JOURNAL OF BACTERIOLOGY, June 1967, p. 2029-2030
Vol. 93, No. 6

Influence of *p*H on the Inhibitory Activity of Formic and Acetic Acids for *Shigella*

DAVID J. HENTGES

Department of Microbiology, The Creighton University School of Medicine, Omaha, Nebraska 68131

Received for publication 17 March 1967

In a previous paper (D. J. Hentges, J. Bacteriol. **93**:1369, 1967), I reported that the inhibition of *Shigella flexneri* by *Klebsiella* (*Aerobacter aerogenes*) in mixed culture is due to an effect of formic and acetic acids produced by *Klebsiella* as end products of metabolism. The mechanisms by which these acids exert their antibacterial effect are not understood, although many investigators are of the opinion that undissociated molecules are responsible for toxicity (C. E. A. Winslow and E. E. Lockridge, J. Infect. Diseases **3**:547, 1906; A. S. Levine and C. R. Fellers, J. Bacteriol. **39**:499, 1940; O. Bergeim, J. Infect. Diseases **66**:222, 1940; O. Bergeim et al., J. Infect. Diseases **69**:155, 1941). If this is true, the activity of the acids is dependent on *p*H, which determines the degree of dissociation. At low *p*H, the proportion of undissociated molecules is greater than at *p*H values approaching neutrality. Theoretically, then, the toxicity of formic and acetic acids for *Shigella* should be more pronounced at low *p*H, when the number of undissociated molecules is greater, than at high *p*H. The experiments reported here demonstrate that the inhibitory activity of formic and acetic acids for *Shigella* increases as the *p*H of the medium is lowered. This increased toxicity of the acids cannot be attributed to a hydrogen ion effect alone.

The liquid synthetic medium used consisted of 1% glucose, 1% glutamic acid, 0.4% NH_4Cl, 2% $NaHPO_4$, 0.5% KH_2PO_4, and 0.01% niacin. In one series of experiments, 0.128% acetic acid and 0.023% formic acid, the inhibitory concentrations of the acids produced by *Klebsiella* in mixed culture (Hentges, J. Bacteriol. **93**:1369, 1967), were added to the synthetic medium. The *p*H of the medium was adjusted to 5.5, 6.0, 6.5, or 7.0 by adding either 1 N HCl or 1 N NaOH. Tubes of the medium were then inoculated with *Shigella* to give approximately 5.0×10^4 viable organisms per ml. In another series of experiments, the *p*H of the synthetic medium, without formic or acetic acids, was adjusted as described above, and the medium was similarly inoculated with *Shigella*. All culture tubes were incubated in a water bath (37 C) for 24 hr. To determine population sizes, saline dilutions were made of 24-hr cultures, and 1-ml portions of the dilutions were flooded on the surface of dried veal infusion-agar plates (D. J. Hentges, Am. J. Clin. Pathol. **38**:304, 1962). The plates were incubated at 37 C. Colonies that developed on the surface of the agar were counted the next day.

Results of the experiments are illustrated in Fig. 1 and 2. The lines in the figures are not meant to represent growth curves, but rather to depict differences in viable populations after 24 hr of incubation.

Fig. 1 illustrates the influence of *p*H on the toxicity of formic and acetic acids for *Shigella*. The lines show that, as the *p*H of the medium was lowered with HCl, the toxicity of the acids for *Shigella* increased and the *Shigella* populations became smaller. At *p*H 6.5, there was only slight evidence of toxicity when compared with medium adjusted to *p*H 7.0. At *p*H 6.0, formic and acetic acids were strongly inhibitory for *Shigella*. After 24 hr of incubation, the population increase at *p*H 6.0 was about 4-fold, compared with a 9,000-fold increase at *p*H 7.0. At *p*H 5.5, the acids exerted a slight bactericidal effect. There was an approximately twofold decrease in viable *Shigella* population at this *p*H.

Experiments were conducted next to determine whether the increased toxicity of formic and acetic acids at low *p*H was due to a hydrogen ion effect. *Shigella* was inoculated into tubes of synthetic medium, containing neither formic nor acetic acids, adjusted to *p*H values ranging from 5.5 to 7.0. Results of these experiments are illustrated in Fig. 2. The lines in Fig. 2 show that, after 24 hr of incubation, *Shigella* populations were the same at *p*H 6.0, 6.5, or 7.0. Only at *p*H 5.5 was there any evidence of toxicity due to hydrogen ions. At this *p*H, the *Shigella* population was about one-fourth the size attained in medium at *p*H 6.0, 6.5, or 7.0.

