The Effect of pH on the Toxicity of Weak Acids and Bases[1]

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Many of the most important substances which are toxic to bacterial and other living cells belong to one of the groups of weak acids or weak bases, and it has been realized for many years that if these toxic agents were applied to the organism at each of two pH levels the extent of the response might be widely different.

For example one might quote studies on the toxicity of phenylacetic and benzoic acids to *Escherichia coli* and *Staphylococcus aureus* which showed that these acids were much more toxic when applied at acid pH levels than when applied at a neutral pH (1). Conversely, using quinine alkaloids, i.e., weak bases, Michaelis (2) and others showed that the bactericidal action was much greater in slightly alkaline solutions, and similarly, increases in the pH value of the medium has been found to increase the toxicity of atabrine (3) in experiments on *E. coli.* Comparable effects of pH on the activity of weak acids and bases on a wide variety of biological processes have frequently been observed (4, 5, 6, 7). In general it is found that if a certain concentration of a toxic weak acid is applied at a series of pH levels and percent inhibition (of controls at the same pH) plotted against pH, one obtains a sigmoid curve which falls from 0% at one pH to 100% at a pH level a few units lower. The curve becomes displaced along the pH axis when different concentrations of inhibitor are chosen; if the concentration is a higher one the curve will be displaced to the right, and if it is lower, to the left. Such graphs show qualitatively that the inhibitor is more toxic in acid solution, and any single curve might be taken to indicate that the acid is not toxic at a high pH, but this conclusion is not justified because toxic effects could be readily observed at higher pH levels by increasing the concentration of the inhibitor.

The quantitative effect of pH can readily be shown in the manner used extensively by Simon and Blackman in work on the toxicity of nitrophenols to *Trichoderma viride* (4). This organism grows reasonably well at any pH between 3 and 8, and it was cultured at several levels within this range in the presence of finely graded concentrations of the toxic agent. For each pH level a graph was constructed on which per cent inhibition of growth was plotted against Log. concentration of inhibitor, and that concentration which induced a 50% response (L. D. 50) was read off from the steeply falling curve. These equi-effective concentrations were then plotted against pH on a further graph, and

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a curve of the type A shown in Figure 1 was obtained. It can be seen that at a low pH, say 4.0, the L. D. 50 was about 10^{-5}M. but at pH 8.0 the concentration of nitrophenol required to produce the same response was more than 100 times greater. When 2:4 dinitrophenol, which has a pK (pH at which it exists equally in the dissociated and

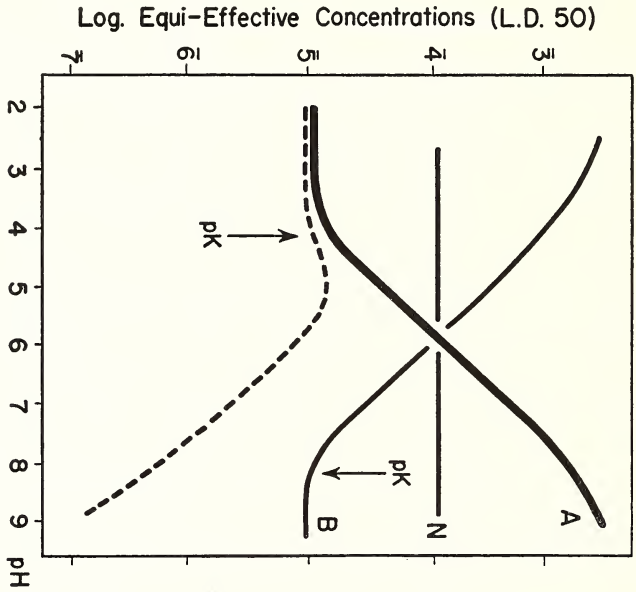

Fig. 1. The determinative effect of pH on the concentration of A, a weak acid, B, a weak base, and N, a non-electrolyte required to induce a standard response. (See text).

undissociated forms) of 4.0 was used, the rise in the curve A began, as shown, when the pH was raised to levels beyond this. With p-nitrophenol, we get the same type of curve but again, the rise does not begin until the pK (7.1) is exceeded; if picric acid (pK 0.8) was used there would be no level part to the curve in the working range pH 3-8.

In general then, at pH levels below the pK of the particular acid the curve levels out; changes in pH in this range have little effect on the concentration of inhibitor required to produce a standard response. As the pH is raised above pK increasingly greater concentrations of inhibitor are required to produce this same response.

Conversely, for weak bases, a mirror image of curve A is obtained, and, as for acids, its placement along the pH axis depends on the pK of the particular compound being used. The bases are most active at alkaline pH levels above pK, where the curve is flat, and as the pH is lowered past pK the curve of L. D. 50 rises sharply.

