STUDIES ON THE INFLUENCE OF VARIOUS ORGANIC SUBSTANCES UPON THE PHENOL COEFFICIENT

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The efficacy of disinfectants may not always be appraised from their behaviour in pure state. Most often they will have to exert their effect in the presence of other substances. The following will present some studies that were carried out in order to illustrate the influence of various substances which may conceivably be present where disinfectants are applied. For this purpose we selected ascites fluid, faeces, horse serum, albumin (hen's egg), milk, sputum, and urine (as a protein-free substance).

We have taken up this question for investigation in connexion with the method elaborated by us (Jensen & Jensen, 1933) for determination of the phenol coefficient of disinfectants. After this cover-slip method, the strength of the disinfectant is estimated by placing a suspension of *Staphylococcus aureus*, letting it dry and then stand for 2 min. in disinfectants of different strength; comparison is made with a phenol solution of known strength. The phenol coefficient is the numerical expression of the proportion between the two solutions that kill within 2 min. the dried cocci on the cover-slip.

The present studies concern the addition of different organic materials in varying amounts to phenol and other disinfectants. Finally, a tabular survey is given of the phenol coefficients of some disinfectants with and without addition of the above-mentioned organic substances in order to estimate their effect upon the phenol coefficient, which is the numerical expression of the bactericidal power of the disinfectant under analysis.

Undoubtedly, when a disinfectant is employed in practice, there will always be present some organic material varying in nature and in different amounts. It may be that a method which is based, for instance, on the addition of faeces is quite worthless for estimation of the efficacy of a disinfectant if it is applied to materials containing, for example, serum or urine.

According to our findings, addition of the latter substances gave such variations in the apparent efficacy of the disinfectants that it is not justified from the effect of one substance to draw any conclusion about the effect of another. It is evident from a review of the literature that most of the previous investigators who have tried to estimate the phenol coefficient of a disinfectant, calculated after addition of one substance or another, have not been very successful. The addition of yeast appears particularly absurd, as yeast is not a substance that is likely to be present under any normal conditions of disinfection.

Review of literature

Winter Blyth (1906), who used both dissolved and suspended organic substances, emphasizes the effect of suspended material (faeces) with reference to reduction in the bactericidal power of the disinfectant when this has the character of an emulsion. But he does not consider faecal material constant enough in composition to be employed in a standard method, and he suggests instead the use of milk as standard material, since it is easy to work with and fairly constant in composition.

Blyth employed *Bacillus coli communis*, and the tests were made at a temperature of 12° C. From his findings it appears that pure phenol is affected only to a slight extent, whereas there is a rather marked reduction in the bactericidal power of the cresols, increasing with increased addition of organic material.

Kenwood (1905–6) reports that the disinfecting power of phenol was affected but little by the presence of urine and faecal material in tests after Rideal and Walker's method, whereas emulsified disinfectants were reduced to two-thirds or one-half of their original bactericidal value.

Somerville & Walker (1906–8) assert that faecal material is too irregular in composition and hence too uncertain in its influence upon disinfectants to be serviceable in such tests. These authors performed tests with the addition of 1% gelatin, casein, mucin, peptone, serum and blood, and they found that the disinfecting power of hypochlorites and potassium permanganate is greatly impaired, whereas cresols are affected relatively little. Later these authors suggested the use of 0·5% gelatin and 0·5% rice starch in solution as the organic substance.

The Admiralty test (Gibson, 1932) is made with finely powdered rice starch and gelatin.

The Chick-Martin test (Chick & Martin, 1908) is made with dried, sterilized and powdered faeces (3%) as addition to the disinfectant, but not to the phenol solution. Instead, the factor 1·2 is employed in the calculation of the coefficient, as the fall in the disinfecting power of phenol on addition of faecal material is said to be constant. Moreover, glucose broth is used for the subculture—an excellent culture medium which undoubtedly contributes to reduce the relatively low value obtained by this method. Coefficients over 4 are rare findings; as a rule they are considerably lower. In her mention of the method, M. M. Barratt (1931) emphasizes the uniform results it gives, and she points out that it would be desirable to find some substitute for faecal material as it is difficult to prepare it properly.

