BACTERICIDAL ACTIVITIES OF SOAP-PHENOL MIXTURES*

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The modification of bactericidal activities of phenols when dissolved in varying concentrations of soaps has been the subject of several communications during the past few years. These reports differ in the postulated mode of action of the soap, and the experimental data are conflicting. The present investigations were undertaken in an attempt to relate the previous findings.

Agar and Alexander[1] and Alexander and Tomlinson[2] measured the extinction times of *Bacterium coli* when exposed to phenol and chlorinated phenols dissolved in aqueous solutions of anionic and cationic surface-active agents. In all cases, the extinction times were minimal at the critical micellar concentration of the surface-active agent. When the phenol concentration was kept constant and the concentration of surface-active agent varied, extinction times rapidly increased at concentrations either below or in excess of the critical micellar concentration of the surface-active agent. The concentration exponent of the phenol-soap mixture was found to remain virtually constant and Alexander and Tomlinson were led to postulate that, as the soap concentration was increased beyond the critical concentration, the extinction times of the mixtures would increase until a toxic concentration of the surface-active agent was reached, the only activity remaining being due to the surface-active agent alone.

Enhanced activity when the soap concentration was increased to the critical concentration was ascribed to the formation of an interfacial "complex" at the bacterium-water interface, the effect being similar to that observed by Alexander and Trim[3] in the anthelmintic activity of hexyl-resorcinol-soap mixtures. At soap concentrations in excess of the critical, they considered, the phenol passed into the micelles. Reduction in bactericidal activity was therefore due to a phenol depletion of the aqueous phase. At very high concentrations of soap, nearly all of the phenol would be dissolved in the micellar phase and any activity of the mixture would then reside only in the activity of the soap itself.

The studies undertaken by Bean and Berry[4,5] employed two phenols of lower water solubility than those investigated by Alexander and his colleagues. They used only one soap, potassium laurate, and they used mixtures which contained not a constant phenol concentration, but a constant phenol/soap ratio.

In agreement with the findings of Alexander and Tomlinson, Bean and Berry observed that increases in soap concentration up to the critical

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micellar concentration resulted in increased bactericidal activity. But as soap concentrations increased in excess of the critical, the bactericidal activity at first decreased sharply and then increased. The soap concentration of minimal activity was also that at which the relative amount of solubilisation of the phenol began to decrease. The activities of mixtures containing micellar concentrations of soap were explained in terms of saturation of the micelles with the phenol, in contrast to Alexander's explanation in terms of "free" phenol concentration in the aqueous phase.

It is difficult to correlate the findings of both groups. The phenol concentrations used were different and the soaps employed were of differing bactericidal activity: the potassium laurate used by Bean and Berry was much more toxic than the aerosol MA and aerosol OT used by Alexander and his colleagues. In the present communication one soap, potassium laurate, and three phenols of widely differing solubility were used. Mixtures containing a constant phenol concentration and mixtures containing a constant phenol/soap molar ratio were separately studied.

Experimental Details

Materials. The soap solutions were prepared from a lauric acid of high purity (m.pt. 42·5–43·5° C.; acid value 279; iodine value, nil). 0·5M solutions of potassium laurate were adjusted to pH 9·6–9·8 and stored under nitrogen until required for use. The phenols used were 4-benzylphenol (m.pt. 83·5–84·0° C.; soluble in 6400 parts of water at 20° C.); 2-hydroxydiphenyl (m.pt. 56–57° C.; soluble in 1400 parts of water at 20° C.); and phenol (analytical reagent quality). All soap-phenol mixtures were prepared with water free from carbon dioxide and were stored under nitrogen.

Test Organism. Bacterium coli (*Escherichia coli*), laboratory strain, type I, 44° C.-positive, formerly N.C.T.C. No. 5933.

Media. The medium used in the determination of extinction times was of the same composition as that employed by Berry and Bean[6]. The solid medium used for cultivation of the test organisms contained 1 per cent. "Oxoid" peptone and 0·5 per cent. sodium chloride, solidified with 2 per cent. Davis bacteriological agar, and was adjusted to pH 7·2.

Experimental Technique. This closely followed the extinction method described by Berry and Bean[6]. The test suspension was adjusted to a density of 2×10^9 organisms per ml., and the inoculum consisted of 10 drops of this suspension, delivered with the improved dropping pipette described by Cook and Yousef[7] and Cook[8]. An experiment consisted of between 15 and 20 replicate determinations, each at 8 or 9 different contact times. The results were analysed by the loglog analysis of Mather[9] as described by Cook and Wills[10]. In a trial series of experiments, the extinction times to varying concentrations of potassium laurate were determined. The first and second calculated approximations to the loglog regression gave estimates with limits of error within which the visual estimate of the mean single survivor time easily lay. Hence, all

subsequent estimations of extinction times of soap-phenol mixtures could be made visually from the regression line best fitted by inspection.

Determinations of extinction times to mixtures containing 2-hydroxydiphenyl or 4-benzylphenol were made at 20° C. Mixtures of phenol with potassium laurate developed so heavy a turbidity due to hydrolysis of the soap that determinations could be undertaken only at a higher temperature.

The Bactericidal Activities of Solutions of Potassium Laurate

In order to assess the contribution of the soap alone to the bactericidal activities of soap-phenol mixtures, determinations of extinction times of *Bact. coli* on exposure to solutions of potassium laurate were made over a wide range of concentration. The effect of varying the pH of the soap solutions was also studied. Figure 1 shows the relation between logarithms of extinction times and logarithms of potassium laurate concentrations. The four relations, A, B, C, and D, were obtained from the use of stock 0·5M soap solutions of pH 10·4, 9·9, 9·7 and 9·7 respectively. Batches C and D are represented by a common regression. Curve E of Figure 1 was obtained from determinations with batch D of soap solution at 25° C.

