RESEARCH PAPERS

THE INFLUENCE OF SOAPS ON THE BACTERICIDAL ACTIVITY OF A SPARINGLY WATER-SOLUBLE PHENOL

By H. Berry and A. Briggs

From the Department of Pharmaceutics, School of Pharmacy, University of London

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The fact that soaps modify the bactericidal activity of phenols has been known for many years, but it is only comparatively recently that attempts have been made to determine the exact extent of this modification and the manner by which it is effected.

As the published results of the earlier workers appeared to be contradictory, Bean and Berry[1-4] investigated the mechanism of the reaction. Taking into account the present theories of the micellar structure and properties of aqueous solutions of soaps[5] they determined the bactericidal activity of benzylchlorophenol and chloroxylenol in aqueous solutions of potassium laurate, using solutions containing a constant benzylchlorophenol or chloroxylenol to potassium laurate molar ratio. This activity was shown to be related to the concentration of the phenol in the soap micelles and independent of the total concentration in the solutions.

Alexander and Tomlinson[6] investigated the effect of soaps on the bactericidal activity of phenol. At soap concentrations above the critical, they were able to show a relation between the time for complete disinfection and the concentration of phenol in the aqueous phase. They did not observe any increase in activity at the higher soap concentrations, as did Bean and Berry[3], though it must be noted that Alexander and Tomlinson used solutions containing a constant concentration of phenol. Similarly Allawala and Riegelman[7] found that the LT99 of iodine dissolved in solutions of a non-ionic detergent was related to the concentration of iodine freely dispersed in the aqueous phase and was not necessarily dependent upon the total amount of iodine present.

This paper is a continuation of the work originated by Bean and Berry and is an investigation into the effect of different soaps on the bactericidal activity of a sparingly water-soluble phenol (benzylchlorophenol). Of the few soaps more than slightly soluble at 20° C., three were chosen, namely: potassium laurate, a straight chain saturated soap, potassium oleate, a monoethenoid soap, and potassium ricinoleate, a hydroxymonoethenoid soap. It was also intended to study the effect of potassium elaidate, the trans-isomer corresponding to potassium oleate, but it was found to be only slightly soluble at room temperature, thus precluding its use.

The solutions investigated were those containing (*a*) a constant benzylchlorophenol to soap molar ratio and varying concentrations of soap and (*b*), a fixed soap concentration and varying benzylchlorophenol to soap molar ratios.

EXPERIMENTAL

Materials

The phenol. The phenol used was benzylchlorophenol (5-chloro-2-hydroxy-diphenyl methane), m.pt. 48·5° C. water solubility 1 in 8650 at 20° C.

The Soap Solutions. Potassium laurate and potassium oleate solutions. These were prepared by neutralisation of lauric acid (acid value 278·8, iodine value 0·04, m.pt. 39·2° C.) and oleic acid (acid value 198·3, iodine value 88·10, n_D^{20} 1·4610). The resulting stock solutions of 0·1M potassium laurate and 0·2M potassium oleate were stored under nitrogen in glass-stoppered bottles, and diluted with carbon dioxide free water as required. The former stock solution had a pH of 9·9 and the latter a pH of 10·0 (glass electrode).

Potassium ricinoleate solution. A sample ricinoleic acid of high acid value (179, theoretical value 188) was prepared from castor oil by the method recommended by Berry and Cook[8]. This sample was used for the preparation of a 0·0307M stock solution of potassium ricinoleate which had a pH of 8·6 (glass electrode) and was stored under nitrogen. The phenol and soap mixtures were prepared as required with carbon dioxide free water and stored under nitrogen.

Culture media. The peptone water contained 2 per cent. Oxoid peptone and 0·5 per cent. sodium chloride, the pH after the final autoclaving being between 7·1 and 7·3.

The peptone water agar used for the cultivation of the test organism contained 1 per cent. Oxoid peptone, 0·5 per cent. sodium chloride and 2 per cent. New Zealand agar.

