THE BACTERICIDAL ACTIVITY OF PHENOLS IN AQUEOUS SOLUTIONS OF SOAP

PART II.—THE BACTERICIDAL ACTIVITY OF BENZYLCHLOROPHENOL IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

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AN earlier paper[1] described the solubility of a slightly water-soluble phenol (benzylchlorophenol)* in aqueous solutions of potassium laurate, and showed how such solubility depended upon the presence of micelles in the potassium laurate solution. The present paper is concerned with the bactericidal activity of such solutions, and, in particular, with the changes in bactericidal activity that are associated with changes in the concentration of both components, phenol and soap.

In order to obtain the maximum information from the experiments it would have been ideal if the bactericidal measurements could have been extended over an infinitely wide range of concentrations. Such a range would give a wide range of death-times with a particular strain of organism. It is only practicable, however, to extend bactericidal measurements from about 1 minute to a maximum of about 8 hours. Hence, it was apparent at the outset that it might be necessary to make some modification to the techniques available for the measurement of bactericidal activity, and, in order to cover a sufficiently wide range of concentrations, it might be necessary to employ more than one strain of test organism. Preliminary experiments indicated that it would be convenient to use a strain of *Bacterium coli* with the weaker solutions and a strain of *Pseudomonas pyocyanea* with the more concentrated solutions. *Ps. pyocyanea* was found to possess greater resistance to the bactericide in question than did the spores of several strains of *Bacillus subtilis*, but suffered from the slight disadvantage that it had a tendency to clump in the presence of potassium laurate. This ruled out the possibility of using a counting technique, and led to the development of an extinction method. The latter was designed to be as simple as possible and to minimise sampling errors arising through clumping of the test organism during the reaction. A more detailed account of the method and an indication of its reliability, as deduced from a large number of experiments performed with phenol, has been given elsewhere[2]. Phenol was employed as the bactericide during the development of the method as it has long been used as a reference substance in bactericidal assays, and the behaviour of bacteria in its solutions is well known.

* 5-chloro-2-hydroxydiphenylmethane.

EXPERIMENTAL

1. *The Determination of Bactericidal Activity.*

Essentially, the method consisted of adding a standardised inoculum to a standard volume of a solution of the bactericide, immediately distributing samples of the reacting mixture into sterile tubes and ultimately quenching the reaction by the addition of sterile broth.

(a) *The Preparation of the Bactericide for Inoculation.* 5·0 ml. of the solution of the bactericide to be assayed was pipetted into a 50-ml. "Pyrex" glass-stoppered bottle and placed in a water-bath at 20°C. ± 0·1°C. for 20 minutes.

(b) *Inoculation of the Bactericide.* 0·2 ml. of a standardised suspension of the organisms prepared from a 24-hour growth on agar was delivered below the surface of the bactericide at 20°C., and a stop-watch was started at the moment of contact. The bottle was swirled to aid even mixing, shaking being avoided as it tended to cause frothing.

(c) *Sampling the Inoculated Bactericide.* A portion of the inoculated bactericide was withdrawn as soon as possible after mixing, into a sterile dropping pipette which was clamped vertically and fitted at its upper end with a rubber teat. Six uniform drops were then delivered from the pipette at 1 sec. intervals[3,4] on to the bottom of each of a series of sterile "Pyrex" glass tubes, which had been in a water-bath at 20°C. for about 20 minutes. The tubes were then immediately replaced in the water-bath, and the reaction between the bactericide and the bacteria allowed to proceed at the controlled temperature. The technique of sampling the reacting organisms immediately after mixing them with the bactericide was adopted to minimise sampling errors introduced by any tendency of the organisms to clump during the bactericidal reaction.

(d) *Examination of the Sample for Sterility.* When the reaction between the bactericide and bacteria had been in progress for a known time, one of the tubes containing the sample drops was removed from the water-bath, and the reaction was quenched by the addition of 5·0 ml. of sterile nutrient broth from an all-glass automatic pipette[4]. The tube was then transferred to an incubator at 37°C. for 3 days, at the end of which time it was examined for the presence of bacterial growth. Simi-

TABLE I

THE MEAN DEATH-TIME OF *Bact. coli* AND *Ps. pyocyanea* IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

Organism	Concentration of potassium laurate	Log. Concentration of potassium laurate	Mean death-time	Log. of mean death-time
			minutes	
Bact. coli	0·10 M	$\bar{1}$·0000	40·00	1·6020
,, ...	0·20 M	$\bar{1}$·3010	10·90	1·0374
,, ...	0·30 M	$\bar{1}$·4771	3·16	0·4997
,, ...	0·40 M	$\bar{1}$·6020	1·66	0·2201
Ps. pyocyanea	0·08 M	$\bar{2}$·9031	16·3	1·2122
	0·09 M	$\bar{2}$·9542	6·00	0·7781

larly, after predetermined time intervals, the reaction in the remaining tubes of the series was quenched and the tubes incubated at 37°C. for 3 days.

2. *The Bactericidal Activity of Aqueous Solutions of Potassium Laurate.*

The components of a bactericide containing a halogenated phenol dissolved in an aqueous soap solution, may each be expected to make some contribution to the total bactericidal activity. An attempt was made, therefore, to assess the contributions made separately by the potassium laurate and the benzylchlorophenol in our solutions. Preliminary trials, carried out by the method described above, suggested that the most suitable concentration range of potassium laurate over which bactericidal measurements could be made against *Bact. coli* was between 0·1 and 0·4 M, and between 0·08 and 0·10 M when *Ps. pyocyanea* was used. Such solutions of potassium laurate were prepared by diluting M potassium laurate with freshly boiled and cooled distilled water.

