was formed, the meetings being held at Old Slaughter's Coffee House. Fordyce was also responsible for the foundation of the "Lyceum Medicis Londinense" in 1785. Finally, the "Abernethtian Society" was established in 1795, the "Medico-Chirurgical" in 1805, and the "Hunterian" in 1819. A society of Physicians to Dispensaries met in the Borough about 1780, but the exact date of its foundation is uncertain.

It will thus be seen that twelve medical societies were formed during the reign of George III, and three only before that period. Many of them came to an end after a few years of usefulness, but six remain, and are flourishing at the present time. Among those who did so much to encourage the formation of these societies, the names of Simmons, Fordyce, William Hunter, Fothergill, and Lettison must for ever be honoured. They toiled, not unsuccessfully, for the advancement of medicine and for a wider medical polity where men holding diverse opinions could meet and exchange their views.

(To be continued.)

THE RELATIVE GERMICIDAL EFFICIENCY OF ANTISEPTICS OF THE CHLORINE GROUP AND ACRIFLAVINE AND OTHER DYES,

WITH OBSERVATIONS OF THE RATIONAL TESTING OF ANTISEPTICS.

BY

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(Report to the Medical Research Committee.)

THE object of the following paper is to point out certain common errors in the testing of antiseptics of the chlorine group which lead to totally inaccurate inferences as to their germicidal potency; secondly, to compare the results obtained by methods eliminating these errors with those furnished by other antiseptics; thirdly, to examine the claims made by Browning and his colleagues¹ for the alleged vastly superior germicidal potency of acriflavine and certain other dyes; and, lastly, to indicate how important the matter of the velocity of disinfection is in forming a judgement of the utility of antiseptics in surgical practice, and also the methods suitable for their successful employment.

The Testing of Antiseptics of the Chlorine Group for Germicidal Action.

The instability and reactivity of the hypochlorites and other antiseptics of the chlorine group has long been known. Their ability to react with proteins, peptones, and amino acids to furnish products some of which still possess germicidal properties (chloramines), and others which are entirely inert (chlorides and compounds containing chlorine united to carbon), has been definitely determined. When a relatively large excess of protein acts on minimal quantities of hypochlorite, no chloramine derivatives persist, and the whole of the chlorine is converted into germicidally inactive forms. Notwithstanding these well established facts current literature contains countless examples of experiments in which workers have taken protein-rich media, then added minute quantities of the chlorine antiseptic; subsequently, after an unstated interval, when all or most of the antiseptic has been decomposed, the mixture is inoculated with organisms, and surprise is expressed at their ability to grow unchecked. Under such circumstances the observed results depend much more on the rate of reaction between the antiseptic and the protein medium than upon germicidal action.

In making tests of the germicidal efficiency of any antiseptic there seems to be no good reason for not following the order of mixing the materials indicated by the conditions of their practical use—namely, to add the antiseptic last to the inoculated medium. Under these conditions very different results are obtained with unstable antiseptics, compared with those observed when the organisms are added last. Perhaps the most naive alleged demonstration of the inutility of hypochlorites as antiseptics was that offered by Delbet, who took egg albumen, added a little hypochlorite of undetermined strength, next heated

the mixture for a considerable time, so that all the antiseptic would be decomposed with certainty, and then found, to his evident satisfaction, that streptococci could grow in the residue. Equally enlightening experiments have led various workers into statements as to the excellence of hypochlorite solutions as culture media for *B. capsulatus* and *B. pyocyaneus*, but these entertaining speculations need not be seriously considered.

More recently, however, Browning and his colleagues have published a series of experiments in which the germicidal potency of various antiseptics of the chlorine group is compared with various dyestuffs, with results which apparently indicate an enormous superiority for the latter. Numerical ratios for the relative antiseptic potency of the various substances are given, in which the lethal concentration of chloramine-T tested against staphylococci in serum is taken as unity. The lethal concentration of chloramine-T in serum is stated to be 1:250 without reference to much lower concentrations already published by others. The discrepancy is due, in the main, to the fact that the antiseptic was added first to the medium and the organisms last. No indication is given by Browning of the all-important interval of time elapsing between the two additions. From what has been already stated, it is clear that by this method of procedure irregular results are inevitable.

