this information has proved of value in providing new methods of local therapy with sulfonamide derivatives.[7]

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Mechanism of Sulfonamide Action. I. Acidic Dissociation and Antibacterial Effect.*

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The antibacterial efficiency of sulfonamides as measured by their ability to overcome *p*-aminobenzoic acid (PAB) has previously been shown to be dependent upon the pH of the medium.[1] On the other hand, since the relative efficiency of sulfanilamide and sulfathiazole is nearly constant at different pH values and independent of the bacterial species tested, the antibacterial property of each sulfonamide may be regarded as an inherent characteristic.[2]

The steric resemblance of sulfonamides and PAB and the lack of activity of the ortho and meta derivatives have been accepted as indicating a specific spatial charge distribution. In view of this specificity, one possible explanation of the difference in efficiency of the sulfonamides on the one hand, and of the significantly large ratios of sulfonamide to PAB on the other, might lie in wide variations in their acidic dissociation. This would lead to the conclusion that the effective constituent of sulfonamide solutions is either the anion or the zwitterion, rather than the undissociated acid or the cation.

Preliminary values of the acidic dissociation constants of sulfanilamide, sulfapyridine and sulfathiazole have been obtained using a

[7] Fox, C. L., Jr., in press.

* The authors are grateful to Dr. William Mansfield Clark for advice on the mathematical interpretation of the data.

[1] Schmelkes, F. C., and Wyss, Orville, *J. Bact.*, 1942, **43**, 71.

[2] Wyss, Orville, Grubaugh, K. K., and Schmelkes, F. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 618.

TABLE I.
Acid Dissociation Constants of Sulfonamides.

Sulfanilamide	2.2×10^{-11}
Sulfapyridine	5.1×10^{-9}
Sulfathiazole	6.2×10^{-8}
p-aminobenzoic acid[3]	1.2×10^{-5}

solubility method. The constants calculated for the dissociation

$$NH_2RH \rightleftharpoons NH_2R^- + H^+$$

are listed in Table I.

The most widely accepted explanation of sulfonamide activity is based on its competition with PAB. On the hypothesis presented above it seemed reasonable to conclude that at a high pH, where a proportionately larger fraction of the dissolved sulfonamide exists in an anionic species, it will compete on a more favorable basis with *p*-aminobenzoate ion than at a low pH where acidic dissociation is relatively lower for the weaker acid. The following experimental data show that this is actually the case.

TABLE II.
Effect of [H⁻] upon Sulfonamide Bacteriostasis.

Inhibition of Growth of *E. coli* in a Synthetic Medium.			
pH	7.6	6.8	6.0
20 mg% sulfanilamide	95%	71%	54%
pH	6.7	5.7	4.6
5 mg% sulfadiazine	95%	89%	17%
Inhibition of Growth of *Strep. pyogenes* (1896 Lockwood) in Heart Infusion Broth.			
pH	7.6	7.1	6.7
Sulfanilamide mg%			
5%	40%	19%	0%
10%	49%	39%	8%

Using the dissociation constants it is possible to express the ratio of anion concentrations of two acids of different strength as a function of $[H^+]$, as follows:

$$\frac{C_B}{C_S} = \frac{[B^-]}{[S^-]}\left\{\frac{K_S[H^+]}{([H^+] + K_S)K_B} + \frac{K_S}{[H^+] + K_S}\right\} \qquad (1)$$

where $[S^-]$ and $[B^-]$ denote the concentration of the sulfonamide and PAB anions, C_S and C_B the total concentrations and K_S and K_B the respective dissociation constants. When $[H^+]$ is at least 100 times as large as K_S then $([H^+] + K_S)$ may be simplified to $[H^+]$ thereby introducing an error not exceeding 1%. For such ranges of $[H^+]$ the equation can therefore be simplified to

$$\frac{C_B}{C_S} = \frac{[B^-]}{[S^-]}\left(\frac{K_S}{K_B} + K_S \cdot \frac{1}{[H^+]}\right) \qquad (2)$$

[3] Walker, *Z. physik. Chem.*, 1905, **51**, 709, and Holmberg, *Z. physik. Chem.*, 1908, **62**, 728.

The hypothesis that an anionic form is the effective agent requires that $[B^-]/[S^-]$ is a constant at any selected bactericidal effectiveness, for instance at one-half maximum growth. In $[H^+]$ ranges where Equation 2 applies, C_B/C_S plotted against $1/[H^+]$ should be a straight line. Also sulfonamides characterized by a higher K_S should give a plot with a steeper slope. On the other hand, as $[H^+]$ becomes significant with respect to K_S equation (1) applies. Accordingly the slope should decrease and become zero where dissociation is substantially complete.

