THE COMPARATIVE ANTIBACTERIAL ACTIVITY OF *O*-CHLOROMERCURIPHENOL AND PHENYLMERCURIC ACETATE

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Of the organomercurial antiseptics, phenylmercuric acetate occupies an established place in both medicine and pharmacy and is favoured as a bacteriostatic agent of high efficiency and low relative toxicity. In common with other mercurial antiseptics, owing their mode of action to combination with sulphydryl groups, its functional efficiency may be revealed by discolouration of the product containing it by mercuric sulphide. Recently *o*-chloromercuriphenol appears to have established itself in spite of the lack of published work relating its worth to that of already established members.

The first reports of the antiseptic activity of *o*-chloromercuriphenol, synthesised by Whitmore and Hanson[1] and Neogi and Chatterji[2], were by Fargher, Galloway and Probert[3,4], who used it for protecting textile materials from fungoid attack. Hart and Anderson[5] showed that the mercury derivatives of hydrocarbons and phenols were effective bacteriostatic agents, claiming *o*-chloromercuriphenol to be the most powerful of a series studied and, later, reported on the bactericidal activity of its derivatives[6]. On the other hand, however, McClusky and Swingle[7] claimed that *sec*-amylcresol preparations were more effective than *o*-chloromercuriphenol, in the presence of plasma, against Gram-positive cocci. Its use in antiseptic paper and fabrics is the subject of a patent[8].

The antiseptic properties of the phenylmercuric salts, however, have been more extensively reported. Weed and Ecker[9], for example, reported on the utility of the nitrate, synthesised by Otto[10], owing to its lack of odour, colour, taste, staining and corroding properties, non-selectivity and non-inhibition in the presence of tissues and that aqueous solutions could be administered either *per os*, or parenterally. They also showed[11] that there was a decreasing activity from the nitrate through the chloride and bromide to the iodide. Birkhaug[12] confirmed Weed and Ecker's results on the nitrate, but Pyman and Stevenson[13] showed that the "nitrate" previously used was the basic salt "merphenyl nitrate," $PhHgOH.PhHg.NO_3$. Phenylmercuric acetate and nitrate were introduced as contraceptives by Baker, Ranson and Tynen[14]. Berry, Jensen and Siller[15] showed that 0·001 per cent. of phenylmercuric nitrate provides a wide margin of safety for sterilising thermolabile substances, a concentration safe for use, as shown by the toxicological studies of Wien[16]

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while, according to Fust[17], 0·1 per cent. of phenylmercuric borate is adequate for the sterilisation of fæces, urine, sputum and linen.

EXPERIMENTAL

Direct comparisons of the traditional type between the two compounds are illustrated in Tables I and II. These are derived by exposing constant inocula of the selected organisms to falling concentrations which vary by increments of 10 to 100 per cent. Evidence of bacteriostatis, at dilutions no longer lethal, is seen with both compounds in the case of Gram-positive but not with the Gram-negative organisms.

TABLE I

INHIBITING CONCENTRATION, IN MG./100 ML. OF NUTRIENT BROTH MEDIUM, OF PHENYLMERCURIC ACETATE AND *O*-CHLOROMERCURIPHENOL WHEN EXPOSED TO CONSTANT INOCULA OF THE ORGANISMS AND INCUBATED AT 37·5°C.

		Inhibiting dilution in mg./100 ml.			
		Phenylmercuric acetate		*o*-Chloromercuriphenol	
		18 hours	48 hours	18 hours	48 hours
Streptococcus pyogenes...	CN 10	<0·00012	0·0012	0·0040	0·031
Staphylococcus aureus ...	CN 491	0·00025	0·00195	0·0010	0·015
Eberthella typhosa ...	CN 512	0·062	0·062	0·125	0·125
Bacterium coli	CN 348	0·125	0·125	0·250	0·250
Pseudomonas æruginosa	CN 200	>0·250	>0·250	>0·250	>0·250

TABLE II

INHIBITING DILUTION IN NUTRIENT BROTH AT WHICH GROWTH OF *E. TYPHOSA* OCCURRED AT 2½ MINUTES BUT NOT AT 5 MINUTES AND THE RATIOS OF BACTERICIDAL ACTIVITY OF *O*-CHLOROMERCURIPHENOL AND PHENYLMERCURIC ACETATE IN TERMS OF PHENOL

	Phenol	*o*-Chloromercuriphenol	Phenylmercuric acetate
Inhibiting dilution...	1/100	1/800	1/3200
Ratio with respect to phenol ...	1	8	32

Much more information, however, may be derived from estimates relating the time of exposure to per cent. mortality, or its probit. In this way time-response curves may be derived and compared with the isolated observations on an unknown curve (Tables I and II). A convenient expression of the estimate is the LT50, or the time required to kill 50 per cent. of the inoculum (Withell[18]) and, where the slopes of the lines (b) are not parallel, use may be made of the concentration exponent (n) of Phelps[19] which expresses, numerically, the power to which the concentration must be raised in order to give comparable rate of kill.