The results show that the toxicity of formic and acetic acids for *Shigella* is greatly influenced by the *p*H of the medium. They also show that the enhanced toxicity of the acids at low *p*H cannot be due to a hydrogen ion effect alone. In synthetic

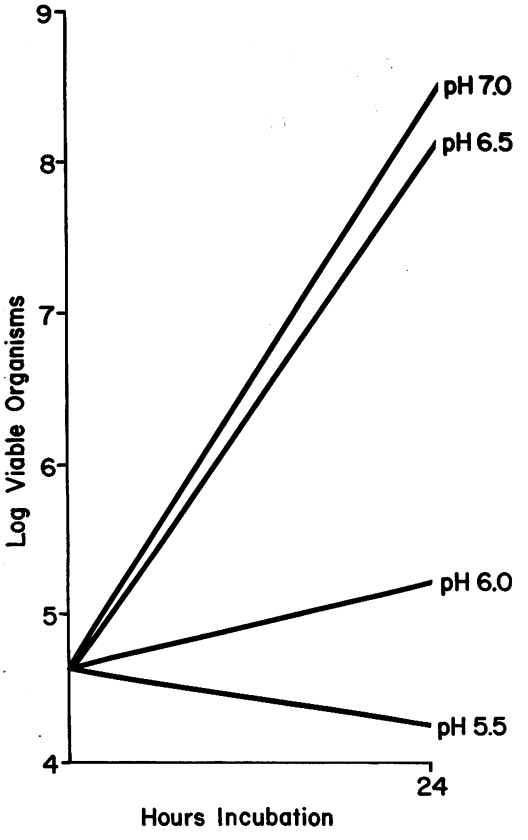

FIG. 1. *Influence of pH on the toxicity of formic and acetic acids for Shigella in synthetic medium.*

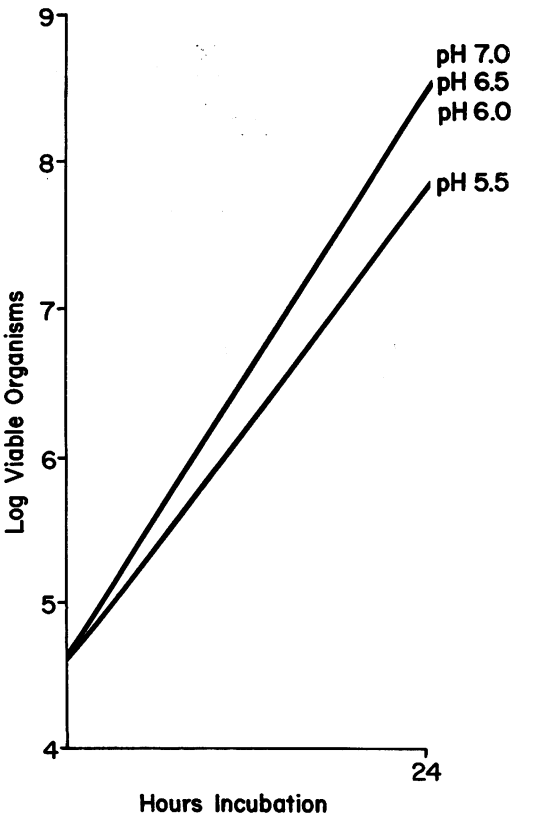

FIG. 2. *Influence of pH on the growth of Shigella in synthetic medium without formic or acetic acids.*

medium without formic and acetic acids, for example, the *Shigella* population was the same size at *p*H 6.0 as at *p*H 7.0 (Fig. 2). The addition of hydrogen ions (HCl) to lower the *p*H of the medium to 6.0 had no effect on *Shigella* growth. Medium containing formic and acetic acids at *p*H 6.0, on the other hand, strongly inhibited *Shigella* growth. The antibacterial activity of the organic acids manifested itself at a much higher *p*H than with HCl. This indicates that hydrogen ion activity alone cannot account for the toxicity of formic and acetic acids for *Shigella*. The evidence presented supports the conclusion that undissociated formic and acetic acid molecules, which increase in proportion as the *p*H of the medium is lowered, are responsible for the inhibition of *Shigella* growth.

This investigation was supported by contract DA DA 17-67-C-0024, U.S. Army Medical Research and Development Command.