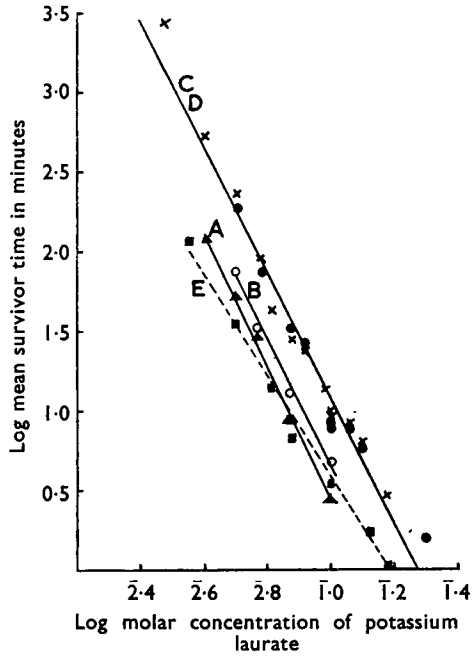

FIG. 1. The relationship between extinction times of *Bacterium coli* and concentration of potassium laurate. Curves A, (▲——▲), B (○——○), C (×——×) and D (●——●) were obtained from determinations at 20° C., using batches of stock 0·5M soap solution of pH 10·4, 9·9, 9·7 and 9·7 respectively. Curve E (■——■) was obtained from determinations undertaken at 25° C., using batch D of stock soap solution.

It is clear that batches C and D had almost equal bactericidal activity; batch B, which was only 0·2 pH unit more alkaline in reaction, was much more toxic; and batch A was the most highly bactericidal. A further batch of stock solution adjusted to pH 9·4 could not be used for reliable bactericidal determinations because of the very pronounced hydrolysis on dilution. The precipitated hydrolysis products occluded dropping pipettes to such an extent that neither the rate of delivery nor the volumes delivered could be controlled.

Solubilities of Phenols in Aqueous Solutions of Potassium Laurate

The concentrations of the phenols present in phenol-saturated solutions of varying potassium laurate concentration were determined spectrophotometrically. All determinations were performed in duplicate. 100 ml. of each concentration of potassium laurate solution was prepared with water free from carbon dioxide. Half of the solution was placed in each of two 60 ml. glass-stoppered bottles. An excess of the phenol was added to each bottle, which was heated in a boiling water bath with frequent vigorous shaking. The bottles were removed after 15 minutes, and placed in a water bath maintained at 20° C. for at least 48 hours.

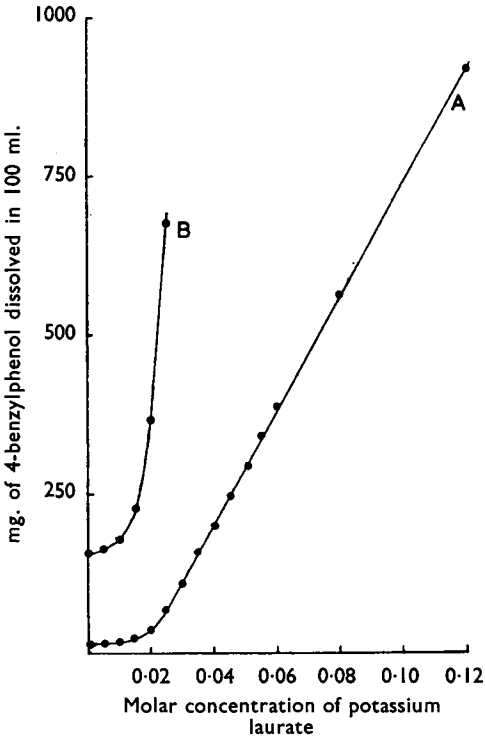

FIG. 2. The solubility of 4-benzylphenol in aqueous solutions of potassium laurate at 20° C. Curve B, for which the values of ordinates have been divided by 10, is an enlargement of curve A.

The contents of each bottle were separately filtered and an aliquot suitably diluted so that the final dilution for estimation contained between 50 and 100 mg. per litre of the phenol, together with 50 per cent. ethanol and 0·05N hydrochloric acid. The E (1 per cent. 1 cm.) of 4-benzylphenol at 277 mμ was determined from eight separately prepared solutions over a tenfold range of known concentration as $89{\cdot}65 \pm 0{\cdot}84$ ($P = 0{\cdot}99$) with coefficient of variation of 0·756. Similarly 2-hydroxydiphenyl gave a value for E (1 per cent. 1 cm.) of $612{\cdot}5 \pm 4{\cdot}8$ ($P = 0{\cdot}99$) with a coefficient of variation of 0·629 at 245 mμ.

The saturation weights of 4-benzylphenol are shown plotted against potassium laurate concentrations in Figure 2. It is seen that up to a soap concentration of 0·015M, very little more of the phenol was dissolved than in water alone. Over a concentration range exceeding 0·02M, however, the weight of the phenol dissolved increased linearly with increasing soap concentration. A change in solubilising properties occurred between 0·015M and 0·020M, the increased solubility being due to the presence of micelles at that concentration. A small increase in solubility occurred over the premicellar range of concentration, similar

behaviour to which was reported by Heller and Klevens[11] for the solubility of ethylbenzene in potassium laurate solutions.