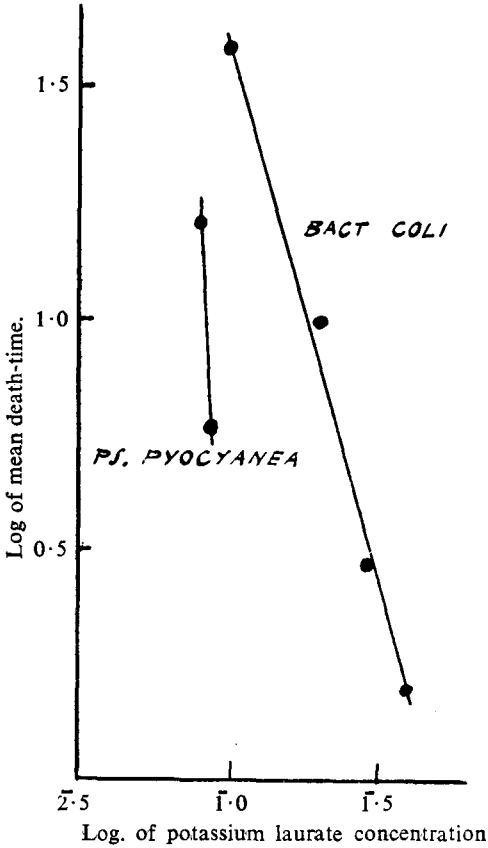

FIG. 1. Relation between the concentration of potassium laurate and the death-time of *Bact. coli* and *Ps. pyocyanea.*

The relatively high concentration of soap in the incubated broth produced turbidity in all the tubes. In order to differentiate between the turbidity produced by the soap and that produced by bacterial growth, it was necessary to subculture the contents of each tube into fresh broth after 24 hours incubation, and incubate a further 48 hours. The mean death-times of *Bact. coli* and *Ps. pyocyanea* in solutions of potassium laurate are shown in Table I, and in each case they are the mean of 6 identical experiments.

The exponential relation between the concentration of potassium laurate in the solutions and the mean death-times of the organisms is shown in Figure 1.

The bactericidal activity of 0·015 M to 0·04 M solutions of potassium laurate is of special interest because it is between these concentrations that the soap micelles develop. Unfortunately, it was not possible to extend bactericidal measurements down to this comparatively low soap concentration, but it can be shown by extrapolation of Figure 1, that the death-time of *Bact. coli* would be about 280 minutes in 0·04 M potassium laurate, about 1,260 minutes in 0·02 M and about 5,500 minutes in 0·015 M potassium laurate. The death-time of *Ps. pyocyanea* in solutions of potassium laurate of the same concentration would be much longer. Thus, it may be concluded that 0·015 M to 0·04 M solutions of potassium laurate have negligible bactericidal action on the test organisms, as far as these experiments are concerned.

3. *The Bactericidal Activity of a Saturated Aqueous Solution of Benzylchlorophenol.*

A saturated aqueous solution of benzylchlorophenol was prepared by boiling approximately 5 g. with 1 l. of distilled water for about 15 minutes. The solution, together with the undissolved material, was allowed to cool, and set aside in a stoppered bottle, which was shaken frequently during 1 week. Then 0·2 ml. of the standard suspensions of *Bact. coli* or *Ps. pyocyanea* were added to separate 5·0 ml. portions of the supernatant. Samples of the mixture of the supernatant liquid and the bacterial suspension were removed at regular intervals for 10 hours, transferred to nutrient broth, and incubated at 37°C. for 3 days. None of the samples was sterile. It may, therefore, be concluded that no significant contribution to the total activity of a bactericide consisting of benzylchlorophenol in aqueous solution of potassium laurate is made by

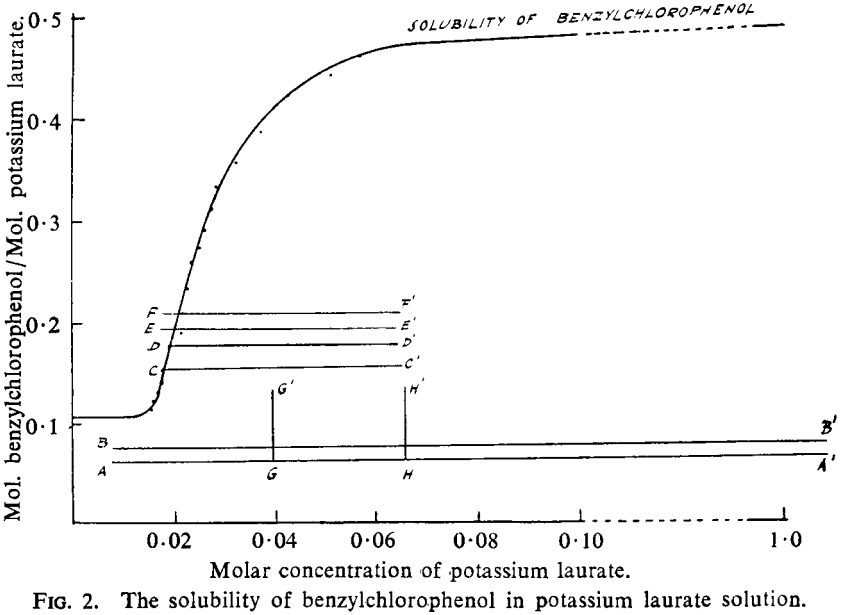

FIG. 2. The solubility of benzylchlorophenol in potassium laurate solution.

the benzylchlorophenol in the water alone, even though the latter be saturated with it.