Test organism. A 24 hour culture of *Escherichia coli*, Type I (formerly Lister Institute No. 5933) in tryptic digest broth was freeze-dried in sterile tubes and stored at 4° C. Tubes of freeze-dried culture were reconstituted at approximately monthly intervals and cultivated on peptone water agar slopes. Four slopes were prepared from each freeze-dried culture and incubated at 37° C. for 24 hours. Afterwards three of them were sealed with paraffin wax and the fourth used to start a series of subcultures, four slopes being prepared each day. The daily slopes were incubated at 37° C. for 24 hours and only slopes from the 4th to the 14th subculture were used in the experiments.

The Solubility of Benzylchlorophenol in Water and Aqueous Solutions of Potassium Laurate, Potassium Oleate and Potassium Ricinoleate, at 20° C.

Solubility in aqueous solutions of potassium laurate. The method consisted of preparing a series of solutions of benzylchlorophenol in potassium laurate, such that each solution contained a smaller proportion of benzylchlorophenol to potassium laurate than the preceding one. Each solution was then diluted with freshly boiled and cooled distilled water until precipitation of the benzylchlorophenol occurred.

Figure 1 shows the relation between the weight of benzylchlorophenol solubilised per ml. of solution and the concentration of the potassium laurate solutions, whilst in Figure 3 the solubility, expressed as number of molecules of benzylchlorophenol solubilised per molecule of soap, is plotted against potassium laurate concentration.

Solubility in aqueous solutions of potassium oleate. The critical concentration of potassium oleate has been recorded[9] as 7–12 $\times$ 10^{-4}M and the results of McBain and Merrill[10] indicate that the micelles are fully formed at 0·016M. The range over which the micelles are increasing in size occurs, therefore, at a very low soap concentration. Attempts to determine the solubility of benzylchlorophenol in the way used for potassium laurate, failed because of the small quantity of benzylchlorophenol crystallising out. The method used consisted of accurately weighing a fixed quantity of benzylchlorophenol into a series of wide-mouthed, screw-capped bottles and adding gradually increasing volumes of the appropriate strength of potassium oleate solution to each one. The bottles were then heated to about 60° C. and shaken to dissolve the benzylchlorophenol. When cool, each solution was seeded with a crystal of benzylchlorophenol and, after being maintained at 20° C. for five days, examined for crystals.

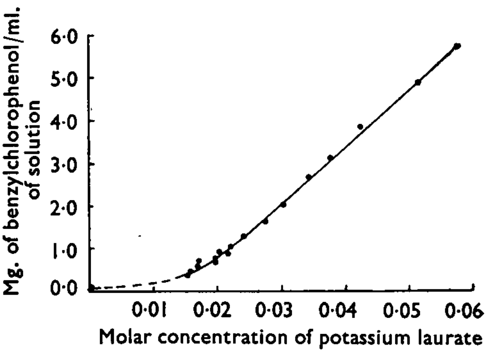

FIG. 1. The solubility of benzylchlorophenol in solutions of potassium laurate.

The relation between the solubility, expressed as number of molecules of benzylchlorophenol solubilised per molecule of soap, and the concentration of potassium oleate is shown in Figure 3.

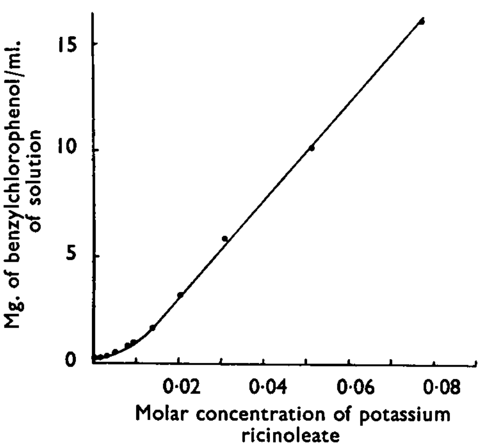

FIG. 2. The solubility of benzylchlorophenol in solutions of potassium ricinoleate.

Solubility in water and aqueous solutions of potassium ricinoleate. As a spectrophotometer became available for use the solubility of benzylchlorophenol in water and aqueous solutions of potassium ricinoleate was determined spectrophotometrically.

Water solubility. The E (1 per cent. 1 cm.) of benzylchlorophenol, at 283 mμ, was 104·3. The solvent used to prepared the standard solutions and the final dilution for testing contained 4 per cent. v/v ethanol and 0·05 N hydrochloric acid. The solubility was found to be 1 in 8650.