Figure 3 shows the saturation phenol/soap molar ratios, i.e., saturation molar concentration of 4-benzylphenol/molar concentration of potassium laurate, plotted against potassium laurate concentrations. The shape of the curve, at concentrations in excess of the critical, is in general agreement with those of other workers who used a variety of solutes and soap: Hartley[12], McBain and Johnson[13], Bean and Berry[14]. At concentrations below 0·015M, however, the saturation molar ratio again increased, since the weight of phenol solubilised decreased only slightly with decreasing soap concentration over the premicellar range. All previous illustrations of solubility in soap solutions, save that of Heller and Klevens[11], have failed to show this initial fall in the curve, either because the solute employed had an extremely low water solubility or because determinations at soap concentrations below the critical were not undertaken. The ethylbenzene employed by Heller and Klevens was of comparable solubility in water to 4-benzylphenol. Figure 3 also shows saturation 2-hydroxydiphenyl/potassium laurate molar ratios plotted against potassium laurate concentrations. The relationship is generally similar to that obtained for 4-benzylphenol.

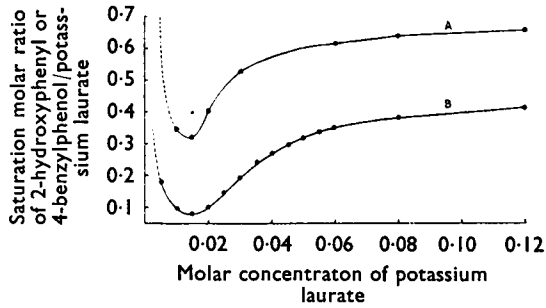

FIG. 3. The solubilities of 2-hydroxydiphenyl (A) and 4-benzylphenol (B) in aqueous solutions of potassium laurate at 20° C. Solubilities are expressed as saturation 4-benzylphenol or 2-hydroxydiphenyl/potassium laurate molar ratios.

THE BACTERICIDAL ACTIVITY OF 2-HYDROXYDIPHENYL IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

Extinction times were determined when (*a*) a constant concentration of the phenol was maintained with variation of soap concentration, after the scheme of Alexander and Tomlinson[2]; (*b*) with a constant phenol to soap molar ratio with varying soap concentrations; (*c*) with varying phenol concentrations but constant soap concentration, as was adopted by Bean and Berry[4,5]. In addition, it was necessary to determine the bactericidal activity of the phenol in aqueous solutions without soap.

Regression line A of Figure 4 was obtained by plotting logarithms of mean single survivor times against logarithms of 2-hydroxydiphenyl concentrations in solutions containing no soap. Curves B, C, D, E and F in the same graph were obtained from the use of test solutions of varied phenol content and of potassium laurate molar concentrations 0·010, 0·015, 0·020, 0·030 and 0·040 respectively. It can be seen that the presence of soap greatly enhanced the bactericidal activity of the phenol with all

the concentrations of phenol and of soap which were studied. The batch of soap solution used in these determinations (batch B), gave an extinction time of 76 minutes for a 0·05M solution, so that here the soap alone did not exert any appreciable bactericidal effect.

Curve A of Figure 5 represents the relation between logarithms of extinction times and concentrations of potassium laurate for soap-phenol

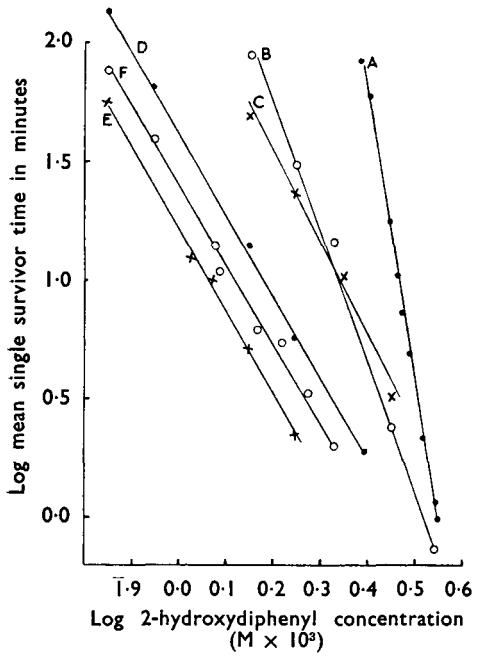

FIG. 4. The bactericidal activity of 2-hydroxydiphenyl against *Bacterium coli* at 20° C. in aqueous solution and in aqueous solutions of potassium laurate. Logarithmic relationships between concentrations of 2-hydroxydiphenyl and extinction times of *Bact. coli*, using five concentrations of potassium laurate: 0·01M (curve B ○——○), 0·015M (curve C ×——×), 0·02M (curve D ●——●), 0·03M (curve E ×——×) and 0·04M (curve F ○——○). Curve A (●——●) represents the activities of solutions of 2-hydroxydiphenyl containing no added soap.

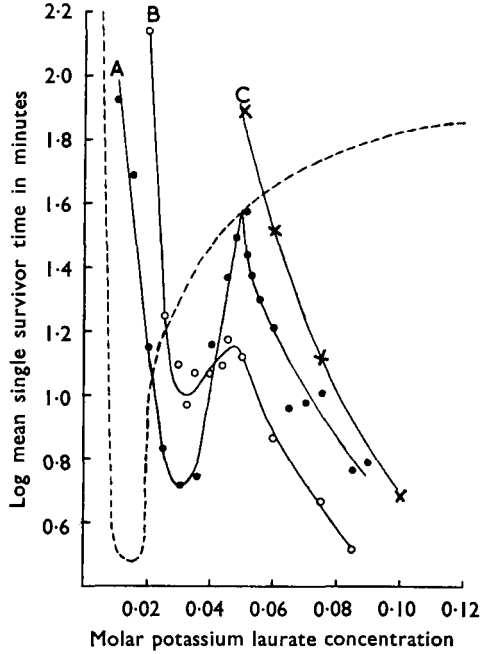

FIG. 5. The bactericidal activities of 2-hydroxydiphenyl-potassium laurate mixtures against *Bacterium coli* at 20° C. Curve A (●——●): mixtures containing a constant phenol concentration of 0·00141M. Curve B (○——○): mixtures containing a constant 2-hydroxydiphenyl/potassium laurate molar ratio of 0·0353. Curve C (×——×): solutions of potassium laurate (batch B) containing no phenol. The broken curve represents the solubility of 2-hydroxydiphenyl in potassium laurate solutions (see Fig. 3).