On the basis of the Chick-Martin test, L. P. Garrod (1934, 1935) has elaborated a method for determination of the bactericidal power of disinfectants in the presence of organic substance. He uses yeast instead of faecal material, stating that yeast is uniform in composition and gives uniform results. This

investigation is a part of the work carried out by the Committee on British Standards, whose task it was to devise a technique for a British standard method for the determination of the bactericidal power of disinfectants.

TECHNIQUE

For details of the methods used in this work the reader is referred to the paper by Jensen and Jensen (1933). Here it will suffice to outline the technique.

From a 24 hr. agar-slant culture of *Staphylococcus aureus* a suspension is made in a few c.c. of sterile physiological salt solution, and a loopful of this suspension is placed on cover-slips (one loopful on each) and then allowed to dry. It is then placed in phenol and disinfectant solutions of varying strength. The cover-slips with the dried bacterial suspension are then twice washed in sterile distilled water and placed in test-tubes (one cover-slip in each tube) containing 10 c.c. of sterile veal infusion broth. The diameter of the test-tubes is such that the cover-slips can stand on edge in them. The subculture is incubated at 37° C. for 48 hr.

ORGANIC MATERIALS EMPLOYED

As already mentioned, the organic substances used in these tests were: ascites fluid, faecal material, horse serum, albumin (hen's egg), milk, sputum, and urine (as protein-free material).

Both the ascites fluid and the horse serum were withdrawn under aseptic precautions. The albumin was withdrawn from fresh eggs under aseptic precautions and mixed with 3 parts of sterile physiological salt solution. Urine was filtered through a Chamberland filter. Skimmed milk was mixed with equal parts of sterile distilled water and pasteurized at 60° C. for 2 hr. Attempts were made to work with pasteurized sputum in order to avoid possible changes in the sputum from autoclaving, but subsequent sterility tests showed that all the sputum specimens (originating from different places) were non-sterile, so that it was necessary to autoclave the sputum. The faeces were dried by smearing them in a thin layer upon a glass plate, which was placed over a steam-heat radiator; after drying, this material was ground finely in a mortar and sifted through a very fine sieve; then it was weighed and ground with 10 times its weight of physiological salt solution, forced through fine gauze and autoclaved. Thus the size of the particles became fairly uniform, even though a good deal of the dry substance was retained by the gauze. But, without this filtration, it would not have been possible to pipette the emulsion with sufficient accuracy. The various substances (withdrawn aseptically, sterilized by filtration, or pasteurized) were all observed for sterility by incubating for 1 week before use.

Behaviour of phenol on addition of organic substances

The same strain of *Staphylococcus aureus* was employed in all the tests and the limit of bactericidal action was established by adding respectively 5, 10, 25, and 50% of organic substance to the phenol solution, making concurrent tests with a pure phenol solution in order to follow the usual variations, which can always be ascertained from day to day. Probably these variations are due to differences in the age of the various organic elements and to the resulting variation of resistance. Besides, it may also happen that a single bacterium which has escaped killing is carried over into the subculture.

Several experiments have been made with each of the substances, not only to establish the limit, but also to follow the changes within this limit. The pure phenol solution serves as control. Table I gives the result obtained in one of these experiments for each substance and also the control test with pure phenol solution.

Table I. 24 *hr. agar-slant culture of* Staph. aureus *at* 37° C. *Subcultures incubated at* 37° C. *for* 48 *hr.*