4. *The Bactericidal Activity of Aqueous Solutions of Benzylchlorophenol and Potassium Laurate.*

Having shown that neither saturated aqueous solutions of benzylchlorophenol, nor aqueous solutions of potassium laurate in the region of the critical concentration, possesses any significant bactericidal activity, the activity of solutions containing both components was studied. The composition of the solutions examined may be represented by points within the curve showing the solubility of benzylchlorophenol in potassium laurate. This is reproduced in Figure 2.

The experiments were planned to illustrate the influence on the bactericidal activity of:—(i) increasing the concentration of both the benzylchlorophenol and the potassium laurate in the same proportion, (ii) increasing the proportion of benzylchlorophenol to potassium laurate without increasing the concentration of the latter.

(a) *The effect on the bactericidal activity of increasing the concentration of both the benzylchlorophenol and the potassium laurate by the same proportion.* To determine the effect of increasing the concentration of both the benzylchlorophenol and the potassium laurate, several series of solutions were prepared and their bactericidal activity measured. Their composition may be represented by points along the horizontal

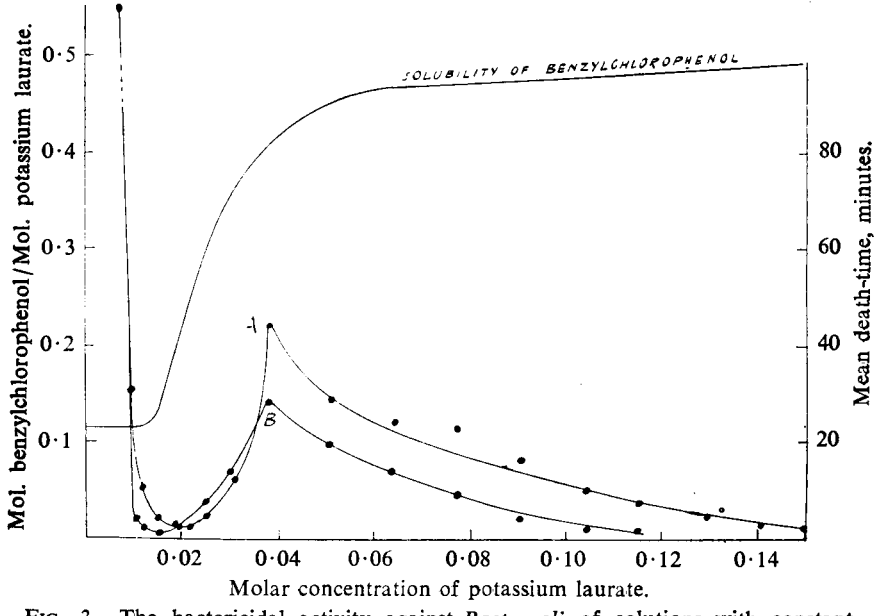

FIG. 3. The bactericidal activity against *Bact. coli* of solutions with constant benzylchlorophenol/potassium laurate ratio and increasing potassium laurate concentration, and the relation of the activity to the solubility of benzylchlorophenol.

A. 0·066 Mol. benzylchlorophenol/Mol. potassium laurate.
B. 0·078 Mol. benzylchlorophenol/Mol. potassium laurate.

lines in Figure 2. For solutions, the composition of which may be represented by points along the lines A – A′ and B – B′, *Bact. coli* was used as the test organism. For solutions containing higher proportions of benzylchlorophenol to potassium laurate (i.e., along the lines C – C′, D – D′, E – E′ and F – F′) the more resistant *Ps. pyocyanea* was used. The limits of the composition range of the solutions examined in each of the series were determined by the ability to measure the death-times of the test organisms in the solutions. For example, it is not possible to measure a death-time of less than about 1 minute, nor is it convenient to continue an experiment over much more than about 8 hours.

(i) *The preparation of the solutions.* The required volumes of "working solution"[1], 0·1M potassium laurate and freshly boiled and cooled distilled water were mixed to provide solutions of the composition shown in Table II. 5·0 ml. portions of the prepared solutions were taken, and their bactericidal activity assessed by the method described previously. The mean death-times of a standard suspension of *Bact. coli*

TABLE II

MEAN DEATH-TIMES OF *B. coli* IN SOLUTIONS OF BENZYLCHLOROPHENOL AND POTASSIUM LAURATE

Concentration of potassium laurate	Mean death-time of *B. coli* when $\frac{\text{Mols benzylchlorophenol}}{\text{Mol. soap}} = 0{\cdot}0666$ (*i.e.*, solns. along line A–A[1] of Fig. 2)	Mean death-time of *B. coli* when $\frac{\text{Mols. benzylchlorophenol}}{\text{Mol. soap}} = 0{\cdot}0778$ (*i.e.*, solns. along line B–B[1] of Fig. 2)
	minutes	minutes
0·0065 M	>480	>480
0·0081 M	—	190
0·0097 M	31·6	3·8
0·0113 M	—	2·3
0·0130 M	11·0	2·2
0·0162 M	4·6	1·3
0·0194 M	3·6	2·8
0·0227 M	2·5	2·1
0·0259 M	4·6	8·6
0·0324 M	13·3	13·3
0·0389 M	44·1	29·1
0·0520 M	28·3	19·6
0·0648 M	23·6	14·6
0·0776 M	22·6	10·3
0·0908 M	16·6	3·3
0·1037 M	9·0	3·2
0·1042 M	—	3·0
0·1167 M	6·3	2·6
0·1296 M	4·2	—
0·1426 M	3·0	—
0·1555 M	2·5	—

in solutions of which the composition is represented by points along the lines A – A′ and B – B′ are tabulated in Table II, each result recorded being the mean of that obtained in 6 experiments. In Figure 3 the death-times are shown superimposed on the solubility curve.

