A STANDARDIZED METHOD OF DISINFECTING INFECTED BLOOD-CLOTS

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(With 1 Figure in the Text)

In spite of its deficiencies, which have long been known to experts (Garrod, 1935, 1940), the Rideal-Walker test, with its Chick-Martin modification, remains the only standardized test for disinfectants. Official warnings (Brit. Stand. Instn, 1934) have made it clear that the phenol-coefficient, determined as it is in a special arbitrary set of conditions, gives no solid information about the relative disinfecting powers of various substances under the diverse conditions of practice.

Such information could only be afforded if all disinfectants were chemically similar and therefore acted in the same way; but in reality their natures and modes of action are so different that a substance may appear stronger than phenol when tested in one way, and weaker when tested in another.

We may take as an example the superiority of the detergents, Zephiran and CTAB, to phenol in the rapid simple bactericidal test, and their inferiority in the clot-disinfection test.

Even the Chick-Martin (1908) and Garrod (1935) modifications of the Rideal-Walker principle, which allow for the neutralizing effect of proteins, do not by any means eliminate all the variables.

Some disinfectants act rapidly but not in high dilution, others act slowly but, if given plenty of time, in very high dilution. An example of the former type is phenol; of the latter, mercuric chloride. Thus the activity of mercury compounds relative to that of phenol will be very different according to whether minutes or hours are allowed for the action.

Again, diffusibility or penetrating power, as has often been pointed out (von Gutfeld, 1944), governs the rate at which the chemical reaches bacteria embedded in a gel. Most authors who have investigated this have used materials such as agar or horn, which are not very like the infected materials of medical practice. Inman (1916) used blood-clot in a single experiment but did not try to standardize the method. The clot-disinfection test described herein shows clearly enough how important is the very variable penetrating power of different chemicals.

Because the Rideal-Walker type of test, and indeed any single test, is unable to tell us what a disinfectant can really do, we are left with the necessity of devising a number of standardizable tests which will estimate the power of the substance to perform definite tasks substantially resembling those it will have to do in practice. And in these various reactions the potency will be measured absolutely, though comparisons with phenol, giving a series of different phenol-coefficients, each specific for a given standard test, may also be useful.

The work described herein was undertaken in the period of the first bombing of our cities, when it seemed desirable to find out what chemicals would quickly disinfect bowls or instruments fouled with infective matter under conditions which precluded the use of heat or even free washing with water. They would have to act quickly in the cold; to penetrate flakes of clotted blood or pus; and to resist neutralization by the constituent proteins.

It seemed that the quick disinfection of a piece of fresh blood-clot of constant dimensions carrying in its substance a constant dose of bacteria would give a fair indication of the power of the chemical to penetrate an organic gel, to resist neutralization by concentrated proteins and to kill the bacteria inside. A distinct advantage would be that instead of the customary small sample the whole dose of bacteria exposed to the disinfectant would be transferred into the culture medium inside its blood-clot. In this way a better proof of complete sterility would be obtainable.

On these principles the method described below was eventually adopted. A standard period of 5 min. exposure to the disinfectant was originally chosen as being about the maximum time a hurried doctor or nurse would be able to give, but when it transpired that no feasible disinfectant would completely sterilize heavily infected clots of standard size in so short a time, further observations were made with a 30 min. period. The size of the clot was chosen to represent about the largest flake of infected blood or pus a rushed or careless assistant would be likely to overlook while washing or wiping a vessel or instrument.

PRINCIPLES OF THE METHOD

Small blood-clots of uniform size and shape in which a measured constant dose of the microbe has been incorporated are immersed in the disinfectant at room temperature (17–22° C.), removed at the end of a measured time, placed in sterile water for a momentary rinsing and then removed into an optimal fluid culture medium, in which the clot is crushed to release the bacteria and to prevent prolongation of the action of the disinfectant absorbed by the clot. The tubes or bottles are incubated and the presence or absence of viable cocci is recorded at intervals up to a week.