mixtures containing a constant 2-hydroxydiphenyl concentration of 0·00141M. Extinction times of mixtures containing a constant 2-hydroxydiphenyl/potassium laurate molar ratio of 0·0353 are represented by curve B of Figure 5, and extinction times of the soap alone are plotted logarithmically against soap concentration to give curve C. Curves A and B intersect at a potassium laurate concentration of 0·040M, the solutions represented by each curve possessing the same phenol concentration at this soap concentration.

THE BACTERICIDAL ACTIVITY OF 4-BENZYLPHENOL IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

Saturated aqueous solutions of 4-benzylphenol at 20° C. were found to be devoid of measurable bactericidal activity. The scheme for the investigations undertaken with this phenol closely followed that employed with 2-hydroxydiphenyl. However, difficulties were encountered when a new batch of stock soap solution was introduced midway through the investigations.

Using the same batch of soap solution as that used in the investigations of 2-hydroxydiphenyl, two series of determinations were carried out in which the test bactericide contained constant 4-benzylphenol/potassium laurate molar ratios of 0·0326 and 0·0652. The results are shown as logarithms of extinction times plotted against potassium laurate concentrations (curves 1 and 2 respectively of Figure 6). A third series of determinations, using a phenol/soap molar ratio of 0·0489, yielded curve 3 of Figure 6. A new batch of stock 0·5M potassium laurate had to be used for this series, also for preparing test solutions containing a constant 4-benzylphenol concentration of 0·00163M, but varying concentrations of potassium laurate (curve 1, Fig. 7). The bactericidal activity of this second batch of soap solution (batch C) is represented by curve 5 in Figure 6 and curve 2 in Figure 7, and that of the first batch (batch B)—the values having been obtained shortly after preparation—is represented by curve 4 of Figure 6.

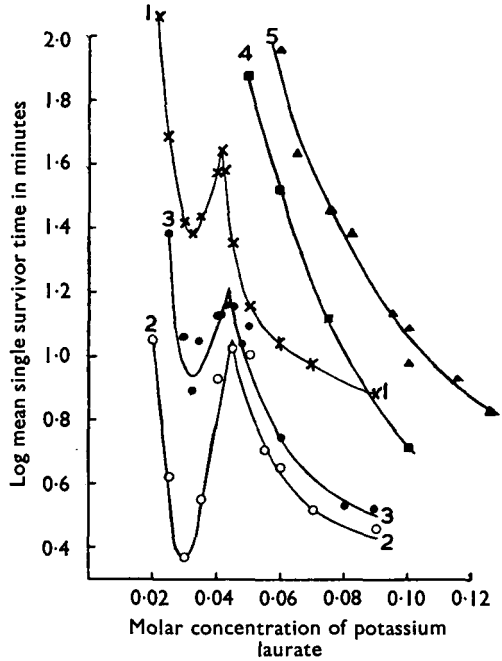

FIG. 6. The bactericidal activities of 4-benzylphenol-potassium laurate mixtures at 20° C. Curves 1 (×——×), 2 (○——○) and 3 (●——●) relate to mixtures containing constant 4-benzylphenol/potassium laurate molar ratios of 0·0326, 0·0652 and 0·0489 respectively. Curves 4 (■——■) and 5 (▲——▲) represent the bactericidal activities of batches B and C of potassium laurate solution in the absence of the phenol.

From the data represented in curves 1–3 of Figure 6 and curve 1 of Figure 7, eight potassium laurate concentrations could be found at each of which had been made four determinations of extinction times in the

presence of varying concentrations of 4-benzylphenol. Logarithms of extinction times are plotted against logarithms of 4-benzylphenol concentrations in Figures 8 and 9, the relations being shown on two graphs in order to avoid confusion in relating the plotted values to the appropriate regression lines. The correlations are poor because of the variations between the two batches of soap solution.

After completion of the work described above, when little of batch B of stock soap solution remained, its bactericidal activity was found to have declined: a concentration of 0·10M, which initially gave extinction after exposure for 4·8 minutes now required 26·3 minutes. No appreciable loss of activity on storage of further batches of potassium laurate solutions under a variety of conditions could be demonstrated.

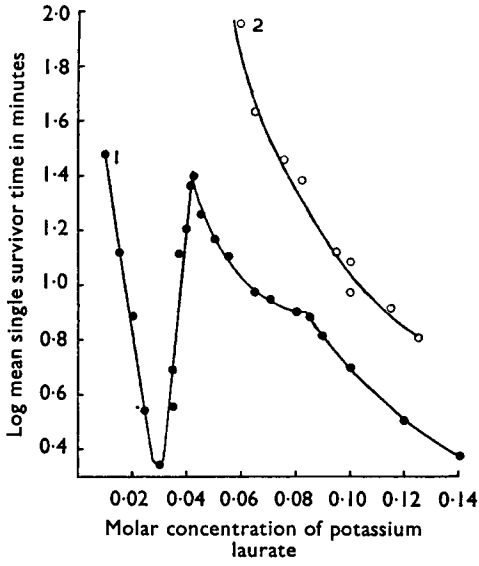

FIG. 7. The bactericidal activities of 4-benzylphenol-potassium laurate mixtures at 20° C. Curve 1 (●——●) relates to mixtures containing a constant 4-benzylphenol concentration of 0·00163M. Curve 2 (○——○) represents the bactericidal activity of batch C of potassium laurate solution in the absence of the phenol.