Phenol with addition of	5 %	10 %	25%	50 %	Pure phenol
Ascites fluid	1 : 50 0	1 : 40 0	1 : 50 0	1 : 50 0	1 : 55 0
	1 : 55 0	1 : 45 0	1 : 55 0	1 : 55 +	1 : 60 0
	1 : 60 +	1 : 50 0	1 : 60 +	1 : 60 +	1 : 65 +
	1 : 65 +	1 : 55 +	1 : 65 +	1 : 65 +	1 : 70 +
Faeces	—	—	—	1 : 50 0	1 : 55 0
	—	—	—	1 : 55 0	1 : 60 0
	—	—	—	1 : 60 +	1 : 65 +
	—	—	—	1 : 65 +	1 : 70 +
Horse serum	1 : 40 0	1 : 40 0	1 : 40 0	1 : 40 0	1 : 55 0
	1 : 45 0	1 : 45 0	1 : 45 0	1 : 45 0	1 : 60 +
	1 : 50 0	1 : 50 0	1 : 50 0	1 : 50 +	1 : 65 +
	1 : 55 +	1 : 55 +	1 : 55 +	1 : 55 +	1 : 70 +
Egg albumin + salt solution (1 + 3)	—	1 : 55 0	—	1 : 40 0	1 : 55 0
	—	1 : 60 0	—	1 : 45 0	1 : 60 0
	—	1 : 65 +	—	1 : 50 0	1 : 65 +
	—	1 : 70 +	—	1 : 55 +	1 : 70 +
Milk and water	1 : 30 0	1 : 45 0	1 : 45 0	1 : 40 0	1 : 55 0
	1 : 40 0	1 : 50 0	1 : 50 0	1 : 45 0	1 : 60 0
	1 : 50 0	1 : 55 0	1 : 55 0	1 : 50 0	1 : 65 0 +
	1 : 60 +	1 : 60 +	1 : 60 +	1 : 55 +	1 : 70 +
Sputum	—	1 : 45 0	1 : 45 0	1 : 50 0	1 : 50 0
	—	1 : 50 0	1 : 50 0	1 : 55 +	1 : 60 +
	—	1 : 55 0	1 : 55 +	1 : 60 +	1 : 65 +
	—	1 : 60 +	1 : 60 +	1 : 65 +	1 : 70 +
Urine	1 : 55 0	1 : 55 0	1 : 55 0	1 : 55 0	1 : 55 0
	1 : 60 0	1 : 60 0	1 : 60 0	1 : 60 0	1 : 60 0
	1 : 65 +	1 : 65 0	1 : 65 0	1 : 65 +	1 : 65 +
	1 : 70 +	1 : 70 +	1 : 70 +	1 : 70 +	1 : 70 +

https://doi.org/10.1017/S0022172400010998 Published online by Cambridge University Press

From Table I it is evident that the disinfectant power of phenol is affected but slightly by the addition of the various organic substances, and although its germicidal power is attenuated a little by addition of ascites fluid, horse serum, milk, sputum, and albumin, the addition of urine (the only protein-free substance in these tests) causes no weakening of the disinfectant power of phenol—rather a reverse tendency. But it has to be kept in mind that within the established limits of this method there are variations from day to day in the resistance of the bacteria (*Staph. aureus*) to phenol, as is also evident from the figures in the column headed "Pure phenol". We see, then, that among the results recorded, and they are only a small part of those we have done, killing took place 4 times in 1 : 60, and twice in 1 : 55 dilution. That urine does not affect the disinfecting power is probably due to the fact that it is a protein-free substance. That the presence of urine in a few instances increases the germicidal power of the disinfectant is probably attributable to some special chemical conditions that have not been examined yet. From Table I it is further evident that the amount of the added substances has no influence upon the disinfecting power of phenol, as the variations in the result fall inside the normal limits of daily variations.

In order to ascertain whether it makes any difference to the result if the bacteria are suspended in organic substance instead of suspension in physiological salt solution and addition of organic substance to the phenol solution, tests were made, as shown in Table II, with suspension of *Staph. aureus* in horse serum + salt solution (1 + 3), in egg albumin + salt solution (1 + 3), and in sputum. Tests were also made with suspensions of the bacteria in undiluted horse serum, but the results varied greatly; presumably the albumin coagulates by drying on the cover-slips, forming clumps of bacteria which are thus prevented from contact with the phenol.