APPARATUS AND MATERIALS

Gauged capillary pipettes for making the standard clots are drawn from no. 4 glass tubing of 5–7 mm. diameter. It is convenient to cut 4 or 5 in. lengths of the tubing and plug both ends with wool; after dry-sterilization these are ready to be drawn out. With a fish-tail burner and a little practice a technician soon learns to draw out about 50 % of the capillaries to the required external diameter, viz. between 1·35 and 1·49 mm., the mean being 1·42 mm., which is the size of no. 17 of the British standard wire gauge. Five or six inches of the capillary should conform to these measurements, which are best controlled by using a metal gauge consisting of a stout strip of brass in which two slots have been cut representing the maximum and minimum diameters stated above.* On each capillary the length that will enter the maximum slot and will not enter the minimum one is marked at both ends with a grease-pencil, and only that length must be used for making a standard clot. Other apparatus and materials comprise a bottle of sterile oxalated horse blood (Burroughs, Wellcome and Co.); enough small sterile Petri dishes (2–3 in. diameter) for each dilution of disinfection to be used; one or two larger ones (4 in. or more) to receive the clot worms from the pipettes; some tubes of sterile water, for rinsing the clot segments; screw-cap McCartney cylindrical bottles of about 20 c.c. capacity containing 10 c.c. of heart broth to which 0·5 c.c. of sterile horse blood has been added; some sterile saline solution; and a wire hook for manipulating the clot segments, which is easily made by bending the tip of a bacteriological nichrome or platinum wire set in the customary handle; also a nichrome spatula, which is a 5 in. length of thick (about 1 mm.), rigid nichrome wire hammered flat for about 1 in. at one end and held in a needle holder at the other. The corners of the flattened part are rounded. A 24 hr. agar culture

* Gauges can be obtained from S. W. Bush, School of Pathology, Oxford.

of a coagulase-positive *Staphylococcus aureus* is required for making the bacterial suspension which will be incorporated in the clot; and some sterile 1 % solution of $CaCl_2$ in physiological saline, for mixing with the blood to cause coagulation.

Just before the test a thick suspension of the *Staphylococcus* is made from the agar culture in a few drops of saline solution in the bottom of a sterile test-tube, and this is diluted with $CaCl_2$ solution until it has the opacity of Brown's no. 1 opacity tube, i.e. 300–400 million cocci per c.c.

PROCEDURE

The tip of a gauged capillary pipette is cut off; a grease-pencil mark is made about 2 cm. from the end and a rubber teat is fitted on the barrel.

Nine volumes (up to the mark) of the sterile blood and one volume of the bacterial suspension are drawn up and the whole is blown out into a small sterile tube and well mixed. The mixture is then drawn up again so as nearly to fill the gauged part of the capillary.

A little air is now allowed to enter the tip, so that the latter can be sealed off in a flame without heating the contents. After sealing, the teat is removed.

The blood-bacteria mixture is now allowed to clot in the capillary, which takes about 10 min. at room temperature. During this time the pipette should be frequently rotated to prevent sedimentation of the corpuscles. To help in judging that coagulation has taken place it is useful to watch the residue of the blood-bacteria mixture in the small tube in which it was mixed; for when that coagulates it may be assumed that the blood in the capillary has also clotted.

The next step is to cut off the tip of the capillary and to blow the clot out into some sterile saline solution in a good-sized Petri dish. The layer of saline should be fairly deep, otherwise surface tension makes the next procedure difficult. The dish is now placed on white paper which has been marked with a line divided into lengths of 1 cm. The cover is removed, and with a flamed scalpel in the right hand and a stout needle or nichrome spatula in the left the clot worm is manipulated so as to lie along the centimetre line, when it can be cut into roughly centimetre lengths with the scalpel.

In this way ten or a dozen standard clot segments containing a constant dose of cocci are prepared.

The disinfectant solution is poured into a small Petri dish, and a clot segment is hooked out and placed in the disinfectant, where it is left for the desired time (in these experiments either 5 or 30 min.). Then it is removed with the hook, rapidly rinsed in a tube or vessel of sterile water, and then placed in a bottle of blood broth.

The next manipulation, which is the only one that presents any difficulty, is the crushing of the clot segment to release the bacteria and quickly dilute any disinfectant remaining in the clot. With the nichrome spatula the segment is drawn up on to the inner wall of the bottle above the medium, and with the bottle tilted the clot is crushed against the glass by pressing and twirling movements of the spatula until no large fragments remain. This is easy when protein-coagulating disinfectants have been used, but in other cases may be rather difficult.

The culture bottle containing the crushed clot is now incubated, and records of any growth that may develop are made after 1, 2–3 and 7 days' incubation.