We note here that a straight line would be obtained for a plot of L. D. 50 values against pH in the range 3-8 if the substance under

investigation was a weak acid with a very high pK (greater than 8) or a weak base with a very low pK (lower than 3). Similarly, changes in pH would have little effect on the activity of non-electrolyte inhibitors. The foregoing considerations show that widely different values for the toxicity of a compound can be obtained at different pH levels and emphasize the need for choice of a suitable pH for toxicity measurement. It is also necessary to maintain rigid control of pH during the measurements because a change of one unit may conceivably make the observed result different from the true value by a factor of some 6-8 times. The pH of the medium is also a factor in experiments in which materials are added which overcome the toxic effect or modify it in some way. These materials may have acidic or basic properties, their effect may well be quite an indirect and unspecific one, if the pH of the medium changes due to their addition.

pH considerations are of great importance too in work on comparative toxicities of a group of compounds. This may best be illustrated by reference to a hypothetical case in which the toxicity of 3 different substances are being compared (Fig. 1). Curve A is that of a weak acid with pK of 4, and B of a weak base with pK of 8. N represents (a) a non-electrolyte, (b) a weak acid with pK greater than 8, or (c) a weak base with pK less than 3. From the graph we can read off the L.D. 50's of these compounds at any pH level, and if we chose three such levels arbitrarily we get the following results.

(1)	At pH 4.0	L. D. 50	A	10^{-5}M.
		L. D. 50	B	10^{-4}M.
		L. D. 50	N	10^{-3}M.

A is 100 times as effective as C.

(2) At pH 6.0 L. D. 50 A, B, N is 10^{-4}M.

All are equally toxic.

(3)	At pH 8.0	L. D. 50	A	10^{-3}M.
		L. D. 50	B	10^{-4}M.
		L. D. 50	N	10^{-5}M.

C is 100 times as effective as A

We see that no valid general statement of comparison is possible; it must be qualified by information on pH and on the pK of the compounds concerned. To overcome this difficulty it is suggested (4, 7) that values for maximum toxicity should be compared. This entails finding L.D. 50 values at pH levels below pK for acids and above pK for bases, where the toxicities are greatest and where small changes in pH have little effect on the result. This suggestion is practical enough for compounds with pK values in the range 3-8 but may be difficult for compounds with pK values outside this range, e.g. it would not be feasible to investigate the reaction of an organism to a substance having a pK of less than 2-3 in this way because the organism would not tolerate such a low pH.

However, on the basis of data from a number of different fields of inquiry Simon (6) has now constructed what we call a general curve (A) which shows the relation between activity (toxicity) and pH for any weak acid. From this relation, if the toxicity at one pH is known, for a particular acid of known pK, the toxic level at pH levels below pK can be calculated. This is really a means of calculating the potential or maximum toxicity of a compound. It might be more important in a particular case to know the toxic concentration at a particular pH, but in seeking for an interpretation of toxicity in terms of biochemical reactions the potential or maximum toxicity is a very useful measure.

It might be asked what is the explanation of these rising curves and why it is that acids are more toxic at low pH levels and bases at high ones. In other words, why does pH of the medium affect the amount of toxic material entering the cell and reacting inside? In the first place we can discount the hypothesis that as the pH level is changed the permeability of the cell to the inhibitor is changed, because wherever in the pH scale the pK occurs, changes in pH beyond this have no effect on the L.D. 50, (and for non-ionizing substances equi effective concentrations are the same at all pH values.)

We have then to consider the effect of pH on the extent of the ionization of the weak acid or base. From qualitative or semi-quantitative studies it has been suggested that since toxicity of acids increases in acid solution where there is a higher concentration of undissociated molecules of the acid, it is this concentration, and not the total concentration of applied inhibitor which produces the response. There seems to be little doubt that the extent of dissociation of the added acid is a major one in determining the toxicity, since it is well established that undissociated acid molecules penetrate more readily that acid anions. The hypothesis that only undissociated molecules are active infers that the ions do not penetrate at all, and this has been assumed to be completely true in analysis of pH effect of phenols on bacteria by Cowles and Klotz. We can test this hypothesis directly on any or all of the curves shown where L.D. 50 is plotted against pH.

We know that the following relation holds between pK and pH and the concentration of molecules and ions of the acid or base.

$$pH = pK + \log \frac{[\text{dissociated acid}]}{[\text{undissociated acid}]}$$

From this equation, since we know the total concentration of acid for any point on the L.D. 50 curve we can calculate what concentration of undissociated molecules exists at that total concentration.

Now if the hypothesis that only the external concentration of undissociated acid determines the response is true, we would expect that the plots would be in a straight line, since the same response is observed in each case.

The broken line in the figure shows the result of such a plot and it is clear that we find a curve which falls sharply as the pH is changed beyond pK. Since we do not get the straight line required by the above

hypothesis, it is clear that the concentration of undissociated acid molecules is not the only factor determining the response. Higher concentrations of inhibitor are needed to produce the effects at higher pH levels, but these concentrations are not so much higher as one would have expected on the hypothesis referred to.

It must therefore be allowed that some other factor, probably the concentration of acid anions has an increasing effect as the pH is altered beyond pK. The possible interpretations are discussed elsewhere by the present authors (7).

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