Solubility in aqueous solutions of potassium ricinoleate. The *E* (1 per cent. 1 cm.) of benzylchlorophenol at 283 mμ, in the presence of varying concentrations of potassium ricinoleate, 50 per cent v/v ethanol and 0·05 N hydrochloric acid was 113·4. The large amount of ethanol was necessary to dissolve the precipitated ricinoleic acid.

Saturated solutions in varying strengths of potassium ricinoleate were prepared, the supernatant liquid filtered and a portion of the filtrate diluted to give a concentration suitable for measurement. The diluent contained 50 per cent. v/v ethanol and 0·05 N hydrochloric acid.

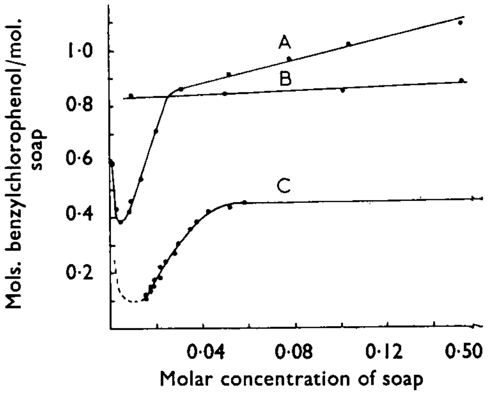

FIG. 3. The solubility of benzylchlorophenol in solutions of: A, potassium ricinoleate prepared from acid having an acid value of 179; B, potassium oleate and C, potassium laurate.

Figure 2 shows the relation between the weight of benzylchlorophenol solubilised and the concentration of potassium ricinoleate, whilst in Figure 3 the solubility, expressed as number of molecules of benzylchlorophenol solubilised per molecule of soap, is plotted against potassium ricinoleate concentration.

The Determination of Bactericidal Activity

The method used was similar to that of Berry and Bean[11]. Five ml. quantities of the phenol and soap solution were used and the inoculum consisted of five drops of a suspension of *E. coli.* The suspension was previously adjusted to contain 2×10^9 organisms per ml., and was delivered by means of a standard dropping pipette made by the method described by Withell[12].

With any phenol and soap solution each experiment was repeated six times, where possible, simultaneously, or failing that on the same day, with as short a time interval as possible between each. The mean death-time was calculated from the individual observed times, adopting the convention that if no growth was obtained after a given period of exposure in all replicates, then any growth thereafter was neglected.

The Bactericidal Activity of the Soap Solutions

The contribution made by the soap solutions themselves towards the bactericidal activity of the benzylchlorophenol and soap solutions was assessed by determining the death-time of *E. coli* in various concentrations of the soaps. The results obtained are shown in Table I.

TABLE I

THE MEAN DEATH-TIME OF *E. coli* IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE, POTASSIUM OLEATE AND POTASSIUM RICINOLEATE

Soap	Concentration of soap (Molar)	Mean death-time (Minutes)	Time of determination
Potassium laurate	0·06	41·6	During work
	0·08	25·7	
	0·10	17·7	
	0·15	8·3	
	0·20	4·8	
Potassium oleate	0·20	300+	At beginning of work
	0·20	300+	At end of work
Potassium ricinoleate	0·307	240	At beginning of work
	0·307	300+	At end of work

Both potassium oleate and potassium ricinoleate made no contribution to the bactericidal activity of the benzylchlorophenol and soap mixtures used but potassium laurate was toxic. An exponential relation was obtained when the concentration of this soap was plotted against the mean death-times and extrapolation of the curve showed that the death-time of *E. coli* would be about 100 minutes in 0·04 M potassium laurate. Thus the contribution made by potassium laurate to the mixtures is small at the soap concentrations used.

The Bactericidal Activity of a Saturated Aqueous Solution of Benzylchlorophenol

A saturated solution of benzylchlorophenol was prepared in sterile distilled water and the mean death-time at 20° C. found to be 75 minutes.

The Bactericidal Activity of Benzylchlorophenol in Aqueous Solutions of Soap

These experiments were arranged to show the influence on the bactericidal activity of the following changes in the composition of the solutions. (i) Maintaining a constant benzylchlorophenol/soap molar ratio whilst increasing the concentration of soap from below to well above the critical concentration. (ii) Maintaining a constant concentration of soap and varying the benzylchlorophenol/soap molar ratios.