In cases where potent antiseptics are compared difficulties arise in estimating the low orders of time involved. An obvious solution is the

choice, in the case of the mercurial antiseptics, of a resistant organism such as *Bacterium coli* (Stark and Montgomery[20]), grown on a synthetic medium to minimise the reversal effects of proteins (Anderson and Hart[6], and Mirimanoff and Masset[21]). MacLeod's medium[22], with the addition of 0·2 per cent. of vitamin-free casein hydrolysate and 1·5 per cent. New Zealand agar gave readily counted colonies of *Bact. coli* after 48 hours' incubation at 37·5°C.

A second difficulty, caused by "carry-over" of bacteriostatic concentrations, is met by the inclusion of specific reversal agents such as glutathione (Fildes[23]) and, of the substances available for this purpose, which include thioglycollic acid (Graydon and Biggs[24], Heinemann[25]), cysteine hydrochloride (Smith, Czarnetsky and Mudd[26]), cysteine hydrochloride and 2:3-dimercaptopropanol (B.A.L., or British Anti-Lewisite[27-30]), glutathione and 2:3-dimercaptopropanol are the most satisfactory (Table III).

TABLE III

ANTIBACTERIAL CONCENTRATIONS OF PHENYLMERCURIC ACETATE AND *o*-CHLOROMERCURIPHENOL IN THE PRESENCE OF FALLING CONCENTRATIONS OF POTENTIAL REVERSING AGENTS USING *BACT. COLI* AS TEST ORGANISM

Concentration of reversing agent →	Inhibiting concentration in mg./100 ml.					
	1/1T	1/10T	1/100T	1/1M	1/10M	None
Glutathione :—						
Phenylmercuric acetate ...	>2·50	1·25	0·31	0·075	0·018	0·0045
o-Chloromercuriphenol ...	2·5	2·5	0·62	0·15	0·037	0·009
Thioglycollic Acid :—						
Phenylmercuric acetate ...	<0·001	0·075	0·018	0·018	0·018	0·0045
o-Chloromercuriphenol ...	<0·001	0·31	0·037	0·037	0·037	0·0045
Cystine HCl :—						
Phenylmercuric acetate ...	0·62	0·075	0·018	0·009	0·002	0·0045
o-Chloromercuriphenol ...	0·31	0·018	0·009	0·0045	0·002	0·002
Cysteine HCl :—						
Phenylmercuric acetate ...	0·15	0·075	0·018	0·009	0·0045	0·002
o-Chloromercuriphenol ...	0·62	0·009	0·009	0·018	0·0045	0·002
2,3-*Dimercaptopropanol* (*B.A.L.*):-						
Phenylmercuric acetate ...	<0·001	0·62	0·31	0·15	0·037	0·018
o-Chloromercuriphenol ...	<0·001	2·50	2·5	0·62	0·31	0·037

Calculation of the LT50 and concentration exponents using different organisms, substrates and temperatures justifies the conclusions that *o*-chloromercuriphenol is more active than phenylmercuric acetate in protein-free medium, but that this relationship is reversed in the presence of proteins (Table IV).

Acute Toxicity. The acute toxicity of the two compounds was determined by the intravenous and subcutaneous administration to groups of 10 mice of fresh solutions in distilled water. The estimates of LD50 were derived by plotting the logarithm of the dose against probit of mortality and the errors of the estimates evaluated graphically according

to Gaddum[31]. The results (Table V) show that, although phenylmercuric acetate is more toxic than *o*-chloromercuriphenol, the toxicities are of the same order.