Figure 3 shows that as the concentration of the solutions was increased (i.e., as the lines were traversed from A to A^1 and from B to B^1 respectively) a very marked increase in bactericidal activity was observed until a potassium laurate concentration of approximately 0·015 M was reached. From about 0·015 M to about 0·04 M, potassium laurate there was an equally marked fall in activity, though the concentration of both components was increased. As the potassium laurate concentration was increased beyond 0·04 M, there was again an increase in activity, but it was more gradual than the previous increase.

The experiments performed with *Ps. pyocyanea* using the solutions containing the higher proportions of benzylchlorophenol to potassium laurate (i.e., along the lines C – C′, D – D′, E – E′ and F – F′ of Figure 2) were generally less satisfactory. The spread of the end-points in the several experiments performed with each solution was much greater than that experienced when using *Bact. coli* with the more dilute solutions. The inconsistencies were no doubt due in part to the marked tendency of *Ps. pyocyanea* to clump in solutions of potassium laurate. The clumping inevitably produced larger sampling errors. It gave rise, on the one hand to false "sterile" samples, and, on the other, to clumps containing organisms which were partially protected from the action of the bactericide by virtue of their being in the centre of the clump. Nevertheless, the use of *Ps. pyocyanea* made it possible to assess approximately the activity of solutions which would have killed *Bact. coli* too rapidly for death-time measurements to have been made. Another factor which must have contributed to the erratic results was the unstable nature of of some of the solutions. The benzylchlorophenol they contained was on the borderline of being just completely soluble in the potassium laurate and of failing to be completely soluble. Further, the potassium laurate concentration was between the limits at which there is initial and complete micelle formation. In view of the above factors, it would be surprising if the results were not somewhat erratic. The results obtained did indicate a definite trend in the manner in which the bactericidal activity changed with changes in the composition of the solutions. A few of the results (i.e., those relating to solutions the composition of which may be represented by points along the line F – F′ of Figure 2) are reproduced in Table III.

The results show again that the effect of increasing the concentration of the solutions. At first the increase in activity is marked, but it is proportion is, in general, to produce an increase in the bactericidal activity of the solutions. At first the increase in activity is marked, but it is arrested when the soap concentration reaches approximately 0·015 M. This maximum of activity is followed by a minimum at approximately 0·04 M. potassium laurate, and then again by a gradual increase. The general changes in activity with changes in the concentration of the

solutions follow closely those already described for solutions containing a lower proportion of benzylchlorophenol per molecule of potassium

TABLE III

MEAN DEATH-TIMES OF *Ps. pyocyanea* IN SOLUTIONS CONTAINING BENZYLCHLOROPHENOL AND POTASSIUM LAURATE

Concentration of potassium laurate	Mean death-time of *Ps. pyocyanea* when Mol. benzylchlorophenol / Mol. soap = 0·2121 (*i.e.*, solutions along line F–F¹ of Fig. 2)
	minutes
0·0134 M	430·0
0·0188 M	31·0
0·0216 M	2·3
0·0270 M	2·0
0·0324 M	1·8
0·0431 M	3·3
0·0540 M	1·2
0·0648 M	1·1

laurate. The significance of the changes in activity will be discussed later.

(b) *The effect on the bactericidal activity of increasing the proportion of benzylchlorophenol to a fixed concentration of potassium laurate.* The second effect examined was that of increasing the concentration of benzylchlorophenol in solutions containing a fixed concentration of

TABLE IV

THE EFFECT ON THE MEAN DEATH-TIMES OF *Bact. coli* OF INCREASING THE CONCENTRATION OF BENZYLCHLOROPHENOL IN SOLUTIONS HAVING A FIXED CONCENTRATION OF POTASSIUM LAURATE

Mol. Benzylchlorophenol / Mol. potassium laurate	Mean death-time of *Bact. coli* when concentration of potassium laurate is 0·0389 M	Mean death-time of *Bact. coli* when concentration of potassium laurate is 0·0648 M
	minutes	minutes
0·0666	44·1	23·6
0·0778	29·2	14·6
0·0933	17·6	4·5
0·1167	8·5	2·8
0·1244	5·5	2·0
0·1334	2·5	——

potassium laurate. The composition of the solutions examined bacteriologically may be represented by points along the vertical lines G – G′ and H – H′ in Figure 2. In each case *Bact. coli* was used as the test organism. The mean death-times of each solution are tabulated in Table IV.

In Figure 4 the logarithm of the mean death-times of *Bact. coli* in the solutions is plotted against the logarithm of mol. benzylchlorophenol/mol. potassium laurate. A straight line results with each concentration of potassium laurate.