(*a*) *The Bactericidal Activity of Benzylchlorophenol in Aqueous Solutions of Potassium Laurate*

The results obtained when solutions containing 0·0653 molecules of benzylchlorophenol per molecule of potassium laurate were used are shown in Figure 4 where the mean death-times are plotted against soap concentration, the points being superimposed on the solubility curve. The concentration of potassium laurate was varied from 0·0065 to 0·12 M, that is, from just below to well in excess of the critical concentration.

The effect on the bactericidal activity of keeping constant the potassium laurate concentration and varying the benzylchlorophenol/potassium laurate molar ratio is shown in Figure 5. Concentrations of 0·025, 0·04 and 0·075 M potassium laurate were used.

(b) The Bactericidal Activity of Benzylchlorophenol in Aqueous Solutions of Potassium Oleate

It was impossible to carry out experiments using benzylchlorophenol/potassium oleate solutions of constant molar ratio owing to the large changes in death-time that occurred with change in soap concentration. The investigation was restricted to the effect on the bactericidal activity of keeping constant the concentration of the soap and varying the benzylchlorophenol to potassium oleate molar ratio. The results are shown in Figure 6.

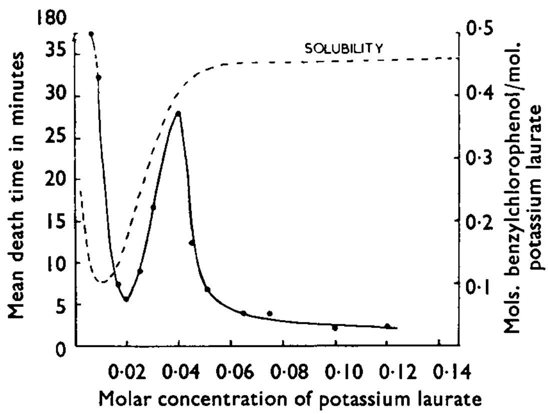

FIG. 4. The bactericidal activity against *E. coli* of solutions with constant benzylchlorophenol/potassium laurate molar ratio (0·0653) and increasing potassium laurate concentration, and the relation of the activity to the solubility of benzylchlorophenol.

(c) The Bactericidal Activity of Benzylchlorophenol in Aqueous Solutions of Potassium Ricinoleate

Two series of solutions were used containing benzylchlorophenol in the ratio of 0·383 and 0·331 molecules per molecule of potassium ricinoleate. The latter molar ratio was used to show the effect on bactericidal activity of solutions having a potassium ricinoleate concentration below the critical value (0·005 M). Had this been attempted with the former ratio it would have necessitated the use of unstable solutions in the region of 0·005 M potassium ricinoleate concentration. The results are shown in Figure 7.

The effect of varying the benzylchlorophenol/potassium ricinoleate molar ratio at soap concentrations of 0·025, 0·04 and 0·075 M potassium ricinoleate was also investigated. The results are shown in Figure 8.

DISCUSSION

The Solubility of Benzylchlorophenol

The weight of benzylchlorophenol solubilised per ml. of potassium laurate and potassium ricinoleate solution has been plotted against soap concentration in Figures 1 and 2 respectively. The curves are of similar shape. As the soap concentration is increased from zero to approximately 0·005 M potassium ricinoleate and 0·01 M potassium laurate there is but little increase in the solubility of benzylchlorophenol. Above these concentrations the weight of benzylchlorophenol solubilised increases sharply with increase in soap concentration due to the formation of micelles.

Figure 3 shows the solubility of benzylchlorophenol expressed as the number of molecules solubilised per molecule of soap, plotted against soap concentration. The benzylchlorophenol/potassium oleate curve could not be completed as the method used was not sufficiently sensitive to determine solubilities at very low soap concentrations. The critical concentration of potassium oleate has been recorded as 7–12 × 10^{-4}M. The curves plotted in Figure 3 indicate that the critical concentration of potassium ricinoleate is 0·005 M and potassium laurate 0·01 M.