The growth of *Staph. aureus* in the blood broth is characteristic, developing in definite colonies either on the plaque of erythrocytes on the bottom of the bottle or suspended in a fibrinous coagulum which forms in the fluid. It is even possible to make a rough estimate of the number of viable cocci in the clot segment according to the number of colonies that develop. Growth is often delayed till the 2nd or 3rd day, and occasionally till the 6th or 7th.

For preliminary approximate estimations of the disinfecting power of a substance two or three segments should be tested in each (two-fold) dilution of the disinfectant. For full, final estimations ten or twelve segments should be treated in each dilution, and the whole experiment should be repeated once or twice.

Controls

(a) *Viability of the bacteria in the untreated clot.* A segment or part of one is crushed in a bottle of the medium at the end of the experiment and incubated with the others. A rich growth should occur.

(b) *Sterility of the blood.* An uninoculated blood-broth bottle, incubated with the others, should remain sterile throughout the experiment.

(c) *'Bacteriostatic' control.* With highly 'bacteriostatic' chemicals, such as mercury or the detergents, it has been the practice to put up a 'bacteriostatic' control for the strongest concentration of the substance used in the test. This is done by transferring into a culture bottle a clot segment which has been exposed to the disinfectant for the standard time, crushing it and then inoculating the medium with a minimal inoculum (e.g. a needle dipped about 0·5 cm. into the bacterial suspension). If a 'bacteriostatic' dose of the disinfectant has been carried over it will inhibit both the viable bacteria, if any, in the clot and also those of the inoculum. In this case the test is a failure; but it should never happen if the clot has been properly rinsed.

(d) *Phenol-controls.* In the later stages of the work each experiment was controlled by exposing segments to two or more phenol-dilutions for 30 min. For clots containing 400,000 cocci phenol 1 in 20, which, though it does not regularly sterilize, always prevents growth in 24 hr. of incubation, and 1 in 160 which never disinfects (see Table 4) were used; and for 4000 cocci 1 in 40 and 1 in 160 dilutions. Unless the results with these were in accordance with prediction, the test was considered invalid, but this hardly ever happened.

Number of tests and degree of accuracy

Early hopes that constant results would be obtained by tests of single segments were not realized, and it gradually became clear that different clots and different segments of a single clot were sufficiently variable in such factors as size, density, bacterial content and dispersion to necessitate multiple tests and the calculation of averages.

Whereas the approximate potency of a substance in this test can be estimated by exposing one or two segments to each (two-fold) dilution of the disinfectant, a precise measurement can only be obtained with numerous segments. In Table 4 and Fig. 1 the clot-disinfecting activity of phenol is shown under varying conditions. The individual figures will be considered later (under 'Phenol'), and we need only note here that they show a decreasing proportion of 'kills' as the dilution increases, and that the concentration that regularly kills is four times greater than that which regularly fails to kill. With other disinfectants this ratio may be greater or less than four.

A similar variability governs the results of exposing clots for variable times to a single concentration. Thus a precisely reproducible result can only be obtained by a large expenditure of energy; yet the approximation which is more easily achieved may be all we need for practical application, since we can allow for a wide margin of error by recommending for practical use a concentration greater than the minimal one found effective in our tests.

The method will also give a reasonably true comparison of two disinfectants, so that we can, if we wish, express the activity of a substance in relation to that of phenol as a 'clot-disinfection phenol-coefficient' always specifying the temperature and time of action.

Preliminary tests of antibacterial action. Before testing its clot-disinfecting power, each substance was tested for its (a) *rapid bactericidal titre,* i.e. the lowest dilution that will sterilize 10–20 million bacteria per c.c. in water in 5 min. at 17–20° C., and (b) *slow bactericidal or 'bacteriostatic' titre,* i.e. the lowest dilution that will prevent the multiplication of about 2000 cells per c.c. in heart-extract peptone broth incubated at 37° C. for about 20 hr. The results of these tests are shown in Table 1.

TESTS OF CLOT-DISINFECTING POWER

(1) *Ethyl alcohol* and other volatile solvents. The rapid bactericidal titre (as defined above) of ethyl alcohol is about 1 in 3·3, or 30 %, and its 'bacterio-

centration. One can say that the small dose is nearly always killed in 30 min. and at least sometimes in 5 min. but as the larger dose so often survives, even 30 min. ethyl alcohol cannot be recommended as a safe clot disinfectant.