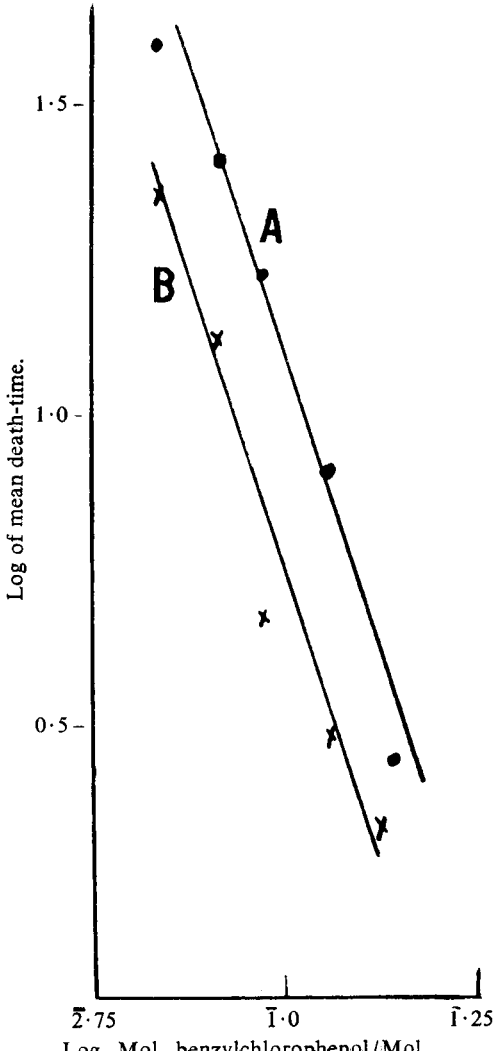

FIG. 4. The effect on the death-time of *Bact. coli* of increasing the proportion of benzylchlorophenol to potassium laurate in solutions with constant potassium laurate concentration.

A. 0·039 M Potassium laurate.
B. 0·065 M Potassium laurate.

DISCUSSION

We have shown earlier[1] that the solubility of benzylchorophenol is very much greater in certain aqueous solutions of potassium laurate than it is in water. There is a marked increase in its solubility in those solutions of potassium laurate which are micellar, and we have shown that the micelles are responsible for solubilising it.

When benzylchlorophenol is dissolved in aqueous solutions of potassium laurate which are more concentrated than the "critical concentration," most of the benzylchlorophenol will be in solution in the micelles. A very small proportion of it will be in equilibrium in the continuous aqueous phase. It will in fact be distributed between the micelles and the water in accordance with the partition coefficient. When bacteria are introduced into such a system, it is postulated that the potassium laurate micelles containing the benzylchlorophenol will be adsorbed on the bacterial surfaces, and benzylchlorophenol will diffuse from the micelles to the bacteria. Thus the bactericidal activity of the system containing

benzylchlorophenol and potassium laurate in excess of its critical concentration must be dependent almost entirely upon the concentration of the benzylchlorophenol in the micelles. The present communication presents evidence in support of this hypothesis. It examines the effect on the bactericidal activity of the systems of increasing the proportion of benzylchlorophenol to potassium laurate, and of increasing the concentration of both the benzylchlorophenol and the potassium laurate in the same proportion.

In the experiments designed to show the effect on the bactericidal activity of increasing the concentration of both the benzylchlorophenol and the potassium laurate by the same proportion (i.e., the reverse of diluting with water), two series of solutions were prepared. One series contained 0·0566 mol. benzylchlorophenol/mol. potassium laurate and the other 0·0778 mol. benzylchlorophenol / mol. potassium laurate. The proportions of benzylchlorophenol to potassium laurate were not significant in themselves, but were convenient in that they provided measurable death-times of *Bact. coli* over a very wide range of potassium laurate concentration, and were easily prepared from the "working solution"[1].

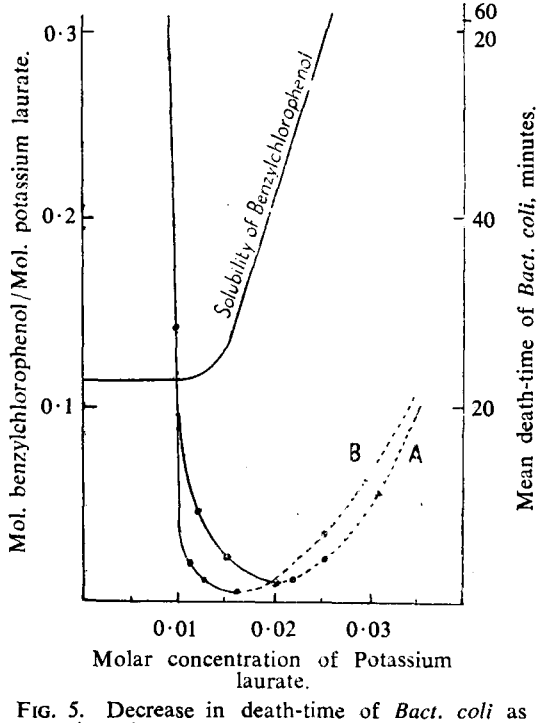

FIG. 5. Decrease in death-time of *Bact. coli* as potassium laurate concentration is increased up to that at which micelles form.

A. 0·066 Mol. benzylchlorophenol/Mol. potassium laurate.

B. 0·078 Mol. benzylchlorophenol/Mol. potassium laurate.

Table II and Figure 5 show that as the concentration of both components was increased from a potassium laurate concentration of 0·0065 M to approximately 0·020 M (i.e., along the lines A – A′ and B – B′ of Figure 2), the bactericidal activity of the system as shown by the decrease in the death-time of *Bact. coli* increased markedly. These solutions were non-micellar but did contain benzylchlorophenol in excess of its solubility in water. An explanation of this phenomenon has been

referred to in our previous paper[1]. The great increase in the bactericidal activity with increase in concentration of these solutions may be due to one of two causes. It is probably due to the increase in the concentration of the benzylchlorophenol which occurs as the potassium laurate is increased from about 0·0065 M to about 0·020 M, and to the reduction in the surface tension of the solutions which accompanies the increase in the potassium laurate concentration. A similar observation has been described by Alexander and Trim[7], who found that the addition of non-micellar soap to an aqueous solution of hexylresorcinol lowered the surface tension of the latter and thus increased the rate of its penetration into nematodes.