TABLE IV

CONCENTRATION EXPONENTS FOR PHENYLMERCURIC ACETATE AND *o*-CHLOROMERCURIPHENOL UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Compound	Concentration	LT50 (sec.)	Concentration exponent (n)	Experimental conditions
Phenylmercuric acetate...	1/100T	32·0	0·0631	Using *Bact. coli*, a temperature of 16°C, sterile distilled water + 0·01 per cent. thioglycollic acid for dilutions, and broth agar, with 0·01 per cent. thioglycollic acid for the counts.
	1/1M	37·0		
o-Chloromercuriphenol...	1/100T	31·0	0·0271	
	1/1M	33·0		
Phenylmercuric acetate...	1/10T	39·0	0·4175	Using *Staph. aureus*, a temperature of 16·5°C., sterile distilled water for the dilutions and broth agar for the counts.
	1/20T	102·0		
o-Chloromercuriphenol...	1/10T	33·0	0·2931	
	1/20T	64·8		
Phenylmercuric acetate...	1/100T	30·6	0·9001	Using *E. typhosa*, a temperature of 16·5°C., sterile distilled water for the dilutions and broth agar for the counts.
	1/1M	243·6		
o-Chloromercuriphenol...	1/100T	31·2	0·5041	
	1/1M	99·6		
Phenylmercuric acetate...	1/1M	225·0	0·0694	Using *Bact. coli*, a temperature of 17·0°C., sterile distilled water for the dilutions and MacLeod's medium + agar for the counts.
	1/10M	264·0		
o-Chloromercuriphenol...	1/1M	33·0	0·5714	
	1/10M	123·0		
Phenylmercuric acetate...	1/1M	172·0	0·0591	Using *Bact. coli*, a temperature of 27·5°C., sterile distilled water + 0·01 per cent. glutathione for the dilutions and MacLeod's medium + agar + 0·01 per cent. glutathione for the counts.
	1/10M	180·0		
o-Chloromercuriphenol...	1/1M	212·0	0·2545	
	1/10M	512·0		

TABLE V

TOXICITY OF PHENYLMERCURIC ACETATE AND *o*-CHLOROMERCURIPHENOL WHEN ADMINISTERED BY THE SUBCUTANEOUS AND INTRAVENOUS ROUTES

Compound	Route	LD50 (g./kg.)	Limits of error (P=0·99)
Phenylmercuric acetate	intravenously	0·019	86—116
	subcutaneously	0·027	87—120
o-Chloromercuriphenol	intravenously	0·023	84—117
	subcutaneously	0·036	93—116

DISCUSSION

Solubility, odour, staining and corrosive action together with direct comparisons of antibacterial activity of the traditional type (Tables I and II) have been used as criteria for the evaluation of potential antiseptics. The differentiation of closely related antiseptics possessing similar properties and the comparative assessment of bactericidal action when they are rapidly effective in high dilution presents many problems, one of which, for example, concerns the extent to which the comparative bacteriostatic activity with constant inocula (Table I) is applicable to those ratios used in pharmaceutical preparations[32].

The greater amount of information which may be derived from estimates relating time of exposure to percentage mortality and its convenient expression in the LT50 is of particular use in evaluating functionally similar antiseptics. In those cases where the slopes of the regression lines are not parallel, the Phelps' concentration exponent provides information as to the comparable rate of increase in antibacterial action with increasing concentration. In the case examined here phenylmercuric acetate is superior to *o*-chloromercuriphenol, but the reverse occurs when the assessment is carried out in the absence of protein.

The problem of "bacteriostatic carry-over" may be met by the presence of specific reversing agents, contained in both the diluting and counting media. The insolubility of mercuric sulphide indicated that "thiol" compounds were potential inactivators in the case of the mercurial antiseptics and, of the several compounds examined, glutathione and 2:3-dimercaptopropanol are the most active. Glutathione is the more preferable of the two, however, owing to its greater convenience in manipulation.

Estimates of the acute toxicity (LD50) show that phenylmercuric acetate is more toxic than *o*-chloromercuriphenol by both the intravenous and subcutaneous routes. The toxicities are, however, of the same order and correlate with those of Wien[16] for the nitrate. The difference is unlikely to be of significance in pharmaceutical preparations incorporating either of the two compounds.

Summary

1. Phenylmercuric acetate possesses a greater *in vitro* antibacterial activity than *o*-chloromercuriphenol.

2. Glutathione and 2:3-dimercaptopropanol (BAL) are more effective inactivators of mercurial antiseptics than thioglycollic acid and cystine or cysteine hydrochlorides.

3. The rate of increase of antibacterial efficiency with increasing concentration is high for both compounds, but phenylmercuric acetate is to be preferred in view of its greater activity in the presence of proteins.

4. The use of concentration exponents is suggested for the comparative evaluation of potential antiseptics of similar properties or chemical structure.

5. Phenylmercuric acetate is more toxic than *o*-chloromercuriphenol following intravenous or subcutaneous administration.

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