In the following table, a few new experiments are given, showing the results of the action of chloramine-T when tested against *Staphylococcus aureus* in blood serum and in 0.7 per cent. Witte's peptone, the antiseptic being added last as in actual practice.

In these experiments the medium (2 c.cm.) was inoculated with a suspension in saline of an agar slant growth of *Staphylococcus aureus*. The chloramine-T solution (1 c.cm.) in varying concentration was added last, and at regular time intervals the number of surviving bacteria was estimated by plating. In every case any unchanged antiseptic was destroyed before plating by the addition of sterile sodium thiosulphate solution.

TABLE I.—Chloramine-T.

Medium used: Horse serum. Concentration of chloramine-T in mixture, 1:1000.		Bacterial Count.*	
Time of Action.			
0	...	...	1,750
5 minutes	...	...	0
Medium: Horse serum. Concentration of chloramine-T in mixture, 1:2,000.			
0	...	...	1,831
5 minutes	...	...	9
15 "	...	...	15
45 "	...	...	49
90 "	...	...	85
3 hours	...	...	307
Medium: Horse serum. Concentration of chloramine-T in mixture, 1:3,000.			
0	...	...	1,509
5 minutes	...	...	82
15 "	...	...	95
45 "	...	...	211
90 "	...	...	627
3 hours	...	...	1,308
Medium: Witte's peptone, 0.7 per cent. in saline. Concentration of chloramine-T in mixture, 1:4,000.			
0	...	...	82,090
5 minutes	...	...	0
Medium: Witte's peptone, 0.7 per cent. in saline. Concentration of chloramine-T in mixture, 1:5,000.			
0	...	...	82,400
5 minutes	...	...	14,520
15 "	...	...	0
Medium: Witte's peptone, 0.7 per cent. in saline. Concentration of chloramine-T in mixture, 1:6,000.			
0	...	...	1,323
5 minutes	...	...	13
15 "	...	...	12
45 "	...	...	14
90 "	...	...	16
3 hours	...	...	37

* 1 drop = $\frac{1}{40}$ c.cm.

From these results it is clear that under the above conditions the lethal concentration of chloramine-T in blood serum is slightly under 1:2,000, and in 0.7 per cent. Witte's peptone between 1:5,000 and 1:6,000. These figures contrast with 1:250 and 1:2,000 respectively given by Browning and his colleagues. The use by Browning of the figure 1:250 for the lethal concentration of chloramine-T against *Staphylococcus aureus* as a standard for calculating the relative antiseptic potency of other substances is clearly misleading.

The fundamental error in Browning's figures for the antiseptic potency of chloramine-T is again encountered in

the case of the still more reactive hypochlorite preparations. A later section of this paper contains experimental results illustrating germicidal action of these and other substances acting in a blood serum-muscle extract medium, and also in defibrinated blood.

Some Factors concerning the Rational Testing of Antiseptics.

It is generally recognized that the testing of substances for their antiseptic or germicidal properties is fraught with innumerable pitfalls, and that by varying the conditions of testing almost any kind of result may be obtained. In the case of disinfectants intended for hygienic purposes, the Walker-Rideal method in one or other of its forms gives a reasonably satisfactory basis for comparison, but with substances intended for wound disinfection, no such standard methods exist. Experiment for the determination of the lethal concentration of the germicide acting on various organisms in water, blood serum, blood, or pus usually are regarded as sufficient, and but little attention is paid to such important factors as the time of action, temperature, and number of organisms present.

A great advantage in the use of serum as a medium for the determination of the germicidal properties of an antiseptic intended for surgical use is the fact that it presents a homogeneous medium of fairly constant composition, but it is important to bear in mind that many organisms are very susceptible to the action of blood serum, even though previously heated to 58-60°. To avoid this complication, large numbers of the organism should be used, and fairly resistant types, such as *Staphylococcus aureus*, should be chosen. The use of blood as a standard medium for the testing of the germicidal properties of antiseptics has been suggested by Emery, and this would seem to be a rational procedure in the case of substances intended for intravenous use. Great difficulties are encountered in securing uniform laboratory experiments on the germicidal action of substances upon pus, largely owing to extreme variations in its composition and physical condition, and whether the infecting organisms are free or ingested in the leucocytes. As a substitute for pus, the use of blood as a test medium is of practical value in throwing light on the action of antiseptics in the presence of cellular elements, though the results will be particularly unfavourable to those substances, such as hypochlorites, which are readily decomposed by haemoglobin.