Alternatively, the increase in the activity of our solutions in the range under discussion may possibly be due to adsorption of the laurate ions on the bacterial surface in much the same way that water soluble bactericides are adsorbed[8]. Such an adsorption and local concentration of laurate ions would undoubtedly lead to the local formation of micelles on the bacterial surface, even though none existed in the solution. The benzylchlorophenol would leave the water to distribute itself between the micelles and thus cause a local concentration of benzylchlorophenol much higher than that obtaining in the water.

The activity of the solutions reached a maximum when the potassium laurate concentration was between 0·015 M and 0·020 M. In the series of solutions containing 0·0666 mol. benzylchlorophenol/mol. potassium laurate the maximum activity (i.e., minimum death-time of *Bact. coli* was observed at a potassium laurate concentration of approximately 0·020 M (Fig. 5) and in the series containing 0·0778 mol. benzylchlorophenol/mol. potassium laurate at a potassium laurate concentration of 0·016 M. It is unlikely that there was any real difference in the concentration of potassium laurate at which maximal activity occurred in the two series of solutions. The apparent difference can be attributed to the experimental errors that are always associated with the type of biological assay involved.

As the potassium laurate concentration was increased from that at which the maxima were observed to approximately 0·04 M, the bactericidal activity decreased markedly in both series of solutions (Fig. 6). The proportion of benzylchlorophenol to potassium laurate was kept constant in each of the two series of solutions. As the concentration of the potassium laurate in the solutions was increased, so the concentration of the benzylchlorophenol increased. Notwithstanding these increases in concentration the activity of the solutions fell. Clearly then, the concentration of benzylchlorophenol in the solutions as a whole does not alone control their bactericidal activity. The activity of the solutions must be largely independent of the total concentration of the benzylchlorophenol, even though it must be the latter that endows the solutions with activity, since aqueous solutions of potassium laurate alone and at the same concentration are without bactericidal activity.

An explanation of the fall in activity that is associated with an increase in the concentration of both the benzylchlorophenol and the potassium

laurate, when the latter is between 0·015 M and 0·04 M, may be derived from an examination of the changes in the concentration of the benzylchlorophenol in the potassium laurate micelles.

The solubility of benzylchlorophenol in aqueous solutions of potassium laurate rises sharply as the concentration of the latter is increased from

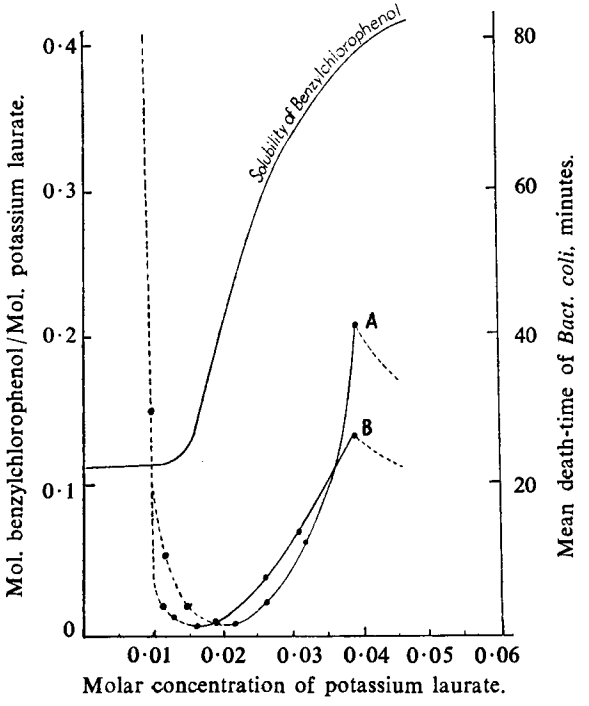

FIG. 6. Increase in death-time of *Bact. coli* as potassium laurate concentration is increased over range of micelle formation.

A. 0·066 Mol. benzylchlorophenol/Mol. potassium laurate.
B. 0·078 Mol. benzylchlorophenol/Mol. potassium laurate.

about 0·015 M to 0·06 M (Fig. 2 and 3). Below about 0·015 M, potassium laurate is ionised in aqueous solution. At this "critical concentration" the ions agglomerate into small groups or micelles, which increase in size with increase in concentration of the soap until the latter approximates 0·06 M, when they reach maximal size. As the potassium laurate concentration is increased over this range, the amount of benzylchlorophenol necessary to saturate the solutions increases rapidly. In our solutions, however, the proportion of benzylchlorophenol to potassium laurate was kept constant as the concentration of the latter was increased. Hence, the degree of saturation of the micelles decreased as the potassium laurate concentration increased from about 0·015 M to about 0·06 M.

