of prompt measures to reduce the temperature. But the heat regulating centres are so much deranged that relapses are common, and there can be no

* Part I was published on July 14th, page 33.