The curves (Fig. 3) describing the solubility of benzylchlorophenol in solutions of potassium laurate and potassium ricinoleate are, except for the initial decrease at low soap concentrations, of the same general shape as those described by Hartley[13], McBain, Merrill and Vinograd[14], and McBain and Johnson[15] for the solubilisation of water insoluble substances—usually dyes—by aqueous soap or synthetic detergent solutions. Only occasionally has this initial decrease been noted[16,17] and then it has usually been attributed to the suspending action of the soap solution. One exception is the work of Heller and Klevens[18] who determined the solubility of ethylbenzene in potassium laurate solutions and obtained a curve very similar in shape to those in Figure 3 for the solubility of benzylchlorophenol in potassium laurate and potassium ricinoleate. Both ethylbenzene and benzylchlorophenol have approximately the same water solubility. Failure to demonstrate this initial fall in the solubility curve is probably due to the fact that water-insoluble substances were used or that the method was not sufficiently sensitive to detect solubilisation at concentrations below the critical.

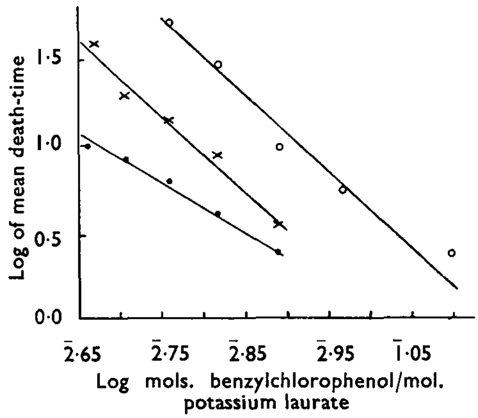

FIG. 5. The effect on the death time of *E. coli* of varying the benzylchlorophenol/potassium-laurate molar ratio in solutions with constant potassium laurate concentration.

×——×, 0·025 M potassium laurate.
○——○, 0·04 M ,, ,,
●——●, 0·075 M ,, ,,

The Bactericidal Activity of Solutions containing a Constant Benzylchlorophenol/Soap Molar Ratio and Varying Concentrations of Soap

It is seen from Figures 4 and 7 that when solutions containing a constant molar ratio of benzylchlorophenol to potassium laurate or potassium ricinoleate are used, changes in the death-time curve and changes in the slope of the solubility curve can be correlated.

Over the first part of the curves, where the death-time decreases rapidly as the soap concentration is increased up to the critical, the saturation of the solution increases whilst the interfacial tension decreases, reaching

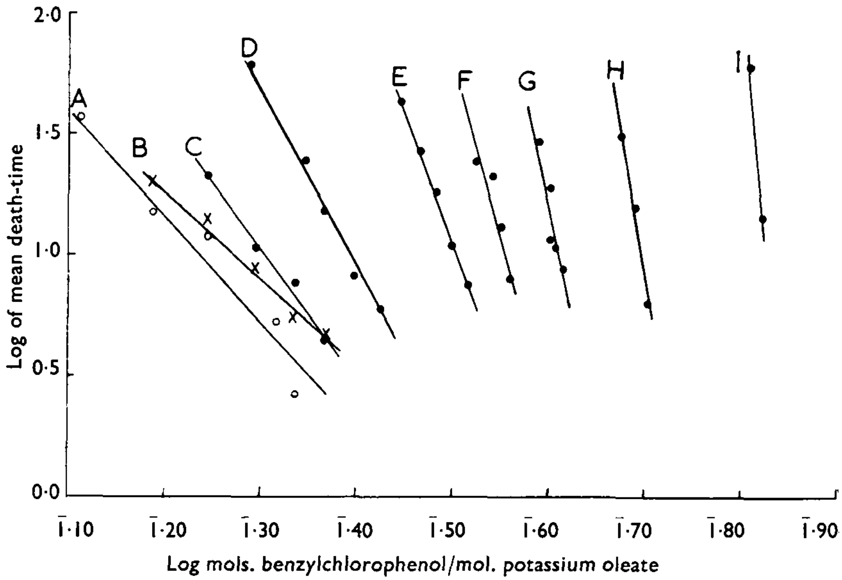

FIG. 6. The effect on the death-time of *E. coli* of varying the benzylchlorophenol/potassium oleate molar ratio in solutions with constant potassium oleate concentration of A, 0·10 M; B, 0·075 M; C, 0·05 M; D, 0·025 M; E, 0·01 M; F, 0·0075 M; G, 0·005 M; H, 0·0025 M and I, 0·001 M.