The use of a very slightly water-soluble substance, such as benzylchlorophenol, makes it possible to estimate the degree of saturation of

TABLE V

THE RELATION BETWEEN THE PERCENTAGE SATURATION OF POTASSIUM LAURATE MICELLES BY BENZYLCHLOROPHENOL AND THEIR BACTERICIDAL ACTIVITY AS INDICATED BY THE DEATH-TIME OF *Bact. coli*

Mol. benzylchlorophenol / Mol. potassium laurate	Mol. concentration of potassium laurate	Mol. benzylchlorophenol / Mol. potassium laurate to saturate solution	Percentage saturation of micelles	Log. of percentage saturation of micelles	Death-time of *Bact. coli* minutes	Log. of death-time of *Bact. coli*
0·0666	0·0227	0·233	$\frac{0{\cdot}0666 \times 100}{0{\cdot}233} = 0{\cdot}287$	1·4578	2·5	0·3979
0·0666	0·0259	0·275	$\frac{0{\cdot}0666 \times 100}{0{\cdot}275} = 0{\cdot}240$	1·3802	4·6	0·6628
0·0666	0·0324	0·359	$\frac{0{\cdot}0666 \times 100}{0{\cdot}359} = 0{\cdot}184$	1·2644	13·3	1·1239
0·0666	0·0388	0·395	$\frac{0{\cdot}0666 \times 100}{0{\cdot}395} = 0{\cdot}170$	1·2229	44·1	1·6318
0·0778	0·0227	0·233	$\frac{0{\cdot}0778 \times 100}{0{\cdot}233} = 0{\cdot}338$	1·5292	2·1	0·3222
0·0778	0·0259	0·275	$\frac{0{\cdot}0778 \times 100}{0{\cdot}275} = 0{\cdot}283$	1·4516	3·6	0·5563
0·0778	0·0324	0·359	$\frac{0{\cdot}0778 \times 100}{0{\cdot}359} = 0{\cdot}217$	1·3359	13·3	1·2939
0·0778	0·0388	0·395	$\frac{0{\cdot}0778 \times 100}{0{\cdot}395} = 0{\cdot}197$	1·2944	29·1	1·4639

the micelles by benzylchlorophenol at any potassium laurate concentration, provided the amount of benzylchlorophenol to saturate the solution and the amount actually in the solution are known. The former is obtainable from the solubility curve[1] and the latter from the known composition of the solutions. The percentage saturation of the micelles in several of the solutions has been calculated from the equation:

$$\text{Per cent. saturation of micelles} = \frac{\dfrac{\text{Mol. benzylchlorophenol}}{\text{Mol. potassium laurate}} \text{ in solution}}{\dfrac{\text{Mol. benzylchlorophenol}}{\text{Mol. potassium laurate}} \text{ to saturate solution}} \times 100$$

The result is set out in Table V, which emphasises that, as the concentration of the potassium laurate was increased, the percentage saturation of the micelles by the benzylchlorophenol fell, even though the total concentration of it in the solutions increased. The relation between the calculated percentage saturation of the micelles and the death-time of *Bact. coli* in the solutions is plotted in Figure 7. It will be seen that a decrease in the degree of saturation of the micelles is accompanied by a marked decrease in the bactericidal activity of the solutions. The relation between the percentage saturation of the micelles and the activity of the solutions is logarithmic. This is the normal relation between the concentration of a bactericide and its activity. Thus, the bactericidal activity of the solutions must be determined by the concentration of the benzylchlorophenol in the micelles alone, and not by the overall concentration in the system.

In both series of solutions the bactericidal activity increased again as the potassium laurate concentration was increased beyond approximately 0·04 M (Fig. 8). The cause of this second increase is not clear and must be the subject of further investigation. In the meantime, a probable explanation is offered.

Reference to the solubility curve of benzylchlorophenol in potassium laurate will show that the curve is beginning to flatten out at 0·04 M potassium laurate. Above 0·04 M there is a marked fall in the rate of production of micellar soap with increase in concentration of the potassium laurate, due to the fact that the micelles are approaching maximal size. We have shown above that a rapid increase in the amount of micellar soap leads to a reduction in the degree of saturation of the micelles by the benzylchlorophenol and a consequent fall in activity. It is therefore to be expected that a reduction in the rate of production of micellar soap with increase in concentration of the potassium laurate, would arrest the fall in the bactericidal activity of the solutions.

An additional factor appears to operate when the potassium laurate concentration exceeds 0·04 M. It has just been stated that at 0·04 M potassium laurate the micelles are approaching maximal size. When they have reached maximal size, the addition of further potassium laurate to the solution must lead to an increase in the number of micelles per unit volume, rather than to an increase in their size. Because the proportion of benzylchlorophenol to potassium laurate in the solutions was kept constant, the additional micelles produced when the soap concentration

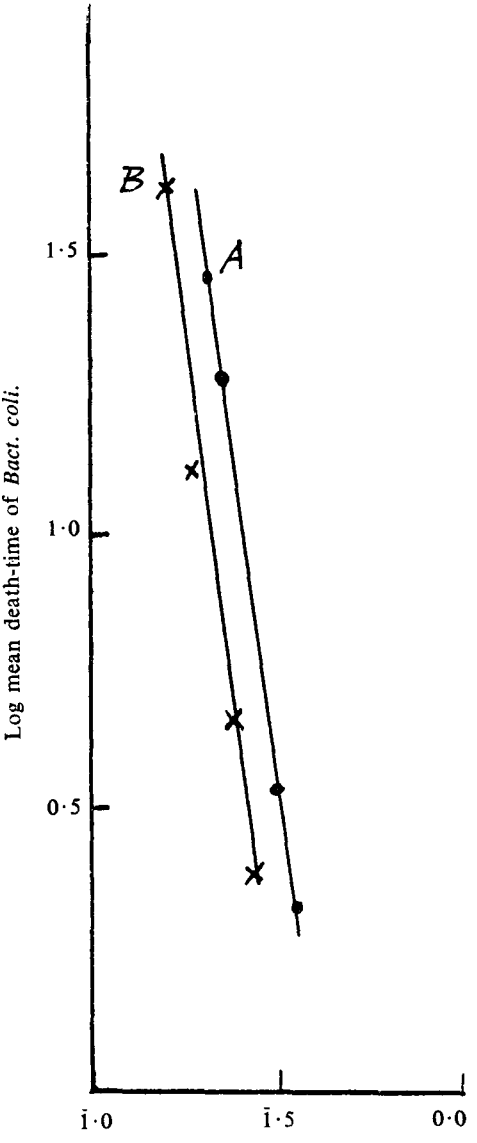

FIG. 7. Relation between percentage saturation of micelles by benzylchlorophenol and the log mean death-time of *Bact. coli.*