Table II. 24 *hr. agar-slant culture of* Staph. aureus *at* 37° C. *Subcultures incubated at* 37° C. *for* 48 *hr.*

	Bacteria suspended in			
	Horse serum + salt solution (1 + 3)	Egg albumin + salt solution (1 + 3)	Sputum	Physiological salt solution
Phenol	1 : 55 0	1 : 40 0	1 : 55 0	1 : 55 0
	1 : 60 +	1 : 45 0	1 : 60 0	1 : 60 0
	1 : 65 +	1 : 50 0	1 : 65 +	1 : 65 +
	1 : 70 +	1 : 55 +	1 : 70 +	1 : 70 +

Comparing Table I with Table II it is seen that the results in Table II are quite in line with the corresponding values in Table I, so that from this we may draw the conclusion that the disinfecting power of phenol is affected in the same way, whether the bacteria are suspended directly in the organic substance and dried with this on the cover-slip, or the substance is added in various amounts to the phenol.

Age of organic substance

The question may be asked: does the age of the organic substance play any role in its influence upon the bactericidal power of the disinfectant? This question was investigated with horse serum to which phenol solution had been added in equal amount. As is evident from Table III, the age of the serum had no influence upon the result.

Table III. 24 *hr. agar-slant culture of* Staph. aureus *at* 37° C. *Subcultures incubated at* 37° C. *for* 48 *hr.*

	Horse serum from			
	October 1934	September 1935	March 1936	Pure phenol
Phenol with serum. Equal parts	1 : 45 0	1 : 45 0	1 : 45 0	1 : 55 0
	1 : 50 +	1 : 50 +	1 : 50 +	1 : 60 0
	1 : 55 +	1 : 55 +	1 : 55 +	1 : 65 +
	1 : 60 +	1 : 60 +	1 : 60 +	1 : 70 +

Behaviour of other disinfectants on addition of organic substances

We may now turn to the behaviour of various types of disinfectants in the presence of organic substances. For this purpose we have selected three commonly used trade cresols, namely: Desol, Kilcrobe and Lysol; besides, phenosalyl (40% C_6H_5OH) and, as a representative of the chlorine-containing disinfectants, paramonochlorphenol ($C_6H_4Cl\,OH$); and also Caporit and Caporitol (hypochlorites). The two last-mentioned disinfectants showed a cover-slip phenol coefficient of 8·0 and 9·0 respectively; but it was weakened so markedly by addition of organic material, that the tests would fall below the solubility of the disinfectant, and hence these two hypochlorites had to be excluded from further studies. Finally, Alcorcin (hexylresorcinol), which has an unusually high cover-slip phenol coefficient, is included in these comparative tests.

Dyes are not included in the present studies, as one of us (Elsa Jensen, 1935) has already shown that the cover-slip method is unsuitable for the determination of the bactericidal power of dyes, partly because in some instances the dye absorbed by the bacteria does not take any effect in the first 24 hr. and partly because the diffusion of the dye into the subculture sometimes inhibits the growth of the bacteria and finally kills them.

As shown in Table IV, the cover-slip phenol coefficient of these substances was determined after addition of 10 and 50% of the various organic substances, and on direct suspension of the bacteria in sputum, horse serum + salt solution (1 + 3) and egg albumin + salt solution (1 + 3), and drying with this on cover-slips. The cover-slip phenol coefficient is calculated both after pure phenol solution and after phenol solution with addition of organic material.

Table IV. *Phenol coefficient of various disinfectants in the presence of various organic substances*