Usually the lethal concentration for germicidal substances tested under various conditions against different organisms is alone considered. But it is becoming constantly clearer that much more than this is needed to form a judgement of the possible value of an antiseptic for wound treatment. A higher concentration of an otherwise inoffensive germicide may easily prove more useful practically than a lower concentration of a more active compound. The fundamental work of Miss Chick,² following that of Paul and Krönig and Madsen and Nyman, has clearly shown that the act of disinfection resembles in many fundamental respects an ordinary chemical reaction in which the two reacting components are represented by bacterial protoplasm and disinfectant. The rate of disinfection varies enormously with different germicides. The mass of antiseptic and that of the bacteria and other reacting substances, as well as the temperature and intimacy of contact between the reacting substances, all influence the result. A knowledge of the speed of disinfection is important in judging of the most suitable manner in which to employ different antiseptics in actual practice. Thus, as will be seen later, the hypochlorites, chloramine-T, and similar substances in concentrations such as are used in practice act with extraordinary speed, completing in a few minutes or even seconds an amount of disinfection which dyes, such as acriflavine, require six hours or more to effect. But in actual use the hypochlorites and chloramine-T are chemically unstable and need frequent renewal unless disinfection has been completed, while frequent renewal of the dyestuffs would add but little to their effect, and actually they are found to be most useful employed as wet dressings, and not for irrigating. This point has been convincingly discussed by Parry Morgan.³

In order to obtain information as to the relative speed of disinfection exerted by various antiseptics, we have determined the progressive change in the number of bacteria in

suitable mixtures after varying lengths of time of action. We have made use of two media—one a mixture of horse blood serum (1 c.cm.) with muscle extract (1 c.cm.), obtained by soaking fresh veal with an equal weight of 0.7 per cent. salt solution and straining through gauze, but not filtering. The other medium was sterile fresh defibrinated rabbit's blood (2 c.cm.). These mixtures were heavily inoculated with a *Staphylococcus aureus* emulsion obtained by shaking a twenty-four-hour agar slant growth with 8 c.cm. of salt solution. After determining the total number of organisms in the mixture by plating an aliquot part, 1 c.cm. of the antiseptic solution was usually added and samples withdrawn from time to time and the surviving bacteria estimated. In most cases the antiseptic was added at about the concentration commonly used for wound treatment, though in the case of some of the more active substances lower concentrations were also employed and occasionally higher ones with the less active substances.

In the case of antiseptics of the chlorine group, their further action in the sample withdrawn for plating was at once checked by addition of an excess of sterile sodium thiosulphate solution, while potassium sulphide was used as antidote with the salts of the heavy metals. No antidote was used in the case of the dyes, and in some cases evidence of inhibition of growth on the plates was noted, due to the mechanical carrying over of some of the dye. The use of the blood serum-muscle extract medium was chosen as bearing some similarity to that in which the antiseptics would be used in wound treatment. It has the further advantage of being easily reproduced in fairly uniform quality. For many antiseptics the addition of muscle extract makes the tests more severe than when blood serum is used alone. The experiments were all carried out at 32-35° C., and the results are all expressed as the number of bacteria present in one standard drop of the mixtures = $\frac{1}{20}$ c.cm. For convenience, the antiseptics are grouped in three classes—namely, those of the chlorine group, dyes, and metallic salts.

TABLE II.—Chlorine Group of Antiseptics.

(Blood Serum-Muscle Extract Medium. *Staphylococcus aureus*.)

Antiseptic Used.