The influence of the variations in toxicity of soap solutions upon the activities of their mixtures with the phenol was investigated as follows. The bactericidal activities of solutions of the phenol prepared from four different batches of potassium laurate solution known to possess differing bactericidal activity were measured. Two soap concentrations were examined: 0·025M and 0·030 M. Two batches, B and C, had already been tested; the others had initial reactions of pH 10·4 and 9·4. The data were analysed by testing the adequacy of common slope and coincident regression line from a knowledge of the residual sums of squares of regressions separately fitted to each batch soap data and computing the residual sum of squares of a common regression. The analysis is described by Tippett[15]. It was found that the four relations could be regarded as being represented by a common coincident regression. Thus alteration in batch of 0·5M stock soap solution had so little effect on extinction times of 4-benzylphenol-soap mixtures, relative to variations between estimates of extinction times with individual batches of stock solution, that the differences between the stock solutions at the concentrations examined could be neglected.

The Bactericidal Activity of Phenol in Aqueous Solutions of Potassium Laurate

Logarithms of extinction times of *Bact. coli* when exposed to soap-phenol mixtures containing a constant phenol concentration of 0·0159M are shown plotted against potassium laurate concentrations in curve A of Figure 10. Curve C relates logarithms of extinction times to molar concentrations of potassium laurate in solutions containing no added phenol. Additional estimations were made with solutions of corresponding soap concentrations with and without phenol; a precaution which was adopted in the hope that day-to-day variations in extinction time estimates would not lead to the postulation of fallacious relationships between the soap-phenol and soap systems. Curve B of Figure 10 relates to mixtures containing a constant phenol/potassium laurate molar ratio of 0·399.

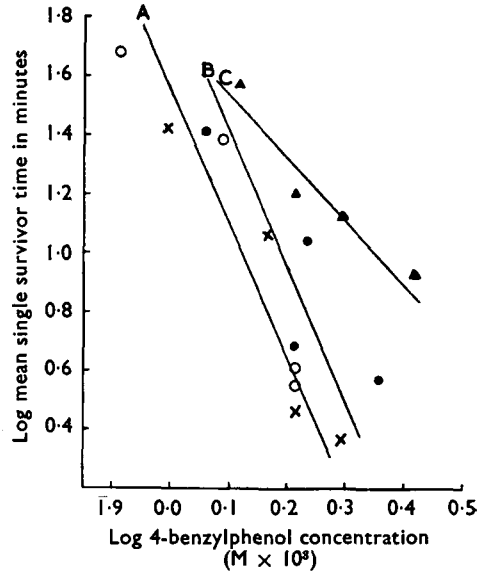

FIG. 8. The bactericidal activity of 4-benzylphenol in aqueous solutions of potassium laurate at 20° C. Curves A (○——○: ×——×), B (●——●) and C (▲——▲) represent phenol-soap mixtures containing 0·025, 0·030, 0·035 and 0·040M potassium laurate respectively.

Also, the phenol contents of the test solutions were varied at the following molar potassium laurate concentrations: 0·028, 0·0325, 0·040, 0·050 and 0·080. The observed extinction times are plotted logarithmically against phenol concentrations to give curves B, C, D, E, and F in Figure 11. Curve A in the same graph relates to solutions of phenol containing no added soap. As explained earlier, all determinations with phenol were made at a temperature of 25° C.

Discussion

Estimates of bactericidal activities of potassium laurate-phenol mixtures containing a *constant phenol concentration*, using three phenols of widely differing solubility, lead to the principal inference that, over the range of concentration studied, activity is governed by the existence of three soap concentrations of limiting activity.

The First Concentration of Limiting Activity

At a potassium laurate concentration of about 0·03M, the phenols exerted maximal bactericidal activity in soap solutions over a range 0 to 0·1M. This characteristic effect was observed with all phenols at all

concentrations and was probably independent of the initial pH of the stock soap solution from which the mixtures were prepared. This concentration of limiting activity must be identified with the critical concentration for the formation of micelles, and throughout the remainder of this discussion the critical concentration of potassium laurate will be taken to be 0·03M. As the soap concentration is increased to this point, the formation of an interfacial "complex" of soap and phenol appears to be responsible for the logarithmic increase in bactericidal activity (curve A, Fig. 5; curve 1, Fig. 7; curve A, Fig. 10). The adsorbed film may promote penetration of the phenol by supplying a high phenol concentration at the surface of the organism—considerably higher than would be found in the bulk solution. Adsorption of the soap may also cause a breakdown of the lipoid cell surface, so that phenol may more readily penetrate the cell, a view which has been put forward by Alexander[16]. Gale and Taylor[17] showed that a variety of surface-active agents and phenol separately bring about damage of the bacterial cell wall and it has been argued that soaps and phenols possess a synergistic action, the surface activity of the complex being greater than that of either soap or phenol alone.

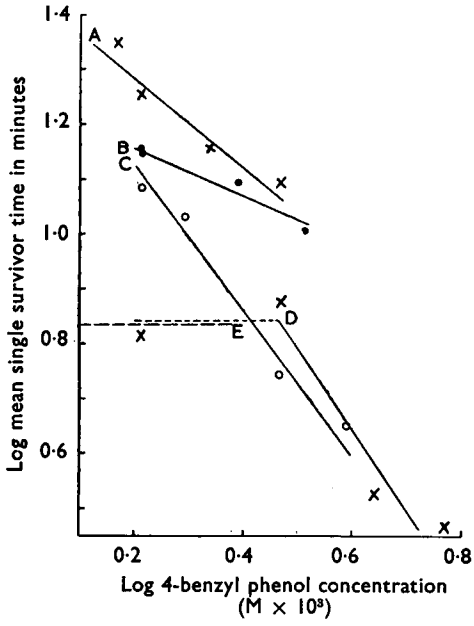

FIG. 9. The bactericidal activity of 4-benzylphenol in aqueous solutions of potassium laurate at 20° C.—continued. Curves A (×——×), B (●——●), C (○——○) and D (×——×) represent phenol-soap mixtures containing 0·045, 0·05, 0·06 and 0·09 M potassium laurate respectively. E represents the extinction time of 0·09M potassium laurate (batch B stock solution) in the absence of the phenol.