A. 0·066 Mol. benzylchlorophenol/Mol. potassium laurate.

B. 0·078 Mol. benzylchlorophenol/Mol. potassium laurate.

exceeded 0·04 M contained the same concentration of benzylchlorophenol, irrespective of the soap concentration. The number of organisms added per ml. to our solutions was also kept constant—within the limits of experimental error. Thus, as the potassium laurate concentration was increased beyond 0·04 M, the solutions contained gradually increasing numbers of micelles per bacterium, and the bacterial surfaces would have gradually increasing numbers of benzylchlorophenol-containing micelles adsorbed on them. Inevitably, this would lead to an increased rate of penetration of the bacteria by the benzylchlorophenol; that is, the bactericidal activity would increase. A few preliminary experiments performed with potassium laurate concentrations in excess of 0·04 M did suggest that a relation does exist between the number of micelles per bacterium and the death-time of the bacteria.

Two further series of experiments were performed. In each series the potassium laurate concentration was kept constant and the concentration of benzylchlorophenol increased. Since the potassium laurate was in the micellar state (Table IV), the addition of benzylchlorophenol increased the concentration of the latter in the micelles. This pro-

duced a marked increase in activity, emphasising again that the bactericidal activity of solution of benzylchlorophenol in aqueous potassium laurate is determined by the concentration of the benzylchlorophenol in the soap micelles.

The experiments indicate that a bactericide consisting of a sparingly water-soluble phenol solubilised by an aqueous solution of a soap will exhibit maximal activity when the micelles are saturated with the phenol. The composition of the bactericide can be arranged so that the micelles

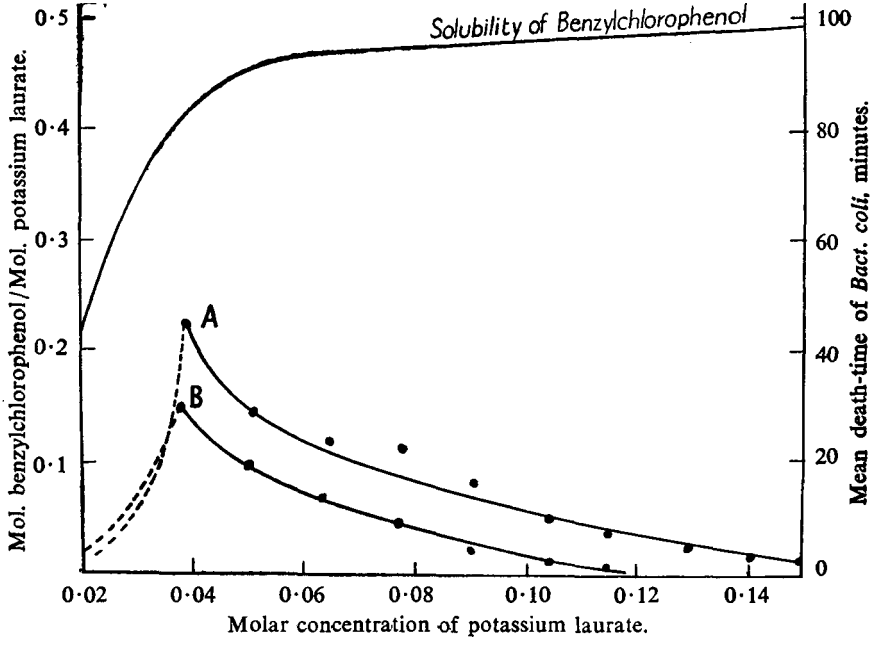

FIG. 8. Decrease in death-time of *Bact. coli* as potassium laurate concentration increased beyond that of complete micelle formation.

A. 0·066 Mol. benzylchlorophenol/Mol. potassium laurate.
B. 0·078 Mol. benzylchlorophenol/Mol. potassium laurate.

are saturated with the phenol at any soap concentration. For a given bactericide, this condition will be most nearly attained when the bactericide has been diluted so that the soap is at its critical concentration.

An interesting practical point arising from the present work is that if the soap concentration lies between the limits corresponding with initial and complete formation of micelles, dilution of the bactericide with water will result in an increase in bactericidal activity. Conversely, concentration will result in a decrease in activity. Further, no less than 3 different dilutions of the bactericide will have the same activity.

SUMMARY

1. A method is described for assessing the bactericidal activity of solutions containing a sparingly water-soluble phenol (benzylchlorophenol) and a soap (potassium laurate).

2. Separate solutions of each of the two components are shown to possess negligible bactericidal activity.

3. When the concentration of both components of the solutions is increased from a low value by the same proportion, the activity of the solutions at first increases considerably. The increase is followed by an abrupt fall in activity and finally by a second gradual increase.

4. An increase in the proportion of benzylchlorophenol to potassium laurate in the solutions produces a marked increase in bactericidal activity.

5. The bactericidal activity of the solutions is shown to be related to the concentration of the benzylchlorophenol in the micelles of the potassium laurate, and independent of the overall concentration in the solutions.

6. The maximal activity of a bactericide consisting of a halogenated phenol solubilised by an aqueous solution of a soap, is exhibited at the "critical concentration" of the soap.

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