I. Sodium hypochlorite. Concentration: As added, 1:200; in mixture, 1:600.

Time of Action.	Bacterial Count.*
0 ...	1,966,000
2 minutes ...	405
5 " ...	0 ¹

II. Sodium hypochlorite. Concentration: As added, 1:200 (0.2 c.cm.); in mixture, 1:2,200.

0 ...	1,966,000
5 minutes ...	311,200
15 " ...	157,400
45 " ...	98,170
90 " ...	16,120
3 hours ...	1,651
6 " ...	1,587
24 " ...	1,294,000 ²

III. Eusol (HClO). Concentration: As added, 1:400; in mixture, 1:1,200.

0 ...	2,150,000
2 minutes ...	496
5 " ...	0 ¹
15 " ...	0 ¹

IV. Eusol. Concentration: As added, 1:400; in mixture, 1:4,400.

0 ...	2,129,000
5 minutes ...	1,310,000
15 " ...	507,900
45 " ...	606,200
90 " ...	641,400
3 hours ...	868,300
6 " ...	983,000
24 " ...	2,310,000 ²

V. Chloramine-T. Concentration: As added, 1:50; in mixture, 1:150.

0 ...	1,365,000
5 minutes ...	0 ¹

VI. Chloramine-T. Concentration: As added, 1:200; in mixture, 1:600.

0 ...	725,300
5 minutes ...	998
15 " ...	191
45 " ...	7
90 " ...	4
3 hours ...	3
6 " ...	0 ²

VII. Dichloramine-T in oil. Concentration: As added, 1:50; in mixture, 1:150.³

0 ...	2,020,000
0.5 minute ...	0

TABLE II (continued).

VIII. Dichloramine-T in oil. Concentration: As added, 1:50; in mixture, 1:150.⁴

Time of Action.	Bacterial Count.*
0	1,157,000
5 minutes	458,700
15 "	294,900
45 "	0

IX. Iodine in potassium iodide. Concentration: As added, 1:50; in mixture, 1:150.

0	1,463,000
5 minutes	0

* 1 drop = $\frac{1}{20}$ c.cm.¹ Active chlorine present. ² Active chlorine absent. ³ Mixed with platinum wire. ⁴ Not mixed, left to diffuse.

The results contained in Table II indicate clearly the extraordinarily rapid action of the antiseptics of the chlorine group. All of the solutions of strengths used in wound treatment affected sterilization in the course of five minutes or less. The experiments in which insufficient quantities of sodium hypochlorite or eusol were used (II and IV) show that when their immediate action is over, and no undecomposed antiseptic remains, growth of the surviving organisms readily takes place. The efficiency of chloramine-T added to the extent of 1:600 in the mixture illustrates its more potent action than roughly equivalent quantities of chlorine in the form of hypochlorite or eusol. This is, no doubt, largely due to its slower rate of decomposition in the medium.

TABLE III.—Metallic Salts, Phenol, Hydrogen Peroxide.

(Blood Serum-Muscle Extract Medium. *Staphylococcus aureus*.)

Antiseptic Used.

I. Mercuric chloride. Concentration: As added, 1:1,000; in mixture, 1:3,000.

Time of Action.	Bacterial Count.*
0	1,894,000
5 minutes	254,400
15 "	44,320
45 "	12,300
90 "	7,258
3 hours	2,985
24 "	0

II. Silver nitrate. Concentration: As added, 1:100; in mixture, 1:300.

0	786,400
5 minutes	720,900
15 "	651,700
45 "	530,600
90 "	425,900
3 hours	175,600
6 "	38,270
24 "	2,643

III. Argylol. Concentration: As added, 1:6.7; in mixture, 1:20.

0	917,500
5 minutes	753,500
15 "	655,300
45 "	622,500
90 "	327,600
3 hours	9,792
6 "	4,693
24 "	0

IV. Zinc chloride. Concentration: As added, 1:33; in mixture, 1:100.

0	1,223,000
5 minutes	868,300
15 "	819,200
45 "	491,500
90 "	211,900
3 hours	51,580
6 "	3,667
24 "	704

V. Hydrogen peroxide. Concentration: As added, 1:35; in mixture, 1:105.

0	1,201,000
5 minutes	589,800
15 "	819,200
45 "	778,300
90 "	802,800
3 hours	1,087,000
6 "	1,135,000

VI. Phenol. Concentration: As added, 1:50; in mixture, 1:150.

0	1,409,000
5 minutes	1,471,000
15 "	819,200
45 "	192,700
90 "	34,900
3 hours	13,700
24 "	2,985

* 1 drop = $\frac{1}{20}$ c.cm.