a minimum at the critical concentration. This decrease in interfacial tension will favour an increase in the adsorption of potassium laurate and benzylchlorophenol causing the concentration of benzylchlorophenol at the bacterial surface to be much higher than in the bulk solution, thus leading to a decrease in the death-time. This surface active effect of soap solutions has been noted by previous workers[3,6,19], and put forward as the cause of the increase in bactericidal activity of soap-phenol solutions in the pre-micellar range of soap concentrations. A phenomenon which has not previously been reported is that, over the pre-micellar range of soap concentration, the saturation of the solution is actually increasing. Thus the decrease in death-time will be due to both these factors.

The second part of the curve commences at approximately the critical micellar concentration and both the solubility and death-times increase rapidly. The interfacial tension remains approximately constant at all concentrations above the critical and therefore changes in bactericidal activity can no longer be ascribed to changes in interfacial tension. It is at the critical concentration that the micelles begin to form and they increase rapidly in size over the range of soap concentration now under consideration. The rate of increase in size is much more rapid than the rate of increase of benzylchlorophenol concentration and therefore the total saturation of the solutions will decrease. Bean and Berry[3] related the activity of the solutions in this range of soap concentration to the percentage saturation of the micelles. It has been shown[6], however, that the activity of phenol-soap solutions is related to the concentration of phenol in the aqueous phase rather than that in the micelles. These two views are not at variance as in any given solution an equilibrium will be set up between the concentration of benzylchlorophenol in the micelles and that in the surrounding water. Variation of the concentration of phenol in one phase will be accompanied by a similar variation in the other phase. Thus the decrease in saturation of the solutions over the second part of the curve will cause a decrease in the concentration of benzylchlorophenol in the aqueous phase and will explain the increase in the death-times that are obtained.

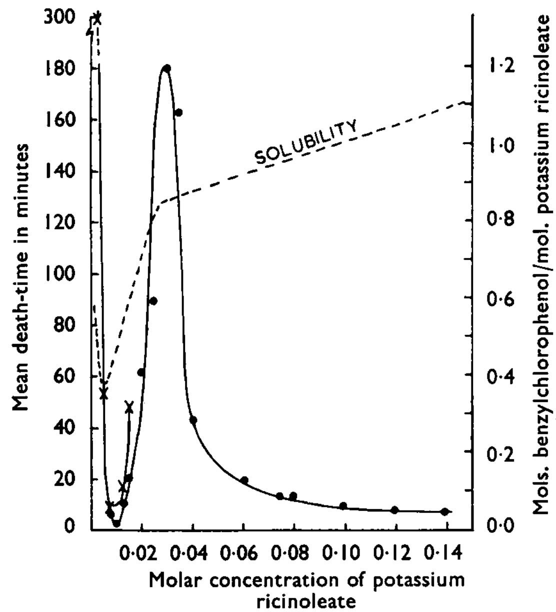

FIG. 7. The bactericidal activity against *E. coli* of solutions with constant benzylchlorophenol/potassium ricinoleate molar ratio and increasing potassium ricinoleate concentration, and the relation of the activity to the solubility of benzylchlorophenol.

●——● 0·383 Mol. benzylchlorophenol/mol. potassium ricinoleate.

×——× 0·331 Mol. benzylchlorophenol/mol. potassium ricinoleate.

The death-time curve reaches a peak at a point which coincides approximately with the change of slope of the solubility curve—the point at which the rapid increase in the number of molecules of benzylchlorophenol solubilised per molecules of soap ceases, and becomes but a relatively slight increase with increasing soap concentration. Above this concentrations the death-times commence to decrease rapidly, finally reaching an approximately constant value at the higher soap concentrations. Thus, over the third part of the curve, the percentage saturation of the solution is decreasing slightly with increasing soap concentration, whilst the death-time falls rapidly before attaining a constant value.