Organic substance added	%	Alcorcin 43·4 Coefficient calculated with: Phenol (pure)	Alcorcin 43·4 Coefficient calculated with: Phenol + organic substance	Desol 3·8 Coefficient calculated with: Phenol (pure)	Desol 3·8 Coefficient calculated with: Phenol + organic substance	Kilcrobe 3·6 Coefficient calculated with: Phenol (pure)	Kilcrobe 3·6 Coefficient calculated with: Phenol + organic substance	Lysol 1·3 Coefficient calculated with: Phenol (pure)	Lysol 1·3 Coefficient calculated with: Phenol + organic substance	Paramonochlorphenol 3·1 Coefficient calculated with: Phenol (pure)	Paramonochlorphenol 3·1 Coefficient calculated with: Phenol + organic substance	Phenosalyl 2·8 Coefficient calculated with: Phenol (pure)	Phenosalyl 2·8 Coefficient calculated with: Phenol + organic substance
Ascites fluid	10	—	—	2·0	2·2	1·8	2·5	1·1	1·2	3·3	3·6	1·6	1·8
,,	50	—	—	1·6	1·8	1·1	1·2	1·1	1·2	2·5	2·8	0·7	0·8
Faeces	50	—	—	1·3	1·3	0·7	0·7	1·0	1·0	2·0	2·0	0·7	0·7
Horse serum	10	4·5	5·0	2·2	2·4	2·3	3·1	1·1	1·2	2·9	3·2	1·3	1·4
,,	50	<0·5	<0·5	1·2	1·3	0·5	0·7	0·7	0·9	1·5	1·8	0·5	0·7
Egg albumin	10	—	—	1·7	1·7	2·7	2·7	1·2	1·2	3·0	3·0	1·5	1·5
,,	50	—	—	1·3	1·8	1·7	2·2	0·8	1·1	1·8	2·4	0·7	0·9
Milk and water	10	6·0	7·7	2·3	2·5	1·8	2·0	1·3	1·6	3·7	4·1	1·8	1·8
,,	50	0·7	0·9	0·7	0·9	<0·5	<0·5	0·7	0·9	1·8	2·4	0·5	0·6
Sputum	10	—	—	2·5	3·3	1·7	2·0	1·0	1·3	3·0	4·0	2·3	3·1
,,	50	—	—	2·3	3·0	1·7	2·0	1·2	1·4	3·0	3·6	1·5	1·8
Urine	10	—	—	1·9	1·9	1·9	1·9	1·2	1·2	3·4	3·4	1·5	1·5
,,	50	—	—	3·0	3·0	2·3	2·3	1·0	1·0	3·7	3·7	1·7	1·7
Bacteria suspended in:													
Horse serum + salt solution (1 + 3)		—	—	2·0	—	1·2	—	1·1	—	2·8	—	0·8	—
Egg albumin + salt solution (1 + 3)		<1·0	—	0·4	—	<1·0	—	1·0	—	2·9	—	1·2	—
Sputum		—	—	3·0	—	2·3	—	1·3	—	3·3	—	2·2	(4·2) Phenosalyl with 70% C_6H_5OH

The number affixed to the name of the disinfectant gives the original cover-slip phenol coefficient.

In contrast to the tests with pure phenol where the amount of organic substance added to the phenol solution had no particular influence upon the disinfecting power of the phenol solution, we see in Table IV, as might be expected, that the addition of 50% organic substance causes a considerable greater reduction of the phenol coefficient than does a 10% addition of the substance—with the exception of urine, which shows the opposite effect, and sputum which gave no particular difference, except in combination with phenosalyl.

A particularly striking effect from the presence of organic substance is found in the numerical reduction in the phenol coefficient of Alcorcin—from 43·4 to 4·5 on addition of 10% horse serum, and under 0·5 on 50% addition. A similar result is obtained on addition of milk water, and also on suspension of the bacteria in egg albumin + salt solution (1 + 3), the coefficient falling below 1·0. While suspension of the bacteria in sputum gives no significant reduction of the phenol coefficient (except for Kilcrobe), a very considerable reduction is brought about by suspension in egg albumin + salt solution (except for paramonochlorphenol and Lysol). Something similar applies to suspension of the bacteria in horse serum + salt solution.

From these studies we have formed the conclusion that it is not justifiable to select any given organic material as an addition to disinfectants in the determination of the phenol coefficient merely because the substance is a good material from an analytical point of view (giving uniform results, being easy to work with, etc.).

As the various disinfectants are affected differently by the presence of various organic substances, the examiner who employs such laboratory methods may easily contribute to false advertising, as both the manufacturer and the distributor employs the result for commercial purposes and hence are not likely to point out to the buyer that the disinfectant in question is effective just under the particular circumstances employed in the test, and is perhaps quite ineffective under other conditions.

Summary

Studies on the phenol coefficient after the cover-slip method were made on some disinfectants with the addition of different amounts of various organic materials. The results show that the phenol coefficient varies with the nature and amount of the different organic substances added to the disinfectant in question.

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(*MS. received for publication* 4. VI. 37—Ed.)