Table 1. *Bactericidal power of chemicals on naked bacteria, expressed as the denominator of the highest effective dilution*

Chemical in aqueous solution	5 min. at 20° C. in water		'Bacteriostatic' (or slow bactericidal) in broth at 37° C. for 24 hr.			
	Staph. aureus	*Ps. pyocyanea*	*Strep. pyog.*	*Staph. aureus*	*Salm. typhi*	*Ps. pyocyanea*
Phenol	100	150	400	500	400	800
Lysol	500	200	800	1,000	800	·400
Parachormetacresol	500	1,000	5,000	10,000	5,000	5,000
Osyl*	100	10	1,600	1,600	100	100
Chloros (= 10 % Cl)	15,000	15,000	20	40	10	15
Iodine (+ KI)	200,000	200,000	1,000	1,000	1,000	1,000
Mercuric chloride	0†	40	160,000	20,000	100,000	20,000
Merthiolate	0	0	2,000,000	4,000,000	2,000,000	250,000
Phenyl merc. nitrate	0	0	2,000,000	5,000,000	1,000,000	8,000
CTAB (Cetavlon)‡	16,000	4,000	50,000	150,000	10,000	400
Zephiran§	3,000	2,000	30,000	200,000	3,000	400
Dettol‖	50	25	2,500	2,500	1,000	100
Pot. permanganate	1,500	1,500	200	100	800	100
Formalin	5	10	10,000	20,000	30,000	10,000
Proflavine sulphate	0	0	80,000	40,000	40,000	3,000

* Osyl = benzyl cresol 3·15 %, monochlorxylenol 1·25 % and a little comp. thyme oil.
† 0 in each case means negative at the highest procurable concentration.
‡ CTAB = cetyltrimethylammonium bromide, a kationic detergent.
§ Zephiran = a 10 % sol. of alkyldimethylbenzylammonium chlorides; a kationic detergent.
‖ Chlorxylenol basis.

Table 2. *Ethyl alcohol. Clot disinfection* (Staph. aureus)

The figures show the number of clot segments which gave the result indicated. The total number tested = D + L.

No. of cocci ...	400,000				4000			
Time (min.) ...	5		30		5		30	
Ethyl alc. % (vol.)	D	L	D	L	D	L	D	L
97	11	15	9	6	1	0	20	1
70	0	2	1	11	—	—	18	0
50	0	1	2	1	1	0	9	0

D, dead (or disinfected); L, living (not disinfected).

static' titre is known to be about 1 in 5 (20 %) for *Salm. typhi*. It differs therefore from most disinfectants in so readily losing its power on dilution.

Although ethyl alcohol with little or no water in it is inactive against dry bacteria, in the clot-disinfection test there is enough water in the clot to activate the alcohol, and no certain difference in effect between 97, 70 and 50 % has been established.

Table 2 shows that the larger dose (400,000) of cocci per segment is not regularly killed either in 5 or 30 min., whereas the smaller dose is killed in 30 min. with considerable regularity, only a single failure having been registered with the 97 % con-

Methyl alcohol, propyl alcohol, acetone and *ether* were only tested for the 5 min. period on the larger dose of cocci. None of them disinfected more than half of the ten or more segments treated.

(2) *Iodine in aqueous* KI *solution* (KI twice the strength of I). Its rapid bactericidal titre is 1 in 200,000, and its 'bacteriostatic' titre (owing to neutralization by broth) is 1 in 1000.

Table 3 shows that iodine at 1 in 40 was effective in 30 min. against clots containing both the larger and the smaller numbers of cocci, but that in 5 min. it often failed with the larger number and occasionally with the smaller. 1 in 80 gave variable results,

and the same is true down to 1 in 640, while 1 in 1280 failed in the only two tests done. This is a longer spread of partially effective dilutions than is seen with most disinfectants. Iodine is rapidly neutralized by blood, but its penetrating power seems very good, and its rapid bactericidal power is so great that a fairly high dilution is needed to abolish the effect completely. This stands in contrast with phenol (Table 4), the bactericidal activity of which is much lower. Comparison of the two tables shows that while the activities of iodine and phenol in 30 min. on the smaller dose of bacteria

shorter times would be effective in practice if no appreciable masses of infected material were to be disinfected.