The reason for this rapid decrease in death-time is not apparent. If one accepts that it is the benzylchlorophenol in the aqueous phase that determines the bactericidal activity, then as the solution is becoming less saturated with respect to benzylchlorophenol, it would be expected that the death-time would continue to increase. Results in accordance with this expectation were obtained by Alexander and Tomlinson[6] who investigated the effect of various concentrations of Aerosol MA on the bactericidal activity of a 0·5 per cent. solution of phenol. They stated that, where the soap is not bactericidal, then the death-time will continue to increase until activity is abolished. These workers were, however, using a constant percentage of phenol, whereas in this work the amount of benzylchlorophenol was increased in proportion to the increase in soap concentration.

The rapid decrease in death-times cannot be due to the toxicity of the soaps as it has been shown that they possess little or no activity at the concentrations concerned. It is significant that the percentage saturation of the solution decreases only slightly with increasing soap concentration. The amount of micellar material per unit volume will, however, increase in ratio to the increase in soap concentration. Thus as the soap concentration is increased, there will be an increasing number of micelles, of approximately the same degree of saturation, available to replace the benzylchlorophenol adsorbed from the water by the bacteria. This might be expected to lead to a more rapid replenishment of the water with benzylchlorophenol and hence to shorter death-times.

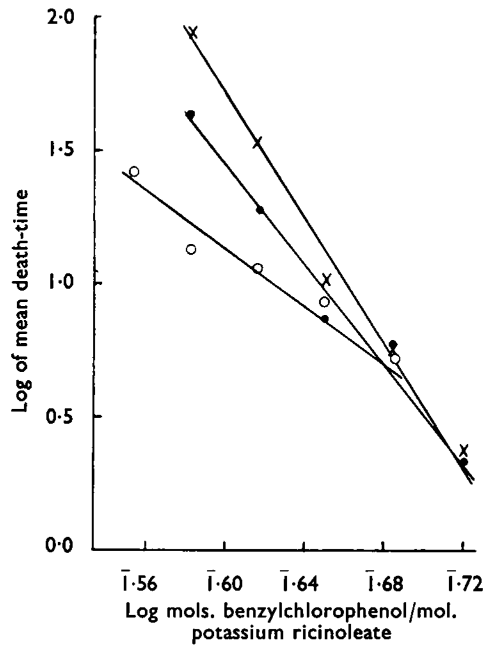

FIG. 8. The effect on the death-time of *E. coli* of varying the benzylchlorophenol/potassium ricinoleate molar ratio in solutions with constant potassium ricinoleate concentrations of: ×——×, 0·25 M; ●——●, 0·04 M; ○——○, 0·075 M.

The death-time falls to an approximately constant value at the higher soap concentrations. This would seem to indicate a limiting factor in the reaction between the soap-phenol solution and the bacteria, this being in all probability the rate of diffusion of the benzylchlorophenol from the micelle to the bacterial cell via the water.

It has already been stated that it was impossible to carry out experiments using benzylchlorophenol/potassium oleate solutions of constant molar ratio owing to the large changes in death-time that occurred with change in soap concentration. Some indication of the effect can, however, be obtained from the results shown in Figure 6. For a constant molar ratio of benzylchlorophenol to potassium oleate, for example 0·2, the death-time increases as the soap concentration is decreased from 0·10 to 0·025 M, as it does with the corresponding decrease in soap concentration for benzylchlorophenol/potassium laurate and benzychlorophenol/

potassium ricinoleate solutions (Figs. 4 and 7), over soap concentration ranges of 0·12 to 0·04 M and 0·14 to 0·03 M respectively.

The Bactericial Activity of Solutions Containing a Fixed Soap Concentration and Varying Benzylchlorophenol/Soap Molar Ratios

The results obtained by using solutions where the soap concentration was kept constant whilst the proportion of benzylchlorophenol to soap was increased are shown in Figures 5, 6 and 8 where the logarithms of the mean death-times are plotted against the logarithms of mols. benzylchlorophenol/mol. soap. A linear relation was obtained in every case, although the slope of these lines varied with soap concentration. Thus, once the soap concentration has been fixed, the mean death-time becomes a function of the concentration of benzylchlorophenol. It is to be expected that the slopes of the lines would not be similar as each soap concentration would affect the mechanism of the bactericidal reaction in a different manner. In the pre-micellar range the composition of the interfacial film would be changing, whilst above the critical concentration the partition coefficient for benzylchlorophenol between micelles and water probably changes with increasing soap concentration, as also will the structure of the micellar material and the interfacial film.

