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LETTERS TO THE EDITOR

The effect of pH on the bactericidal activity of pentafluorophenol

It is generally accepted that the undissociated molecule is the predominantly toxic form of antimicrobially active weak acids (Ordal, 1941; Winsley & Walters, 1965; Albert, 1968). However, pentachlorophenate ions have been shown to have high bactericidal activity (Allawala & Riegelman, 1954; Hueck, Adema & Wiegmann, 1966). During a study of the activities of ring-substituted fluorine derivatives of phenol (Pinney & Walters, 1967, 1969), we have investigated pentafluorophenol solutions of varying pH to determine whether ionic activity also occurred in this pentahalogenated phenol.

Solutions of 0·15% pentafluorophenol were prepared in 0·04M McIlvaine citric acid-phosphate buffer (McIlvaine, 1921), and 0·1% survivor times of *Escherichia coli* NCTC 5933 in these solutions were determined as before (Pinney & Walters, 1967, 1969). Buffer alone at pH 3·60 produced a 70% kill of the organisms after 5 h, but at pH 5·46, the loss of viability was only 20% after the same period (Fig. 1a). The percentages of ionized molecules at each pH tested were calculated from the Henderson-Hasselbalch equation, the pK_a of pentafluorophenol being 5·33 (Birchall & Haszeldine, 1959). Results are presented in Fig 1b and c.

It is apparent from Fig. 1b, that the intrinsic bactericidal activity of pentafluorophenol in solution decreases markedly with increasing ionization. If the bactericidal

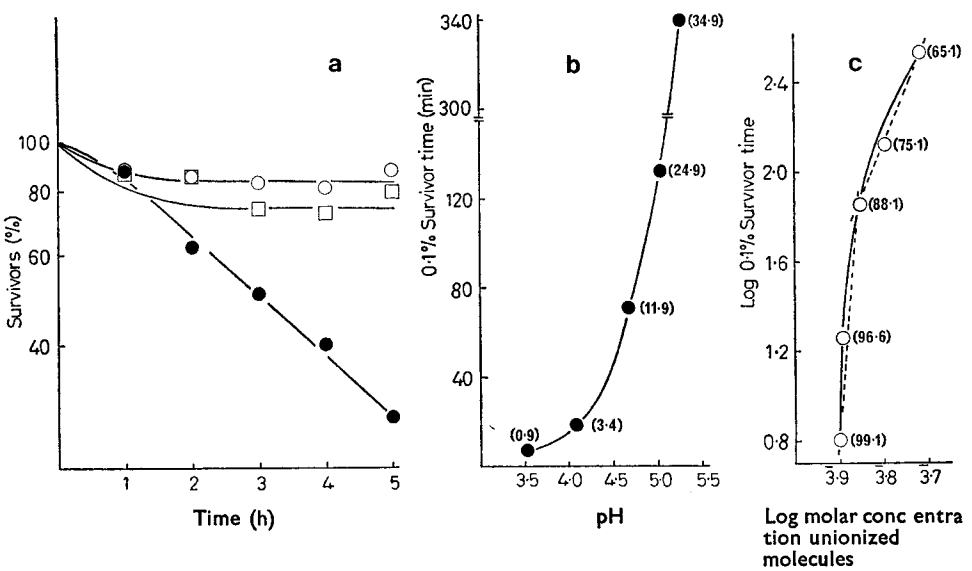

FIG. 1. a. Log survivor-time curves for *E. coli* in water and in citric acid-phosphate buffer. ○ = Water. □ = Buffer, pH 5·46. ● = Buffer, pH 3·60.
b. Relation between pH and 0·1% survivor time for *E. coli* in solutions of 0·15% pentafluorophenol. Figures in brackets are the percentages of ionized molecules.
c. Concentration exponent plot of unionized molecules of 0·15% pentafluorophenol at various pH values against *E. coli*. Figures in brackets are the percentages of unionized molecules. Dashed lines correspond to calculated regressions (slope = n). For 99·1% to 88·1% unionized molecules n = 19·2; for 88·1% to 65·1% unionized molecules n = 5·7.

activity is due only to the undissociated molecules, the concentration exponent plot for unionized molecules (Fig. 1c) should be linear with a slope corresponding to the dilution coefficient (n) of the compound in water. The calculated slope of the regression of log 0·1% survivor time on log molar concentration of unionized molecules (Fig. 1c) for pH 3·54–4·66 (99·1%–88·1% unionized molecules) is 19·2; for pH 4·66–5·22 (88·1%–65·1% unionized molecules) it is 5·7. This latter figure corresponds well with the n value of 5·1 for pentafluorophenol in water (Pinney & Walters, 1969), from which it may be concluded that over this pH range, the antibacterial activity is due to the unionized molecules. Over the lower pH range, the bactericidal effect is greater than would be expected if the unionized molecules alone were responsible for toxicity. The 0·1% survivor time for 0·15% pentafluorophenol in buffer at pH 3·54 (99·1% unionized molecules) is 6·4 min, and for the same concentration in water (pH 3·80 = 98·2% unionized molecules) it is 24·5 min. It is therefore evident that the activity is increased by the presence of buffer as was found with the monofluorophenols (Pinney & Walters, 1967). However, for the buffer to be completely responsible for the enhanced activity at the lower pH, it would need to exert an unequal synergistic toxic effect over the pH range 3·5 to 5·3. Such a mechanism is less likely to be the case than that the increased activity is due to a combination of both the buffer effect and the greater concentration of hydrogen ions at the lower pH values; thus Fig. 1a shows that the toxicity of buffer alone is greater at pH 3·60 than at pH 5·46.

Therefore whilst the precise cause of the biphasic nature of Fig. 1c is not known, it is clear from the results that unlike pentachlorophenol, the toxic form of pentafluorophenol is the unionized molecule; pentafluorophenate ions have little activity.

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