(3) *Chlorine.* This was used in the form of 'Chloros', a commercial disinfectant said to contain the equivalent of some 10% free Cl. In strong concentration it has a powerful solvent action on blood-clot.

The 5 min. *bactericidal titre* of *chlorine* in this form is about 1 in 150,000, but its '*bacteriostatic*' titre is only 1 in a few hundreds, because chlorine is so much inactivated by the constituents of broth.

Table 3. *Iodine in aqueous* KI *solution. Clot-disinfecting activity at* 17–20° C.

Figures in brackets indicate tests incubated only for 3 days. A few of the D's might have become L's in 7 days.

No. of cocci ...	400,000				4000			
Time (min.) ...	5		30		5		30	
Dilution of iodine	D	L	D	L	D	L	D	L
1 in 20	(4)	(3)	—	—	23	1	—	—
40	(4)	(5)	24	0	—	—	6	0
80	(1)	(8)	7	2	—	—	2	2
160	0	(2)	5	4	—	—	4	2
320	0	(2)	5	4	—	—	3	5
640	—	—	0	2	—	—	3	3
1280	—	—	—	—	—	—	0	2

D, dead (disinfected); L, living (not disinfected).

Table 4. *Phenol. Clot-disinfecting activity at* 17–20° C. (Staph. aureus)

No. of cocci ...	400,000						4000					
Time (min.) ...	5			30			5			30		
Dilution of phenol	D	L	D per 10	D	L	D per 10	D	L	D per 10	D	L	D per 10
1 in 20	10	15	4	11	6	6·5	34	2	9·5	6	0	10·0
40	—	—	—	8	9	4·7	4	8	3·3	26	0	10·0
80	—	—	—	3	14	1·8	—	—	—	22	9	7·1
160	—	—	—	0	15	0·0	—	—	—	0	28	0·0

D, disinfected; L, living (not disinfected).

The total number of clot segments tested in each way is D + L. 'D per 10' $= \dfrac{D}{D+L} \times 10$.

are about equal (i.e. the clot-disinfection phenol-coefficient of iodine *in these conditions* is about 1), on the larger number of bacteria the 30 min. coefficient is more than 2. It is clear enough that a phenol-coefficient which varies with the number of bacteria must be used with very great reserve.

To sum up, to leave a good margin of safety in practice, iodine in KI solution would have to be used at about 1 in 20 (5%) and applied for some 30 min., which makes it not very practicable as a rapid disinfectant. It must always be remembered that the standard clot disinfection (with 400,000 cocci) is a severe test. Lower concentrations for
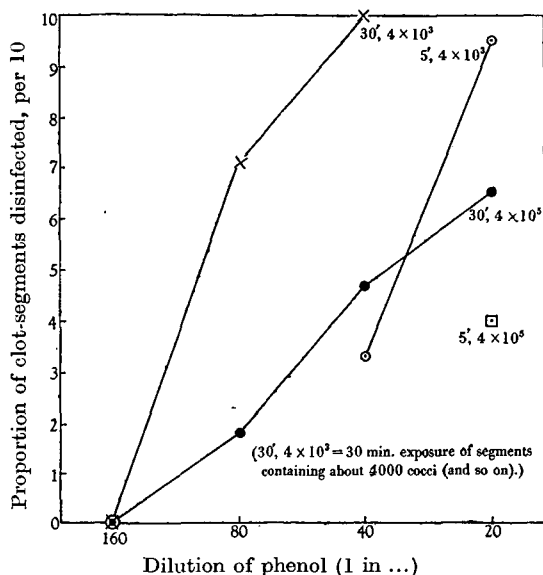
The few *clot-disinfection tests* that were done showed that even undiluted Chloros (chlorine 1 in 10) failed to disinfect either the large or the small number of bacteria in 5 min.; but that in 30 min. it disinfected the large number at a 1 in 2 (Cl 1 in 20) dilution and the small number at 1 in 4 (Cl 1 in 40).

Thus although the number of tests was insufficient to justify definite statements, the potency of chlorine in this form is clearly of the same order as that of iodine. In short, Chloros at 1 in 2 sterilizes blood-clots of standard size in 30 min. but is not effective in 5 min.