The results of experiments with metallic salts, phenol, and hydrogen peroxide, are recorded in Table III. Mercuric chloride, added as a 1:1,000 solution, is seen to be distinctly more rapid and complete in its action than silver nitrate or zinc chloride, and, though the latter were used in much higher concentration, they did not effect sterilization in twenty-four hours. Argylol was used in very high

concentration (1:20), and, while it eventually sterilized completely, its action was by no means rapid, and only slightly superior to phenol (1:50). The experiment with hydrogen peroxide illustrates its rapid but very transitory effect, which reached its maximum in a few minutes, with subsequent unrestricted growth.

TABLE IV.—Dyes.

(Blood Serum-Muscle Extract Medium. *Staphylococcus aureus*.)

Antiseptic Used.

I. Malachite green (Grübler). Concentration: As added, 1:333; in mixture, 1:1,000.

Time of Action.	Bacterial Count.*
0	821,600
5 minutes	182,700
15 "	60,920
45 "	8,920
90 "	5,574
3 hours	78,200
6 "	1,015,000
24 "	3,706,000
48 "	0

II. Malachite green (Grübler). Concentration: As added, 1:1,000; in mixture, 1:3,000.

0	2,097,000
5 minutes	1,359,000
15 "	369,100
45 "	211,500
90 "	272,300
3 hours	557,000
6 "	1,096,000
24 "	6,553,000
48 "	0

III. Brilliant green (Grübler). Concentration: As added, 1:333; in mixture, 1:1,000.

0	810,200
5 minutes	29,950
15 "	27,410
45 "	20,160
90 "	14,300
3 hours	8,871
6 "	1,946
24 "	255,600
48 "	658,900

IV. Acriflavine. Concentration: As added, 1:333; in mixture, 1:1,000.

0	589,800
5 minutes	358,400
15 "	249,300
45 "	179,200
90 "	78,210
3 hours	704
6 "	0
24 "	0

V. Acriflavine. Concentration: As added, 1:1,000; in mixture, 1:3,000.

0	557,100
5 minutes	218,600
15 "	121,900
45 "	100,800
90 "	81,540
3 hours	474
6 "	0
24 "	0

VI. Acriflavine. Concentration: As added, 1:3,333; in mixture, 1:10,000.

0	755,400
5 minutes	524,300
15 "	319,300
45 "	308,200
90 "	106,500
3 hours	94,840
6 "	22,780
24 "	1,113,000

VII. Proflavine. Concentration: As added, 1:333; in mixture, 1:1,000.

0	888,100
5 minutes	794,500
15 "	744,500
45 "	308,200
90 "	104,800
3 hours	66,560
6 "	53,250
24 "	1,242

VIII. Proflavine. Concentration: As added, 1:1,000; in mixture, 1:3,000.

0	888,100
5 minutes	744,500
15 "	655,400
45 "	326,100
90 "	169,900
3 hours	154,800
6 "	84,900
24 "	4

IX. Proflavine. Concentration: As added, 1:3,333; in mixture, 1:10,000.

0	855,400
5 minutes	761,800
15 "	744,500
45 "	468,500
90 "	304,500
3 hours	196,400
6 "	93,750
24 "	183,600

* 1 drop = $\frac{1}{20}$ c.cm.

The action of the dyes on staphylococci in the blood serum-muscle extract was, on the whole, disappointing. Malachite green and brilliant green, even when added at 1:333 concentration, so that 1:1,000 was present in the

mixture, failed to effect sterilization in twenty-four hours, and by the end of that time the organisms were growing freely. Similar mixtures on exposure at 37° readily underwent thorough putrefaction, so that it is clear that unchanged persistence of colouring matter cannot be regarded as evidence of continuing germicidal action. The experiments with acriflavine indicate a relatively slow action, and even in the higher concentrations a fair number of viable organisms were present after six hours' action. The lower concentration (1:3,333 as added) failed to sterilize in twenty-four hours, and permitted growth so that the final number of organisms had risen above that present at the start.