Comparison of the Effect of Different Soaps

The percentage saturation of the three soaps by benzylchlorophenol required to kill a standardised suspension of *E. coli* in ten minutes, has been calculated from the experimental results for three different soap concentrations and tabulated in Table II.

TABLE II

THE PERCENTAGE SATURATION OF POTASSIUM LAURATE, POTASSIUM OLEATE AND POTASSIUM RICINOLEATE BY BENZYLCHLOROPHENOL REQUIRED TO KILL THE STANDARDISED SUSPENSION OF *E. coli* IN TEN MINUTES

Soap	Percentage saturation		
	At 0·025 M	At 0·04 M	At 0·075 M
Potassium laurate	25	22	10
Potassium oleate	30	25	22
Potassium ricinoleate	56	51	44

It is realised that had a different death-time been used at which to make the comparison, different values for the percentage saturation of the solutions would have been obtained. A similar difference between the soaps would still have been illustrated.

From the above figures it is evident that each soap influences the bactericidal activity of benzylchlorophenol to a different degree. The amount of benzylchlorophenol needed to bring about a given effect increases in the order potassium laurate, potassium oleate, potassium ricinoleate. Conversely, equally saturated solutions of these soaps would show differing bactericidal activity. This variation between soaps is attributed to the difference in the structure of the soap molecules and its influence on the structure of the micelles formed in aqueous solutions. It also indicates that the affinity of these micelles for benzylchlorophenol varies from soap to soap, thus influencing the degree of saturation of the aqueous phase and hence the bactericidal activity of the solution.

Summary

1. The solubility of benzylchlorophenol in solutions of potassium laurate and potassium ricinoleate, when expressed as molecules of benzylchlorophenol solubilised per molecule of soap, decreases as the soap concentration is increased up to the critical. Thereafter the shape of the solubility curve is similar to those previously reported for the solubility of water-insoluble dyes in aqueous solutions of soaps.

2. When mixtures containing a constant benzylchlorophenol to soap molar ratio and varying soap concentration are used, changes in the bactericidal activity can be correlated with changes in the slope of the solubility curve.

3. The effect of varying the benzylchlorophenol to soap molar ratio has been determined at various constant soap concentrations.

4. It has been shown that the three soaps used each influence the bactericidal activity of benzylchlorophenol to a different degree. For a given weight of benzylchlorophenol the highest level of activity is obtained when it is dissolved in potassium laurate solution, the lowest in potassium ricinoleate solution.

References

1. Bean and Berry, *Nature, Lond.*, 1948, 161, 396.
2. Bean and Berry, *J. Pharm. Pharmacol.*, 1950, **2**, 484.
3. Bean and Berry, *ibid.*, 1951, **3**, 639.
4. Bean and Berry, *ibid.*, 1953, **5**, 632.
5. Berry and Bean, *J. Pharm. Pharmacol.*, 1950, **2**, 473.
6. Alexander and Tomlinson, *Surface Chemistry*, Butterworth's Scientific Publications, London, 1949.
7. Allawala and Riegelman, *J. Amer. pharm Ass., Sci. Ed.*, 1953, **42**, 396.
8. Berry and Cook, *J. Pharm. Pharmacol.*, 1950, **2** 217
9. Corrin Klevens and Harkins *J. chem. Phys.* 1946 **14** 480.
10. McBain and Merrill, *Industr. Engng Chem.*, 1942, **34**, 915.
11. Berry and Bean, *J. Pharm. Pharmacol.*, 1954, **6**, 649.
12. Withell, *Quart. J. Pharm. Pharmacol.*, 1938, **11**, 736.
13. Hartley, *J. chem. Soc.*, 1938, 1968.
14. McBain, Merrill and Vinograd, *J. Amer. chem. Soc.*, 1941, **63**, 670.
15. McBain and Johnson, *ibid.*, 1944, **66**, 9.
16. McBain and Huff, *J. colloid. Sci.*, 1949, **4**, 383.
17. McBain, Wilder and Merrill, *J. phys. Colloid Chem.*, 1948, **52**, 12.
18. Heller and Klevens, *J. chem. Phys.*, 1946, **14**, 567.
19. Frobisher, *J. Bact.*, 1927, **13**, 163.