(4) *Phenol and cresols* (Table 4 and Fig. 1). Phenol has a 5 min. *bactericidal titre* of 1 in 100, and a 'bacteriostatic' titre of 1 in 500. The numerous clot-disinfecting tests that were done show that when 400,000 cocci are contained in the segment, the strongest available concentration, 1 in 20, did not disinfect more than a proportion of clot-segments in 5 min. or even in 30 min. With 4000 cocci, however, 1 in 20 disinfected nearly (but not quite) all segments in 5 min.; and both 1 in 20 and 1 in 40 were completely successful in 30 min.

The figures show how the proportion of successes (expressed as the number per 10 trials) decreases as the dilution of the phenol is increased, until none is obtained at 1 in 160.

Clot disinfection (*Staph. aureus*) by phenol at 17–20° C.

(30', 4 × 10³ = 30 min. exposure of segments containing about 4000 cocci (and so on).)

Dilution of phenol (1 in ...)

Thus phenol could not be relied on to disinfect heavily fouled apparatus in a few minutes at room temperature, but would be effective at 1 in 20 or more on relatively clean apparatus if given upwards of half an hour to act.

Lysol (Marshall's) was not so thoroughly investigated, but it was found that with 400,000 cocci even at 1 in 10 it will not disinfect all segments either in 5 min. or in 30 min. With 4000 cocci, however, it was successful in 30 min. down to 1 in 80, or possibly 1 in 160.

Its bactericidal and bacteriostatic powers (Table 1) are about twice as great as those of phenol, and its clot-disinfection phenól-coefficient (30 min., 4000 cocci) would probably work at about 2·0; but like phenol, it did not prove equal to the maximum requirements of this investigation.

Chlorocresol (*p*-chlor-*m*-cresol), which has a

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bactericidal *titre* (5 min.) of about 1 in 1000 and a 'bacteriostatic' titre of 1 in 10,000, is only soluble in water at 1 in 250 or more. At that concentration it fails to disinfect the majority of clot segments with 400,000 cocci in 5 min. Other times and doses were not tried; but a 5 % solution in (*a*) acetone and (*b*) 97 % alcohol was also unsuccessful in about half the trials (5 min., 400,000 cocci).

For this purpose, therefore, it does not seem better than phenol, or lysol, if as good.

Izal and Cyllin also failed at all concentrations in the 5 min., 400,000 clot-disinfection test, and the same is true of *Osyl*, a proprietary disinfectant containing 3·15 % of benzyl cresol and 1·25 % of monochlorxylenols. No doubt all of these substances would be effective in a longer time or against fewer bacteria.

(5) *The cationic detergents, Zephiran* and *CTAB* (Cetavlon), proved disappointing in spite of their highly selective action on Gram-positive cocci. Bactericidally active in water to a titre of 1 in 15,000 to 1 in 30,000 (N.B. Zephiran is about a 1 in 10 solution of its active constituents) and having 'bacteriostatic' titres of 1 in 200,000 or more, they nevertheless penetrate blood-clot so poorly that they cannot at any concentration regularly disinfect the standard clot with 400,000 cocci in 5 or 30 min.

A few tests of Zephiran with 4000 cocci, however, indicated a good disinfection in 30 min. at dilutions up to about 1 in 80. Here again, the number of bacteria acted on is clearly a determining factor.

(6) *Chlorxylenols. Dettol.* Different samples of Dettol gave bactericidal titres against *Staph. aureus* of 1 in 10 to 1 in 100, and 'bacteriostatic' titres around 1 in 1000. Both at full strength (five tests) and diluted from 1 in 2 to 1 in 8 (three tests each) it failed to disinfect in 5 min. clots containing 400,000 cocci. (It should be noted that the mixture of substances sold commercially as Dettol is variable, and its potency may therefore be altered by changes of constituents.) No tests with fewer cocci or longer time were done, so it is impossible to say what conditions it requires to be effective, but enough was done to show that it is not the powerful rapid disinfectant that was being sought. Its merits as a non-irritant skin-antiseptic are not called in question by these tests.