Browning gives the following lethal concentrations for dyes acting in serum on *Staphylococcus aureus*: Malachite green, 1:40,000; brilliant green oxalate, 1:100,000; acriflavine, 1:200,000. Under the conditions of our experiments the lethal concentration for malachite green and brilliant green is greater than 1:1,000, and for acriflavine between 1:3,000 and 1:10,000.

TABLE V.—Chlorine Group of Antiseptics.

(Defibrinated Blood Medium. *Staphylococcus aureus*.)

Antiseptic Used.

I. Sodium hypochlorite. Concentration: As added, 1:200; in mixture, 1:300.

Time of Action.	Bacterial Count.*
0	573,400
5 minutes	563
15 "	282
45 "	2,432

II. Sodium hypochlorite. Concentration: As added, 1:200; in mixture, 1:333.

0	573,400
5 minutes	1,774
15 "	1,485
45 "	19,656

III. Sodium hypochlorite. Concentration: As added, 1:200; in mixture, 1:400.

0	232,900
45 minutes	89,600

IV. Eusol (HClO). Concentration: As added, 1:400; in mixture, 1:600.

0	578,000
5 minutes	1,562
15 "	2,330
45 "	36,600

V. Eusol. Concentration: As added, 1:400; in mixture, 1:700.

0	811,000
5 minutes	36,200
15 "	48,250
45 "	51,580

VI. Eusol. Concentration: As added, 1:400; in mixture, 1:800.

0	260,800
5 minutes	143,300

VII. Chloramine-T. Concentration: As added, 1:50; in mixture, 1:150.

0	224,500
5 minutes	125
15 "	0

VIII. Chloramine-T. Concentration: As added, 1:100; in mixture, 1:200.

0	235,600
45 minutes	0

IX. Chloramine-T. Concentration: As added, 1:133; in mixture, 1:400.

0	1,178,000
5 minutes	20,160
15 "	6,451
45 "	4,032
3 hours	806

X. Dichloramine-T in oil. Concentration: As added, 1:50; in mixture, 1:150.†

0	59,900
2 minutes	0

* 1 drop = $\frac{1}{20}$ c.cm. † Mixture stirred with platinum wire.

The relative inefficiency of sodium hypochlorite and eusol acting in defibrinated blood is shown in Table V. This is apparently due to the ease with which sodium hypochlorite is decomposed by haemoglobin. The fact that an equal volume of the ordinary hypochlorite solutions added to blood infected with streptococci is insufficient to effect sterilization has already been noted by Emery. The superior efficiency of chloramine-T compared with equivalent amounts of hypochlorite acting in blood is very marked, and is doubtless due to its slower rate of decomposition already noted.

TABLE VI.—Dyes.

(Defibrinated Blood Medium. *Staphylococcus aureus*.)

Antiseptic Used.

I. Malachite green (Grübler). Concentration: As added, 1:333; in mixture, 1:1,000.

Time of Action.	Bacterial Count.*
0	1,754,000
5 minutes	107
15 "	88
45 "	66
90 "	82
3 hours	35
6 "	36
24 "	13
72 "	0

II. Malachite green (Grübler). Concentration: As added, 1:1,000; in mixture, 1:3,000.

0	1,693,000
5 minutes	1,310,000
15 "	819,200
45 "	208,000
90 "	146,900
3 hours	86,010
6 "	89,600
24 "	1,219,000

III. Brilliant green (Grübler). Concentration: As added, 1:333; in mixture, 1:1,000.

0	1,966,000
5 minutes	0

IV. Brilliant green (Grübler). Concentration: As added, 1:1,000; in mixture, 1:3,000.

0	1,630,000
5 minutes	1,261
15 "	563
45 "	755
90 "	717
3 hours	857
6 "	2,592
24 "	1,171,000

V. Acriflavine. Concentration: As added, 1:333; in mixture, 1:1,000.

0	654,600
5 minutes	358,400
15 "	108,800
45 "	98,180
90 "	1,472
3 hours	203
6 "	17
24 "	0