The Second Concentration of Limiting Activity

At a potassium laurate concentration of about 0·045M, all of the phenols exhibited a minimal bactericidal activity. The decrease in bactericidal activity at soap concentrations immediately in excess of the critical has been differently explained by Alexander and Tomlinson[2] and by Bean and Berry[4,5]. The presence of a second concentration of limiting activity, although found by Bean and Berry with their constant ratio mixtures, was not reported for mixtures containing a constant phenol concentration by Alexander and Tomlinson.

Extinction time determinations and counts of survivors were undertaken

by Alexander and Tomlinson at five concentrations of soap in excess of the critical concentration (1 per cent. Aerosol MA). The first three determinations showed no change from that at the critical, and the fourth determination, at 2 per cent. Aerosol MA, gave an increase in extinction time from 15 to 40 minutes. The last determination, at Aerosol concentration 4·5 per cent., gave an extinction time of over 480 minutes. The corresponding survivor counts gave an almost constant percentage of survivors (about 25 per cent.) after exposures of from 10 to 60 minutes, after which no further counts were made. Thus no extinction time or true index of activity was in fact determined at this soap concentration. Their soap, without addition of phenol, was almost equally toxic at the same concentration, judging from their recorded counts of survivors, so that one might expect that the extinction times of the mixtures would be falling with increase in soap concentration in this region.

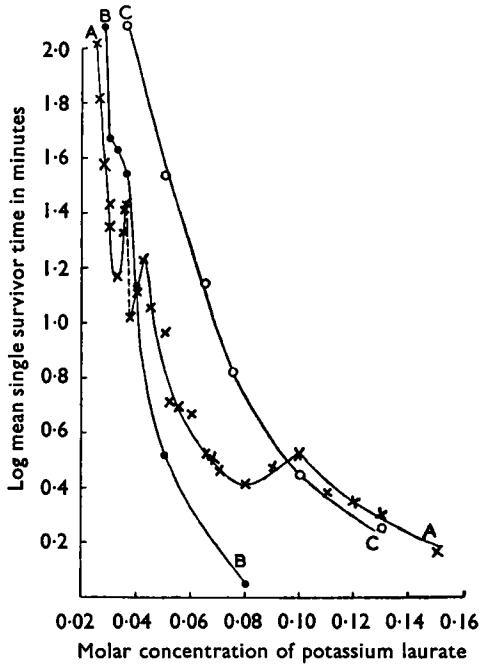

FIG. 10. The bactericidal activities of phenol-potassium laurate mixtures at 25° C. Curve A (×——×) relates to mixtures containing a constant phenol concentration of 0·0159M. Curve B (●——●) relates to mixtures containing a constant phenol/potassium laurate molar ratio of 0·399. The activity of the potassium laurate in absence of phenol at 25° C. is represented by curve C (○——○).

Our results (Figs. 5, 7, 10) cannot be fully explained by the interpretation of Bean and Berry without modification, for we have demonstrated a similar behaviour in solutions containing a constant concentration of the phenol, where the presence of more micelles with increasing soap concentration can result only in a phenol depletion of the aqueous phase and a reduced phenol concentration in the micelles. The second concentration of limiting activity, beyond which activity sharply increases, must be explained either as intervention of the bactericidal activity of the soap itself or as a sudden change in the mode of action of the soap-phenol mixture. Since soap solutions were used which differed considerably in bactericidal activity at 20° C., and determinations at 25° C. giving very much shorter extinction times were undertaken, while the concentration of minimal activity was not substantially changed, it is considered that the soap itself cannot be sufficiently toxic to account for the break in the curve.

That limiting activity was still observed at the same concentration when mixtures of varying toxicity were used, so that the extinction time of the mixture was either only a little shorter or very much shorter than that of the soap alone, may also be taken as an indication of independence of soap toxicity.

A sudden change in the mode of action of the mixtures might be explicable in terms of multilayer adsorption of the soap. The limiting concentration could represent the lowest concentration at which micelles begin to be adsorbed on to the bacterial surface to produce a multilayer. Another possible explanation is that at this concentration, the soap produces more extensive damage to the cell surface, this having the effect either of allowing much easier access of the phenol or of causing such extensive leakage of cellular contents that the time required for disinfection is much reduced at higher soap concentrations. It may be that the adsorbed complex exists in a different state at this concentration of soap—that a new, more highly adsorbable complex is formed between the phenol and the soap or its hydrolysis products, as described by Allawala and Riegelman[18]. It may be impossible to draw either correct or complete conclusions until more is known of the structure of micelles, the structure of bacterial surfaces and the ways in which surface-active agents modify these surfaces.

The Third Concentration of Limiting Activity

At concentrations of potassium laurate exceeding the second concentration of limiting activity, the extinction times of the mixtures decrease, at first rapidly. But when the soap concentration lies in the range 0·065–0·080M there is little decrease in extinction times, which may, in fact, increase. After a concentration of about 0·08M has been reached, the extinction times decrease with increasing soap concentration to the same extent as do those with the soap alone, the activity of the mixtures being only a little greater. With phenol (Fig. 10) the extinction times of the mixtures were a little greater than those for the soap alone, and this concentration of the phenol is sufficiently high to promote hydrolysis of the soap. There were not enough estimations with 2-hydroxydiphenyl to draw conclusions (Fig. 5).