Experimental. Bacterial growth rates were determined at a constant pH by using strongly buffered synthetic media and making measurements early in the log phase of growth where the hydrogen ion concentration was adequately controlled. The inoculated medium was divided into portions which were adjusted to the desired pH values with a few drops of concentrated acid or alkali. After a short incubation period the medium was dispensed into culture tubes containing a sulfonamide concentration well over the minimum required to inhibit growth and containing for each pH a series of PAB concentrations. Incubation was continued and growth was followed by turbidity measurements or plate counts. The growth rates were thus determined and the molar ratio of PAB to sulfonamide permitting a rate of growth one-half that of the control tube was computed for each pH. In the figure this molecular ratio C_B/C_S is plotted against the reciprocal of the hydrogen ion concentration.

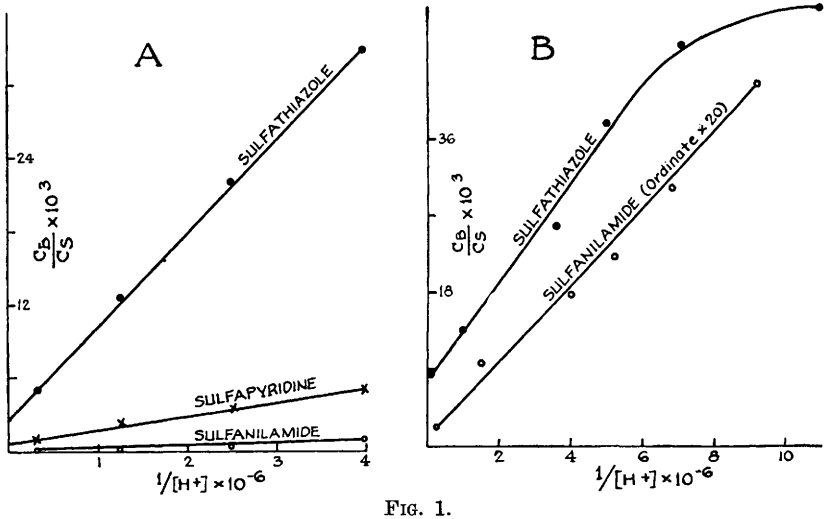

FIG. 1.

Mol ratio of PAB/sulfonamide permitting one-half maximum growth as an inverse function of the hydrogen ion concentration.

Values computed from growth studies on *E. coli* in synthetic medium at controlled pH.

From Fig. 1 A, it is apparent that in $[H^+]$ ranges where equation 2 applies, the predicted straight line plot is obtained, whereas in Fig. 1 B, it is shown that due to its high dissociation constant the sulfathiazole curve bends in accordance with equation 1. Moreover, the predicted relation between slope and K_S obtains.

As shown in Fig. 1 B and in accordance with equation 1 the increase of the efficiency of sulfathiazole with pH becomes progressively smaller, beginning approximately at pH 7. On the other hand, the corresponding point of inflection for sulfanilamide would be in the neighborhood of pH 10.5. If the pH is gradually increased above a value of 7, sulfanilamide should approach sulfathiazole in effectiveness. Preliminary experiments with organisms which grow in synthetic media buffered at slightly above pH 9 indicate that sulfanilamide actually approaches sulfathiazole in efficiency when this high pH is maintained.

Discussion. It should be borne in mind that while the degree of acidic dissociation governs the effectiveness of the active sulfonamides studied, other important factors are involved in the mechanism of sulfonamide action.

Further, this is only one aspect of the clinical problem which is complicated by considerations of absorption, solubility, toxicity, excretion, etc. Nevertheless, it would appear highly advantageous to maintain the pH of such infected loci that are to be treated with sulfonamides at the highest physiologically acceptable value. At pH 9 there is only comparatively little difference in the bactericidal effect of sulfanilamide and sulfathiazole.

Summary. From the nature of the function relating the effectiveness of sulfonamides to the hydrogen ion concentration the conclusion can be drawn that the active agent in a sulfonamide solution is an anionic species.

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Factors Influencing the Choice of Media for *in vitro* Sulfonamide Studies.

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Certain media are reported to inhibit the action of sulfonamides