VI. Acriflavine.† Concentration: As added, 1:333; in mixture, 1:1,000.

5 minutes	835,600
15 "	494,600
45 "	190,500
90 "	139,800
3 hours	10,480
24 "	0

VII. Acriflavine. Concentration: As added, 1:1,000; in mixture, 1:3,000.

0	654,700
5 minutes	411,900
15 "	225,800
45 "	138,100
90 "	30,640
3 hours	1,152
6 "	32
24 "	0

VIII. Acriflavine. Concentration: As added, 1:3,333; in mixture, 1:10,000.

0	900,600
5 minutes	819,200
15 "	508,900
45 "	190,000
90 "	83,260
3 hours	32,260
6 "	768
24 "	3

IX. Acriflavine.† Concentration: As added, 1:3,333; in mixture, 1:10,000.

5 minutes	802,800
15 "	790,900
45 "	587,100
90 "	405,000
3 hours	159,700
24 "	16

X. Proflavine. Concentration: As added, 1:333; in mixture, 1:1,000.

0	819,200
5 minutes	4,710
15 "	1,638
45 "	474
90 "	304
3 hours	113
6 "	12
24 "	0

XI. Proflavine.† Concentration: As added, 1:333; in mixture, 1:1,000.

5 minutes	778,000
15 "	376,800
45 "	186,400
90 "	164,300
3 hours	28,290
24 "	0

TABLE VI (continued).

XII. Proflavine. Concentration: As added, 1:1,000; in mixture 1:3,000.

Time of Action.	Bacterial Count.*
0	819,200
5 minutes	201,340
15 "	208,000
45 "	122,500
90 "	53,220
3 hours	29,670
6 "	2,995
24 "	2

XIII. Proflavine.† Concentration: As added, 1:1,000; in mixture, 1:3,000.

5 minutes	778,000
15 "	376,800
45 "	186,400
90 "	164,300
3 hours	28,290
24 "	0

XIV. Proflavine. Concentration: As added, 1:3,333; in mixture, 1:10,000.

0	819,200
5 minutes	753,700
15 "	652,000
45 "	591,400
90 "	394,200
3 hours	448,000
6 "	48,380
24 "	282

XV. Proflavine.† Concentration: As added, 1:3,333; in mixture, 1:10,000.

5 minutes	933,600
15 "	835,600
45 "	750,900
90 "	208,700
3 hours	204,500
24 "	331

* 1 drop = $\frac{1}{10}$ c.cm.

† The blood and antiseptic were mixed, incubated twenty-four hours and then the suspension of staphylococci added. In these experiments, therefore, the precipitate occasioned by the dye was produced before inoculation. Comparison of X and XI indicates the probability that many organisms are carried down by the relatively slow formation of a flocculent precipitate. The rapid falling off of the cocci found in the fluid was probably due to this cause, and not to the germicidal action of the dye.

The germicidal action of the dyes in defibrinated blood is in most cases distinctly superior to that shown in the blood serum-muscle extract medium. Malachite green is seen to be distinctly inferior to brilliant green, acriflavine, or proflavine. Neither brilliant green nor malachite green when present in 1:3,000 concentration failed to prevent multiplication of the organisms. Acriflavine and proflavine present in 1:3,000 concentration failed to sterilize in six hours, but usually did so in twenty-four hours, while when present in 1:10,000 concentration the mixture was not quite sterile in twenty-four hours.

DISCUSSION AND CONCLUSIONS.

The main facts disclosed by the preceding series of experiments are as follows: (a) The rapid and complete disinfection brought about by solutions (one volume) of members of the chlorine group of antiseptics of the strength commonly used in the treatment of wounds, when added to heavily infected blood serum-muscle extract mixture (two volumes). (b) Under similar circumstances, solutions of acriflavine, proflavine, brilliant green, and malachite green failed to sterilize in six hours mixtures which the chlorine antiseptics sterilized completely in five minutes or less.