The reduction in the rate of increase in activity with increase in soap concentration over the range 0·065–0·080M seems to be due to the effect of the factor responsible for the increase in extinction times at concentrations of soap just above the critical micellar concentration. The rapid increase in activity caused either by adsorption of a more toxic interfacial complex or by increased damage to the cell surface is not maintained because the "free" phenol concentration in the aqueous phase is further reduced by presentation of a much enlarged micellar phase. The activity of the phenol is also reduced by increased dissociation occurring in the more alkaline solutions at these soap concentrations. Only at soap concentrations exceeding this third limiting soap concentration—0·08M—is activity due solely to the soap, the phenol now being largely in solution in the micelles.

Hence the typical extinction time curves for the constant phenol concentration mixtures can be regarded as being made up of two parts, the second part being a repetition of the first part and explicable in similar terms. The first half begins at very high dilutions of the soap and the second half at about 0·045M, ending at that concentration at which the mixture and the soap alone have nearly equal activities. The mechanism is capable of and worthy of further investigation.

Mixtures of Constant Phenol/Soap Molar Ratio

The behaviour of soap-phenol mixtures in which the phenol concentration is increased proportionately to increase in soap concentration varies according to the solubility of the phenol. The least soluble phenol examined, 4-benzylphenol, showed steep increases in extinction times at concentrations in excess of the critical (curves 1–3, Fig. 6), the effect being comparable to that described by Bean and Berry[4] for their chlorinated benzylphenol which was also of comparable solubility in water. The phenol of intermediate solubility, 2-hydroxydiphenyl, exhibited only a slight increase in extinction time over the same soap concentration range (curve B, Fig. 5), whereas the most soluble phenol exhibited only a barely perceptible halt at soap concentrations just above the critical micellar concentration.

The difference in behaviour must be attributed to the differing partitions of the phenol between the micellar and aqueous phases of the solutions over the concentration range. It follows that a phenol of high solubility in water will attain a higher "free" phenol concentration in the aqueous phase in the presence of micelles than will a phenol of low solubility, where the aqueous phase will be much more depleted and the extinction times increased to a much greater extent. Thus constant molar ratio mixtures will exhibit the second limiting concentration effect only when the phenol possesses a partition coefficient permitting low partition in the aqueous phase as compared with the micellar phase.

Concentration Exponents of Soap-phenol Mixtures

The relations between phenol concentrations and extinction times at varying soap concentrations for each of the three phenols (Figs. 4, 8, 9, 11) show certain characteristics which are common to the three systems:

(*a*) Where the bactericidal activity could be determined in aqueous solutions containing no soap, the relationships between extinction time and phenol concentration (curve A, Fig. 4; curve A, Fig. 11) were steeper, i.e., the concentration exponents higher, than in solutions containing soap at any concentration.

(*b*) The more soluble the phenol, the more was its activity increased by addition of all proportions of soap.

(*c*) In the presence of soap, the relationships between phenol concentration and extinction time gradually changed at high dilution of the soap, the slope changing with increase in soap concentration to yield a constant slope over concentrations immediately above and below the critical concentration. Only when the soap concentration was increased beyond

0·04M did the slope become less steep. At that concentration at which activity of the constant concentration mixtures began to increase (about 0·05M) the slope was less than at any other soap concentration. Further increases in soap concentration resulted in a steepening of the curves, the slope probably remaining unchanged over the range 0·06–0·09M.

Thus the concentration exponent of the phenol changes with increasing soap concentration as follows: it is gradually reduced over a concentration range ending at 0·015M, remains constant until that concentration is reached at which extinction times rise towards the maximal value (0·04M), decreases markedly over the range 0·04–0·05M, and then increases to values which, however, are lower than those found around the critical micellar concentration but which probably remain constant from 0·06–0·09M. Hence the second soap concentration of limiting activity is associated with minimal concentration exponents of soap-phenol mixtures.

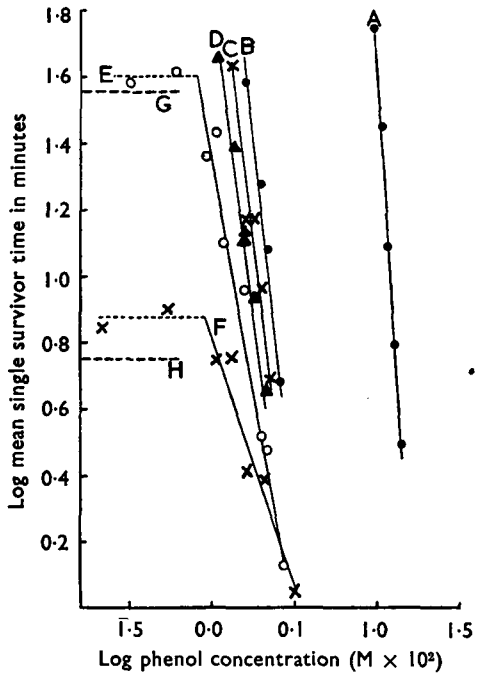

FIG. 11. The bacterical activity of phenol in aqueous solutions of potassium laurate at 25° C. Logarithmic relationships between concentration of phenol and extinction times of *Bact. coli* using five concentrations of potassium laurate: 0·028M (curve B ●——●), 0·0325M (curve C ×——×), 0·040M (curve D ▲——▲), 0·050M (curve E ○——○), and 0·80M (curve F ×——×). Curve A (●——●) represents the activities of solutions of phenol containing no soap, and G and H represent bactericidal activities of potassium laurate alone in concentrations of 0·050 and 0·080M respectively.