(7) *Mercury compounds.* These are well known to be slow in their bactericidal effect, but to exert a rapid 'bacteriostatic' action which is reversible by the addition to the culture medium of mercury-neutralizing substances such as sodium thioglycollate (1 in 1000). With the use of this substance bactericidal titres (5 min.), 'bacteriostatic' titres (24 hr.) and clot-disinfection activities were established for three compounds, with *Staph. aureus* as follows:

Compound	Bactericidal titre (5 min.)	'Bacteriostatic' (slow bactericidal) titre (24 hr.)	Clot disinfection (5 min.) 400,000 cocci
Mercuric chloride	0 (= < 1 in 20)	1 in 20,000	0 (= < 1 in 20)
Merthiolate	0 (= < 1 in 200)	1 in 4,000,000	0 (= < 1 in 200)
Phenyl mercuric nitrate	0 (= < 1 in 8000)	1 in 5,000,000	0 (= < 1 in 8000)

The limiting dilutions shown represent the highest practicable concentrations. Mercury compounds are thus clearly useless for rapid clot disinfection, as indeed they are for any rapid destruction of bacteria; a fact which contrasts with their very strong and irreversible effect if given plenty of time.

(8) *Miscellaneous substances.* (a) *Formalin* (40 % formaldehyde solution) is another slow disinfectant. Its 5 min. *bactericidal titre* is only 1 in 5, while its *bacteriostatic* effect in 24 hr. runs up to 1 in 20,000. At 1 in 4 (10 % formaldehyde) it only disinfected 6 out of 12 segments containing 400,000 cocci in 5 min. A few tests of 30 min. exposure with both 400,000 and 4000 cocci indicated a high degree of disinfection up to a 1 in 8 dilution. Thus formalin, which in strong concentration is unpleasant to handle, is not well suited for rapid disinfection of septic matter.

(b) *Proflavine sulphate.* 5 min. *bactericidal titre* = 0 (at 1 in 500, which is the greatest feasible concentration). 24 hr. 'bacteriostatic' *titre* = 1 in 40,000. *Clot disinfection*, 5 and 30 min., 400,000 and 4000 cocci, three tests each, all failed to disinfect. The flavines have not the solubility, speed or power for rapid disinfection of apparatus; but their slow action and lack of toxicity make them very good wound-antiseptics.

(c) *Potassium permanganate.* 5 min. *bactericidal titre* = 1 in 1000, 24 hr. 'bacteriostatic' *titre* = < 1 in 100 (owing to neutralization by broth). *Clot disinfection*, 5 min., 400,000 cocci, unsuccessful in single trials at 1 in 20 and higher dilutions.

(d) The following substances also failed to disinfect in 5 min. single (or a few) clot segments with 400,000 cocci: H_2O_2, undiluted solution; *Zant* (undiluted) (this is of unknown composition, but seems to contain a xylenol); *Neopantocid and catalysator*, a chlorine-releasing compound of Russian origin, with a 5 min. bactericidal titre of 9000 and a 'bacteriostatic' titre of about 2000 failed to disinfect a single clot segment at 1 in 20, but was successful at 1 in 10. It was not, however, proved that it will regularly disinfect at that strength.

Iodine 2 % in 70 % alcohol constantly failed in 5 min., but succeeded nine times out of twelve in a 30 min. exposure. *Acriflavine at 1 in 1000 in 70 % alcohol* was only tried at 30 min. exposures, which disinfected all of 12 clots (400,000 cocci), indicating a slow synergic action with the alcohol.

SUMMARY AND CONCLUSIONS

1. A method is described for estimating the disinfecting power of chemical substances on small cylindrical blood-clots of standard dimensions (about 1·5 mm. in diameter) containing a measured number of bacteria (*Staph. aureus*). It is capable of giving guidance for the use of disinfectants under the described conditions.

2. No practicable disinfectant regularly sterilized heavily infected clots (400,000 cocci) in 5 min. at 17–20° C.

3. In 30 min. the following were the only substances that were uniformly successful with heavily infected clots: 2·5 % iodine in aqueous KI solution; 50 % Chloros, containing about 5 % chlorine; 70 % alcohol containing 0·1 % acriflavine. (The first of the three was tested on a larger scale than the others, and its potency is therefore the more firmly established.)

4. Reduction of the number of cocci in the clot increased the range of effective disinfectant solutions. Thus with 4000 cocci per clot regular clot disinfection was achieved in 30 min. with the following additional solutions: ethyl alcohol 70 and 50 %; Chloros 25 %; phenol 2·5 % and lysol 1·25 %. A few tests indicated that Zephiran (and probably CTAB) at 1 or 2 % concentration would also pass this less exacting test.

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