Browning concludes from his experiments that acriflavine is 800 times more powerful against staphylococci in serum than chloramine-T. A comparison of our Experiment VI, Table II, and Experiment IV, Table IV, shows that a 1:600 solution of chloramine-T can sterilize in six hours an infected blood serum-muscle extract which was not sterilized in six hours by 1:1,000 acriflavine. The difference in results is striking and deserves comment. One source of error in Browning's experiments has already been indicated, namely, the addition of the chlorine antiseptics to the blood serum before adding the organisms. We believe another source of discrepancy is due to the fact that Browning and his associates used extraordinarily few bacteria in their tests, namely, 0.1 c.cm. of a 1:20,000 dilution in saline of a twenty-four-hour peptone water culture. A loopful of this mixture plated on agar yielded "twenty or more colonies." It appears to us that the use of such small numbers of organisms is unsuited for the determination of the germicidal value of substances, for, in the first place, such a low concentration of organisms in the secretion of a wound of long standing borders on "surgical

sterility"; hence the experimental conditions are not comparable with those in acute septic wounds. Secondly, if 5 per cent. of the organisms in Browning's tests survived they would have a good chance of being entirely overlooked, and sterility might thus be inferred when viable organisms were actually present. Lastly, the bactericidal properties of the serum medium becomes a significant factor when few organisms are used in the presence of native germicidal substances.

Another cause of the marked difference between our own and Browning's results is the fact that he allowed the antiseptics to act for twenty-four to forty-eight hours before testing, whereas in our own experiments subcultures were taken at frequent intervals, which brought under observation the progress of disinfection. The longer period naturally fosters a favourable judgement of the slowly acting dyes, while shorter periods are adverse to them.

We conclude, therefore, that the statements of Browning and his colleagues as to the relative germicidal efficiency of chloramine-T and other antiseptics of the chlorine group compared with brilliant green, malachite green, and acriflavine, are incorrect, and that their mode of testing the germicidal action of the substances studied by them leads to results which are radically misleading.

REFERENCES.

¹ Browning, Gulbransen, Kennaway and Thornton, *BRITISH MEDICAL JOURNAL*, January 20th, 1917. ² *Journal of Hygiene*, 8, p. 92, 1908; 10, p. 237, 1910. ³ *BRITISH MEDICAL JOURNAL*, January 27th, 1917; February 10th, 1917.

DETECTION AND TREATMENT WITH EMETINE BISMUTH IODIDE OF AMOEBIC DYSENTERY CARRIERS AMONG CASES OF IRRITABLE HEART.*

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(Report to the Medical Research Committee.)

AMOEBIC dysentery as a direct or aggravating cause of irritable heart was found amongst soldiers returned from the Mediterranean Force.¹ It was therefore considered important, in dealing with this condition, to determine the number of cases of carriers, and to attempt to cure them. From October, 1916, to February, 1917, we examined the stools of all cases from the Mediterranean Force admitted to the Hampstead Military Hospital suffering from irritable heart. No selection of the men was made in so far as the presence or absence of a history of dysentery was concerned. In all sixty-five cases have been examined, of which twenty-five had a definite history of dysentery.

Infections.

The microscopic examinations of the stools were made in the usual way, and we aimed at six careful examinations at least of each man before discharging him as uninfected. How far we were able to carry this out the following figures will show:

6 or more examinations	...	...	51 cases
5 examinations only	...	...	1 case.
4 "	"	"	8 cases
3 "	"	"	3 "
2 "	"	"	1 case
1 "	"	"	1 "
			65

The following infections were detected:

<i>Entamoeba histolytica</i>	24 cases	= 36.9 %	total cases.
<i>Entamoeba coli</i>	32 "	= 49.2 %	"
<i>Lamblia intestinalis</i>	20 "	= 30.8 %	"
<i>Chilomastix mesnili</i>	3 "	= 4.6 %	"
<i>Trichomonas hominis</i>	1 case.		

Entamoeba nana (Wenyon and O'Connor) was found as the active free amoeba in one case, and its cysts were found in others. Their occurrence was not always recorded, however, so that no indication of the frequency of this infection can be given. The so-called "iodine cysts"

* This work was done at the Hampstead Military Hospital. The treatments were carried out by one of us (J. C. M.), and the examinations made by the other (M. W. J.).

† This was done at the suggestion of Mr. Clifford Dobell.