It is suggested that each range of changing concentration exponent represents an initiation of a new mode of bactericidal action. At high dilutions (below 0·015M), it is the transitional stage between uptake of phenol by the unmodified bacterial surface and uptake through an interfacial soap-phenol complex at the bacterium-water interface. The second change corresponds to the multilayer adsorption of the mixed film or further modification of the bacterial surface with concomitant increase in bactericidal activity of the mixtures.

That the concentration exponent remains constant over limits of about ± 50 per cent. of the critical concentration supports the findings of Alexander and Tomlinson[2] who demonstrated a roughly constant value over rather wider limits of concentration. Bean and Berry[4] were led to consider that relations between phenol concentration and extinction time

were parallel for different soap concentrations as a result of observations at two concentrations: 0·039 and 0·065M. The results shown in Figures 8 and 9, obtained with a phenol of similar solubility, indicate that, although the slopes at these concentrations may not be greatly dissimilar, a large difference will be found at intermediate concentrations.

Comparison of Activities of the Phenols

Examination of the plotted values in Figures 4–11 at once reveals that phenol is by far the least active under all conditions—in solutions containing no soap and in solutions containing all concentrations of soap—whereas 4-benzylphenol and 2-hydroxydiphenyl have much more nearly equal activity, giving nearly equal extinction times at any given phenol concentration.

Molar concentrations of the two phenols required to give the same extinction time in the presence of varying amounts of soap are recorded in Table I, which also expresses the concentrations as percentages saturation of the solutions, i.e.

$$\frac{\text{phenol/soap molar ratio} \times 100}{\text{saturation phenol/soap molar ratio}}.$$

It is seen that the most saturated systems do not give the highest bactericidal activity irrespective of the nature of the phenol, a 22·8 per cent. saturated solution of 4-benzylphenol having the same activity as a 7·24 per cent. saturated solution of 2-hydroxydiphenyl at soap concentration 0·03M.

TABLE I

MOLAR CONCENTRATIONS AND PERCENTAGES SATURATION OF 2-HYDROXYDIPHENYL AND 4-BENZYLPHENOL REQUIRED TO GIVE IDENTICAL EXTINCTION TIMES (20° C.) IN SOLUTIONS OF POTASSIUM LAURATE

Molar concentration of potassium laurate	Mean single survivor time (minutes)	2-Hydroxydiphenyl		4-Benzylphenol	
		Molar concentration ($\times 10^3$)	Percentage saturation	Molar concentration ($\times 10^3$)	Percentage saturation
0·03	10·0	1·15	7·24	1·32	22·8
0·04	10·0	1·31	5·81	2·29	21·4
0·05	12·6	1·76	5·97	2·11	12·6
0·06	7·4	2·11	5·73	2·63	12·5

At the four different soap concentrations the ratios of molar concentrations of 4-benzylphenol/2-hydroxydiphenyl required to produce extinction of *Bact. coli* in the same time were 1·15, 1·75, 1·20 and 1·25. The second value is much higher than the others, which give an estimate of the ratio of activity of 1 : 1·2.

THE CRITICAL MICELLAR CONCENTRATION

Estimation of the critical concentration for micelle formation of potassium laurate by the two methods described previously gave a wide discrepancy. Solubility determinations, in which the soap solutions were saturated with 4-benzylphenol or 2-hydroxydiphenyl, gave estimates of

0·015M with both phenols. The estimates provided by the concentration of maximal bactericidal activity of mixtures with phenols were always between 0·030 and 0·033M. A possible explanation for the lower values obtained from solubility measurements was depression of the critical concentration on saturation of the solutions with phenols. McBain, Merrill and Vinograd[19] reported that solubility measurements generally gave estimates of critical concentration lower than those obtained by other methods, and McBain and Merill[20] maintained that this was found because increase in solubilising power provided a much more sensitive method than measurement of several other properties. On the other hand, Klevens[21] has shown that his estimates from solubility agree well with estimates by most other methods.

Summary

1. Solubilities in solutions of potassium laurate of two phenols, 2-hydroxydiphenyl and 4-benzylphenol, which exhibited a nearly five-fold difference in water solubility, were estimated spectrophotometrically. The solubility curves are similar to those described by Bean and Berry[4,5] for a chlorinated benzylphenol and for a chloroxylenol.

2. Soap-phenol mixtures containing constant concentrations of phenol, 2-hydroxydiphenyl and 4-benzylphenol, with varying concentrations of potassium laurate, have been examined for bactericidal activity against *Bacterium coli*. The characteristic extinction time-soap concentration curve shows three soap concentrations of limiting activity, the significance of which has been discussed.

3. Mixtures containing a constant phenol/soap molar ratio and varying potassium laurate concentrations give changes in bactericidal activity which depend on the aqueous solubility of the phenol.

4. Increased water solubility of the phenol is associated with greater enhancement of bactericidal action in the presence of soap at all concentrations.

5. The concentration exponents of aqueous solutions of phenols containing no soap are greater than those of solutions containing soap. The concentration exponents of solutions of phenols containing potassium laurate are roughly constant over a soap concentration range extending from 0·015 to at least 0·040M. Possible interpretations of deviations at either extreme of this range have been put forward.

6. 4-Benzylphenol and 2-hydroxydiphenyl have been shown to be nearly equally active as bactericides despite their difference in solubility in water and in solutions of potassium laurate. Equally saturated solutions of different phenols are therefore not necessarily equally active. Comparable bactericidal activity depends upon the concentration of the compounds in total solution and, over a range of soap concentrations, 2-hydroxydiphenyl was found to be about 1·2 times more active than 4-benzylphenol.

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